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Relationship of Specific Vaginal Bacteria and Bacterial Vaginosis Treatment Failure in Women Who Have Sex with Women: A Cohort Study

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Abstract

Background—Bacterial vaginosis frequently persists after treatment. The role of newly defined bacterial vaginosis-associated bacteria (BVAB), with specificity $\geq 97\%$ for this condition, has not been assessed.

Objective—Define risks for bacterial vaginosis persistence, including pre-treatment detection of specific vaginal bacteria, among women reporting sex with other women.

Design—Observational cohort study.

Setting—University-based research clinic.

Patients—335 women 16–29 years-old reporting sex with ≥ 1 woman in the prior year recruited through advertisements and provider referral.

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Reproducible Research Statements

Protocol: available to interested readers by contacting Dr. Marrazzo at jmm2@u.washington.edu

Statistical Code: available to interested readers by contacting Ms. Thomas at kkt@u.washington.edu

Data: not available

Intervention—Bacterial vaginosis was treated with intravaginal metronidazole gel (0.75%), 37.5 mg nightly for five nights.

Measurements—Species-specific 16S rDNA polymerase chain reaction (PCR) assays targeting 17 bacteria were applied to vaginal fluid obtained at baseline. Test of cure by clinical criteria and Gram stain analysis and repeat PCR assays of vaginal fluid were performed one month post-treatment, and interim behaviors assessed using computer-assisted self-interview.

Results—Of 335 women, 24% of whom also reported sex with men within 3 months before enrollment, 131 (39%) had bacterial vaginosis. In 120 (92%) with follow-up, incidence of persistent bacterial vaginosis was 26%, and significantly higher in women with baseline detection of *Clostridia*-like bacteria designated BVAB1 (risk ratio (95% C.I.) 2.0 (1.1–4.0), BVAB2 (risk ratio (95% C.I.) 8.7 (2.5–∞), or BVAB3 (risk ratio (95% C.I.) 3.1 (1.7–5.8)), or of *Peptoniphilus lacrimalis* (risk ratio (95% C.I.) 3.5 (1.6–15.5)) or *Megasphaera* phylotype 2 (risk ratio (95% C.I.) 3.4 (1.4–5.5)), and lower with treatment adherence (risk ratio (95% C.I.) 0.4 (0.2–0.9)). Detection of these bacteria at test-of-cure was associated with persistence, while post-treatment sexual activity was not.

Limitations—Findings may not be generalizable to women who have sex only with men, or to women whose bacterial vaginosis is treated with oral antibiotic. The study may be too small and involve too selected a population to draw definitive conclusions about associations of persistent infection with post-treatment sexual behaviors.

Conclusions—Persistent bacterial vaginosis is associated with several *Clostridiales* order bacteria and *Peptoniphilus lacrimalis*, suggesting that vaginal microbiology at diagnosis may define risk for antibiotic failure.

Keywords

bacterial vaginosis; vaginitis; *Lactobacillus*; sexually transmitted diseases; *Clostridiales*; women's health; antimicrobial resistance

INTRODUCTION

Bacterial vaginosis is characterized by depletion of hydrogen peroxide-producing lactobacilli that characterize normal vaginal flora, with profound overgrowth of anaerobic bacteria.(1) Bacterial vaginosis is the most prevalent vaginal infection in reproductive age women, affecting 8% to 29%, and most common etiology of vaginal symptoms prompting medical care.(2) Of 3,739 women enrolled during 2001–2004 in a nationally representative sample of the U.S. civilian non-institutionalized population, almost one in three (29.2%; 95% C.I. 27.2–31.3) had bacterial vaginosis by Gram stain of vaginal fluid.(3,4) Bacterial vaginosis has been consistently associated with adverse outcomes related to the upper genital tract, and with increased risk of HIV acquisition.(5–7)

Treatment of bacterial vaginosis targets the abundance of anaerobes that define this condition. With oral metronidazole used for seven or vaginal metronidazole for five days, symptoms improve in 83%–87% of women by two to three weeks.(8,9) Improvement is similar for women who use vaginal clindamycin regimens; both antibiotics are recommended,(10) and restoration of vaginal lactobacilli at 30 days is similar.(11,12) While short-term treatment response is acceptable, bacterial vaginosis persists or recurs in 11%–29% of women at one month, (8,13, 14) and long-term recurrence rates exceed 70%.(15–17) Few studies have reported factors associated with bacterial vaginosis recurrence after successful treatment, and these have included Black race, older age, higher Nugent score at enrollment,(17) prior bacterial vaginosis, regular sex partner during study, female sex partner, and hormonal contraception. (15) Even fewer studies have assessed risks associated with bacterial vaginosis persistence at one month post-treatment. Available data suggest that condom use in the period immediately following

treatment might support maintenance of normal vaginal flora and thus enhance cure rates. (16,18)

For unknown reasons, women who have sex with women have a high bacterial vaginosis prevalence (25%–52%).(3,19) We hypothesized that this population might provide a unique opportunity to study the response to treatment of bacterial vaginosis because the potentially confounding exposure of unprotected vaginal intercourse was not likely to be common. In a cohort study of vaginal flora in this population, we assessed incidence of bacterial vaginosis persisting at one month after treatment with vaginal metronidazole. In addition to measuring contribution of recognized risk factors for bacterial vaginosis, including race, sexual behaviors, and douching, we assessed the contribution of specific species of bacterial vaginosis-associated bacteria (BVAB) present at the initiation of BV treatment. These bacteria include fastidious anaerobes detected by species-specific polymerase chain reaction (PCR), some of which have not yet been cultivated and include three recently identified bacteria in the *Clostridiales* order (BVAB1, BVAB2, and BVAB3) that are highly specific (>97%) for bacterial vaginosis.(20)

