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Title
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Permalink
https://escholarship.org/uc/item/57b49799

Journal
American Journal of Obstetrics and Gynecology, 210(3)

ISSN
0002-9378

Authors
Foxman, Betsy
Wen, Ai
Srinivasan, Usha
et al.

Publication Date
2014-03-01

DOI
10.1016/j.ajog.2013.10.003

Peer reviewed

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Author Manuscript
Am J Obstet Gynecol. Author manuscript; available in PMC 2015 March 01.

Published in final edited form as:
Am J Obstet Gynecol. 2014 March ; 210(3): 226.e1–226.e7. doi:10.1016/j.ajog.2013.10.003.

Mycoplasma, Bacterial Vaginosis Associated Bacteria BVAB3, Race, and Risk of Preterm Birth in a High Risk Cohort

Betsy Foxman, PhD, **Ai Wen, PhD**, **Usha Srinivasan, PhD**, **Deborah Goldberg, PhD**, **Carl F. Marrs, PhD**, **John Owen, MD**, **Deborah A. Wing, MD**, and **Dawn Misra, PhD**
Department of Epidemiology, University of Michigan School of Public Health (Drs. Foxman, Wen, Srinivasan, and Marrs), the Department of Ecology and Evolutionary Biology, University of Michigan (Dr. Goldberg), the Division of Maternal Fetal Medicine, Department of Obstetrics and Gynecology, The University of Alabama at Birmingham (Dr. Owen), the Division of Maternal Fetal Medicine, Department of Obstetrics and Gynecology University of California at Irvine (Dr. Wing), and the Department of Family Medicine and Public Health Sciences, School of Medicine, Wayne State University (Dr. Misra)

Abstract

OBJECTIVE—Genital tract infection accounts for ~ 25–40% of all pre-term births. We sought to assess the relationship between preterm birth and selected vaginal bacterial taxa associated with preterm birth either directly or through their association with bacterial vaginosis (BV).

STUDY DESIGN—Vaginal fluid for Gram stain was collected between 17 and 22 weeks gestation as part of a randomized trial of ultrasound-indicated cerclage for preterm birth prevention in women at high risk for recurrent spontaneous preterm birth. Bacterial DNA was extracted from the Gram stain slides and analyzed using quantitative PCR.

RESULTS—Among the 499 participants, *Mycoplasma* was positively correlated with increased risk of preterm (RR = 1.83; 95% CI: 1.52,2.22) as was *Mobiluncus* (RR=1.36; 95% CI: 1.07, 1.73) and *Atopobium* (RR=1.44; 95% CI: 1.1, 1.87). However, there were strong interactions between race/ethnic group and the presence of these and other individual taxa on risk of preterm birth. By contrast, BVAB3 was consistently associated with a reduction in risk of preterm birth for all racial/ethnic groups (0.55; 95%CI: 0.39, 0.78).

CONCLUSIONS—BV is characterized by a reduction of *Lactobacillus*, and lactic acid producing bacteria and the presence of *Mobiluncus*; we found these factors and presence of *Mycoplasma* to be associated with increased risk of preterm birth. By contrast, the presence of a recently identified organism sufficient to cause BV, BVAB3, decreased risk of preterm birth. These findings give insight into why treating BV has mixed impact on risk of preterm birth.

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Corresponding author: Betsy Foxman, Hunein F. and Hilda Maassab Professor of Epidemiology, University of Michigan School of Public Health, 1415 Washington Heights, Ann Arbor, Michigan 48109-2029, 734 764 5487, bfoxman@umich.edu.

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The authors report no conflict of interest.

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INTRODUCTION

In 2011, 11.72% of all births in the United States occurred prior to 37 completed weeks gestation[1]. Prematurity was most common among non-Hispanic Blacks (16.75% compared to 10.49% for non-Hispanic White)[1]. The costs to society of preterm birth are large: an estimated $26 billion dollars in 2005[2]. Prematurity is also a major cause of infant mortality[2].

