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Comparisons of vaginal flora patterns among sexual behaviour groups of women: implications for the pathogenesis of bacterial vaginosis

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Abstract

Background: Bacterial vaginosis (BV) is the most common cause of vaginal discharge. The objective was to compare the prevalence of BV by Nugent score among African-American women who have sex with women (WSW) and women who have sex with women and men (WSWM) compared with an age-matched group of women who have sex with men (WSM). Secondary objectives were to correlate low versus high Nugent scores with vaginal symptoms among women with BV and to correlate BV diagnosis with sexual practices.

Methods: A secondary analysis of clinical and laboratory data from African-American WSW ($n = 73$) and WSWM ($n = 68$) participating in the Women's Sexual Health Project (August 2011–October 2013) and a 3 : 1 age-matched group of African-American WSM participating in the Longitudinal Study of Vaginal Flora (August 1999–February 2002) at a sexually transmissible infection clinic ($n = 423$) was performed.

Results: Compared with WSM, WSW and WSWM were significantly more likely to have BV based on the Nugent score (OR 2.63; 95% CI 1.55–4.48; $P < 0.01$ and OR 3.67; 95% CI 2.17–6.21; $P < 0.01$ respectively). WSW and WSWM with BV were not significantly more likely to have higher Nugent scores than WSM with BV. Among women with BV reporting symptoms, there was no significant difference in the proportion of women with low-positive versus high-positive Nugent scores. Women who participated in receptive vaginal sex (penile or digital) within

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Conflicts of interest

The authors declare no conflicts of interest.

the 30 days preceding study enrolment were significantly more likely to have BV (OR 2.50; 95% CI 1.57–3.63; $P < 0.01$).

Conclusions: WSW and WSWM were significantly more likely to have BV than WSM. Further analysis of sexual practices among sexual behaviour groups of women is needed to determine their potential impact on BV rates.

Introduction

Bacterial vaginosis (BV) is the most common cause of vaginal discharge in women.¹ It is associated with multiple adverse outcomes, including pelvic inflammatory disease,^{2,3} preterm birth⁴ and increased risk for acquisition and transmission of sexually transmissible infections (STIs), including HIV.^{2,3} BV is characterised by a change in the vaginal flora, with a decrease in H₂O₂-producing-lactobacilli^{5,6} and an increase in facultative (*Gardnerella vaginalis*) and strict anaerobic bacteria (BV-associated bacteria; BVAB).^{2,7,8} Despite over 50 years of research, the precise aetiology of BV remains unknown. It is well-accepted that BV represents a synergistic relationship between a large number of microorganisms, including *G. vaginalis* and other BVAB. However, the trigger that initiates these alterations is debated. It is unknown whether BV results from acquisition of *G. vaginalis* (present in vaginal fluid in 95% of women with clinically diagnosed BV)^{8,9} as the ‘founder’ organism, which subsequently leads to the complex changes in the vaginal microbiota associated with BV¹⁰ or if BV is transmitted as a polymicrobial consortium of microorganisms.¹¹ It is also unknown if BV is caused by behavioural factors, such as douching, which may influence the vaginal microbiota.^{6,12} BV is very common among women who have sex with women (WSW), likely more so than in women who have sex with men (WSM).^{13,14} Additionally, African-American WSW and WSWM in the southern United States are at particularly high risk for STIs.^{15,16} These women are also considered to be in ‘triple jeopardy’, in which their sex, race and sexual orientation act as stressors, resulting in poor health outcomes.¹⁷

In clinical settings, at least three of four Amsel criteria are necessary for a diagnosis of BV: homogenous, thin, grayish-white vaginal discharge; vaginal pH >4.5, positive whiff-amine test; and clue cells present on a wet mount of vaginal fluid.¹⁸ In research settings, BV is defined by the Nugent score.¹⁹ Scores of 0–3 are graded as lactobacillus-predominate normal vaginal flora, 4–6 as intermediate flora with the emergence of *G. vaginalis*/*Bacteroides* morphotypes and 7–10 as BV flora with disappearance of lactobacillus species, numerous *G. vaginalis*/*Bacteroides* and curved Gram-variable anaerobic rods. The appearance of curved Gram-variable anaerobic rods (*Mobiluncus* spp. or possibly BVAB1) results in Nugent scores of 9–10.^{20,21} Nugent scoring has not routinely been used to compare BV prevalence between sexual behaviour groups of women [WSW; women who have sex with women and men (WSWM); and WSM]. In addition, there are little data on correlations of BV Nugent score with vaginal symptoms among women.^{22,23}

The primary objective of this study was to compare the prevalence of BV by Nugent score among African-American WSW and WSWM to an age-matched group of African-American WSM. We hypothesised that there would be a significant difference in the number of women with BV between sexual behaviour groups, with WSW having the highest number of cases.

