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MAJOR ARTICLE

Group B Streptococci Colonization in Pregnant Guatemalan Women: Prevalence, Risk Factors, and Vaginal Microbiome

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Background. Infection causes 1 of every 5 neonatal deaths globally. Group B *Streptococcus* (GBS) is the most significant pathogen, although little is known about its epidemiology and risk in low-income countries.

Methods. A cross-sectional study in 2015 at a public hospital in Guatemala City enrolled women ≥35 weeks' gestation. Vaginal and rectal swabs were processed using Lim broth and GBS CHROMagar then agglutination testing. Risk factors were assessed using multivariate analysis. Vaginal microbiota were profiled by 16S ribosomal ribonucleic acid sequencing in a subset of 94 women.

Results. Of 896 pregnant women, 155 (17.3%; 95% confidence interval [CI], 14.9–19.9) were GBS colonized. Colonization was associated with history of previous infant with poor outcome (odds ratio [OR], 1.94; 95% CI, 1.15–3.27) and increasing maternal age (OR, 1.05; 95% CI, 1.02–1.09). Multiparity was protective (OR, .39; 95% CI, .21–.72). Four (6%) GBS-exposed infants had early-onset neonatal sepsis. Vaginal microbiome composition was associated with previous antibiotic exposure ($P = .003$) and previous low birth weight infant ($P = .03$), but not GBS colonization ($P = .72$). Several individual taxa differed in abundance between colonized and noncolonized women.

Conclusions. Group B *Streptococcus* is prevalent in pregnant women from Guatemala with different risk factors than previously described. Although the vaginal microbiome was not altered significantly in GBS-colonized women, use of antibiotics had an effect on its composition.

Keywords. colonization; GBS; pregnancy; *Streptococcus agalactiae*; vaginal microbiome.

Neonatal mortality is a critical problem globally resulting in over 3 million deaths per year with two thirds occurring in the first week of life. Neonatal sepsis and pneumonia are responsible for 20% of those deaths [1, 2]. Guatemala has the highest neonatal mortality rate in Central America and second highest in Latin America, estimated at 15.3 deaths per 1000 live births, approximately half of all deaths of children under 5 years of age [1–3]. Group B *Streptococcus* (GBS) is among the leading causes of early-onset sepsis (EOS) worldwide with a 12% global case-fatality rate, which can be 3 times higher in low-income countries [4]. Although EOS from GBS has been successfully reduced by 79% with the use of intrapartum antibiotic prophylaxis (IAP) in most high-income countries [5], these screening and treatment programs are not routinely offered in low- and middle-income countries (LMIC), including Guatemala.

Group B *Streptococcus* colonization of the genitourinary tract during pregnancy is common among women from high-income countries with prevalence rates from 10% to 30%; however, less is known about its epidemiology in LMIC [6]. Among the few studies performed in Latin America, GBS prevalence varies significantly by geographical region from as low as 6% in Peru to 17% to 25% in Brazil [7–10].

Understanding the epidemiology of GBS and its risk factors as a cause of neonatal sepsis in LMIC is important for reducing the global burden of disease. Therefore, we aimed to determine the GBS colonization prevalence in Guatemalan women and assess its associated factors. In addition, as an exploratory objective and given the increasing evidence suggesting that the microbiome is an important determinant of vaginal pathogen colonization (*Escherichia coli* and *Gardnerella* spp) [11], we evaluated the potential relationship of vaginal microbiota composition to GBS colonization in this population.

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METHODS

Study Design

This was a cross-sectional study conducted in the outpatient obstetrics clinic and emergency room of Hospital Roosevelt in Guatemala City from April to November 2015. No screening for GBS or IAP protocol was in place for prevention of GBS disease at the time of the study. Informed consent was obtained from pregnant women or their parents or guardians if under age 18. The study was approved by the Colorado Multiple Institutional Review Board and the Ethics Committee at the Hospital Roosevelt in Guatemala City.

