An interim analysis on the predictive accuracy of strep B carrot broth kit versus lim broth in detecting group B streptococcus colonization among pregnant patients between 35-37 weeks age of gestation in a tertiary hospital*

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INTRODUCTION

Clinical trials and well-designed observational studies have demonstrated that administering intravenous antibiotics to women during labor who are at risk for transmitting Group B Streptococcus (GBS) to newborns can prevent invasive disease in the first week of life.[1-2] The Center for Disease Control (CDC) revised guidelines for the prevention of early-onset GBS disease issued last 2002 recommended universal culture-based screening of all pregnant women at 35-37 weeks age of gestation to improve the identification of women who should receive intrapartum antibiotic prophylaxis.[3]

An estimated > 15,000 cases and > 1,300 deaths due to GBS disease occur annually in the United States before active prevention was initiated.[4] Increased prevention activities were done in the 1990's,[5] which resulted in striking declines in disease incidence and further reduction followed after the issuance of the recommendation for universal screening in 2002.[6] However, GBS disease remains the leading infectious cause of morbidity and mortality among newborns in the United States.[7-8]

Group B streptococcus or *Streptococcus agalactiae* is a gram positive bacterium that causes invasive disease primarily in infants, pregnant or postpartum women, and older adults, with the highest incidence among young infants.[6]

In recent years, CDC has estimated 1,200 cases of early-onset GBS invasive disease per year. Approximately 70% of these cases are among those born at term (≥37 weeks' gestation).[6]

Early-onset infections are acquired vertically through exposure to GBS from the vagina of a colonized woman. Neonatal infection occurs when GBS ascends from the vagina to the amniotic fluid after onset of labor or rupture of membranes. GBS also can invade through intact membranes.[9] During passage through the birth canal, infants also can become infected with GBS. Those who are exposed to the organism through this route can become colonized in the gastrointestinal or respiratory tracts at the mucous membrane sites, but these colonized infants most commonly remain healthy.[10]

Maternal intrapartum GBS colonization is the principal risk factor for infant early-onset disease. In the absence of any intervention, an estimated 1%-2% of infants born to colonized mothers develop early-onset GBS infections.[11] Approximately 10%-30% of pregnant women are colonized with GBS in the vagina or rectum.[12-13] The gastrointestinal tract serves as the primary reservoir for GBS and is the probable source of vaginal colonization.[10] Heavy colonization associated with higher risk for disease, compared to that from selective broth only.[14]

Gestational age < 37 completed weeks, longer duration of membrane rupture, intra-amniotic infection, young maternal age, black race, and low maternal levels of GBS-specific anticapsular antibody are added risk factors for early onset GBS (EOGBS) disease.[15-16] These women had 6.5 times the risk for having an infant with early-onset GBS disease compared with women who were colonized prenatally but had none of the risk factors.[17]

It has been recommended that GBS screening should be performed and intrapartum antibiotic prophylaxis be given but the execution of these has been suboptimal due to limited GBS screening during hospital admission.[18]

A recent systematic review in 2010 confirms the recommendations to screen for GBS at 35-37 weeks' gestation.[19] GBS culture is the standard for the detection of GBS colonization, but its disadvantage is it takes 24-72 hours before the results are released thus culture-based testing is suitable for antepartum screening.

The use of a highly sensitive and specific test with prompt turn-around time can assess intrapartum GBS colonization and thus guide intrapartum antibiotic prophylaxis. Rapid tests such as optical immunoassays and enzyme immunoassays, fluorescence in situ hybridization, and latex agglutination tests were not as sensitive and specific to replace the established culture method.[20-21] Recently, molecular testing methods have been established, including DNA probes[22] and nucleic acid amplification

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tests (NAAT) such as polymerase chain reaction (PCR).[23] Compared to culture, the performance of commercially available NAAT on non-enriched samples was alterable and inadequate. With the use of an enrichment step, the sensitivity of NAAT for GBS can be as high as 92.5-100.0% but the latter will increase the turnaround time. A rapid PCR test can detect non-viable or low-count bacteria and has a rapid turn around time of an hour.[24]

Regardless of the test selected to identify GBS, use of an enrichment broth improves detection markedly. When direct agar plating is done instead of selective enrichment broth, 50% of women who are GBS carriers have false-negative culture results.[25-26] Todd-Hewitt, TransVag broth or Lim broth are examples of selective enrichment broths.[27]