We also explored the prevalence of low^{7,8} versus high^{9,10} BV Nugent scores among the sexual behaviour groups. We hypothesised that WSW and WSWM with BV would be more likely to have higher Nugent scores^{9,10} than WSM with BV due to more efficient transfer of strict anaerobes in infected vaginal secretions compared with penile–vaginal transmission in WSM. A secondary objective of the study was to correlate self-reported vaginal symptoms (i.e. vaginal discharge, odour, etc.) with sexual practices (i.e. penile–vaginal sex, receptive oral sex, etc.) among women with BV in these groups. We hypothesised that self-reported vaginal symptoms would be more likely to be present in women with BV with high Nugent scores,^{9,10} compared with women with low BV Nugent scores.^{7,8} This would be due to the presence of amine-producing anaerobic bacteria, such as *Mobiluncus* spp., which would contribute to the vaginal symptoms (to have a Nugent score of 9–10, *Mobiluncus* spp. must be seen on the vaginal Gram stain). In addition, we hypothesised that specific sexual practices such as receptive oral and/or anal sex would be correlated with a higher likelihood of BV.²⁴

Methods

We conducted a secondary analysis of WSW, WSWM and WSM participating in either of two studies at the Jefferson County Department of Health (JCDH) Sexually Transmitted Disease (STD) clinic in Birmingham, AL, USA. Both parent protocols and this secondary analysis were approved by the University of Alabama at Birmingham Institutional Review Board and by the JCDH. The Longitudinal Study of Vaginal Flora enrolled 3620 women presenting to the JCDH STD clinic from August 1999 to February 2002. Of these, 2773 (76.6%) were African-American women who were aged 19–45 years, who only reported male sexual partners in their lifetime (WSM). The Women's Sexual Health Project enrolled 163 African-American women presenting to the JCDH STD clinic from August 2011 to October 2013. Of these, 141 were aged 19–45 years, of whom 73 reported only female sexual partners (WSW) during the past 12 months, and 68 reported both female and male sexual partners during the same timeframe (WSWM). We performed a 3 : 1 age-matched random selection of the 2773 eligible participants from the enrolment visit in the Longitudinal Study of Vaginal Flora ($n = 423$) to the 141 eligible participants in the Women's Sexual Health Project for a total of 564 participants in this secondary analysis.

All women in both studies underwent a standardised pelvic exam and had vaginal swabs collected at their enrolment visit. Vaginal specimens were obtained for pH, a wet prep for evaluating for clue cells, yeast and *Trichomonas vaginalis*, a potassium hydroxide 'whiff test,' a culture for *T. vaginalis* (InPouch; Biomed Diagnostics, White City, OR, USA) and a Gram stain for Nugent score determination. Cervical specimens were obtained for *Chlamydia trachomatis* testing [by ligase chain reaction (Abbott Laboratories, Abbott Park, IL, USA) in the Longitudinal Study of Vaginal Flora and by transcription-mediated amplification (Aptima Combo 2 assay; Gen-Probe, Inc., San Diego, CA, USA; in the Women's Sexual Health project) and *Neisseria gonorrhoeae* testing (determined by culture in the Longitudinal Study of Vaginal Flora and the Gen-Probe Aptima Combo 2 assay in the Women's Sexual Health Project). Per convention, a Nugent score of 0–3 was considered consistent with lactobacillus-predominant normal vaginal flora, 4–6 with intermediate flora and 7–10 with BV.¹⁹ Inter-rater agreement of Nugent scoring between the two studies was

assessed using a weighted Cohen's kappa coefficient statistic of 30 randomly selected slides. We found a coefficient of kappa = 0.79 (95% CI = 0.74–0.84). All women enrolled in either study completed an interviewer-administered questionnaire that included demographic information, self-reported vaginal symptoms at time of presentation (i.e. vaginal discharge, vaginal odour and/or genital itching) and detailed information about sexual practices [i.e. receptive vaginal sex (penile or digital), receptive oral sex and receptive anal sex (penile or digital) during the 30 days preceding study enrolment].

Statistical analysis

Statistical analysis was performed using SAS software v9.2 (SAS Institute, Cary, NC, USA). Descriptive statistics were performed using Fisher's exact test. Frequencies and column percentages were reported for the total sample and for each sexual behaviour group (WSW, WSWM, WSM). Column percentages were used due to unequal sample sizes of the groups. For the primary study objective, unadjusted analyses were conducted to compare the prevalence of BV by Nugent score between sexual behaviour groups and reported as unadjusted odds ratios (ORs) with 95% Wald confidence intervals (CIs). For the secondary study objective, unadjusted analyses were conducted to compare self-reported vaginal symptoms and sexual practices among women with BV. For both study objectives, logistic regression was used to evaluate potential confounders. The final models for all analyses were created using backward stepwise elimination (performed manually with a significance level to stay of $P = 0.20$) and reported as adjusted odds ratios with 95% Wald CIs. Missing values were excluded from the analyses. A P -value of <0.05 was considered statistically significant.