Study Population

Pregnant women were included if they were ≥35 weeks gestation, between the ages of 15 and 45 years of age, and came for prenatal care at the obstetrics clinic or emergency room. Women were excluded if they were in active labor, significant pain, under influence of narcotic medications, or did not consent.

Data Collection

To assess risk factors for GBS colonization, a standardized questionnaire was administered verbally by a Spanish-speaker physician in a private location to all enrolled participants before specimen collection. Maternal answers on the questionnaire were then cross-referenced with the medical record, which included the Centro Latinoamericano de Perinatologia form ([C.L.A.P.] Montevideo, Uruguay), a standardized prenatal intake widely used in Latin America to collect clinical care data such as height, weight, and medical and obstetric history. In the case of discrepancies, answers were clarified with the mother. All data was entered into a REDCap (Vanderbilt University, Nashville, TN) online database.

To explore labor and postnatal outcomes, a subset of women with positive GBS were contacted by phone within 4 months of enrollment to retrospectively assess maternal and infant outcomes. These data were based on maternal recall, and no review of medical records or comparison with GBS-negative women was done given our limited resources and the cross-sectional nature of the study.

Sample Collection

Sequential lower-vaginal and rectal samples were collected using a rayon-tipped swab following the Centers for Disease Control and Prevention guidelines, then placed into Amies transport medium without charcoal (BBL Culture Swab; Becton Dickinson, Sparks, MD), and transported to the hospital laboratory for either immediate processing or storage at 4°C if samples were received after hours. All swabs were processed within 48 hours of collection. For microbiome analysis, an additional lower-vaginal swab was collected from a convenience sample of the first 94 enrolled women and stored in cryovials at −70°C until shipped to the University of Colorado Microbiome Laboratory for analysis.

Group B *Streptococcus* Detection

All rectovaginal swabs were first plated onto CHROMagar StrepB ([CA] CHROMagar, Paris, France) then inoculated into selective broth medium BBL Lim Broth (Todd Hewitt broth + colistin [10 g/mL] and naladixic acid [15 g/mL]; Becton Dickinson). Broth was incubated for 18–24 hours at 35–37°C then plated onto CA. All direct-CA and broth-CA plates were incubated for 18–24 hours at 35–37°C then inspected for colony growth. Any mauve or darker pink colonies were considered positive. If no growth was visible, plates were reincubated for an additional 24 hours and re-examined. Positive results on CA were then tested using GBS latex agglutination ([LA] Pastorex Strep; Bio-Rad, Hercules, CA) assay. Latex agglutination results were either positive, negative, or indeterminate (defined by light clumping or difficult to interpret). Confirmed GBS equated to a positive GBS LA from a positive colony on 1 or both culture methods. Subjects with only a single culture method completed or indeterminate results on LA (deemed very low colonizers) were removed from the final analysis. All other results (negative on LA or negative on both cultures) were considered negative for GBS. Positive results were reported to physician providers and to study participants before delivery. However, no specific indications or guidance for antibiotic prophylaxis were given to providers.

Microbiome Analysis

Deoxyribonucleic acid was purified from vaginal swabs using the PowerFecal Kit (MO BIO Laboratories, Inc., Carlsbad, CA). Polymerase chain reaction amplification of 16S ribosomal ribonucleic acid (rRNA) genes using barcoded primers [12] specific to the V3V4 variable region (357F, 806R), multiplexed sequencing on the Illumina MiSeq (v3 2x300nt kit), and sequence quality filtering and merging as described in our previous studies [13, 14]. Assembled sequences were aligned and classified with SINA (1.3.0-r23838) using the Silva 115NR99 reference database [15, 16]. This process generated 6 368 288 high-quality 16S sequences (mean of 67 748 sequences/sample; median Goods coverage score of ≥99.7% at the rarefaction point of 8334).

