Association of Bacterial Vaginosis With *Chlamydia trachomatis* Infection Among Women in Mombasa, Kenya: A Nested Case-Control Study

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Abstract

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Objective: Evidence is mixed regarding the relationship between bacterial vaginosis (BV) and *Chlamydia trachomatis* (CT) acquisition; therefore, we assessed the relationship between recent BV and subsequent CT infection.

Study Design: A nested case-control study was conducted using data and samples from cisgender women who reported engaging in transactional sex and who were participating in the Mombasa Cohort, a longitudinal study, in Kenya. BV was assessed monthly and categorized by Nugent score (0-6=Normal, 7-10=BV-positive); CT testing was conducted quarterly by nucleic acid amplification testing. For each CT case, 2 controls were randomly selected using incidence density sampling that tested negative for CT around the time a case tested positive for CT (index visit). Conditional logistic regression was

used to estimate the odds of being a case between participants with and without BV at the prior visit (index-1).

Results: Between September 2010 and November 2021, 89 cases and 178 controls were identified. The median interval between the index and index-1 visits was 61 days (interquartile range [IQR] 35-134) for cases and 33 days (IQR 28-42) for controls. At the index-1 visit, 42.7% of cases and 23.6% of controls had BV. The unadjusted odds of being a case were 2.50 times higher among participants with BV at the index-1 visit compared to those without BV (95% confidence interval (CI): 1.44, 4.33). After adjustment for age, years of sex work, and contraception, there remained two-fold higher odds of CT among those with BV (adjusted OR=2.03; 95% CI 1.07, 3.88).

Conclusion: In this population of individuals at increased risk for CT exposure, recent BV was associated with subsequent CT infection. Further research is needed to assess how BV-associated bacteria and communities may enhance susceptibility to CT infection.

Introduction

Chlamydia trachomatis (CT) is the most prevalent bacterial sexually transmitted infection (STI) worldwide, with approximately 129 million new cases reported in 2020.[1] The global prevalence of CT stands at around 2.9%, with people assigned female at birth exhibiting a higher prevalence compared to those assigned male at birth (3.1% versus 2.6%, respectively).[2] Among cisgender (cis) women, those aged 25 and under bear a significant burden of CT infection.[3] However, the prevalence of CT among cis-women varies across different regions designated by the World Health Organization (WHO). The Americas and sub-Saharan Africa display the highest CT prevalence rates, with 5.3% and 3.8% respectively.[2]

CT infection in cis-women can manifest through various signs and symptoms, including abnormal vaginal discharge, pain during urination and sexual intercourse, and vaginal bleeding.[4] However, it is important to note that CT infections are often asymptomatic leading to underreporting and lack of diagnosis. Only a small percentage, approximately 6%, of women with CT infection experience acute signs and symptoms.[5] It is crucial to address CT infection promptly as untreated cases can result in long-term complications such as pelvic inflammatory disease (PID). Complications like PID can lead to infertility, ectopic pregnancy, and chronic pelvic pain if left untreated.[6]

While highly sensitive testing is available for CT, estimates from low- and middle-income countries may be unreliable due to limited laboratory infrastructure and diagnostic capabilities, resulting in inadequate surveillance. A 2018 meta-analysis reported a pooled CT prevalence of 7.8% among reproductive-age cis-women in sub-Saharan Africa, which is substantially higher than the 3.8% reported in a 2020 meta-analysis.[7] In countries with

limited resources, high rates of CT infection can pose serious consequences due to the challenges in accessing reliable screening and diagnosis.[8] To address this, many of these countries have implemented syndromic management for STIs.[8] Although this approach may be effective for managing self-reported symptomatic infections, it is insufficient for detecting and treating asymptomatic infections.[9] Furthermore, although CT is curable with antibiotics and true antimicrobial resistance appears to be rare, untreated or recurrent infections can increase the risk of serious reproductive health issues.[10] Consequently, the prevention and treatment of chlamydia is a significant public health concern in these regions.

Bacterial vaginosis (BV) is the most common vaginal condition in cis-women of reproductive age.[11] It is characterized by an imbalance in the vaginal microbiota. The global prevalence of BV is substantial, ranging from 23% to 29% in the general population, with variations observed within countries and sub-populations.[12] BV presents with signs and symptoms such as an abnormal off-color discharge accompanied by a fishy odor, increased vaginal pH, and vaginal itching or soreness.[13]

The etiology of BV remains incompletely understood, however, several factors have been identified that may influence its occurrence. Epidemiological evidence suggests that initiation of penile-vaginal sexual intercourse at a younger age[14], a higher number of lifetime sexual partners[15], engaging in sex work[16], having sex with a female partner[17], and regular douching[18] may increase the likelihood of BV. Conversely, the use of certain hormonal contraceptives[19] and consistent use of male condoms[20] has been associated with a reduced likelihood of BV.