Results

Data analysis was restricted to African-American women aged 19–45 years in both parent studies ($n = 564$). Table 1 presents select demographics of this sample stratified by sexual behaviour group (WSW, WSWM, WSM). WSM were significantly more likely than WSW or WSWM to be employed full- or part-time ($P < 0.01$), while WSW were significantly more likely to have never been married ($P < 0.01$). WSWM were significantly more likely than WSW or WSM to have 11 lifetime sexual partners ($P < 0.01$) and have a current diagnosis of trichomoniasis ($P < 0.01$), chlamydia ($P < 0.01$) and gonorrhoea ($P = 0.03$). Although not statistically significant, there was a trend for WSW to be more likely to have a high school or General Equivalency Diploma (GED) education ($P = 0.06$), and for WSWM to be more likely to have a current diagnosis of vaginal candidiasis ($P = 0.07$).

Table 2 presents the prevalence of BV (based on Nugent score) by sexual behaviour group. In the unadjusted analysis, the odds of having a BV diagnosis were significantly higher for WSW than WSM (OR 3.04; 95% CI 1.82–5.07). Similarly, the odds of having a BV diagnosis were significantly higher for WSWM than for WSM (OR 2.52; 95% CI 1.49–4.24). Based on the results in Table 1, employment, education, marital status and number of lifetime sexual partners were included as covariates in logistic regression analysis as was a current diagnosis of trichomoniasis, gonorrhoea, chlamydia and vaginal candidiasis. After backward stepwise elimination, the final adjusted model included only marital status as a

covariate. In this adjusted model, the odds of having a BV diagnosis were significantly higher for WSW than for WSM (OR 2.63; 95% CI 1.55–4.48). Similarly, the odds of having a BV diagnosis remained significantly higher for WSWM than for WSM (OR 3.67; 95% CI 2.17–6.21).

Table 3 shows the results of a subgroup analysis of BV-positive women based on Nugent score ($n = 203$). The prevalence of low BV-positive Nugent scores^{7,8} and high BV-positive Nugent scores^{9,10} stratified by sexual behaviour group, were compared. There was no significant difference in prevalence of the scores between groups on either unadjusted or adjusted analysis.

Table 4 shows self-reported vaginal symptoms among women with BV, stratified by low-positive^{7,8} versus high-positive^{9,10} BV Nugent scores. The majority of women in both groups reported no vaginal symptoms. In both unadjusted and adjusted analysis, there was no significant association of vaginal odour, vaginal discharge or genital itching with low^{7,8} versus high^{9,10} BV Nugent scores. We were unable to evaluate a category for ‘any symptom reported’ due to a lack of consistency between the symptoms evaluated in the questionnaires used in both studies.

Table 5 shows sexual practices within the 30 days preceding enrolment, stratified by BV diagnosis. On unadjusted analysis, there was a significant association between BV diagnosis and participation in receptive vaginal sex (penile or digital) (OR 2.71; 95% CI 1.87–3.92) and receptive oral sex (OR 2.80; 95% CI 0.10–22.29). Employment, education, marital status, number of lifetime sexual partners, sexual risk behaviour group and current diagnosis of trichomoniasis, gonorrhoea, chlamydia, and vaginal candidiasis were included as covariates in logistic regression analysis. After backward stepwise elimination, the final adjusted model controlled for a current diagnosis of trichomoniasis and sexual behaviour group. In this adjusted model, diagnosis of BV remained significantly associated with receptive vaginal sex during the 30 days preceding enrolment (OR 2.40; 95% CI 1.57–3.63); however, the association with receptive oral sex was no longer significant. Receptive anal sex (penile or digital) was not significantly associated with a diagnosis of BV in either unadjusted or adjusted analysis.

Of all analyses conducted, only data on self-reported sexual practices had missing values, which were excluded from the analysis. Missing values affected less than 3% of all observations.

Discussion

In this retrospective study, African-American WSW and WSWM were significantly more likely than African-American WSM to have a diagnosis of BV based on Nugent score. This is consistent with findings from several previous studies.^{13,14} Among a representative sample of USA reproductive aged women, Koumans *et al.* found that BV prevalence was significantly higher among women reporting a history of female sex partners (45.2%) compared with women that did not (28.8%).¹³ Similarly, a community-based UK study found that BV prevalence was significantly higher among self-identified lesbian women

(25.7%) compared with heterosexual women (14.4%).¹⁴ A high level of concordance (87%) of vaginal flora (presence of BV or absence of BV) was found among female partnerships in this study, which is similar to that found in other studies,^{25,26} leading the authors to hypothesise that BV is sexually transmitted between women. Indeed, Marrazzo *et al.* have found that prevalence of BV among WSW is associated with sexual practices that efficiently transmit vaginal fluid.²⁷

