








Prevalence of early neonatal sepsis and positive maternal culture for group B beta-hemolytic *Streptococcus*

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SUMMARY

OBJECTIVE: The aim of this study was to evaluate the prevalence of early neonatal sepsis in pregnant women with a positive culture for group B beta-hemolytic *Streptococcus* in a middle-income city in Southeastern Brazil.

METHODS: A retrospective cohort study was conducted, involving singleton low- and high-risk pregnancies in whom group B beta-hemolytic *Streptococcus* cultures were evaluated between 35 and 37 weeks of gestation using vaginal and anal swabs. A specific medium (Todd-Hewitt) was used for culturing. The pregnant women were divided into two groups based on positive (n=201) and negative (n=420) cultures for group B beta-hemolytic *Streptococcus*.

RESULTS: The maternal colonization rate by group B beta-hemolytic *Streptococcus* was 32.3%. The prevalence of early neonatal sepsis was 1.0% (2/201) among patients with a positive group B beta-hemolytic *Streptococcus* culture and 1.9% (8/420) among patients with a negative culture. Among the patients who underwent adequate prophylaxis, crystalline penicillin G was used in 51.9% (54/104), followed by cefazolin in 43.3% (45/104), ampicillin in 3.8% (4/104), and clindamycin in 1.0% (1/104). A model that included prematurity ($p=0.001$) proved to be an independent risk predictor of early neonatal sepsis [$\chi^2(1)=15.0$, odds ratio: 16.9, 95% confidence interval: 4.7–61.6, $p<0.001$, Nagelkerke $R^2=0.157$].

CONCLUSION: The prevalence of a positive culture for group B beta-hemolytic *Streptococcus* was high. However, the prevalence of early neonatal sepsis was low in pregnant women with both positive and negative group B beta-hemolytic *Streptococcus* cultures and in pregnant women with a positive culture who underwent both adequate and inadequate antibiotic prophylaxis. Prematurity proved to be an independent predictor of early neonatal sepsis, considering the entire study population.

KEYWORDS: *Streptococcus*. Neonatal sepsis. Antibiotic prophylaxis. Pregnancy outcomes.

INTRODUCTION

Group B beta-hemolytic *Streptococcus* (GBS) is an important cause of maternal and neonatal morbidity and mortality. In pregnant women, maternal colonization by GBS is associated with adverse perinatal outcomes, such as low birth weight, preterm birth, and premature rupture of ovular membranes (PROM)¹. In newborns, GBS is an important cause of neonatal sepsis, meningitis, and pneumonia². Neonatal infection by GBS is divided into early (within the first week of life) and late (between 1 week and 3 months of life) onsets³, and maternal colonization by GBS is the leading cause of early neonatal sepsis in newborns⁴.

The rate of maternal GBS colonization was observed to be 13.4% in a study conducted in Saudi Arabia⁵ and 14.6% in a study conducted in Ethiopia⁶. Therefore, universal screening using vaginal and anal swabs between 35 and 37 weeks

of gestation is recommended for GBS detection. The primary risk factor for neonatal GBS early-onset disease is maternal colonization of the genitourinary and gastrointestinal tracts. Vertical transmission usually occurs during labor or after the rupture of membranes^{1,7}.

Intrapartum administration of antibiotics reduces the rate of early neonatal sepsis due to GBS, with crystalline penicillin G being the most commonly used antibiotic⁸; however, high resistance rates have been described for antibiotics such as clindamycin and erythromycin⁹. Inadequate antibiotic prophylaxis for maternal colonization by GBS may lead to increased rates of early neonatal sepsis¹⁰.

Prematurity, low birth weight, and antepartum fetal tachycardia proved to be significant risk factors for pneumonia and sepsis, whereas prematurity, low birth weight, and an anomalous presentation were identified as risk predictors for neonatal

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conjunctivitis. Positive GBS cultures were found in 46% of neonatal sepsis cases¹¹.

The main objective of this study was to evaluate the prevalence of early neonatal sepsis in pregnant women with a positive culture for GBS in a middle-income city in Southeastern Brazil. Secondary objectives were to evaluate the association between positive and negative cultures for GBS, PROM, and preterm birth and to evaluate the best predictors of early neonatal sepsis in patients who underwent culture for GBS.