The conventional measures for identifying GBS after selective enrichment is through isolation on subculture to blood agar plates followed by presumptive identification by the CAMP test[28] or serologic identification with group B streptococcal antisera.[29] Chromogenic agars that undergo color change when beta-hemolytic colonies of GBS are present have recently become accessible but the majority will not detect non-hemolytic strains.[30]

Step B carrot broth, a chromogenic agar, had exceptional diagnostic performance compared to that of Group B Lim broth, because of direct reporting of GBS cases based on an orange color change, thus decreasing overall labor and material costs.[31] Sensitivity, specificity, positive predictive value, and negative predictive value were 92%, 100%, 100%, and 98.3%, respectively.

Despite these facts, still there are concerns on the real-world turnaround time, lack of antimicrobial susceptibility, available 24-hour service, staffing requirements, and their costs. Current evidence does not support their use in replacement of antenatal culture or risk-based assessment of women with unknown GBS status intrapartum.[32]

In the United Kingdom, universal swab based screening is not recommended because of the low prevalence of EOGBS disease (0.5 per 1,000 births) even in the absence of systematic screening or widespread intrapartum antibiotic prophylaxis and high prevalence (60–70%) of clinical risk factors.[33] This risk-based strategy can prevent 67% of EOGBS by giving intrapartum antibiotic prophylaxis to 17% of pregnant women.[34] Thus, universal swab-based strategy was found to be not cost-effective in the UK.[35]

In the developed world, the most common agents of early neonatal sepsis are group B streptococci (GBS) and *Escherichia coli*.[36] Yet here GBS sepsis was conspicuous for its absence, resonating results from the international infections in pregnancy study group, which found GBS colonization rates to be one half compared to those found in Dublin, Ireland, and one third that found in Philadelphia, the United States.[37-38] GBS was found in 2.3% sepsis episodes in those with hospital acquired neonatal sepsis in developing nations.[39]

A 2010 study done showing GBS prevalence in reproductive-age women at a tertiary care center was 8.2 or 16% in Japan, 7.5% in the Philippines (Manila) and 8.0% in Korea.[40] This is challenging given that existing guidelines for neonatal sepsis – such as the WHO's pocket manual for the management of hospital illnesses in resource-poor countries[41] adopt that GBS and fully sensitive enteric gram negative rods are major agents of early neonatal sepsis.

There has been considerable progress in the prevention of early-onset GBS disease. Despite these developments, important challenges still remain. Significant differences persist among racial and ethnic groups, despite the decline in early onset disease. Research aimed at better understanding racial or ethnic differentiations in GBS disease could lead to occasions for more efforts for effectual prevention. This study may possibly be the start of establishing local guidelines regarding GBS screening and subsequent perinatal prevention of GBS disease in our country.

OBJECTIVES

This research had three objectives: to determine the accuracy of Group B streptococcus colonization among pregnant patients between 35-37 weeks age of gestation in a tertiary hospital, to determine the sensitivity, specificity, positive predictive value, negative predictive value and likelihood ratio of Strep B carrot broth kit versus Lim broth in screening for Group B streptococcus colonization and to determine the prevalence of Group B streptococcus colonization among pregnant patient between 35-37 weeks age of gestation in a tertiary hospital.

