Cervicovaginal and rectal colonization by group B *Streptococci* in Mexican women with full-term pregnancies

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Abstract

Background: A pregnant woman rectally or vaginally colonized by group B *Streptococcus* can infect her newborn. **Patients and methods:** Prospective, cross-sectional, analytical 24-month study in pregnant women. Women in labor with ≥ 36 weeks of gestation were included. Pregnancy was classified as normal or high-risk. Main risk factors of the pregnant women were analyzed. Rectal and vaginal samples were obtained, placed in Todd-Hewitt broth and subsequently inoculated in 5% sheep blood agar. Identification was carried out by biochemical tests and latex agglutination. **Results:** 3,347 pregnant women were included. Mean age was 25.6 ± 5.3 years, 95.5% received antenatal care; 2,213 (66%) had normal-risk pregnancies, and in 1,370 (41%), delivery was by cesarean section. Overall colonization was 4.3% (145/3,347), and it was higher in the 30-34 years age group (6.8%). Serotype I (58%) was the most common. **Conclusion:** The percentage of colonization in this population was low. A routine cervicovaginal and rectal culture program in pregnant women and the intrapartum antimicrobial prophylaxis program are controversial in our region.

KEY WORDS: Cervicovaginal colonization. Group B streptococcus. Pregnancy.

Colonización cervicovaginal y rectal por estreptococos del grupo B en embarazadas mexicanas a término

Resumen

Antecedentes: Una mujer embarazada colonizada por estreptococo del grupo B por vía rectal o vaginal puede infectar a su recién nacido. **Pacientes y métodos:** Estudio prospectivo, transversal y analítico, durante 24 meses, en embarazadas. Se incluyeron aquellas en trabajo de parto con ≥ 36 semanas de gestación. El embarazo se clasificó como normal o de alto riesgo. Se analizaron los principales factores de riesgo de las embarazadas. Se tomaron muestras rectales y vaginales, se colocaron en caldo Todd-Hewitt y posteriormente se inocularon en agar sangre de carnero al 5%. La identificación se realizó mediante pruebas bioquímicas y aglutinación con látex. **Resultados:** Se incluyeron 3,347 embarazadas, edad media 25.6 ± 5.3 años, 95.5% con control prenatal; 2,213 (66%) embarazo de riesgo normal y 1,370 (41%) obtenidas por cesárea. La colonización global fue del 4.3% (145/3,347), siendo mayor en el grupo de edad de 30 a 34 años (6.8%). El serotipo I (58%)

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Date of reception: 26-07-2021

Date of acceptance: 12-08-2021

DOI: 10.24875/GMM.M21000607

Gac Med Mex. 2021;157:516-521

Contents available at PubMed

www.gacetamedicademexico.com

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fue el más frecuente. **Conclusión:** El porcentaje de colonización en esta población fue bajo. Un programa sistemático de cultivo cervicovaginal y rectal en mujeres embarazadas y el programa de profilaxis antimicrobiana intraparto son controvertidos en nuestra región.

PALABRAS CLAVE: Colonización cervicovaginal. *Estreptococo* del grupo B. Embarazo.

Streptococcus agalactiae or group B *Streptococcus* (GBS) is a gram-positive coccus that frequently colonizes the lower digestive tract.

In 1938, Fry described its capacity as a human pathogen.¹ Since the 1970s, there has been an increase in perinatal infections by this microorganism in developed countries, and in the 1980s, it was considered the most common cause of neonatal sepsis and meningitis.²⁻⁵ GBS is one of the main etiological agents in perinatal infections, with chorioamnionitis, spontaneous abortions and postpartum infections standing out.⁶

A mother with genital or rectal GBS colonization might transmit this microorganism to her newborn (NB) by hematogenous-transplacental route, by ascending route or during the passage through the cervicovaginal canal.⁷ In developed countries, different preemptive measures have been implemented in order to prevent vertical transmission, including intrapartum antibiotic prophylaxis.^{8,9} To implement these measures, it is necessary to know the frequency of maternal colonization by specific region in order for local perinatal risk to be inferred.

Most studies on GBS colonization and infection have been carried out in developed countries; in Mexico, information is limited with regard to the number of studies and evaluated regions.¹⁰⁻¹⁵

In this study, the frequency of cervicovaginal and rectal colonization by GBS was determined in a sample of pregnant women cared for in a gynecology-obstetrics hospital of Mexico City.