METHODS

A retrospective cohort study was conducted at the Mário Palmério University Hospital (MPHU) in the city of Uberaba, Minas Gerais, Brazil, by analyzing the medical records of pregnant women who attended the hospital from March 2016 to March 2019. According to GBS culture, pregnant women included were separated into two groups: positive GBS culture and negative GBS culture. Subsequently, for secondary analyses, pregnant women with positive culture were subdivided into adequate prophylaxis and inadequate prophylaxis for GBS. The study was approved by the Research Ethics Committee of the University of Uberaba (CAAE: 52299421.7.0000.5145).

The study included all patients with singleton pregnancies and without fetal malformations or chromosomal anomalies who were treated in the MPHU's low- and high-risk prenatal outpatient clinics, delivered in the MPHU's labor ward, and had a GBS culture performed between 35 and 37 weeks of gestation.

In our service, pregnant women are routinely screened for GBS cultures after vaginal and perianal swab collection. Immediately after collection, each swab was individually inserted into a tube containing Stuart transport medium (Biocon®, Belo Horizonte, Brazil) and stored at room temperature before being sent to the laboratory within a maximum period of 3 days.

In the laboratory, the material was cultured in a specific medium (Todd-Hewitt), which provides essential nutrients for the development of the microorganism while partially inhibiting other microorganisms. At a temperature of 35°C–37°C, the reading was taken manually to identify the growth and count of GBS colonies after 24 h in this environment.

In our service, prophylactic antibiotics are indicated for all pregnant women with a positive culture for GBS upon admission for induction of labor or labor management, except for those undergoing cesarean section with intact membranes (prophylactic antibiotics are administered prior to skin incision). According to the institutional protocol, prophylactic antibiotics are administered to all pregnant women with a negative

culture taken 5 weeks or more ago in labor with rupture of membranes lasting more than 18 h. If crystalline penicillin is unavailable, a 2-g intravenous (i.v.) dose of ampicillin (i.v.) is given as a loading dose, followed by 1 g (i.v.) every 6 h until delivery. In case of penicillin allergy, clindamycin 900 mg (i.v.) is given every 8 h until delivery. In patients undergoing cesarean section, cefazolin 2 g (i.v.) may also be given as a loading dose, followed by 1 g (i.v.) every 6 h until delivery. Administration of two doses of any antibiotic within 4 h of delivery is considered adequate prophylaxis¹².

The following outcomes were considered adverse perinatal outcomes: neonatal sepsis, maternal admission to the intensive care unit (ICU), chorioamnionitis, neonatal ICU admission, Apgar score <7 at 1st minute, and early neonatal death (up to 48 h of life). Maternal and infant ICU admission was based on any clinical instability that warranted intensive care. Not all cases admitted to the ICU as a result of GBS infection were included in the study.

The presence of any of the following abnormalities was considered early neonatal sepsis: leukocytes <5,000/mm³ or >25,000/mm³ at birth, >30,000/mm³ at 12–24 h of life, or ≥21,000/mm³ at 2 days of life; increased immature neutrophils; platelets <150,000/mm³; immature/total neutrophil ratio >0.3; and the presence of signs such as lethargy, irritability, thin pulse, cyanotic extremities, and tachypnea¹³.

Quantitative variables were subjected to a normality test (D'Agostino-Pearson). The mean and standard deviation were used to represent variables with a normal distribution. The median and interquartile range were used to represent variables with a non-normal distribution. Categorical variables were described in absolute and percent frequencies and represented in Tables 1–3. To study the difference between categorical variables and their proportions, the chi-square test was used. Mann-Whitney and Student's t-tests were used to study the impact of the study group on continuous variables. Stepwise binary logistic regression analysis was used to assess the best predictors for early neonatal sepsis. The significance level for all the tests was set at $p < 0.05$.

