Interventions to Improve Vaginal Health in Kenyan Women

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Abstract

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Introduction: Vaginal infections, including bacterial vaginosis (BV), vulvovaginal candidiasis (VVC) and *Trichomonas vaginalis* (TV), are highly prevalent among reproductive-aged women and are associated with a number of adverse reproductive health outcomes. Interventions that improve vaginal health could have a substantial impact on reproductive health.

Methods: Data from women enrolled in a randomized trial of periodic presumptive treatment (PPT) for vaginal infections were used to assess the effect of PPT on: (1) establishing and sustaining a healthy vaginal environment; and (2) BV and *Lactobacillus* colonization in the 3-month interval following completion of the trial. Data from women enrolled in an open cohort study of female sex workers were used to compare the effect of single-dose 2g oral metronidazole on TV infection in HIV-1-seropositive versus HIV-1-seronegative women.

Results: The incidence of a healthy vaginal environment was 608 per 100 person-years in the intervention arm and 454 per 100 person-years in the placebo arm (hazard ratio [HR]=1.36; 95% confidence interval [CI] 1.17–1.58). Sustained vaginal health (healthy vaginal environment for ≥3 consecutive visits) was also more frequent in the intervention arm (HR=1.69; 95% CI 1.23–2.33).

The post-trial incidence of BV was 260 per 100 person-years in the intervention arm versus 358 per 100 person-years in the placebo arm (HR=0.76; 95% CI 0.51–1.12). The post-trial incidence of *Lactobacillus* colonization was 180 per 100 person-years in the intervention arm versus 127 per 100 person-years in the placebo arm (HR=1.42; 95% CI 0.85–2.71).

There were 42 of 282 (15%) persistent infections among HIV-seropositive women versus 35 of 288 (12%) among HIV-seronegative women (adjusted odds ratio [aOR]=1.26; 95% CI 0.75-2.12). TV infection with concurrent BV by Gram stain was associated with an increased likelihood of persistent TV (aOR=1.82; 95% CI 1.11-2.99).

Conclusions: Periodic presumptive treatment is effective at establishing and sustaining a healthy vaginal environment. However, its effect on BV and *Lactobacillus* colonization was not sustained after cessation of the intervention. The frequency of persistent TV following treatment with single-dose metronidazole was similar by HIV-1 status. Alternative regimens may be necessary to improve cure rates for women with TV and concurrent BV.

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Introduction

Vaginal infections, including bacterial vaginosis (BV), vulvovaginal candidiasis (VVC) and *Trichomonas vaginalis* (TV), are highly prevalent among HIV-1-seronegative and HIV-1-seropositive reproductive-aged women.[1-8] Among HIV-1-seronegative women, multiple prospective studies have demonstrated associations between vaginal infections and an increased risk of HIV-1 acquisition[7,9-17], other sexually transmitted infections (STIs)[18-23], and adverse reproductive health outcomes.[24-31] Conversely, women colonized with *Lactobacillus* species, in particular hydrogen peroxide (H_2O_2) producing species, may have a reduced risk of acquiring HIV-1 and other STIs.[12,32,33] Vaginal infections are also commonly diagnosed among HIV-1-seropositive women[7,34-39] and are associated with adverse reproductive health outcomes[40] as well as increases in genital shedding of HIV-1 RNA.[34,41-43] Treatment of vaginal infections can decrease genital HIV-1 shedding[42,44], potentially reducing the risk of transmission to HIV-1-uninfected partners.[45]

The vaginal microenvironment plays an important role in mediating HIV-1 and STI susceptibility and transmission potential.[46-56] Interventions that improve and sustain vaginal health by increasing the prevalence and quantity of *Lactobacillus* species and reducing the incidence of BV, VVC and TV could have a substantial impact on the reproductive health of both HIV-1-seronegative and HIV-1-seropositive women. In order to improve our understanding of the vaginal environment and factors associated with vaginal health, the analyses presented in this dissertation utilized data and samples collected from female commercial sex workers in Mombasa, Kenya enrolled in two research studies. These data were analyzed to accomplish the following specific aims: (1) To assess the effect of periodic presumptive treatment (PPT) on establishing and sustaining a healthy vaginal environment among women enrolled in a randomized trial; (2) To assess the post-trial effect of PPT on BV and *Lactobacillus* colonization in the 3-month interval following completion of the randomized trial; and (3) To compare the

effect of single-dose 2g oral metronidazole on TV infection in HIV-1-seropositive versus HIV-1-seronegative women among women enrolled in a prospective open cohort study. The information generated from completion of these specific aims contributes to our overall understanding of vaginal health and will inform the development of interventions to reduce susceptibility to HIV-1, STIs and adverse reproductive health outcomes.

Chapter 1.

Establishing and sustaining a healthy vaginal environment: analysis of data from a randomized trial of periodic presumptive treatment for vaginal infections

1.1 Background

Vaginal conditions including BV, VVC, TV, and abnormal vaginal flora are highly prevalent among reproductive-aged women. Mixed infections, where more than one vaginal condition is present at the same time, are also common. Disturbances in the vaginal environment due to abnormal vaginal flora and vaginal infections have been associated with increased risk of STIs, including HIV-1.[13,15,17,18,57] Conversely, the presence of normal vaginal flora has been associated with the lowest risk of HIV-1 and STI acquisition.[12-14]

Interventions that improve vaginal health by establishing and sustaining a healthy vaginal environment, defined as the absence of any vaginal infections or abnormal vaginal flora, may reduce susceptibility to HIV-1 and other STIs. Recently, a randomized controlled trial (RCT) assessed the effect of monthly periodic presumptive treatment with 2g of oral metronidazole plus 150mg of fluconazole versus placebo on the incidence of BV, VVC and TV (each analyzed as separate outcomes) among Kenyan women.[58] The intervention reduced the incidence of BV and increased colonization with *Lactobacillus* species. In this secondary analysis, we take an additional step, assessing the effect of PPT on establishing and sustaining a healthy vaginal environment.

1.2 Methods

Detailed methods for the RCT have been published.[58] Briefly, the trial was conducted in Mombasa, Kenya between May 2003 and December 2006. Female sex workers enrolled in an open cohort study of risk factors for HIV-1 acquisition[59] were eligible to participate if they were

HIV-1-seronegative, 18-45 years old, and non-pregnant. To avoid open-label treatment at enrollment, women with abnormal vaginal discharge or itching were ineligible to enroll. The study was approved by the institutional review boards at the University of Nairobi/Kenyatta National Hospital (Nairobi, Kenya) and the University of Washington (Seattle, USA). All participants provided written informed consent.