Infection, both overt and subclinical, is thought to account for ~ 25–40% of all pre-term births[3]. One condition, bacterial vaginosis (BV), is associated with a significant risk of preterm birth with estimates ranging as high as an eightfold increase [4]. However, the effects of BV treatment on reducing rates of preterm birth have been disappointing; vaginal metronidazole therapy has been associated with an *increased* risk of preterm birth in some groups [5]. One significant limitation of previous studies is their focus on the association of BV, diagnosed by Nugent or Amsel criteria, with preterm birth[6], rather than on the multiple specific taxa found in conjunction with BV, which include *Mobiluncus*[7] and the recently identified bacterial vaginosis-associated bacteria in the order *Clostridiales:* BVAB1, 2 and 3. While it is clear is that vaginal microbes associated with BV are correlated with an increased risk of preterm birth [8], the role of specific microbial taxa is much less clear. Application of genetic techniques for bacterial identification has substantially eased the difficulties of screening vaginal specimens for the presence of multiple different taxa. We used quantitative PCR to analyze vaginal specimens collected during the second trimester of pregnancy from women at high risk of recurrent preterm birth for the presence and relative load of bacterial taxa either associated with BV or that have been previously associated with preterm birth. These included the recently identified BVAB1, 2 and 3 [9, 10].

MATERIALS AND METHODS

Study Population and Sample collection

Vaginal fluid for Gram stains and clinical data were collected as part of a multi-center, randomized trial of ultrasound-indicated cerclage for preterm birth prevention[11]. Women with a singleton gestation and at least one previous spontaneous preterm birth of 17–33 weeks' gestation were eligible for enrollment. Cervical length was measured at the initial sonographic cervical length evaluation, which was scheduled between 16 and 21 weeks gestation. At the visit, a sterile speculum examination was performed to collect vaginal fluid from the upper one-third of the vaginal sidewalls for pH and Gram stain. Serial transvaginal ultrasound was conducted throughout the study. If the cervical length shortened to less than 25mm, the participant became eligible for randomization to cervical cerclage or a no-cerclage cohort. The study protocol was approved by the Human Subjects Committee at the University of Alabama at Birmingham (X991227014), and similar committees at all participating sites[11]. The use of deidentified data for this study was deemed exempt and unregulated by the Human Subjects Committee at the University of Michigan because the original consent form provided for the collection and analysis of biologic specimens as it related to studying the etiology of preterm birth. This microbiological study includes samples from participants whose cervical length shortened to < 25 mm and were randomized to **not** receive cerclage, and samples from participants not eligible for the intervention trial because their cervical length remained at least 25 mm. We defined preterm birth as birth < 37 weeks' gestation.

For this study we identified 608 women with associated Gram stain slides and ultrasound measurement. Women with an inconclusive ultrasound due to a poorly developed lower segment (n=76) were included, because their cervical length was longer than the 25mm cutoff we used to defined cervical shortening, although not measureable precisely. Of the 608, 109 were excluded for various reasons: inability to extract and test bacterial DNA

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(n=32), self-reported racial/ethnic group was 'other' (n=46), or missing critical data on sample collection (n=31), leaving a total of 499 for analysis.

Quantitative PCR assay for targeted bacteria taxa

This technique enables estimation of the number of bacterial cells present, and classification of bacteria based on the sequence of a region of a gene coding for the ribosome, which is present in all cells. Resolution to the species level is not always possible (some species in the same genus may have the same genetic sequence in the region of interest), therefore we use the term 'bacterial taxon' throughout the manuscript.

Bacterial DNA was extracted from the gram stain slides as described by Srinivasan et al. [12]. Preamplification was done using 8F-1492R universal bacterial primers based on 16s rRNA. Total bacterial and species specific bacterial ribosomal gene copy numbers were identified using total bacteria primers and primers specific to each bacteria taxa. The targeted bacteria genera and species include: *Atopobium* spp., bacterial vaginosis-associated bacterium (*BVAB*) types 1, 2 and 3 in the order *Clostridiales*, *Escherichia coli*, *Gardnerella vaginalis*, Group B *Streptococcus*, *Lactobacillus spp.*, *Mobiluncus spp.*, *Mycoplasma spp*., and *Ureaplasma spp*. We also used a primer set for Lactic Acid Bacteria (LAB) that includes lactic acid producing bacteria of the genera *Lactobacillus*, *Pediococcus*, *Leuconostoc*, and *Weissella*. Total bacterial load was estimated using a broad coverage universal bacterial primer for the 16S rrn gene. Primer sequences and conditions used for individual taxa were reported previously [13]. The limit of detection was 100 copies and readings lower than the limit were considered negative. We calculated the relative proportion of 16S rrn copies from each bacterial taxon using the bacterial copy number measured by each specific bacteria assay divided by the total number of 16S rrn bacterial copies measured by the universal bacterial assay [14].