RESULTS

During the study period, 857 pregnant women were admitted, 229 of whom were excluded because they did not collect the swab for GBS culture and 7 cases were excluded due to missing information in the medical records. For the final statistical analysis, 201 cases with a positive culture for GBS and 420 cases with a negative culture were considered, indicating a 32.3% maternal colonization rate by GBS.

Table 1. Clinical characteristics of the study population.

	Positive culture (n=201)	Negative culture (n=420)	p-value
Age (years)	28.0 (23.0–34.5)	28.0 (24.0–34.0)	0.531 [†]
Weight (kg)	78.0 (71.2–84.0)	77.5 (68.2–86.0)	0.532 [†]
Height (m)	1.65 (1.62–1.68)	1.65 (1.60–1.69)	0.163 [†]
BMI (kg/m ²)	28.0 (26.3–31.2)	28.4 (25.9–31.3)	0.678 [†]
Ethnicity			0.523 [§]
White	46.2% (92/199)	49.3% (203/412)	
Black	11.1% (22/199)	8.3% (34/412)	
Mixed	41.2% (82/199)	41.7% (172/412)	
Asian	1.5% (3/199)	0.7% (3/412)	
Smoking	6.5% (13/200)	7.7% (32/417)	0.600 [§]
Number of pregnancies	2.0 (1.0–3.0)	2.0 (1.0–3.0)	0.281 [†]
Number of deliveries			0.030 [§]
None	3.0% (6/201)	7.6% (32/420)	
≥1	97.0% (195/201)	92.4% (388/420)	
Gestational age at vaginal collection (weeks)	36.1 (35.8–36.4)	36.0 (35.6–37.0)	<0.001 [†]
High-risk pregnancy	32.3% (65/201)	39.5% (166/420)	0.083 [§]
Premature rupture of ovular membranes	13.9% (28/201)	20.0% (84/420)	0.066 [§]
Preterm birth	4.5% (9/201)	7.1% (30/420)	0.200 [§]
Gestational age at delivery (weeks)	39.4 (38.6–40.0)	39.3 (38.4–40.0)	0.332 [†]
Antibiotic use prophylaxis	65.7% (132/201)	20.7% (87/420)	<0.001 [§]
Types of delivery			0.006 [§]
Vaginal	39.9% (79/198)	51.0% (212/416)	
Cesarean section	59.1% (117/198)	49.0% (204/416)	
Forceps	1.0% (2/198)	0.0% (0–416)	
Birth weight (g)	3270.0 (2933.0–3573.0)	3240.0 (2915.0–3520.0)	0.402 [†]
Apgar score 1st minute	8.0 (8.0–9.0)	9.0 (8.0–9.0)	0.007 [†]
Apgar score 5th minute	8.0 (8.0–9.0)	9.0 (9.0–10.0)	<0.001 [†]

[†]Mann-Whitney test; median (interquartile range); [§]chi-square; percentage (N/n); p<0.05.

Pregnant women with a positive culture for GBS had a lower prevalence of nulliparity (3.0% vs. 7.6%, $p=0.030$), a higher prevalence of prophylactic antibiotic use (65.7% vs. 20.7%, $p<0.001$), and a higher prevalence of cesarean sections (59.1% vs. 49.0%, $p=0.006$) than pregnant women with negative GBS cultures (Table 1). Among pregnant women with negative GBS cultures, 71.3% (62/87) received prophylactic antibiotics due to cesarean section, 24.1% (21/87) due to PROM>18 h, and 4.6% (4/87) for urinary infection treatment.

There was no significant association observed between positive and negative cultures for GBS and the presence of early neonatal sepsis ($p=0.399$), APGAR score<7 at the 1st minute ($p=0.081$), neonatal ICU admission ($p=0.802$), neonatal death

in the first 48 h ($p=0.148$), chorioamnionitis ($p=0.489$), and maternal ICU admission ($p=0.645$). The prevalence of early neonatal sepsis was 1.0% (2/201) among patients with a positive culture and 1.9% (8/420) among patients with a negative culture ($p=0.399$).