BV represents a significant public health concern due to its association with adverse health outcomes, including PID, infertility, and endometriosis.[21] Furthermore, BV has been associated with an increased risk of STIs such as human immunodeficiency virus (HIV)[22], *Neisseria gonorrhoeae* (GC)[23], *Trichomonas vaginalis* (TV)[24], and CT.[23] While several studies have explored the association between BV and CT, many of them have been cross-sectional in nature, limiting the ability to establish temporal relationships between BV and CT.

Given the high rates of both CT infection and BV recurrence, it is crucial to gain a comprehensive understanding of their relationship for effective chlamydia control efforts.[25] Investigating the role of BV in CT susceptibility is vital as it could pave the way for targeted interventions aimed at disrupting this association. Therefore, it represents a critical area of research. To address this knowledge gap, this nested case-control study aims to evaluate the relationship between BV and CT, using established methods to characterize BV, as part of a larger study assessing the impact of vaginal bacteria on CT acquisition. The larger study will also examine the involvement of key metabolites necessary for CT survival.

Methods

Study design and population

This study constitutes a secondary analysis of longitudinal data from an open cohort study of adult women engaging in transactional sex in Mombasa, Kenya. The eligibility criteria for cohort inclusion encompassed individuals between 18 to 50 years old, residing in Mombasa, engaging in transactional sex, and providing informed consent.

Although gender identity was not specifically assessed in the Mombasa cohort, it was assumed based on the study protocol that all participants identified as cisgender women. Ethical approval for the study was obtained from the ethical review committee at Kenyatta National Hospital and the University of Washington. The analysis utilized data collected between September 2010 and November 2021 from eligible participants who were not pregnant and tested negative for HIV.

Study procedures and data collection

During the enrollment process and subsequent monthly follow-up visits, study staff conducted standardized interviews to gather comprehensive information on participants' demographic characteristics, medical history, gynecologic history, and sexual history. Additionally, study clinicians performed physical examinations, including pelvic speculum examinations, during quarterly visits. Swabs of cervical and vaginal secretions were collected during these visits for laboratory testing to diagnose STIs. However, for the evaluation of BV, swabs were collected monthly. All participants received free outpatient medical services, including STI treatment adhering to WHO guidelines and Kenyan national protocols. Syndromic management was administered during examination visits as needed. Any additional required treatment was provided during the participant's subsequent visit if laboratory tests identified any infections that had not been treated syndromically during the previous visit.

Laboratory methods

The collected cervical and vaginal secretion samples underwent nucleic acid amplification-based testing (Aptima Combo-2, Hologic, San Diego, CA) to detect *Chlamydia trachomatis* (CT) and other STIs. The presence of BV was assessed using

two approaches. First, the Nugent and Hillier score[26] was utilized, with scores ranging from 0 to 3 indicating BV-negative/normal, scores from 4 to 6 indicating intermediate, and scores from 7 to 10 indicating BV-positive. Additionally, Amsel's criteria[27] were applied, which required the presence of at least three out of the following four criteria: 1) thin, homogenous, or milky discharge, 2) vaginal fluid pH greater than 4.5, 3) positive whiff test (detecting a fishy amine odor upon adding 10% potassium hydroxide to the sample), and 4) at least 20% clue cells (vaginal epithelial cells with obscured borders due to adherent coccobacilli on wet-mount preparation).

Data Analysis

Cases were defined as participants who tested positive for CT (and negative for GC and TV) at a quarterly study visit. Controls were selected in a 2:1 ratio using incidence density sampling and were defined as participants who tested negative for all STIs (CT, GC, and TV) at the time of CT diagnosis in cases ideally within 60 days. Individual participants could be represented as both cases and controls by design and could be presented more than once in the analysis. There were 182 unique individuals in the study and 22 of them contributed as cases and controls. The visit where CT infection status was determined was referred to as the "index" visit, and the preceding visit was the "index-1" visit. Figure 1 illustrates the sampling strategy for cases and controls at the index and index-1 visits and the BV status at the index-1 visit.

The primary exposure of interest was BV status, determined by the Nugent score. BV status based on Amsel's criteria served as the secondary exposure of interest. BV status was initially categorized into two groups: Nugent score 0-6 (BV-negative) and 7-10 (BV-positive), while Amsel's criteria were categorized as the presence of ≤2 criteria

(negative) and ≥3 criteria (positive). BV status was further categorized into three groups: Nugent score 0-3 (BV-negative), 4-6 (intermediate), and 7-10 (BV-positive).

