Differences in Bacterial Vaginosis-associated organisms among pregnant Foreign-born and US-Born black women

By

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Differences in Bacterial Vaginosis-associated micro-organisms among pregnant US-Born and Foreign-born black women

ABSTRACT

Objectives: The purpose of this study was to determine whether there are differences in vaginal colonization with bacterial vaginosis (BV) associated organisms between pregnant US-born and Foreign-born African American women.

Study Design: We conducted a cross-sectional study on 2890 U.S born and 349 Foreign-born Black women at gestational age of 14.8 ±0.2 weeks. BV and associated microorganisms were diagnosed by Nugent’s criteria.

Results: US-born Black women were more likely to be BV positive compared to Foreign-born Black women (52% vs. 42.1%; Pearson's Chi-Square test: p <0.05). Among women with BV, US born status was associated with specific vaginal microflora including Gardnerella Vaginalis (OR 1.6, 95%CI 1.2-2.2); Mobiluncus spp (OR 1.5, 95%CI 1.2-2.2); Prevotella/Bacteroides (OR 1.5, 95%CI 1.2-2.2) after adjusting for age, age of sexual debut, marital status, education, income, number of lifetime partners, recent douching, recent smoking, receipt of oral within a year of pregnancy. Among women with BV intermediate status, US born women were 6 times more likely to have Gardnerella vaginalis 95%CI (2.0-17.8); and 2.5 times more likely to have Mobiluncus. Among women with no BV; US-born women were 4.0 times more likely to have Mobiluncus 95% CI (1.6-9.5).
Conclusions: Higher rates of BV and BV associated organisms, especially *G. vaginalis* and *Mobiluncus* among US-born black women as compared to Foreign-born black women, may be a potential contributor to the higher rates of preterm birth seen in the US-born black women.
Introduction

Preterm birth (PTB), defined as delivery before 37 weeks of gestation, is the second leading cause of death among infants in the United States and the leading cause of neonatal deaths among African Americans. African American women are twice as likely as Caucasian women to experience preterm delivery; this disparity has persisted over the past four decades.

Several studies have demonstrated that foreign-born black women have better birth outcomes than US born black women and similar outcomes to US-born non-Hispanic Whites. The apparent protective effect of foreign nativity has been postulated to be due to selective migration of healthy women or high socioeconomic status and more favorable health behaviors. Another plausible explanation is the unique sociocultural and political background of US-born African Americans compared to Foreign-born blacks. US-born Blacks have experienced long-term exposure to socioeconomic and structural discrimination, foreign-born women, on the other hand, may not have had similar experiences. Psychosocial stress from discrimination, mediated through the weathering hypothesis has been associated with adverse pregnancy outcomes among black women.

One possible mechanism which has not been explored in the literature is the role of maternal infections in pregnancy outcomes of US-born vs. Foreign-born black women.

*Maternal Infections and Preterm birth*
Maternal infections have increasingly been associated with adverse pregnancy outcomes including preterm birth. There is good evidence in the medical literature linking preterm birth and systemic and ascending genital tract infections. The relationship between genital tract infections and preterm birth is evidenced by the presence of microorganisms within the chorioamniotic membranes or between the decidua and membranes which can penetrate the membranes and infect the amniotic fluid. It is estimated that intra-amniotic infection may be responsible for up to 50% of preterm birth occurring before 30 weeks' gestational age.

The maternal and or fetal inflammatory response to intrauterine infection is hypothesized to be one of the mechanisms through which preterm labor is triggered. Elevated levels of proinflammatory mediators such as cytokines, matrix metalloproteinases and white blood cells have been found in the sera, cervical secretions, and amniotic fluid of women in preterm labor. Another possible mechanism is the production of phospholipase A2 and endotoxins by certain bacteria which stimulate uterine contractions and preterm labor. These mechanisms are further supported by animal models that have placed live bacterial organisms or bacterial endotoxins inside animal uterus resulting in a precipitation of preterm labor.
Bacterial Vaginosis and Preterm Birth

More than 50 organisms have been associated with intrauterine infections and preterm labor. Some of the micro-organisms commonly found in the uterus before membrane rupture include *Ureaplasma, Mycoplasma, Gardnerella vaginalis, Mobiluncus, Peptostreptococcus* and *Bacteroides*. It is noteworthy that *Mycoplasma, G. vaginalis, Mobiluncus, and Bacteroides* are associated with the clinical syndrome Bacterial Vaginosis (BV). Several researchers have found associations between BV and preterm birth. In addition, there is evidence in the literature of an association between preterm delivery and some of the specific organisms associated with BV, namely the presence of *Mobiluncus* species, high concentrations of *G. vaginalis* or *Bacteroides*, and low concentrations of *Lactobacillus* species, especially the hydrogen peroxide-producing species. While the evidence is unclear on the efficacy of screening for BV in high risk women, there is at least one meta-analysis which illustrates that screening for BV in a subset of high risk women may decrease the incidence of preterm birth.

It is unclear whether there is a difference in vaginal colonization by BV associated microorganisms between US born and foreign born Black women. A Medline search using the MESH terms (Bacterial Vaginosis/Microbiology) or (vaginal flora) AND (pregnancy or pregnancy complications) AND (African American) OR (Nativity) did not identify any studies which investigated the relationship between bacterial
vaginosis/organisms during pregnancy and nativity status of African American Women. The search was limited to articles in English published after 1990.