Pregnant women who received adequate prophylaxis had a significantly higher median number of doses than pregnant women who received inadequate prophylaxis (4.0 vs. 0.0, $p<0.0001$). Among the patients who received adequate prophylaxis, crystalline penicillin G was used in 51.9% (54/104), followed by cefazolin in 43.3% (45/104), ampicillin in 3.8% (4/104), and clindamycin in 1.0% (1/104). Antibiotic prophylaxis was not used in 70.1% (68/97) of patients with

Table 2. Clinical characteristics of pregnant women with a positive culture for B beta-hemolytic *Streptococcus* who received adequate or inadequate prophylaxis during labor.

	Adequate prophylaxis (n=104)	Inadequate prophylaxis (n=97)	p-value
High-risk pregnancy	35.6% (37/104)	28.9% (28/97)	0.309 ^s
Number of antibiotic doses	4.0 (4.0–4.0)	0.0 (0.0–1.0)	<0.0001 [†]
Antibiotic use			<0.001 ^s
Crystalline penicillin G	51.9% (54/104)	20.6% (20/97)	
Ampicillin	3.8% (4/104)	3.1% (3/97)	
Clindamycin	1.0% (1/104)	2.1% (2/97)	
Cefazoline	43.3% (45/104)	4.1% (4/97)	
None	0.0% (0/104)	70.1% (68/97)	
Gestational age at delivery (weeks)	39.4 (38.9–40.1)	39.3 (38.3–40.0)	0.130 [†]
Preterm birth	1.92% (2/104)	7.22% (7/97)	0.091 ^s
Types of delivery			0.701 ^s
Vaginal	42.7% (44/103)	36.8% (35/95)	
Cesarean section	56.3% (58/103)	62.1% (59/95)	
Forceps	1.0% (1/103)	1.1% (1/95)	
Birth weight (g)	3282 (489.7)	3197 (553.0)	0.253 ^f
Apgar score at 1st minute	8.0 (8.0–9.0)	8.0 (8.0–9.0)	0.779 [†]
Apgar score at 5th minute	9.0 (9.0–9.75)	9.0 (9.0–9.0)	0.728 [†]

^fStudent's t-test: mean (standard deviation); [†]Mann-Whitney test: median (interquartile range); ^schi-square test: percentage (N/n); p<0.05.

Table 3. Prediction of early neonatal sepsis, considering all cases included in the study, using positive culture for group B beta-hemolytic *Streptococcus*, inadequate prophylaxis, and prematurity as covariants.

	OR	CI 95%	p-value
GBS-positive	0.16	0.10–2.50	0.408
Inadequate prophylaxis	3.78	0.47–30.2	0.209
Prematurity	16.9	4.7–61.4	<0.001

OR: odds ratio; CI: confidence interval; GBS: group B beta-hemolytic *Streptococcus*; stepwise binary logistic regression; p<0.05.

inadequate prophylaxis. Among pregnant women with inadequate prophylaxis who received at least one dose of antibiotic, 20.6% (20/97) used crystalline penicillin G, 4.1% (4/97) used cefazolin, 3.1% (3/97) used ampicillin, and 2.1% (2/97) used clindamycin (Table 2).

There was no significant association between adequate and inadequate prophylaxis and adverse perinatal outcomes in pregnant women with a positive culture for GBS. There was no significant association between the groups regarding the presence of early neonatal sepsis (p=0.170), Apgar score at 1st minute<7 (p=0.671), neonatal ICU admission (p=0.654), neonatal death within first 48 h (p=0.333), chorioamnionitis, and maternal ICU admission (p=0.141). The prevalence of early neonatal sepsis was 1.9% (2/104) in patients with

adequate prophylaxis and 0.0% (0/97) in patients with inadequate prophylaxis for GBS.

Considering all cases included in the study, a stepwise binary logistic regression model was created to assess whether positive culture for GBS, inadequate prophylaxis, and prematurity are predictors of early neonatal sepsis. The models including positive culture for GBS (p=0.408) and adequate prophylaxis for GBS (p=0.209) were not adequate to predict early neonatal sepsis, whereas prematurity proved to be an independent predictor (p=0.001). This model was significant in predicting the risk of early neonatal sepsis [χ^2 (1)=15.0, odds ratio (OR): 16.9, 95%CI 4.7–61.6, p<0.001, Nagelkerke R²=0.157], with 98.4% prediction capacity (Table 3).