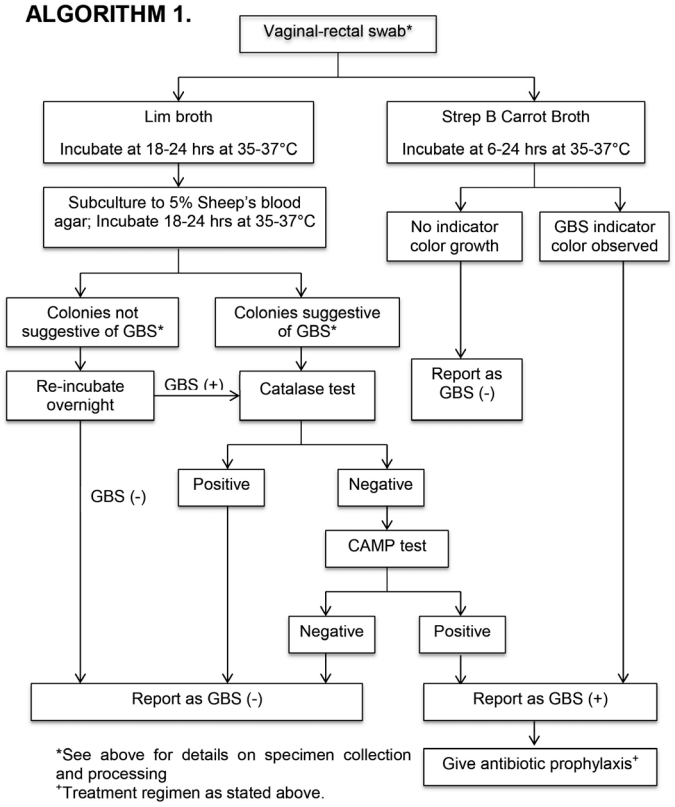
METHODS

Study population

This is a cross-sectional study conducted at a tertiary hospital from April 2016 to September 2016. The population was composed of pregnant patients between 35-37 weeks age of gestation during a prenatal check-up. The inclusion Criteria were the following: pregnant patients between 35-37 weeks age of gestation during a routine prenatal check-up who were not in labor, with intact bag of waters and with informed consent. The exclusion criteria were pregnant patients with vaginal discharge that had an undiagnosed vulvovaginal infection, those with current infection such as pneumonia and viral exanthems, currently receiving antimicrobial treatment for any infection, with ruptured bag of waters, fever, signs of infections and those who withdrew consent.

This study is ongoing and the data presented here

ALGORITHM 1.



are based on interim results including 40 patients but for completion of this study, a minimum of 112 samples are required based on a level of significance of 5%, a sensitivity of 92% with a confidence interval of 80.77 to 97.78%, and prevalence of 35% as noted from the reference articles by Church DL et al[31] and Schuchat[42].

Data Collection

All pregnant patients between 35-37 weeks age of gestation, fulfilling the inclusion/exclusion criteria and who gave informed consent were included in the study. For every patient recruited into the study, the lead investigator and resident in charge completed the patient case report form and patient data sheet which included demographic data, personal, social, medical, obstetric, and prenatal history.

The culture-based technique in isolating GBS used in this study was adapted from the protocol of the Centers for Disease Prevention and Control released in 2010.

The principal investigator or resident in charge collected two rectovaginal swabs from each patient. (Algorithm 1) The specimens were collected by swabbing the lower vagina and rectum through the anal sphincter using 2 sterile cotton pledget swabs. The swabs were processed within 24 hours upon collection. If the swabs were processed beyond 24 hours, the samples were refrigerated at a temperature of 2-6^{0}C.

The specimen bottle was sent to the Microbiology section. One swab was placed in Lim broth and the tube gently mixed. The tube was then incubated for 18-24 hours at 35-37°C. After incubation, a sterile wire loop was dipped into the Lim broth to obtain approximately 0.01 ml of the inoculum and was streaked in 5% sheep's blood agar. The plates were incubated for 18-24 hours at 35-37 degrees C in an aerobic atmosphere. If no growth was observed, the plates were re-incubated and inspected after 48 hours.

The medium was inspected and organisms suggestive of GBS identified (examples are: narrow zone of beta hemolysis on blood agar, gram-positive cocci/chains). A well-isolated colony was picked using a sterile wire loop and placed on a sterile, clean, glass slide and a drop of 3% H_2O_2 added to the slide. The colony was mixed with the hydrogen peroxide and observed for bubble formation. The presence of this reaction was indicative of catalase formation of the bacteria present in the sample and was indicative of the presence of staphylococci bacteria. A negative catalase result did not show bubble formation and was indicative of the presence of streptococcus species.