Statistical analysis

The prevalence of each bacterial taxon was calculated overall and stratified by cervical length. To evaluate the association between the presence of each bacterial taxon and cervix shortening to < 25 mm, we used the ratio of the prevalence among women with shortened relative to normal cervices. In addition, we estimated the risk ratio (RR) of preterm birth relative to term birth, associated with the presence of each bacterial taxon, stratified by self-reported racial/ethnic group, and estimated the 95% confidence intervals around the ratio[15].

To test for an effect of abundance or "load" of each bacterial taxon (that is, a dose response) we fit a set of logistic regression models. The models included preterm birth as the dependent variable, and the participant's self-reported racial/ethnic group (White, Black, Hispanic), cervical length status (< 25 mm vs. ≥25 mm), and the proportion of a given bacterial taxon relative to the total bacteria load as covariates; separate models were fit for each bacterial taxon. Because of observed interactions, for *Lactobacillus*, we fit separate models by race, and included the presence/absence of *Mycoplasma* and cervical length status as covariates. Statistical tests were conducted using the software program R, version 2.15.2 [16].

RESULTS

As expected due to the entry criteria of the study (at least one prior preterm birth, see Methods), the proportion of women with a pre-term birth < 37 weeks in this population was very high, with Blacks having significantly higher rates of pre-term birth than Hispanics or Whites, as well as significantly higher rates of cervical shortening (< 25mm). Smoking and douching habits also differed significantly by race (Table 1).

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The overall prevalence of selected bacterial taxa varied by racial/ethnic group, with significant differences for *Atopobium* (Chi-square test p=0.001), BVAB1 (p=0.00004), BVAB2 (p=0.001), BVAB3 (p=0.008), *Gardnerella* (p=0.004), Group B *Streptococcus* (p=0.004) and *Mycoplasma* (p=0.00008) (Figure 1). The prevalence of BVAB1 and *Mycoplasma* were positively and significantly associated with a shortened cervix, and *Ureaplasma* negatively associated with a shortened cervix, but the prevalence of other taxa tested did not vary by cervical length (Table 2).

Race/ethnicity strongly modified the crude associations between an individual bacterial taxon during the second trimester and risk of subsequent pre-term birth (Table 3). Several taxa were overall positively associated with preterm birth but the risk ratios differed considerably among racial/ethnic groups, sometimes even shifting from hazardous (risk ratio >1) to protective (risk ratio < 1). Modification by racial/ethnic group was especially apparent for bacterial taxa BVAB3, Group B *Streptococcus*, *Mobiluncus* and *Mycoplasma*. Group B *Streptococcus* was statistically significantly associated with risk of pre-term birth among Hispanic women (Risk Ratio (RR)=2.38; 95% CI 1.14,4.98), but among Black or White women the risk ratios did not differ from 1. *Mobiluncus* – whose presence is generally considered diagnostic for BV – was associated with a significant nearly twofold increase in risk of preterm birth among Hispanic women (RR=1.91; 95% CI 1.00, 3.65), but there was no statistically significant association (hazardous or protective) for the other racial/ethnic groups. The association of *Mycoplasma* with preterm birth was significant among Black (RR= 1.97; 95% CI 1.50, 2.57) and Hispanic women (RR=2.81; 95% CI 1.76, 4.50) but among Whites the RR was less than 1.0 and the association was not significant. Finally, the presence of BVAB3 was *negatively* associated with preterm birth overall (RR=0.55; 95% CI: 0.39, 0.78) – significantly so for Black women (0.55; 95% CI: 0.36, 0.83).

Because *Mycoplasma* was the only taxon associated with both cervical shortening and preterm birth, we further examined the association of *Mycoplasma* with preterm birth by cervical length (<25mm and ≥25mm). *Mycoplasma* was positively associated with increased risk of preterm birth regardless of cervical length, although the association was not statistically significant for those with a shortened cervix, perhaps because of the smaller sample size (≥25mm RR=1.99 [1.58, 2.52], p= 3.13e-08; <25mm RR=1.47 [0.84, 2.55], p=0.24). When stratified by racial/ethnic group, the association between the presence of *Mycoplasma* and preterm birth remained only among Black and Hispanic participants who had cervical length ≥25 mm (Black participants RR=2.04 [1.51,2.76], p=3.66e-06; Hispanic participants RR=2.74 [1.70,4.43], p=0.00023; White participants RR=0.82 [0.42,1.61], p=0.65). (Only one Hispanic woman and 7 White women experienced cervical shortening).