At enrollment and each of 12 monthly follow-up visits, a brief face-to-face interview was conducted to collect information on medical and sexual history. A physical examination, including speculum-assisted pelvic examination, was performed with collection of specimens for diagnosis of genital tract infections. Blood was collected for HIV-1 testing and a urine pregnancy test was performed. Participants were randomized to receive 2g of metronidazole plus 150mg of fluconazole or identical placebo monthly. At monthly visits, study product was administered orally as directly observed treatment. Women who reported abnormal vaginal discharge or vulvovaginal itching were treated syndromically with a single 2g dose of oral metronidazole plus clotrimazole 200mg vaginal suppositories nightly for 3 nights. When this treatment was dispensed, study product was withheld. Other genital infections were treated according to World Health Organization guidelines.[60]

All laboratory procedures were performed in Mombasa. A Gram stain of vaginal fluid was evaluated for diagnosis of BV by Nugent criteria.[61] A vaginal saline wet mount was examined microscopically for the presence of motile trichomonads and fungal elements. A drop of 10% potassium hydroxide was added to the slide and evaluated again for the presence of yeast buds or hyphae. Culture for TV was performed in Diamond's modified medium. HIV-1 testing was performed using an ELISA (Detect-HIV [BioChem ImmunoSystems]). A positive ELISA result was confirmed using a second ELISA (Recombigen [Cambridge Biotech] or Vironostika [bioMerieux]).

The objectives of this analysis were to assess the effect of the intervention on the presence of a healthy vaginal environment (defined as a Nugent score of 0-3 with no yeast on

wet mount and no TV on wet mount or culture) and the presence of sustained vaginal health (defined as the presence of a healthy vaginal environment for ≥3 consecutive visits). Women were included in the analysis if they were randomized and returned for at least one follow-up visit. We conducted an intent-to-treat analysis using an Andersen-Gill proportional hazards model that allows for recurrent events to estimate the effect of the intervention versus placebo on the frequency of a healthy vaginal environment during follow-up. Participants were censored if they became pregnant, HIV-1-seropositive, or at 420 days following date of enrollment (administrative censoring).

Since the outcome for the sustained vaginal health analysis was a composite variable based on vaginal health status at three consecutive visits, this analysis was restricted to women who were returned for ≥3 follow-up visits. We compared baseline characteristics by study arm using chi-squared tests for categorical outcomes and Wilcoxon rank sum tests for continuous outcomes. We used Kaplan-Meier survival analysis, the log-rank test, and a Cox proportional hazards model to estimate the effect of the intervention on the incidence of sustained vaginal health during follow-up. We conducted sensitivity analyses in which we repeated our analysis using the definitions of ≥2 and ≥4 consecutive follow-up visits with a healthy vaginal environment. All statistical tests used a 2-sided alpha of 0.05. Analyses were conducted using Stata version 11.0 (StataCorp, Inc., College Station, TX).

1.3 Results

This trial screened 378 women of whom 310 were enrolled. Demographic and clinical characteristics of enrolled participants have been presented previously, and were similar between study arms [8]. Briefly, the median age, duration of sex work, and number of sexual episodes in the past week were: 32 years (interquartile range [IQR]: 27-38), 4 years (IQR: 1-9), and 1 episode (IQR: 0-2), respectively. Condom use was high, with a median of 100% use (IQR: 0-100) among those who reported sex in the past week. The majority of women (266 women

[94%]) reported vaginal washing in the past week, 88 (29%) reported using hormonal contraception, and 106 (35%) had a healthy vaginal environment at enrollment. Three hundred and two women returned for ≥1 follow-up visit (151 women in each arm). The number of follow-up visits attended was also similar between study arms (median [IQR] in the intervention arm = 12 [7-12] versus 12 [9-12] in the placebo arm; p = 0.7). The intervention was well tolerated with similar rates of adverse events reported by arm.[58]

The frequency of a healthy vaginal environment is presented in Table 1.1. The proportion of women who had a healthy vaginal environment at every visit was identical between the study arms (6 [4%] women in each arm, p=1.0). Conversely, the proportion of women who never had a healthy vaginal environment was lower in the intervention arm than the placebo (9 [6%] versus 24 [16%]; p=0.006). Women in the intervention arm were more likely to have a healthy vaginal environment at any visit than women in the placebo arm (hazard ratio [HR] 1.36, 95% confidence interval [CI] 1.17–1.58) (Table 1.1).

We also conducted an exploratory analysis stratifying by vaginal conditions present at enrollment (Table 1.1). Among women who had a healthy vaginal environment at enrollment, the intervention had no effect on the presence of a healthy vaginal environment during follow-up. However, among women who did not have a healthy vaginal environment at enrollment, those in the intervention arm had an increased incidence of a healthy vaginal environment during follow-up compared to placebo.

The analysis of sustained vaginal health included 284 women who attended ≥ 3 follow-up visits, comprising 142 women in each arm. Women included in this analysis were slightly older (median age: 32 versus 28 years; p=0.002) and reported a longer duration of sex work (median duration: 4 versus 2 years; p<0.001) compared to women who were excluded due to <3 follow-up visits. Baseline characteristics were similar by study arm among the sub-group who attended ≥3 follow-up visits (data not shown). There were 151 episodes of sustained vaginal health (Table 1.1). Median time to sustained vaginal health was 30 weeks in the intervention

arm versus 56 weeks in the placebo arm (log-rank test p=0.001) (Figure 1.1). Women in the intervention arm were more likely to have sustained vaginal health than women in the placebo arm (HR 1.69; 95% CI 1.23–2.33). Results were similar in sensitivity analyses assessing the effect of the intervention using shorter (≥2 months) or longer (≥4 months) periods to define sustained vaginal health (data not shown).

1.4 Discussion

In this secondary analysis, monthly oral treatment with metronidazole and fluconazole increased both the frequency of having a healthy vaginal environment and the incidence of sustained vaginal health compared to placebo. In addition, we observed that the impact of the intervention was greatest among women lacking a healthy vaginal environment at enrollment. This analysis complements the primary RCT analysis that reported a decrease in the incidence of BV and an increase in *Lactobacillus* colonization.[58] Interventions that promote the establishment and maintenance of a healthy vaginal environment are important for potentially reducing susceptibility to HIV-1 and STIs.