Due to the COVID-19 pandemic and mandated lockdown measures, clinic and study visit activities were suspended from March to July 2020, resulting in longer intervals between the index-1 and index visits during that period. To explore the impact of these longer intervals on the association between BV status and CT infection, a sensitivity analysis was conducted to exclude participants with longer intervals between the index and index-1 visit (i.e., the highest quartile of days between visits). In addition, we conducted an additional sensitivity analysis to evaluate the association between BV status at both the index and index-1 visit and CT infection. BV status was classified into four exposure categories: 1) BV-negative at both visits, 2) BV-positive only at the index-1 visit, 3) BV-positive only at the index visit, and 4) BV-positive at both the index and index-1 visits. It also allowed for the examination of a potential dose-response effect of discordant and concordant BV status and CT infection.

Statistical analysis

To estimate the odds of being a case, a comparison was made between BV status at the index-1 visit among cases and controls. Additionally, the odds of being a case were also assessed by comparing BV status at both the index and index-1 visits between cases and controls. Age, contraceptive use, sexual behavior, and socioeconomic status variables were evaluated for potential inclusion as adjustment and confounding variables in the multivariate model (see Tables 1a, 1b, and 1c). The selection of these variables was based on their established associations with both BV and CT acquisition, and their

inclusion in the model was determined by observing at least a 10% change in the crude odds ratio when each covariate was incorporated into the univariate model.

Regarding missing data, there were two cases with missing Nugent scores at the index-1 visit; however, data on Amsel's criteria were available at this visit. Based on Amsel's criteria and Nugent score data from the index visit, we assigned a Nugent score category for these visits. Similarly, six cases and twenty controls were missing individual components of Amsel's criteria. Where possible, Amsel's criteria category was assigned based on a subset of the available criteria (i.e. if a participant was missing whiff test results, but was negative for the three other available criteria they were classified as not having BV). If a determination could not be made based on the available Amsel's criteria data, we used Nugent score to make a final determination for BV status. Conditional logistic regression was employed to estimate the odds of being a case based on BV status at the index-1 visit, with a significance level set at 5% (alpha = 0.05). The analysis was conducted using Stata/BE version 17.0.

Results

Among the participants enrolled between September 2010 and November 2021, a total of 267 individuals met the inclusion criteria for this analysis, comprising 89 cases and 178 controls. Tables 1a, 1b and 1c provide the demographic, sexual, and other behavioral characteristics of participants at the index and index-1 visits. The median age of cases was 31 years (IQR, 26-37), while for controls it was 38 years (IQR, 32-47) (Table 1a). Regarding sexual behavior, 55.1% of cases and 50.0% of controls reported having two or more different sexual partners in the past working week at the index visit, while at the index-1 visit, these percentages were 59.6% for cases and 49.4% for controls (Table

1b). Furthermore, 29.2% of cases and 32.6% of controls reported having two or more new sexual partners in the past month at the index visit, while at the index-1 visit, these percentages were 51.7% for cases and 26.4% for controls (Table 1b). At the index-1 visit, 93.3% of cases and 93.8% of controls reported engaging in vaginal douching, and 21.4% of cases and 18.5% of controls used lubrication during sex (Table 1c). In terms of contraception, 87.4% of cases and 84.3% of controls used a method of contraception, with 39.3% of cases and 47.8% of controls exclusively relying on condoms (Table 1c).

The median interval between the index and index-1 visits was 61 days (interquartile range [IQR], 35-134) for cases and 33 days (IQR, 28-42) for controls. The range of intervals between the index and index-1 visits was 28 to 603 days for cases and 26 to 157 days for controls. The prevalence of BV, as determined by Nugent score and Amsel's criteria at both visits, is presented in Table 2. Using the binary categorization of BV status, 43.8% of cases and 23.6% of controls were BV-positive at the index-1 visit. Unadjusted and adjusted odds ratios from univariate and multivariate conditional logistic regression models are presented in Table 3. The unadjusted odds of being a case were 2.50 times higher among participants who were BV-positive at the index-1 visit compared to those who were BV-negative (95% CI: 1.44, 4.33). After adjusting for age, years of sex work, and contraceptive use, the adjusted odds ratio was 2.03 (95% CI: 1.07, 3.88).

Similar results were observed when using the three-level categorization of Nugent score, comparing participants with a score of 7-10 to those with a score of 0-3 (adjusted OR=2.04; 95% CI 1.07-3.91). Notably, 10.1% of cases and 11.2% of controls had an intermediate score at the index-1 visit. The unadjusted odds of being a case were 1.06 times higher among participants with an intermediate score compared to those who were

BV-negative (95% CI: 0.38, 2.97). When BV status was categorized by Amsel's criteria, 21.4% of cases and 7.9% of controls were BV-positive at the index-1 visit. The unadjusted odds of being a case were 3.10 times higher among participants who were BV-positive compared to those who were BV-negative (95% CI: 1.46, 6.58), while the adjusted odds ratio was 2.18 (95% CI: 0.86, 5.53).