In order to estimate the effect of bacterial load, we fit a set of logistic regression models with birth status as the dependent variables and racial/ethnic group, cervical shortening and proportion of a selected taxon (relative to total bacterial load) as independent variables. These analyses were limited to women where the taxon was present. This enabled us to examine the effects of lactic acid producing bacteria, *Lactobacillus* and *E. coli*, which were present in almost all participants. These models showed increasing relative abundance decreased the risk of pre-term birth for three of the 12 taxa tested, lactic acid bacteria, *Lactobacillus* and *E. coli*. The models predicted that for each 10-fold increase in the bacteria load, the odds of preterm birth decreased by 28.9%, 29.9% and 46.5%, respectively. However, there was no association of total bacterial load and gestational age at birth (p=0.61).

As *Lactobacillus* load had a significantly negative and *Mycoplasma* presence a significantly positive association with preterm birth, we further examined the interaction between the

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two, taking into account racial/ethnic group and cervical length. (This analysis was limited to the 497 women where *Lactobacillus* was detected.) Figure 2 shows the observed values and those predicted by the logistic regression models. Increasing the proportion of *Lactobacillus* relative to total bacterial load significantly decreased the probability of preterm birth among White participants (the model predicts that a 10-fold increase of *Lactobacillus* the odds of preterm birth decreased by 58.8%, p=0.0047). This was true regardless of presence of *Mycoplasma*. By contrast, for Black and Hispanic participants the *Lactobacillus* levels did not significantly change the risk of preterm birth, but in the model *Mycoplasma* significantly increased the odds of preterm birth (Black OR=4.65 [2.45, 9.09]; Hispanic OR=5.43 [2.36, 12.9]). Cervical shortening alone only marginally increased the odds of preterm birth among Black (OR=3.71 [0.92, 18.44]) participants. (Only one Hispanic woman and 7 White women experienced cervical shortening). Results were very similar when using lactic acid bacteria instead of *Lactobacillus*.

As *E. coli* previously has been reported to increase risk of preterm birth[8, 17] (see trend in Table 3), we sought further explanation for the apparently protective effect with increasing *E. coli* load identified in the logistic regression model. We hypothesized that the protective effect with increased dose might be explained by the overall positive correlation between *E. coli* and lactic acid bacteria (linear regression, β=0.05 [0.02, 0.08], r^2=0.018, p=0.0024). A more detailed analysis using quantile regression[18] found the positive correlation between *E. coli* and lactic acid bacteria depended on the *E. coli* load: the correlation was significant among participants whose *E. coli* load was in the lower quantiles (quantile 0.25 β=0.06 [0.03,0.09]; quantile 0.50β=0.07 [0.04, 0.09]) but not toward the extreme low (quantile 0.05 β_1 = 0.06 [−0.0006,0.09]); nor in the upper quantiles (quantile 0.75 β_1 = 0.04 [−0.002,0.08]; quantile 0.95 β_1 = −0.003 [−0.07,0.0]). The overall correlation, and the results using quantile suggest the apparent protective effect on risk of preterm of increasing proportion of *E. coli* is an artifact.

COMMENT

In an analysis of vaginal specimens collected from Black, White and Hispanic women at high risk of preterm birth between 17 and 22 weeks gestation, we made several observations that give insight into the effect of selected vaginal taxa on the risk of preterm birth. First, we identified a protective effect of BVAB3 on risk of preterm birth; BVAB3, was previously associated with BV[7]. The effect was robust to adjustment for racial/ethnic group and cervical shortening to <25 mm. We found no similar reports in the literature. If confirmed, this finding may suggest a possible explanation for the inconsistent effects of BV treatment on preventing preterm birth, as acquisition of BVAB3 is strongly associated with developing BV [7]. Increasing levels of lactic acid producing bacteria and *Lactobacillus* species also were associated with decreasing risk of preterm birth, but these taxa were present in essentially all participants. We found no dose effect for *Atopobium*, which also produces lactic acid. There was a significant association of *Atopobium* and increased risk of preterm birth (RR 1.44; 95% CI: 1.1, 1.87), but this was modified strongly by racial/ethnic group: among Black women the RR was 1.02 (95% CI: 0.72, 1.43).