The issue of sustained vaginal health is of critical importance given the prevalence of vaginal infections and the frequency with which they recur. A prospective study of 121 women treated for symptomatic BV reported that 23% experienced another episode of symptomatic BV within 1 month and 58% experienced a recurrence by month 12.[62] In addition, vaginal infections can occur in combination (mixed infections) or sequentially. Bacterial vaginosis and TV are frequently detected together [7], and several studies have reported an association between BV treatment with metronidazole and subsequent symptomatic VVC.[62,63] Finally, when considering the question of vaginal health, it is important to note that the absence of vaginal infections does not guarantee the presence of healthy vaginal flora; abnormal vaginal flora (Nugent score 4-6) has also been associated with increased risk of HIV-1 acquisition.[12]

We observed equal proportions of women in both arms who experienced a healthy vaginal environment at every follow-up visit. It is possible that microbiologic, immunologic, behavioral, or other host factors that are unique to these women may contribute to reduced susceptibly to abnormal flora and vaginal infections. Identifying factors that are associated with prolonged vaginal health could inform the development of future vaginal health interventions.

With repeated use of antibiotic regimens, it is important to consider the issue of antimicrobial resistance. A study of resistance patterns associated with BV treatment reported metronidazole resistance in <1% of anaerobic bacterial isolates.[64] While resistance was not measured in our trial, these results suggest that metronidazole resistance is likely to be infrequent.

The high rate of retention in the trial, combined with monthly measurement of biological outcomes, allowed us to gain a substantial level of precision in this longitudinal characterization of vaginal health. Nonetheless, these findings should be interpreted in the context of several limitations. First, this is a secondary analysis in which our sustained vaginal health analysis uses a sub-group of participants from the RCT, potentially introducing bias. Because the endpoint of sustained vaginal health required 3 months of follow-up, the analysis necessarily restricted the population to women who had at least three follow-up evaluations. Second, the selection of our definition of sustained vaginal health was somewhat arbitrary. We hypothesized that 3 consecutive visits with a healthy vaginal environment reflected a more substantial shift in the vaginal flora compared to 2 visits. While the clinical implications of having sustained vaginal health have not yet been explored, we are reassured by the consistency of the findings observed in the sensitivity analyses. Third, wet mount microscopy alone was used to diagnose VVC. The combination of wet mount with culture may provide greater sensitivity and specificity. Lastly, behavioral characteristics that are unique to the study population could limit generalizability. Women in our study reported high rates of vaginal washing and condom use. It is possible that the effect of the intervention may differ in other populations.

There is mounting evidence that disruptions in the vaginal environment due to vaginal infections and abnormal flora increase susceptibility to HIV-1 infection. Among women at risk for HIV-1 infection, PPT could be used to promote vaginal health and potentially reduce susceptibility to HIV-1. As antiretroviral-based HIV-1 prevention strategies such as tenofovir gel continue to be evaluated[65], interventions that promote or preserve a healthy vaginal environment could be used to augment the effect of these HIV-1 prevention strategies, especially if antiretroviral-based strategies provide only partial protection. Additional studies are needed to assess the effects of a healthy vaginal environment and sustained vaginal health on risk of HIV-1 and STI acquisition.

Table 1.1 Frequency of a healthy vaginal environment and sustained vaginal health by study arm

	Occurrences	Rate per 100 person-years (95% CI)	Hazard Ratio (95% CI)
Healthy vaginal environment [a]			
All women			
Intervention (n=151)	815	608 (568 – 652)	1.36 (1.17 - 1.58)
Placebo (n=151)	635	454 (420 – 491)	1.0 (Reference)
Women with a healthy vaginal environment at enrollment			
Intervention (n=59)	365	676 (610 – 749)	0.99 (0.84 – 1.17)
Placebo (n=47)	293	679 (605 – 761)	1.0 (Reference)
Women without a healthy vaginal environment at enrollment			
Intervention (n=92)	450	563 (513 – 617)	1.63 (1.32 - 2.02)
Placebo (n=104)	342	354 (318 – 393)	1.0 (Reference)
Sustained vaginal health [b]			
Intervention (n=142)	86	102 (83 – 126)	1.69 (1.23 – 2.33)
Placebo (n=142)	65	63 (49 – 80)	1.0 (Reference)

[a] Nugent score of 0-3 with no yeast on wet mount and no *T. vaginalis* on wet mount or culture
[b] Healthy vaginal environment at ≥3 consecutive follow-up visits