Statistical Methods

Prevalence was calculated based on positive GBS results within the study population. Group B *Streptococcus* recovery on both CA methods was assessed using McNemar test for correlated percentages [18]. Sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV) for both CA methods were determined using a composite gold standard, which included all positives on LA. Baseline characteristics were compared using χ^2 or Fisher's exact test for categorical variables and Student *t* test for continuous variables. Normal distribution of continuous variables was assessed using Kolmogorov-Smirnov test.

Potential risk factors associated with GBS colonization were selected a priori based on previous reports and tested using

unadjusted and adjusted odds ratios (ORs) derived from univariate and multivariate logistic regression. These included age, diabetes, body mass index (BMI), tobacco use, douche/enema use, number of sexual partners in lifetime, parity, history of an infant with prematurity, low birth weight ([LBW] <2500 grams), or a poor infant outcome. A woman's parity was defined as either nulliparous, primiparous, or multiparous, indicating whether the pregnant woman had no living children, 1 living child, or more than 1 living child, respectively. Poor infant outcome was defined as any previous infant hospitalized or dead within the first 3 months of life. Backwards elimination was used to reduce the number of variables not significantly associated with GBS and not confounding other risk estimates (>10% change in estimates approach). Differences between hierarchal models were assessed using the likelihood ratio test. Colinearity was assessed with Pearson's correlation coefficient (significant correlation was considered if greater than 0.4) and variance inflation factors (none being greater than 4.0). Predictive power of the model was assessed with receiver operating characteristic curves. The final multivariate model adjusted for age, parity, education, marital status, literacy, ethnicity, home location, tobacco use, BMI, diabetes, prenatal care utilization, number of sexual partners, previous infant with low birth weight, prematurity, and previous poor infant outcome. Income, douche/enema, or antibiotic use during the month before enrollment were not significantly associated with GBS colonization status and did not confound other variables; thus, they were removed from the final model. All tests were 2-tailed and $P < .05$ was considered statistically significant. All statistical analyses were completed using STATA software package, version 12 (StataCorp, College Station, TX).

Associations between overall microbiome composition (using Bray-Curtis dissimilarities and 100 000 resampling) and GBS colonization as well as other independent variables as described above were assessed with Wilcoxon rank-sum tests of relative abundance and permutation-based multivariate analysis of variance (PERMANOVA) tests using R statistical software package [19].

Labor and postpartum maternal and infant outcomes were assessed as count data and presented as percentages. This included the following: delivery location, method, gestational age, antibiotic administration during delivery, and complications such as maternal fever or signs of infection; neonatal peripartum information such as need for antibiotics, duration of hospitalization, disposition, complications such as intubation, and significant anomalies; and neonatal information up to 3 months of age such as need and reason for antibiotics, hospitalization, or death.

RESULTS

From 1100 women enrolled, 990 (90%) women had both culture methods completed (Figure 1). Of those, 896 (90.5%) had definitive LA, 90 (9%) had indeterminate LA, and 4 (0.5%) had no growth on 1 culture. Of the 896 included in the final analysis, 155 women were confirmed colonized with GBS for a prevalence of 17.3% (95% CI, 14.9–19.9). No difference in GBS recovery was found between direct-CA (14.5%) and broth-CA (13.1%) ($P = .102$). For direct-CA and broth-CA, the sensitivities were 83.9% and 75.5%, specificities were 95.5% and 97.6%, PPV were 79.8% and 86.7%, and NPV were 96.6% and 95.0%, respectively.

