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Socio-Behavioral Predictors of Vaginal *Lactobacillus* Dominance in Pregnant African American Women

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An abstract of
A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health
in Global Epidemiology
2018

Abstract

Socio-Behavioral Predictors of Vaginal *Lactobacillus* Dominance in Pregnant African American Women

By Anya Cutler

Adverse birth outcomes disproportionately affect African American women compared to women of other races and ethnicities. Socioeconomic and lifestyle factors do not fully explain these racial disparities. Recent advances in bacterial sequencing technology have revealed the importance of *Lactobacillus* species in maintaining an acidic and stable vaginal microbiome. Vaginal dysbiosis, which occurs most often in African American women, is characterized by low levels of *Lactobacillus* and high levels of anaerobic bacteria and has been linked to increased risks of sexually transmitted infections, bacterial vaginosis, and preterm birth. This study sought to identify the socioeconomic and behavioral predictors of *Lactobacillus* dominance and preterm birth among pregnant African American women. 184 women were recruited from a public and private hospital in Atlanta. Vaginal swabs and demographic/lifestyle questionnaires were collected at 8-14 weeks (T1) and 24-30 weeks (T2) gestational age. Hierarchical clustering was performed to characterize a *Lactobacillus*-dominant community state type (L-CST) and a diverse community state type (D-CST) among vaginal microbiome samples. Women had significantly higher levels of *Lactobacillus* at T2 compared to T1 ($p=0.038$). Vaginal dysbiosis at T1 was significantly associated with young maternal age, single marital status, and low education, whereas vaginal dysbiosis at T2 was significantly associated with a short cervical length and recent receipt of oral sex. The odds of having a D-CST at T2 was 2.87 (95% CI = 1.014, 8.132) times higher among women who received oral sex in the past month compared to those who did not after controlling for socio and behavioral variables. Additionally, the risk of preterm birth (<36 weeks) and early birth (<38 weeks) was significantly higher among women who transitioned from a L-CST to D-CST microbiome between T1 and T2 compared to women who maintained a L-CST microbiome throughout pregnancy. I concluded that oral sex and short cervical length in late pregnancy may predispose women to vaginal dysbiosis and subsequently cause inflammation and premature rupturing of the amniotic sac. Further work on the relationship between *Lactobacillus* levels, cervical length, and sexual practices could identify clinical recommendations that would improve vaginal health and decrease preterm birth risk in African American women.

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Background/ Literature Review

Minority populations suffer from a disproportionately high burden of adverse health outcomes across the globe. In the United States, African American race is one of the major risk factors for morbidity and mortality (1). Ever since the Centers for Disease Control and Prevention (CDC) began collecting data on maternal and infant morbidity and mortality in 1981, the data has consistently shown large racial divides in the health of pregnant mothers and their newborns. In particular, African Americans experience far higher rates of maternal and infant mortality, preterm birth, low birth weight, and cesarean section than other races (2). Differential health care access and treatment, chronic stress from racial discrimination, and poor education have been proposed as dominant drivers of the higher rates of adverse birth outcomes in African Americans. However, despite hundreds of studies showing significant associations between socioeconomic factors and racial differences in birth outcomes, there is little understanding of the biological mechanisms underlying these associations. This review will cover the details of racial disparities in adverse birth outcomes in the United States and discuss several variables that could partially explain the disparities. It will focus ultimately on the association between race and the genera composition of the vaginal microbiome, which is an important factor potentially underpinning the racial dissimilarities in pregnancy outcomes. Finally, there will be a discussion on the need for epidemiologic and geospatial modeling to describe how racially-correlated variables are connected to an vaginal dysbiosis and its consequences on birth outcomes.

I. Racial differences in adverse pregnancy and birth outcomes

Low birth weight (LBW) infants are defined by a birth weight below 2500 grams, and very low birth weight (VLBW) infants by a birth weight below 1500 grams (3). Compared to infants with normal birth weight, infants born with LBW and VLBW are 40 times and 200 times more likely to die during the neonatal period, respectively (4). Among African American infants, 13% are born LBW and 2.8% are born VLBW, in contrast to only 7% and 1.1% among White infants (3). Berg *et al.* (5) calculated an OR of 3.3 for VLBW in African American infants versus White infants after adjusting for several potential confounders, including gestational age, maternal education, marital status of parents, drug and alcohol use during pregnancy, financial support from father, and income. LBW can be caused by several conditions, including premature rupture of the membranes, spontaneous preterm labor, hypertensive disorders, and hemorrhage. Pregnant African American women experience higher rates of all of these, with no one clinical condition accounting for the majority of the racial disparity. Genetic factors do not cause these differences, given that genetics only accounts for 27% of the variation in birth weight (6). In addition to an increased risk of mortality within the first year of life, infants born with LBW who survive to adulthood are more likely to develop cardiovascular disease, hypertension, and Type II diabetes as adults, all of which occur at much higher rates in African American adults than White adults (7). The "fetal origins hypothesis" suggests that these morbidities are programmed *in-utero* when malnourishment of the fetus and other environmental stressors cause changes in blood circulation, insulin resistance, vascular structure, and liver function that persists into adulthood (8). The association between LBW and adult morbidity persists after controlling for several demographic and lifestyle variables, further supporting the fetal origins

hypothesis. Therefore, the intrauterine environment may be a major contributing factor to high LBW rates in African Americans. However, we are still in early stages of understanding how the African American experience can modify intrauterine environments.

Preterm birth (PTB), while related to low birth weight, carries its own independent set of risks. PTB is defined as any birth occurring at or before 36 weeks of gestation. PTB is a strong predictor of infant mortality, as well as several neurocognitive disorders later in life (9,10). In 2015, African American women had a preterm birth rate of 13.4%, compared to 8.8% in White women (3). There has been some indication of a genetic contribution, with a higher rate of polymorphisms linked to preterm birth in African Americans than other races (11). However, genetic variance accounts for only a small portion of the racial discrepancy in PTB and several socioeconomic and sociobehavioral factors likely contribute.

Infant mortality has been consistently declining in the United States over the last several decades, but African American infants are still twice as likely to die as White infants (12). While LBW and PTB partially account for the racial difference in infant mortality, African American infants have actually shown a survival advantage at young gestational ages compared to White infants, likely due to genetic factors. African American fetuses have faster maturation on average than other races, particularly of the lungs (13). As technology has developed to combat infant mortality, it has focused primarily on clinical conditions that affect White infants more than African American infants. For example, surfactant therapy has greatly decreased infant mortality among White infants, who have higher rates of lung immaturity than African American infants (14). In African American infants, infant mortality primarily occurs from preterm related deaths, congenital malformations, SIDS, and unintentional injuries (10). To decrease infant deaths among African Americans, prevention

and research efforts should focus on identifying the specific causes of African American preterm birth and teaching safe parenting practices during infancy.

Despite the exceptional technology and high health care expenditure, the United States ranks 46th in the world for maternal mortality and is one of only 14 countries in which the maternal mortality rate is on the rise (2,15). Additionally, maternal morbidity is increasing, likely due to higher maternal age, c-section rates, and obesity leading to life-altering procedures and diagnoses during delivery (16). There are 43.5 maternal deaths out of 100,000 live births among African American women, compared to only 12.7 deaths for White women (2). Similar to the birth outcomes previously discussed, this disparity persists after accounting for education and socioeconomic status (17).

II. Socioeconomic and sociobehavioral contributions to adverse birth outcomes

In the United States, healthcare is privatized and coverage relies on employers and individuals, which makes poverty the largest risk factor for poor health outcomes in the country. Poverty is associated with low education, poor access to health services, malnutrition, and dangerous living and work conditions, all of which translate to high rates of acute and chronic clinical conditions (1). Women in the lowest income bracket suffer from the highest rates of preterm birth, low birth weight, and stillbirth (18). African American women are twice as likely to live below the federal poverty level than White women, and as a result have much higher rates of unemployment, uninsurance, and staggeringly low levels of prenatal care in the first trimester (19). Even those who do receive early prenatal care are less likely to receive necessary medical advice, information about health risks and the associated complications, and options for prenatal treatments (20).

African Americans are also the most highly segregated racial group, which stems from housing policies of the Civil War era and onward that limited the choices of African Americans and forced most of them to live in the poorest neighborhoods. These areas were, and still are, highly vulnerable to crime, pollution, toxic waste, overcrowding, inaccessible health care, food deserts, poor education, and limited employment opportunities. As a consequence, African Americans who live in these neighborhoods still suffer from extremely high infant mortality and other adverse birth outcomes (19,20). Among African Americans who are able to live in areas with higher quality education, the economic benefit of educational degrees is still lower than what White citizens achieve with the same degree. In fact, the average net worth of the average college graduate African American is equal to that of the average high-school graduate White (7). All of these racial disadvantages collectively contribute to socioeconomic disparities, with several related factors, including obesity, maternal age, and smoking having well-studied consequences on maternal health.

Obesity is not only more common in African American women than White women, but its negative effects on birth outcomes are stronger (21). Extremely obese (BMI $\geq$ 40) African American women have the strongest association between weight and stillbirth than any other racial demographic (22). While obesity is independently associated with long, difficult deliveries and cesarean sections, it is also a risk factor for hypertension and diabetes, both of which increase the risk for several adverse birth outcomes (21,23). Maternal Type I, Type II, and gestational diabetes increase the chance of preterm birth and are associated with an increased risk of the infant developing diabetes later in life, even after controlling for genetics (21). Additionally, obesity and diabetes are thought to act synergistically to increase the risk of non-congenital defects in newborns (24).

Rates of teenage pregnancy and unintended pregnancy are far higher among African American women than White women (3,19). After controlling for several socioeconomic variables, teenage mothers still have much higher rates of preterm birth and low birth weight than mothers in their mid to late twenties (25). Furthermore, the known effects of older maternal age on low birth weight and preterm birth are strongest in African American women after controlling for confounding socioeconomic factors (26,27). Despite several studies demonstrating an interaction between race and maternal age on the effect of birth weight and preterm birth, there is little understanding of the mechanisms behind this association.

Smoking during pregnancy is most common among adults living in poverty and teenage girls (28). Tobacco use during pregnancy has been associated with several adverse birth outcomes, including intrauterine growth retardation, SIDS, preterm birth, placental abruption, and stillbirth (29,30,31). Because poverty and the teenage pregnancy rate is higher among African American women, smoking has been implicated as a potential cause of racial disparities in preterm birth. However, the rate of smoking during pregnancy is estimated as 9-10% among African American women, compared to 16% among White women (32). It is therefore unlikely that smoking explains any portion of the racial gap.

III. Stress from racial discrimination and adverse birth outcomes

Although socioeconomic and sociobehavioral factors undoubtedly contribute to racial disparities in health, studies have repeatedly shown that the disparities persist even after controlling for these variables (33). In fact, there is evidence that the gap actually widens at higher income and education levels (34). African Americans, at all levels of

socioeconomic status, often experience oppression, alienation, and exclusion based on their race, which in turn can deflate an individual's self-esteem and worldview (7,35,36,37). As these experiences accumulate over one's lifetime, chronic stress can cause weathering, as evidenced by increasing racial disparities in health with older age groups (38). Racism can occur on an individual and institutional level. Individual racism occurs from differential assumptions about one's abilities and motives or differential treatment based on race. Examples include lack of respect, suspicion, devaluation, scapegoating, and dehumanization. Institutional racism is defined as "differential access to goods, services, and opportunities of society by race" and occurs on a system-wide level (39). Approximately 96% of African Americans report an experience of some type of racism in the past year and 98% report an experience of racism during their lifetime, with many of these experiences causing depression, anger, and anxiety (40). Within clinical care, African American patients are often treated differently than White patients with the same symptoms, receiving fewer diagnostic and therapeutic interventions and less pain medication (19). Pregnant African American women have reported indifference and disrespect from clinicians, as well as assumptions that they are young, unmarried, multiparous, on welfare, and have poor health habits based on their race (41). This distrust of care will often lead to a lower likelihood of following through with screenings and lower adherence to treatment regimens (42). Indeed, there are several studies that have found a link between acute and chronic stress related to racial discrimination and adverse birth outcomes (33,43,44,45). While many pregnant women experience stress from the financial and emotional burden that accompanies a child, Klonoff *et al.* (40) showed that racism contributed a distinct type of stress that caused unique symptoms and risks among African American women.