A second novel finding of our study was the observed interaction between *Mycoplasma*, racial/ethnic group, cervical length, and relative proportion of *Lactobacillus* species present. Among White women there was a decreasing risk of preterm birth with increasing levels of *Lactobacillus*, and this effect was true regardless of presence of *Mycoplasma*, which did not have a significant effect on risk of preterm birth. By contrast, there was essentially no association with proportion of *Lactobacillus* on risk of preterm birth among Black or Hispanic women after stratifying for presence of *Mycoplasma* and cervical length. However, *Mycoplasma* was strongly associated with risk of preterm birth among Black and Hispanic

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women. This strong modification by racial/ethnic group suggests that *Mycoplasma* may not be a cause of preterm birth, but a marker of the presence of other factors that cause preterm birth. We note, however, that results from a study in a Belgium cohort suggest an increased risk of preterm birth with presence of *Mycoplasma hominis* [19, 20]. Our results are not directly comparable as our detection method only allowed us to identify *Mycoplasma* spp., rather than specifically *M. hominis*.

Third, our study adds to the growing literature suggesting that some of the increased risk of preterm birth among Black Americans is attributable to differences in immune response to specific vaginal bacteria, rather than differences in carriage rates [21, 22]. The strong modification of the effects of selected taxa on the risk of preterm birth by racial/ethnic group suggests that the observed associations with preterm birth are contingent on the presence of co-factors, not only host behaviors, but the other members of the microbial community and overall host response to that community. Studies by Ravel et al. demonstrated considerable variation on vaginal microbial community structure by self-reported race [23]. *In vitro* studies of fetal membrane immune response to selected bacteria found cytokine responses varied by racial/ethnic group [21, 22]. Differences in response to specific taxa by racial/ethnic group also potentially explain the inconsistent results reported for risk of preterm birth and specific bacterial taxa across studies conducted in different populations [reviewed in[10]].

In our data, *E. coli* load was *negatively* associated with preterm birth, but *E. coli* presence was positively – but not significantly – associated with increased risk of preterm birth. The negative association of *E. coli* dose with preterm birth is probably an artifact: two previous studies, that cultured specimens collected in the third trimester, reported an increasing risk for preterm birth with heavier *E. coli* loads [8, 17]. Further, at least in non pregnant populations, *E. coli* presence in the vaginal cavity (detected using culture) is correlated with day since sexual activity, so in our population increased dose may reflect better overall health [24]. We also found a marginally significant association of *Mobiluncus* with preterm birth, that was strongly modified by racial/ethnic group, but found no other reports in the literature. *Mobiluncus* is generally diagnostic for BV.

Our findings are not without limitation. We detected the presence and relative proportion of the selected bacteria taxa using qPCR applied to the DNA extracted from Gram stain slides. The amount of material present on the Gram stains varies, and whether it represents the entire vaginal community is subject to sampling variation. Further, qPCR is very sensitive – able to accurately detect as few as 100 copy numbers of the ribosomal gene; this may account for the high prevalence rates of Group B *Streptococcus*. Study participants all experienced a previous preterm birth, putting them at high risk of a recurrent preterm birth; whether our observed associations with specific bacterial taxa might be present in the general population is uncertain.

In conclusion, we observed several novel associations between BV associated bacteria and risk of preterm birth. BV is characterized by a reduction of *Lactobacillus*/lactic acid producing bacteria and the presence of *Mobiluncus*; we found both of these factors to be associated with risk of preterm birth. The presence of *Mycoplasma* was also associated with risk of preterm birth, but there were interactions between this association and cervical length and race/ethnicity. By contrast, the presence of a recently identified organism sufficient to cause BV, BVAB3, decreased risk of preterm birth. These findings give insight into why treating BV has mixed impact on risk of preterm birth and suggest that more taxon-specific treatments should be developed and tested for effectiveness in reducing risk of pre-term birth.

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Acknowledgments

SPONSOR/GRANT/FINANCIAL SUPPORT FOR THE STUDY

Source of Funding: R01 HD038098

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CLINICAL IMPLICATIONS

- Treating bacterial vaginosis has a mixed impact on risk of preterm birth because different taxa can cause the same clinical syndrome, and these taxa have different propensities to cause preterm birth.
- Taxon-specific treatments should be developed and tested for effectiveness in reducing risk of pre-term birth among women with bacterial vaginosis.