Table 4 illustrates the concordant and discordant BV status at the index and index-1 visits using Nugent score (binary categorization) and Amsel's criteria. The unadjusted odds of being a case were 3.37 times higher among participants who were BV-positive at both visits compared to those who were BV-negative at both visits (95% CI: 1.72, 6.60). After adjusting for potential confounders, the adjusted odds ratio was 2.67 (95% CI: 1.20, 5.93). The adjusted odds of being a case were 2.36 times higher among participants who were BV-positive only at the index visit (95% CI: 0.80, 6.98), and 1.76 times higher among participants who were BV-positive only at the index-1 visit (95% CI: 0.69, 4.51). When using Amsel's criteria, the adjusted odds of being a case were 3.47 times higher among participants who were BV-positive only at the index-1 visit (95% CI: 1.07, 11.25).

To evaluate the impact of longer visit intervals on our findings, we performed a sensitivity analysis, restricting cases and controls to participants with visit intervals within the upper quartile. This resulted in the selection of 67 cases and 137 controls (Table 5). Using the binary categorization of BV status, 46.3% of cases and 21.9% of controls were BV-positive. The unadjusted odds of being a case were 2.95 times higher among participants who were BV-positive compared to those who were BV-negative (95% CI: 1.46, 5.96). After adjustment, the results were slightly attenuated, with an adjusted odds ratio of 2.89 (95% CI: 1.18, 7.10).

When using the three-level categorization of BV status, the unadjusted odds of being a case were 2.82 times higher among participants who were BV-positive compared to those who were BV-negative (95% CI: 1.39, 5.72). The adjusted odds ratio was 2.82 (95% CI: 1.14, 7.00). Using Amsel's criteria, 23.9% of cases and 8.0% of controls were BV-positive. The unadjusted odds of being a case were 4.17 times higher among participants who were BV-positive compared to those who were BV-negative (95% CI: 1.61, 10.78), and the adjusted odds ratio was 3.81 (95% CI: 1.03, 14.16).

Discussion

In this secondary analysis of the Mombasa Cohort, we investigated the association between BV and CT. Our results showed that BV-positive status at the prior visit (index-1) was associated with CT infection at the subsequent visit (index). Furthermore, participants who were BV-positive at both visits had even higher odds of being CT cases compared to those who were BV-negative at both visits. While the prevalence of BV by Nugent score was higher, the magnitude of the association was higher with Amsel's criteria although the confidence intervals are much wider and in some cases included 1 (adjusted OR 2.18; 95% CI 0.86, 5.53). A possible reason for this stronger association may be that although BV is frequently asymptomatic, women with BV are more likely than those without BV to report vaginal odor and discharge which are assessed clinically for Amsel's criteria.[28] This may indicate a more inflammatory state of the vaginal environment that may be more conducive to CT infection. Our sensitivity analysis, which included participants with shorter intervals between visits and closer proximity to CT assessment also revealed a stronger association between BV and CT.

These findings add to the existing evidence supporting the consistent association between BV and subsequent CT infection. Similar associations have been reported in previous studies. For instance, the cross-sectional analysis by Kaul *et al* demonstrated that the CT infection rate among cis-women who were BV-positive was 2.1-fold higher than the rate among cis-women who were BV-negative.[29] Also, the secondary analysis of another longitudinal cohort study by Abbai *et al* observed a nearly 2-fold higher incidence of CT infection among women with baseline BV when compared to women who were BV-negative.[30] In contrast, Masese *et al* reported a 1.1-fold higher association between abnormal vaginal microbiota or BV and CT infection, which was also not statistically significant.[31] It is worth noting that the prospective study by Mases *et al* only had 16 baseline CT cases while the present study, using the same Mombasa cohort, included 89 cases, a much larger number of CT cases, which may explain the stronger association observed.

This nested case-control study aimed to examine the relationship between BV and CT using established BV characterization methods within a larger study investigating the impact of vaginal bacteria on CT acquisition. Our analysis provided robust evidence of a positive association between BV and CT. Moving forward, the next steps involve investigating the underlying mechanisms of this association at a biological level. This includes identifying the specific BV-associated bacteria, bacterial metabolites, and bacterial communities that increase susceptibility to CT infection. One proposed mechanism suggests that since urogenital CT requires the amino acid tryptophan to proliferate and the immune system generally guards against CT infection[32] by producing an enzyme that breaks down tryptophan,[33] urogenital CT may be able to circumvent this

defense mechanism by producing tryptophan on its own using indole,[34,35] a metabolite produced by BV-associated bacteria such as *Prevotella spp*.[36,37] However, further research is needed to fully explore this theory, which is ongoing in the larger nested case-control study.[38]

Strengths and limitations

This analysis has several strengths that contribute to the understanding of the association between BV and CT infection. First, the cases and controls were selected from a longitudinal study, allowing for the assessment of BV status prior to the visit where CT was evaluated. This longitudinal follow-up design enabled the evaluation of the temporal ordering of BV and subsequent CT infection within the Mombasa cohort. Furthermore, this analysis helps address the limited literature on CT infection in Kenya, considering the scarcity of CT screening and diagnosis in resource-limited settings.

