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Preterm labor and bacterial vaginosis-associated bacteria among urban women

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Abstract

Aims—Bacterial vaginosis (BV) affects millions of women, is extremely prevalent and is frequently chronic. We recognize numerous microbiologic variations among women with BV and this variability may explain the limited effectiveness of metronidazole in curing BV and/or reducing the risk of spontaneous preterm birth (SPTB) among BV-positive pregnant women. We assessed the independent role of seven common BV-associated bacteria on the risk of spontaneous preterm birth (SPTB) among urban pregnant women.

Methods—This prospective cohort study was conducted within an urban obstetrics practice at Temple University Hospital in Philadelphia, PA. Fifty pregnant women with documented singleton pregnancies between 25–36 weeks' gestation from February 2007 through June 2007 who presented to the Labor and Delivery Unit for evaluation of uterine contractions/preterm labor were enrolled.

Results—We found that high median levels of *Gardnerella vaginalis* and low median levels of *Lactobacillus crispatus* were significantly predictive of SPTB. Slightly higher levels of *Megasphaera*-like species were also found among the group of women experiencing a SPTB during the follow-up period.

Conclusions—Further identification of the individual attributable risk for separate BV-associated bacteria may be most useful in developing successful treatments to prevent SPTB among BV positive women.

Keywords

Bacterial vaginosis; *Gardnerella vaginalis*; *Lactobacillus crispatus*; spontaneous preterm birth

Introduction

Bacterial vaginosis (BV) affects millions of women, is extremely prevalent among low-income, urban pregnant women and is frequently chronic [13]. In a healthy lower genital tract, *Lactobacillus crispatus* constitutes 95% of the bacteria present; among cases of BV, the levels of *Lactobacillus crispatus* are dramatically reduced and an overgrowth of various

anaerobic bacteria exists [2, 5, 15]. Several authors have recognized numerous microbiologic variations among women with BV and this variability may explain the limited effectiveness of metronidazole in curing BV and/or reducing the risk of spontaneous preterm birth (SPTB) among BV-positive pregnant women [14, 16, 18]. In fact, BV treatment itself is only partially effective with high rates of BV recurrence, discontinuation of therapy due to metronidazole side effects, and metronidazole resistance of some BV-associated bacteria. Recently, a PCR-based strategy was developed to identify hard-to-culture bacteria involved in complex microbial diseases, such as BV, by characterizing ribosomal RNA genes (rDNA) [5]. Using these methods, Fredricks et al. described the most common bacteria among women with BV and identified BV-associated bacteria responsible for chronic, recurrent and hard-to-treat BV [5]. Fredrick et al. found that *Lactobacillus crispatus* was rarely detected among women with BV but *Leptotrichia/Sneathia* species and Megasphaera-like species were frequently detected among women with BV. *Gardnerella vaginalis* was present in all subjects with BV and one-half of subjects without BV. In addition, *Leptotrichia amnionii* and *Gardnerella* were linked to persistent and relapsed cases of BV. Three new Clostridia-like bacteria were very specific for BV and were designated bacterial vaginosis-associated bacterium (BVAB) 1, 2 and 3 based on their distant phylogenetic relationships with previously known bacteria and with each other [5]. In this study, we assessed the independent role of each of these common BV-associated bacteria on the risk of SPTB.

Material and methods

This study was conducted within an urban obstetrics practice at Temple University Hospital in Philadelphia, PA. We enrolled 50 pregnant women with documented singleton pregnancies between 25–36 weeks' gestation from February through June 2007 who presented to the Labor and Delivery Unit for evaluation of uterine contractions/preterm labor. Women with incompetent cervix or cerclage and reporting minimal vaginal bleeding were not enrolled in the study as were women presenting with ruptured membranes. Dry vaginal swabs were collected at enrollment and immediately stored in a –80°F freezer and shipped to the laboratory at the Fred Hutchinson Cancer Research Center. The samples were subjected to DNA extraction with MoBio Ultra-clean soil DNA extraction method, following the manufacturer's directions. Successful DNA extraction and the absence of PCR inhibitors were documented using a human 18S rDNA quantitative PCR assay. Eight real-time quantitative PCR assays were run which target different bacterial species. This technique to quantify the level of bacterial species is reproducible [5]. These assays employ a TaqMan format in which species specific primers and probes are used to detect the amount of bacterial DNA from each taxonomic group. Known amounts of cloned bacterial 16S rDNA were added to standards in each qPCR in order to generate a standard curve and thus assess the amount of bacterial DNA in vaginal samples. These assays all have a detection threshold of 1–10 16S rDNA molecules per reaction, and a specificity wherein addition of one million copies of non-target vaginal bacterial 16S rDNA from 50 vaginal bacteria results in no detectable amplification. No-template PCR controls and sham digest DNA extraction controls were also run to monitor for bacterial contamination [5].