Patients and methods

Design and patients

A 24-month observational, prospective, cross-sectional, analytical study was carried out in pregnant women. Pregnant women admitted for the care of labor were included, with those with ≥ 36 weeks of pregnancy and those with 35-36 weeks of pregnancy on active labor and no response to labor inhibition being chosen. Those who had received antibiotics (penicillin, cephalosporin, trimethoprim-sulfamethoxazole and/or erythromycin) within the last trimester of

pregnancy and those with medical contraindications (placenta previa, premature birth, placental abruption) were excluded. Pregnancies were classified as normal or high-risk according to Coopland's classification.¹⁶

Sampling

A group of trained nurses, at first examination in the delivery room or in the operating room, placed each patient in gynecological position. Wearing sterile gloves, a 15-cm plastic cotton-tipped swab was inserted in the vagina, turned, and moved laterally; the sample was placed in a tube with Todd-Hewitt (TH) broth (Becton-Dickinson) with nalidixic acid (15 $\mu\text{g/mL}$) and gentamicin (8 $\mu\text{g/mL}$). In the same position, a rectal sample was taken by inserting the swab 2 cm into the rectal ampulla, turning it on its own axis and placing it in another tube with HT. The tubes remained in the delivery area at room temperature (25 °C) for 18 to 24 hours and were taken to the laboratory every morning.

Bacterial identification

The TH tubes that showed turbidity were inoculated on 5% sheep blood agar plates (SBA5%); those that had no turbidity were incubated for 48 h more, and those that showed turbidity were inoculated on SBA5%. The plates were incubated for 24 hours at 37 °C, and β -hemolytic, gram-positive, catalase test-negative, hippurate hydrolysis-positive and CAMP-positive colonies were isolated. Identification of group and soluble antigens for serotypes I, II and III was carried out with a latex agglutination test (Pastorex B Streptococci®, Sanofi Diagnostic Pasteur, France). In the absence of agglutination, the isolate was considered non-typeable (NT).

The study was approved by the Ethics and Research Committee of the Tlatelolco Gynecology-Obstetrics Hospital (registration no. 98-701-0010). Written informed consent was obtained from all participants.

Statistical analysis

Descriptive statistics were used for general characteristics of the sample. The chi-square test (X^2) was

used to compare the prevalence of GBS colonization between women with normal and high-risk pregnancies. A p -value < 0.05 was considered to be significant.

Results

Over a 24-month period, 3,347 pregnant women were included; 2,213 had a usual risk pregnancy (66%), and 1,134, a high-risk pregnancy (34%). Mean age was 25.6 ± 5.3 years (range: 14-43 years), 95.5% had prenatal care. Cesarean section was carried out in 1,370 (41%), with its use being higher in the high-risk pregnancy group (62 vs. 32%, $p < 0.001$). Urinary infection occurred in 27.5%, and 15.5% had premature rupture of membranes (PROM), while preeclampsia was observed in 10.8% and anemia in 1.6%. There were significant differences in some characteristics: age, diabetes, preeclampsia, obesity, fever and PROM > 12 hours. The remaining characteristics are presented in table 1.

Maternal colonization

Vaginal and rectal cultures were obtained in all 3,347 pregnant women, with GBS being isolated from one or both sites in 145, for a colonization rate of 4.3%. This rate was higher in women aged between 30 and 34 years (6.8%) with a significant difference with those younger than 19 years (3.6 vs. 6.8%, $p = 0.033$) and those younger than 25 and 30 years (Table 2). GBS was isolated only from the vagina in 85/145 (59%), from the rectum in 36/145 (25%), and from both sites in 24/145 (17%); this corresponds to 60/145 (42%) women rectally and 109/145 (75%) vaginally colonized (Fig. 1). GBS colonization was higher in the high-risk pregnancy group than in the usual risk group (5.9 vs. 3.4%, $p = 0.001$) and in married women, with fever and a history of urinary infection. The frequency of PROM was higher in non-colonized pregnant women. There was no significant difference in any of the other evaluated characteristics (Table 3).

Serotypes

Serotype I (58%) was the most common in both groups. Although higher identification of serotype III (24 vs. 21%) and lower frequency of serotype II (9 vs. 16%) was observed in the high-risk group, there were no significant differences ($p = 0.42$) (Table 4).