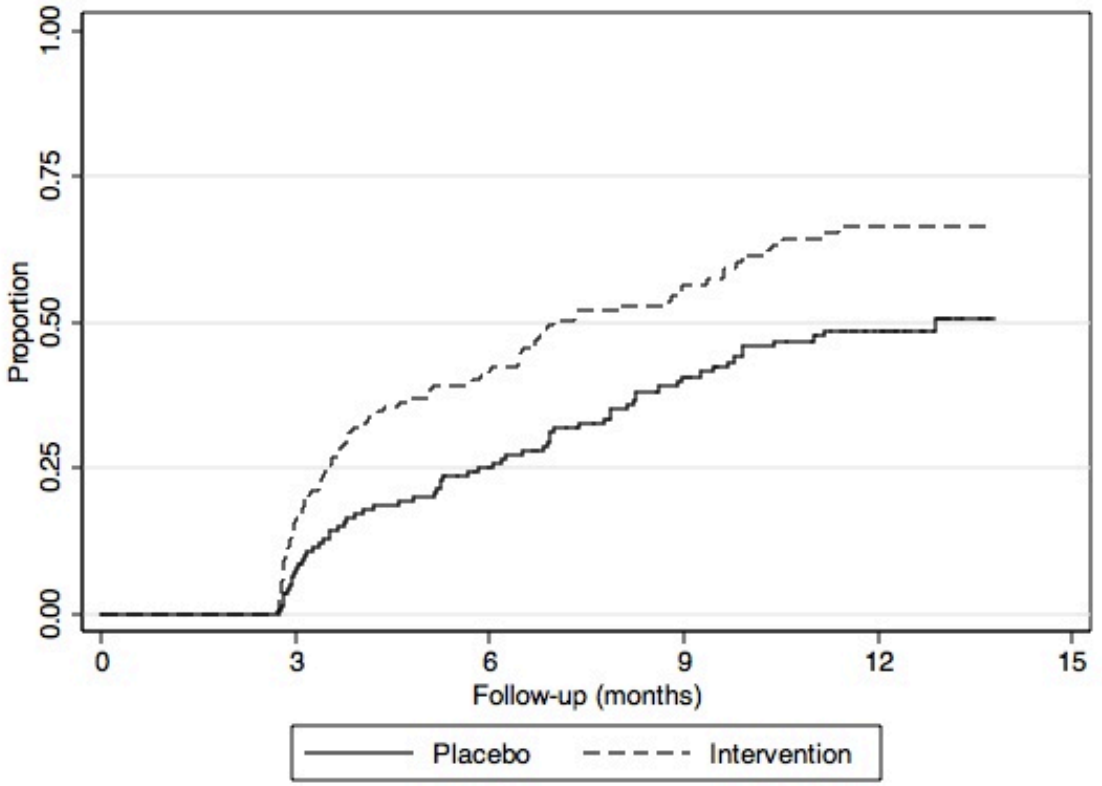

Figure 1.1 Cumulative incidence of sustained vaginal health

Chapter 2.

The post-trial effect of periodic presumptive treatment for vaginal infections on the incidence of bacterial vaginosis and *Lactobacillus* colonization

2.1 Background

Bacterial vaginosis is a common vaginal infection that is associated with an increased risk of HIV-1 acquisition, other STIs, and adverse reproductive health outcomes.[15,18,24,25,31] Although there are several bacterial species that are frequently detected among women with BV, the exact etiology of this condition remains unknown. Standard antibiotic regimens for treatment of BV result in cure rates >75%.[66,67] However, approximately one-third of women will experience another episode of BV within 3 months of treatment and more than half will have a recurrence within one year.[62,63,68] Given the high prevalence of BV and the frequency with which it recurs, alternative treatment regimens are needed that reduce the incidence and recurrence of BV.

Suppressive antibiotic therapy has been shown to effectively reduce BV recurrence. A clinical trial compared topical twice-weekly administration of 0.75% metronidazole vaginal gel versus placebo among women who were successfully treated for BV at enrollment.[63] This trial demonstrated significant reductions in BV among women in the intervention arm after 16 weeks of study product use. Following the completion of suppressive therapy, the probability of recurrent BV by Amsel's criteria[69] was 30% in the intervention arm versus 61% in the placebo arm (p=0.001). Unfortunately, the effect of the intervention did not persist in the absence of treatment. After 12 weeks of post-intervention follow-up, the probability of recurrent BV was 66% in the intervention arm versus 82% in the placebo arm.

It is possible that longer durations of suppressive therapy are required to facilitate changes in the vaginal microbiota that will persist following the completion of treatment,

resulting in lower rates of BV recurrence. Previously, we conducted a randomized controlled trial of suppressive therapy using oral PPT to reduce vaginal infections among Kenyan women.[58] We observed a decrease in BV (hazard ratio [HR] = 0.55; 95% confidence interval [CI], 0.49–0.63) and an increase in *Lactobacillus* colonization (HR = 1.47; 95% CI, 1.19–1.80) among women randomized to receive oral 2g metronidazole plus 150mg fluconazole monthly for 12 months. After the trial, women were invited to continue follow-up in an open cohort study. Given the significant reduction in BV and increase in *Lactobacillus* colonization achieved after 12 months of PPT, we wondered if this intervention might have created vaginal conditions that would result in continued normal vaginal flora after the intervention was completed. Therefore, we analyzed post-trial data to test the hypothesis that the treatment effect would persist in the absence of PPT.

2.2 Methods

Female sex workers enrolled in a prospective, open cohort study of risk factors for HIV-1 acquisition were recruited to participate in the RCT.[59] Detailed methods for the RCT have been published.[58] Briefly, the trial was conducted in Mombasa, Kenya between May 2003 and December 2006 and enrolled women if they were 18-45 years of age, not pregnant, HIV-1-seronegative and had no abnormal vaginal discharge or itching at enrollment. Both the open cohort study and the RCT received approval from the institutional review boards at Kenyatta National Hospital/University of Nairobi (Nairobi, Kenya) and the University of Washington (Seattle, USA). All participants provided separate written informed consent for the cohort study and the RCT.

At enrollment in the RCT and each of 12 monthly follow-up visits, a face-to-face interview was conducted to collect information on medical history, sexual history and vaginal washing practices. A physical examination, including speculum-assisted pelvic examination, was performed with collection of specimens for diagnosis of genital tract infections. A urine

pregnancy test was performed and blood was collected for HIV-1 testing. Participants were randomized to receive oral 2g of metronidazole plus 150mg of fluconazole or identical placebo. At monthly visits, study product was administered orally as directly observed treatment. Women reporting abnormal vaginal discharge or vulvovaginal itching were treated syndromically with a single dose of 2g oral metronidazole plus clotrimazole 200mg vaginal suppositories nightly for 3 nights. Study product was withheld when this treatment was dispensed. Other genital tract infections were treated according to World Health Organization and Kenya Ministry of Health guidelines. At completion of the RCT, women were invited to resume follow-up in the open cohort study. Women who resumed follow-up continued with standard procedures for the cohort, which were similar to those conducted during the RCT.

All laboratory procedures for the RCT and the cohort study were performed in Mombasa, Kenya. A Gram stain of vaginal fluid was evaluated for diagnosis of BV by Nugent criteria.[61] A vaginal saline wet mount was examined microscopically for the presence of motile trichomonads and fungal elements. A drop of 10% potassium hydroxide was added to the slide and evaluated again for the presence of yeast buds or hyphae. *Trichomonas vaginalis* culture was performed in Diamond's modified medium. *Lactobacillus* culture was performed on Rogosa agar.[12] Sub-culture of *Lactobacillus* isolates on tetramethylbenzdine agar containing horseradish peroxidase was performed to H_2O_2 production.[70] Endocervical secretions were cultured on modified Thayer–Martin media for *N. gonorrhoeae.* A Gram stain of endocervical secretions was evaluated for cervicitis, defined as the presence of an average of ≥30 polymorphonuclear leukocytes per high-power field on microscopic examination (original magnification X100). HIV-1 testing was performed using an ELISA (Detect-HIV [BioChem ImmunoSystems]). Positive ELISA results were confirmed using a second ELISA (Recombigen [Cambridge Biotech] or Vironostika [bioMerieux]).