Stress can cause adverse birth outcomes through either behavioral or physiological mechanisms. Anxiety or depression from discrimination can result in poor nutrition, increased tobacco or drug consumption, and inadequate physical activity, which will subsequently lead to adverse birth outcomes. However, after the behavioral effects of stress are adjusted for, stress is still associated with a high risk of health effects (7). Chronic release of stress hormones during pregnancy can create a wear and tear effect on the body that leads to preterm birth and low birth weight (34,46,47). Among pregnant African American women, those reporting high levels of discrimination have much higher rates of preterm birth and low birth weight infants than those reporting low levels of discrimination (48). There have been three primary physiological mechanisms proposed to explain the effect of stress on preterm birth and low birth weight: the neuroendocrine pathway, the vascular pathway, and the immune-inflammatory pathway. In the neuroendocrine pathway, corticotropin-releasing hormone (CRH) stimulates the release of cortisol, which will in turn decrease the production of CRH through a negative feedback loop. However, in cases of chronic stress, the negative feedback loop is inhibited, causing CRH and cortisol to build up in the body (49). CRH is involved with fetal cellular development and maturation and plays a regulatory role during labor. Chronically elevated CRH levels can stimulate early labor, ultimately resulting in preterm birth (7,50). In the vascular pathway, stress causes increased blood pressure (hypertension), which leads to vasoconstriction and decreased blood flow to the fetus. Decreased blood flow from maternal hypertension is a major risk factor for fetal hypoxia, growth retardation, preterm birth, and low birth weight (7,51). Hypertension is much more common in both pregnant and non-pregnant African American women than White women (52). Lastly, chronic stress can also cause excessive immune suppression, leaving the mother vulnerable to infections (53). High levels of chronic stress are associated

with urogenital infections, which increase the risk of amniotic infection, premature rupture of the membranes, and preterm birth (54). African American women have much higher rates of urogenital infections than women of other races, even after controlling for lifestyle factors (7). In addition to urogenital infections, there has been a cascade of studies finding associations between race and clinical conditions related to dysbiosis in the vaginal microbiome. The remainder of this review will concentrate on current understanding of race and the microbiome, and how social, environmental, genetic, and behavioral conditions related to race may be linked to adverse birth outcomes through microbiological routes.

IV. The vaginal microbiome and its role in health

Prior to genetic sequencing, knowledge of the vaginal microbiome was limited to organisms that could be cultured. Recent developments in 16S ribosomal sequencing have allowed for the identification of several unculturable organisms that appear to be regular inhabitants of the vaginal microbiome (55). As a result, the last decade has been a turning point in understanding the role of the vaginal microbiome in health and disease. Unlike the microbiome of other body sites, the healthy vaginal microbiome has relatively low alpha diversity and skewed evenness, with *Lactobacillus* species comprising 60-99.9% of the total microbial makeup (56, 57, 58). Furthermore, dominance by *Lactobacillus* is usually restricted to one *Lactobacillus* species, giving rise to four primary community state types (CSTs) dominated by *Lactobacillus*. These CSTs were coined CST I, II, III, and V for *L. crispatus, L. gasseri, L. iners,* and *L. jensenii* dominance, respectively (58). The byproducts of vaginal glycogen are fermented by *Lactobacillus* into lactic acid, which lowers the pH of the vagina to below 4.5 and prevents the colonization and growth of potentially pathogenic organisms

(59). Because estrogen regulates levels of vaginal glycogen, estrogen level is associated with *Lactobacillus* dominance (56). *Lactobacillus* are also thought to protect against infection by bacteriocin and hydrogen peroxide production and host immune modulation (56).

In approximately one third of women, the vaginal microbiome has little to no *Lactobacillus*, and is instead characterized by a much more diverse community, often including *Prevotella, Megasphaera, Gardnerella, Sneathia,* and *Atopobium* species (58, 60). This diverse community state type (CST), coined by Ravel *et al.* (58) as CST-IV, is associated with bacterial vaginosis (61, 62, 63), sexually transmitted infections (64, 65, 66, 67), vulvovaginal atrophy (68), mother-to-child transmission of HIV (69), reduced efficacy of antiretroviral prevention for HIV (70), and preterm birth (62,71). Bacterial vaginosis (BV) is the most common vaginal disorder among reproductive aged women, occurring at least once in 30% of women between the ages of 14 and 49 (72). It is characterized by a vaginal pH above 4.5, a creamy white vaginal discharge with a fishy odor, and the presence of superficial squamous cells surrounded by clumps of bacteria (73). When BV occurs in pregnant women, it is associated with an increased risk of preterm birth, miscarriage, and postpartum endometritis (74,75). Pregnant women who have the diverse CST-IV vaginal microbiome, which is often indicative of BV, are at particularly high risk of preterm birth if the bacterium *Gardnerella vaginalis* is present (62). Women can switch between *Lactobacillus*-dominant and diverse CSTs within their lifetime and even within a pregnancy. However, the likelihood of transitioning from a *Lactobacillus*-dominant CST to CST-IV changes depending on the specific *Lactobacillus* species present. DiGuilio *et al.* (62) found that pregnant women with a vaginal microbiome dominated by *L. iners* were more likely to transition to CST-IV than women with a *L. crispatus, L. gensenni,* or *L. gasseri* dominant microbiome. In contrast, Romero *et al.* (76) found

that women commonly transitioned between dominance by different *Lactobacillus* species but rarely transitioned between a *Lactobacillus*-dominant CST and CST-IV.

V. Race and the vaginal microbiome

In addition to the several health outcomes previously discussed, African American women have higher rates of vaginal pathogens, including *Chlamydia trachomatis*, *Neisseria gonorrhoeae, Bacteroides,* and *Gardnerella vaginalis* compared to women of other races (6,77). Likewise, African American women also have a higher vaginal pH on average, a much higher prevalence of CST-IV, and three times the rate of BV compared to White women (58,63,71,78,79). While the most common vaginal CST in White Women is dominated by *L. crispatus,* the most common CST's in African American women are CST-IV and the *L. iners*-dominant CST (80). Additionally, communities in sub-Saharan Africa show a similar distribution of CST's as African Americans (81). There appears to be a global phenomenon in which vulnerable populations overall have disproportionately high rates of BV, and likely higher rates of CST-IV, even after controlling for sexual behaviors. For example, Afro-Caribbean populations in the United Kingdom, Gypsy populations in Spain, and Tibetan populations in portugal all have higher rates of BV compared to the other local populations (82). The difference in BV rates persists after controlling for sexual behaviors (83,84). Known risk factors for BV include African American race, unmarried status, low income, low education, young maternal age, and stress (54,85). While it is assumed that CST-IV and BV contribute to racial disparities in preterm birth by predisposing African American women to intrauterine infections (6), it is not understood why African American women have higher rates of CST-IV in the first place.

Several studies have found associations between sexual behaviors and CST-IV, including pessary use (86), douching, and use of lubricants (56). Sexual behaviors, hygiene practices, hormonal fluctuations, stress, vaginal bleeding, insertion of foreign objects, contraception devices, smoking, substance abuse, antibiotics, and antifungals can all affect the vaginal flora (7,54,87,88). Additional dietary factors, such as iron and vitamin D deficiency, dietary fat, high glycemic load, and overall low nutritional density, have been linked to BV (89,90,91,92). In contrast, folate, Vitamin E, and calcium intake are inversely related to BV (92). It is unknown, however, whether any of these dietary or behavioral factors could explain racial differences in the vaginal microbiome makeup. Douching has been suggested as a likely contributor, given that African American women are twice as likely to douche as White women (93,94). Douching can both increase the vaginal pH and introduce pathogens (95). However, douching may cause the altered flora or may simply be a behavioral result of an a preexisting dysbiosis in the vaginal microbiome (7). To date, there has not been a study focusing on the sexual, socioeconomic, and stress related determinants of diverse vaginal microbiomes among African American women.

VI. Need for further research

Health care research often relies on predictive regression models to identify risk factors for and estimate the likelihood of health outcomes (96). When assessing complex relationships that involve several independent and codependent factors, such as that between vaginal flora and race, modeling can help to hone in on the strongest predictors of risk. However, predictive modeling has not yet been done to determine likely risk factors for a diverse microbiome strictly among African American women. Because vaginal microbiome

community state types have only recently been discovered, the majority of the research connecting sociodemographic and biobehavioral factors to vaginal flora has used BV as an outcome. Fettweis *et al.* (80) used logistic regression to assess intrinsic and extrinsic factors associated with BV. They found that pregnancy status and race were the only significant predictors. Meis *et al.* (97) ran separate regressions for African American and White women, and found that a recent episode of vaginal bleeding, alcohol use during pregnancy, and lack of a home phone were associated with BV among African American women. Both of these studies looked at a small number of predictors, none of which were stress-related or sexual behaviors. Because both stress and sexual behavior is correlated with race and vaginal flora, it is crucial that these variables be considered in such an analysis. Additionally, the sensitivity and specificity of BV diagnosis can be low and many women are asymptomatic (56, 98). Therefore, using a more sensitive measure, such as CST, would be a more reliable outcome for modeling.

VII. Aims of this study

This study was conducted to: 1) Characterize *Lactobacillus* dominant and diverse CSTs at 8-14 and 24-30 weeks gestation among a cohort of pregnant African American women in Atlanta; 2) Assess differences in demographic, behavioral, and sexual characteristics between women with a *Lactobacillus*-dominant vaginal microbiome and women with a diverse vaginal microbiome; 3) Regress CST against several predictor variables to identify potentially important risk factors for vaginal dysbiosis; 4) Determine if a change in CST through pregnancy is correlated with a change in antibiotic use, infection diagnosis, or behavior; 5) Determine if a relationship exists between CST and preterm or early term birth.

Methods

I. Participant recruitment

The data for the study was part of the ongoing Emory University African American Microbiome in Pregnancy study (99), which was approved by the Emory University Internal Review Board and the appropriate review councils for each hospital. Pregnant women who self-identified as African American were recruited from Grady Memorial Hospital, a public hospital serving primarily low-income patients, and Emory University Midtown Hospital, a private hospital serving a diverse patient population. The women were recruited for the study during prenatal visits between 8-14 weeks gestational age, which was determined by the last menstrual period or by ultrasound. Other than self-identifying as African American, women had to be expecting a singleton birth, comprehend written and spoken English, be between 18-40 years of age, have no chronic medical condition or take prescribed chronic medications as verified by medical records, and live within 20 miles of the Emory laboratory to preserve the quality of biological samples during transportation.

II. Biological sample collection and processing

Vaginal swabs were self-collected during visits at 8-14 weeks (T1) and 24-30 weeks (T2) gestation after participants were provided with verbal and pictorial instructions by trained clinicians. Self-sampling for vaginal microbiome analysis has shown to yield similar quality samples as physician collection (100). Two swabs were used for DNA sequencing for microbiome analysis, one was used for Gram stain, and one was used for pH determination. Swabs for microbiome analysis were Sterile Catch-All™ Sample Collection Swabs (Epicentre Biotechnologies, Madison WI) which were stored in MoBio bead tubes (MoBio Laboratories Inc., Carlsbad, CA) at -80°C until DNA extraction. Swabs for pH assessment were applied

to pH strips (Merck, Darmstadt, Germany) and scored from 4.0-7.7. Swabs for Gram staining were rolled onto glass slides to determine Nugent Score criteria.

DNA was extracted from swabs with the MoBio Isolation kit using the HMP Standard Operating Protocol (101). Samples were PCR amplified in duplicate using multiplexed primers targeting the V3-V4 region of the 16S rRNA gene (102). Pooled amplicons were sequenced on an Illumina MiSeq with up to 50% phiX174 control added to each lane. Only reads with a Q score >30 at each base were kept, leaving an average of 158,501 reads per sample (5th percentile = 57,037, 95th = 332,641). Data quality control, demultiplexing, and operational taxonomic unit (OTU) clustering was accomplished using the QIIME pipeline (103). Taxonomy of OTUs was assigned de novo using GreenGenes 13.8. After taxonomic assignment, reads were glommed at the genus level.