CAMP test was performed to differentiate Group B streptococcus with a positive result. Using an inoculating loop a beta-lysin-producing *Staphylococcus aureus* is streaked into a straight line across the center of a sheep's blood agar plate. The test organism was streaked in a straight line perpendicular to the *S. aureus* leaving 1 cm space between the two streaks. The plate was incubated at 37 degrees Celsius in ambient air for 18-24 hours. A positive result showed enhanced hemolysis by an arrow head-shaped zone of beta-hemolysis at the junction of the two organisms and was indicative of GBS. A CAMP negative result showed no enhanced hemolysis.[43]

The second swab was placed in a tube of Carrot Broth (SBCB) containing a SBCB tile, which was previously dropped into the broth tube. The tube cap was replaced and screwed down tightly. The inoculated Strep B Carrot Broth tube with swab and tile was incubated for 6 to 24 hours at 35 degrees C. The tubes were examined for an orange to red color change and/or spots typical of group B streptococci. If no color was observed, the tubes were re-incubated and inspected after 48 hours.[44]

If the results showed that GBS was positive, penicillin prophylaxis was given to these patients when in labor until delivery.[15]

All data gathered from patients and results obtained from this investigation were recorded in the patient data sheet and treated as confidential information, privy only to the research team and the patient.

RESULTS

Descriptive statistics was used to summarize the demographic and clinical characteristics of the patients included in the study. Frequency and proportion was used to describe the categorical variables, while mean and standard deviation was used for interval/ratio variables. Missing variables were neither replaced nor estimated. Sensitivity, specificity, positive and negative predictive values, and their corresponding 95% confidence intervals were determined. Data analyses were performed using STATA 12 and MedCalc.

A total of 40 pregnant patients were included in this study. The mean age of patients was 26.05 + 4.72. More than half the patients (21/40, 52.5%) were multiparous. The demographic profile of the patients were tabulated (Table 1).

The prenatal profile of the patients enrolled in the study were computed (Table 2). Majority of the patients (33/30, 82.5%) were at 35 to 36 6/7 weeks age of gestation. All patients had no previous GBS screening. One patient had a previous baby possibly with GBS infection and was

Table 1. Demographic profile of 40 pregnant patients who underwent Group B streptococcus (GBS) screening

	Frequency (%); Mean + SD
Age	26.05 + 4.72
Status	
Single	26 (65)
Married	14 (35)
Gravidity	
G2 and up	21 (52.5)
G1	19 (47.5)
Parity	
P1 and up	17 (42.5)
P0	23 (57.5)
Past medical history	
Previous surgery	5 (12.5)
Hepatitis B infection	2 (5)
DM/GDM	2 (5)
Pancreatitis	1 (2.5)
Asthma	1 (2.5)
VDRL	1 (2.5)
Education status	
College graduate	9 (22.5)
College undergrad/ vocational	9 (22.5)
High school graduate	21 (52.5)
Below High School	1 (2.5)

Table 2. Prenatal profile of 40 pregnant patients who underwent Group B streptococcus (GBS) screening

	Frequency (%)
Age of gestation	
35 to 36 6/7 weeks	33 (82.5)
37 weeks	7 (17.5)
Without previous GBS screening	40 (100)
Without previous baby with GBS	39 (97.5)
Number of prenatal check-ups	
0-5	38 (95)
6-10	2 (5)
Illnesses during pregnancy	
UTI	6 (15)
Asymptomatic bacteriuria	4 (10)
Preterm labor	1 (2.5)
Pancreatitis	1 (2.5)
Medications taken	
Prenatal meds	29 (72.5)
Cefuroxime	6 (17.5)
Metronidazole	1 (2.5)
Unrecalled	4 (10)

Table 3. Prenatal laboratory workup results of 40 pregnant patients who underwent Group B streptococcus (GBS) screening

	Frequency (%); Mean + SD
Culture positive for GBS	1 (2.5)
Urine culture (n=27)	
No growth	15 (55.56)
Positive growth	10 (37.04)
None	2 (7.41)
STD workup (n=34)	
Normal STD workup	31 (91.18)
HBSAg normal	1 (2.94)
Normal FBS	1 (2.94)
(+) RPR, (+) syphilis, RPR quanti 1:4	1 (2.94)
Pap smear (n=32)	2 (6.25)
Marked inflammation	7 (21.88)
Moderate inflammation	2 (6.25)
Mild inflammation	21 (65.63)
Normal pap smear	10.43 + 2.74
WBC (n=32)	

Table 4. Diagnostic accuracy of Strep B carrot broth kit versus Lim broth among 40 pregnant participants that were screened for GBS colonization.