Table 1. Characteristics of 3,347 pregnant women cared for in a gynecology-obstetrics hospital of Mexico City

	Pregnancy risk		p
	Usual	High	
Age, years (mean $\pm$ SD)	24.9 $\pm$ 4.9	26.9 $\pm$ 5.7	0.0001 [†]
Marital status			
Single	178 (8%)	57 (5%)	0.003*
Married	2,036 (92%)	1,076 (95%)	
Number of pregnancies			0.0001*
≤ 2	1,703 (76.9%)	775 (68.3%)	
≥ 3	510 (23%)	357 (31.4%)	
Prenatal care	2,110 (95%)	1,085 (96%)	0.28
Diabetes	1 (0.04%)	7 (0.6%)	0.003*
Obesity	317 (14%)	467 (41%)	0.0001*
Preeclampsia	46 (2%)	316 (28%)	0.0001*
Fever	3 (0.1%)	11 (1%)	0.001*
Urinary tract infection	8 (0.4%)	8 (0.7%)	0.13
Chorioamnionitis	0	4 (0.4%)	0.013*
PROM > 12 h	18 (0.8%)	60 (5.3%)	0.0001*
Cesarean section	681 (32%)	689 (62%)	0.0001*
BGS positive culture	77 (3.47%)	68 (5.99%)	0.001*
Total	2,213 (66%)	1,134 (34%)	

*Fisher's exact test.

[†]Student's t-test.

SD: standard deviation; PROM: premature rupture of membranes; GBS: group B *Streptococcus*.

Figure 1. B-group *Streptococcus* (BGS) isolation site in 145 pregnant women cared for in a gynecology-obstetrics hospital of Mexico City.

Discussion

GBS is considered normal microbiota of the intestine, vagina and rectum; however, it is identified as an important pathogen of the NB and the pregnant woman. Worldwide, approximately one in every five

Table 2. Colonization by group B *Streptococcus* in pregnant women from a gynecology-obstetrics hospital of Mexico City

Age (years)	Colonized pregnant women	Total pregnant women	p
≤ 19*	16 (3.6%)	442	
20-24	33 (3.1%)	1,052	0.64
25-29	51 (4.7%)	1,093	0.007†
30-34	38 (6.8%)	556	0.033‡
> 35	7 (3.4%)	204	0.026§
Total	145 (4.3%)	3,347	

*First stratum was taken as reference.

†Fisher's exact test, when < 25 years vs. > 25 years are contrasted.

‡Fisher's exact test, when 30-34 vs. ≤ 19 years are contrasted.

§Fisher's exact test, when > 35 years vs. < 30 years are contrasted.

pregnant women is colonized,¹⁷ which is a known risk factor for early invasive neonatal disease.^{18,19} The frequency of this phenomenon varies according to the colonized site, gestational age and route of delivery, as well as to intrinsic differences and geographic location of the studied populations.

There is no established international standard for maternal GBS colonization sampling, which generates variations when studies from different countries are compared. These variations may be due to the sampling method and timing, sampled anatomical sites and laboratory techniques used for sample transportation and culture.^{20,21}

In the present study, the frequency of colonization (4.3%) was lower than that reported in the world (17.9%, 95% confidence interval [CI]: 16.2-19.7%),²² with a range of 11.1% (95% CI: 6.8-15.3%) in South-east Asia to 22.4% in Africa (95% CI: 18.1-26.7%). Some countries such as Brazil (14%), China (14%), Nigeria (19.5%) and Saudi Arabia (19%) have a high frequency.²²⁻²⁷ In Mexico there are also differences with previous studies: in 1981, Collado et al. found 4% of colonization;¹⁰ however, in a tertiary care center in Mexico City, 10.3% was reported; in a rural population in the south of the country, 8.6% was found,¹² and in a secondary care hospital in western Mexico, 13%.¹³ Colonization in the high-risk population of the present study was lower than that found in 1989 in Mexico City (6 vs. 10.3%).¹¹ Perhaps the differences in the present study could be explained by the sample size and by the fact that the sampling was carried out in pregnant women who were in labor, who had been recently admitted, with the majority of them having full-term pregnancies. GBS colonization was higher in women with high-risk pregnancy, with frequency increasing at

Table 3. Characteristics of pregnant women colonized and not colonized by group B *Streptococcus* (GBS)