Based upon our results and those from prior studies, one possibility that may be contributing to differences in BV prevalence among sexual behaviour groups is differential participation in at-risk sexual practices (i.e. penile–vaginal sex, digital–vaginal sex, oral–vaginal sex, use of sex toys, etc.).²⁸ A direct dose–response relationship between increasing numbers of episodes of receptive oral–vaginal sex and incident BV has been found among WSW.²⁹ Another study of WSW found that vaginal insertive use and sharing of sex toys was associated with decreased quantities of H₂O₂-producing lactobacilli and a higher risk of *G. vaginalis* colonisation,³⁰ while a study of primarily heterosexual women found an increasing BV risk among women engaging in more frequent penile–vaginal sex.³¹ It is not known whether transmission of infected vaginal fluid among women (WSW, WSWM) is a more efficient mechanism for BV acquisition than transmission from the male genitourinary tract among WSM. Future research is needed to explore this important topic.

Contrary to our initial hypothesis that strict anaerobes would be more efficiently transferred in infected vaginal secretions during female–female sex, WSW and WSWM with BV were not significantly more likely to have higher Nugent scores^{9,10} than WSM with BV in this study. This could potentially favour the *G. vaginalis* hypothesis in the pathogenesis of BV because if BV were sexually transmitted as a polymicrobial consortium of microorganisms in infected vaginal secretions, WSW and WSWM with BV should be more likely to have very high Nugent scores.^{9,10} One *G. vaginalis* hypothesis states that BV is initiated by the sexual transmission of this microorganism, which has the appropriate virulence factors to adhere to the host vaginal epithelium, create a biofilm community, and successfully compete with lactobacilli for dominance in the vaginal environment.¹⁰ Symbiotic relationships with normally dormant strict vaginal anaerobes would then lead to overgrowth of these organisms leading to the complex microbial community found in BV. Because this secondary analysis was cross-sectional in nature, we cannot make any causal conclusions regarding the events leading up to the development of BV among women in this study. Future studies are needed to determine the sequence of events culminating in BV among sexually active WSW, WSWM and WSM.

Our secondary objective was to correlate self-reported vaginal symptoms and sexual practices among WSW, WSWM and WSM with BV based on Nugent score. Interestingly, 77.8% of women with BV in our overall sample ($n = 203$) reported no vaginal symptoms, while 13.3% reported vaginal discharge, 11.3% vaginal odour and 7.9% genital itching. Several studies, including The Longitudinal Study of Vaginal Flora, have also found that many women with BV do not report symptoms, even upon direct questioning.^{32,33} In addition, women with BV in this study were not significantly more likely to have high-positive (9–10) Nugent scores. We had originally hypothesised that women with high (9–10) Nugent scores would be more likely to have vaginal symptoms (such as vaginal odour) due

to a larger predominance of strict anaerobic bacteria, including *Mobiluncus* spp. This was based on an association between *Mobiluncus* spp. and a positive amine odour after potassium hydroxide (KOH) preparation ('whiff test') among BV-positive women (by clinical criteria) attending an STD clinic³⁴ and in a study of 1756 BV-positive (by Nugent criteria) pregnant women.²² Seven amines have been found to contribute to the 'fishy odour' in women with BV.³⁵ These amines have been produced by mixed anaerobic growth in patients with BV, but not in pure cultures of *G. vaginalis* isolated from women with BV.³⁶ Inherent limitations associated with self-report of vaginal symptoms may have contributed to the lack of an association of vaginal symptoms and high BV-positive Nugent scores in this study. With regards to the sexual practices data, we found that women who reported participating in receptive vaginal sex within the 30 days preceding study enrolment were significantly more likely to be BV-positive by Nugent score. This finding is corroborated by that of others;²⁹ however, future research on the role of oral sex in BV acquisition is needed.

This study has several limitations. Nugent score reads from both parent studies were performed by different personnel during different time periods. Despite a relatively good interrater agreement calculated by the weighted Cohen's kappa in a random sample of 30 slides, this study may be subject to interrater disagreement. Second, as previously mentioned, this was a cross-sectional study and no definitive conclusions regarding the pathogenesis of BV can be made based on this data; future prospective studies on the pathogenesis of incident BV among WSW, WSWM and WSM are needed and may be guided by our findings. Third, our secondary study objective was limited by differences between the two parent study questionnaires. Because of this, we were unable to be more specific in our evaluation of specific sexual practices, such as the inability to distinguish between penile and digital vaginal sex and specific sex toy practices. Additionally, we were limited by the low number of respondents reporting some of the specific vaginal symptoms and sexual practices in the analyses shown in Tables 4 and 5, which precluded stratifying by sexual behavior groups. Future studies evaluating specific sexual practices, stratified by sexual behavior group, are warranted to investigate whether the disproportionate prevalence of BV in WSW and WSWM is indeed influenced by differential participation in at-risk sexual practices. In addition, the use of behavioral controls (WSM) enrolled 10 years previously could limit comparisons of sexual behavior and BV between groups, given changes in epidemiology and social norms over this period. It is also unknown if a woman was a participant in both studies, as the databases used for analysis were de-identified. Finally, due to the retrospective nature of this study, it is unknown why a very large proportion of women with BV, based on Nugent score, reported no symptoms (77.8%). The prevalence of asymptomatic BV among women has traditionally been ~50%;¹⁸ however, more recent studies have found varying proportions (35–85%) of asymptomatic BV among their study participants.^{13,37,38} Additional research is needed to clarify this important topic.