METHODS

Subjects and Clinical Definitions

The study population was composed of women aged 16 to 30 years who reported sex with at least one other woman in the previous year who responded to recruitment through advertisements, media, and community referral between October 2004 and December 2006. Subjects completed an extensive computer-assisted self-interview on demographics and medical, reproductive and sexual history and underwent standardized examination including collection of vaginal fluid for Gram stain, saline microscopy, pH measurement, potassium hydroxide evaluation, and culture of *Trichomonas vaginalis*. All were asked to return for four quarterly visits or at any time if genital symptoms developed. To obtain specimens for bacterium-specific PCR assays, a polyurethane foam swab (Catch-All, Epicentre Biotechnologies, Madison, WI) was brushed against the lateral vaginal wall and re-sheathed and frozen immediately in a –80° freezer until DNA extraction. Bacterial vaginosis was diagnosed if three of four clinical (Amsel) criteria (vaginal pH >4.5, clue cells on saline microscopy >20% of epithelial cells, amine odor on addition of potassium hydroxide, and homogeneous vaginal discharge) were present(21) and Gram stain of vaginal fluid confirmed abnormal flora (Nugent score >3).(4) Women with bacterial vaginosis were treated with vaginal metronidazole gel (37.5 mg nightly for 5 days) and asked to return in one month for test-of-cure, at which time all women had repeat examination and collection of vaginal fluid for Gram staining. Initially, vaginal fluid samples for bacteria-specific polymerase chain reaction assays were collected at test-of-cure visit for all women whose vaginal pH was greater than 4.0 and thus suspicious for bacterial vaginosis or trichomoniasis; after approximately half the study subjects were enrolled, these samples were collected routinely on all subjects at this visit.

For the analysis, the first visit at which a woman was found to have bacterial vaginosis (whether at the initial enrollment visit, at a later quarterly routine visit, or at a visit self-initiated for vaginal symptoms) was used. Test-of-cure visits following a woman's first bacterial vaginosis positive visit and completed before March 31, 2007 were used to examine incidence of persistent bacterial vaginosis and abnormal vaginal flora. Persistent bacterial vaginosis was defined by Amsel criteria and confirmed by Nugent score of vaginal fluid >6 at the one-month follow up visit, and abnormal vaginal flora by Nugent score >3. Written informed consent was obtained from all subjects. Conduct of the study adhered to standard guidelines for research involving human subjects, and was approved by the University of Washington and Fred Hutchinson Cancer Center Human Subjects Review Committees.

Microbiology

For DNA extraction, vaginal swabs for bacterial PCR were placed in 15 mL conical vials with 2 mL of saline and vortex mixed for 1 minute to dislodge cells. The solution was centrifuged at 14,000 rpm for 10 minutes, and the pellet resuspended in 100µl supernatant. DNA was extracted from the pellet using the Ultra Clean Soil DNA Kit (MoBio, Carlsbad, CA) according to manufacturer's instructions. DNA was eluted from silica columns in a volume of 150 µL buffer. Sham digests using a swab without human contact were performed with each round of DNA extraction (every 10–25 samples) to control for contamination that may arise from kit reagents or collection swabs.

Bacterium specific PCR assays were developed based on detection of species-specific regions of the 16S rRNA gene. 16S rDNA sequences from vaginal bacteria detected by broad range 16S rDNA PCR were aligned.(22) Primers were designed to target highly variable regions of the bacterial 16S rRNA gene that appear unique for each species. PCR assays were developed for 17 bacterial species that were commonly detected in vaginal samples.(23) Each 50µl PCR reaction contained 1X PCR Buffer II, 2mM magnesium chloride, 0.8 mM deoxyribonucleotide triphosphate mix, 1 unit AmpliTaq Gold DNA polymerase (all from Applied Biosystems, Foster City, CA), 0.2µM each of forward and reverse primer, and 1 µL of template DNA. PCR conditions included a pre-melt at 95°C for 10 minutes; then 40–45 cycles of 95°C for 30 seconds (melt), 53–62°C for 30 seconds (annealing), and 72°C for 30 seconds (extension); followed by a final extension at 72°C for 7 minutes. PCR products were visualized after electrophoresis in 2% agarose gels and staining with ethidium bromide. Bacterial PCR assays were optimized so that each assay was capable of detecting ≤100 molecules of cloned 16S rDNA per reaction, though most assays could detect 1–10 molecules. Every PCR with a visible band of the expected size on gel electrophoresis was sequenced (BigDye version 3, Applied Biosystems) to confirm that the PCR product had at least 99% similarity with the expected bacterial target, thereby assuring bacterial specificity. PCR reactions without visible bands on gel electrophoresis or without confirmed sequence homology to the expected target were considered negative. No-template PCR controls (consisting of master mix, primers, and water) and sham digest controls (template consisting of water subjected to DNA extraction) were run with each PCR assay to monitor for contamination.

Each subject's extracted DNA was subjected to a human β-globin PCR to assure that amplifiable DNA was successfully extracted from the sample and to monitor for PCR inhibitors.(24) The β-globin PCR protocol used is the same as listed for bacterial PCR, with the exception that the following primers were employed: GH20-5'-GAAGAGCCAAGGACAGGTAC-3' and PCO4-5'-CAACTTCATCCACGTTACC-3'. In addition, an internal amplification control quantitative PCR assay was performed using DNA from each vaginal sample in order to detect more subtle PCR inhibitors by monitoring the amplification of an exogenously added template (jellyfish aequorin gene target) at known concentration (25).

Subjects were tested at enrollment for *C. trachomatis* and *N. gonorrhoeae* using the APTIMA-COMBO 2 assay (Gen-Probe, San Diego, CA) on urine, and at follow-up visits if they reported interim risk behavior (new sex partner, >1 partner) or genitourinary symptoms.