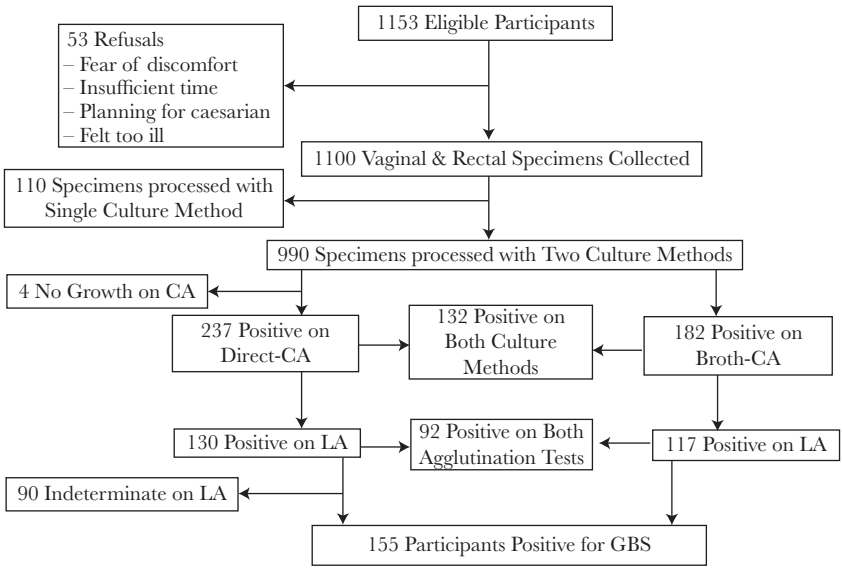

Figure 1. Flow-chart of samples from pregnant 1100 women enrolled for Group B *Streptococcus* (GBS) colonization at Hospital Roosevelt, Guatemala City. CA, CHROMagar; Direct-CA, specimens plated directly to CHROMaga; Broth-CA, specimens inoculated in Lim broth then plated to CHROMagar; LA, latex agglutination.

Baseline characteristics including age, marital status, parity, prenatal care use, BMI, and diabetes were similar between GBS-positive and GBS-negative women (Table 1). On univariate analysis, only having a previous infant with poor outcome and having a previous infant with prematurity were significantly associated with GBS colonization (Table 2). On multivariate analysis, maternal age and history of previous infant with poor outcome were independently associated with GBS colonization (Table 2). History of previous infant with prematurity showed a trend toward increased odds of colonization. Increased parity had an inverse relationship with the risk of GBS colonization. All other variables were not significant. Subgroup analysis of nulliparous women demonstrated age as the only associated risk factor for GBS colonization (OR, 1.10; 95% CI, 1.04–1.17; P = .002). In a subgroup analysis among parous women, we found that age was not a risk factor (OR, 1.03; 95% CI, 1.00–1.08; P = .077), but increased parity maintained an inverse relationship with risk of GBS colonization (OR, .56; 95% CI, .33–.96; P = .034). Receiver operating characteristic curve analysis of the multivariate model was 0.65.

Table 1. Baseline Characteristics of Pregnant Women According to GBS Status in Guatemala City

Characteristic	GBS Positive (n = 155) n (%)	GBS Negative (n = 741) n (%)	*P* Value
Age, mean (range, SD)	26.2 (15–45, 7.0)	25.1 (15–44, 6.5)	.074
Ethnicity			.410
Nonindigenous	137 (88.4)	671 (90.6)	
Native Mayan	18 (11.6)	70 (9.5)	
Marital Status			.734
Married	49 (31.6)	224 (30.2)	
Partnership	88 (56.8)	392 (52.9)	
Single	18 (11.6)	125 (16.9)	
Education			.482
None	14 (9.0)	47 (6.3)	
Primary	97 (62.6)	459 (61.9)	
Secondary	39 (25.2)	207 (27.9)	
University	5 (3.2)	28 (3.4)	
Literate			.324
No	12 (7.7)	42 (5.7)	
Yes	143 (92.3)	699 (94.3)	
Income			.654
≤$200/month	145 (93.6)	700 (94.5)	
>$200/month	10 (6.5)	41 (5.5)	
Dwelling			.079
Urban	114 (73.6)	592 (79.9)	
Rural	41 (26.5)	149 (20.1)	
Parity			.659
Nulliparous	48 (31.0)	243 (32.8)	
Primiparous	66 (42.6)	272 (36.7)	
Multiparous	41 (26.5)	226 (30.5)	
Prenatal Visits			.285
Adequate (≥4)	121(78.1)	548 (74.0)	
Inadequate (<4)	34 (21.9)	193 (26.0)	
BMI			.245
<30 kg/m^2	114 (73.5)	510 (68.8)	
≥30 kg/m^2	41 (26.5)	231 (31.2)	
Diabetes (all types)			.764
No	153 (98.7)	729 (98.4)	
Yes	2 (1.3)	12 (1.6)	

Abbreviations: BMI, body mass index; GBS, Group B *Streptococcus*; SD, standard deviation.