III. Sociodemographic, psychosocial, and clinical measures

During the initial visit at 8-14 weeks gestation, participants completed a socio-demographic survey to obtain information on age, marital status, education, and income. At 8-14 weeks and 24-30 weeks gestation, participants completed a health survey that asked about sexual, hygiene, and substance-use practices in the month prior to the visit. Participants also were assessed on psychosocial measures at the 10-14 weeks. The Childhood Trauma Questionnaire (CTQ) asks subjects to rate 21 questions about neglect and abuse during childhood on a 5-point Likert scale, which yields a total score ranging from 21-105. The Stressful Life Events Index asks about 13 stressful events occurring in the past year, yielding a total score ranging from 0-13. Krieger Experiences of Discrimination Scale measures exposure to discrimination in nine settings on a 4-point Likert scale, yielding a total score ranging from 0-27. The Brief Jackson-Hogue Stress Scale asks 36 questions about

gendered racism among African American women. For the purpose of this analysis, the only item used from the Jackson-Hogue stress scale stated "Racism is a problem in my life", in which participants who stated "strongly agree" or "agree" were coded as 1 and participants who stated "unsure", "disagree", or "strongly disagree" were coded as 0. The Edinburgh Depression Scale, which was completed at both 10-14 weeks and 24-30 weeks, asks about 10 symptoms of depression in the past seven days and is rated on a 4-point Likert scale, yielding a total score of 0-30. All of these instruments are frequently used in minority populations of pregnant women (104, 105, 106, 107, 108, 109).

Trained clinicians on the research team used medical chart abstraction after delivery to ascertain BV infection, Gonorrhea infection, Chlamydia infection, antibiotic use, BMI, cervical length, anemia, hypertension, and birth outcome.

IV. Statistical analysis

All DNA sequences were mapped to genus-level OTUs. Shannon Diversity was assessed on the unrarefied dataset and a dataset rarefied to the smallest number of reads in a sample (7,614). Because the Shannon Diversity measures remained the same after rarefaction, analysis was completed on the unrarefied dataset.

Samples were grouped into clusters using complete hierarchical clustering of Bray-Curtis similarity indices. Clustering was performed separately for samples at T1 and samples at T2 to observe if differences in community state types (CSTs) existed at different time points in pregnancy. Samples were assembled into the minimum number of clusters that separated *Lactobacillus* dominant CSTs (L-CST) from diverse CSTs (D-CST) (2 clusters for T1 and 4 for T2), with *Lactobacillus* dominance determined by visualization on a heat map. Redundancy analysis was performed to produce a constrained ordination plot. *Lactobacillus*

proportion was calculated as the proportion of reads belonging to the *Lactobacillus* genus divided by the total number of reads in the sample that mapped to a taxa.

To determine if demographic differences existed between women with L-CSTs and D-CSTs or between women with preterm or early births and term births, one way ANOVAs were carried out for continuous variables and chi-squared tests were carried out for categorical variables. For the purpose of crude univariate differences in demographic variables, multiple comparisons were not accounted for. If expected cell counts of continuous variables were less than five, Fisher's Exact Tests were performed instead of chi-squared tests. Multivariate logistic regression was performed to obtain adjusted ORs for each predictor using either D-CST, preterm birth (≤ 36 weeks), or early birth (≤ 38 weeks) as the outcome. Preterm birth and early birth were analyzed in two ways: 1) Including all births and 2) Restricting to only spontaneous births. Multivariate linear regression was performed to assess if changes in behavior, infection, or antibiotic use between T1 and T2 could explain variation in the change in *Lactobacillus* proportion between T1 and T2. Cervical length and income were omitted from the multivariate models due to high numbers of missing values.

All diversity analysis and figures were conducted in R v. 3.3. Shannon diversity and redundancy analysis was performed using the *vegan* package, heat maps were created using the *gplots* package, and bar plots were created using the *ggplots2* package. ANOVAs, chi-squared tests, and regressions were run in SAS v. 9.4.

Results

I. Composition of the vaginal microbiome at 8-14 and 24-30 weeks gestation

Of the 47,507,163 reads that mapped to a taxa, 50.4% of them belonged to the *Lactobacillus* genus. At 8-14 weeks gestational age (T1), there was a clear bimodal distribution of the proportion of reads belonging to *Lactobacillus* in the samples, indicating two distinct community state types (CSTs) either dominated by *Lactobacillus* (L-CST) (54.4%) or containing a diverse community of other genera (D-CST) (45.6%) (Figure 1A). In contrast, a higher proportion of samples were dominated by *Lactobacillus* at 24-30 weeks gestation (T2) (66.4% at T1 vs. 33.6% at T2), and there was no distinct community state type with extremely low levels of *Lactobacillus* (Figure 1B). Instead, when the proportion of *Lactobacillus* fell below 90% of the reads, there was a relatively even spread of *Lactobacillus* proportion in the remaining samples. Overall, the *Lactobacillus* proportion of the participants' vaginal microbiomes significantly increased between T1 and T2 ($p=0.0382$).

Hierarchical clustering of samples from T1 separated the participants into one L-CST and one D-CST (Figure 2). Most T1 samples categorized as a L-CST had very few reads belonging to other genera, with a mean Shannon Diversity Index of 0.38 (95% CI = 0.18, 1.05). The D-CST in T1 had higher relative abundance of *Shuttleworthia, Dialister, Atopobium, Megasphaera, Gardnerella,* and *Prevotella* and a mean Shannon Diversity Index of 1.93 (95% CI = 1.65, 2.16). Approximately half of the samples belonging to the D-CST group were dominated by *Shuttleworthia.* Hierarchical clustering of T2 samples had two distinct L-CST's and two distinct D-CST's (Figure 3). One of the L-CSTs had almost no other genera present, while the other had relatively high numbers of genera other than *Lactobacillus*, including *Gardnerella, Prevotella, Shuttleworthia,* and *Megasphaera.* One of the T2 D-CST's was similar in composition to that of T1, while the other was primarily dominated by *Prevotella,*

Bacteroides, and Streptococcus. Overall, the D-CSTs had an average Shannon Diversity index of 1.89 (95% CI = 1.59, 2.14). The L-CSTs, when grouped together, had a mean Shannon Diversity Index of 0.46 (95% CI = 0.19, 1.14) and were therefore more diverse at T2 than at T1.

Despite T2 having more than one L-CST and D-CST, redundancy analysis revealed that differences in community composition were primarily driven by the relative abundance of *Lactobacillus* to other genera (Figure 4). The D-CST group was influenced exclusively by the first principal component, and the L-CST influenced exclusively by the second for the T1 samples, and vice versa for the T2 samples.

Several participants switched CST type between T1 and T2. Of the 146 participants that had samples from both visits, 67 had a L-CST on both visits, 19 transitioned from a L-CST to a D-CST, 30 transitioned from a D-CST to a L-CST, and 30 had a D-CST on both visits. However, CST, as determined by hierarchical clustering, did not perfectly coincide with relative proportion of reads belonging to *Lactobacillus*. For example, some of the participants whose samples clustered in the L-CST group for both visits had substantial increases or decreases in the proportion of the reads that belonged to *Lactobacillus* between visits (Figure 5). There were slight discrepancies between categorization of L-CST by hierarchical clustering and categorization of *Lactobacillus* dominance by *Lactobacillus* proportion alone (in which *Lactobacillus* dominance was defined as a *Lactobacillus* proportion greater than 60%). Of the 99 T1 samples characterized as L-CST, 16 had a *Lactobacillus* proportion below 60%. Of the 97 T2 samples characterized as L-CST, 16 had a *Lactobacillus* proportion below 60%. Of the 49 T2 samples characterized as D-CST, one had a *Lactobacillus* proportion above 60%. Because of this, regression of CSTs yielded slightly different results than regression of *Lactobacillus* proportion.

II. Demographic differences between L-CST and D-CST

Both T1 and T2 samples classified as D-CST came from participants with a significantly higher vaginal pH and Nugent score than participants with samples in the L-CST group (Tables 1 and 2). Additionally, participants with T1 D-CST samples were significantly younger, had a lower marriage rate, and were less educated than participants with T1 L-CST samples (Table 1). In contrast, participants with T2 D-CST samples had a significantly higher BV diagnosis rate prior to sampling, a shorter cervical length, lower marriage rate, and reported more oral sex than participants with T2 L-CST samples (Table 2). Participants with D-CST samples also tended to report lower incomes than those with L-CST samples, although many participants did not report income and this difference was not statistically significant ($p=0.0565$ and $p=0.1619$ for T1 and T2, respectively).

When all of the demographic variables were included together in a logistic model to predict CST, none of them had a significant association with T1 CST (Table 3). However, maternal age and oral sex were associated with an increased odds of having a T2 D-CST vaginal microbiome, after controlling for age, marital status, education, tobacco use, alcohol use, marijuana use, stress, depression, childhood trauma, racism, chronic hypertension, BMI, anemia, and antibiotic use. In contrast, higher levels of reported depression was associated with a decreased odds of having a T2 D-CST vaginal microbiome.

When *Lactobacillus* dominance was defined by *Lactobacillus* proportion rather than hierarchical clustering of CSTs, a high proportion of *Lactobacillus* (>= 60%) at T1 was associated with reporting of racism and a low proportion of *Lactobacillus* (<60%) at T2 was associated with single marital status and oral sex, after controlling for all other demographic variables (Table 4).

III. Microbiome and demographic associations with preterm birth

Of the 170 women who had a live birth, 114 delivered term births, 33 delivered early (36-38 weeks), and 23 delivered preterm (<36 weeks). Birth outcomes did not differ significantly by CST, when assessed statically at each visit (Tables 1 and 2). This also held true after adjusting for other demographic variables and analyzing birth outcomes as either spontaneous births only or inclusion of all births (Tables 5 and 6). Both preterm and early birth were, however, significantly associated with lower BMI after controlling for all other variables. Additionally, all-inclusive preterm birth was associated with alcohol consumption at T1 (Table 5). Preterm birth was inversely associated with reporting of childhood trauma (Tables 5 and 6). Preterm birth was also associated with oral sex at T2 (Table 6).

While birth outcomes did not associate with static differences in CST at T1 or T2, they did associate with the temporal change in *Lactobacillus* proportion between T1 and T2. Of the women who delivered spontaneous term births, the mean change in *Lactobacillus* proportion between T1 and T2 was 0.1165 (SD = 0.3510). Of women who delivered spontaneous preterm (<36 weeks), the mean change in *Lactobacillus* proportion was 0.1040 (SD = 0.5321). Women who delivered spontaneous early term, which includes those who delivered preterm, had a mean decrease in *Lactobacillus* proportion of -0.0745 (SD = 0.4546) between T1 and T2. The difference in *Lactobacillus* change between term and early births was significant ($p = 0.0299$). Compared to women who had a L-CST for both T1 and T2, women who transitioned from L-CST to D-CST between visits had a higher risk of any early birth (RR = 1.94, 95% CI = 1.14, 3.30), spontaneous PTB (RR = 4.83, 95% CI = 1.27, 18.41), and spontaneous early birth (RR = 2.24, 95% CI = 1.36, 3.71). Outcomes did not

differ significantly between women who transitioned either from D-CST to L-CST or women who stayed as D-CST and women who stayed as L-CST (Figure 6).

IV. The effect of vaginal infection and antibiotic use on the vaginal microbiome

Among all women sampled, 45 received a BV diagnosis, 21 received a Chlamydia diagnosis, and 3 received a Gonorrhea diagnosis. Receiving a diagnosis of any infection and antibiotic prescription prior to microbiome sampling was not associated with the CST of the sample (Tables 1 and 2). However, D-CST samples at T1 were associated with a BV diagnosis after sampling (Table 2).

Participants who did not take any prenatal antibiotics had the smallest change in *Lactobacillus* proportion between T1 and T2, with a mean change of only 0.0176 (SD = 0.3609) (Figure 7). Those who took prenatal antibiotics prior to T1 had a mean change of 0.1030 (SD = 0.4189), those who took antibiotics between T1 and T2 had a mean change of 0.1152 (SD = 04187), and those who took antibiotics after T2 had a mean change of 0.0202 (SD = 0.4316). The difference in change between groups was not statistically significant ($p = 0.3814$).