Statistical analysis

Bivariate associations between subjects' characteristics and detection of bacterial vaginosis at the one-month post-treatment visit were measured by calculating risk ratios and 95% confidence intervals were produced using bootstrap percentiles.(26) These characteristics included demographics, sexual and genital hygiene behaviors in the interim period between diagnosis and assessment at one-month, and PCR detection of individual bacteria in vaginal

fluid at the baseline visit. We estimated adjusted risk ratios using Poisson regression, which provides unbiased estimates of the log relative risks and bootstrap confidence intervals.(27) Adjustment was performed for other statistically significant covariates and for adherence to treatment. We performed these analyses among all women for whom follow-up visits were available regardless of time to follow-up, then on a limited “per protocol” group (follow up between 21 and 44 days post-treatment). To examine whether bacteria found to be associated with persistence when present at the baseline visit were also preferentially present in vaginal fluid of women with persistent bacterial vaginosis, we applied bacterium-specific polymerase chain reaction assays to vaginal fluid obtained at the follow-up visits for which these specimens were available. Because the population with samples available for this analysis was enriched with BV positive women, odds ratios are presented in lieu of risk ratios. All statistical tests for statistical significance were two-sided and a level of $P < 0.05$ was considered statistically significant. Analyses were performed using R 2.4.1 and Stata 9.2 (College Station TX, USA). No funding agency had any role in the design or conduct of the study or in the collection, management, or statistical analysis of the data or in the submission of the manuscript for publication.

RESULTS

The characteristics of 335 women enrolled in the yearlong prospective study are summarized in Table 1. Two women (0.6%) had *C. trachomatis*. None had *N. gonorrhoeae*, trichomoniasis, or clinically evident genital herpes. As depicted in Figure 1, 96 women (28.7%) had bacterial vaginosis at enrollment, and an additional 35 women had bacterial vaginosis at either routine quarterly follow-up visit ($N=28$) or self-initiated visit for vaginal symptoms ($N=7$). Of these 131, 120 returned for follow-up (adherence to follow up for all subjects, 92%). Median time to follow-up was 34 days (range 21–78 days); 75% returned within 36 days. Of the 120 women for whom we report test-of-cure findings, 119 returned for scheduled visits, and one returned for a self-initiated symptom visit at 42 days post-treatment. The per protocol group, who returned between 21 and 44 days, included 108 women. Beta-globin was amplified from every vaginal sample, confirming contact of swabs with a human surface. There was no evidence of PCR inhibition in any sample using the internal amplification control PCR assay.

At one-month follow-up, persistent bacterial vaginosis defined by Amsel criteria occurred in 31 (25.8%) subjects; all were confirmed by Nugent’s score. An additional four women had bacterial vaginosis by Nugent’s score and were not included as persistent bacterial vaginosis. Abnormal flora (Nugent score >3) was observed in 44 subjects (36.7%). Table 2 displays relationships between subjects’ characteristics and detection of either persistent bacterial vaginosis or abnormal flora. For bacteria detected by PCR at initial diagnosis, *Atopobium* species *G. vaginalis*, *Megasphaera* phylotype 1 and *Lactobacillus iners* were found in most subjects ($>97\%$). Thus, we could not examine whether rates of persistence differed by presence or absence of these bacteria. However, women with each of BVAB1, BVAB2, or BVAB3 at baseline had significantly higher rates of persistence relative to those without that bacterium (15 of 38 (39%) with BVAB1 vs. 16 of 82 (19%) without; 30 of 93 (32%) with BVAB2 vs. 1 of 26 (3%) without, and 18 of 38 (47%) with BVAB3 vs. 13 of 82 (16%) without). Those with *Peptoniphilus lacrimalis* were also more likely to have persistent bacterial vaginosis (26 of 72 (36%) with vs. 5 of 48 (10%) without) as were those with *Megasphaera* phylotype 2 (4 of 5 (80%) with vs. 27 of 115 (23%) without, while those who adhered to metronidazole therapy were less likely. Of the 110 women who indicated whether they had completed treatment with vaginal metronidazole therapy, all but eight (92%) completed the full course of treatment. Twenty-five (25%) of those completing treatment had persistent bacterial vaginosis versus five (63%) of eight who did not. Women with *Mobiluncus curtisii* had an increased incidence of persistent bacterial vaginosis, as others have reported,(26) but this did not reach statistical

significance. These findings did not differ when women in the per-protocol group were analyzed separately

Characteristics that significantly predicted persistent bacterial vaginosis at one month also predicted detection of abnormal flora; also, Black race approached statistical significance. Only five women seen at follow-up were Black; two had persistent bacterial vaginosis, but four had abnormal flora. Most women (78%) who did not complete metronidazole therapy had abnormal flora, versus 32% of those who did. Although most subjects reported sexual activity in the month following bacterial vaginosis diagnosis, no specific activity was related to persistence, including condom or vaginal lubricant use, sharing sex toys, or genital to genital contact (data not shown; see Table 2 for other variables).

Although the number of subjects was too small to perform extensive multivariable analysis, we assessed associations between detection of specific bacteria at baseline adjusted for non-adherence to treatment (Table 3). Detection of either BVAB3 or *P. lacrimalis* at baseline remained statistically significantly associated with likelihood of bacterial vaginosis persistence.

Vaginal fluid samples were available from the test-of-cure visit for 78 of the 120 women who returned. Other than having a higher likelihood of bacterial vaginosis, women for whom post-treatment vaginal fluid was collected did not differ from those for whom it was not in demographics (age, race) or post-treatment sexual behavior (data not shown). As shown in Table 4, risk of persistent bacterial vaginosis was higher among women who were positive at 30 days for any of the bacteria associated with persistence, whether analyzed regardless of detection of these bacteria at baseline (Group 1) or limited to women who had these bacteria at baseline (Group 2), except for *Megasphaera* phylotype 2, where numbers were too small to examine the relationship.

DISCUSSION

Among women seen one month after treatment of bacterial vaginosis with vaginal metronidazole, predictors of treatment failure included detection of bacterial vaginosis associated bacteria (BVAB) at baseline, including the Clostridia-like bacteria BVAB1, BVAB2 and BVAB3, *Peptoniphilus lacrimalis* and *Megasphaera* phylotype 2, as well as failure to adhere to five days of vaginal metronidazole. Importantly, report of no specific sexual practices with either male or female partners in the month after treatment predicted either persistent bacterial vaginosis or abnormal flora. The latter finding is notable, as others have reported that unprotected vaginal intercourse is associated with recurrent bacterial vaginosis. However, as we note below, our ability to detect such associations was limited by the relatively small number of outcomes we observed and the selected population we studied. Importantly, the bacteria we found to be associated with persistence were detectable at the time that treatment failure was diagnosed, and were detected rarely in women whose bacterial vaginosis was cured.