	Lim broth positive	Lim broth negaive	Total
	Frequency (%)		
SBCB positive	1 (100)	2 (5.13)	3 (7.5)
SBCB negative	0	37 (94.87)	37 (92.5)
Total	1 (100)	39 (100)	40 (100)

Sensitivity	100% (2.5-100)	Positive LR	19.5 (05.06- 75.21)
Specificity	94.87% (82.68-99.37)	Negative LR	0
PPV	33.33% (0.84-90.57)	Accuracy	97.44%
NPV	100% (90.51-100)		

PPV, positive predictive value; NPV, negative predicted value; LR, likelihood ratio.

given antibiotics for several days. Nearly all patients had at least five prenatal check-ups (38/40, 95%). The most common illness during pregnancy among the participants were UTI (n=6) and asymptomatic bacteriuria (n=4).

Table 3 shows the summary of the laboratory and workup results of the patients enrolled in the study. The mean WBC was 10.43 + 2.74 (mg/dl). Urine culture, STD workup, and pap smear were performed on most of the patients. Of the forty patients, there was one patient who was culture positive for Group B streptococcus, giving a prevalence of 2.5%.

To determine the diagnostic accuracy of using strep B carrot broth in screening patients for GBS colonization, the sensitivity, specificity, positive/negative predictive values and positive/negative likelihood ratios were computed (Table 4).

The sensitivity of the strep B carrot broth, or the proportion of pregnant patients who are correctly identified as positive for GBS colonization was 100%, (95% CI, 2.5-100). The specificity of the strep B carrot broth, or the proportion of those who do not have the GBS colonization that are correctly identified by the strep B carrot broth as negative was 94.87% (95% CI, 82.68-99.37).

The positive predictive value (PPV), or the proportion of strep B carrot broth positives (Figure 1) that are truly GBS colonized, was at 33.33% (95% CI 0.84-90.57). The negative predictive value (NPV), or the proportion of strep B carrot broth negatives who are not GBS colonized, was at 100% (95% CI 90.51-100).

The positive likelihood ratio (LR+) was 19.5. This value tells us that the strep B carrot broth is 19.5 times more likely to give a positive screening test if a patient has GBS colonization than in patients who do not have GBS colonization. As a rule, the higher is the computed positive LR from 1 as a reference point, the stronger is the evidence for predicting the presence of the disease, and in this case, of a patient having GBS colonization. The overall diagnostic accuracy of strep B carrot broth for our study participants was 97.44%.

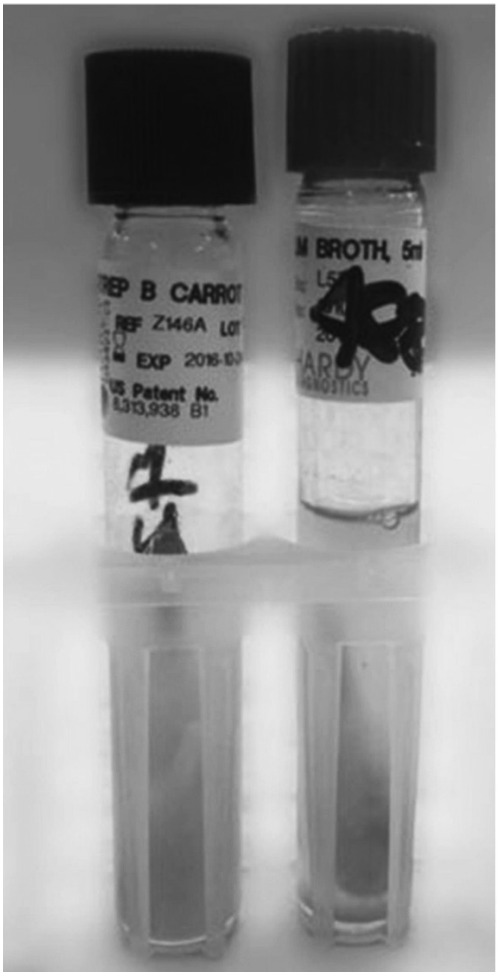


Figure 1. SBCB showing orange color change positive for GBS.

DISCUSSION

There are not enough studies in our country intended to better understand GBS disease in order to be able to come up with better preventive efforts. We then begin to wonder if GBS screening is really requested by the private and government based obstetricians on all their pregnant patients during the prenatal period or do they individualize screening based on the patients' risk factors.