In summary, our study demonstrated that African-American WSW and WSWM were significantly more likely than WSM to have a BV diagnosis based on Nugent score. Both WSW and WSWM with BV were not significantly more likely to have higher Nugent scores^{9,10} than WSM with BV. Among women with BV reporting symptoms, there was no significant difference in the proportion of low-positive^{7,8} and high-positive^{9,10} BV Nugent scores. Finally, women participating in receptive vaginal sex within the 30 days preceding

study enrolment were significantly more likely to have a diagnosis of BV by Nugent score. Future prospective studies are needed to determine the precise aetiology of BV.

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References

1. Allsworth JE, Peipert JF. Prevalence of bacterial vaginosis: 2001–2004 National Health and Nutrition Examination Survey data. *Obstet Gynecol* 2007; 109(1): 114–20. doi:10.1097/01.AOG.0000247627.84791.91 [PubMed: 17197596]
2. Hillier SLMJ, Holmes KK. Bacterial vaginosis In: Holmes KKSP, Sparling PF, Mardh PA, Lemon SM, Stamm WE, Piot P, and Wasserheit JN, editors. *Sexually transmitted diseases*. New York: McGraw-Hill; 2008 pp. 737–68.
3. Martin HL, Richardson BA, Nyange PM, Lavreys L, Hillier SL, Chohan B, Mandaliya K, Ndinya-Achola JO, Bwayo J, Kreiss J. Vaginal lactobacilli, microbial flora, and risk of human immunodeficiency virus type 1 and sexually transmitted disease acquisition. *J Infect Dis* 1999; 180(6): 1863–8. doi:10.1086/315127 [PubMed: 10558942]
4. Hillier SL, Nugent RP, Eschenbach DA, Krohn MA, Gibbs RS, Martin DH, Cotch MF, Edelman R, Pastorek JG 2nd, Rao AV, McNellis D, Regan JA, Carey JC, Klebanoff MA. Association between bacterial vaginosis and preterm delivery of a low-birth-weight infant. The Vaginal Infections and Prematurity Study Group. *N Engl J Med* 1995; 333(26): 1737–42. doi:10.1056/NEJM199512283332604 [PubMed: 7491137]
5. Eschenbach DA, Davick PR, Williams BL, Klebanoff SJ, Young-Smith K, Critchlow CM, Holmes KK. Prevalence of hydrogen peroxide-producing *Lactobacillus* species in normal women and women with bacterial vaginosis. *J Clin Microbiol* 1989; 1989(27): 251–6.
6. Hawes SE, Hillier SL, Benedetti J, Stevens CE, Koutsky LA, Wolner-Hanssen P, Holmes KK. Hydrogen peroxide-producing lactobacilli and acquisition of vaginal infections. *J Infect Dis* 1996; 174(5): 1058–63. doi:10.1093/infdis/174.5.1058 [PubMed: 8896509]
7. Thorsen P, Jensen IP, Jeune B, Ebbesen N, Arpi M, Bremmelgaard A, Møller BR. Few microorganisms associated with bacterial vaginosis may constitute the pathologic core: a population-based microbiologic study among 3596 pregnant women. *Am J Obstet Gynecol* 1998; 178(3): 580–7. doi:10.1016/S0002-9378(98)70442-9 [PubMed: 9539529]
8. Hill GB. The microbiology of bacterial vaginosis. *Am J Obstet Gynecol* 1993; 169(2): 450–4. doi:10.1016/0002-9378(93)90339-K [PubMed: 8357043]
9. Catlin BW. *Gardnerella vaginalis*: characteristics, clinical considerations, and controversies. *Clin Microbiol Rev* 1992; 5(3): 213–37. doi:10.1128/CMR.5.3.213 [PubMed: 1498765]
10. Schwebke JR, Muzny CA, Josey WE. Role of *Gardnerella vaginalis* in the pathogenesis of bacterial vaginosis: a conceptual model. *J Infect Dis* 2014; 210(3): 338–43. doi:10.1093/infdis/jiu089 [PubMed: 24511102]
11. Srinivasan S, Fredricks DN. The human vaginal bacterial biota and bacterial vaginosis. *Interdiscip Perspect Infect Dis* 2008; 2008: 750479. doi:10.1155/2008/750479 [PubMed: 19282975]
12. Ness RB, Hillier SL, Richter HE, Soper DE, Stamm C, McGregor J, Bass DC, Sweet RL, Rice P. Douching in relation to bacterial vaginosis, lactobacilli, and facultative bacteria in the vagina. *Obstet Gynecol* 2002; 100: 765. [PubMed: 12383547]
13. Koumans EH, Sternberg M, Bruce C, McQuillan G, Kendrick J, Sutton M, Markowitz LE. The prevalence of bacterial vaginosis in the United States, 2001–2004; associations with symptoms, sexual behaviors, and reproductive health. *Sex Transm Dis* 2007; 34(11): 864–9. doi:10.1097/OLQ.0b013e318074e565 [PubMed: 17621244]

