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Group B streptococci colonization in pregnant women: risk factors and evaluation of the vaginal flora

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Abstract
Objective To determine the prevalence of group B streptococci (GBS) in our population, and to assess the association between risk factors and vaginal flora with maternal rectovaginal colonization.
Method Samples were obtained from 405 patients between 35 and 37 weeks of gestation. Swabs from the vaginal and perianal regions were cultured in Todd Hewitt and subcultured in blood agar. Colonies suggestive of GBS were submitted to catalase and CAMP test. The vaginal flora was evaluated on Gram stain vaginal smears. Socio-demographic and obstetric data were obtained by designed form. Considering maternal GBS colonization as the response variable, a logistic regression model was fitted by the stepwise method with quantitative and qualitative explanatory variables.
Results The prevalence of GBS colonization was 25.4%. The most frequent vaginal flora abnormalities were cytolytic vaginosis (11.3%), followed by bacterial vaginosis (10.9%), candidosis (8.2%) and intermediate vaginal flora II (8.1%). Logistic regression analysis revealed that maternal age, number of sexual intercourse/week, occurrence of previous spontaneous abortion, presence of candidosis and cytolytic vaginosis were associated with streptococcal colonization.
Conclusion The prevalence of GBS is high in pregnant women and is associated with sexual intercourse frequency, previous spontaneous abortion and the presence of candidosis or cytolytic vaginosis.

Keywords Group B streptococci · Pregnancy · Vaginal flora · Risk factors

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Introduction

During the last few decades, neonatal group B streptococcal (GBS) disease has been associated with significant rates of morbidity and mortality in the perinatal period [1]. GBS is the leading cause of early neonatal sepsis [2]. Maternal streptococcal colonization is also associated with increased risk of urinary tract infection and pregnancy complications, such as endometritis [3] and chorioamnionitis [3, 4], premature delivery and intrauterine death [5]. The main source of neonatal infection is maternal genital tract colonization [6]. GBS is transmitted vertically during labor and delivery and occurs in up to 80% of neonates born to colonized mothers [7].

The Centers for Disease Control and Prevention (CDC) currently recommend screening of all pregnant women for *Streptococcus agalactiae* between 35 and 37 weeks of gestation [8]. For those with rectovaginal GBS-positive cultures, intravenous penicillin prophylaxis during labor has been recommended and 70% reduction in neonatal

early-onset sepsis observed since this strategy was implemented [9].

According to the literature, between 6.5% [10] and 43.6% [11] of pregnant women have *S. agalactiae* colonized in the vagina or rectum. Maternal colonization rate may vary with population characteristics such as age, parity, socio-economic status, geographic location [12], presence of sexually transmitted diseases [13] and sexual behavior [14]. Differences in colonization rates can be attributed to variation in the culture methods employed, including the media selected and collection sites used [15].

Another important factor associated with pregnancy outcome is the vaginal flora pattern [16]. Lower rates of maternal and perinatal complications are associated with *Lactobacillus* sp. predominance in the vaginal flora, which plays a key role in protecting the genital tract against infections [17].

Previous studies have demonstrated that vaginal flora composition differs between GBS-positive and GBS-negative women. Women with GBS colonization show a lactobacilli-reduced flora [18, 19] and greater *Candida* species isolation [19].

Considering the varying rates of maternal GBS colonization in different populations and the high prevalence of vaginal flora alteration during pregnancy, the purpose of this study was to evaluate GBS prevalence in our population and its relationship with risk factors and the vaginal flora pattern.

Materials and methods

This prospective study was conducted at Botucatu Medical School, São Paulo State University, UNESP, between February 2006 and January 2007. Samples were obtained from 405 women at 35 and 37 weeks of gestation and with a singleton pregnancy.

Exclusion criteria were refusal to participate, symptoms of urinary infection or antibiotic use within the preceding 60 days and sexual intercourse within 72 h before examination. The study was approved by the institutional ethics review board, and written informed consent was obtained from all patients. Gynecological and socio-economic data, as well as information on previous gestations, were obtained with a form specifically designed for this study.

For the assessment of GBS status, samples were collected from the vaginal introitus (VI), upper lateral vaginal vault (LV) and perianal region (PR) using different sterile swabs samples were inoculated into non-nutritive Amies transport medium and transported to the microbiology research laboratory. Swabs were removed from the transport medium and inoculated into Todd Hewitt broth supplemented with colistin (10 μg/mL) and nalidixic acid (15 μg/mL). The inoculated broth was incubated for 24 h at 37°C. The broth was subcultured on sheep blood Columbia agar base under the same incubation conditions. Colonies suggestive of *S. agalactiae* by a narrow zone of beta-hemolytic colonies were submitted to catalase and CAMP test. Negative subculture plates were reincubated for another 18–24 h and reexamined.

Another swab was collected from the mid-third of the vaginal wall for flora evaluation. Gram-stained slides were evaluated and graded to determine the vaginal flora pattern according to Nugent's criteria [20]. Altered vaginal flora was defined either as flora in which lactobacilli did not predominate (for example, bacterial vaginosis, intermediate flora, aerobic vaginitis) or flora positive for *Candida* species by microscopy (presence of pseudo hyphae and leukocytes). Mixed infections were defined as those positive for both BV and vaginal candidosis. The presence of more than 25 leukocytes/field on a Gram stain in the absence of trichomonads, candidosis, leptothrix or characteristics of aerobic vaginitis received a designation of "other abnormal flora". All stained slides were read by experienced observers at the Botucatu Medical School, UNESP, who were blinded to the clinical data.

Considering the presence or absence of maternal GBS colonization as the response variable, a logistic regression model was fitted by the stepwise method with quantitative and qualitative explanatory variables. Statistical analyses were performed with the SAS software, version 9.1. Significance level was set at $P < 0.05$.