This paper will investigate whether there is a difference in rates of BV and vaginal colonization with BV-associated organisms among pregnant Foreign-born vs. US-born Black women. Our hypothesis is that US-born women will have higher rates of BV and BV-associated organisms compared to Foreign-born women.

Materials and Methods

Study Question/Hypothesis. The purpose of this study is to determine whether there are differences in vaginal colonization with BV-associated micro-organisms among pregnant US-born and Foreign-born African American women. We hypothesized that US-born women will have higher rates of BV and BV-associated organisms compared to Foreign-born women.

Study Design and Study Population This is a secondary data analysis of a longitudinal study of clinical prevalence of maternal stress, BV and preterm birth, based on women who received prenatal care from public health centers in Philadelphia and met inclusion criteria for the Stress Pregnancy Evaluation and Community Project (SPEAC). Included in the study were women with singleton intrauterine pregnancies, who were English or Spanish speaking, were at less than 20 weeks of gestation and
receiving prenatal care at one of eight public health centers in Philadelphia. The women were recruited, screened and enrolled for participation in the SPEAC study between February 1, 1999 and April 20, 2003, during their first prenatal care visit at the mean gestational age of 14.8 ± 0.2 weeks. We conducted our analysis on data from a sample of 3239 African American women; 89% of whom are US-born and 11% foreign-born. This study was approved by the Institutional Review Board of the University of North Carolina Chapel Hill (IRB# 05-PUB/HLTH-1087).

**Specimen collection and processing.** Clinical practitioners collected 2 vaginal specimens from each study participant during routine pelvic exam at initial prenatal visit; one of which was used for gram stain and the other for wet mount. Practitioners also assessed for the clinical signs of BV including vaginal discharge, positive whiff-amine test, clue cells on wet mount and high pH. All the data collected was recorded by study personnel.

BV was diagnosed by Gram-stained vaginal fluid specimens using Nugent’s criteria. Scoring of Gram-stained slides was performed by a microbiologist who was blinded to clinical findings and demographics of study participants.

Vaginal morphotypes including large uniform gram-positive rods (*Lactobacillus spp*), small pleomorphic gram-variable rods (*Gardnerella vaginalis* or *G Vaginalis*), small gram-negative rods (*Prevotella/Bacteroides morphotypes*), and curved gram-variable rods (*Mobiluncus spp*) were
quantified over 5 nonadjacent fields under oil immersion (100X magnification). Each of the morphotypes was quantified on a scale of 0 to 4 based on the number of organisms seen on 5 nonadjacent fields. A gram-stain score of 1+ was assigned for less than 1 organism per field; 2+ for 1 to 4 per field; 3+ for 5 to 30 per field; and 4+ for more than 30 per field. Using the Nugent criteria, points were awarded by Gram-stain score based on the relative proportions of the different bacterial morphotypes; *Lactobacillus* spp and *G. Vaginalis* or *Prevotella/Bacteroides* spp were awarded points ranging from 0 to 4 while *Mobiluncus* spp was scored from 0 to 2. *Lactobacillus* was inversely scored with the highest number of morphotypes awarded the least score e.g. 4+ *Lactobacillus* assigned 0 points, 3+ *Lactobacillus* spp assigned 1 point and so on. *G. Vaginalis* or *Prevotella/Bacteroides* spp and *Mobiluncus* spp were scored by giving those with the highest number of organism the highest score e.g. 4+ *G Vaginalis* or *Prevotella/Bacteroides* spp. was assigned 4 points and 3 to 4+ *Mobiluncus* spp was awarded 2 points. Points were totaled to give a score of 0 to 10 using the following formula: *Lactobacillus + G vaginalis or Prevotella/Bacteroides + Mobiluncus = total score*. A score of 7-10 was defined as BV positive, a score of 4 to 6 was defined as BV intermediate and a score of 0 to 3 was considered normal.

**Dependent variables.** In this analysis the 2 dependent variables are BV and BV-associated organisms namely: *Lactobacillus* (decreased),
Gardnerella vaginalis; Mobiluncus species; Prevotella/Bacteroides species. BV was categorized using the Nugent criteria: A score of 7-10 was defined as BV positive, a score of 4 to 6 was defined as BV intermediate and a score of 0 to 3 was defined as BV negative. Lactobacillus, G Vaginalis, Mobiluncus and Prevotella/Bacteriodes Species were coded as positive (1) if their Gram-stain scores were 1+ and higher and negative (0) if their Gram-stain scores were equal to 0.

Independent variable. Nativity Status among Black women was coded as a binary variable, defined as US born or Foreign born. Nativity and race were determined by asking women in a questionnaire respectively about whether there were born in the US (yes/no) and their race (Black/African American; White/Caucasian; Latino; Asian or Pacific Islander).

Covariates. Sociodemographic and behavioral variables that have been identified in several studies as important risk factors for BV were assessed in an interview conducted by trained research staff by a questionnaire. Women were asked about sociodemographic factors such as maternal age, education, maternal income, marital status, insurance status and parity; and behavioral risk factors such as age of sexual debut, number of lifetime partners, douching, oral sex, recent smoking, and history of sexually transmitted diseases (STD).

Maternal age, a continuous variable, was created from the date of birth of the study participant.
Education, a categorical variable, was defined as the highest grade in school that was completed. For purposes of analyses, we recoded education as a binary variable, High school graduate/higher (1) or less than high school (0).

Maternal income, a continuous variable, was defined as each study participant's yearly income which included reported income from all of the following sources: pay checks, under-the-table/off-the books, Social Security income, Department of Public Assistance/Aid to Families with Dependent Children checks, food stamps, unemployment compensation, and money from baby's father, relatives, and friends. We recoded as, a binary variable, less that $9800 (2006 Federal Poverty guideline for a one person household or one person in a family) (1) or greater than $9800 (0).

