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BRIEF REPORT

Vaginal and Extra-Vaginal Bacterial Colonization and Risk for Incident Bacterial Vaginosis in a Population of Women Who Have Sex With Men

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Background. Bacterial vaginosis (BV) is a common cause of vaginal discharge and associated with vaginal acquisition of BV-associated bacteria (BVAB).

Methods. We used quantitative polymerase chain reaction assays to determine whether presence or concentrations of BVAB in the mouth, anus, vagina, or labia before BV predict risk of incident BV in 72 women who have sex with men.

Results. Baseline vaginal and extra-vaginal colonization with *Gardnerella* spp, *Megasphaera* spp, *Sneathia* spp, BVAB-2, *Dialister* sp type 2, and other BVAB was more common among subjects with incident BV.

Conclusions. Prior colonization with BVAB is a consistent risk for BV.

Keywords. anal; bacterial vaginosis; microbiota; oral; sexual behaviors.

Bacterial vaginosis (BV) is a highly prevalent condition associated with increased risk of sexually transmitted infections, preterm birth, pelvic inflammatory disease, and other sequelae [1]. Bacterial vaginosis is characterized by a shift in the vaginal bacterial biota from one composed largely of *Lactobacillus* species to a microbiota with diverse anaerobic and facultative bacteria, here designated BV-associated bacteria (BVAB) [2]. It remains unclear how BVAB are acquired in the vagina and from what source. There is ongoing debate as to whether BV is a sexually transmitted infection with acquisition of BVAB from sexual partners [3–5], reflects vaginal inoculation from endogenous reservoirs in women [6, 7], both, or some other process. A previous study in a cohort of women who have sex with women (WSW) demonstrated that colonization of extra-vaginal reservoirs with certain BVAB was associated with increased risk for incident BV [7]. However, different sexual practices and exposures in women who have sex with men (WSM) could lead to different patterns of vaginal and extra-vaginal bacterial colonization that impact BV risk. We sought to examine the link between vaginal or extra-vaginal bacterial colonization and risk of incident BV in a second cohort focusing on WSM to assess generalizability of these findings. Quantitative polymerase chain reaction (qPCR) methods were used to detect bacterial colonization in the mouth, anus, vagina, and external genitalia (labia) of women who did not have BV at time of initial sampling; women were observed prospectively to assess subsequent incidence of BV and association with colonization status.

METHODS

The study population consisted of 72 women recruited from the community and seen at the University of Washington Infectious Disease Research Clinic or Public Health-King County Sexually Transmitted Diseases Clinic during the period from October 2012 through March 2015 who did not have prevalent BV (visit 1). Inclusion criteria included women ages 18–50, with or without history of BV. All women were seen in clinic 1 month later for assessment of incident (new) BV (visit 2). The analysis included 24 women (cases) who subsequently developed BV at this follow-up visit ~1 month later (visit 2) and 48 women (controls) who did not develop BV in this interval. Of the 24 cases, 13 had no previous diagnosis of BV, and 11 had a previous diagnosis but were negative at the baseline visit. Controls were randomly selected from a group of women who did not have BV (both Amsel's criteria negative and Nugent score <7) at these 2 visits and had samples collected at extra-vaginal sites. Written informed consent was obtained from all participants. The study was approved by the Institutional Review Board at the Fred Hutchinson Cancer Research Center.