Our findings have several possible explanations. First, bacterial vaginosis is a heterogeneous syndrome characterized by diverse microflora, some of which might harbor complete or relative resistance to antibiotics commonly used for treatment. Cultivable bacteria associated with bacterial vaginosis, particularly *Mobiluncus*, can be resistant to either metronidazole or clindamycin.(28–30) Nyirjesy assessed bacterial vaginosis-associated morphotypes on Gram stain at test-of-cure visits in women treated with either vaginal metronidazole or clindamycin. (31) Women treated with metronidazole were less likely to clear *Mobiluncus* morphotypes than those treated with clindamycin (95.5% vs. 66.7%, respectively; $P = 0.047$), and those with *Mobiluncus* morphotypes at baseline were more likely to experience cure when treated with clindamycin than with metronidazole; in our study, women with *M. curtisii* at baseline were

also more likely to have persistent BV, though this association did not reach statistical significance. Data from our study indicates that detection of all three novel *Clostridiales* Order bacterial vaginosis-associated bacteria (BVAB1-3) by specific PCR is positively associated with detection of *Mobiluncus* morphotypes on Gram stain, especially BVAB1.(32) *Mobiluncus* species and BVAB1 have curved rod morphologies when visualized using fluorescence in situ hybridization;(20) conceivably, BVAB1 may be confused with *Mobiluncus* on Gram stain, although the Gram stain characteristics of BVAB1 are currently unknown. Other data support that the choice of antibiotic for bacterial vaginosis can have measurable effects on the population of cultivatable vaginal flora. In one study, women with bacterial vaginosis who were treated with clindamycin (but not metronidazole) had high frequencies (80%) of clindamycin-resistant anaerobic bacteria persisting for 90 days after treatment.(33) *Atopobium vaginae* has also been associated with bacterial vaginosis, and many isolates are metronidazole resistant *in vitro* (34) and may predict treatment failure.(35,36) Molecular characterization of the genus *Peptostreptococcus* has resulted in the subdivision of this genus into several different genera, including the *Peptoniphilus* genus consisting of non-saccharolytic butyrate-producing bacteria such as *Peptoniphilus lacrimalis*;(37,38) these bacteria may be more susceptible to clindamycin than to metronidazole.(39,40) Finally, persistence of a tenacious biofilm that hinders access of antibiotic to bacteria associated with bacterial vaginosis may further increase women's risk of treatment failure.(41)

In our subjects, bacterial vaginosis was not associated with risk factors that others have identified, including failure to use condoms with male partners.(15–18) This may partly be explained by a low incidence of sexual contact with men among our subjects in the month after treatment. However, given the high prevalence of bacterial vaginosis among lesbians, the frequency of concordant bacterial vaginosis in female sex partners, and the observation that report of sex with another woman was associated with increased risk of recurrence in one prospective study,(15) we were surprised to find no association between persistent bacterial vaginosis and sexual behaviors with female partners in the month after treatment. This could be explained by small numbers of women in these subgroups, or by an overriding role of baseline vaginal flora associated with bacterial vaginosis promoting persistence in this group—rather than persistent “reinfection” or exposure to another causative factor through sex(15–18). One hypothesis is that some *Clostridia*-like bacteria associated with bacterial vaginosis may produce spores that result in rapid vaginal recolonization after antibiotic therapy, analogous to the process underlying relapsing *Clostridium difficile* colitis.

Our study has limitations. First, our subjects were selected on the basis of reporting sex with other women. Although 24% also reported sex with men in the three months prior to enrollment, they are unlikely to be representative of exclusively heterosexual women, a group that should be studied in similar fashion and to whom our findings may not necessarily apply. However, the fact that our subjects infrequently reported vaginal intercourse with male partners in the month after treatment might actually be advantageous in helping to define the role of vaginal microbiology in determining response to treatment. Second, we have not yet performed these analyses with data derived from quantitative vaginal cultures obtained in our subjects; this analysis is underway. Third, our findings may not apply to women whose bacterial vaginosis is treated with an oral antibiotic regimen.

Our findings raise several important areas for future research. First, comparative assessments of risks for persistent bacterial vaginosis, including molecular characterization of baseline microbiology of BVAB, need to be performed in heterosexual women; these efforts should help to define whether the molecular epidemiology of bacterial vaginosis differs from that of lesbians, and whether unprotected vaginal intercourse with male partners promotes persistence. Second, our BVAB-specific assays were qualitative. Quantitative polymerase chain reaction assays applied to vaginal fluid samples obtained in our subjects should offer additional insight

into the natural history of this condition. For example, if key BV-associated bacteria are resistant to antibiotic therapy, then vaginal levels of these bacteria may never decline, resulting in persistent BV. Alternatively, vaginal levels of these BV-associated bacteria may decline with antibiotic therapy but eradication may not be achieved, setting the stage for future relapse, a condition that could also conceivably arise from re-inoculation with these bacteria. Because prolonged therapy with twice weekly suppressive vaginal metronidazole significantly reduced recurrence rates relative to placebo over four months in one study,⁽¹⁷⁾ more intensive antibiotic therapy might offer one approach to overcome the problem of persistence. Finally, the establishment of consensus definitions for persistence and recurrence of bacterial vaginosis would greatly assist in progress towards describing the natural history of this condition. Whether risk factors that promote persistence are the same as those that promote recurrence is not clear.