Marital status, a categorical variable, was defined as single, married, living as married. We recoded as a binary variable defined as single (1) or married/living as married (0).

Parity was a continuous variable defined as number of other pregnancies besides the index pregnancy. We recoded as a binary variable, defined as nulliparous(1) or multiparous(0).

Age of sexual debut was a continuous variable, defined as age at first intercourse.

Number of lifetime partners was a continuous variable, defined as number of people with whom participants had had vaginal intercourse in their lifetime. We recoded as a binary variable, lifetime partners greater than four (1) or less than or equal to four (0).
Douching, a binary variable, was defined as douching the year before getting pregnant (yes (1), no (0)).

Recent smoking, a binary variable, was defined as smoking the year before pregnancy (yes(1), no(0)).

Oral sex, a continuous variable, was defined as frequency of receipt of oral sex the year before current pregnancy. We recoded as a binary variable (receipt of oral sex of greater than 0 times before current pregnancy (1) or equal to 0 (0)).

History of STD, a binary variable created from recoding several binary variables and defined as presence of at least one of the following STDs: Gonorrhea, Chlamydia, Trichomonas, Herpes, HIV/AIDS, syphilis or genital warts.

**Statistical Analysis.** We compared US-born vs. Foreign-born women on several demographic and behavioral characteristics in order to describe important similarities and differences between the populations and to identify possible confounders. The differences between the two groups were analyzed with Pearson Chi-square tests for categorical variables and Student t tests for continuous variables.

We determined if covariates were actual confounders of the relationship between the independent variable, nativity status and the dependent variables—BV and BV-associated microflora (*Lactobacillus, Gardnerella vaginalis, Mobiluncus spp, Prevotella/ Bacteroides spp*). We tested each covariate in separate logistic regressions models. Covariates that had been identified as potential risk factors for BV on the basis of our literature search
and preliminary univariate analysis were included in the models. Parity and private insurance were not included in the models because there was no statistically significant variation in parity among US-born and Foreign born women. For the sake of consistency, we considered covariates potential confounders if the adjusted OR differed from the crude OR by at least 10% in at least one of the dependent variables. We eliminated variables which were not statistically significant in the models (history of BV and history of STD). Our final model was adjusted for the following: age (continuous); age at sexual debut (continuous); education (high school graduate or higher versus less than high school graduate; yearly income (<$9800 vs. >$9800); number of lifetime sexual partners (>4 vs. <4), douching within a year of pregnancy (yes/no), smoking within a year of pregnancy (yes/no), and receipt of oral sex within a year of pregnancy (yes/no).

Multivariate logistic regression analyses were used to determine if there was an increased odds of colonization with BV and BV associated microorganisms among US-born women compared to Foreign-born women. Logistic regression models were adjusted for identified confounders.

Two-sided statistical significance was set at \( p \leq 0.05 \). All analyses were conducted using STATA 9.1 \(^6\).

Results

Of the 3243 women included in this analysis, 2890 (89%) were US-born and 349 (11%) were foreign-born. Table I shows that many characteristics
differed between US-born women and Foreign-born women including that US-born women were more likely to be single, have greater than 4 lifetime partners, to have smoked within a year of pregnancy, to have douched recently and to have a history of an STD. US-born women were less likely to have any education past high school compared to Foreign-born women. The mean age of US-born women in the sample was 24 years compared to 27 years among Foreign-born women; the mean age of sexual debut for US-born women was 15 years compared to 17.6 years among Foreign-born women.

Table II shows the frequency of BV and BV-associated microflora among US-born and Foreign-born women. Overall, there was a high prevalence of BV within this study population. Rates of BV varied across the two groups, with US-born women having statistically higher rates compared to Foreign-born women (52% vs. 42.1%; Pearson’s Chi-Square test: $p < 0.05$). In addition, US-born women tended to have statistically significant higher rates of colonization with BV-associated organisms compared to foreign born women. US-born women had slightly lower rates of *Lactobacillus* (54.8% vs. 60.7%, Pearson’s Chi-Square test: $p < 0.05$) and higher rates of *Gardnerella vaginalis* (72.8% vs. 59%, Pearson’s Chi-Square test: $p < 0.05$); *Mobiluncus spp* (35.6% vs. 26.4%, Pearson’s Chi-Square test: $p < 0.05$) and *Prevotella/Bacteroides spp* (69.3% vs. 54.9%, Pearson’s Chi-Square test $p < 0.05$). Particularly noteworthy is that the frequency of
vaginal colonization with *Gardnerella vaginalis* was 72.8% among US-born women compared to 59.0% among Foreign-born women.

Table III shows the unadjusted odds ratio and the 95% confidence interval for US-born women compared with Foreign-born women who have colonization with BV and each microorganism. US-born women were 1.7 times more likely to have BV positive flora (95%CI: 1.3-2.2) compared to Foreign-born women; and 1.8 times of having BV intermediate flora (95%CI: 1.2-2.8). This association was maintained after adjusting for age, age of sexual debut, marital status, education, income, number of lifetime partners, recent douching, recent smoking and receipt of oral within a year of pregnancy. US born status was associated with specific vaginal microflora including *Gardnerella Vaginalis* (OR 1.6, 95%CI: 1.2-2.2); *Mobiluncus spp* (OR 1.5, 95%CI: 1.2-2.2); *Prevotella/Bacteriodes* (OR 1.5, 95%CI 1.2-2.2) after adjusting for confounders. US born women had lower rates of *Lactobacillus* (OR 0.8, 95%CI: 0.6-1.0), however this difference disappeared after adjusting for confounders (OR 1.0, 95%CI: 0.7-1.3).