V. Behavioral and biological associations with change in *Lactobacillus* proportion

Linear regression of change in *Lactobacillus* proportion with change in antibiotic use, infection diagnosis, and behaviors revealed an association between a decrease in tobacco use with an increase in *Lactobacillus* proportion ($p=0.036$). Although not statistically significant, an increase in *Lactobacillus* proportion may also be associated with a decrease in anal sex ($p=0.0601$) and a decrease in alcohol consumption ($p=0.0622$). BV diagnosis had a close to significant association with a decrease in *Lactobacillus* proportion ($p=0.0543$).

Discussion

I. The composition of the diverse CST is similar to CST-IV.

The diverse CSTs at both T1 and T2 were similar in composition to CST-IV documented in previous studies (58). *Gardnerella, Prevotella, Megasphaera, Atopobium,* and *Sneathia* were among the overlapping genera common to the D-CST in this study and CST-IV (60). *Shuttleworthia,* in contrast, was the predominant non-*Lactobacillus* genus in this study but is not typically found in CST-IV, with the exception of one study of South African women (110). *Shuttleworthia* may be found primarily in women of African descent. Alternatively, *Shuttleworthia* has a similar sequence to the BV-associated genus BVAB-1 (111) and could be incorrectly classified by GreenGenes.

As expected, women with a D-CST had a higher vaginal pH and higher Nugent score than women with a L-CST, further corroborating that *Lactobacillus* dominance allows for the maintenance of a low vaginal pH (63, 56). However, D-CST, specifically at T1, was not associated with BV diagnosis. BV diagnosis was determined by medical charts and was not standardized across care providers. Therefore, this result may reflect variability in BV diagnosis. Nugent Score, however, is a reliable proxy for BV and was much higher in the D-CST group compared to the L-CST group, so we believe that there is a likely a true difference in BV between the D-CST and L-CST groups at 8-14 weeks gestation.

II. *Lactobacillus* tends to increase later in pregnancy, and decreases may predict preterm and early birth.

The average proportion of reads belonging to *Lactobacillus* was higher at T2 than at T1. Estrogen increases throughout pregnancy, which stimulates an increase in vaginal glycogen production and, in turn, provides more nutrients for *Lactobacillus* (56). While

Lactobacillus dominance is typically stable during pregnancy, women often experience a substantial decrease in *Lactobacillus* relative abundance and increase in vaginal microbiome diversity in the initial postpartum period (76,112). The rise in *Lactobacillus* during pregnancy has been repeatedly documented and is assumed to be evolutionarily advantageous in preventing pathogens from harming the vulnerable fetus (62,112).

Although there was an increase in *Lactobacillus* proportion in the majority of the participants, several of the women experienced a decrease. These women were at an increased risk of both preterm and early birth compared to women whose *Lactobacillus* levels were stable or increased throughout pregnancy, which includes those who had relatively low *Lactobacillus* levels at both time points. This suggests that women who have a diverse vaginal microbiome for longer-term periods may be protected from inflammatory responses resulting in premature rupture of the membranes when subsequently colonized with non-*Lactobacillus* genera. In contrast, women who experience a decrease in *Lactobacillus* during pregnancy may have experienced an acute event that introduced foreign pathogens into the vaginal canal, resulting in inflammation and preterm birth. This is the first study to capture differences in birth outcomes by temporal changes in the vaginal microbiome.

III. Anatomic, demographic, and bio-behavioral factors associated with CST differ between early and late pregnancy.

At T1, D-CST was associated with young maternal age, single marital status, and low education. The association between BV and socioeconomic status (SES) has been well documented (54,85,113), but there are few studies that assess the demographic risk factors for vaginal dysbiosis, particularly among African American women. This study suggests that African American women of low SES are at higher risk of vaginal dysbiosis than African

American women of higher SES, particularly in early pregnancy. However, as previously discussed, vaginal dysbiosis in early pregnancy may be unrelated to risk of PTB.

In contrast, D-CST at T2 was associated with a short cervix length and receiving oral sex within one month prior to sampling. Although *Lactobacillus* dominance typically stabilizes in later pregnancy, it is possible that the vaginal microbiome may be more vulnerable to acute disturbances than in early pregnancy if a woman has a shortened cervix. Short cervical length is a well-known risk factor for PTB (114). The cervix serves as a protective barrier from ascending pathogenic bacteria. A shortened cervix increases the risk of bacteria reaching the amniotic cavity, which can stimulate inflammation and premature rupturing of the membranes (115). Kindinger *et al.* (116) found that short cervical length was associated with *Lactobacillus iners* dominant microbiomes, but not vaginal dysbiosis, at 16 weeks gestation. However, The *L. iners* dominant CST has been shown to easily transition to CST-IV during pregnancy (62), and it is possible that many of the women with *L. iners* dominant CSTs would have transitioned to CST-IV if they were sampled in the third trimester. Unfortunately, in this study, cervical length had several missing values and was therefore not included in the regression models. However, oral sex was significantly associated with CST both in univariate and multivariate regression. While oral sex has been associated with BV previously (117), it has not been associated with vaginal dysbiosis. Oral sex could certainly introduce bacteria that would alter the vaginal microbiome. Additionally, women in the study are screened for cervical length at 20 weeks of pregnancy and, if found to have a shortened cervix, are told to avoid vaginal penetration for the remainder of pregnancy and they may therefore increase oral sex frequency. It is possible that the association between oral sex and CST is confounded by cervical length, which was not controlled for in the multivariate regression models. Alternatively, there could have been an interaction between cervical

length and oral sex, in which oral sex had the strongest impact on the vaginal microbiome in women with a short cervix.

Additionally, the linear regression of change in *Lactobacillus* proportion suggested that a decrease in tobacco use between visits could increase *Lactobacillus*. It has been previously shown that smokers have a higher risk of vaginal dysbiosis (56) and it is well-documented that smoking increases the risk of PTB (83). Smoking is also likely correlated with other risky behaviors not captured in the this study. While acute events, such as oral sex, may introduce pathogenic bacteria, quitting smoking during pregnancy may decrease the chance of successful colonization by pathogens.

IV. Stress and depression were either inversely or not associated with D-CST and PTB.

Most of the stress measures used in the study did not correlate with D-CST or PTB. In the multivariate regression model, the Edinburgh depression score was inversely associated with D-CST at T2. Similarly, reporting of childhood trauma was inversely associated with PTB. Because stress is typically associated with BV, these results were unexpected. However, *Lactobacillus iners* is often the dominant *Lactobacillus* species in African American women and can potentially predispose women to vaginal dysbiosis and BV (80). It is quite possible that the links between the microbiome and stress rely on the identification of *Lactobacillus* species and grouping all *Lactobacillus* species together masks more nuanced relationships between the African American experience and the vaginal microbiome.

V. Strengths and limitations of this study

This is the first study to focus exclusively on the microbiome of pregnant African American women. Additionally, it is one of the largest microbiome in pregnancy studies, including 184 women at two time points, compared to an average of 40-80 women in other studies (62,71,112,118). It is also among the first studies to correlate microbiome composition with several socio-behavioral variables, and the first to correlate microbiome to race-related experiences among African Americans. Although other studies have looked at the change in microbial composition through pregnancy (62), none of them have assessed the relationships between changes in microbiome with change in behaviors or pregnancy outcomes.

The sequencing data from this study was processed through the QIIME 1 pipeline and referenced against the GreenGenes database. Unfortunately, the QIIME 1 pipeline does not have as stringent data quality control as the recent QIIME 2 pipeline, and GreenGenes lacks several *Lactobacillus* species sequences. For this reason, we analyzed the microbiome at the taxonomic level of genus rather than species. This prevented the ability to ascertain potentially important differences in the dominant *Lactobacillus* species and limited the scope of the analysis.

The study was also limited by the high numbers of missing data for income and cervical length. Although education can serve as a proxy for SES, income is overall a much more accurate measure but often goes unreported (119). Because cervical length had a significant association with CST, it would have been helpful to include in the multivariate regression model, particularly because it may confound or interact with the relationship between oral sex and CST.

Future Directions

Lactobacillus species are increasingly recognized for differential effects on vaginal health. Therefore, the analysis of this study should be applied to the relationships between the primary dominant *Lactobacillus* species, CST-IV, and sociobehavioral variables as a logical next step. It has also been shown that both gut and oral microbiome are likely involved in pregnancy health and outcomes (120, 121). Therefore, these methods can be adapted for the analysis of other body site microbiomes. Furthermore, the results from this study suggest that a high amount of the variability in vaginal microbiome composition remains unexplained by the sociodemographic and sociobehavioral variables assessed. Several studies have found that host genetics can predict microbiome composition (122, 123). GWAS analysis of the women in this study may help to identify genotypes that are particularly susceptible to vaginal dysbiosis.

This is the first study to assess sociobehavioral risk factors of vaginal dysbiosis in pregnant African American women. Low socioeconomic status appears to correlate with low *Lactobacillus* and high diversity, but does not seem to be a risk factor for preterm birth. However, women who experience a decrease in *Lactobacillus* dominance between 8-14 weeks gestation and 24-30 weeks gestation appear to be at greater risk of preterm or early birth compared to women who maintain similar levels of *Lactobacillus* throughout pregnancy or who experience an increase in *Lactobacillus* during pregnancy. A decrease in *Lactobacillus* levels may be caused by a short cervical length, receiving oral sex, or an interaction of the two. Further work on the relationship between *Lactobacillus* levels, cervical length, and sexual practices could help to identify clinical recommendations that would increase vaginal health and decrease preterm birth risk in African American women.

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Tables

Table 1: Demographic differences between 8-14 week vaginal CSTs.
Significant associations are in bold.

		L-CST (n= 99)	D-CST (n= 83)	p-value
Recruiting site [n (%)]	Emory	48 (48.48)	32 (38.55)	0.2303
	Grady	51 (51.52)	51 (61.45)	
Shannon diversity [Median (IQR)]		0.3814 (0.1810, 1.055)	1.9270 (1.6471, 2.1574)	**<0.0001**
Vaginal pH [n (%)]	4.5	81 (81.82)	33 (39.76)	**<0.0001[A]**
	5	12 (12.12)	28 (33.73)	
	5.5	2 (2.02)	18 (21.69)	
	6	2 (2.02)	3 (3.61)	
	6.5	1 (1.01)	0 (0)	
	Missing	1 (1.01)	0 (0)	
BV diagnosis [n (%)]	prior to visit 1 sampling	7 (7.07)	9 (10.84)	0.3707
	post visit 1 sampling	15 (15.15)	14 (16.87)	0.7527
Chlamydia diagnosis [n (%)]	prior to visit 1 sampling	7 (7.07)	4 (4.82)	0.5255
	post visit 1 sampling	4 (4.04)	6 (7.23)	0.3471
Gonorrhea diagnosis [n (%)]	prior to visit 1 sampling	0 (0.00)	0 (0.00)	N/A
	post visit 1 sampling	3 (3.03)	0 (0.00)	0.2517 [A]
Received prenatal antibiotics prior to visit 1 sampling [n (%)]		20 (20.20)	21 (25.30)	0.4121
Nugent score [Median (IQR)]		2 (1, 4)	8 (7, 9)	**<0.0001**
Birth weight [n (%)]	Normal, >= 2500 g	78 (78.79)	67 (80.72)	0.8815 [A]
	Low, <2500 g, >=1500 g	10 (10.10)	6 (7.23)	
	Very Low, <1500 g	4 (4.04)	3 (3.61)	
	Missing	7 (7.07)	7 (8.43)	
Birth Outcome [n (%)]	Preterm	12 (12.12)	11 (13.25)	0.5169
	Early Term	21 (21.21)	12 (14.46)	
	Term	60 (60.61)	54 (65.06)	
	Spontaneous or Elective Abortion	6 (6.06)	6 (7.23)	
Age [Median (IQR)]		24 (22, 27)	22 (20, 25)	**0.0073**
BMI [Median (IQR)]		25.7 (22.45, 33.07)	25.23 (21.61, 32.47)	0.9375
Cervical length in cm. [Median (IQR)], # *missing*		3.80 (3.32, 4.28), *31*	3.80 (3.28, 4.31), *31*	0.6486
Gestational diabetes [n (%)]		4 (4.04)	1 (1.20)	0.3779 [A]
Anemia [n (%)], # *missing*		7 (7.07)	5 (6.02)	0.9999 [A]