The objective of this analysis was to test the hypothesis that the treatment effect would persist following completion of one year of PPT. The study population consisted of non-

pregnant, HIV-1-seronegative women who completed all 12 RCT visits and attended ≥1 cohort study visit within 120 days of their final RCT visit. We decided *a priori* to include only the first 3 visits that occurred within the 120-day post-trial period. We used descriptive statistics, chi-squared tests for categorical outcomes and Wilcoxon rank sum tests for continuous outcomes to summarize and compare demographic and clinical characteristics by study arm. The same methods were used to compare women included in the analysis versus those excluded (factors evaluated are listed in Table 2.1). Andersen-Gill proportional hazards models that allow for recurrent events were used to estimate the post-trial effect of the intervention versus placebo on our primary outcomes: the incidence of BV by Gram stain (Nugent score ≥7) and any *Lactobacillus* species by culture. Secondary outcomes included abnormal vaginal flora (Nugent score >3) and H_2O_2-producing *Lactobacillus* species by culture. Demographic and clinical characteristics that were unbalanced by study arm at the final RCT visit ($p \leq 0.10$), which served as the baseline visit for this analysis, were included in the final statistical models. To better understand the effect of the intervention on BV during and after the trial, we conducted a descriptive analysis in which we calculated the incidence of BV at each RCT and post-trial follow-up visit. All statistical tests were assessed using a 2-sided alpha of 0.05. Analyses were conducted using Stata version 11.0 (StataCorp, Inc., College Station, TX).

2.3 Results

The RCT enrolled 310 participants (155 per arm), of whom 208 completed all 12 RCT visits. Of these 208, 165 participants (83 intervention and 82 placebo) returned for a cohort study visit within 120 days of their last RCT visit and were included in this analysis. Participants who returned within 120 days were slightly older (median (IQR): 34 years (30 – 40) versus 31 year (27 – 36); $p<0.001$) and reported a longer duration of sex work (median (IQR): 7 years (3-12) versus 4 years (2 – 7); $p<0.001$) compared to those who did not return following completion of the RCT.

All participants included in this analysis had at least one post-trial follow-up visit within 120 days of their last RCT visit, 147 participants had two visits (72 intervention versus 75 placebo) and 103 had three visits (56 intervention versus 47 placebo). The minimum time to the first post-trial visit was 25 days in the intervention arm and 26 days in the placebo arm. At the final RCT visit, demographic and behavioral characteristics were generally similar by study arm (Table 2.1). However, there was a trend towards a higher proportion of women in the intervention arm reporting vaginal washing in the last week compared to women in the placebo arm (94% versus 85%; p=0.10). Clinical factors were similar by arm, with the exception of BV and abnormal vaginal flora. The prevalence of BV at the final RCT visit was 17% in the intervention arm and 43% in the placebo arm ($p<0.001$) and the prevalence of abnormal vaginal flora was 33% in the intervention arm and 61% in the placebo arm ($p<0.001$)

The post-trial incidence of BV, abnormal vaginal flora, any *Lactobacillus* colonization and H_2O_2+ *Lactobacillus* colonization are presented in Table 2.2. In the post-trial period, we observed a lower incidence of BV (260 per 100 person-years versus 358 per 100 person-years) and a higher incidence of any *Lactobacillus* colonization (180 per 100 person-years versus 127 per 100 person-years) among women receiving the intervention compared to women receiving the placebo, respectively. However, the differences were not statistically significant (Table 2.2). The findings were similar for abnormal vaginal flora (420 per 100 person-years versus 536 per 100 person-years) and H_2O_2+ *Lactobacillus* colonization (77 per 100 person-years versus 61 per 100 person years). The results did not differ in multivariable models adjusting for vaginal washing in the past week. To better understand the effect of the intervention over time in the study population, we calculated the incidence of BV at each visit interval during the RCT and in the post-trial interval by study arm. Although we observed an overall decrease in the incidence of BV in the intervention arm during the trial, following the cessation of the intervention, the incidence of BV in the intervention arm increased during the post-trial period (Figure 2.1). Thus, the effect of the intervention appears to wane following cessation of the intervention.

2.4 Discussion

In this analysis of post-trial data, the effect of monthly PPT with 2g metronidazole plus 150mg fluconazole was not sustained during the 120 days following cessation of the intervention. In the RCT, we observed a 45% reduction in the risk of BV and a 47% increase in the likelihood of *Lactobacillus* colonization in the intervention arm.[58] Though the incidence of BV was lower in the intervention arm than the placebo during the post-trial period, the magnitude of the difference was less than what was observed during the RCT. In addition, we observed that the incidence of BV in the placebo arm decreased following the completion of the trial (Figure 2.1). This decrease might be due to chance and could reflect normal fluctuations in the vaginal microbiota. More importantly, we observed a gradual increase in the post-trial incidence in the intervention arm, which suggests that the suppressive effect of the intervention on BV diminished following cessation. Conversely, the magnitude of the difference in *Lactobacillus* colonization was similar to what was observed during the RCT. However, fewer participants and a shorter duration of follow-up contributed to a reduction in study power resulting in a difference in *Lactobacillus* colonization that was not statistically significant. Vaginal washing, which has been associated with an increased risk of BV,[71,72] differed slightly by arm in the post-trial period, although adjustment for this behavior produced similar results. Our findings are similar to those of a trial of suppressive therapy using biweekly 0.75% metronidazole vaginal gel.[63] The reduced effect of PPT and suppressive therapy following the completion of treatment highlights the need for new interventions that reduce BV recurrence and promote *Lactobacillus* colonization without the need for ongoing treatment.