In summary, our findings suggest that vaginal colonization with key BVABs at diagnosis is an independent risk factor for persistent bacterial vaginosis after standard antibiotic therapy. Because bacterial vaginosis may confer an increased risk of poor pregnancy outcome and HIV acquisition and predict upper genital tract disease, defining reasons for treatment failure and, conversely, effective treatment should be a priority. If vaginal microbiology at diagnosis predicts persistent bacterial vaginosis, then tailored treatment approaches based on initial vaginal microflora are one possible strategy for optimizing treatment outcomes. Our findings support the need for more rigorous study of bacterial vaginosis treatment that should pair alternative antibiotic strategies with subsequent PCR-based monitoring of bacterial populations in the vagina. Further study is also needed to cultivate the bacteria we found to be associated with treatment failure in order to measure complete or partial antibiotic resistance as one possible explanation for persistent bacterial vaginosis.

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References

1. Hillier, SL.; Marrazzo, JM.; Holmes, KK. Bacterial vaginosis. In: Holmes, KK.; Sparling, PF.; Mardh, P-A., et al., editors. Sexually Transmitted Diseases. 4th ed. New York: McGraw-Hill; 2008. (in press)
2. Sobel J. Current Concepts: Vaginitis. *N Engl J Med* 1997;337:1896–1903. [PubMed: 9407158]
3. Koumans EH, Sternberg M, Bruce C, et al. The Prevalence of Bacterial Vaginosis in the United States, 2001–2004; Associations With Symptoms, Sexual Behaviors, and Reproductive Health. *Sex Transm Dis.* 2007
4. Nugent RP, Krohn MA, Hillier SL. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. *J Clin Microbiol* 1991;29(2):297–301. [PubMed: 1706728]
5. Leitich H, Bodner-Adler B, Brunbauer M, Kaider A, Egarter C, Husslein P. Bacterial vaginosis as a risk factor for preterm delivery: A meta-analysis. *Am J Obstet Gynecol* 2003;189(1):139–147. [PubMed: 12861153]
6. Myer L, Denny L, Telerant R, Souza M, Wright TC Jr, Kuhn L. Bacterial vaginosis and susceptibility to HIV infection in South African women: a nested case-control study. *J Infect Dis* 2005;192(8):1372–1380. [PubMed: 16170754]
7. Sweet RL. Gynecologic conditions and bacterial vaginosis: implications for the non-pregnant patient. *Infect Dis Obstet Gynecol* 2000;8(3–4):184–190. [PubMed: 10968604]

8. Hanson JM, McGregor JA, Hillier SL, et al. Metronidazole for bacterial vaginosis. A comparison of vaginal gel vs. oral therapy. *J Reprod Med* 2000;45(11):889–896. [PubMed: 11127100]
9. Livengood CH 3rd, Soper DE, Sheehan KL, et al. Comparison of once-daily and twice-daily dosing of 0.75% metronidazole gel in the treatment of bacterial vaginosis. *Sex Transm Dis* 1999;26(3):137–142. [PubMed: 10100770]
10. Centers for Disease Control and Prevention. Sexually transmitted disease treatment guidelines, 2006. *MMWR* 2006;51(RR11)
11. Agnew KJ, Hillier SL. The effect of treatment regimens for vaginitis and cervicitis on vaginal colonization by lactobacilli. *Sex Transm Dis* 1995;22(5):269–273. [PubMed: 7502179]
12. Nyirjesy P, McIntosh MJ, Gattermeir DJ, Schumacher RJ, Steinmetz JI, Joffrion JL. The effects of intravaginal clindamycin and metronidazole therapy on vaginal lactobacilli in patients with bacterial vaginosis. *Am J Obstet Gynecol* 2006;194(5):1277–1282. [PubMed: 16647910]
13. Hillier SL, Lipinski C, Briselden AM, Eschenbach DA. Efficacy of intravaginal 0.75% metronidazole gel for the treatment of bacterial vaginosis. *Obstet Gynecol* 1993;81(6):963–967. [PubMed: 8497364]
14. Livengood CH 3rd, McGregor JA, Soper DE, Newton E, Thomason JL. Bacterial vaginosis: efficacy and safety of intravaginal metronidazole treatment. *Am J Obstet Gynecol* 1994;170(3):759–764. [PubMed: 8141197]
15. Bradshaw CS, Morton AN, Hocking J, et al. High recurrence rates of bacterial vaginosis over the course of 12 months after oral metronidazole therapy and factors associated with recurrence. *J Infect Dis* 2006;193(11):1478–1486. [PubMed: 16652274]
16. Sanchez S, Garcia P, Thomas KK, Catlin M, Holmes KK. Intravaginal metronidazole gel versus metronidazole plus nystatin ovules for bacterial vaginosis: a randomized controlled trial. *Am J Obstet Gynecol* 2004;191:1898–1906. [PubMed: 15592270]
17. Sobel JD, Ferris D, Schwebke J, et al. Suppressive antibacterial therapy with 0.75% metronidazole vaginal gel to prevent recurrent bacterial vaginosis. *Am J Obstet Gynecol* 2006;194(5):1283–1289. [PubMed: 16647911]
18. Schwebke JR, Desmond R. Risk factors for bacterial vaginosis in women at high risk for sexually transmitted diseases. *Sex Transm Dis* 2005;32(11):654–658. [PubMed: 16254538]
19. Marrazzo JM, Koutsky LA, Eschenbach DA, Agnew K, Stine K, Hillier SL. Characterization of vaginal flora and bacterial vaginosis in women who have sex with women. *J Infect Dis* 2002;185(9):1307–1313. [PubMed: 12001048]
20. Fredricks DN, Fiedler TL, Marrazzo JM. Molecular identification of bacteria associated with bacterial vaginosis. *N Engl J Med* 2005;353(18):1899–1911. [PubMed: 16267321]
21. Amsel R, Totten PA, Spiegel CA, Chen KC, Eschenbach D, Holmes KK. Nonspecific vaginitis. Diagnostic criteria and microbial and epidemiologic associations. *Am J Med* 1983;74(1):14–22. [PubMed: 6600371]
22. Fredricks DN, Marrazzo JM. Molecular methodology in determining vaginal flora in health and disease: its time has come. *Curr Infect Dis Rep* 2005;7(6):463–470. [PubMed: 16225785]
23. Fredricks DN, Fiedler TL, Thomas KK, Oakley BB, Marrazzo JM. Targeted PCR for detection of vaginal bacteria associated with bacterial vaginosis. *J Clin Microbiol* 2007;45(10):3270–3276. [PubMed: 17687006]
24. Fredricks DN, Relman DA. Paraffin removal from tissue sections for digestion and PCR analysis. *Biotechniques* 1999;26(2):198–200. [PubMed: 10023524]
25. Limaye AP, Jerome KR, Kuhr CS, et al. Quantitation of BK virus load in serum for the diagnosis of BK virus-associated nephropathy in renal transplant recipients. *J Infect Dis* 2001;183(11):1669–1672. [PubMed: 11343217]
26. Efron B, Tibshirani RJ. *An Introduction to the Bootstrap*. 1986
27. Liang K-Y, Zeger SL. Longitudinal data analysis using generalized linear models. *Biometrika* 1986;73:13–22.
28. Skarin A, Holst E, Mardh PA. Antimicrobial susceptibility of comma-shaped bacteria isolated from the vagina. *Scand J Infect Dis Suppl* 1983;40:81–84. [PubMed: 6582590]
29. Spiegel CA, Eschenbach DA, Amsel R, Holmes KK. Curved anaerobic bacteria in bacterial (nonspecific) vaginosis and their response to antimicrobial therapy. *J Infect Dis* 1983;148(5):817–822. [PubMed: 6631073]

