Prevalence of Maternal group B Streptococcal Colonization and Related Risk Factors in a Brazilian Population

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The objective of this study was to determine the prevalence of maternal group B Streptococcal (GBS) colonization and compare risk factor data related to GBS colonization. A prospective surveillance study of 598 pregnant women was conducted in two socioeconomically diverse maternity hospitals in Ribeirão Preto, Brazil between June and October 1999. Swabs from the lower vagina were obtained between 35 and 37 weeks gestation and cultured on selective media. Risk factor data were obtained by patient interview and chart review. The overall maternal GBS colonization prevalence rate was 17.9%. There was no association of GBS colonization with maternity hospital and no association of GBS colonization with previously identified risk factors, such as age, race, martial status, maternal education, parity, smoking, or alcohol use. There is a relatively high prevalence of maternal GBS colonization in this Brazilian population, although previously-identified-risk factors were not found to be important. This study provides baseline data for the creation of community-based GBS disease prevention protocols.

Key Words: Group B Streptococcus (GBS), colonization, neonatal sepsis, Brazil, Latin America, risk factors.

In 1996, consensus guidelines for prevention of perinatal Group B *Streptococcus* (GBS) disease were issued in the United States by the Centers for Disease Control and Prevention (CDC), the American College of Obstetricians and Gynecologists, and the American Academy of Pediatrics [1]. According to this protocol, providers should administer intrapartum antibiotics to mothers, using either a GBS risk factor-based approach or a prenatal screening-based approach. The 2002 updated guidelines recommend universal screening for all pregnant women at 35 to 37 weeks gestation and reserve a risk-factor-based approach for women who have no prenatal culture result [2]. Since the early 1990's, implementation of this prevention protocol across the United States has reduced the incidence of GBS disease among newborns up to 80% [3-5].

Only a few epidemiological studies have produced comprehensive data on GBS disease in the developing world, including Latin America [6-11]. Rates of GBS colonization remain unpredictable and vary geographically, while rates of GBS disease are less often reported from other countries [12]. To develop effective preventive measures at the community level, it is essential to know incidence rates of early-onset and late-onset GBS disease, as well as rates of sepsis caused by

other bacterial pathogens. However, maternal GBS colonization continues to be the most important risk factor for developing disease in the newborn [13]. No comprehensive data on maternal GBS colonization have been collected in Brazil.

We examined the prevalence of maternal GBS colonization in Ribeirão Preto, Brazil and compared maternal and newborn data on GBS colonization in two economically diverse populations. This information will contribute to the design of an optimal public health prevention strategy for neonatal sepsis due to GBS infection in Brazil.

Material and Methods

This prospective surveillance study was conducted from May 28, 1999 through October 11, 1999 at Mater and Sinha Junqueira maternity hospitals in Ribeirão Preto. During the study period, neither maternity unit had established protocols for prevention of GBS disease, such as routine testing of pregnant women or administration of intrapartum chemoprophylaxis. Mater, where approximately 250 patients deliver per month, serves a largely indigent population that receives little prenatal care. Complicated pregnancies and deliveries are transferred to the university-affiliated hospital, Sinha Junqueira, where approximately 220 patients deliver per month. It serves a private paying population. In this hospital, there is a full service neonatal intensive care unit (NICU).

Participants were enrolled in the study as they presented to either hospital for a prenatal visit, in labor, or for planned cesarean delivery. The institutional review board of Yale University approved the study, and oral informed consent was obtained from the participants before collection of the

sample and again prior to the interview. In Ribeirão Preto, where greater than 90% of deliveries occur in hospitals, these two maternity hospitals accounted for approximately 53% of all deliveries for this time period [14]. A prevalence rate for maternal GBS colonization was calculated using results from the 598 specimens collected, while risk factor analysis was based on a subset of 529. Due to the short period pregnant women stayed in the hospital recovery room, 69 women were not interviewed.