DISCUSSION

The universal bacteriological screening for GBS is controversial according to some scientific entities. The National Screening Committee of the United Kingdom does not recommend universal bacteriological screening for GBS¹⁴. Their view is that there is no clear evidence to show that testing for GBS routinely would do better than harm. The American College of Obstetricians and Gynecologists recommends a universal culture-based screening strategy for identifying candidates

for GBS intrapartum antibiotic prophylaxis, which has been demonstrated to be superior to risk-based screening protocols for the prevention of GBS early-onset disease¹. In our study, the prevalence of a positive GBS culture was high. Even using antibiotic prophylaxis in pregnant women with a positive culture for GBS, we did not find a significant difference in early neonatal sepsis between groups. Prematurity was an independent predictor of early neonatal sepsis in our study population.

In a meta-analysis of maternal colonization rates by GBS in Africa, 83 articles were evaluated, of which 57 studies were conducted in 5 sub-regions in 21 countries (22,206 pregnant women). The overall rectovaginal colonization rate was estimated at 19.3%. The highest estimate was observed in South Africa (23.8%), followed by North Africa (22.7%), while the lowest was found in East Africa (15.4%)¹⁵. In Germany and Uruguay, maternal colonization rates by GBS were 16 and 67.3%, respectively^{16,17}. In Brazil, a review of 21 articles found that the prevalence of maternal colonization by GBS ranged from 4.2 to 28.4% between 2008 and 2018, considering 3 geographical regions (South, Southeast, and Northeast) and 8 states¹⁸.

In the present study, a high rate of maternal colonization by GBS was observed, probably due to the institution's protocols for universal screening using anal and vaginal swabs between 35 and 37 weeks and a high proportion of black and mixed people in the state of Minas Gerais (53.5%). The rate of maternal colonization by GBS is directly related to the screening rate. Out of six Latin American countries studied, Nicaragua presented the lowest rate (0.8%), while Uruguay had the highest rate (67.3%)¹⁷. In a study with 526 pregnant women with positive screening for GBS, black African ethnicity and sexually transmitted diseases were the only independent risk factors for maternal colonization by GBS¹⁹. Even within the same country, the prevalence of GBS colonization can vary widely. The main reason for this difference may be related to local economic levels and environmental factors. Another important factor is the neglect of the detection method for GBS.

In the present study, there was no significant association between positive and negative cultures for GBS and the presence of early neonatal sepsis. The prevalence of early neonatal sepsis was 1.0% (2/201) among patients with a positive culture for GBS and 1.9% (8/420) among patients with a negative culture. In a study conducted in South Korea, the prevalence of early neonatal sepsis was 1.5% (2/134) among patients with a positive culture for GBS and 0.3% (3/1,024) among patients with a negative culture²⁰.

Regarding adverse perinatal outcomes, no significant statistical differences were observed between the groups with positive and negative cultures for GBS. In a retrospective American

study, Edwards et al.²¹ estimated the prevalence of GBS colonization, compared the risk of adverse pregnancy outcomes by GBS colonization status, and estimated the incidence of invasive GBS disease. They found that overall 21.6% of the population was GBS colonized. In the adjusted analyses, there was an increased risk of gestational diabetes in colonized pregnancies and a decreased incidence of short cervix, chorioamnionitis, wound infection, and operative delivery. In a study in South Korea, pregnant women with a positive culture for GBS presented lower rates of preterm births without differences in PROM and intrauterine infection than those with a negative GBS culture²⁰. In our study, the majority of pregnant women with GBS colonization routinely receive prophylactic intravenous antibiotics during labor. The resulting reduction in bacterial burden likely decreases the incidence of chorioamnionitis and wound infection rates and may decrease the risk of short cervix associated with subclinical infection.