30. Tohill BC, Heilig CM, Klein RS, et al. Vaginal flora morphotypic profiles and assessment of bacterial vaginosis in women at risk for HIV infection. *Infect Dis Obstet Gynecol* 2004;12(3–4):121–126. [PubMed: 15763911]
31. Nyirjesy P, McIntosh MJ, Steinmetz JI, Schumacher RJ, Joffrion JL. The effects of intravaginal clindamycin and metronidazole therapy on vaginal mobiluncus morphotypes in patients with bacterial vaginosis. *Sex Transm Dis* 2007;34(4):197–202. [PubMed: 17179772]
32. Thomas, KK.; Fredricks, DN.; Agnew, KJ.; Fiedler, TL.; Marrazzo, JM. Associations between bacterial morphotypes seen on Gram stain and infection with fastidious bacteria associated with bacterial vaginosis; 17th Meeting of the International Society of Sexually Transmitted Disease Research; Seattle, WA. 2007.
33. Beigi RH, Austin MN, Meyn LA, Krohn MA, Hillier SL. Antimicrobial resistance associated with the treatment of bacterial vaginosis. *Am J Obstet Gynecol* 2004;191(4):1124–1129. [PubMed: 15507930]
34. De Backer E, Verhelst R, Verstraelen H, et al. Antibiotic susceptibility of *Atopobium vaginae*. *BMC Infect Dis* 2006;6:51. [PubMed: 16542416]
35. Bradshaw CS, Tabrizi SN, Fairley CK, Morton AN, Rudland E, Garland SM. The association of *Atopobium vaginae* and *Gardnerella vaginalis* with bacterial vaginosis and recurrence after oral metronidazole therapy. *J Infect Dis* 2006;194(6):828–836. [PubMed: 16941351]
36. Ferris MJ, Masztal A, Aldridge KE, Fortenberry JD, Fidel PL Jr, Martin DH. Association of *Atopobium vaginae*, a recently described metronidazole resistant anaerobe, with bacterial vaginosis. *BMC Infect Dis* 2004;4(1):5. [PubMed: 15018635]
37. Ezaki T, Kawamura Y, Li N, Li ZY, Zhao L, Shu S. Proposal of the genera *Anaerococcus* gen. nov., *Peptoniphilus* gen. nov. and *Gallicola* gen. nov. for members of the genus *Peptostreptococcus*. *Int J Syst Evol Microbiol* 2001;51(Pt 4):1521–1528. [PubMed: 11491354]
38. Song Y, Liu C, McTeague M, Vu A, Liu JY, Finegold SM. Rapid identification of Gram-positive anaerobic coccal species originally classified in the genus *Peptostreptococcus* by multiplex PCR assays using genus- and species-specific primers. *Microbiology* 2003;149(Pt 7):1719–1727. [PubMed: 12855723]
39. Freeman CD, Klutman NE, Lamp KC. Metronidazole. A therapeutic review and update. *Drugs* 1997;54(5):679–708. [PubMed: 9360057]
40. Ng LK, Dillon JA. Plasmid analysis and antimicrobial susceptibilities of *Peptostreptococcus* species. *FEMS Microbiol Lett* 1989;52(1–2):47–51. [PubMed: 2599360]
41. Swidsinski A, Mendling W, Loening-Baucke V, et al. An adherent *Gardnerella vaginalis* biofilm persists on the vaginal epithelium after standard therapy with oral metronidazole. *Am J Obstet Gynecol*. 2007

Figure 1. Derivation of the study population

* Vaginal fluid is not available on all 120 women because post-treatment vaginal fluid collection was not instituted until approximately 1/3 of the way through the study. Initially, vaginal fluid was selectively collected for women with suspected BV at follow-up due to limited resources. Collection of vaginal fluid at the follow-up visit was later broadened to all women. Other than having bacterial vaginosis, women for whom post-treatment vaginal fluid was collected did not differ from those for whom it was not in demographics (age, race) or post-treatment sexual behavior with male or female partners.