Information concerning host factors associated with maternal GBS colonization were collected using standardized maternal interview forms, while labor and delivery outcomes, including premature rupture of membranes (PROM), birth weight, gestational age, duration of labor, and five minute APGAR score were obtained from patient charts. Pregnant women between 35 and 37 weeks gestation had vaginal swabs collected for microbiological analysis. The author (SNSF) or the infection control nurse performed maternal interviews in Portuguese during a prenatal visit, or after delivery.

One swab (Starplex Scientific, Ontario, Canada) was collected from the lower vagina before a vaginal examination was performed. A speculum was not used and samples were not collected from the cervix or rectum. Swabs were placed in non-nutritive Aimes transport media and transported to a remote laboratory at room temperature for microbiological analysis.

Swabs were placed into a selective broth medium [Lim's + colistin (10g/ml) and naladixic acid (15g/ml)] within 72 hours of collection. They were incubated for approximately 24 hours in the selective broth medium and then cultured on 4% sheep's blood agar plates (tryptic soy agar base). Culture plates were incubated at 37°C with 5% CO_2 for 48 hours. All suspected GBS colonies (beta-hemolytic, or non-hemolytic, Gram-positive, catalase negative) were sub-cultured and isolated for confirmatory testing. A positive Christie, Atkins, and Munch-Petersen (CAMP) test was considered presumptive identification of a positive GBS culture. Ambiguous CAMP test culture results were re-tested using a GBS latex agglutination assay [1].

Univariate statistical analysis was performed on all continuous and categorical variables for the total population and for each hospital population. The chi-square and Student's T-tests were used to compare the two maternity hospital populations. Frequency tables were used for bivariate analysis of maternal risk factors for GBS colonization as well as labor and delivery outcomes for each hospital. Odds ratio, 95% confidence intervals, and P-values were calculated for each dichotomous variable using the chi-square test. Differences at the P= 0.05 level were considered significant.

Results

One hundred seven of 598 (17.9%) women tested positive for GBS (Table 1). There was no significant difference in the prevalence of GBS colonization between the two hospitals (P = 0.345).

Mothers from *Mater* were typically younger (P< 0.0001) and less likely to be in a married relationship (41.6% *vs*. 83%, P<0.001). They also were more likely to identify themselves as black or mixed skin color (48.2% *vs*. 10.6%, P<0.001). Women attended at *Mater* reported a lower average monthly income than women delivering in *Sinha* and fewer years of maternal education (P< 0.001, Table 2).

A significant difference between hospital populations was observed for the variables gravidity, prior cesarean deliveries, mean number of ultrasound examinations for the current pregnancy, current smoking, and PROM. No significant relationship was found between hospital populations for the variables consumption of alcohol, or preterm birth (< 37 weeks). Among the delivery and newborn variables, only birth weight and 5 minute APGAR score were not significantly different. Mode of delivery was found to be significantly different (P< 0.001), with a cesarean delivery rate at *Sinha* of 84.5% *vs*. 23% at *Mater*. Additional significant differences between the two hospitals were noted for PROM (hours), and duration of labor adjusted for type of delivery (Table 3).

For the total population and in each of the hospitals, none of the following factors that might contribute to colonization were found to be significantly associated with GBS colonization status. Bivariate analysis included the variables age, monthly income, race, marital status, education, gravidity >3, parity >3, prior abortions, prior cesareans, alcohol consumption, current smoker, urinary tract infection during the current pregnancy, and use of vaginal cream or other antibiotics in the last three months (data not shown).

GBS colonization as a predictor of labor and delivery outcome variables was also studied. GBS colonization was not associated with PROM, gestational age < 37 weeks, duration of labor > 360 minutes, or birth weight < 3000 grams (data not shown). Adjusting for age and race did not yield any positive associations between host risk factor or labor and delivery outcomes and GBS colonization.