To ascertain whether there were nativity differences in the presence of each morphotype within the group of women classified as having bacterial vaginosis or not, we stratified by bacterial vaginosis status and compared the distributions of morphotypes. We conducted subgroup analyses by stratifying our logistic models by BV status and testing for the association of BV-associated organisms within nativity status. Among women with BV positive status, US born women were more likely to have
*Gardnerella vaginalis* OR 1.9; 95% C (1.2-18.5); and 1.2 times as likely to have *Mobiluncus* but this relationship was not statistically significant. Among women with BV intermediate status, US born women were 6 times more likely to have *Gardnerella vaginalis* 95%CI (2.0-17.8); and 2.5 times as likely to have Mobiluncus compared to foreign-born women. US-born women of BV intermediate status were 7.4 times as likely to have *Lactobacillus* (95% CI: 2.4-22.4) as BV-negative women. Among women with no BV; US-born women were 4.0 times more likely to have *Mobiluncus* 95% CI (1.6-9.5).

**Discussion**

Foreign-born women face unique challenges in accessing adequate health care largely due to economic, legal, linguistic and cultural barriers. Despite these barriers, foreign born black women have better pregnancy outcomes compared to their US born counterparts after adjusting for sociodemographic factors and health status. The role of maternal infections in this paradox has not been explored in the literature.

Demba et al (2005) reported BV prevalence of 47.2% among nonpregnant women in the Gambia. In that study, the prevalence of BV-associated bacteria were: *G vaginalis* 44.4%; *Prevotella/Bacteroides* 31.9%; 15.2%; *Peptostreptococcus* 1.5%; *Mobiluncus* 0%; other anaerobes 3.1%. While Dovender et al (1995) found BV in 52% of black underprivileged pregnant women in South Africa higher than the BV positive rate of 42.1% in our foreign-born cohort.

Our study is the first to investigate whether there are differences in rates of BV and BV-associated microflora between US-born and Foreign-born African
American women. Our findings supported our hypothesis that US-born women have higher rates of BV and BV-associated organisms compared to Foreign-born women. US-born status was associated with the occurrence of higher rates of BV positive and BV intermediate flora. US-born women were more likely to have higher rates of BV-associated microflora. Adjusting for potential confounders did not erase the association of nativity status and BV and G vaginalis, Mobiluncus and Prevotella/Bacteroides. Most remarkable, among women with BV intermediate status, US-born women were 6 times as likely to be colonized with G vaginalis compared to foreign-born women. Among women with BV negative status, US-born women were 4 times as likely to be colonized with Mobiluncus compared to foreign-born women. These findings suggest that US-born women have higher rates of BV associated organisms irrespective of whether they are BV positive or not. Royce et al (1999) reported similar findings with a nine fold difference in colonization with Mobiluncus among black women compared to whites.

Differential exposure to social stressors may be a possible explanation for the higher prevalence of BV among US-born women. Culhane et al (2001) reported that chronic social stressors were positively associated with BV among low-income pregnant women. US-born blacks have been exposed to unique psychosocial stressors compared to other ethnic groups in the US.

Another possible explanation for the difference in rates of BV associated microflora especially G vaginalis and Mobiluncus could be differences in the inflammatory cytokines and toll-like receptor genes. Goepfert et al (2005) reported higher rates of polymorphisms in IL6-174 locus among BV positive black women.
compared to white women. It will be interesting to investigate if there are differences in IL-6 among black women by nativity status.

An important limitation to our study is that BV-associated organisms were identified only on the basis of Gram stain without the use of cultures. This approach could be problematic because differentiating Gram-variable, pleomorphic bacillus (G. Vaginalis) from uniform Gram-negative bacillus (Prevotella/Bacteroides spp) can be difficult and is quite subjective. Our analysis is further limited by the process of confounder determination. For the sake of consistency, we included in our final models covariates which did not significantly alter the crude OR of all our dependent variables; this approach widened our confidence intervals and decreased the robustness of our results. In our preliminary analyses, some variables acted as confounders in the analysis with some of the microorganisms as dependent variables, while they were not confounders in others. We included any variable that was identified as a confounder in all sub analyses.

Our study population is not representative of the US population as a whole as most of the women were low-income inner city residents and at high risk for sexually transmitted disease. Therefore, our study findings may only be generalizable to other similar urban settings in the US with populations comparable to the group of women used in our analysis.

Strengths of our study include the large number of women studied, the use of consistent data collection protocols and the blinding of our microbiologist to the clinical findings, demographic and behavioral characteristics of study participants.
Our review of the medical literature shows that the health outcomes, especially obstetric outcomes, of Foreign-born black women have received very little attention. Our study hoped to fill the gaps in the literature. Further research needs to be done on the role of intermediate BV status on preterm birth and the role if any of Mobiluncus and preterm birth among BV negative black women.

The public health importance of our findings is that the presence of BV increases the risk for adverse obstetric and gynecological diseases including preterm birth. The increased rates of BV and associated organisms among US born women may be a significant contributor to the high rates of PTB seen in this population compared to foreign-born women. It is not known why non BV positive US born black women have higher rates of BV associated organisms and whether these higher rates result in higher risk of PTB in this non-BV group. It is possible that some unidentified physiological factors render this group more susceptible to colonization with Gardnerella vaginalis and Mobiluncus spp which subsequently puts them at risk for PTB.