Eligible women who agreed to participate in the study provided consent for collection of vaginal swabs and review of their medical record following delivery. A brief baseline questionnaire to collect demographic, prior and current reproductive risk factors, and substance use information was also completed. This study was conducted in accordance with the guidelines established by Temple University Institutional Review Board.

Statistical analysis

The analysis is based on 48 women with complete pregnancy outcome information. Demographic and reproductive risk factors of women experiencing a SPTB and women maintaining their pregnancy beyond 37 completed weeks' gestation were compared using Fisher's exact tests for categorical variables and Mann-Whitney *U*-tests for continuous variables. Simple logistic regression models were generated for SPTB using log transformed continuous levels of each BV-associated bacteria. The SPSS statistical package version 14.0 was used. A two-sided *P*-value of ≤ 0.05 was considered statistically significant.

Results

Forty-four percent of women experienced a SPTB during the follow-up period ($n = 21$). The remaining women ($n = 27$) delivered after 37 completed weeks' gestation (56%). As shown in Table 1, no significant differences exist in demographic and/or behavioral risk factors between the two groups and both groups were enrolled at the same mean gestational age (32 weeks). Although not statistically significant, women experiencing a SPTB were more likely to report vaginal bleeding at enrollment (29% vs. 15%), a prior PPRM (10% vs. 4%) and were more likely to have a history of lower genital tract infections compared to controls. One woman was treated for BV and one woman was treated for trichomonads at the time of enrollment; both delivered at term and were included in the control group.

Seventy six percent of cases compared to 67% of controls were *Gardnerella vaginalis* positive at enrollment. We found that Megasphaera phylotype 1 (RR = 2.00, 95% CI: 0.94–4.26), *Gardnerella vaginalis* (RR = 1.21, 95% CI: 0.73–2.01) and *Leptotrichia/Sneathia* (RR = 1.44, 95% CI: 0.75–2.79), measured at 32 weeks gestation, were more prevalent among women with a subsequent SPTB (Table 2). Of note, 47% of women later experiencing a SPTB were positive for Megasphaera phylotype 1 compared to 19% of controls. In addition, the prevalence of BVAB 1 and BVAB 2 were also higher among the case group, though, again, these differences were not statistically significant. *Lactobacillus crispatus*, (RR = 0.78, 95% CI: 0.48–1.29) was less prevalent among the group of women experiencing a SPTB.

Among the group of women positive for the BV-associated bacteria of interest, the median level of *Gardnerella vaginalis* was significantly higher among the SPTB group ($P = 0.007$) and the mean/median level of *Lactobacillus crispatus* was significantly lower ($P = 0.02$) (Table 2). BVAB2, *Leptotrichia amnionii*, and Megasphaera levels were also higher among the cases, although not statistically significant. In a simple logistic regression analysis, high levels of *Gardnerella vaginalis* measured 2–4 weeks before the SPTB were predictive of SPTB (RR = 1.30; 95% CI: 1.07–1.59). In addition, over a tenfold increased risk of SPTB was found when comparing the highest to lowest tertile of log levels of *Gardnerella vaginalis* (RR = 10.5, 95% CI: 1.36–81.05). High log transformed *Lactobacillus crispatus* levels were still protective of SPTB although significance was borderline (RR = 0.78, 95% CI: 0.59–1.01). Continuous or tertile comparisons for the other BV-associated bacteria were not related to SPTB risk. We also found that *Gardnerella vaginalis* levels were negatively correlated with *Lactobacillus crispatus* levels ($P = 0.01$) and positively correlated with BVAB1 ($P = 0.05$) and BVAB2 levels ($P = 0.004$).

