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Prevalence of *Streptococcus agalactiae* colonization in pregnant women from the 18th Health Region of *Paraná State*

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ABSTRACT

Introduction: The aim of this study was to determine the prevalence of GBS colonization in pregnant women in a public health service. Methods: A study of 496 pregnant women at 35-37 gestational weeks was conducted from September 2011 to March 2014 in 21 municipalities of the 18th Health Region of *Paraná* State. Vaginal and anorectal samples of each woman were plated on sheep blood agar, and in HPTH and Todd-Hewitt enrichment broths. Results: Of the 496 pregnant women, 141 (28.4%) were positive for GBS based on the combination of the three culture media with vaginal and anorectal samples. The prevalence was 23.7% for vaginal samples and 21.9% for anorectal ones. Among the variables analyzed in this study, only urinary infection was a significant factor (0.026) associated with GBS colonization in women. Conclusions: Based on these results, health units should performs universal screening of pregnant women and hospitals should provide adequate prophylaxis, when indicated.

KEYWORDS: *Streptococcus agalactiae.* Colonization. Urinary infections. Pregnant women. Public health.

INTRODUCTION

Maternal colonization by Group B Streptococcus (GBS) is the main risk factor for neonatal GBS infection. GBS or *Streptococcus agalactiae* may be part of the human microbiota, mainly colonizing the gastrointestinal and genitourinary tract[1]. About 50 to 75% of newborns exposed to intravaginal GBS become colonized, and 1 to 2% of newborns of carrier mothers will develop early-onset invasive disease[1,2]. In the mother, GBS may cause abortion, urinary infection, preterm birth, chorioamnionitis or puerperal endometritis[3].

A hypothesis of this occurrence might be the hormonal changes occurring during the gestational period and the consequent microbiota imbalance, increasing the chances of GBS infections which can trigger maternal and child complications[1].

In Brazil, some studies have shown different colonization rates by GBS (24.5%, 16.6%, 27.6%)[4-6]. In other countries researches have also shown different rates (3.3% to 22.76%)[7-9].

Variations in prevalence of GBS colonization in women found in literature can be attributed to both, differences in the characteristics of the studied populations and the employed bacteriological methodologies[2].

Samples for culture obtained up to four weeks before delivery have greater

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sensitivity and specificity to identify maternal colonization by GBS[10]. Studies have shown a high rate of transmission to newborns during childbirth, as well as increased infection and neonatal mortality rate. Given this pathogenic and relatively frequent role of vaginal colonization by GBS, screening strategies should be adopted between the 35th and 37th gestational weeks[11].

GBS screening in pregnant women and antimicrobial prophylaxis (when indicated) may reduce neonatal morbidity and mortality; therefore, future studies should evaluate the effects and costs of introducing a universal infection screening program.

In this sense, this study aimed to verify the prevalence of *Streptococcus agalactiae* colonization in pregnant women from public health services.

METHODS

A cross-sectional study was conducted in 21 municipalities belonging to the 18th Regional Health Department of *Paraná State*, Brazil. The number of live births in this region in 2010 was 2,848. The sample was calculated using Epidata software by the population proportion method, with the proportion of the expected outcome being 50%, a sample error of 4%, and a 95% confidence level. Thus, the calculated sample was 496 individuals. The following calculation was used for stratification of the number of pregnant women in each municipality: minimum size of the previously calculated/ total sample of live births from the 18th Regional Health Department multipled by the total number of live births from each municipality (496/2,848 x number of live births from each municipality).

Inclusion criteria of the study were: pregnant women with gestational age between 35 and 37 weeks, determined from the date of the last menstruation period (DLM) or by the fetal ultrasound performed in the first trimester of gestation. All participants signed the informed consent form. A guardian signed the informed consent form when pregnant women were under the age of 18. Pregnant women who had been using antimicrobials in the last seven days or who used vaginal ointment at the time of collection were excluded from the study.

Data collection was performed by the researcher between September 2011 and March 2014. When tests were scheduled, the researcher went to the municipalities to collect the biological samples and to help women to fill out a form that contained the following information: pregnant woman's identification, ethnicity, age, educational level, marital status, family income, number of pregnancies, current gestational data, gestational age, occurrence of urinary tract infection during the current gestation, sexually transmitted disease prior to or during the current gestation, prior miscarriage and number of sexual partners.