Foam swabs were used to collect oral, anal, vaginal, and labial samples from all participants in clinic as previously described [7]. Swabs were stored at −80°C. Material was eluted from swabs by vortex mixing the tip in 500 µL saline. The saline was centrifuged at 18 000 ×*g* for 10 minutes, and the pellet was subjected to deoxyribonucleic acid (DNA) extraction using the MoBio/QIAGEN BiOstic Bacteremia kit (Hilden, Germany). Extracted DNA was subjected to an amplification control qPCR assay to assess for PCR inhibitors [8]. In addition, 15 taxon-specific

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qPCRs targeting the 16S ribosomal ribonucleic acid (rRNA) gene of key vaginal bacteria were performed using a TaqMan format. Bacteria targeted included the following: BVAB-2, *Aerococcus christensenii*, a vaginal *Eggerthella* sp, *Gardnerella* spp, *Porphyromonas uenonis/Porphyromonas asaccharolytica*, *Porphyromonas* sp type 1, *Prevotella amnii, Prevotella buccalis*, vaginal *Megasphaera* spp, vaginal *Sneathia* spp, *Lactobacillus crispatus*, *Lactobacillus jensenii*, *Prevotella timonensis*, *Dialister micraerophilus*, and *Dialister* sp type 2 (93% similar to *Dialister propionicifaciens* and *D micraerophilus*). Assay conditions have been described previously [9, 10].

Diagnosis of BV was made by Gram-stain of vaginal fluid smears on glass slides with Nugent scoring [11] and Amsel clinical criteria [12]. Demographic and behavioral data were obtained in clinic and via home diaries, which included information on frequency and types of sex behaviors.

Statistics

Proportions of women with individual bacteria present at each site were compared using a Wald test for a quasi-Poisson regression model coefficient representing the relative risk (RR) of bacterial colonization between cases and controls (unadjusted or adjusted for age and race); bacterial DNA levels were compared using Wilcoxon rank-sum tests. Fisher's exact test was used for associating colonization with sex behaviors. The principal outcome measure was detection of BV at the 1-month visit (visit 2) by Amsel criteria or Nugent score. The Benjamini-Hochberg method was used for false discovery rate (FDR) correction.

RESULTS

Demographic and behavioral characteristics of the 72 women enrolled in this study are displayed in Supplementary Table 1, noting baseline variables in the 24 women who developed BV during observation (cases) and the 48 women who did not develop BV (controls). Twenty-one cases had BV by Nugent score, 16 of whom were also positive for BV by Amsel clinical criteria. Three cases had BV by Amsel with 4 of 4 criteria present and Nugent scores in the intermediate range. Over half (56.5%) of women with BV were symptomatic with discharge or odor. Women without BV were negative by both Amsel criteria and Nugent score. In the 30 days before visit 1 and 30 days after visit 1 leading to visit 2, 83% of women reported sex with men, 8% reported having a female partner, and 9% did not respond to the question. Overall, 25% of women enrolled were black, and there was a larger percentage of black women among cases (38%) compared with controls (19%).

The RR of BV based on bacterial "detection" of 15 taxa in vaginal and extra-vaginal niches at visit 1 is displayed in Supplementary Table 2 using unadjusted and adjusted (reported below) quasi-Poisson regression. Anal and vaginal colonization with *Gardnerella* (RR = 1.75 anal and 1.77 vaginal) and presence of *Dialister* sp type 2 (RR = 23.3 oral and 2.51 vaginal) were significantly associated with increased risk of subsequent BV after FDR correction. Several additional bacteria were significantly associated with elevated risk of BV when detected in anal swabs, including *Sneathia* spp (RR = 4.34), *Aerococcus* (RR = 1.8), *Eggerthella* (RR = 3.81), and *Porphyromonas* type 1 (RR = 2.39). Additional bacteria were significantly associated with increased risk of BV when detected on labial swabs, including *Sneathia* spp (RR = 8.11), *Megasphaera* spp (RR = 4.55), and *Aerococcus* (RR = 1.83). Seven BVABs detected in the vagina were significantly associated with increased risk of subsequent BV as noted in Supplementary Table 2. None of the vaginal lactobacilli detected were significantly associated with reduced risk of BV.