Maternal and Infant Outcomes

Seventy-three of 155 (47%) GBS-positive women were contacted within 4 months of enrollment. Seventy-three (100%) women delivered at a hospital and 52 (71%) delivered via Caesarian section (Table 3). Only 19 (26%) women reported receiving antibiotics during delivery. Twelve (16%) reported meeting GBS risk-based screening criteria at time of delivery including prolonged rupture of membranes (PROM), chorioamnionitis, and premature delivery <37 weeks gestational age; of those, 6 (50%) received antibiotics during delivery. Seven (10%) infants had complications during the first 7 days of life including 4 (6%) developing sepsis within 24 hours of delivery requiring intensive care unit admission and prolonged intravenous antibiotics (all with recovery). Three of the 4 mothers of these infants met 1 GBS risk-screening criteria at time of delivery including PROM (1), chorioamnionitis (1), and prematurity (1), with only 1 receiving antibiotics. The 2 reported deaths were due to noninfectious causes.

Vaginal Microbiome and Group B *Streptococcus* Colonization

To explore the hypothesis of a relationship between GBS carriage and the microbiome composition of the vaginal tract, a subset of 94 consecutive women provided samples for 16S rRNA sequencing. No significant difference in overall bacterial microbiome composition was detected between GBS-colonized and -noncolonized women (PERMANOVA, P = .72), but selected individual genus-level taxa, such as *Corynebacterium* (Wilcoxon rank-sum test, P = .03), *Aerococcus* (Wilcoxon rank-sum test, P = .03), and *Staphylococcus* (Wilcoxon rank-sum test, P = .06), were significantly different or trended towards significance among these groups (Figure 2). A higher nonsignificant median abundance of *Lactobacillus* (89.9% vs 51.0%; Wilcoxon rank-sum test, P = .8) and lower median abundance of *Gardnerella* (2.2% vs 11.0%; Wilcoxon rank-sum test, P = .3) were observed in the GBS-colonized versus -noncolonized individuals. In contrast, both antibiotic exposure (PERMANOVA, P = .003) and previous infant with LBW (PERMANOVA, P = .03) were independently associated with differences in overall vaginal microbiome composition (Figure 2). Women with a previous LBW infant exhibited a lower abundance of lactobacilli compared with those with a previous normal-weight birth (39.2% vs 68.9% relative abundance; Wilcoxon rank-sum test, P = .07). Other variables found as risk factors for GBS colonization in this population including age, parity, and history of infant with poor outcome within 3 months of delivery were not associated with microbiome structure.

Table 2. Univariate and Multivariate Analysis of Risk Factors for GBS Colonization in Guatemalan Pregnant Women