Biological samples were collected from the distal third of the vagina by the introduction of sterile swabs through the vaginal introitus without speculum. This procedure was repeated three times and the samples were labeled as vaginal swab 1, 2, and 3. Anorectal samples were collected by the introduction of sterile swabs through the anorectal region three times and samples were labeled as anorectal swabs 1, 2, and 3. Vaginal swab 1 and anorectal swab 1 were cultured in HPTH culture medium (Hitchens - Pike - Todd - Hewitt) supplemented with 100 µL of sterile defibrinated sheep blood (Laborclin, *São José dos Pinhais, Paraná*, Brazil) and incubated at 35 °C for 18 to 24 h. After this period of incubation, the sample was subcultured into blood agar and incubated at 35 °C for 24 to 48 h. Vaginal swab 2 and anorectal swab 2 were cultured in Todd-Hewitt culture medium (Himedia, Curitiba, *Paraná*, Brazil) supplemented with 8 µg/mL of gentamicin (Inlab, *São Paulo*, Brazil) and 15 µg/mL of nalidixic acid (Inlab, *São Paulo,* Brazil), and incubated at 35 °C for 18 to 24 h. The material was then subcultured into blood agar (Himedia, Curitiba, *Paraná*, Brazil) and incubated at 35 °C for 24 to 48 h. Vaginal swab 3 and anorectal swab 3 were immediately cultured in 1/3 of blood agar medium (Himedia, Curitiba, *Paraná*, Brazil) and incubated at 35 °C for 18 to 24 h.

Streptococcus identification was carried out in the Clinical Bacteriology laboratory of the Department of Clinical Analysis and Biomedicine (DAB) of the *Universidade Estadual de Maringá.* Colonies that were suggestive of GBS (beta- and non-hemolytic) were subjected to microscopy (Gram stain), biochemical identification (catalase, bile esculin, and hippurate hydrolysis) and latex agglutination using a streptococcal grouping kit (Oxoid, Hampshire, UK) according to the manufacturer's instructions.

Data were entered into the Microsoft Office Excel 2007 program and analyzed using the Statistical Analysis System (SAS) version 7.3. The chi-squared test was used to analyze the relationship between the values with a 5% significance level (p=0.05). Results were presented in tables, and discussed according to the implemented theoretical framework.

The research was approved by the Research Ethics Committee of the *Universidade Estadual de Maringá*, Process Nº 236/2011.

RESULTS

Of the 496 pregnant women at 35-37 gestational weeks who participated in the study, 141 (28.4%) were positive for

GBS based on the combination of the three culture media results from the two clinical specimens. The detected GBS colonization rate was 22.2% (IC- 18.52 -25.84) for HPTH medium, 21.2% (IC- 17.57- 24.77) for SBA, and 13.1% (IC- 10.13 – 16.07) for Todd-Hewitt enrichment broth (Figure 1). The prevalence for vaginal samples was 23.7%, and 21.9% for anorectal samples.

Regarding socio-demographic data, 72.8% of pregnant women were white, 88.1% had one partner, and the age group ranged from 14 to 41 years old with an average of 24.8 years. Evaluation of the educational level followed the functional illiteracy criterion adopted by the Brazilian Institute of Geography and Statistics (IBGE), showing that 65.1% of women had studied more than eight years, and 19.4% had monthly family income of up to two minimum wages (Table 1). No statistically significant relationship was observed between socio-demographic data and GBS colonization (Table 2).

Regarding the gynecological-obstetric characterization, the majority was of multiparous (59.7%) with gestational age of 35th week (43.1%). The age of first gestation ranged from 12 to 39 years; 27.2% were less than 19 years old; 84.7% had no previous abortion; 3.0% reported a sexually transmitted disease in life (among those cited were: HIV, Trichomoniasis, HPV, Syphilis, and Chlamydia); 40.1% reported urinary tract infection in the current pregnancy; and 40.0% had only one sexual partner in life. The predominant type of delivery was vaginal (59.9%) (Table 3). There was only a statistically significant relationship between urinary infection during the current gestation and GBS colonization in women (0.026) (Table 4).

Table 1 - Distribution of pregnant women according to socio-demographic variables, 18th Regional Department of Health, 2015

Variables	N	%
Ethnicity		
White	361	72.8
Non-White	135	27.2
Marital Status / Relationship Status		
With a partner	437	88.1
Without a partner	59	11.9
Current age		
< 20 years	135	27.2
≥ 20 years	361	72.8
Educational level		
Up to 8 years of study	173	34.9
More than 8 years of study	323	65.1
Family Income		
Up to 1 minimum wage	400	80.6
Up to 2 minimum wages	96	19.4
TOTAL	**496**	**100**

DISCUSSION

In this study, the frequency of colonization by *S. agalactiae* was 28.4%, and it was higher than that found in