14. Evans AL, Scally AJ, Wellard SJ, Wilson JD. Prevalence of bacterial vaginosis in lesbians and heterosexual women in a community setting. *Sex Transm Infect* 2007; 83(6): 470–5. doi:10.1136/sti.2006.022277 [PubMed: 17611235]
15. Muzny CA, Sunesara IR, Martin DH, Mena LA. Sexually transmitted infections and risk behaviors among African American women who have sex with women: does sex with men make a difference? *Sex Transm Dis* 2011; 38(12): 1118–25. doi:10.1097/OLQ.0b013e31822e6179 [PubMed: 22082722]
16. Muzny CA, Sunesara IR, Austin EL, Mena LA, Schwebke JR. Bacterial vaginosis among African American women who have sex with women. *Sex Transm Dis* 2013; 40(9): 751–5. doi:10.1097/OLQ.0000000000000004 [PubMed: 23949590]
17. Greene B. African American Women In Comas-Díaz L, Greene B, editors. *Women of color: Integrating ethnic and gender identities in psychotherapy*. New York: Guilford Press; 1994 p. 10–29.
18. 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. doi:10.1016/0002-9343(83)91112-9 [PubMed: 6600371]
19. 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]
20. Zozaya-Hinchliffe M, Lillis R, Martin DH, Ferris MJ. Quantitative PCR assessments of bacterial species in women with and without bacterial vaginosis. *J Clin Microbiol* 2010; 48: 1812–9. doi:10.1128/JCM.00851-09 [PubMed: 20305015]
21. Srinivasan S, Morgan MT, Liu C, Matsen FA, Hoffman NG, Fiedler TL, Agnew KJ, Marrazzo JM, Fredricks DN. More than meets the eye: associations of vaginal bacteria with gram stain morphotypes using molecular phylogenetic analysis. *PLoS One* 2013; 8(10): e78633. doi:10.1371/journal.pone.0078633 [PubMed: 24302980]
22. Pereira L, Culhane J, McCollum K, Agnew K, Nyirjesy P. Variation in microbiologic profiles among pregnant women with bacterial vaginosis. *Am J Obstet Gynecol* 2005; 193(3): 746–51. doi:10.1016/j.ajog.2005.01.069 [PubMed: 16150269]
23. 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. doi:10.1097/01.olq.0000235152.98601.d7 [PubMed: 17179772]
24. Gorgos LM, Marrazzo JM. Sexually transmitted infections among women who have sex with women. *Clin Infect Dis* 2011; 53(Suppl. 3): S84–91. doi:10.1093/cid/cir697 [PubMed: 22080273]
25. 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: 1307–13. doi:10.1086/339884 [PubMed: 12001048]
26. Berger BJ, Kolton S, Zenilman JM, Cummings MC, Feldman J, McCormack WM. Bacterial vaginosis in lesbians: a sexually transmitted disease. *Clin Infect Dis* 1995; 21(6): 1402–5. [PubMed: 8749623]
27. Marrazzo JM, Thomas KK, Agnew K, Ringwood K. Prevalence and risks for bacterial vaginosis in women who have sex with women. *Sex Transm Dis* 2010; 37(5): 335–9. [PubMed: 20429087]
28. Muzny CA, Sunesara IR, Kumar R, Mena LA, Griswold ME, Martin DH, Lefkowitz EJ, Schwebke JR, Swiatlo E. Characterization of the vaginal microbiota among sexual risk behavior groups of women with bacterial vaginosis. *PLoS One* 2013; 8(11): e80254. doi:10.1371/journal.pone.0080254 [PubMed: 24236175]
29. Marrazzo JM, Thomas KK, Fiedler TL, Ringwood K, Fredricks DN. Risks for acquisition of bacterial vaginosis among women who report sex with women: a cohort study. *PLoS One* 2010; 5(6): e11139. doi:10.1371/journal.pone.0011139 [PubMed: 20559445]
30. Mitchell C, Manhart LE, Thomas KK, Agnew K, Marrazzo JM. Effect of sexual activity on vaginal colonization with hydrogen-peroxide producing *Lactobacilli* and *Gardnerella vaginalis*. *Sex Transm Dis* 2011; 38(12): 1137–44. doi:10.1097/OLQ.0b013e31822e6121 [PubMed: 22082725]