Discussion

Group B *Streptococcus* (GBS) is an important cause of infection in pregnant women and their newborns; however, it has been little studied in Latin America. Implementation of rational GBS disease prevention protocols must be preceded by collection of data at the community level, due to variations in maternal GBS colonization and disease incidence rates in different populations [15]. The six published studies from Latin America provide evidence of geographical variation in maternal GBS colonization, but they give little information on disease incidence or risk factors that affect maternal colonization [16]. In Brazil, Benchetrit et al. [7] reported a GBS maternal colonization rate of 25.6% in 86 pregnant women in Rio de Janeiro in 1982. Unlike this earlier study, our methods did not include collection of rectal samples; therefore, our rate of 17.9% may be an underestimate of GBS colonization. Brazil maintains

Table 1. Prevalence of maternal group B Streptococcal (GBS) colonization in two maternity hospital populations, Ribeirão Preto, Brazil, 1999.

Variable	Mater Hospital (n= 293)	Sinha Hospital (n= 305)	Total (n= 598)	Hospital Comparison P-value
GBS positive	48 (16.4%)	59 (19.3%)	107 (17.9%)	0.345
GBS negative	245 (83.6%)	246 (80.7%)	491 (82.1%)	

Table 2. Socioeconomic characteristics of the two maternity hospital populations

Variable	N	Mater Hospital	Sinha Hospital	Total	Hospital P-value
Mean age (SD)	488	23.1 (5.4)	27.5 (5.6)	25.6 (5.9)	<0.0001*
Marital status (Married)	485	41.6%	83.0%	65.8%	<0.001
Race	416				<0.001
White	51.8%	89.4%	74.0%		
Black and mixed	48.2%	10.6%	26.0%		
Mean monthly income (Reais)	249	572.54	1645.23	951.64	<0.0001*
Maternal education	265				<0.001
Basic schooling	81.9%	33.4%	64.9%		
Advanced schooling	13.1%	66.7%	35.1%		

* Student's T-test for Mean Age and Monthly Income; SD = standard deviation; reais is plural for real, the Brazilian currency (1 real = approximately US$0.48 in August 2006).

Table 3. Obstetric and health characteristics for the two maternity hospital populations

Variable	N	Mater	Sinha	Total	Hospital P-value
Gravity	471				0.001
1		39.9%	38.4%	39.0%	
2		20.7%	41.4%	32.4%	
≥3		39.4%	20.2%	28.4%	
Type of birth	496				0.001
Vaginal		63.0%	15.5%	35.9%	
Forceps		14.1%	0%	6.1%	
Cesarean		23.0%	84.5%	58.1%	
Mean prenatal visits (SD)*	266	6.3 (2.0)	8.4 (1.1)	7.07 (2)	0.0001**
Mean ultrasound exams (SD)*	264	2.2 (1.2)	3.0 (1.1)	2.53 (1)	0.0001**
Current smoker (Yes)	284	26.0%	8.1%	19%	0.001
PROM (Yes)	503	22.9%	16.5%	19.5%	0.013
Mean hours PROM (SD)	91	11.9 (14)	4.79 (11)	8.66 (12)	0.0068
Duration of labor (min.)	449				0.001
Vaginal mean (SD)	288 (370)	658 (416)	83 (28.6)		
Cesarean mean (SD)	102 (69)	751 (407)	82 (17.6)		

* For the current pregnancy; ** Student's T-test; PROM = premature rupture of membranes; SD = standard deviation.

a relatively high maternal GBS colonization rate when compared to reports from other Latin American cities, such as Lima, Peru (6%) and Mexico City, Mexico (4%) [9,10].

Mussi-Pinhata MM et al. [17] studied 261 infants with respiratory distress from a neonatal intensive care unit in Ribeirão Preto, Brazil and concluded that Gram-positive flora,

specifically GBS (19.4%), are the most common etiologic agents cultured from this population. Miura and Martin [18] described 15 cases of neonatal GBS disease in Porto Alegre, Brazil over a 3.5-year period; they reported an incidence of 1/ 1000 newborns, indicating that GBS is an important pathogen in the etiology of early-onset sepsis in this region as well.