Chronic hypertension during pregnancy [n (%)], # missing		3 (3.03)	4 (4.82)	0.7083
Married [n (%)]		17 (17.17)	3 (3.61)	**0.0036**
Education [n (%)]	High school or less	41 (41.41)	51 (61.45)	**0.0069**
	Some college or technical school	35 (35.35)	25 (30.12)	
	College graduate or higher	23 (23.23)	7 (8.43)	
Income	<150% FPL	39 (39.39)	41 (49.40)	0.0565
	150-299% FPL	15 (15.15)	11 (13.25)	
	>=300% FPL	17 (17.17)	5 (6.02)	
	Missing	28 (28.28)	26 (31.33)	
Douched in the last month [n (%)], # *missing*		4 (4.04), *4*	4 (4.82), *3*	0.9999 Λ
Taken a bath or shower in the past 2 days [n (%)], # *missing*		95 (95.96), *3*	81 (97.59), *2*	0.9999 Λ
Had vaginal sex in the past month [n (%)], # *missing*		75 (75.76), *3*	69 (83.13), *2*	0.2295
Received oral sex in the past month [n (%)], # *missing*		33 (33.33), *4*	32 (38.55), *3*	0.4729
Had anal sex in the past month, [n (%)], # *missing*		4 (4.04), *4*	0 (0), *2*	0.1255 Λ
Smoked cigarettes or cigars in the past month, [n (%)], # *missing*		10 (10.10), *3*	9 (10.84), *2*	0.8818
Consumed alcohol in the past month, [n (%)], # *missing*		5 (5.05), *3*	3 (3.61), *2*	0.7284 Λ
Smoked marijuana in the past month, [n (%)], # *missing*		15 (15.15), *3*	17 (20.48), *4*	0.3155
Perceived stress REDCAP score [Median (IQR)]		30 (24, 33), *7*	29 (22, 32), *5*	0.1737
Edinburgh Depression Scale Score [Median (IQR)]		5 (3, 10), *7*	6 (3, 9), *7*	0.5782
Experienced Childhood Trauma [n (%)]		65 (65.66)	56 (67.47)	0.7963
Total Stressful Life events [Median (IQR)]		4 (2,5), *6*	4 (2,5), *8*	0.8276
Krieger Experiences of Discrimination Score [Median (IQR)]		3.5 (0, 9)	2.5 (0, 8.5)	0.7617
Feels that racism is a problem in their life [n (%)], # *missing*		11 (11.11), *6*	5 (6.02), *10*	0.2807

ΛFisher's exact used instead of Chi squared because of sparse data. Otherwise, reported p-values are either from chi-squared test for categorical variables or from ANOVA for continuous variables.

Table 2: Demographic differences between 24-30 week vaginal CSTs.
Significant associations are in bold.

		L-CST (n= 97)	D-CST (n= 49)	p-value
Recruiting site [n (%)]	Emory	47 (48.45)	19 (38.78)	0.2672
	Grady	50 (51.55)	30 (61.22)	
Shannon diversity [Median (IQR)]		0.4625 (0.1872, 1.1353)	1.8936 (1.5888, 2.1417)	**<0.0001**
Vaginal pH [n (%)]	4.5	69 (71.13)	25 (51.02)	**<0.0001[A]**
	5	20 (20.62)	12 (24.49)	
	5.5	6 (6.19)	7 (14.29)	
	6	1 (1.03)	3 (6.12)	
	6.5	0 (0)	1 (2.04)	
	7	1 (1.03)	0 (0)	
	Missing	1 (1.03)	0 (0)	
BV diagnosis [n (%)]	prior to visit 2 sampling	14 (14.43)	17 (34.69)	**0.0047**
	post visit 2 sampling	4 (4.12)	4 (8.16)	0.3112
Chlamydia diagnosis [n (%)]	prior to visit 2 sampling	9 (9.28)	6 (12.24)	0.5772
	post visit 2 sampling	2 (2.06)	1 (2.04)	0.9999[A]
Gonorrhea diagnosis [n (%)]	prior to visit 2 sampling	2 (2.06)	0 (0)	0.5510[A]
	post visit 2 sampling	1 (1.03)	0 (0)	0.9999[A]
Received antibiotics prior to visit 2 sampling [n (%)]		41 (42.27)	26 (53.06)	0.2165
Nugent score [Median (IQR)]		3 (2, 7)	7 (4, 8)	**<0.0001**
Birth weight [n (%)]	Normal, >= 2500 g	86 (88.66)	42 (85.71)	0.4613[A]
	Low, <2500 g, >=1500 g	6 (6.19)	6 (12.24)	
	Very Low, <1500 g	2 (2.06)	1 (2.04)	
	Missing	3 (3.09)	0 (0)	
Birth Outcome [n (%)]	Preterm	12 (12.37)	4 (8.16)	0.3109
	Early Term	16 (16.49)	13 (26.53)	
	Term	69 (71.13)	32 (65.31)	
	Spontaneous or Elective Abortion	0 (0)	0 (0)	
Age [Median (IQR)]		24 (21, 28)	23 (21, 26)	0.2189
BMI [Median (IQR)]		26.15 (22.46, 33.64)	24.50 (21.09, 32.28)	0.4845
Cervical length in cm. [Median (IQR)], # *missing*		3.81 (3.40, 4.33)	3.63 (3.15, 4.00)	**0.0303**
Gestational diabetes [n (%)]		5 (5.15)	0 (0)	0.1685[A]
Anemia [n (%)]		7 (7.22)	3 (6.12)	0.9999[A]
Chronic hypertension during pregnancy [n (%)], # missing		4 (4.12)	0 (0)	0.1008[A]
Married [n (%)]		16 (16.49)	0 (0)	**0.0026**
Education [n (%)]	High school or less	45 (46.39)	28 (57.14)	0.3122
	Some college or technical school	31 (31.96)	15 (30.61)	
	College graduate or higher	21 (21.65)	6 (12.24)	
Income	<150% FPL	37 (38.14)	23 (46.94)	0.1619
	150-299% FPL	16 (16.49)	6 (12.24)	
	>=300% FPL	16 (16.49)	3 (6.12)	

Missing	28 (28.87)	17 (34.69)	
Douched in the last month [n (%)], # *missing*	1 (1.03), *1*	2 (4.08), *1*	0.2577[A]
Taken a bath or shower in the past 2 days [n (%)], # *missing*	92 (94.85), *3*	47 (95.92), *1*	0.9999[A]
Had vaginal sex in the past month [n (%)], # *missing*	69 (71.13), *1*	32 (65.31), *1*	0.5197[A]
Received oral sex in the past month [n (%)], # *missing*	28 (28.27), *1*	21 (42.86), *2*	**0.0063**
Had anal sex in the past month, [n (%)], # *missing*	2 (2.11), *2*	1 (2.13), *2*	0.9999[A]
Smoked cigarettes or cigars in the past month, [n (%)], # *missing*	6 (6.19), *1*	2 (4.08), *1*	0.7190[A]
Consumed alcohol in the past month, [n (%)], # *missing*	7 (7.22), *4*	4 (8.16), *1*	0.9999[A]
Smoked marijuana in the past month, [n (%)], # *missing*	5 (5.15), *2*	4 (8.16), *1*	0.4841[A]
Perceived stress REDCAP score [Median (IQR)]	30 (23, 32)	30 (24, 33)	0.8142
Edinburgh Depression Scale Score [Median (IQR)]	5.5 (3, 10)	6 (3.5, 9)	0.6541
Experienced Childhood Trauma [n (%)]	59 (60.82)	33 (67.35)	0.4408
Total Stressful Life events [Median (IQR)]	4 (2, 5)	4 (2, 5)	0.7305
Krieger Experiences of Discrimination Score [Median (IQR)]	2 (0, 7.5)	2.5 (0, 7)	0.8281
Feels that racism is a problem in their life [n (%)], # *missing*	8 (8.25), *6*	4 (8.16), *1*	0.9999[A]

[A]Fisher's exact used instead of Chi squared because of sparse data. Otherwise, reported p-values are either from chi-squared test for categorical variables or from ANOVA for continuous variables.

Table 3: Multivariate logistic regression odds ratios (95% CI) with D-CST as the outcome. Significant associations are in bold.

Variable *(Coding)*	**8-14 weeks gestation (n=157)**	**24-30 weeks gestation (n=127)**
Hospital *(Grady = 1, Emory = 0)*	0.631 (0.244, 1.635)	1.263 (0.426, 3.739)
Maternal age *(Continuous)*	0.938 (0.841, 1.046)	**1.157 (1.008, 1.328)**
Marital status *(Single = 1, Married = 0)*	4.342 (0.927, 20.327)	>999.999 (<0.001, >999.999)
Low education *(1 = High school degree or lower, 0 = Other)*	2.244 (0.962, 5.235)	1.628 (0.56, 4.733)
Douched in the last month *(1 = Yes, 0 = No)*	0.547 (0.105, 2.858)	3.649 (0.237, 56.134)
Had vaginal sex in the last month *(1 = Yes, 0 = No)*	1.86 (0.697, 4.964)	0.38 (0.117, 1.231)
Received oral sex in the last month *(1 = Yes, 0 = No)*	1.297 (0.579, 2.906)	**2.871 (1.014, 8.132)**
Had anal sex in the last month *(1 = Yes, 0 = No)*	<0.001 (<0.001, >999.999)	1.419 (0.066, 30.63)
Smoked cigarettes or cigars in the last month *(1 = Yes, 0 = No)*	0.854 (0.227, 3.205)	0.245 (0.027, 2.234)
Drank alcohol in the last month *(1 = Yes, 0 = No)*	0.942 (0.144, 6.143)	1.25 (0.249, 6.28)
Smoked marijuana in the last month *(1 = Yes, 0 = No)*	1.337 (0.474, 3.769)	2.289 (0.347, 15.09)
Perceived Stress REDCAP score *(Continuous)*	0.995 (0.95, 1.042)	1.052 (0.992, 1.116)
Edinburgh Depression Scale Score *(Continuous)*	0.952 (0.872, 1.039)	**0.868 (0.779, 0.968)**
Experienced Childhood trauma *(1 = Yes, 0 = No)*	0.971 (0.424, 2.221)	2.341 (0.84, 6.52)
Stressful life events score *(Continuous)*	1.055 (0.889, 1.252)	0.973 (0.795, 1.191)
Krieger experiences of discrimination *(Continuous)*	1.026 (0.956, 1.102)	1.033 (0.951, 1.122)
Experiences racism in daily life *(1 = Yes, 0 = No)*	0.268 (0.061, 1.169)	2.083 (0.387, 11.209)
BMI *(Continuous)*	1.027 (0.98, 1.077)	0.979 (0.921, 1.041)
Chronic hypertension during pregnancy *(1 = Yes, 0 = No)*	2.85 (0.391, 20.758)	<0.001 (<0.001, >999.999)
Anemic *(1 = Yes, 0 = No)*	1.626 (0.395, 6.698)	1.07 (0.195, 5.865)
Prenatal antibiotic use prior to sampling date (*1 = Yes, 0 = No*)	0.818 (0.346, 1.933)	1.42 (0.565, 3.574)

Table 4: Multivariate logistic regression odds ratios (95% CI) with Lactobacillus proportion <60% as outcome.
Significant associations are in bold.