However, our findings should be interpreted in light of certain limitations. Due to the nature of case-control studies, we cannot establish causality based solely on the association between BV at a prior visit and subsequent CT infection. Moreover, there may be measurement errors in key covariates, including self-reported sexual risk behaviors, due to potential recall bias and social desirability bias. Although efforts were made to minimize recall bias by focusing on sexual risk behaviors in the past week, some misreporting remains a possibility. Additionally, unmeasured behavioral factors, such as a history of STIs, partner characteristics, and social and cultural factors, may introduce residual confounding. Furthermore, misclassification of BV status may occur in participants with longer visit intervals, as the vaginal microbiota at the prior visit may not be representative of the microbiota preceding CT acquisition at the current visit.

It is important to note that the study participants were cis-women engaged in transactional sex work, which may limit the generalizability of the findings to the broader population of women in the Mombasa region. Additionally, this analysis focused exclusively on cis-women, but the public health implications of BV extend to all individuals with a vagina, including non-binary and transgender men. Further research is needed to encompass a more diverse population beyond the scope of this analysis.

Conclusion

In conclusion, our study found a significant association between bacterial vaginosis (BV), as assessed by Nugent score and Amsel's criteria, and an increased likelihood of acquiring *Chlamydia trachomatis* (CT). These findings are consistent with previous similar studies, reinforcing the importance of exploring this relationship further. Our analysis emphasizes the need for an in-depth investigation into the underlying biochemical pathways, potentially involving indole salvage, which may facilitate CT infection. To gain a mechanistic understanding, the aforementioned larger nested case-control study is warranted. Future research should focus on elucidating the intricate interactions and involvement of specific BV-associated bacteria, metabolites, and microbial communities, ultimately enhancing our understanding of how they contribute to the heightened susceptibility and proliferation of *Chlamydia trachomatis*.