Advances in our ability to characterize the vaginal microbiota using cultivation-independent methods, such as fluorescent in situ hybridization (FISH) and 16S rRNA polymerase chain reaction (PCR) assays, have shed light on microbiological factors associated with BV recurrence and persistence.[73-76] Recent evidence suggests that the high rate of recurrent BV could be due to the formation of adherent biofilms of BV-associated bacteria.[73,74]

An evaluation of vaginal biopsies from women with and without BV by Amsel's criteria showed that a dense bacterial biofilm was observed using FISH among 90% of biopsy specimens from subjects with BV and 10% of subjects without BV ($p<0.001$).[73] These biofilms were composed primarily *Gardnerella* and *Atopobium*, two species highly associated with BV.[76-78] A follow-up study among women with BV (positive by both Amsel's criteria and Nugent's score) who were treated with 500mg metronidazole twice daily for 7 days reported a decrease in biofilms in the 7 days following treatment. However, despite decreases in Nugent score (<7) and the absence of symptoms among all participants, the biofilm re-emerged in the 5 weeks following treatment.[74] In addition, cultivation-resistant species identified through species-specific 16S rRNA PCR assays have been associated with BV persistence.[75] Among women with symptomatic BV who were treated with vaginal metronidazole gel, women with persistent BV one month post-treatment were more likely to have BVAB1, BVAB2, BVAB3, *Megasphaera*, and *Peptoniphilus lacrimalis* detected in vaginal samples at baseline compared to women who experienced clinical cure (defined as < 3 Amsel's criteria).

Eradication or more profound reduction in the concentrations of certain critical BV-associate species might be required to significantly reduce rates of BV recurrence after treatment. This conceivably could be accomplished by higher doses and/or longer courses of antibiotic treatment. Low cure and high recurrence rates of BV were observed with single dose oral 2g metronidazole, leading to a change in treatment guidelines to multi-day regimens that deliver a higher total dose over a longer duration.[66] However, the cure and recurrence rates with the currently recommended regimens remain unacceptably low and high, respectively. As a result, alternative antibiotic regimens continue to be evaluated. A trial of topical therapy demonstrated that metronidazole 500 mg plus nystatin 100,000 units (co-formulated suppositories) administered for 5 nights was superior to metronidazole 0.75% gel (containing 37.5 mg of metronidazole per dose) also administered for 5 nights.[79] In this study, BV (positive by both Amsel's criteria and Nugent's score) was detected at the first follow-up visit among 7%

of women in the metronidazole plus nystatin arm compared to 26% in the metronidazole gel arm (relative risk = 0.28 [95% CI: 0.11-0.71]). The authors also assessed BV-free survival following treatment. At 104 days following treatment, 33% of women in the metronidazole plus nystatin arm had experienced a recurrent episode of BV compared to 52% of women in the metronidazole arm (p=0.01). Though the rate of BV recurrence was lower in the intervention arm, it is similar to what has been reported by others at approximately 3 months post-treatment with oral metronidazole.[62]

Non-antibiotic approaches to improve BV cure rates and reduce recurrence have also been investigated. With the depletion of lactobacilli commonly observed among women with BV, there is great interest in the use of probiotic regimens to facilitate re-colonization of the vagina with *Lactobacillus* species.[80] It is hypothesized that *Lactobacillus* colonization, in particular colonization with H_2O_2-producing strains, may be responsible for maintaining a low vaginal pH and controlling the growth of other bacteria through their ability to produce lactic acid and H_2O_2.[70,81] A number of small studies have evaluated probiotic regimens administered either alone or in combination with antibiotics or estriol for BV treatment;[82] however, additional evidence is needed to support the use of probiotics for BV treatment. Continued development and rigorous evaluation of treatment regimens, both antibiotic and probiotic, that further improve the cure rate and reduce the recurrence rate of BV are necessary.

The findings from this analysis should be interpreted in the context of several limitations. This is a secondary analysis in which we selected a sub-group of participants from the RCT. Our selection criteria for this analysis resulted in the exclusion of a large number of women either due to incomplete RCT follow-up (<12 visits) or no follow-up visit within the post-trial period. We chose to restrict our analysis to women who completed all 12 follow-up visits, since we hypothesized that if there was a true post-trial effect of the intervention we would be most likely to observe it in this population. By design, our selection criteria resulted in a smaller sample size. In addition, this analysis included a relatively short duration of post-trial follow-up.

As a result, we cannot rule out the persistence of small differences in the incidence of BV and *Lactobacillus* colonization by study arm in the post-trial period. There is the potential for selection bias in sub-group analyses. However, we are reassured by the fact that women who were included in the analysis were generally similar to those excluded. Moreover, among women included in this sub-group analysis, there were minimal differences by study arm. Behavioral characteristics that are unique to the study population could also limit the generalizability of our findings. Women in our study reported high rates of vaginal washing. The post-trial effect of the intervention may differ in populations who report less vaginal washing. Our characterization of the vaginal microbiota was limited to detection of *Lactobacillus* by culture. Therefore, we were unable to assess the effect of the intervention in the post-trial period on other vaginal bacterial species, including BV-associated bacteria. Lastly, we used a single dose metronidazole regimen, which was the recommended treatment during the time that the study was conducted. Currently, single dose regimens are no longer recommended and have been replaced by multi-day regimens as the preferred treatment for BV.[66]

Our findings raise several important points for consideration in future research studies. First, future studies of periodic presumptive or suppressive treatment for BV should continue to assess the effect of intervention following cessation of the treatment regimen. It is unlikely that interventions that require continued treatment will be feasible or acceptable to patients. Therefore, it is critical to evaluate the sustained effect of the intervention following an extended course of suppressive therapy. Second, our study employed culture methods alone to characterize the vaginal microbiota since cultivation-independent methods (16S rRNA PCR) were not widely available at the time the RCT was conducted. With the increasing availability of cultivation-independent methods, future studies may be strengthened through the use of both culture and cultivation-independent methods. Due to the polymicrobial nature of BV and the importance of cultivation-resistant species in this condition, it is critical to understand the immediate and long-term effect of treatment on both cultivatable and cultivation-resistant vaginal

bacterial species. Culture and cultivation-independent methods each have strengths and weakness.[83] The use of combined methods allows for a more complete characterization of the vaginal microbiota and may provide new insights into mechanisms of BV cure, persistence and recurrence.

In summary, PPT is a promising approach for reducing the incidence of BV and increasing *Lactobacillus* colonization. However, the regimens evaluated to date have demonstrated a reduced effect following the cessation of therapy. New interventions that decrease BV recurrence and promote long-term *Lactobacillus* colonization without the need for ongoing PPT or suppressive therapy are needed.