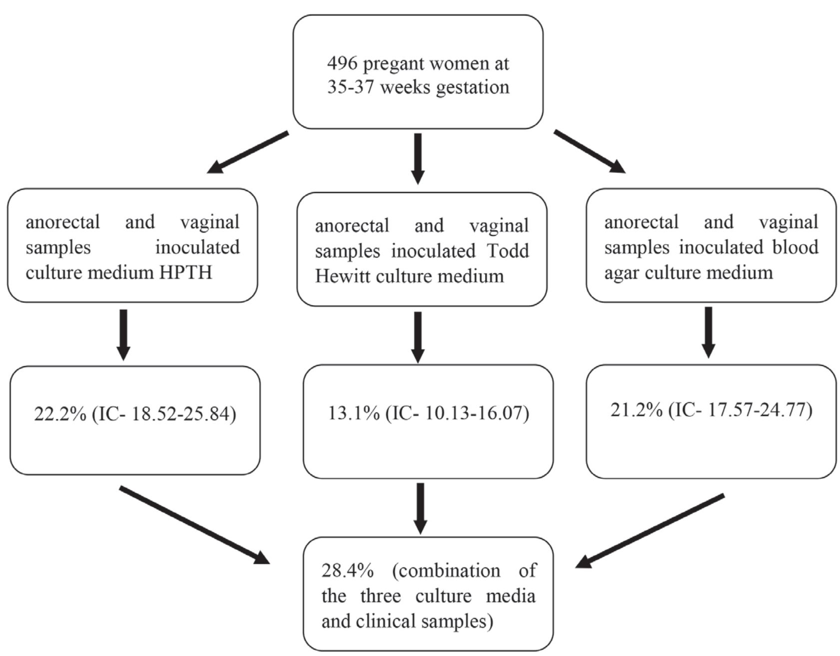

Figure 1 – Flowchart of culture results, from vaginal and anorectal specimens, in pregnant women at 35-37 gestational weeks to detect *Streptococcus agalactiae* by Hitchens-Pike-Todd-Hewitt (HPTH), Todd-Hewitt enrichment broth and sheep blood agar (SBA) culture media

Table 2 - Distribution of pregnant women colonized by GBS according to socio-demographic variables, 18th Regional Department of Health, 2015

Variables	n	%	IC 95%	p
Ethnicity				**0.635**
White	100/361	27.70	22.95 – 32.46	
Non-White	41/135	30.37	22.24 – 38.50	
Marital Status / Relationship Status				**0,485**
With a partner	127/437	29.06	24.69 – 33.43	
Without a partner	14/59	23.73	12.06 – 35.43	
Current age				**0,520**
< 20 years	35/135	25.93	18.16 – 33.69	
≥ 20 years	106/361	29.36	24.53 – 34.20	
Educational level				**0,947**
Up to 8 years of study	50/173	28.90	21.86 – 35.95	
More than 8 years of study	91/323	28.17	23.11 – 33.23	
Family Income				**0,069**
Up to 1 minimum wage	106/400	26.50	22.05 – 30.95	
Up to 2 minimum wages	35/96	36.46	26.31 – 46.61	

Table 3 - Distribution of pregnant women according to gynecological and obstetric variables, 18th Regional Department of Health, 2015

Variables	N	%
Gestational Age		
35	214	43.1
36	138	27.8
37	144	29.1
Number of pregnancies		
Primiparous	200	40.3
Multiparous	296	59.7
Sexually Transmitted Disease		
Yes	15	3.0
No	481	97.0
Urinary Infection during Pregnancy		
Yes	199	40.1
No	297	59.9
Type of delivery*		
Vaginal	173	59.9
Cesarean	116	40.1
Number of partners		
1	198	40.0
2	113	22.8
3 or more	185	37.2
Use of condom		
Yes	23	4.6
No	473	95.4
Abortion		
Yes	76	15.3
No	420	84.7
TOTAL	**496**	**100**

* The values do not total 496 pregnant women because primiparous and abortions were excluded from this sum, because the risk factor for GBS is for women who have had childbirth, regardless of the route.

national studies conducted in a Primary Health Units (UBS) in *São Paulo* (17.4%), as well as in private (15.2%) and public (22.5%) centers in Rio Grande do Sul[10,12,13]. Some studies performed in other countries have also found lower frequencies, ranging from 2.3% to 8.3%[14-16].

Prevalence variations of GBS colonization in women found in the literature can be attributed to both, differences in the characteristics of the studied populations (such as age, parity, ethnic group, socioeconomic level and geographical location) and to the employed diagnostic methods[14].