Variable *(Coding)*	8-14 weeks gestation (n=157)	24-30 weeks gestation (n=127)
Hospital *(Grady = 1, Emory = 0)*	1.164 (0.442, 3.068)	0.541 (0.183, 1.599)
Maternal age *(Continuous)*	0.932 (0.835, 1.04)	1.039 (0.918, 1.176)
Marital status *(Single = 1, Married = 0)*	3.925 (0.908, 16.967)	**21.34 (2.073, 219.644)**
Low education *(1 = High school degree or lower, 0 = Other)*	1.739 (0.734, 4.123)	2.827 (0.989, 8.081)
Douched in the last month *(1 = Yes, 0 = No)*	0.271 (0.048, 1.529)	>999.999 (<0.001, >999.999)
Had vaginal sex in the last month *(1 = Yes, 0 = No)*	1.412 (0.526, 3.793)	1.091 (0.375, 3.173)
Received oral sex in the last month *(1 = Yes, 0 = No)*	1.893 (0.799, 4.486)	**3.075 (1.121, 8.435)**
Had anal sex in the last month *(1 = Yes, 0 = No)*	<0.001 (<0.001, >999.999)	<0.001 (<0.001, >999.999)
Smoked cigarettes or cigars in the last month *(1 = Yes, 0 = No)*	0.846 (0.221, 3.245)	0.564 (0.121, 2.628)
Drank alcohol in the last month *(1 = Yes, 0 = No)*	0.425 (0.056, 3.219)	0.15 (0.01, 2.29)
Smoked marijuana in the last month *(1 = Yes, 0 = No)*	1.194 (0.403, 3.537)	2.42 (0.723, 8.1)
Perceived Stress REDCAP score *(Continuous)*	0.996 (0.949, 1.045)	1.001 (0.946, 1.06)
Edinburgh Depression Scale Score *(Continuous)*	0.945 (0.863, 1.036)	0.928 (0.836, 1.031)
Experienced Childhood trauma *(1 = Yes, 0 = No)*	1.16 (0.487, 2.761)	1.983 (0.781, 5.032)
Stressful life events score *(Continuous)*	1.119 (0.937, 1.335)	0.976 (0.798, 1.194)
Krieger experiences of discrimination *(Continuous)*	1.03 (0.957, 1.109)	1.055 (0.969, 1.148)
Experiences racism in daily life *(1 = Yes, 0 = No)*	**0.175 (0.04, 0.76)**	0.614 (0.132, 2.848)
BMI *(Continuous)*	1.022 (0.973, 1.073)	0.997 (0.944, 1.053)
Chronic hypertension during pregnancy *(1 = Yes, 0 = No)*	6.561 (0.44, 97.912)	0.282 (0.019, 4.25)
Anemic *(1 = Yes, 0 = No)*	0.801 (0.193, 3.33)	1.563 (0.333, 7.335)
Prenatal antibiotic use prior to sampling date (*1 = Yes, 0 = No*)	1.144 (0.456, 2.871)	1.992 (0.749, 5.299)

Table 5: Multivariate logistic regression odds ratios (95% CIs) with birth outcomes regressed against 8-14 week gestational age predictors.
Significant associations are in bold. PTB = preterm birth (≤ 36 weeks), EB = early birth (≤ 38 weeks), Sp. = spontaneous

Variable *(Coding)*	All PTB vs. All Term (n = 119)	All EB vs. All Term (n = 102)	Sp. PTB vs. Sp. Term (n = 65)	Sp. EB vs. Sp. Term (n = 88)
Visit 1 Vaginal CST (*Diverse = 1, Lactobacillus-dominant = 0*)	0.451 (0.066, 3.062)	0.485 (0.2, 1.18)	1.8 (0.091, 35.604)	0.563 (0.168, 1.885)
Hospital *(Grady = 1, Emory = 0)*	9.821 (0.799, 120.694)	1.192 (0.403, 3.519)	3.724 (0.022, 642.781)	0.855 (0.165, 4.437)
Maternal age *(Continuous)*	1.063 (0.843, 1.34)	1.046 (0.929, 1.177)	1.055 (0.711, 1.565)	1.137 (0.964, 1.341)
Marital status *(Single = 1, Married = 0)*	1.258 (0.035, 45.879)	1.964 (0.375, 10.282)	>999.999 (<0.001, >999.999)	2.173 (0.276, 17.137)
Low education *(1 = High school degree or lower, 0 = Other)*	2.802 (0.333, 23.583)	1.951 (0.71, 5.361)	11.546 (0.211, 631.33)	2.982 (0.691, 12.866)
Douched in the last month *(1 = Yes, 0 = No)*	2.95 (0.064, 136.799)	1.108 (0.131, 9.391)	21.337 (0.037, >999.999)	3.06 (0.115, 81.239)
Had vaginal sex in the last month *(1 = Yes, 0 = No)*	0.295 (0.033, 2.653)	0.603 (0.201, 1.813)	0.227 (0.012, 4.246)	0.57 (0.143, 2.264)
Received oral sex in the last month *(1 = Yes, 0 = No)*	5.66 (0.829, 38.639)	1.596 (0.649, 3.922)	7.227 (0.195, 267.498)	1.987 (0.6, 6.575)
Had anal sex in the last month *(1 = Yes, 0 = No)*	7.535 (0.052, >999.999)	1.355 (0.055, 33.21)	>999.999 (<0.001, >999.999)	>999.999 (<0.001, >999.999)
Smoked cigarettes or cigars in the last month *(1 = Yes, 0 = No)*	2.288 (0.126, 41.501)	1.163 (0.236, 5.72)	0.137 (0.002, 10.493)	0.295 (0.036, 2.418)
Drank alcohol in the last month *(1 = Yes, 0 = No)*	**37.777 (1.536, 929.292)**	6.477 (0.811, 51.729)	508.871 (0.148, >999.999)	13.876 (0.381, 505.946)
Smoked marijuana in the last month *(1 = Yes, 0 = No)*	0.095 (0.006, 1.473)	0.781 (0.222, 2.746)	5.731 (0.062, 533.208)	1.848 (0.31, 11.032)
Perceived Stress REDCAP score *(Continuous)*	1.044 (0.922, 1.183)	1.004 (0.951, 1.06)	0.935 (0.798, 1.096)	0.971 (0.892, 1.058)
Edinburgh Depression Scale Score *(Continuous)*	0.979 (0.801, 1.198)	0.956 (0.863, 1.059)	1.045 (0.755, 1.445)	0.974 (0.845, 1.124)
Experienced Childhood trauma *(1 = Yes, 0 = No)*	**0.06 (0.007, 0.506)**	0.547 (0.221, 1.354)	**0.007 (<0.001, 0.369)**	0.436 (0.127, 1.491)
Stressful life events score *(Continuous)*	0.767 (0.497, 1.181)	0.877 (0.717, 1.073)	0.535 (0.25, 1.145)	0.836 (0.652, 1.074)
Krieger experiences of discrimination *(Continuous)*	1.129 (0.939, 1.358)	1.013 (0.927, 1.107)	1.133 (0.881, 1.457)	1.009 (0.889, 1.146)
Experiences racism in daily life *(1 = Yes, 0 = No)*	<0.001 (<0.001, >999.999)	0.923 (0.201, 4.241)	<0.001 (<0.001, >999.999)	1.073 (0.173, 6.664)
BMI *(Continuous)*	**0.835 (0.715, 0.976)**	**0.9 (0.846, 0.957)**	**0.762 (0.591, 0.982)**	**0.903 (0.826, 0.988)**
Chronic hypertension during pregnancy *(1 = Yes, 0 = No)*	4.108 (0.036, 463.748)	1.417 (0.141, 14.202)	1.347 (<0.001, >999.999)	2.103 (0.026, 170.21)

Anemic *(1 = Yes, 0 = No)*	<0.001 (<0.001, >999.999)	0.156 (0.015, 1.6)	0.003 (<0.001, >999.999)	0.801 (0.043, 14.93)

Table 6: Multivariate logistic regression odds ratios (95% CIs) with birth outcomes regressed against 24-30 week gestational age predictors.

Significant associations are in bold. PTB = preterm birth (≤ 36 weeks), EB = early birth (≤ 38 weeks), Sp. = spontaneous

Variable *(Coding)*	All PTB vs. All Term (n=104)	All EB vs. All Term (n = 127)	Sp. PTB vs. Sp. Term (n = 60)	Sp. EB vs. Sp. Term (n = 60)
Visit 2 Vaginal CST (*Diverse = 1, Lactobacillus-dominant = 0*)	0.037 (0.001, 1.262)	1.045 (0.369, 2.961)	0.117 (0.002, 6.612)	1.105 (0.277, 4.404)
Hospital *(Grady = 1, Emory = 0)*	19.203 (0.913, 403.784)	1.004 (0.33, 3.055)	7.234 (0.058, 906.138)	1.165 (0.241, 5.624)
Maternal age *(Continuous)*	0.925 (0.675, 1.266)	1.021 (0.887, 1.176)	0.859 (0.509, 1.449)	1.181 (0.974, 1.432)
Marital status *(Single = 1, Married = 0)*	1.116 (0.015, 85.603)	3.964 (0.453, 34.719)	>999.999 (<0.001, >999.999)	8.045 (0.472, 137.16)
Low education *(1 = High school degree or lower, 0 = Other)*	1.842 (0.188, 18.018)	1.522 (0.486, 4.765)	5.233 (0.229, 119.494)	2.32 (0.455, 11.839)
Douched in the last month *(1 = Yes, 0 = No)*	305.93 (0.866, >999.999)	9.213 (0.533, 159.382)	>999.999 (<0.001, >999.999)	>999.999 (<0.001, >999.999)
Had vaginal sex in the last month *(1 = Yes, 0 = No)*	0.025 (<0.001, 1.134)	0.46 (0.138, 1.531)	0.166 (0.003, 9.37)	0.263 (0.047, 1.469)
Received oral sex in the last month *(1 = Yes, 0 = No)*	**116.892 (1.742, >999.999)**	1.654 (0.555, 4.931)	2.049 (0.019, 216.699)	3.239 (0.712, 14.731)
Had anal sex in the last month *(1 = Yes, 0 = No)*	<0.001 (<0.001, >999.999)	<0.001 (<0.001, >999.999)	<0.001 (<0.001, >999.999)	<0.001 (<0.001, >999.999)
Smoked cigarettes or cigars in the last month *(1 = Yes, 0 = No)*	16.159 (0.256, >999.999)	1.082 (0.112, 10.439)	<0.001 (<0.001, >999.999)	<0.001 (<0.001, >999.999)
Drank alcohol in the last month *(1 = Yes, 0 = No)*	<0.001 (<0.001, >999.999)	0.971 (0.179, 5.277)	<0.001 (<0.001, >999.999)	2.669 (0.148, 48.222)
Smoked marijuana in the last month *(1 = Yes, 0 = No)*	0.001 (<0.001, 0.55)	0.079 (0.004, 1.474)	<0.001 (<0.001, >999.999)	<0.001 (<0.001, >999.999)
Perceived Stress REDCAP score *(Continuous)*	1.127 (0.993, 1.279)	1.03 (0.966, 1.099)	1.038 (0.874, 1.232)	1.03 (0.935, 1.135)
Edinburgh Depression Scale Score *(Continuous)*	0.897 (0.71, 1.133)	0.978 (0.868, 1.103)	1.194 (0.789, 1.805)	0.988 (0.823, 1.186)
Experienced Childhood trauma *(1 = Yes, 0 = No)*	**0.006 (<0.001, 0.306)**	0.42 (0.149, 1.185)	0.028 (0.001, 0.759)	0.515 (0.137, 1.937)
Stressful life events score *(Continuous)*	1.387 (0.775, 2.482)	0.906 (0.727, 1.13)	0.88 (0.42, 1.846)	0.752 (0.554, 1.02)
Krieger experiences of discrimination *(Continuous)*	1.218 (0.997, 1.489)	1.051 (0.958, 1.152)	1.23 (0.917, 1.65)	1.06 (0.893, 1.257)

Experiences racism in daily life *(1 = Yes, 0 = No)*	<0.001 (<0.001, >999.999)	1.725 (0.339, 8.78)	<0.001 (<0.001, >999.999)	1.189 (0.18, 7.852)
BMI *(Continuous)*	**0.733 (0.54, 0.997)**	**0.897 (0.834, 0.965)**	**0.75 (0.51, 1.104)**	**0.885 (0.794, 0.987)**
Chronic hypertension during pregnancy *(1 = Yes, 0 = No)*	>999.999 (4.423, >999.999)	9.957 (0.538, 184.216)	>999.999 (<0.001, >999.999)	>999.999 (<0.001, >999.999)
Anemic *(1 = Yes, 0 = No)*	<0.001 (<0.001, >999.999)	0.156 (0.016, 1.532)	0.002 (<0.001, >999.999)	1.006 (0.037, 27.62)

Table 7: Linear regression coefficients with change in Lactobacillus proportion between visits as outcome.

Negative beta estimates indicate an association with a decrease in Lactobacillus proportion between visits 1 and 2. Positive beta estimates indicate an association with an increase in Lactobacillus proportion between visits 1 and 2.