	GBS culture		p
	Positive n (%)	Negative n (%)	
Age, years (Mean ± SD)	26.6 ± 5	25.5 ± 5.2	0.01*
Marital status			
Single	5 (3.4%)	232 (7.2%)	0.047†
Married	138 (95.1%)	2,920 (91.2%)	
Unknown	2 (1.4%)	50 (1.5%)	
Number of pregnancies			
< 3	105 (72.4%)	2,374 (74.1%)	0.35†
≥ 3	40 (27.6%)	828 (25.9%)	
Gestational age, weeks			
≤ 36	2 (1.3%)	132 (4.1%)	0.06‡
≥ 37	143 (98.6%)	3,070 (95.9%)	
Obesity	40 (28%)	745 (23%)	0.13†
Diabetes	0	8 (0.2%)	0.7†
PROM	15 (10.3%)	505 (16%)	0.04†
Preeclampsia	16 (11%)	346 (11%)	0.50†
Fever	2 (1.3%)	11 (0.3%)	0.02†
Recent urinary infection	2 (1.3%)	14 (0.4%)	0.15†
Past urinary infection	50 (34.4%)	870 (27%)	0.03†
Chorioamnionitis	1 (0.7%)	3 (0.1%)	0.16†
Total	145 (100%)	3,202 (100%)	

*Student's t-test.

†Chi-square test.

‡Fisher's exact test.

SD: standard deviation; PROM: premature rupture of membranes.

Table 4. Group B *Streptococcus* (GBS) serotypes in women with usual and high-risk pregnancy

Serotype	Pregnancy risk		Total
	Usual n (%)	High n (%)	
I	42 (55%)	42 (62%)	84 (58%)
II	12 (16%)	6 (9%)	18 (13%)
III	16 (21%)	17 (25%)	33 (22%)
NT	7 (9%)	3 (5%)	10 (7%)
GBS positive culture	77 (100)	68 (100)	145

NT: non-typeable.

older age; however, this frequency is lower than that observed in other populations of the world.²²⁻²⁷

It has been suggested that vaginal and rectal cultures should be carried out simultaneously; in this study, 25% of isolates were obtained only from the rectum; therefore, if only vaginal cultures would have been taken, a significant percentage of colonized women would have been missed. This supports sampling at both sites.²⁸

Worldwide, five GBS serotypes (Ia, Ib, II, III and V) represent 98% of predominant serotypes.¹⁷ In our study, serotype I predominated, as previously reported in Mexico¹¹⁻¹⁴ and in other regions of the world.¹⁷ Serotype V was not searched in this study because it was considered of very low frequency in Mexico according to previous studies.¹¹⁻¹⁴

Given the frequency of colonization and perinatal infections by GBS, in the United States and other developed countries, systematically searching for it is recommended in all pregnant women at between 35 and 37 weeks of gestation, and administering antibiotic prophylaxis to colonized women since four hours before delivery. This strategy has achieved a drastic decrease in the incidence of early-onset neonatal disease, without the incidence of late-onset infections being modified.²⁹ In other countries, this policy has not been adopted due to the lack of information on colonization and transmission to the NB. In underdeveloped countries there are limitations for having epidemiological data available due to the lack of easy, rapid and low-cost tests. The performance of cultures is an expensive and time-consuming practice. Screening based on maternal risk has not offered good results.²⁸

In the population of Mexican women, cervicovaginal and rectal colonization by GBS is low in comparison with other countries; therefore, the performance of cultures in all pregnant women appears not to be justified in Mexico, considering the high cost of this practice and the low probability of detecting colonized women. For now, available information is insufficient for establishing an intrapartum antimicrobial prophylaxis program.

One weakness of this study is that only pregnant women from Mexico City were included; however, available information from other states of the country suggests that this low colonization percentage is real.^{12,13} The factors that may be determining this low colonization rate in the Mexican population are still unknown and, therefore, constitute a field that requires future research.

Funding

This study received funding from Instituto Mexicano del Seguro Social participating institutions.

Conflict of interests

The authors declare that they have no conflicts of interest.

Ethical disclosures

Protection of human and animal subjects. The authors declare that the procedures that were followed adhered to the ethical standards of the responsible committee for experimentation on human beings and were in agreement with the World Medical Association and the Declaration of Helsinki.

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

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