31. Bradshaw CS, Morton AN, Garland SM, Morris MB, Moss LM, Fairley CK. Higher-risk behavioral practices associated with bacterial vaginosis compared with vaginal candidiasis. *Obstet Gynecol* 2005; 106(1): 105–14. doi:10.1097/01.AOG.0000163247.78533.7b [PubMed: 15994624]
32. Klebanoff MA, Schwebke JR, Zhang J, Nansel TR, Yu K-F, Andrews WW. Vulvovaginal symptoms in women with bacterial vaginosis. *Obstet Gynecol* 2004; 104(2): 267–72. doi: 10.1097/01.AOG.0000134783.98382.b0 [PubMed: 15291998]
33. Yen S, Shafer M-A, Moncada J, Campbell CJ, Flinn SD, Boyer CB. Bacterial vaginosis in sexually experienced and non-sexually experienced young women entering the military. *Obstet Gynecol* 2003; 102(5, Part 1): 927–33. doi:10.1097/00006250-200311000-00009 [PubMed: 14672465]
34. Hallén A, Pålsson C, Forsum U. Bacterial vaginosis in women attending STD clinic: diagnostic criteria and prevalence of *Mobiluncus* spp. *Genitourin Med* 1987; 63: 386–9. [PubMed: 3323027]
35. Chen KC, Forsyth PS, Buchanan TM, Holmes KK. Amine content of vaginal fluid from untreated and treated patients with nonspecific vaginitis. *J Clin Invest* 1979; 63: 828–35. doi:10.1172/JCI109382 [PubMed: 447831]
36. Chen KC, Amsel R, Eschenbach DA, Holmes KK. Biochemical diagnosis of vaginitis: determination of diamines in vaginal fluid. *J Infect Dis* 1982; 145(3): 337–45. doi:10.1093/infdis/145.3.337 [PubMed: 7061879]
37. Eschenbach D, Hillier S, Critchlow C. Diagnosis and clinical manifestations of bacterial vaginosis. *Int J Gynaecol Obstet* 1989; 28(2): 195. doi:10.1016/0020-7292(89)90483-9
38. Nelson DB, Bellamy S, Nachamkin I, Ruffin A, Allen-Taylor L, Friedenberg FK. Characteristics and pregnancy outcomes of pregnant women asymptomatic for bacterial vaginosis. *Matern Child Health J* 2008; 12(2): 216–22. doi:10.1007/s10995-007-0239-7 [PubMed: 17546484]

Table 1.

Select characteristics of study participants (*n* = 564)

WSW, women who have sex with women; WSWM, women who have sex with women and men; WSM, women who have sex with men. Results are presented as *n* (%)

Characteristic	All (<i>n</i> = 564)	WSW (<i>n</i> = 73)	WSWM (<i>n</i> = 68)	WSM (<i>n</i> = 423)	<i>P</i>
Age (years)					0.44
19–24	264 (47)	38 (52)	28 (41)	198 (47)	
25–45	300 (53)	35 (48)	40 (59)	225 (53)	
Employment status					<0.01
Unemployed, disabled, student	248 (44)	43 (59)	36 (53)	169 (40)	
Employed, full- or part-time	316 (56)	30 (41)	32 (47)	254 (60)	0.06
Education					
<High school	117 (21)	8 (11)	17 (25)	92 (22)	
High school or GED	447 (79)	65 (89)	51 (75)	331 (78)	<0.01
Marital status					
Never married	412 (73)	68 (93)	49 (72)	295 (70)	
Married, separated, divorced, widowed	152 (27)	5 (7)	19 (28)	128 (30)	<0.01
Number of lifetime sexual partners					
<3	104 (18)	6 (8)	3 (4)	95 (22)	
4–10	318 (56)	41 (56)	19 (28)	258 (61)	
11	142 (25)	26 (36)	46 (68)	70 (17)	
Diagnosis of trichomoniasis	46 (8)	11 (15)	16 (24)	19 (4)	<0.01
Diagnosis of chlamydia	7 (1)	3 (4)	4 (6)	0 (0)	<0.01
Diagnosis of gonorrhoea	2 (0.4)	0 (0)	2 (3)	0 (0)	0.03
Diagnosis of vaginal candidiasis	46 (7)	8 (11)	8 (12)	30 (6)	0.07

Table 2.
Bacterial vaginosis (BV) diagnosis by Nugent Score in WSM, WSW and WSWM (*n* = 564)

WSW, women who have sex with women; WSWM, women who have sex with women and men; WSM, women who have sex with men; OR, odds ratio; CI, confidence interval. Results are presented as *n* (%)