**Conclusion**

Our findings of higher rates of BV and BV associated organisms especially *G. vaginalis* and *Mobiluncus* among US-born black women as compared to Foreign-born black women may be potential contributor to the higher rates of preterm birth seen in the US-born black women. Further research should investigate the independent roles of Mobiluncus spp and Gardnerella Vaginalis in the
pathogenesis of preterm birth (irrespective of BV status) amongst pregnant women.

Acknowledgement

I will like to thank Dr Jennifer Culhane for providing me with the data for this analysis. I will like to thank Dr Vijaya Hogan and Dr Margaret Gourlay for their expertise and comments during the writing process. I will also like to thank Dr Julius Atashili for his assistance with the statistical analysis. Finally, thanks to Dr. Russ Harris and Dr. Diane Calleson for guidance throughout the writing process.
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§ Significance test for comparisons based on 2-sample t-test for continuous variables and Pearson's chi-square test for categorical variables.

† Significantly different compared with US born women p<0.05

* S.D (Standard Deviation)

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<td>No</td>
<td>718</td>
<td>27.2</td>
</tr>
<tr>
<td>Mobiluncus sp</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1031</td>
<td>35.6</td>
</tr>
<tr>
<td>No</td>
<td>1859</td>
<td>64.3</td>
</tr>
<tr>
<td>Prevotella sp/Bacteriodes sp</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1828</td>
<td>69.3</td>
</tr>
<tr>
<td>No</td>
<td>808</td>
<td>30.7</td>
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</table>

* Significantly different compared with US born women p<.05
### Table III Multivariate Odds Ratio (OR) for BV and associated vaginal microflora among US-born and Foreign-born Black pregnant women in Public Health Centers in Philadelphia, SPEAC Study, 1999-2003, n= 3239

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>Unadjusted OR(95% CI)</th>
<th>Adjusted OR(95% CI)</th>
<th>Adjusted OR(95% CI)</th>
<th>Adjusted OR(95% CI)</th>
<th>Adjusted OR(95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BV Positive only</td>
<td>BV Intermediate Only</td>
<td>BV Negative Only</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BV Positive</td>
<td>1.7(1.3-2.2)</td>
<td>1.4(1.0-1.8)</td>
<td>1.5(1.1-2.37)</td>
<td>1.5(1.1-2.37)</td>
<td>1.5(1.1-2.37)</td>
</tr>
<tr>
<td>BV Intermediate</td>
<td>1.5(1.1-2.37)</td>
<td>1.8(1.2-2.8)*</td>
<td>1.5(1.1-2.37)</td>
<td>1.5(1.1-2.37)</td>
<td>1.5(1.1-2.37)</td>
</tr>
<tr>
<td>Lactobacillus</td>
<td>0.8(0.6-1.0)†</td>
<td>1.0(0.7-1.3)</td>
<td>0.8(0.5-1.5)</td>
<td>0.8(0.5-1.5)</td>
<td>0.8(0.5-1.5)</td>
</tr>
<tr>
<td>G. Vaginalis</td>
<td>1.9(1.5-2.4)*</td>
<td>1.6(1.2-2.2)*</td>
<td>1.9(1.2-1.85)</td>
<td>1.9(1.2-1.85)</td>
<td>1.9(1.2-1.85)</td>
</tr>
<tr>
<td>Mobiluncus sp</td>
<td>1.6(1.2-2.0)*</td>
<td>1.5(1.2-2.0)*</td>
<td>1.2(0.8-1.9)</td>
<td>1.2(0.8-1.9)</td>
<td>1.2(0.8-1.9)</td>
</tr>
<tr>
<td>Prevotella sp/</td>
<td>1.7(1.5-2.4)*</td>
<td>1.5(1.2-2.0)*</td>
<td>0.6(0.1-3.2)</td>
<td>0.6(0.1-3.2)</td>
<td>0.6(0.1-3.2)</td>
</tr>
<tr>
<td>Bacteriodes sp</td>
<td>1.7(1.5-2.4)*</td>
<td>1.5(1.2-2.0)*</td>
<td>1.3(0.5-3.3)</td>
<td>1.3(0.5-3.3)</td>
<td>1.3(0.5-3.3)</td>
</tr>
</tbody>
</table>

* Adjusted for age, age of sexual debut, marital status, education, income, number of lifetime partners, recent douching, recent smoking, receipt of oral within a year of pregnancy.

† Every study participant without BV had lactobacillus.

* p<0.005
† p<0.05
Addendum to Masters Paper

Background

Epidemiology and Diagnosis of BV

BV is the most common form of vaginitis among women of reproductive age, with a global prevalence of 5% to 60%\(^1\)\(^2\). The occurrence of BV varies by population studied, with a prevalence of 17 to 19 percent in family-planning or student health clinics\(^3\); 24 to 37 percent in sexually transmitted disease clinics\(^4\); and 10 to 35 percent among pregnant women in the United States\(^5\).