Vaciloto et al. [19] retrospectively reviewed all cases of early-onset sepsis due to GBS from 1991 to 2000 in a Brazilian hospital, reporting an incidence of 0.39/1000 newborns. Considering that GBS colonization is the most important risk factor for GBS disease in the newborn, these results are consistent with our finding of a relatively high maternal GBS colonization rate in Ribeirão Preto.

The role of host factors, such as age, race, socioeconomic standing, obstetric history, antibiotics, co-morbid infections, consumption of alcohol and smoking, in GBS colonization in this population is not entirely clear. It was found, however, that GBS colonization was not significantly associated with being a patient at a particular hospital. Given the significant differences in socioeconomic status of the two hospital populations (Table 2), GBS colonization does not appear to be directly related to socioeconomic factors in Ribeirão Preto, Brazil. The multicenter Vaginal Infections and Prematurity (VIP) Study Group in the United States also reported a weak association between GBS colonization and socioeconomic standing [20].

The influence of race on GBS colonization is a particularly interesting question in the Brazilian population, which has a high degree of racial mixing and a relatively high rate of GBS colonization compared to other Latin American countries. In the United States, black and Hispanic women are disproportionately colonized by GBS, 21.2% and 20.9% respectively, when compared to 13.7% in whites [20]. In addition, other studies have shown that Hispanics of Caribbean or African descent are at higher risk when compared to Hispanics of other ethnic origins [20]. Consequently, we predicted that the racial mixing of the Brazilian population would diminish the effect of race as a risk factor for GBS colonization. In our study, race was not associated with GBS colonization in either maternity hospital, even after adjusting for age. Racial mixing may explain both why our prevalence rate of 17.9% is higher than for other Latin American countries with less racial mixing, such as Peru (6%) [9], and why race is a poor predictor for GBS colonization in Brazil.

Maternal GBS colonization is a risk factor for adverse pregnancy outcomes, including prematurity (< 37 weeks), low birth weight, longer duration of labor, and PROM [21,22]. No significant relationships between GBS colonization status and delivery outcomes were noted for either of the maternity hospitals in this study. Transfer of women with preterm gestations (< 37 weeks) from Mater to a "high-risk" facility may have confounded the observed relationship between GBS colonization and preterm delivery in this population. Failure to associate GBS colonization with these delivery outcomes may also be due in part to the unusually high cesarean delivery rate (84.5%) recorded for Sinha. Many cesarean deliveries in this hospital were elective in nature, not high-risk, and not medically indicated. Since the mode of delivery is highly correlated with all the outcome variables, there is a potential confounding effect on the relationship between GBS colonization and delivery outcomes. The role of cesarean delivery in maternal GBS colonization remains largely unexplored [23].

As more data regarding GBS in Brazil become available, it is important to consider implementation of already-proven neonatal GBS disease prevention plans using intrapartum antibiotics. Use of GBS management protocols in other countries, including Canada and Australia, have resulted in up to 80% reduction in cases of early-onset GBS disease [24]. The relatively high maternal GBS colonization rate found in Ribeirão Preto, together with culture positive GBS in neonates with bacterial infections, may contribute to higher than necessary rates of neonatal infection.

Determinants of GBS prevention policies depend on disease incidence, health care delivery infrastructure, cost-effectiveness, and cultural attitudes [25]. We found that GBS colonization was not affected by socioeconomic standing or host risk factors, nor did GBS colonization status influence the labor and delivery outcomes. Therefore, a prevention strategy in this population cannot safely rely on a single-risk-factor approach for the identification of GBS-colonized mothers. Rather, a culture-based screening approach would be the most accurate method for identifying colonized women. Future studies in Ribeirão Preto should be prospective and collect follow-up data on newborns to substantiate our conclusions on disease. Along with accurate microbiological assessment of infants with sepsis, future studies focusing on the effects of mode of delivery on neonatal bacterial infections, including GBS, could take advantage of the unusually high rate of cesarean delivery in these populations for comparative analysis.

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