Population	BV negative ^A (<i>n</i> = 361)	BV positive ^B (<i>n</i> = 203)	Unadjusted OR (95% CI)	<i>P</i>	Adjusted OR (95% CI)	<i>P</i>
WSW	32 (44)	41 (56)	3.04 (1.82–5.07)	<0.01	2.63 (1.55–4.48)	<0.01
WSWM	33 (49)	35 (51)	2.52 (1.49–4.24)	<0.01	3.67 (2.17–6.21)	<0.01
WSM	296 (70)	127 (30)	Reference		Reference	

^ANugent Score 0–6 (231 participants had a normal Nugent score of 0–3 and 130 participants had an intermediate Nugent score of 4–6).

^BNugent Score 7–10; row percentages reported; adjusted for marital status.

Table 3.
Low-BV positive and high-BV positive Nugent Scores among WSM, WSW and WSWM (*n* = 203)

BV, bacterial vaginosis; WSW, women who have sex with women; WSWM, women who have sex with women and men; WSM, women who have sex with men; OR, odds ratio; CI, confidence interval. Results are presented as *n* (%)

Population	BV Nugent score 7–8 (<i>n</i> = 103)	BV Nugent score 9–10 (<i>n</i> = 100)	Unadjusted OR (95% CI)	<i>P</i>	Adjusted OR (95% CI)	<i>P</i>
WSW	20 (49)	21 (51)	1.10 (0.47–2.09)	0.79	0.74 (0.33–1.65)	0.46
WSWM	18 (51)	17 (49)	0.99 (0.47–2.09)	0.98	0.83 (0.37–1.87)	0.67
WSM	65 (51)	62 (49)	Reference	Reference	Reference	Reference

Row percentages reported; adjusted for trichomoniasis diagnosis and education status.

Table 4.

Self-reported vaginal symptoms among WSM, WSW and WSWM women with BV, stratified by low-positive and high-positive BV Nugent Score ($n = 203$)

Column percentages reported; estimated using [Exact logistic regression]. BV, bacterial vaginosis; WSW, women who have sex with women; WSWM, women who have sex with women and men; WSM, women who have sex with men; OR, odds ratio; CI, confidence interval. Results are presented as n (%)

Population	BV Nugent score 7–8 ($n = 103$)	BV Nugent score 9–10 ($n = 100$)	Unadjusted OR (95% CI)	P	Adjusted OR (95% CI)	P
Asymptomatic ^A	75 (73)	83 (83)	1.82 (0.92–3.59)	0.11	1.82 (0.90–3.69)	0.13
Vaginal odour ^B	15 (15)	8 (8)	0.51 (0.21–1.26)	0.20	0.517 (0.20–1.34)	0.26
Vaginal discharge ^C	16 (16)	11 (11)	0.67 (0.30–1.53)	0.46	0.61 (0.26–1.42)	0.35
Genital itching ^D	12 (12)	4 (4)	0.32 (0.10–1.02)	0.07	0.32 (0.10–1.06)	0.10

^A Adjusted for trichomoniasis diagnosis and education level.

^B Adjusted for sexual risk behaviour group, education level and employment status.

^C Adjusted for trichomoniasis diagnosis and education level.

^D Adjusted for trichomoniasis diagnosis, education level and total number of lifetime partners.

Table 5.
Sexual practices in WSW, WSWM and WSM, stratified by BV diagnosis (*n* = 564)

BV, bacterial vaginosis; WSW, women who have sex with women; WSWM, women who have sex with women and men; WSM, women who have sex with men; OR, odds ratio; CI, confidence interval. Results are presented as *n* (%)

Population	BV negative ^A (<i>n</i> = 361)	BV positive ^B (<i>n</i> = 203)	Unadjusted OR (95% CI)	<i>P</i>	Adjusted OR (95% CI)	<i>P</i>
Receptive vaginal sex (penile or digital) ^C	89 (25)	94 (46)	2.71 (1.87–3.92)	<0.01	2.40 (1.57–3.63)	<0.01
Receptive oral sex ^D	49 (14)	62 (31)	2.80 (0.10–22.29)	<0.01	0.19 (0.18–1.15)	0.29
Receptive anal sex (penile or digital) ^E	8 (2)	8 (4)	1.75 (0.60–5.13)	0.45	0.76 (0.24–2.37)	0.84

^A Nugent Score 0–6.

^B Nugent Score 7–10; column percentages reported.

^C Adjusted for trichomoniasis diagnosis and sexual risk behaviour group; missing values = 5.

^D Adjusted for trichomoniasis diagnosis, and sexual risk behaviour group; missing values = 10.

^E Adjusted for trichomoniasis diagnosis, sexual risk behaviour group and marital status; missing values = 14; estimated using [Exact logistic regression].