Variable	Univariate/Unadjusted Odds Ratio (95% CI)	*P* Value	Multivariate/Adjusted Odds Ratio[a] (95% CI)	*P* Value
Previous Infant With Poor Outcome				
No	Ref		Ref	
Yes	1.82 (1.14–2.90)	.012	1.95 (1.16–3.28)	.012
Previous Infant With Prematurity				
No	Ref		Ref	
Yes	1.83 (1.09–3.08)	.023	1.81 (0.97–3.39)	.063
Age	1.03 (1.00–1.05)	.061	1.05 (1.02–1.09)	.002
Parity				
No living child	Ref		Ref	
1 living child	1.23 (0.82–1.85)	.326	0.79 (0.50–1.26)	.327
>1 living child	0.92 (0.58–1.45)	.714	0.38 (0.21–0.72)	.003
Ethnicity				
Nonindigenous	Ref		Ref	
Native Mayan	1.12 (0.85–1.48)	.411	1.05 (0.77–1.43)	.761
Dwelling				
Urban	Ref		Ref	
Rural	1.20 (0.98–1.46)	.080	1.18 (0.95–1.47)	.136
History of Sexual Partners				
1	Ref		Ref	
2	0.91 (0.61–1.35)	.622	0.99 (0.65–1.52)	.969
≥3	0.57 (0.30–1.07)	.082	0.53 (0.27–1.05)	.068
BMI				
<30	Ref		Ref	
≥30	0.79 (0.54–1.17)	.246	0.78 (0.52–1.18)	.234

Abbreviations: BMI, body mass index; CI, confidence interval; GBS, Group B *Streptococcus*.

[a]Adjusted for age, parity, education, marital status, literacy, ethnicity, dwelling, smoking, prenatal care, diabetes, BMI, sexual partners, previous infant with poor outcome, prematurity, or low weight.

DISCUSSION

Our study demonstrates that pregnant women in Guatemala have high rates of GBS colonization, which translates into potential risk for perinatal morbidity and mortality. The detected prevalence of 17.3% is similar to a global prevalence rate of 17.9% determined in a recent meta-analysis [20], lower than rates found in Brazil (17.9%–25.6%) [8–10], but relatively high compared with rates reported in Peru (6%) [7], Argentina (7.6%) [21], and Mexico (4%–10%) [22, 23]. Previous smaller descriptive studies in Guatemala found GBS colonization rates as low as 2.5% among predominately native Mayan, rural populations [24] to 14.4% among predominately urban, nonindigenous populations [25]. The large variation in prevalence found throughout Latin America and within Guatemala may be influenced by different methods of sample collection (vaginal-only swabs or perineal swab instead of anorectal) and processing (all swabs processed with enrichment broth, use of traditional laboratory methods for GBS identification) between studies. Vaginal-rectal sampling can increase the yield of GBS by as much as 50% [26]. Although enrichment broth may facilitate GBS growth for women with lighter colonization [26, 27], it may also decrease detection of GBS when using rectal samples due to fecal flora growth competition [28]. Finally, small sample sizes may leave prevalence rates prone to sampling error because 8 of the 9 aforementioned studies based in Latin America used study populations of ≤500 women. This reinforces the need for sufficiently powered population-based studies to inform local epidemiology of GBS before implementation of GBS prevention strategies.

In the United States before IAP, 50% of infants born to GBS-colonized women were colonized and 1%–2% suffered neonatal infection. Hospital Roosevelt cares for ~8000 deliveries per year, and at the detected colonization rates, approximately 1400 infants will be born to a GBS-colonized woman, potentially resulting in 14 cases (1.75 cases per 1000 live births) of preventable EOS. However, even if only 2 of the cases of EOS identified among our cohort of neonates are due to GBS, this gives an incidence rate 2.23 cases per 1000 live births. These estimates are comparable to a recent study identifying an early-onset GBS incidence rate of 2.35 per 1000 live births in the Dominican Republic [29]. Our findings suggest that GBS may be a significant contributor to neonatal morbidity and mortality in Guatemala.

Aside from host and population health risk factors, the role of the vaginal microbiome as a determinant of GBS colonization remains largely unknown. Vaginal pH and the vaginal

Table 3. Descriptive Characteristics of 73 of 155 GBS-Positive Mothers and Their Neonates Within 4 Months of Enrollment in Guatemala City