According to CDC, the use of vaginal/rectal swabs improves GBS isolation by 40%, compared with vaginal specimens alone[17]. Marconi *et al.*[18] collected material from the vaginal introitus, the lateral recess of the vagina and the perianal region. Among the patients with positive culture, 28.1% were positive at only one collection site, 24.2% at two sites and 47.5% at the three sites cited. In the present study, the vaginal colonization rate was 23.7%, and the anorectal rate was 21.9%. When the two sites were combined, the rate was of 28.4%. Thus, cultures performed with samples from more than one site allow the identification of the site from which more GBS can be recovered, resulting in more reliable results.

No significant association was found in this study regarding socio-demographic data and GBS colonization in women. The same can be observed in the work of other researchers[7,15].

The Center for Disease Control (CDC)[19] recommends a gestational age from the 35th week for sample collection because there is a greater risk of vertical transmission in this period. Colonization of pregnant women at the beginning of gestation has no predictive value regarding

Table 4 - Distribution of pregnant women colonized by GBS according to gynecological and obstetric variables, 18th Regional Department of Health, 2015

Variables	n	%	IC%	p
Gestational Age				**0.387**
35	54/214	25.23	19.18 - 31.29	
36	43/138	31.16	23.07 - 39.25	
37	44/144	30.56	22.68 - 38.43	
Number of pregnancies				**0.783**
Primiparous	55/200	27.5	21.06 - 33.94	
Multiparous	86/296			
Sexually Transmitted Disease				**1.00**
Yes	4/15	26.67	7.78 - 55.1	
No	137/481	28.48	24.34-32.62	
Urinary Infection during Pregnancy				**0.026**
Yes	68/199	34.17	27.33-41.01	
No	73/297	24.58	19.51-29.64	
Type of delivery*				**0.963**
Vaginal	52/173	30.06	22.94-37.18	
Cesarean	36/116	31.03	22.18-39.88	
Number of partners				**0.961**
1	55/198	27.78	21.28-34.27	
2	33/113	29.20	20.38-38.03	
3 or more	53/185	28.65	21.86-35.43	
Use of condom				**0.649**
Yes	8/23	34.78	13.14-56.42	
No	133/473	28.12	23.96-32.28	
Abortion				**0.977**
Yes	21/76	27.63	16.92-38.34	
No	120/420	28.57	24.13-33.01	

* The values do not total 496 pregnant women because primiparous and abortions were excluded from this sum, because the risk factor for GBS is for women who have had childbirth, regardless of the route.

neonatal infection. This period was determined because it is considered that GBS colonization can be transient, and it is relevant to know the colonization frequency in the period near birth[20]. In the present study, the majority (43.1%) of women was in the 35th week of gestation, and 38.3% of these were colonized by GBS; however, the gestational age at the time of collection was not associated with the presence of GBS (p = 0.387).

Socio-demographic and clinical factors were not associated with GBS colonization, but urinary infection at some point in the current gestation was a significant risk factor. A similar result was found in the study by Mitima *et al*.[21] ($p < 0.05$), but it differs from the study by Kruk *et al*.[20], in which urinary infection was not significant in GBS colonization in women (p = 0.191). It was not possible to identify which pathogen caused urinary tract infections in pregnant women because this variable was not reported by the interviewees. It is known that urinary tract infections can cause complications during the gestation period.

CONCLUSION

Although screening is affordable and sample collection is simple, GBS culture is still not routinely performed during prenatal care in many cities in the country. Public policies in the area of maternal and child health have been organized in recent decades focusing on expanding and improving the quality of obstetric care. However, a lack of information regarding the occurrence of infection may be responsible for the lack of attention given by responsible agencies, both in the prenatal screening and in the correct prophylaxis at the time of delivery of colonized women.

Since pregnant women colonization was shown to be frequent in this study, and GBS can cause neonatal sepsis, miscarriage and endometritis, it is recommended that health units perform universal screening of pregnant women and that hospitals perform prophylaxis, when indicated.

AUTHORS' CONTRIBUTIONS

SCCSM: design, material collection, tabulation, data interpretation, writing of the article, approval of the review to be published; ABC, FTRS, NMMGS and CMT: review of the article and approval of the review to be published; RFC: design, data interpretation, writing of the article, approval of the review to be published; RAFP: test development; IP: statistical analysis and data interpretation; MDBC: approval of the review to be published; SMP: design, data interpretation, writing of the article, approval of the review to be published.

CONFLICT OF INTERESTS

The authors explicitly state that there are no conflicts of interest in connection with this article.

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