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Tables And Figures

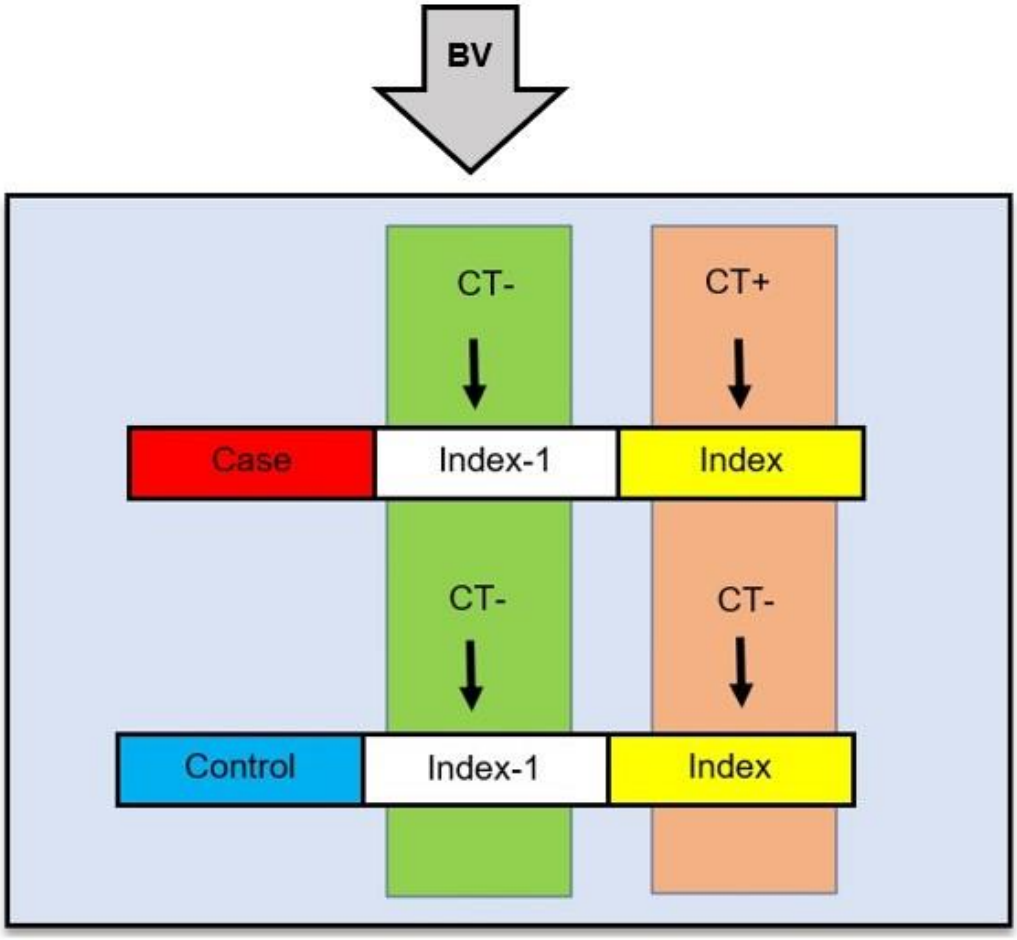

Figure 1: The sampling strategy for cases and controls at the Index and index-1 visits. Index visit = CT+ visit preceded by CT- visit (orange box). Index-1 = Visit prior to the index visit (green box). BV status compared at the index-1 visit (grey arrow)

Table 1a: Demographic characteristics of participants with *Chlamydia trachomatis* infection (cases) and matched controls at the index and index-1 visits reported at enrollment

	Cases (CT+) n = 89	**Controls (CT-) n = 178**
	n (%) or Median (IQR)	**n (%) or Median (IQR)**
Demographic characteristics		
Age[1]	31 (26–37)	38 (32–47)
Nationality		
Kenyan	82 (92.1)	167 (93.8)
Other	7 (7.9)	11 (6.2))
Religion		
Christian	68 (76.4)	142 (79.8)
Muslim	21 (23.6)	32 (18.0)
Other	0 (0.0)	1 (0.6)
Marital status		
Never married	44 (49.4)	78 (43.8)
Currently married	0 (0.0)	1 (0.6)
Widowed/Divorced	45 (50.6)	99 (55.6)
Years of education	8 (7–10)	8 (6–11)
Age at first sex	17 (15–18)	16 (14–18)
Years engaged in sex work	2 (0–4)	3 (1–5)
Cigarette consumption		
≥ 1 cigarette smoked per day	22 (24.7)	35 (19.7)
Number of cigarettes per day among those that smoke ≥ 1	4 (3–10)	4 (1–10)
Alcohol consumption		
≥ 1 drinks per day	70 (78.7)	150 (84.3)
Number of drinks per day among those that drink ≥ 1	5 (2–12)	8 (3–14)
1 Age data was collected at enrollment and calculated longitudinally for follow-up visits		

Data in the table was only collected at enrollment
"Religion," "Cigarette consumption," and "Alcohol consumption" had ≤5% missingness between cases and controls
"Other" specific responses are either purposely omitted or currently unavailable

Table 1b: Sexual behavior characteristics of participants with *Chlamydia trachomatis* infection (cases) and matched controls at the index and index-1 visits reported at enrollment, follow-up visit, and the past working week.

	Cases (CT+) n = 89		**Controls (CT-) n = 178**	
	Index	**Index-1**	**Index**	**Index-1**
	n (%) or Median (IQR)	**n (%) or Median (IQR)**	**n (%) or Median (IQR)**	**n (%) or Median (IQR)**
Sexual behaviors				
Sexual partnerships in the past working week				
No new sexual partners	15 (16.9)	15 (16.9)	40 (22.5)	37 (20.8)
1 different sexual partner	25 (28.1)	20 (22.5)	48 (27.0)	53 (29.8)
≥ 2 different sexual partners	49 (55.1)	53 (59.6)	89 (50.0)	88 (49.4)
Number of different sexual partners in the past working week among those with ≥ 2	3 (3–5)	3 (3–5)	3 (2–5)	3.5 (2.5–5)
Frequency of vaginal intercourse during the past working week[1]	3 (1–5)	3 (2–6)	3 (1–4)	2 (1–5)
Frequency of vaginal intercourse with condoms during the past working week[1]	2 (1–5)	3 (2–5)	2 (1–4)	2 (1–5)
Frequency of anal intercourse during the past working week[1,2]	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)
New sexual partnerships in the past month				
No new sexual partners	48 (53.9)	36 (40.5)	95 (53.4)	102 (57.3)
1 new sexual partner	15 (16.9)	7 (7.9)	25 (14.0)	29 (16.3)
≥ 2 new sexual partners	26 (29.2)	46 (51.7)	58 (32.6)	47 (26.4)
Number of new partners in the past month among those with ≥ 2	4 (3–5)	3 (2–5)	3 (2–5)	3 (2–4)

1 Among those who reported 1 or more different sexual partners during the past working week
2 There were no responses to “Frequency of anal intercourse during past working week” and therefore no responses to “Frequency of anal intercourse with condoms during past working week” so the variable for the latter was omitted from the table
“Sexual partnerships in the past working week” and “New sexual partnerships in the past month” had ≤5% missingness between cases and controls
Index = CT+ visit preceded by CT- visit
Index-1 = Visit prior to the index visit