Characteristic	N = 73 n (%)
Hospital delivery	73 (100%)
Type of delivery	
Vaginal	21 (29%)
Caesarian	52 (71%)
Previous C/S	27 (52%)
Eclampsia/Pre-eclampsia	8 (15%)
Breech/CPD	6 (12%)
Fetal distress	2 (4%)
Other	9 (17%)
Antibiotics administered during delivery	19 (26%)
Met GBS risk-based screening	12 (16%)[a]
GA <37 weeks	7 (10%)
PROM	2 (3%)
Chorioamnionitis	5 (7%)
Low birth weight (<2500 g)	14 (19%)
Infant complication first 7 DOL	7 (10%)
Death	2 (3%)
Sepsis	4 (6%)
Seizure	1 (1%)
Complication >7 to 90 DOL	19 (26%)
Pneumonia	3 (4%)
Other bacterial infection	6 (8%)
Common cold	10 (14%)

Abbreviations: CPD, cephalopelvic disproportion; C/S, Caesarian section; DOL, days of life; GA, gestational age; GBS, Group B *Streptococcus*; PROM, prolonged rupture of membranes.

[a]Two women had multiple risk factors.

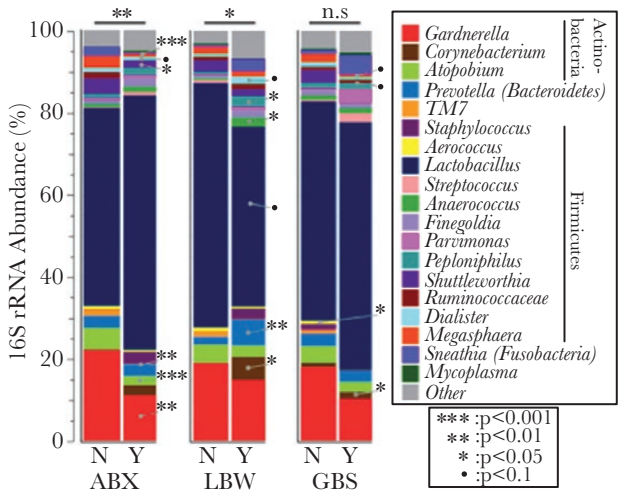

Figure 2. Variability in vaginal microbiota among subset of 94 pregnant women in Guatemala City. The height of each bar represents the mean abundance of taxa within each subject group. ABX, antibiotic use before L&D (N = 53; Y = 41 subjects; low birth weight (LBW), previous birth of LBW infant (N = 47; Y = 11 subjects); for Group B *Streptococcus* (GBS), GBS carriage (N = 86; Y = 8 subjects); n.s., not significant. Association of variables with overall vaginal microbiome composition is denoted above bar charts, whereas association with individual taxa are indicated by lines and symbols adjacent to barcharts.

microbiome, which varies by region, may influence GBS colonization and infection in certain populations [10, 30]. In a large population study in Denmark, women who had bacterial vaginosis were half as likely to be colonized by GBS than women without bacterial vaginosis [31]. Our study explored this hypothesis and observed a similar trend where there was a lower relative abundance of *Gardnerella* (2.2% vs 11.0%) among GBS-colonized women. In contrast, we found a higher relative abundance of lactobacilli among the GBS-colonized women, compared with GBS-noncolonized women (89.9% vs 51.0% median abundance). Additional, larger studies are needed to better understand the relationship between the vaginal microbiome, maternal colonization, and impact on neonatal pathogen colonization.

Our study demonstrates an overall 5% increased odds of GBS colonization for each year increase in maternal age. However, our subgroup analysis suggests that the association with older age may be dependent on parity where age is a significant risk factor for women who are nulliparous but not for women who are parous. Association with older age has been identified in multiple other studies including in Latin America [6, 10, 27]. The implication of this remains unclear. It is possible that older age or parity may serve as surrogate markers for some yet-unidentified risk factors. Although our limited vaginal microbiome analysis did not show any relationship between its composition and increased parity or age affecting GBS colonization, further studies are encouraged.

Our finding of a 2-fold increased odds of GBS colonization for women with a prior infant with poor outcome in the first 3 months of life may suggest previous neonatal GBS infection and disease. Quantifying the risk associated with a previous infant with invasive GBS disease has been difficult due to its low incidence, but mothers of these infants have lower GBS antibody levels and are more likely to be chronic GBS carriers [32–34].