Guidelines for the prevention of perinatal *S. agalactiae* disease have evolved from a paradigm based in part, on maternal risk factors, to one centered on culture, mainly as a result of data indicating the superiority of the latter approach.[23]

The development of relatively rapid laboratory tests to identify GBS moves us closer to the possibility of an intrapartum test for GBS colonization screening. A highly sensitive, less complex test and a quick processing time could be used to establish intrapartum GBS colonization, thereby overcoming some of the fundamental limitations in late antenatal screening. Using selective enrichment broth that includes chromogenic pigments, such as Strep B Carrot Broth, has lately been included in the CDC's Prevention of Perinatal Group B Streptococcal Disease.[10] Strep B Carrot Broth demonstrates increased sensitivity and specificity, reduced incubation time, and reduced need for additional plated media.[23, 31]

Lim broth enrichment, based on latest studies, have shown 18 to 35% more clinical isolates of *S. agalactiae* compared to culture with primary solid medium. On the other hand, carrot broth, a modification of Granada medium, is 8 to 15% more sensitive than Lim broth for detection of the organism.[23] Our data indicate that the sensitivity of Strep B carrot broth, was 100% and the specificity 94.87%. Among the 3 SBCB positive results, only 1 also showed positive results with Lim broth. The positive predictive value of SBCB was at 33.33% (95% CI 0.84-90.57) while the negative predictive value was at 100% (95% CI 90.51-100).

A culture-based approach for the detection of *S. agalactiae*, especially one that requires sub culturing to solid medium, possess a number of limitations beyond the increased time to detection. Not all *S. agalactiae* strains exhibit beta-hemolysis. Competing saprophytic flora, feminine hygiene products and the site of specimen collection can compromise recovery of the organism, as well as circumstances related to specimen transport.[23]

The production of orange, red, or brick red pigment is a distinctive characteristic of hemolytic GBS due to its reaction with substrates such as starch, peptone, serum, and folate pathway inhibitors. These components serve as the basis for culture media used to distinguish and identify these organisms. GBS detection with Granada media is only possible with beta-hemolytic colonies, thus providing evidence of a direct genetic linkage between pigment production on Granada media and hemolysin production. Beta-hemolytic, pigment-producing GBS occurs with 95.3 to 99.5% of all GBS strains isolated from clinical specimens.[45]

Enrichment broth procedures are known to be more sensitive than plate methods in their ability to detect GBS colonization. Strep B Carrot Broth is used to detect beta-hemolytic GBS without the need for further testing and, as an enrichment broth, has greater sensitivity. The overall diagnostic accuracy of strep B carrot broth for our study participants was 97.44%.

Studies regarding GBS screening and its prevalence in the Philippines has been challenging. The lack of interest, funding, and readily available culture media are contributing factors to such, thus culture-based guidelines for GBS screening among Filipinos have not been established. This study is a forerunner in establishing our own culture-based guidelines for GBS colonization in pregnancy.

Based on the interim results of this study, the incidence of GBS colonization is 2.5 % based on Lim broth and 7.5% based on SBCB. In a study where 61 vaginal/rectal swabs from pre-natal patients were investigated for GBS using three different methods, it was concluded that Carrot broth was the best performing antepartum assay and was superior to Lim broth standard culture for detection of GBS at 35-37 weeks gestation.[46]

In summary, the high negative predictive value and increased sensitivity associated with SBCB makes it comparable to or even superior to the standard Lim broth. Being a third world country, it is inevitable that cost becomes a factor in the routine screening of all women during the prenatal period. The accuracy of SBCB that is shown here, as well as its availability, affordability and rapid turn around time makes it feasible for all women to be screened, thereby decreasing neonatal morbidity and mortality.

CONCLUSION

The prevalence of GBS colonization among pregnant women between 35-37 weeks age of gestation in our country is 7.5%. Strep B Carrot Broth is superior to and can thus be used as an alternative for standard culture in GBS screening and also significantly reduces labor and cost.

LIMITATIONS

This study is a preliminary study and is for completion by September 2016. This study also does not intend to identify the growth of non-beta hemolytic streptococci.

The Strep B carrot broth chromogenic agar inoculation step only comprises the presence or absence of color change and no further subculture is done.

RECOMMENDATIONS

It is recommended that the neonatal outcomes, length and course of hospital stay of those whose born of mothers who were found to be positive for GBS be taken into account and thus correlated with the prevalence of neonatal morbidity and mortality.

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