In Supplementary Table 3, we present data on associations between "concentrations" of bacterial taxa at extra-genital sites at visit 1 and risk of BV (visit 2). These results are generally concordant with the presence and/or absence analysis noted above. Figure 1 summarizes the prevalence of key bacteria in each niche (swab type) in cases and controls before onset of BV. Figure 2 and Supplementary Figure 1 show differences in bacterial concentrations for select taxa between cases and controls for oral, anal, labial, and vaginal swabs. Note the higher concentrations of *Gardnerella* in BV cases compared with controls in oral, anal, labial, and vaginal swabs before onset of BV, with concentrations in the anus that approach 10^5 and labia 10^7 16S rRNA gene copies per swab. *Sneathia* spp were detected at higher concentrations in anal, labial, and vaginal swabs among cases compared with controls, whereas *L crispatus* concentrations were higher in labial and anal swabs from controls compared with BV cases.

A study participant was deemed to engage in a particular sexual behavior if reported either at study entry using a questionnaire or in the interval between visits 1 and 2 using a daily diary. Seventy-six percent of women engaged in oral sex, 27% engaged in anal sex, and 81% engaged in vaginal sex. Report of anal sex was not associated with increased anal colonization with any BV-associated bacteria measured when combining bacterial data for visit 1 and 2. Rather, anal sex was associated with decreased anal colonization with *Sneathia* spp, *Dialister* type 2, and *Eggerthella* (Supplementary Table 5). Likewise, report of oral sex was not associated with oral colonization of women with vaginal bacteria. Women reported receiving oral sex on 7% of daily diary entries and giving oral sex to partner on 9% of daily diary entries between visits 1 and 2.

Although all study participants did not have BV at first visit, some cases and controls had a history of BV. We sought to determine whether history of BV was associated with vaginal and extra-vaginal colonization status at visit 1 (Supplementary Table 6). These data show that history of BV was not consistently associated with vaginal or extra-vaginal colonization with most bacteria assayed here, except that women with a history of BV were