Major limitations to prevention programs in LMIC include the cost of testing, laboratory infrastructure, and personnel. Chromogenic agar has the potential to reduce these challenges through simplification of testing and result interpretation. Direct plating of vaginal-rectal swabs to CA optimizes GBS recovery by limiting overgrowth of fecal bacteria (eg, *Enterococcus faecalis*) promoted in enrichment broth [28, 35, 36]. Removal of the enrichment step also reduces cost, time, and human resources, all of which are scarce in LMIC. Furthermore, direct-CA demonstrated similar GBS recovery and sensitivity compared with broth-CA, a finding comparable to other studies examining the use of direct-CA [35]. Therefore, direct-CA followed by LA could be an acceptable GBS recovery method in a resource-constrained setting. However, an important consideration is that approximately 10% of subjects had indeterminate results on LA. Many of these likely reflect light GBS colonization

with resultant low-colony growth on CA. Weak agglutination due to low inoculum has been commonly described as a reason for indeterminate results on different LA assays. For analysis purposes, these results were excluded because including them as negatives would have underestimated GBS prevalence and positive confirmation was not obtained.

Given the prevalence of GBS colonization in Guatemala and its potential contribution to the burden of the high neonatal mortality, an IAP program could produce significant results if designed and implemented appropriately. However, in order for IAP to be effected, LMICs need to establish GBS screening programs that can be either universal culture-based (all women ≥35 weeks' gestation) or targeted risk-based screening (PROM, chorioamnionitis, and premature delivery). Although cost-effectiveness studies between these 2 screening methods have not been completed in most LMICs, in high-income countries, universal culture-based screening is slightly more cost effective [37] and identifies a greater proportion of GBS-positive women, resulting in better coverage of IAP [5, 38]. If a screening and prevention program were to be implemented in Guatemala where there are 480 000 live births per year, assuming an early-onset GBS incidence rate of 2 cases per 1000 live births with a 30%–50% mortality rate, a prevention program with 80% coverage could lead to 230 to 380 neonatal deaths averted annually.

Important considerations for a screening program in Guatemala include the ongoing challenges in healthcare delivery during the prenatal and delivery periods. Although 93% of Guatemalan women have at least 1 prenatal visit during their pregnancy, the majority of these visits take place during the first and second trimester, likely as a means to confirm pregnancy, and only 51% of deliveries are attended by a skilled birth attendant [39]. Therefore, innovative screening and prevention programs that can be adapted to local and regional barriers are necessary.

The administration of a GBS vaccine to women during pregnancy could be a feasible and more practical approach to prevention given the challenges of a GBS screening program and IAP in LMIC. However, a recent study in South Africa estimates that a joint vaccination/risk-based screening program would be more effective in reducing GBS disease compared with vaccine alone while remaining cost effective [40]. This reiterates the importance and need for LMICs to identify GBS burden and implement screening and prevention strategies while awaiting results of the best vaccine strategy for GBS prevention.

Some limitations of our study include the following: (1) its external validity because we limited our findings to a tertiary care center in the capital; (2) enrollment of women from the emergency room without controlling for use reason beyond exclusion criteria; (3) limitations of LA as a confirmatory test due to possible weak agglutination; (4) lack of a control comparison group for our exploration of retrospective maternal and neonatal outcomes and its possible recall bias; and (5) the small sample size for the vaginal microbiome analysis.

CONCLUSION

Pregnant women in Guatemala City have high rate of GBS colonization and with possible high incidence of sepsis in their neonates. Older age at first pregnancy was associated with increased GBS colonization. In light of the above findings, GBS screening and antibiotic prophylaxis could impact maternal and neonatal morbidity and mortality. Furthermore, upcoming GBS vaccines could ultimately be the most cost-effective intervention.

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