Table 2.1 Demographic and clinical characteristics of participants at baseline visit for this analysis (corresponding to final RCT visit)

	Intervention N=83		Placebo N=82		P-value
Age (years)	34	(29 – 40)	35	(30 - 40)	0.76
Education duration (years)	8	(6 – 8)	8	(7 – 10)	0.13
Duration of sex work (years)	7.6	(4 – 13)	6.2	(3 – 11)	0.17
Sex in the last week	57	(69)	47	(57)	0.13
Unprotected sex in the last week[1]	16	(19)	23	(28)	0.19
Hormonal contraceptive use[2]	23	(28)	24	(29)	0.83
Vaginal washing in the last week	78	(94)	70	(85)	0.10
Bacterial vaginosis	13	(16)	35	(43)	<0.001
Abnormal vaginal flora[3]	27	(33)	50	(61)	<0.001
Vaginal candidiasis	5	(6)	4	(5)	0.75
T. vaginalis infection	0	(0)	1	(1)	0.31
Lactobacillus colonization					
Any species	14	(17)	15	(18)	0.81
H_2O_2 producers	5	(6)	9	(11)	0.25
N. gonorrhoeae infection	0	(0)	1	(1)	0.31
Cervicitis[4]	2	(2)	2	(2)	0.98

N (%) or median (IQR) presented

[1]Unprotected sex in the past week versus protected sex or no sex in the past week. Among 104 women reporting sex in the last week, 28% of women in the intervention arm reported unprotected sex versus 49% in the placebo arm (p=0.03).

[2]Injectables or oral contraceptive pill use

[3]Nugent score >3

[4]Defined as the presence of an average of ≥30 polymorphonuclear leukocytes per high-power field on microscopic examination of Gram-stained cervical secretions (original magnification X100)

Table 2.2 Post-trial frequency of BV, abnormal flora, and *Lactobacillus* colonization by study arm

	Intervention		Placebo		Unadjusted Hazard Ratio (95% CI)		Adjusted Hazard Ratio[2] (95% CI)	
	Frequency	Rate[1] (95% CI)	Frequency	Rate[1] (95% CI)				
Bacterial vaginosis[3]	54	260 (199 – 340)	76	358 (286 – 448)	0.76	(0.51 – 1.12)	0.75	(0.51 – 1.11)
Abnormal vaginal flora[3]	87	420 (340 – 518)	114	536 (446 – 645)	0.81	(0.61 – 1.08)	0.80	(0.60 – 1.07)
Any *Lactobacillus*	39	188 (137 – 257)	27	127 (87 – 185)	1.42	(0.85 – 2.71)	1.57	(0.87 – 2.82)
H_2O_2+ *Lactobacillus*	16	77 (47 – 126)	13	61 (36 – 105)	1.26	(0.54 – 2.95)	1.29	(0.54 – 3.12)

[1] Rate per 100 person-years
[2] Adjusted for vaginal washing in the last week.
[3] BV = Nugent score ≥ 7; Abnormal flora = Nugent score > 3

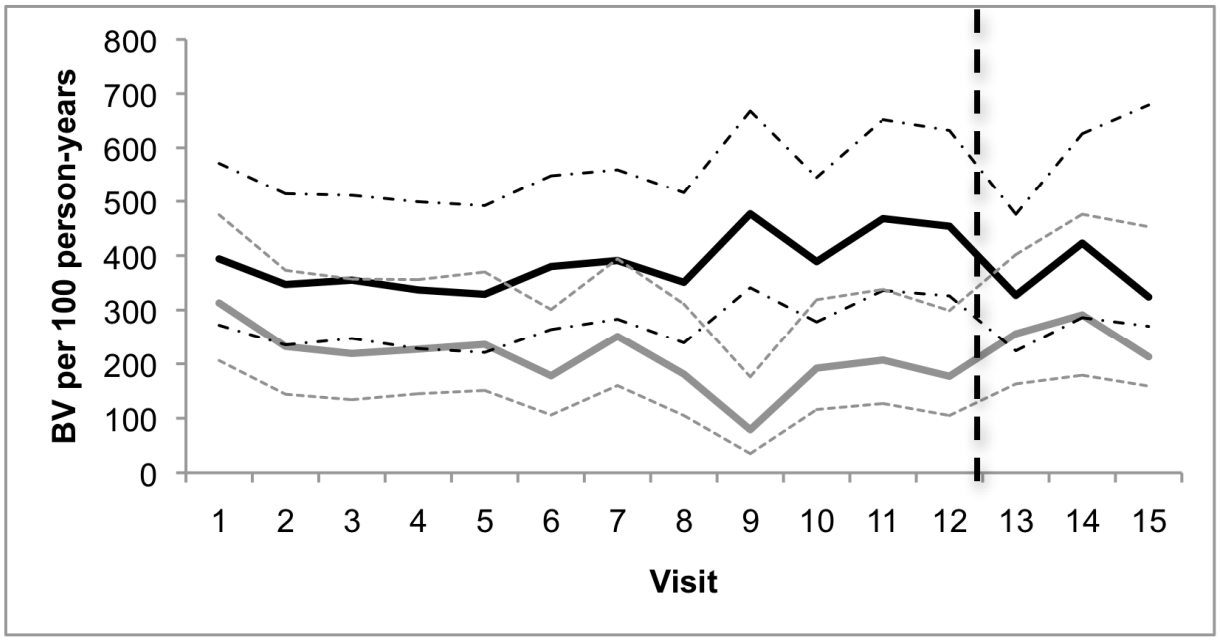

Figure 2.1. Incidence of BV at each RCT visit and at the first three post-trial monthly study visits by RCT study arm[1]

[1]The incidence of BV at each study visit among the 165 women who completed all 12 RCT visits and had at least one cohort study visit within 120 days of completing the RCT

Placebo — 95% Confidence Interval

Intervention — 95% Confidence Interval

The dashed vertical line represents the end of the RCT. Visits to the right of the dashed line occurred during the 120-day post-trial period.

Chapter 3.