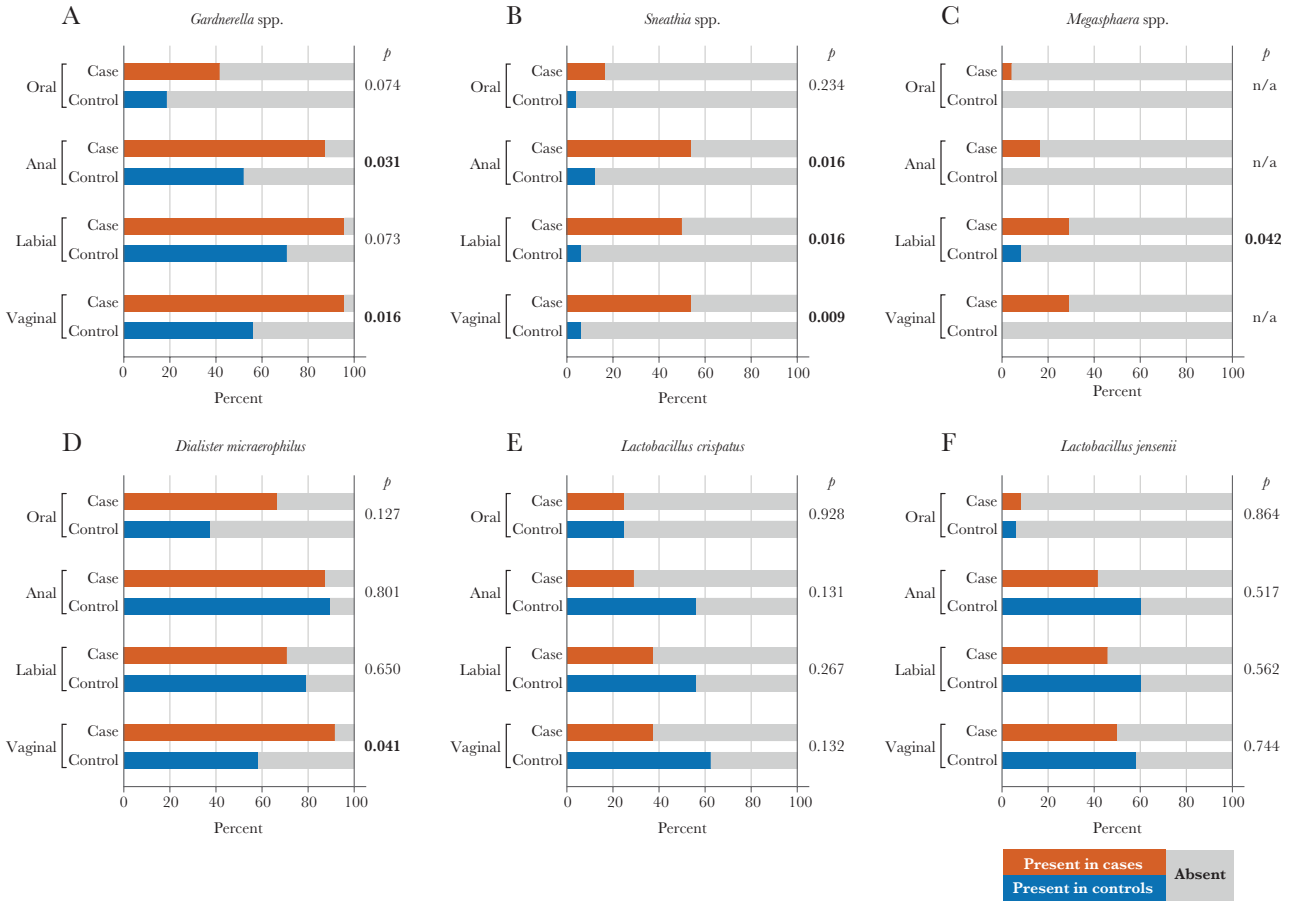

Figure 1. Prevalence of key vaginal bacteria at body sites (oral, anal, labial, vaginal) in cases and controls at visit 1 (which is before onset of bacterial vaginosis in cases). *Gardnerella* species and *Dialister micraerophilus* are commonly found on the labia and in the anus of cases and controls. *Sneathia* spp and *Megasphaera* spp are more commonly detected in labial and anal swabs from cases compared with controls. *P* values are based on age- and race-adjusted quasi-Poisson regression analysis.

significantly more likely to have *Gardnerella* colonization of the mouth, anus, labia, and vagina.

DISCUSSION

This study provides evidence that women who develop BV are more likely to have previous colonization of vaginal and extra-vaginal sites with key BVAB such as the following: *Gardnerella* (anus, vagina); *Sneathia* spp (anus, labia, vagina); BVAB-2 (vagina); *Eggerthella* sp (anus, vagina); *Dialister* sp type 2 (mouth, vagina); *A christensenii* (labia, vagina); and *Porphyromonas* type 1 (anus). Higher concentrations of *Megasphaera* species were found in the anus and on the labia of women who developed BV, whereas higher anal and labial concentration of *L crispatus* was linked to reduced risk of BV. This study of WSM is concordant with a previous study associating extra-vaginal BVAB colonization with risk of BV in WSW [7]. This consistent association highlights the potential to identify women who are at higher risk of developing BV based on presence of bacteria in the vagina and extra-vaginal sites. For example, the adjusted RR for BV when *Dialister* sp type 2 was detected in the mouth was 23.3. This study was not designed to determine whether extra-vaginal bacterial colonization precedes vaginal colonization and BV, but, if this is true, a potential pathway to reduce risk of BV may be eradicating extra-vaginal colonization. Likewise, given the high rate of recurrence in BV [13], eradication of extra-vaginal reservoirs could be explored to reduce recurrence. If extra-vaginal bacterial colonization is important in BV recurrence, one might predict that oral metronidazole could help eradicate BVAB from extra-vaginal reservoirs resulting in lower recurrence, whereas intra-vaginal metronidazole gel would not. No published studies have examined the effect of antibiotic therapy on colonization of extra-vaginal sites with BVAB. Few studies have directly compared oral and intravaginal metronidazole, and the focus has not been BV recurrence [14, 15].