Variable	Beta estimate (p-value)
Antibiotic use prior to visit 1	-0.00529 (0.9666)
Antibiotic use between visits	0.17276 (0.1094)
BV diagnosis prior to visit 1	0.00057839 (0.9971)
BV diagnosis between visits	-0.27045 (0.0543)
Chlamydia diagnosis prior to visit 1	-0.02944 (0.8723)
Chlamydia diagnosis between visits	-0.11524 (0.6134)
Gonorrhea diagnosis between visits	0.31887 (0.4321)
Increase in douching between visits	0.72896 (0.0833)
Decrease in douching between visits	-0.2939 (0.4687)
Increase in bathing between visits	0.30054 (0.2194)
Decrease in bathing between visits	-0.67276 (0.0912)
Increase in vaginal sex between visits	0.04806 (0.6465)
Decrease in vaginal sex between visits	0.15416 (0.2936)
Increase in oral sex between visits	-0.02864 (0.799)
Decrease in oral sex between visits	0.16371 (0.1891)
Increase in anal sex between visits	-0.20169 (0.4966)
Decrease in anal sex between visits	0.74996 (0.0601)
Increase in tobacco use between visits	0.24173 (0.077)
Decrease in tobacco use between visits	**0.62175 (0.036)**
Increase in alcohol use between visits	-0.05835 (0.7441)
Decrease in alcohol use between visits	-0.26442 (0.0622)
Increase in marijuana use between visits	-0.06161 (0.5788)
Decrease in marijuana use between visits	-0.09619 (0.7477)

Figures and Figure Legends

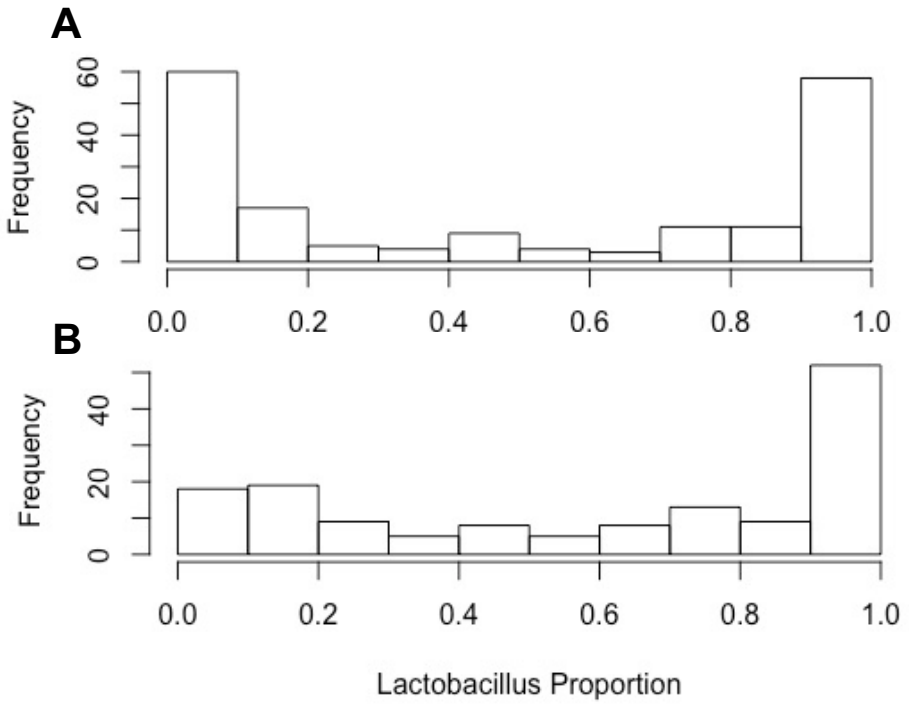

Figure 1: Distribution of Lactobacillus proportion among vaginal swabs at 8-14 weeks (A) and 24-30 weeks (B) gestational age.

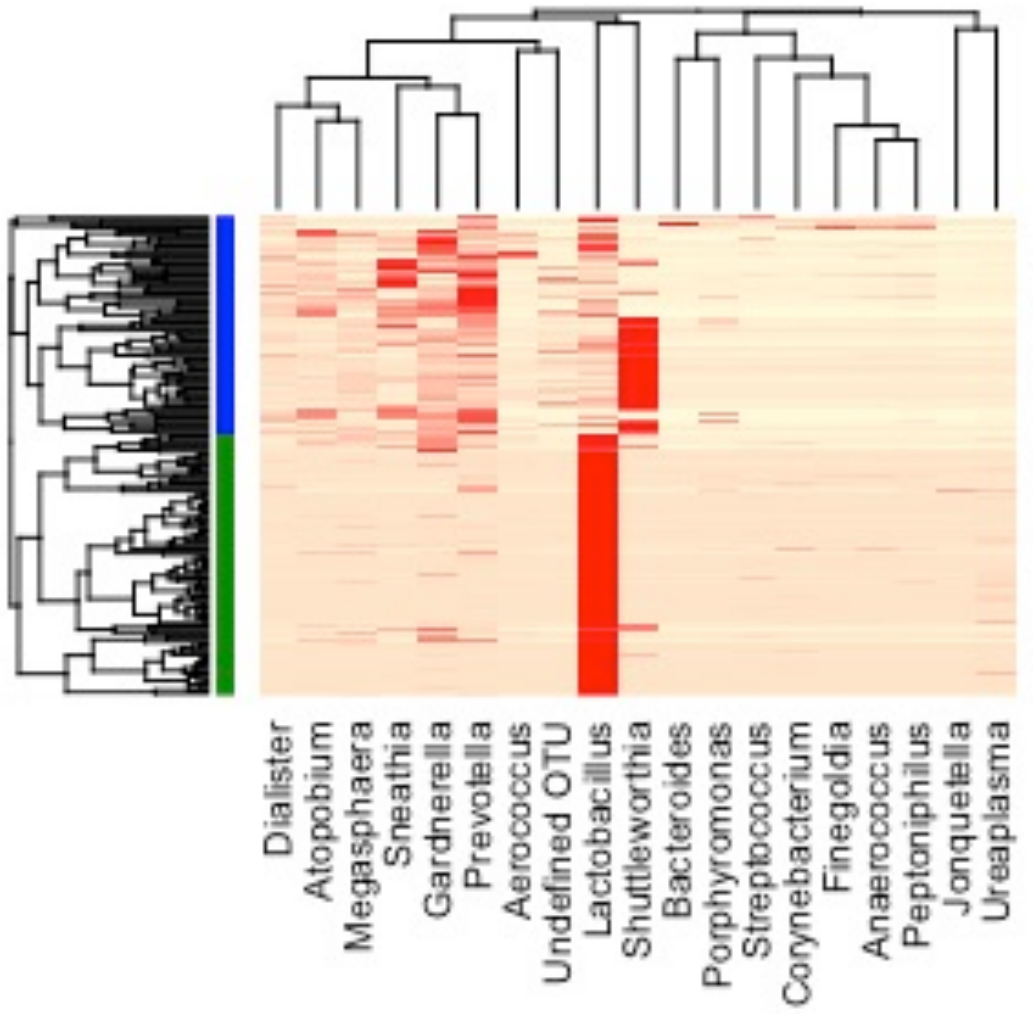

Figure 2: Heat map of genera found in vaginal swabs at 8-14 weeks gestational age.

Rows represent individual samples, which are clustered by Uni-Frac distance and characterized as a L-CST (green) or D-CST (blue).

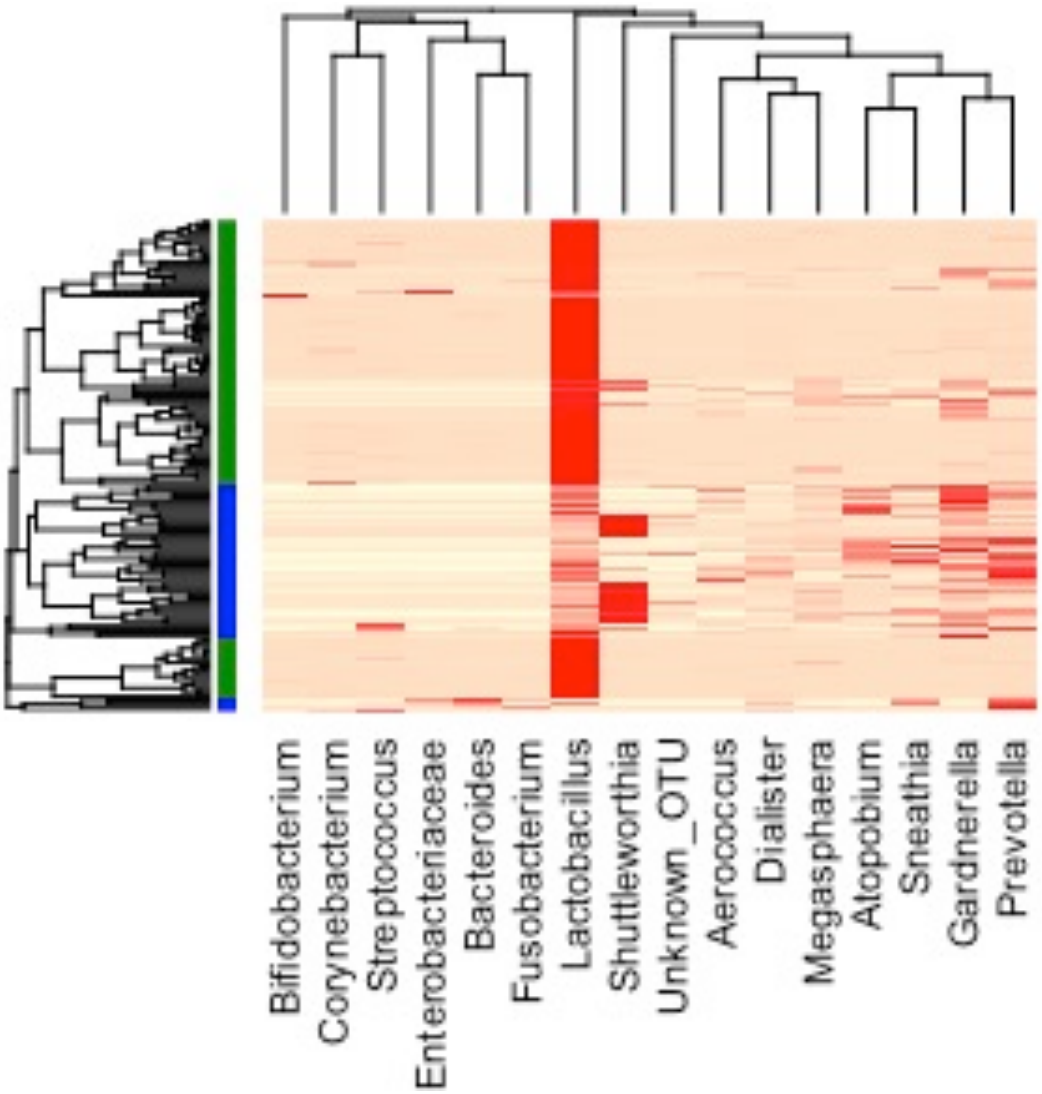

Figure 3: Heat map of genera found in vaginal swabs at 24-30 weeks gestational age.