Table 1c: Additional behavioral characteristics of participants with *Chlamydia trachomatis* infection (cases) and matched controls at the index and index-1 visits reported at enrollment and follow-up

	Cases (CT+) n = 89		**Controls (CT-) n = 178**	
	Index	**Index-1**	**Index**	**Index-1**
	n (%) or Median (IQR)	**n (%) or Median (IQR)**	**n (%) or Median (IQR)**	**n (%) or Median (IQR)**
Additional behaviors				
Have you used anything to wash the inside of your vagina?	85 (95.5)	83 (93.3)	165 (92.7)	167 (93.8)
What do you use to clean your vagina?[1]				
Water alone	41 (48.2)	39 (47.0)	69 (41.8)	70 (41.9)
Detergents & other substances	44 (51.8)	44 (51.8)	96 (58.2)	97 (58.8)
What have you used to clean inside your vagina?[1]				
Finger	52 (61.2)	53 (63.9)	120 (72.7)	123 (73.7)
Douche bag	3 (3.5)	2 (2.4)	3 (1.8)	3 (1.8)
Bathing flannel	28 (32.9)	27 (32.5)	38 (23.0)	37 (22.2)
Other	2 (2.4)	1 (1.2)	4 (2.4)	4 (2.4)
Put anything not for washing into vagina?	3 (3.4)	3 (3.4)	3 (1.7)	3 (1.7)
Used vaginal lubricant for sex	18 (20.2)	19 (21.4)	32 (18.0)	33 (18.5)
Method of contraceptive use				
None	18 (20.2)	11 (12.4)	35 (19.7)	28 (15.7)

Condoms only	31 (34.8)	35 (39.3)	81 (45.5)	85 (47.8)
Oral contraceptive pills	2 (2.3)	3 (3.4)	4 (2.3)	5 (2.8)
DMPA[2]	13 (14.6)	16 (18.0)	23 (12.9)	24 (13.5)
Permanent & Long-Acting Reversible Contraception	25 (28.1)	23 (25.8)	33 (18.5)	36 (20.2)
Other	0 (0.0)	0 (0.0)	1 (0.6)	0 (0.0)

1 Among those who reported Yes to “Have you used anything to wash the inside of their vagina?”
2 Contraceptive injection most commonly known as Depo Provera (depot-medroxyprogesterone acetate)
“Have you used anything to wash the inside of your vagina,” “What do you use to clean your vagina?,” “Used vaginal lubricant for sex,” and “Method of contraceptive use” had ≤5% missingness between cases and controls. “Other” specific responses are either purposely omitted or currently unavailable
Index = CT+ visit preceded by CT- visit
Index-1 = Visit prior to the index visit

Table 2: The prevalence of bacterial vaginosis by Nugent score and Amsel's criteria of participants with *Chlamydia trachomatis* infection (cases) and matched controls at the index and index-1 visits

	Cases (CT+) n=89		**Controls (CT-) n=178**	
Nugent score				
Categorization	**Index**	**Index-1**	**Index**	**Index-1**
2 categories				
0-6 (BV-)	50 (56.2)	50 (56.2)	140 (78.7)	136 (76.4)
7-10 (BV+)	39 (43.8)	39 (43.8)	38 (21.4)	42 (23.6)
3 categories				
0–3 (BV-)	39 (43.8)	41 (46.1)	119 (66.9)	116 (65.2)
4–6 (intermediate)	11 (12.4)	9 (10.1)	21 (11.8)	20 (11.2)
7–10 (BV+)	39 (43.8)	39 (43.8)	38 (21.4)	42 (23.6)
Amsel's criteria				
Categorization	**Index**	**Index-1**	**Index**	**Index-1**
BV-	77 (86.5)	70 (78.7)	161 (90.5)	164 (92.1)
BV+	12 (13.5)	19 (21.4)	17 (9.6)	14 (7.9)

1 Adjusted for age, years of sex work, and contraceptive use
Index = CT+ visit preceded by CT- visit
Index-1 = Visit prior to the index visit

Table 3: Multivariate conditional logistic regression analysis describing odds of *Chlamydia trachomatis* infection, by bacterial vaginosis status at index-1 visit