In the present study, most pregnant women who underwent adequate prophylaxis used crystalline penicillin G (51.9%), followed by cefazolin (43.3%). However, antibiotic prophylaxis was not given to 70.1% of patients with inadequate prophylaxis. In a study in Ethiopia, most isolated GBS were sensitive to crystalline penicillin G and ampicillin, but erythromycin and clindamycin resistance were found in 50.0 and 40.9% of the isolated samples, respectively²². Of 3,494 GBS-positive cultures through a vaginal swab, penicillin resistance was observed in only 6 (0.2%). In a Chinese study, 636 (8.2%) of 7,726 pregnant women who were screened for GBS were positive, and 100% of this sample was sensitive to penicillin, which is recommended as the first choice for treatment and prevention of early neonatal sepsis²³. These results are consistent with the findings of the present study, in which most patients received prophylaxis with crystalline penicillin G.

In our study, no reduction in the rate of neonatal sepsis was observed in patients who underwent prophylaxis for GBS. Most patients who underwent prophylaxis for GBS used crystalline penicillin and cefazolin, followed by ampicillin and clindamycin. We speculate that the lack of reduction in neonatal sepsis may be explained by different regimens of antibiotic prophylaxis used in our institution. The time-dependent bactericidal mechanism of action of β -lactam antibiotics supports the efficacy of ampicillin and penicillin administered at least 4 h before delivery²⁴. No data specifically inform the clinical effectiveness of intrapartum antibiotic prophylaxis with cefazolin, but the pharmacokinetics and mechanism of bactericidal action for cefazolin are similar to those of penicillin and ampicillin that administration of cefazolin can be considered adequate prophylaxis against early-onset GBS. Although data on the

pharmacokinetics of clindamycin and vancomycin have been published, evidence on their clinical efficacy is more limited. Therefore, when non- β -lactam antibiotics of any duration are administered for intrapartum antibiotic prophylaxis of GBS, such treatment should be considered not fully adequate for neonatal risk assessment purposes²⁴.

Regarding inadequate treatment, in an Italian study, out of 136 pregnant women with an indication for antibiotic prophylaxis use, only 68 (50%) received adequate treatment¹⁰. This inadequate prophylaxis rate is very similar to that observed in the present study, which was 48.2%. In a French study, 5,997 pregnant women were evaluated between 2006 and 2008, and the GBS colonization rate ranged from 13 to 18%. In that study, it was observed that the percentage of pregnant women who received correct antibiotic prophylaxis remained stable during the period²⁵. In the present study, despite the high rate of inadequate prophylaxis, there were no cases of early neonatal sepsis in this group. Inadequate prophylaxis may contribute to an increased early neonatal sepsis rate and may be explained by the higher incidence of women in advanced labor, making it difficult to fully implement the antibiotic therapy protocol.

Failure to diagnose neonatal sepsis quickly, primarily due to its vague signs and symptoms, makes the disease more deadly and destructive. A blood culture report, as the only main solution, takes practically 2 days to generate a result. Therefore, there is a need to look into novel approaches that can help in the rapid prediction of neonatal sepsis. In the present study, a binary logistic regression model was created,

which showed that prematurity is an independent predictor of early neonatal sepsis. Spaans et al.¹¹ studied 8,215 births between 1983 and 1988 and observed 104 cases of pneumonia and 50 cases of sepsis. Cultures for GBS were positive in 46% of neonatal sepsis cases. After testing all risk factors identified by univariate analysis in a logistic regression model, tachycardia remained an independent predictor of neonatal pneumonia or sepsis.

CONCLUSION

The prevalence of positive culture for GBS was high. However, the prevalence of early neonatal sepsis was low in pregnant women with both positive and negative GBS cultures and in pregnant women with a positive culture who underwent both adequate and inadequate antibiotic prophylaxis. Prematurity proved to be an independent predictor of early neonatal sepsis, considering the entire study population.

AUTHORS' CONTRIBUTIONS

ABP: Conceptualization, Formal Analysis, Project administration, Supervision, Visualization. **JAVR:** Conceptualization, Visualization. **CGCS:** Data curation, Visualization, Writing – original draft. **MJAL:** Data curation, Visualization, Writing – original draft. **APMF:** Investigation, Visualization. **JTM:** Methodology, Visualization. **EAJ:** Validation, Visualization, Writing – review & editing.

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