Discussion

These findings among women presenting with symptoms of preterm labor suggest that high levels of *Gardnerella vaginalis* and low levels of *Lactobacillus crispatus* are predictive of later SPTB. These results support other studies which suggest that the presence of *Lactobacillus crispatus* may be protective for SPTB [9, 10]. In addition, these data suggest a

closer examination of the role of BVAB2, *Leptotrichia* and *Sneathia* species, and Megasphaera-like species on SPTB risk.

Bacterial vaginosis, diagnosed by Gram stain, has been linked with an increase risk of spontaneous preterm birth in numerous studies [4, 9, 11, 19]. Recent clinical trials examining the effectiveness of BV treatment to reduce the risk of preterm delivery among symptomatic and asymptomatic BV positive women have found no reduction in SPTB risk and perhaps an increase in preterm labor or preterm birth among treated asymptomatic BV positive pregnant women [3, 8, 10, 12]. These null findings may have been driven by the ineffectiveness of metronidazole in treating the BV-associated bacteria most likely contributing to SPTB risk. In this study, we examined seven BV-associated bacteria that have a high overall prevalence among pregnant women and have been linked to overall BV positivity as well as recurrent and hard-to-treat BV. We found the presence and high level of *Gardnerella vaginalis* and low levels of *Lactobacillus crispatus* to be the most important predictors in later SPTB among women presenting with preterm labor.

To date, *Gardnerella vaginalis* has been consistently linked to BV positivity and *Gardnerella vaginalis* is one of the more common pathogens found among women with PPROM [1]. Only a few other studies have linked the presence of *Gardnerella vaginalis* and the lack of *Lactobacillus crispatus* with SPTB among women with preterm labor [17, 20]. Given the public health impact of high rates of infant morbidity and mortality due to SPTB and the lack of information regarding the cause of SPTB, the results from this study may help identify a group of women at highest risk of SPTB based on vaginal flora characteristics during pregnancy. These findings point to the need to understand more fully the role of various BV-associated bacteria in SPTB risk.

These BV-associated bacteria may contribute to SPTB through localized inflammation of the endometrium creating an environment incompatible with proper placental formation and growth and/or by circulating cytokine production resulting in PPROM or preterm labor and subsequent SPTB. It is clear that lower genital tract bacteria can invade the choriodecidual space, the amnion and chorion, the placenta and the amniotic fluid and the bacteria associated with BV have been isolated from both the lower and upper genital tract of pregnant women [6]. Among women with SPTB with intact membranes, one of the most commonly identified uterine bacteria was *Gardnerella vaginalis* and studies have reported a twofold increased risk of SPTB among women found to have an excess of these organisms [6]. Invasion of the choriodecidual space by BV-associated bacteria and the release of endotoxins and exotoxins, activate the production of cytokines and subsequent inflammation [6, 7]. Cytokine, endotoxins, and exotoxins stimulate prostaglandin and metalloproteases synthesis and release. BV positive women have also been found to have a higher level of proinflammatory cytokines which promote chronic endometrial inflammation [15]. Among studies of SPTB, prostaglandins stimulate uterine contractions and metalloproteases attack the chorioamniotic membranes leading to PROM and softening of the collagen in the cervix. Higher levels of the BV-associated bacteria identified in this study may increase the risk for SPTB through increased cytokine production, endometrial inflammation, and improper implantation with subsequent PPROM or preterm labor. Results such as these, that detect and quantify fastidious members of the vaginal bacterial community, may be critical for determining why only a subset of women with BV are at increased risk of SPTB and why antibiotic treatment for BV fails in reducing SPTB risk.

We do recognize the variability in these results based on the small sample size and the limited assessment of confounding influences. In the future, larger studies are needed to confirm these findings. In addition, we understand that women enrolled in the study are at higher risk for SPTB given the study population characteristics (i.e., women presenting with

signs/symptoms of preterm labor). However, identification of the group of women delivering prior to 37 weeks' gestation among this high-risk group experiencing signs/symptoms of premature labor may be most important; although, these data are not able to assess the role of presence or level of BV-associated bacteria on preterm labor. Lastly, vaginal samples were collected around 32 weeks' gestation among this high-risk group of women which may hamper the long-term prospective assessment of SPTB risk. Future studies are needed to assess the vaginal microbiology earlier in pregnancy and examine their contribution to SPTB risk. Thus, future findings may suggest that first trimester screening and appropriate treatment for specific BV-associated bacteria may be successful in prevention of SPTB.