BV is characterized by a disturbance in vaginal microflora, with a reduction in the prevalence and concentration of the normally occurring lactobacilli (large gram-positive rods) and an overgrowth of mainly anaerobic bacteria such as *Gardnerella vaginalis* (small pleomorphic Gram-variable rods), *Prevotella, Bacteroides* (small gram-negative rods), *Mobiluncus* species, and *Mycoplasma Hominis, Porphyromonas, Peptostreptococcus* species\(^6\). Clinical diagnostic criteria for BV include 3 of 4 of the 1983 Amsel et al criteria: vaginal discharge pH>4.5, homogenous discharge vaginal discharge, amide odor upon mixture with KOH and clue cells on wet mount\(^7\). The reliability of these signs in clinical practice has not been evaluated, especially clue cells which are used most frequently in practice\(^8\). Gram stain is the preferred method for screening in epidemiological studies. Two different gram stain
criteria have been developed for diagnosis of BV; namely the Spiegel et al criteria and the Nugent's criteria\textsuperscript{8}. Comparison of gram stain to the clinical Amsel et al criteria has demonstrated sensitivities ranging from 62%-97% and specificities from 66% to 95% for Spiegel et al criteria. Meanwhile, using the Nugent et al criteria has shown a sensitivity of 89% and specificity of 83%\textsuperscript{8}. Thus the Nugent et al criteria is the preferred method for gram stain evaluation.

\textit{Adverse outcomes associated with BV}

BV carries potentially serious complications and is associated with obstetrics and gynecologic disease. Causal relations have been established between BV and plasma-cell endometritis, postpartum fever, post-hysterectomy vaginal-cuff cellulitis, and postabortal infection\textsuperscript{4}. There may be a relationship between BV and cervical intraepithelial neoplasia\textsuperscript{9}. BV may be a risk factor for HIV acquisition and transmission\textsuperscript{10}.

BV is a significant contributor to PTB. There is good evidence that bacterial vaginosis (BV) is associated with adverse birth outcomes\textsuperscript{3,6,11}; including up to a two to three fold risk for PTB\textsuperscript{12}. BV has a higher prevalence in African American women compared to Whites\textsuperscript{3-7}. Differences in BV prevalence may
contribute to the persistent disparity in preterm birth among African Americans.

The proposed mechanism of action is through the effects of prostaglandins and cytokines released during the immunological response to substances such as endotoxins or peptidoglycans produced by BV-associated organisms or in response to BV induced deciduitis and amnionitis\textsuperscript{13}. However, efforts at prevention of PTB by treatment of BV have yielded mixed results. There is good evidence that screening for BV in average-risk asymptomatic pregnant women does not reduce the incidence of preterm birth\textsuperscript{8, 14}. The evidence is unclear on the efficacy of screening for BV in high risk women, but there is at least one meta-analysis which illustrates that screening for BV in a subset of high risk women may decrease the incidence of preterm birth\textsuperscript{15}. The 3\textsuperscript{rd} US Preventive Services Task Force reported insufficient evidence to recommend for or against screening high-risk pregnant women for BV, but recommends against screening of average-risk asymptomatic pregnant women\textsuperscript{14}. The American College of Obstetricians and Gynecologist reported that "current data do not support the use of BV screening as a strategy to identify or prevent preterm birth\textsuperscript{16}. On the other hand, The 2002 Center for Disease Control and Prevention of STD treatment guidelines recommends that screening for BV may be conducted at the first prenatal visit for
asymptomatic high risk pregnant women i.e. those who have a history of a previous preterm delivery\textsuperscript{17}.

BV is not the same syndrome in every woman. Microflora vary among women who have BV positive status, BV intermediate status or BV negative status. Investigators have found an association between preterm delivery and some of the specific organisms associated with BV, namely the presence of \textit{Mobiluncus} species, high concentrations of \textit{G. vaginalis} or \textit{Bacteroides}, and low concentrations of \textit{Lactobacillus} species, especially the hydrogen peroxide-producing species\textsuperscript{18-21}. Four studies have reported differences in the presences of specific morphotypes by race and ethnicity\textsuperscript{12, 20, 22-23}. Royce et al reported that black women are 9.26 times as likely to have \textit{Mobiluncus} compared with their white counterparts\textsuperscript{20}.

Variation in bacterial morphology among African American women may contribute to the persistent race/ethnic disparity in preterm delivery and racial/ethnic differences in prevalence of BV. This microbiologic variation may also explain some of the inconsistent associations of BV with obstetric and gynecologic outcomes in the literature and the poor effect of BV treatment on preventing adverse obstetric outcomes.
Systematic Review of the Literature

We conducted a systematic review of the literature to identify articles which explore variation in BV-associated organisms in vaginal flora among African American women by nativity status. The literature search using the MESH terms (Bacterial Vaginosis/Microbiology) or (vaginal flora) AND (pregnancy or pregnancy complications) AND (African American) AND (Nativity) did not identified any studies which examined the prevalence of BV among Foreign-born black women of African and Caribbean descent. Neither did our search produce any studies which assessed variation vaginal colonization with BV-associated organism among African American women by nativity status. The search was limited to articles in English published after 1990.

One article was found which examined vaginal flora patterns among women in Gambia. This article was included to provide some background information on BV-associated microorganisms among women in Africa. We subsequently, refined our search to review articles which explore the variation in BV-associated microorganisms among White and Black women.

Selection of Articles

A MEDLINE search was performed using the MESH terms (Bacterial Vaginosis/Microbiology) or (Bacterial Vaginosis AND
Tabah 6

Vaginal flora) AND (pregnancy or pregnancy complications) AND (race/ethnicity). The search was limited to articles with abstracts, articles in English and studies on Humans only published since 1990.