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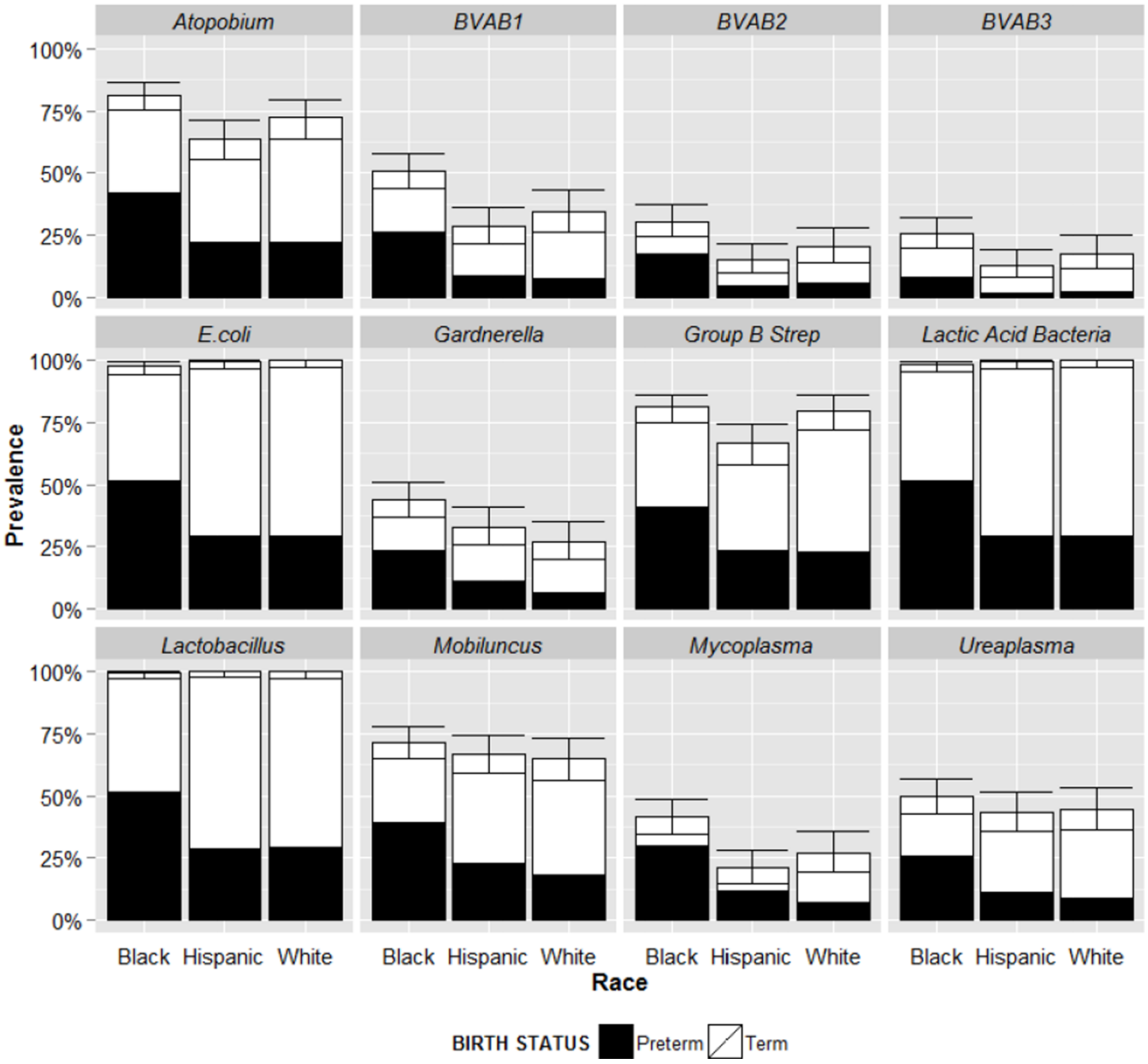

Figure 1.

Prevalence of selected vaginal bacterial tax in vaginal samples collected between 17–22 weeks gestation from 499 women at high risk of pre-term birth, by racial/ethnic groups (Black, Hispanic and White participants). Prevalence is stratified by the birth status (preterm vs. term) of participants. Error bars show 95% CI of each bacteria taxon's overall prevalence, regardless of birth status.