We expected that anal sex would be associated with increased anal colonization with vaginal bacteria, such as might occur after vaginal sex followed by anal sex. We were surprised to find that sexual behaviors were not useful in predicting colonization of extra-vaginal reservoirs except that anal sex was associated with "reduced" colonization with *Sneathia* spp, *Dialister* type 2, and *Eggerthella* species. Why anal colonization with these bacteria would be reduced in women having anal sex is not clear.

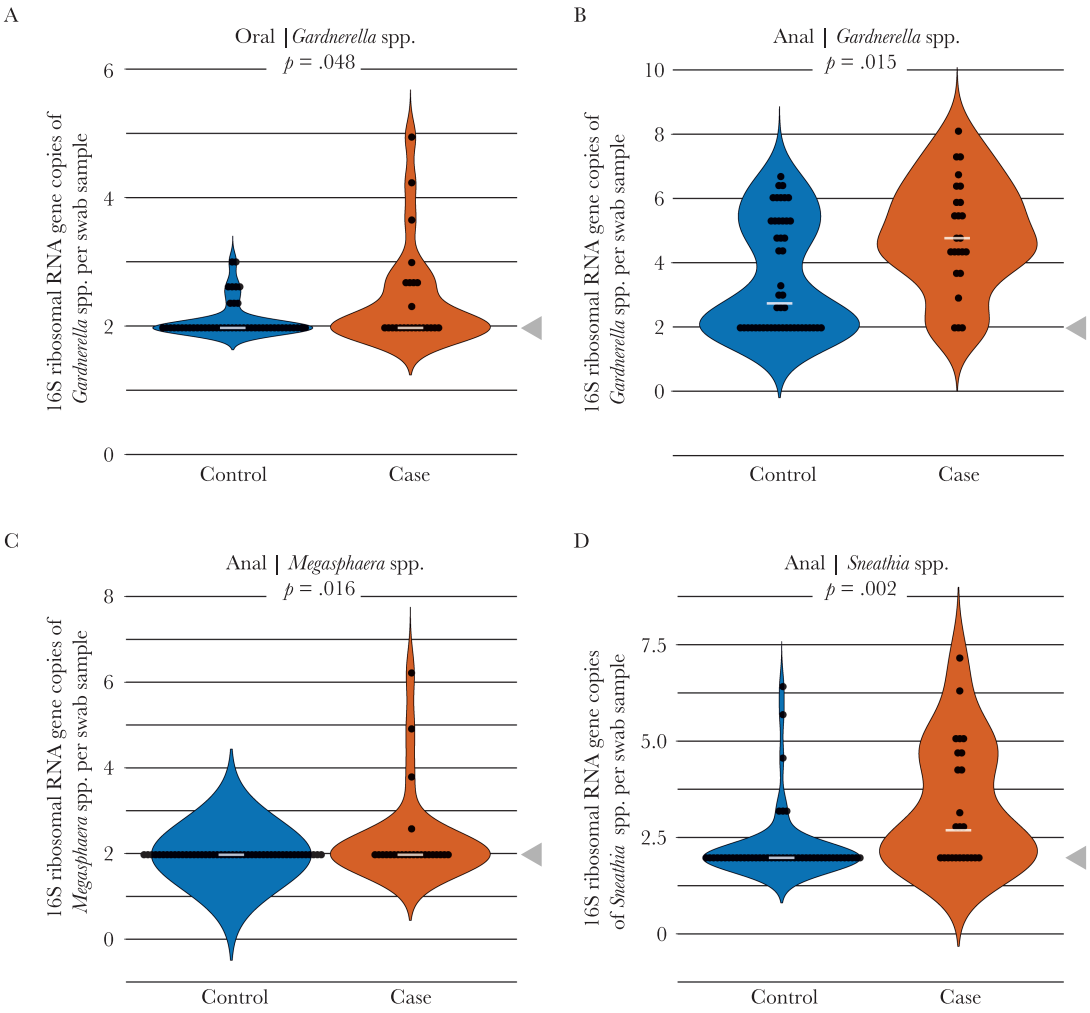

Figure 2. Violin pots depict bacterial deoxyribonucleic acid concentrations from swabs collected from 3 body sites expressed as 16S ribosomal ribonucleic acid (RNA) gene copies per swab in cases of incident bacterial vaginosis (BV) and controls. Each dot represents the concentration in a single participant. Threshold concentrations (arrowhead) and median copies (bar) are indicated in each plot. Oral (A) and anal (B) concentrations of *Gardnerella* spp were higher in cases compared with controls before onset of BV. Anal concentrations of *Megasphaera* spp (C) and *Sneathia* spp (D) were also higher in cases compared with controls. *P* values are based on Wilcoxon rank-sum test.

This study establishes an association between BVAB colonization at vaginal and extra-vaginal sites and risk of BV, but it does not inform on how these bacteria are inoculated to establish colonization at these sites.

Strengths of this study include (1) its longitudinal design to assess BV risk and (2) use of highly sensitive qPCR assays to measure presence and concentrations of vaginal bacteria at vaginal and extra-vaginal sites. Limitations of this study include our inability to conclude how extra-vaginal sites became colonized, the finite set of bacterial taxa assayed based on available qPCR assays, and the limited period of observation.

CONCLUSIONS

Vaginal and extra-vaginal bacterial colonization in women is a consistent risk factor for developing BV in both WSM and WSW. Understanding how BVAB traffic to these extra-vaginal sites, and from these reservoirs to the vagina and back, may provide opportunities for preventing BV and reducing recurrence after treatment.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases online*. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Supplementary Figure 1. Violin plots depict bacterial DNA concentrations from swabs collected at various body sites expressed as 16S rRNA gene copies per swab in cases of incident BV and controls. Each dot represents the concentration in a single participant. Threshold concentrations (arrowhead) and median copies (bar) are indicated in each plot. Concentrations of *L crispatus* on anal swabs were higher in controls than cases of BV, and similarly *L crispatus*

concentrations on labial swabs were higher in controls than cases. In contrast, labial concentrations of *Gardnerella* spp and *Sneathia* spp were higher among BV cases than controls before onset of BV. Cases had higher vaginal concentrations of several bacteria before onset of BV, including BVAB-2, *Megasphaera, Gardnerella*, and *Sneathia* species. *P* values are based on Wilcoxon rank-sum test.

Supplementary Figure 2. Heatmap showing presence and/or absence of bacterial species detected by qPCR at each body site in each study participant, grouped by cases and controls.

Supplementary Table 1. Demographic and behavioral characteristics of the study population, including 24 women who developed BV and 48 controls who did not.

Supplementary Table 2. Association of incident BV with vaginal and extra-vaginal bacterial detection by PCR. Reported values are the relative risk (*P* value) of colonization comparing cases to controls, based on quasi-Poisson regression adjusted for age and race.

Supplementary Table 3. Concentrations of bacteria in vaginal and extra-vaginal sites and risk of subsequent BV as assessed by taxon-specific qPCR. Values that are significant after false discovery rate (FDR) adjustment are in bold.

Supplementary Table 4. Associations between detection of bacteria in extra-vaginal sites of women and report of sexual behaviors.

Supplementary Table 5. Association of past episodes of BV with vaginal and extra-vaginal bacterial concentration. Reported values are the relative risk (*P* value) of colonization comparing individuals with and without a past history of BV, based on quasi-Poisson regression adjusted for age and race. Significant values are in bold.

Notes

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Potential conflicts of interest. D. N. F. and T. L. F. report intellectual property around diagnosis of bacterial vaginosis. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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