All abstracts were reviewed by one author (BT), and articles that focused on variation in BV-associated microorganisms among White versus Black pregnant and nonpregnant were included. Articles that focused on variation of prevalence of BV only or that explored vaginal flora among Asians or Hispanic populations only were excluded. Articles were also excluded if they focused on variation in microbial profile among HIV positive women. Bibliographies were hand-searched, and articles that appeared to be relevant to our search criteria were reviewed. Case reports, review articles and editorials were excluded.

Our search resulted in four articles examining BV-associated microbes among white and black women. One article examined vaginal flora among black women in the Gambia. The five articles are presented in Table 1. All the articles used a cross-sectional design. Three studies examined vaginal flora among pregnant women. Two studies were performed on nonpregnant women.

Appraisal of Literature Exploring BV-associated organism among white women vs black women.
Internal Validity Ratings

Table 2 presents quality ratings for each of the five articles included in the systematic review. The five articles identified in the search were assigned quality ratings by the primary reviewer (BNT). Overall judgment of internal validity of the studies was based on the amount of selection bias, measurement bias and confounding bias and the strength of the results. Potential for selection bias, potential for measurement bias, potential for confounding were assigned ratings of + to +++ in increasing level of severity. The strength of the results and overall internal validity of the studies were assigned quality ratings of poor, fair, good, excellent.

Selection Bias

Selection bias was evaluated based on whether the source population was adequately described and whether the study population was representative of the source population. In addition, selection criteria of the study population were taken into consideration.

Measurement Bias

Measurement bias was evaluated based on means of data collection and identification of data collectors. Studies received low scores for bias if the authors adequately described the methods of data collection, including questionnaires or interview questions.
Reliability and validity of instruments and blinding of investigators during measurement of outcome was an important factor in ascribing potential for measurement bias.

Confounding Bias

Confounding bias was evaluated based on whether the articles named potential confounders and described how each was controlled for.

Strength of Results

The strength of the results was evaluated based on the magnitude and direction (point estimate; 95% confidence interval); statistical significance of the results reported in the articles.

Summary of Internal Validity

The article by Pereira et al\(^23\) was assigned an internal validity rating of good because measurement of outcomes and independent variables was well described and investigators were blinded during measurement of the outcome; there was however a high potential for confounding bias because only unadjusted associations of Mobiluncus to race were reported.

Ness et al\(^22\) received a rating of fair for internal validity because of the potential for high measurement bias because the vaginal specimens were self collected by the study participants and there was no mention in the article if investigators who evaluated
the gram stains were blinded to demographic and behavioral characteristics of the study participants.

The article by Goldenberg et al\textsuperscript{12} received an internal validity rating of excellent because of the low potential for selection bias and measurement bias and the excellent strength of the results. The study however, had a moderate potential for confounding bias because the authors did not adjust for douching; a risk factor for BV identified in the literature\textsuperscript{1, 2, 24, 25}.

**External Validity: Generalizability to other populations**

Generalizability of any of the articles to other populations is very limited to populations outside the respective study population, because of the highly selective group of women used in the studies e.g. pregnant women, women of low-SES or women at high risk for sexually transmitted disease. Demba et al recruited women in the Gambia; these results in this study can not be generalized to Foreign-born black women in the US who are of different cultural backgrounds.
Table 1: Selected studies on Vaginal Flora by ethnicity/race

<table>
<thead>
<tr>
<th>Study Authors, Year</th>
<th>Study Design</th>
<th>Source Population</th>
<th>Study Population</th>
<th>Measurements</th>
<th>Significant Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pereira, Culhane, McCollum, Agnew, Nyirjesy 2005</td>
<td>Descriptive, Cross-sectional</td>
<td>4361 Pregnant women from public health centers in Philadelphia meeting inclusion criteria; recruited between 1996-2003</td>
<td>1756 Pregnant BV positive women at mean gestational age of 14±6 weeks were followed in study</td>
<td>Air-dried vaginal smears collected by practitioners were assessed for BV according to the Nugent et al Criteria. Gram Stain scoring was performed by a microbiologist blinded to clinical findings and demographics of subjects. Demographic and lifestyle behavior information was obtained from study subjects using a questionnaire.</td>
<td>In unadjusted analyses, BV positive women with Mobiluncus spp were significantly more likely to be non-Hispanic black (80.9% vs 66.2%; p&lt;0.0001).</td>
</tr>
<tr>
<td>Dembia, Morison, Van der Loeff, Awassana, Gooding, Bailey, Mayaud, West 2005</td>
<td>Descriptive, C</td>
<td>227 women from a large genito-urinary medicine clinic in Fajara, The Gambia in 2000. Inclusion criteria included 18 and over with self-reported symptoms of vaginal discharge and/or vaginal itching</td>
<td>Same as source population</td>
<td>Vaginal swabs were performed by study participants. BV and associated bacteria was diagnosed by the Nugent’s score and Amsel’s clinical criteria and collaborated with cultures. A standardized questionnaire elicited socio-demographic characteristics, reproductive and sexual health history including vaginal douching and menstrual hygiene practices, and current STD symptoms.</td>
<td>Prevalence of BV-associated bacteria were: G vaginalis 44.4%; Bacteroides 16.7%; Prevotella 15.2%; Peptostreptococcus 1.5%; Mobiluncus 0%; other anaerobes 3.1%; and Mycoplasma hominis 21.4%. BV was positively associated with isolation of G vaginalis (odds-ratio [OR] 18.42, 95%CI 7.91 – 47.6) and anaerobes (P = 0.001 [OR] could not be calculated), but not with M hominis.</td>
</tr>
</tbody>
</table>
### Tabah 11