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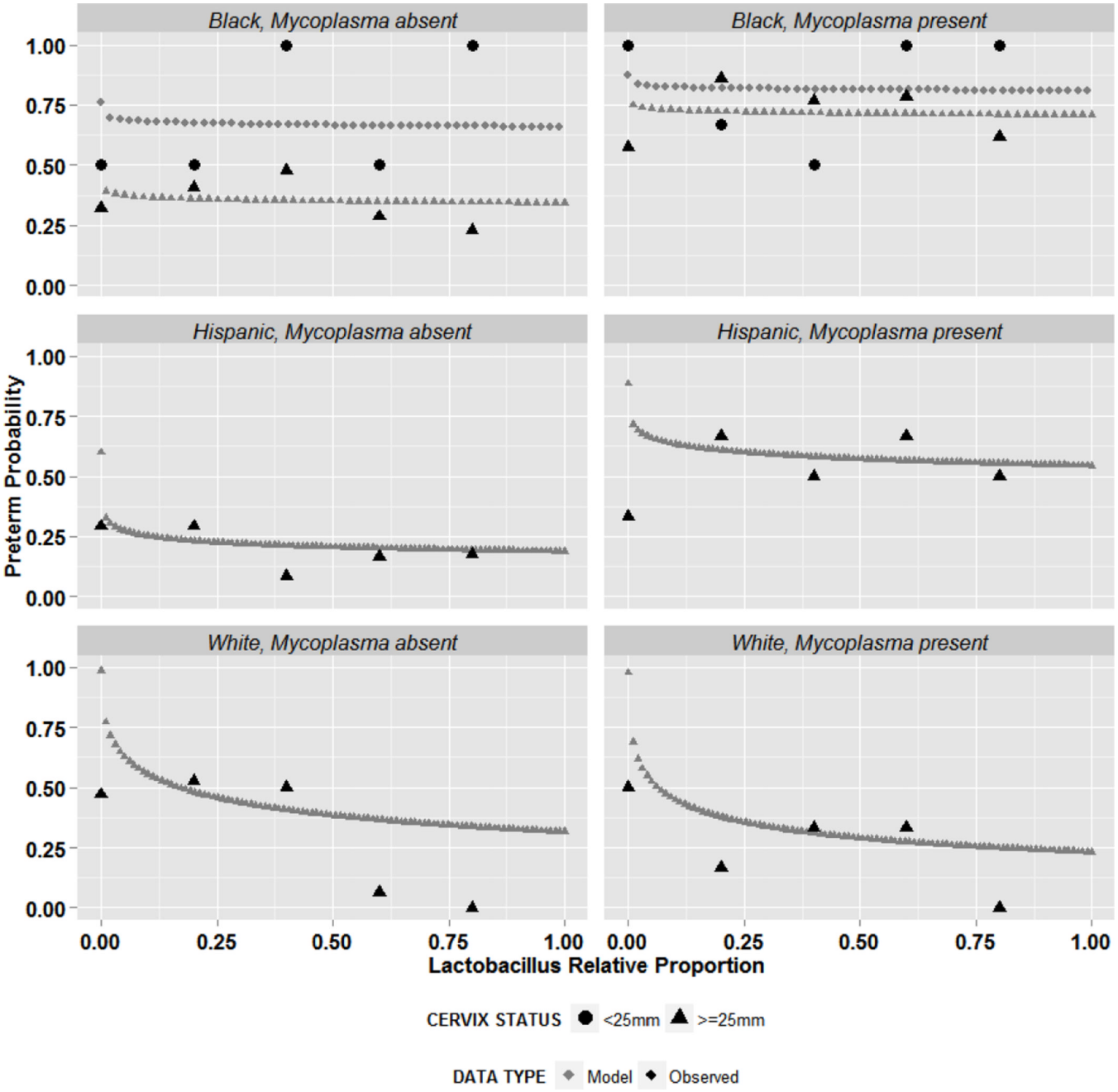

Figure 2.

Interactions of cervical length, race/ethnicity, *Mycoplasma* presence/absence, and the association of proportion of total bacteria that are *Lactobacillus* with risk of preterm birth. Grey symbols display predictions from a logistic regression model while black symbols show observed preterm birth probability among participants, calculated by pooling the relative proportion of *Lactobacillus* into quintiles. Note only 1 Hispanic participant and 7 white participants had cervix length <25mm at sampling, and hence the effect of cervical shortening was not included. 497 women (Black, Hispanic and White participants) at high risk of preterm birth who had *Lactobacillus* present were included in the analysis. (Two

participants had no detectable *Lactobacillus*). Results were very similar using lactic acid bacteria.

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Table 1

Demographic, biological and behavioral characteristics of 499 pregnant women at high risk of preterm birth at 17 to 22 weeks gestation. Cervical length measured using vaginal ultrasound.