Table 1

Characteristics of 335 subjects, according to the presence of bacterial vaginosis diagnosed at any visit

	BV* present N = 131	BV absent N = 204
Age – years		
Median	25	25
Range	15–35	17–34
Race (self-defined) – no. (%) [†]		
White	98 (75)	156 (76)
Black	8 (6)	8 (4)
Nonwhite, other than black	20 (15)	36 (18)
Declined to provide race data	5 (4)	4 (2)
Sex with women, prior 3 months – no. (%)	92 (83)	164 (80)
Female sex partners, prior 90 days		
Median no.	1	1
Range	0–7	0–4
Sex with men, prior 3 months – no. (%)	21 (19)	453 (26)
Male sex partners, prior 90 days [‡]		
Median no.	0	0
Range	0–5	0–4
Consistent condom use – no (%)	5 (28)	14 (31)
Current cigarette smoking	48 (42)	57 (33)
Douching, past month	6 (5)	8 (4)
Hormonal contraception use, past 60 days	10 (8)	25 (12)
Vaginal symptoms [§] present – no (%)	42 (34)	39 (20)
Concurrent genitourinary infection present – no (%)		
Vulvovaginal candidiasis	3 (2)	15 (8)
Trichomoniasis	0 (0)	0 (0)
Chlamydia trachomatis	2 (2)	1 (1)

* BV = bacterial vaginosis; defined by presence of Amsel criteria and confirmed by Nugent score >6

[†] Subjects were permitted to choose more than one category to describe their race. “White” refers to those who chose only ‘White’ to describe their race; “Black” refers to those who chose “Black or African American” even if they also chose another race, and “Nonwhite” refers to those who chose any other race besides white.

[‡] Among women who reported vaginal intercourse with a male partner in the prior three months.

[§] Defined as change in amount, color, or odor of vaginal discharge.

Table 2

Associations between detection of persistent bacterial vaginosis or abnormal vaginal flora* and subjects' characteristics (N (% by bacterial vaginosis or flora status))

Characteristic (Prevalence in all subjects)	Persistent BV [†] N=31	BV cure N=89	Risk Ratio [‡] (95% C.I.)	Abnormal flora N=44	Normal flora N=76	Risk Ratio [§] (95% C.I.)
Demographics & symptoms						
Age > 25 y (48%)	25 (48)	42 (48)	1.0 (0.5–1.8)	21 (48)	36 (48)	1.0 (0.6–1.6)
Black race (4%)	2 (6)	3 (3)	1.6 (0.0–4.2)	4 (9)	1 (1)	2.3 (1.0–3.7)
Vaginal symptoms [¶] (34%)	9 (31)	29 (35)	0.9 (0.6–2.5)	14 (34)	24 (33)	1.0 (0.6–1.7)
Results of bacteria-specific PCR assays performed at baseline visit^{**}						
BVAB1 (32%)	15 (48)	23 (26)	2.0 (1.1–4.0)	20 (45)	18 (24)	1.8 (1.1–2.9)
BVAB2 (78%)	30 (97)	63 (71)	8.7 (2.5–∞)	40 (91)	53 (70)	2.9 (1.4–13.2)
BVAB3 (31%)	18 (58)	19 (21)	3.1 (1.7–5.8)	22 (50)	15 (20)	2.2 (1.4–3.8)
<i>Peptoniphilus</i> sp (80%)	27 (87)	69 (78)	1.7 (0.8–8.3)	38 (86)	58 (76)	1.6 (0.8–4.7)
<i>P. lacrimialis</i> (60%)	26 (84)	46 (52)	3.5 (1.6–15.5)	33 (75)	39 (51)	2.0 (1.2–4.0)
<i>Mobiluncus curtisii</i> (49%)	20 (65)	39 (44)	1.9 (1.0–3.7)	26 (59)	33 (43)	1.5 (0.9–2.4)
<i>Megasphaera</i> -type 2 (4%)	4 (13)	1 (2)	3.4 (1.4–5.5)	4 (9)	1 (1)	2.3 (1.0–3.6)
<i>Atopobium</i> sp (96%)	13 (100)	59 (95)	--	23 (96)	49 (96)	1.0 (0.3–∞)
<i>Megasphaera</i> -type 1 (96%)	13 (100)	59 (95)	--	22 (92)	50 (98)	0.5 (0.2–∞)
<i>Leptotrichia</i> sp (79%)	10 (77)	49 (79)	0.9 (0.3–∞)	19 (79)	40 (78)	1.0 (0.5–3.8)
BVAB-TM7 (24%)	4 (31)	14 (23)	1.4 (0.3–4.0)	5 (21)	13 (25)	0.8 (0.2–1.8)
<i>Eggerthella</i> sp (89%)	12 (92)	55 (89)	1.4 (0.4–∞)	22 (92)	45 (88)	1.3 (0.5–∞)
<i>Lactobacillus iners</i> (97%)	13 (100)	60 (97)	--	23 (96)	50 (98)	∞ (0.2–∞)
<i>Lactobacillus crispatus</i> (16%)	2 (15)	10 (17)	1.0 (0.0–3.2)	4 (19)	8 (16)	1.1 (0.2–2.2)
<i>Gardnerella vaginalis</i> (96%)	13 (100)	59 (95)	--	24 (100)	48 (94)	--
<i>Mobiluncus mulieris</i> (15%)	4 (31)	7 (11)	2.6 (0.4–3.9)	6 (25)	5 (10)	1.9 (0.8–3.0)
<i>Prevotella G1</i> (94%)	11 (85)	47 (76)	1.6 (0.5–∞)	17 (71)	41 (80)	0.7 (0.4–1.9)
BVAB 1 or BVAB3 (47%)	22 (71)	34 (38)	2.8 (1.5–6.6)	28 (64)	28 (37)	2.0 (1.2–3.9)
BVAB1, 2, or 3 (82%)	31 (100)	67 (75)	--	41 (93)	57 (78)	3.1 (1.3–∞)
Interim behaviors reported, enrollment to one-month follow-up visits						
Adherence to treatment (93%)	25 (89)	77 (96)	0.4 (0.2–0.9)	33 (83)	69 (99)	0.4 (0.3–0.6)
Sex partner with BV ^{††} (17%)	5 (16)	15 (17)	0.9 (0.2–2.0)	7 (16)	13 (17)	0.9 (0.4–1.8)
Any sexual activity (78%)	26 (84)	68 (76)	1.4 (0.7–5.2)	38 (86)	56 (74)	1.8 (0.9–5.3)
Any sex with female (68%)	22 (71)	60 (67)	1.1 (0.6–3.1)	31 (70)	51 (67)	1.1 (0.7–2.1)
Vaginal intercourse with male (16%)	6 (19)	13 (15)	1.3 (0.4–2.5)	8 (18)	11 (14)	1.2 (0.5–2.0)
Receptive oral sex ^{‡‡} (56%)	18 (58)	49 (55)	1.1 (0.6–2.2)	28 (64)	39 (51)	1.4 (0.9–2.5)
Receptive anal sex ^{§§} (16%)	3 (10)	16 (18)	0.6 (0.0–1.4)	6 (14)	13 (17)	0.8 (0.3–1.5)