<table>
<thead>
<tr>
<th>Study Title</th>
<th>Study Type</th>
<th>Participants</th>
<th>Study Design</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ness, Hillier, Richter, soper, Stam, Bass, Sweet, Rice 2003²²</td>
<td>Qualitative</td>
<td>Women 13 to 36 years of age recruited from five sites located in eastern, south, and western regions of the US in the GYN Infections follow-through (GIFT) Study</td>
<td>Inclusion: women with a score of three points based on an algorithm developed by the study investigators</td>
<td>1135 women included in the study analyses Study participants self collected vaginal specimens using Q-tip cotton swabs. Swabs were smeared on slides at bedside by study staff and Gram stain assessed for BV and associated organisms using Nugent et al criteria. Participants were asked about demographic information and relevant lifestyle behaviors in a 20 minute interview Black race was independently related to lack of H2O2 lactobacillus (OR 2.0, 95% CI 1.4-2.8); Gardnerella vaginalis (OR 1.9, 95% CI 1.4-2.6); Mycoplasma hominis (OR 2.1, 95% CI 1.5-3.1) Anaerobic Gram negative rods pigmented (OR 2.0 95% CI 1.4-2.8) and nonpigmented (OR 1.7, 95% CI 1.1-2.5); Mobiluncus (OR 4.5. 95% CI 2.4-8.4) Analyses adjusted for clinical site, age, education, history of trichomoniasis, gravidity, current smoking, sex with menses, hormonal contraceptive use, and douching</td>
</tr>
<tr>
<td>Royce, Jackson, Thorp, Hillier, Rabe, Pastore, Savitz 1999²⁰</td>
<td>Descriptive, Cross-sectional</td>
<td>Women enrolled for a study of preterm delivery-the Pregnancy, Infection and Nutrition (PIN) study. PIN participants are recruited among women attending prenatal care at several public and one private prenatal care clinics that deliver in two large tertiary health care centers (Wake Medical Center in Raleigh and University of North Carolina Hospitals in Chapel Hill). Only women with single gestations, ages 16 or older, able to understand English, and able to give informed consent are invited to participate in the study.</td>
<td>842 women at 24 to 29 weeks' gestation were included in the study</td>
<td>Vaginal smear specimens were collected during the routine pelvic exam conducted at approximately 28 weeks' gestation Gram staining was used to evaluate vaginal flora using Nugent et al criteria. Investigators assessing the slides were blinded to the identity and characteristics of the study participants Study participants demographic and behavioral characteristics were obtained via questionnaire. Black women were more likely than white women to have lactobacilli morphotypes be absent from their slide specimens (RP 1.39, 95% CI 1.05, 1.84). Black women were 1.20 times as likely to have G. vaginalis or other small gram-negative rods (95% CI 1.02, 1.41); Black women were 9.0 times as like to have Mobiluncus (OR 9.0 95% CI 3.1-26.3) OR adjusted for age, income, education, marital status, parity, number of sex partners in the 6 months prior to pregnancy, and during the pregnancy whether the woman smoked, engaged in sexual intercourse in the past month, had a sexually transmitted disease (STD) (syphilis, gonorrhea, chlamydia, or trichomones) diagnosed, douching, and used condoms, foam, suppositories, or the contraceptive sponge</td>
</tr>
</tbody>
</table>
| Goldenberg, Klebanoff, Nubent, Krohn, Hallier, Andrews 1996²⁵             | Cross-sectional     | Women recruited for the Vaginal Infections and Prematurity Study from 1984 to 1989 | 13,747 predominantly low SES women at 23 to 26 weeks' gestation recruited from seven urban medical center in the US from 1984 to 1989 | Vaginal specimens were collected by investigators and assessed for BV and microorganisms using the Nugent et al criteria Data was collected regarding demographic and behavioral characteristics of study participants were collected by interview Black women were more likely to be colonized with BV associated organism compared to white women after adjusting for maternal parity, age, education, insurance status, marital status, smoking, age, age at first intercourse, and number of male partners in last year. Black women were 4.5 times as likely to have Mobiluncus (OR 4.5 95% CI 2.4-8.4) Bacteroides sp (OR 2.0 95% CI 1.8-2.2) M. hominis (OR 2.2 95% CI 2.0-2.4) U. urealyticum (OR 1.9 95% CI 1.7-2.2)
Table II: Quality ratings for studies in systematic review. Each study was rated on a scale of + to +++ for potential of bias (+ = low, ++ = moderate, +++ = high); Strength of results and overall internal validity were ascribed ratings of poor, fair, good, and excellent.

<table>
<thead>
<tr>
<th>Study Authors, Year</th>
<th>Potential for Selection Bias (+ to +++</th>
<th>Potential for Measurement Bias (+ to +++</th>
<th>Potential for Confounding Bias (+ to +++</th>
<th>Strength of Results</th>
<th>Overall Internal Validity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pereira et al 2005</td>
<td>+</td>
<td>+++</td>
<td>good</td>
<td>fair</td>
<td></td>
</tr>
<tr>
<td>Demba et al 2005</td>
<td>++</td>
<td>+</td>
<td>N/A</td>
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<td>good</td>
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<tr>
<td>Ness et al 2003</td>
<td>++</td>
<td>+++</td>
<td>+</td>
<td>good</td>
<td>fair</td>
</tr>
<tr>
<td>Royce et al 1999</td>
<td>+</td>
<td>+</td>
<td>++</td>
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<tr>
<td>Goldenberg et al 1996</td>
<td>+</td>
<td>+</td>
<td>++</td>
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