In conclusion, we found that high levels of *Gardnerella vaginalis* and low levels of *Lactobacillus crispatus* are associated with SPTB. Larger studies with more power will be necessary to examine the role of fastidious BV-associated bacteria on adverse pregnancy outcomes including SPTB. Identifying the individual attributable risks for separate BV-associated bacteria might be most useful in developing successful treatments to prevent SPTB among BV positive women.

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Table 1

Demographic/reproductive history by pregnancy outcome.

	SPTB (n = 21)	Term birth (n = 27)	P-value
Demographic information			
Age (mean)	23 years	25 years	0.29
Percent African American	42.9%	55.6%	0.56
Percent Hispanic	63.2%	42.3%	0.23
Percent high school graduate	52.4%	55.6%	1.00
Percent married	9.5%	25.9%	0.26
Reproductive information			
Vaginal bleeding	28.6%	14.8%	0.29
Prior SPTB	9.5%	18.5%	0.45
Prior PPROM	10.0%	4.0%	0.58
Gestational age (weeks)			
At enrollment	32.5 weeks	32.6 weeks	0.83
At delivery	34.3 weeks	38.9 weeks	<0.001
Smoking	19.0%	11.5%	0.68
Infections (ever)			
Bacterial vaginosis	15.0%	11.5%	1.00
Chlamydia	10.0%	7.7%	1.00
Gonorrhea	10.0%	0.0%	0.18
Trichomoniasis	19.0%	15.4%	1.00
Yeast	45.0%	19.0%	0.11

Note: Two women were lost-to-follow-up and not included in this analysis (4%). Fisher's exact test was used for categorical comparisons and Mann-Whitney *U*-test for continuous variables.

Table 2

Prevalence of bacteria and mean concentration of bacteria by pregnancy outcome.

	SPTB (n = 21)	Term birth (n = 27)	RR (95% CI)
Percent positive			
<i>Leptotrichia/Sneathia</i>	38.1%	22.2%	1.44 (0.75–2.79)
Bacterial vaginosis-associated bacterium (BVAB) 1	14.3%	11.1%	1.14 (0.49–2.65)
Bacterial vaginosis-associated bacterium (BVAB) 2	23.8%	18.5%	1.16 (0.59–2.28)
Bacterial vaginosis-associated bacterium (BVAB) 3	4.8%	7.4%	0.83 (0.36–1.93)
Megasphaera phytotype 1	47.6%	18.5%	2.00 (0.94–4.26)
<i>Gardnerella vaginalis</i>	76.2%	66.7%	1.21 (0.73–2.01)
<i>Lactobacillus crispatus</i>	38.1%	51.9%	0.78 (0.48–1.29)
Natural log mean levels among women with a positive sample/standard deviation (16S rDNA copies per swab)			
	SPTB	Term birth	Two-sided P-value (Mann-Whitney U-test)
<i>Leptotrichia/Sneathia</i> (n = 14)	3.62 (std: 2.06)	3.72 (std: 2.43)	0.89
Bacterial vaginosis-associated bacterium (BVAB) 1 (n = 6)	7.27 (std: 5.68)	7.21 (std: 6.88)	0.83
Bacterial vaginosis-associated bacterium (BVAB) 2 (n = 10)	4.04 (std: 2.57)	2.86 (std: 1.98)	0.60
Bacterial vaginosis-associated bacterium (BVAB) 3 (n = 3)	1.96 NA	3.53 (std: 3.42)	1.0
Megasphaera phylotype 1 (n = 15)	9.06 (std: 2.54)	8.99 (std: 5.07)	0.41
<i>Gardnerella vaginalis</i> (n = 34)	11.07 (std: 3.13)	6.79 (std: 4.49)	0.007
<i>Lactobacillus crispatus</i> (n = 22)	6.15 (std: 3.31)	9.47 (std: 3.68)	0.02