Results

During the study period, 567 patients with gestational age between 35 and 37 weeks presented at the Prenatal Care Unit of Botucatu Medical School. A total of 162 patients were excluded; 106 of them were receiving antibiotics and the other 56 refused to participate in the study. Among the 405 women included in the study, maternal *S. agalactiae* colonization rate was 25.4%.

The prevalence of vaginal flora alteration was 42.9%: 45.6% in GBS-positive women and 42.1% in women with negative GBS culture. The vaginal flora abnormalities most frequently observed among all pregnant women were cytolytic vaginosis (11.3%), bacterial vaginosis (10.9%), candidosis (8.2%) and intermediate vaginal flora (8.1%), followed by aerobic vaginitis (2.2%) and other altered vaginal flora (1.5%). Mixed infections were present in 0.7% of the evaluated vaginal smears.

Socio-demographic and gestational data of the patients are shown in Table 1. Median maternal age, parity, schooling level, frequency of sexual intercourse, discharge,

Table 1 Gestational and socio-demographic characteristics of the subjects according to GBS colonization status

Gestational and socio-demographic variables	*Streptococcus agalactiae*		*P* value
	Positive	Negative	
Age (years)	26 (21–33)	25 (20–31)	0.05
Years of schooling	9 (6–11)	9 (6–11)	0.85
Parity			
Nulliparity	37/103	142/302	0.07
Multiparity (II)	38/103	85/302	0.11
Multiparity (≥III)	28/103	75/302	0.17
Number of sexual intercourses/week	1 (1–2)	2 (2–3)	0.06
Spontaneous abortion	20/103	84/302	0.09
Premature rupture of membranes*	8/66	14/160	0.49
Premature labor*	14/66	32/160	0.94
STD*	12/103	21/302	0.17
Vaginal discharge	38/103	79/302	0.05

Values are expressed as median and interquartile range. *Z* test comparison of proportion

STD sexually transmitted diseases

* Previous gestation

premature labor, rupture of membranes, previous abortions, and sexually transmitted diseases did not differ between GBS-positive and GBS-negative women.

Logistic regression analysis showed that maternal age, number of sexual intercourses/week, occurrence of previous spontaneous abortion, presence of candidiasis and cytolytic vaginosis were associated with GBS colonization. OD values for all variables associated with GBS colonization are shown in Table 2.

Discussion

In this study, the prevalence of maternal *S. agalactiae* colonization observed in 405 women was similar to that reported in literature [18, 19, 21]. Given that culture accuracy can be increased depending on the anatomic sites of collection and the microbiologic methods used, *S. agalactiae* detection was performed in accordance with CDC recommendations [22].

There is evidence that GBS colonization varies according to the socio-economic characteristics of the population studied. In this study, median age was slightly higher in GBS-positive women than in the GBS-negative group, in disagreement with other authors who found higher rates in younger women [23, 24].

GBS colonization also seems to correlate with sexual behavior. The rate of positive GBS cultures is reported to be higher in women who have sexual intercourse more frequently [14]. This finding was confirmed by our results. Moreover, GBS has been demonstrated to play a leading role in spontaneous abortion [25]. The fact that women with GBS colonization in previous gestations are more likely to be colonized in subsequent gestations [26] may explain the association between GBS isolation and previous spontaneous abortion found here.

Our data about vaginal flora indicate a high percentage of abnormal vaginal flora in the third trimester of gestation. The importance of this finding lies on the fact that pregnant women with altered vaginal ecosystem detected by Gram

Table 2 Logistic regression analysis of the gestational and socio-demographic characteristics associated with GBS colonization

Variable	Estimation	SE	OR	95% CI	*P* value
Age	0.0424	0.0197	1.043	(1.004–1.084)	0.03
Sexual intercourses/week	−0.3300	0.1542	0.719	(0.531–0.973)	0.03
Spontaneous abortion	0.3142	0.1509	1.875	(1.038–3.387)	0.03
Candidosis	1.1780	0.4161	6.962	(2.193–22.105)	0.005
Cytolytic vaginosis	0.4998	0.2365	2.717	(1.075–6.866)	0.03

SE standard error, *OR* odds ratio, *CI* confidence interval

stain evaluation are at increased risk for adverse gestational outcome [27].

According to literature, vaginal flora composition in pregnant women with GBS colonization correlates inversely with lactobacilli counts [18, 19] and directly with *Candida* species isolation [19]. In this study, Gram smear examination showed that GBS colonization was associated with candidosis and cytolytic vaginosis. On the other hand, Honig et al. [28] observed no correlation between GBS-positive cultures and vaginal flora alteration, such as bacterial vaginosis, candidosis and co-infection by *Trichomonas vaginalis* or *Neisseria gonorrhoeae* and *Chlamydia trachomatis* endocervicitis. Considering that the rates of *Candida* species isolation are higher in women with GBS-positive vaginal flora, development into vulvovaginal candidosis is more likely to occur among them.

No previous study has assessed the association between cytolytic vaginosis and GBS colonization. However, the large number of subjects included in this study allows the establishment of this association. Cytolytic vaginosis is commonly misdiagnosed as vaginal candidosis due to the similarity of symptoms. The symptoms of cytolytic vaginosis are caused by the release of irritant cytoplasmic components, which result from lactobacilli overgrowth [29].

In brief, our results show that the prevalence of GBS in the study population was high; and there was association between maternal GBS colonization and sexual intercourse frequency, previous spontaneous abortion and the presence of candidosis or cytolytic vaginosis.

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Conflict of interest statement We declare that we have no conflict of interest.

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