* Abnormal vaginal flora defined by Nugent score >3

[†] BV = bacterial vaginosis; defined by presence of Amsel criteria and confirmed by Nugent score >6

[‡] Bootstrap resampling used to estimate the 95% confidence interval

[¶] Defined as change in vaginal discharge, including increased or malodorous vaginal discharge

^{**} BVAB = bacterial vaginosis-associated bacteria; N=120 for BVAB1, BVAB2, BVAB3, *Peptoniphilus* sp, *Mobiluncus curtisii*, *P. lacrimialis*, *Megasphaera* type 2 (24 with persistent BV, 35 with abnormal flora); N=75 for *Atopobium* sp, *Megasphaera*-type 1, *Leptotrichia* sp, *Prevotella* sp, *Lactobacillus iners*, and *Lactobacillus crispatus*, *G. vaginalis*, *M. mulieris*, *Prevotella G1* (13 with persistent BV, 22 with abnormal flora).

^{††} Assessed for three most recent female sex partners

[#] Receptive oral sex includes sex performed by either male or female sex partners

^{§§} Receptive anal sex includes any receptive anal sexual behavior (anal intercourse, oral-anal sex, digital-anal sex)

Table 3

Multivariable analysis of factors associated with persistence of bacterial vaginosis, adjusted for non-adherence to treatment course (N = 113 subjects)

BV-Associated Bacteria Detected at Baseline[*]	Risk Ratio (95% C.I.)[†]	Expected Risk of BV Persistence among Adherent Participants (95% C.I.)^{††}
BVAB3	2.6 (1.4–5.45)	0.20 (0.04–0.44)
<i>P. lacrimalis</i>	2.8 (1.2–13.3)	0.22 (0.10–0.36)
Neither BVAB nor <i>P. lacrimalis</i>	Referent	0.08 (0.02–0.15)

^{*} BV= bacterial vaginosis; BVAB= bacterial vaginosis-associated bacteria

[†] Risk ratios and 95% confidence intervals obtained using Poisson regression with bootstrap confidence intervals.

^{††} Expected risks of BV persistence and 95% confidence intervals obtained using Poisson regression with bootstrap confidence intervals. In these 113 women, 34% had neither BVAB3 nor *P. lacrimalis*, 8% had only BVAB3, 37% had only *P. lacrimalis*, and 24% had both bacteria detected at baseline. Adherent participants with both BVAB3 and *P. lacrimalis* detected have an expected risk of BV persistence of 0.58 (95% C.I., 0.37–0.80).

Table 4

Detection of bacterial vaginosis-associated bacteria in vaginal fluid 30 days post-treatment by bacterium-specific polymerase chain reaction assay, stratified by detection of persistent bacterial vaginosis vs. cure

Bacterium-specific PCR result, 30 days	Group 1: All Available Observations (N=59)				Group 2: Limited to subjects with BVAB-specific PCR assay at baseline (pre-treatment) *			
	BV Status at 30 days post-treatment †		BVAB-specific PCR assay at baseline (pre-treatment) ‡		BV Status at 30 days post-treatment †		BVAB-specific PCR assay at baseline (pre-treatment) ‡	
	Persistence n (%) N=31	Cure n (%) N=47	Odds Ratio (95% CI) †	Odds Ratio (95% CI) §	Persistence n (%) N=31	Cure n (%) N=47	Odds Ratio (95% CI) †	Odds Ratio (95% CI) §
BVAB1	Yes 13 (46)	Yes 5 (9)	6.1 (1.7–24.5)	6.1 (1.7–24.5)	12 (83)	4 (30)	8.0 (1.1–66.5)	
	No 18 (54)	No 42 (91)			3 (17)	8 (70)		
BVAB2	Yes 26 (88)	Yes 6 (11)	35.5 (8.6–2159)	35.5 (8.6–2159)	26 (91)	5 (16)	33.8 (7.0–182)	
	No 5 (12)	No 41 (89)			4 (9)	26 (94)		
BVAB3	Yes 14 (42)	Yes 1 (3)	37.9 (4.8–164)	37.9 (4.8–164)	14 (77)	1 (11)	35.0 (2.9–1622)	
	No 17 (58)	No 46 (96)			4 (23)	11 (89)		
<i>P. lacrimalis</i>	Yes 23 (79)	Yes 7 (17)	16.4 (4.7–60.3)	16.4 (4.7–60.3)	22 (86)	3 (14)	38.5 (6.4–271)	
	No 8 (21)	No 40 (83)			4 (14)	21 (82)		
<i>Megasphaera</i>	Yes	Yes			3 (75)	0 (0)		
type-2//	No	No			1 (25)	1 (100)		

* PCR = polymerase chain reaction; BVAB = bacterial vaginosis-associated bacteria

† BV = bacterial vaginosis. Persistent BV defined by Amsel's criteria and confirmed by Gram stain at 30 day visit.

‡ Odds ratio (OR) represents odds of persistent BV in women PCR-positive for bacteria indicated at the 30-day post-treatment visit relative to odds in women not positive for same; confidence intervals (CI) are exact.

§ Denominator varies because not all women were positive by PCR for all of these BVAB at baseline visit.

// PCR assay for *Megasphaera* phylotype 2 was not performed on all samples at the post-treatment visit due to the small numbers of women positive for this bacterium at baseline (N=5).