	Cases (CT+) n=89	**Controls (CT-) n=178**	**Unadjusted**			**Adjusted[1]**		
Nugent score								
Categorization	**Index-1**		**OR**	**95% CI**	***P***	**OR**	**95% CI**	***P***
2 categories								
0-6 (BV-)	50 (56.2)	136 (76.4)	Ref.	–	–	Ref.	–	–
7-10 (BV+)	39 (43.8)	42 (23.6)	2.50	1.44–4.33	0.001	2.03	1.07–3.88	0.031
3 categories								
0–3 (BV-)	41 (46.1)	116 (65.2)	Ref.	–	–	Ref.	–	–
4–6 (intermediate)	9 (10.1)	20 (11.2)	1.13	0.47–2.73	0.781	1.06	0.38–2.97	0.905
7–10 (BV+)	39 (43.8)	42 (23.6)	2.53	1.45–4.43	0.001	2.04	1.07–3.91	0.031
Amsel's criteria								
Categorization	**Index-1**		**OR**	**95% CI**	***P***	**OR**	**95% CI**	***P***
BV-	70 (78.7)	164 (92.1)	Ref.	–	–	Ref.	–	–
BV+	19 (21.4)	14 (7.9)	3.10	1.46–6.58	0.003	2.18	0.86–5.53	0.101

1 Adjusted for age, years of sex work, and contraceptive use
OR = Odds ratio
CI = Confidence Interval
P = p-value
Index-1 = Visit prior to the index visit

Table 4: Discordant and concordant bacterial vaginosis status at the index and index-1 visits using Nugent score and Amsel's criteria & multivariate conditional logistic regression analysis describing odds of *Chlamydia trachomatis* infection, by bacterial vaginosis status

		Cases (CT+) n=89	**Controls (CT-) n=178**	**Unadjusted**			**Adjusted[1]**		
BV status		***Nugent score***							
Index	**Index-1**			**OR**	**95% CI**	***P***	**OR**	**95% CI**	***P***
(–)	(–)	36 (40.4)	121 (68.0)	Ref.	–	–	Ref.	–	–
(–)	(+)	14 (15.7)	19 (10.7)	2.42	1.10–5.32	0.028	1.76	0.69–4.51	0.237
(+)	(–)	14 (15.7)	15 (8.4)	3.28	1.32–8.14	0.010	2.36	0.80–6.98	0.120
(+)	(+)	25 (28.1)	23 (12.9)	3.37	1.72–6.60	0.000	2.67	1.20–5.93	0.016
BV status		***Amsel's criteria***							
Index	**Index-1**			**OR**	**95% CI**	***P***	**OR**	**95% CI**	***P***
(–)	(–)	64 (71.9)	155 (87.1)	Ref.	–	–	Ref.	–	–
(–)	(+)	13 (14.6)	7 (3.9)	4.80	1.79–12.85	0.002	3.47	1.07–11.25	0.038
(+)	(–)	6 (6.7)	9 (5.1)	1.73	0.59–5.05	0.315	2.33	0.63–8.66	0.206
(+)	(+)	6 (6.7)	7 (3.9)	1.82	0.59–5.64	0.302	1.22	0.30–4.93	0.779

1 Adjusted for age, years of sex work, and contraceptive use
OR = Odds ratio
CI = Confidence Interval
P = p-value
Index = CT+ visit preceded by CT- visit
Index-1 = Visit prior to the index visit

Table 5: Sensitivity analysis to address the impact of longer visit intervals on the association between bacterial vaginosis by Nugent score and Amsel's criteria and *Chlamydia trachomatis* infection among cases and matched controls

	Cases (CT+) n=67	**Controls (CT-) n=137**	**Unadjusted**			**Adjusted[1]**		
Nugent score								
Categorization	**≤134 days**	**≤42 days**	**OR**	**95% CI**	***P***	**OR**	**95% CI**	***P***
2 categories								
0-6 (BV-)	36 (53.7)	107 (78.1)	Ref.	–	–	Ref.	–	–
7-10 (BV+)	31 (46.3)	30 (21.9)	2.95	1.46–5.96	0.003	2.89	1.18–7.10	0.021
3 categories								
0–3 (BV-)	30 (44.8)	90 (65.7)	Ref.	–	–	Ref.	–	–
4–6 (intermediate)	6 (9.0)	17 (12.4)	0.56	0.18–1.80	0.334	0.44	0.11–1.86	0.266
7–10 (BV+)	31 (46.3)	30 (21.9)	2.82	1.39–5.72	0.004	2.82	1.14–7.00	0.025
Amsel's criteria								
Categorization	**≤134 days**	**≤42 days**	**OR**	**95% CI**	***P***	**OR**	**95% CI**	***P***
BV-	51 (76.1)	126 (92.0)	Ref.	–	–	Ref.	–	–
BV+	16 (23.9)	11 (8.0)	4.17	1.61–10.78	0.003	3.81	1.03–14.16	0.046

1 Adjusted for age, years of sex work, and contraceptive use.
Cases and controls were restricted to participants with index and index-1 visit intervals which excluded participants with longer intervals between the index and index-1 visit (i.e., the highest quartile of days between visits). Cases were limited to those within 134 days, and controls were limited to those within 42 days.
OR = Odds ratio
CI = Confidence Interval
P = p-value