		Black (N=207)	Hispanic (N=155)	White (N=137)
Birth status*	Pre-term	107 (51.7%)	45 (29%)	40 (29.2%)
	Term	100 (48.3%)	110 (71.0%)	97 (70.8%)
Age (mean±sd)		26.2±4.7	27.3±5.6	27.8±5.8
BMI (mean±sd)		31.1±8.3	27.7±5.4	27.5±6.3
Shortened cervix (<25mm) at sampling*		25 (14.4%)	1 (0.7%)	7 (6.1%)
Shortened cervix (<25mm) between sampling and the end of study*		51 (24.6%)	12 (7.7%)	19 (13.9%)
Smoking during pregnancy*		43 (20.8%)	2 (1.3%)	36 (26.2%)
Douching before or during pregnancy*		66 (31.9%)	26 (16.8%)	26 (19.0%)

*Prevalence was significantly different by racial/ethnic group using the chi square test ($p<0.05$)

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Table 2

Prevalence of selected vaginal bacterial taxa at 17 to 22 weeks gestation among 499 women at high risk of preterm birth, by whether the cervix subsequently shortened to <25 mm (as measured using vaginal ultrasound). Bold indicates p<0.05

Bacteria Taxa	Prevalence			Prevalence Ratio (PR) and 95% confidence interval	
	All Women	Shortened	Not shortened	PR	(95% CI)
Atopobium	73.2%	73.9%	73.0%	1.17	(1.00,1.37)
BVAB1	39.3%	41.7%	38.5%	**1.52**	**(1.11,2.08)**
BVAB2	22.8%	29.6%	20.8%	1.21	(0.68, 2.17)
BVAB3	19.4%	21.7%	18.8%	1.44	(0.80, 2.60)
E.coli	98.8%	100.0%	98.4%	1.00	(0.96, 1.04)
Gardnerella	35.9%	44.3%	33.3%	1.29	(0.87, 1.91)
Group B Streptococcus	76.3%	91.2%	71.5%	1.11	(0.95, 1.30)
Lactic Acid Bacteria	99.0%	99.1%	99.0%	1.00	(0.96,1.04)
Lactobacillus	99.8%	100.0%	99.7%	0.99	(0.95, 1.03)
Mobiluncus	68.5%	73.9%	66.9%	1.14	(0.93, 1.39)
Mycoplasma	31.4%	60.6%	22.8%	**2.02**	**(1.47, 2.78)**
Ureaplasma	46.5%	39.8%	48.5%	**0.59**	**(0.34, 1.03)**

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Table 3

Risk ratios (RR) for preterm birth by presence of selected vaginal bacteria taxa at 17 to 22 weeks gestation. 499 women at high risk of preterm birth, by self reported race/ethnicity group. Bold indicates that the risk ratio is significantly different from 1. Risk ratio and 95% Confidence Intervals were calculated by unconditional maximum likelihood estimation with normal approximation (Wald Method, Rothman & Greenland 1998).

	All Preterm=233 Term=312	**Black Preterm=107 Term=100**	**Hispanic Preterm=45 Term=110**	**White Preterm=40 Term=97**
Atopobium	**1.44 (1.1,1.87)**	1.02 (0.72,1.43)	1.77 (0.97,3.2)	1.24 (0.65,2.37)
BVAB1	1.01 (0.83,1.24)	0.99 (0.76,1.29)	1.02 (0.6,1.76)	0.64 (0.34,1.19)
BVAB2	1.2 (0.97,1.48)	1.16 (0.88,1.52)	1.06 (0.54,2.07)	0.97 (0.51,1.87)
BVAB3	**0.55 (0.39,0.78)**	**0.55 (0.36,0.83)**	0.31 (0.08,1.2)	0.38 (0.13,1.14)
E.coli	1.5 (0.46,4.87)	2.62 (0.45,15.22)	*a*	*b*
Gardnerella	1.08 (0.88,1.31)	1.04 (0.8,1.35)	1.24 (0.75,2.04)	0.78 (0.41,1.49)
Group B Streptococcus	1.08 (0.85,1.37)	0.87 (0.64,1.19)	**2.38 (1.14,4.98)**	0.74 (0.42,1.31)
Lactic AcidBacteria[c]	-	-	-	-
Lactobacillus[c]	-	-	-	-
Mobiluncus	**1.36 (1.07,1.73)**	1.42 (0.99,2.04)	**1.91 (1.00,3.65)**	0.85 (0.5,1.45)
Mycoplasma	**1.83 (1.52,2.22)**	**1.97 (1.50,2.57)**	**2.81 (1.76,4.5)**	0.88 (0.46,1.66)
Ureaplasma	0.84 (0.68,1.02)	1 (0.77,1.31)	0.79 (0.47,1.31)	**0.53 (0.29,0.95)**

[a] Among Hispanic participants only five participants did not have *E. coli*

[b] Among white participants, only one participant did not have *E. coli*

[c] Two participants had no detectable *Lactobacillus* and six participants had no detectable Lactic Acid Bacteria.