Rows represent individual samples, which are clustered by Uni-Frac distance and characterized as a L-CST (green) or D-CST (blue)

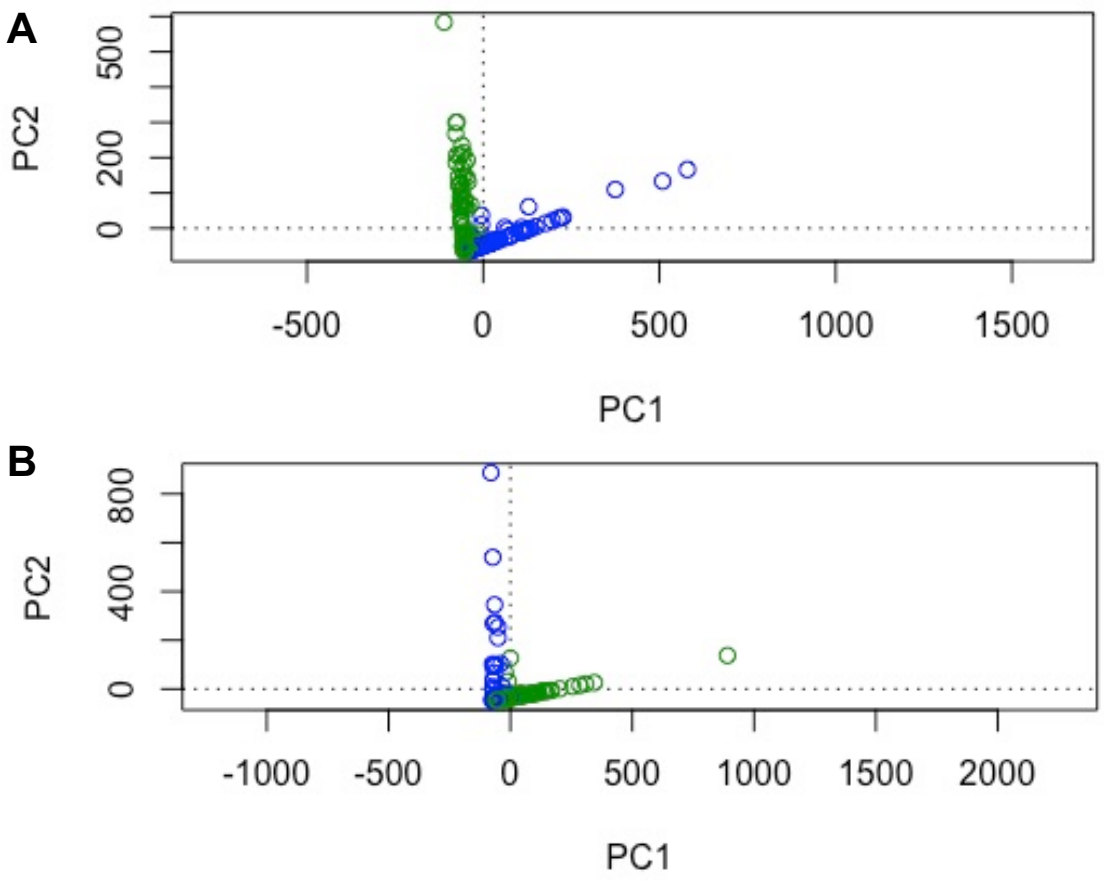

Figure 4: Redundancy Analysis of vaginal microbiome swabs at 8-14 weeks (A) or 24-30 weeks (B) gestational age.

Markers represent individual samples and are colored by CST (L-CST = green, D-CST = blue).

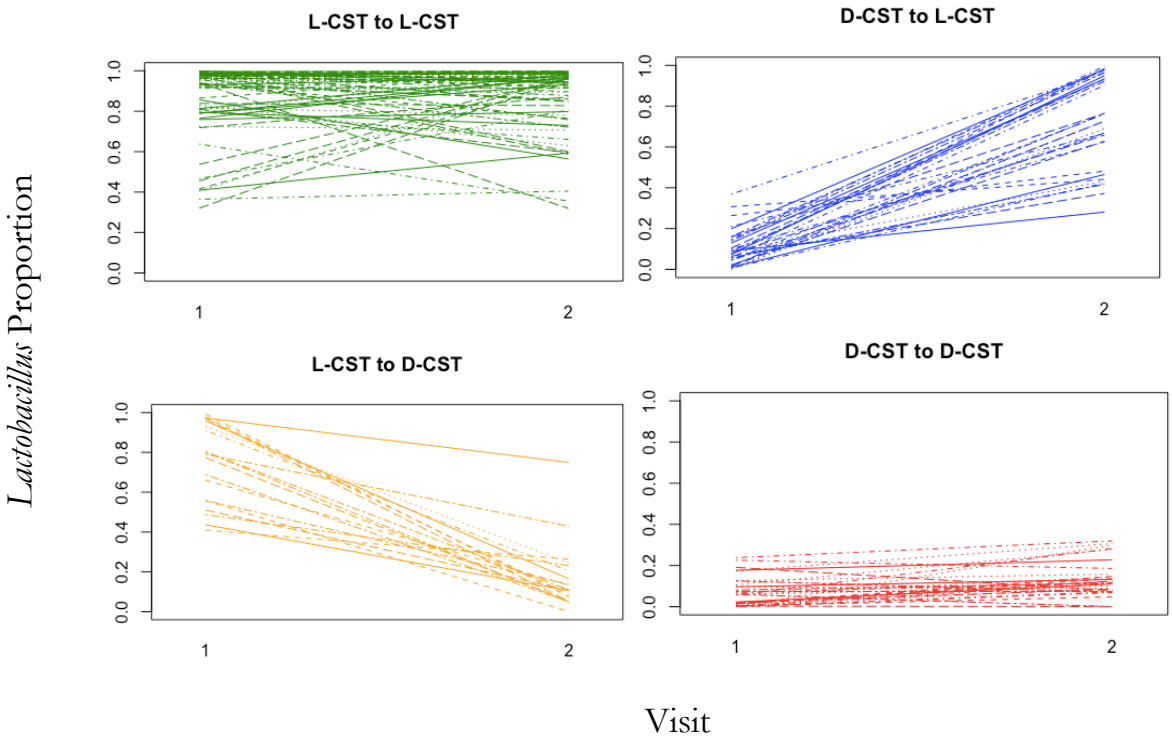

Figure 5: Change in Lactobacillus proportion between 8-14 and 24-30 weeks gestational age.

Participants were grouped by the CST assignment at V1 and V2.

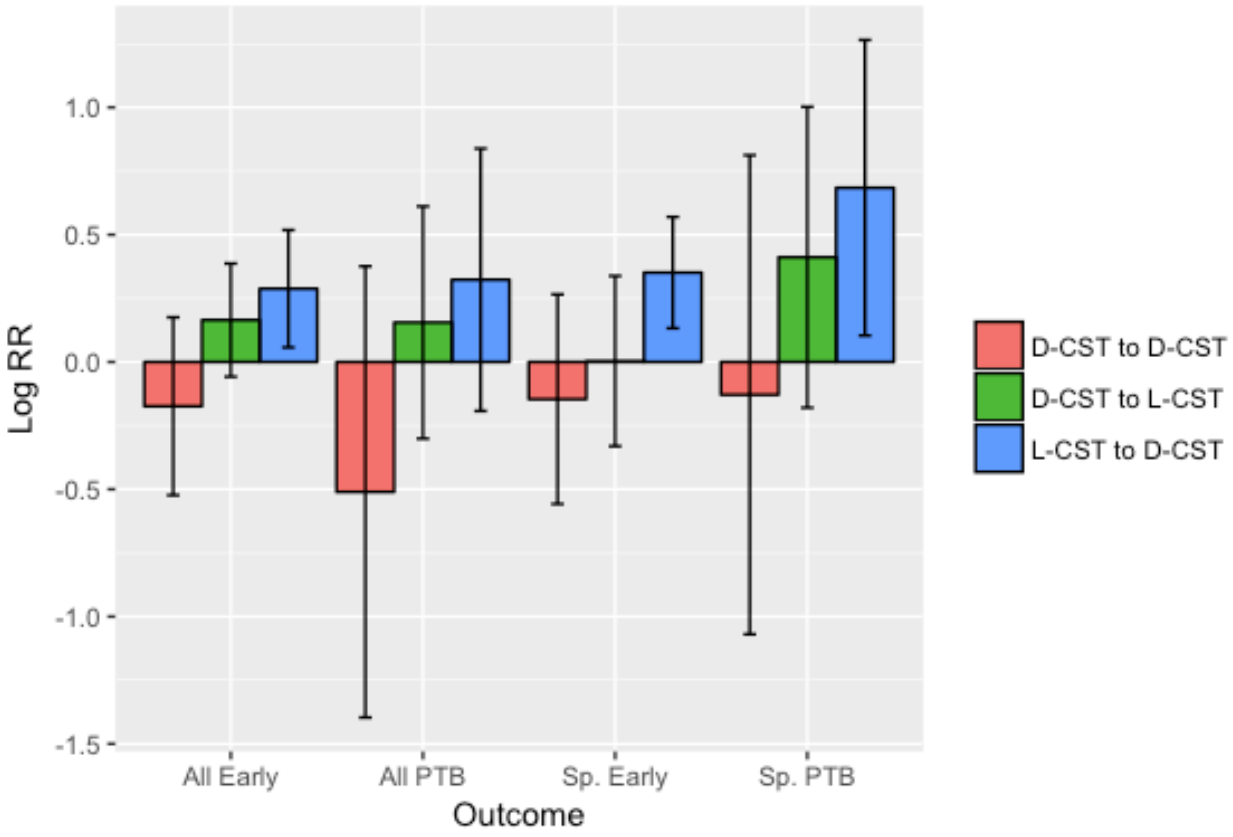

Figure 6: Log risk ratios comparing risks of women who either had a D-CST for both visits (red), transitioned from a D-CST to a L-CST between visits (green), or transitioned from L-CST to D-CST between visits (blue) to women who had a L-CST for both visits.

95% confidence intervals not crossing the y-axis indicate a significantly higher risk of the outcome compared to women who had a L-CST for both visits. Outcomes include all births before 38 weeks gestational age (All Early), all births before 36 weeks gestational age (All PTB), spontaneous births before 38 weeks gestational age (Sp. Early), and spontaneous births before 36 weeks gestational age (Sp. PTB).

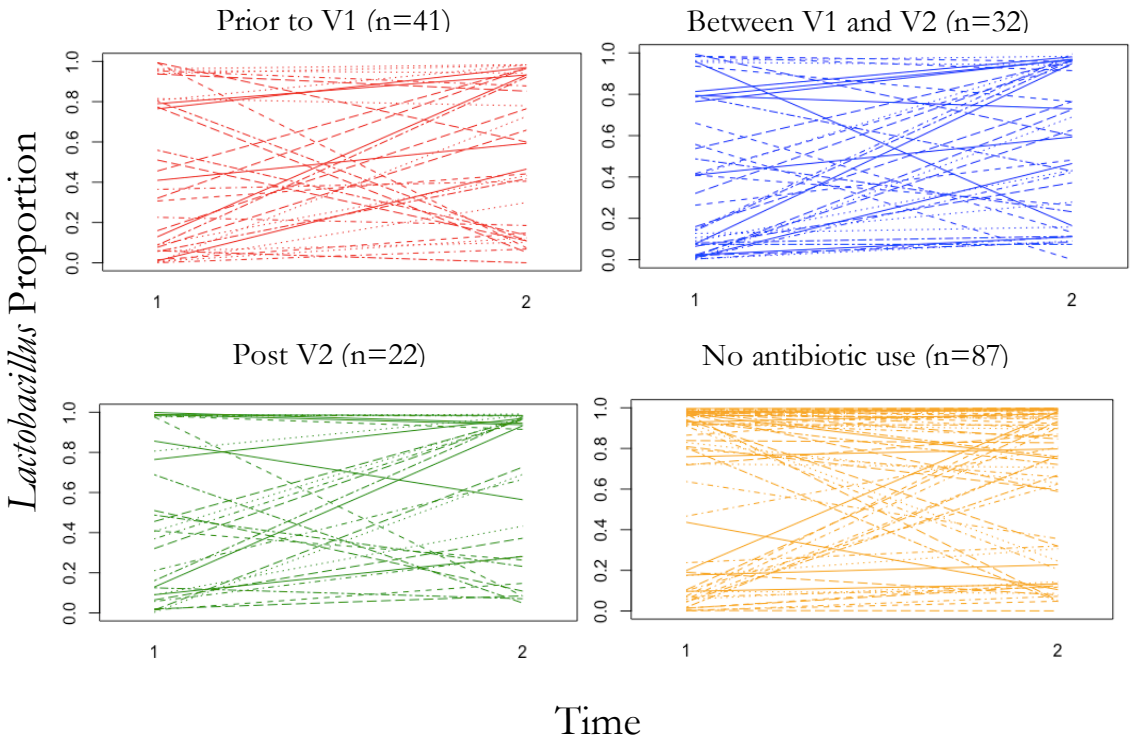

Figure 7: Lactobacillus proportion between 8-14 and 24-30 weeks gestational age by prenatal antibiotic use.

The mean change in *Lactobacillus* proportion is 0.0176 (SD = 0.3609) for no antibiotic use, 0.1030 (SD = 0.4189) for antibiotic use prior to first visit, 0.1152 (SD = 0.4187) for antibiotic use between visits 1 and 2, and 0.0202 (SD = 0.4316) for antibiotic use after visit 2. Differences between groups are not statistically significant (p=0.3814).