A prospective cohort study comparing the effect of single-dose 2g oral metronidazole on *Trichomonas vaginalis* infection in HIV-1-seropositive versus HIV-1-seronegative women

3.1 Background

Trichomonas vaginalis (TV) is a sexually transmitted protozoan that accounts for more than half of all curable sexually transmitted infections (STIs) worldwide.[5] The prevalence of TV is highest among women of reproductive age[4,5,84] and is associated with pelvic inflammatory disease[40,85], adverse pregnancy outcomes[86-88], and increased risk of HIV acquisition.[7,16,17] Vaginal trichomoniasis is also frequently diagnosed among HIV-seropositive women[7,34-39] and has been associated with increased genital shedding of HIV RNA.[34,41,42] Treatment of TV infection can decrease vaginal shedding of HIV[42,44], potentially reducing the risk of transmission to HIV-uninfected partners. [45]

The Centers for Disease Control and Prevention currently recommends single-dose 2g oral metronidazole or tinidazole for treatment of TV infection.[66] However, data are conflicting regarding the effectiveness of single-dose metronidazole treatment among HIV-seropositive women. A study comparing HIV-seropositive women attending an outpatient HIV clinic to HIV-seronegative women at a family planning clinic in the southern United States reported that 11 of 60 (18.3%) HIV-seropositive women with TV infection were still positive 1 month after treatment with 2g single-dose metronidazole compared to 24 of 301 (8%) HIV-seronegative women (p=0.01).[36] Conversely, a study among women attending a primary care clinic in South Africa observed similar proportions of HIV-seropositive and seronegative women who were still positive for TV 8 to 10 days after treatment with 2g oral metronidazole.[35] To improve our understanding of the effectiveness of treatment for TV infection among HIV-seropositive

women, we compared the proportion of HIV-seropositive and seronegative women that were still positive for TV following treatment with single-dose 2g oral metronidazole.

3.2 Methods

Study population and procedures

Data were obtained from women enrolled in an open cohort study of female sex workers in Mombasa, Kenya between February 1993 and December 2010. Detailed methods for the cohort have been described previously.[59] In brief, women 16 years of age or older who presented to the clinic and reported that they engaged in transactional sex underwent confidential HIV counseling and testing. Initially, only HIV-seronegative women were enrolled. Beginning in 2001, HIV-seropositive female sex workers were also invited to enroll. The study received approval from the institutional review boards at Kenyatta National Hospital/University of Nairobi (Nairobi, Kenya), the University of Washington (Seattle, USA), and the Fred Hutchinson Cancer Research Center (Seattle, USA). All women provided informed consent.

At enrollment and each monthly follow-up visit, a standardized face-to-face interview was conducted to collect information on medical history, sexual history, and vaginal washing practices. A physical examination was performed, including speculum pelvic examination with collection of vaginal and cervical secretions for diagnosis of genital tract infections and assessment of vaginal pH. Participants who reported symptoms of a genital tract infection were treated syndromically at the same visit according to the Kenyan Ministry of Health Guidelines (based on current World Health Organization Guidelines).[89] Among HIV-seronegative participants, blood was collected by venipuncture each month for HIV testing. Among HIV-seropositive participants, blood was collected by venipuncture for quarterly CD4 testing. HIV-seropositive participants (identified at screening or during follow-up) were offered a routine package of HIV care at no cost. In addition, women who met the Kenyan National Guidelines[90]

for initiation of antiretroviral therapy (ART) were offered PEPFAR-supported antiretroviral therapy in our clinic beginning in March, 2004. All participants were given a follow-up appointment one week after their visit. At that time, they were provided with additional treatment if STIs were identified by laboratory testing that were not covered by syndromic treatment at the prior visit.

Laboratory procedures

All laboratory procedures were performed in Mombasa, Kenya. *Trichomonas vaginalis* infection was diagnosed by the presence of motile trichomonads on saline microscopy. The saline wet preparation was also assessed for the presence of clue cells and fungal elements. A drop of 10% potassium hydroxide was added to the slide and evaluated again for the presence of yeast buds or hyphae. A Gram stain of vaginal fluid was evaluated for diagnosis of BV by Nugent criteria.[61] *Lactobacillus* culture was performed on Rogosa agar[12], and sub-culture of *Lactobacillus* isolates on tetramethylbenzdine agar containing horseradish peroxidase was performed to evaluate hydrogen peroxide production.[70] A Gram stain of endocervical secretions was evaluated for cervicitis, defined as the presence of an average of ≥30 polymorphonuclear leukocytes per high-power field on microscopic examination (original magnification X100). Throughout the study, endocervical secretions were cultured on modified Thayer–Martin media for *Neisseria gonorrhoeae.* C*hlamydia trachomatis* testing was performed by EIA (Syva [Microtrak, Palo Alto, USA]) from February 1993 – April 1999. Beginning in 2006, the Aptima Combo-2 GC/CT Detection System (Gen-Probe, San Diego, USA) was used for detection of both *N. gonorrhoeae* and *C. trachomatis*. HIV-1 testing was performed using an ELISA (Detect-HIV [BioChem ImmunoSystems, Allentown, USA]). Positive ELISA results were confirmed using a second ELISA (Recombigen [Cambridge Biotech, Cambridge, MA, USA] or Vironostika [bioMerieux, Marcy l'Etoile, France]). CD4 cell counts were assessed by Coulter (Cytosphere,

Haileah, USA), Zymmune (Bartels, Issaquah, USA), and FACSCount (Becton Dickinson, Franklin Lakes, USA) as each method became available over time.

T. vaginalis episode inclusion criteria and outcomes

Episodes of TV infection were included in the analysis if the participant received treatment with single-dose 2g oral metronidazole within 14 days from the time of diagnosis and returned for a follow-up visit with collection of genital specimens within 60 days from the time of diagnosis. Participants were allowed to contribute multiple infections to the analysis. Episodes of TV were excluded if the participant was pregnant or received an alternative metronidazole dosing regimen at the time of diagnosis or within 14 days of treatment with single dose metronidazole. Parasitologic cure was defined as the absence of motile trichomonads by microscopy at the next exam visit within 60 days from the initial diagnosis. The presence of TV by microscopy at the next examination visit within 60 days of diagnosis was classified as persistent TV infection, recognizing the possibility of either treatment failure or early re-infection. Infections detected after parasitologic cure of the first TV infection were classified as repeat infections.

Statistical analysis

We used descriptive statistics to summarize demographic, behavioral and clinical characteristics at diagnosis and the assessment of cure visit. Vaginal washing data were missing for 113 infections at the time of diagnosis. These values were imputed based on vaginal washing status at enrollment into the cohort, as prior analyses of vaginal washing in the cohort have demonstrated a strong correlation between vaginal washing practices at enrollment and follow-up.[91] We assumed an effect window of 85 days for hormonal contraceptive use.[92] Bacterial vaginosis was assessed using Nugent's criteria.[61] Data on the whiff test to detect amines were limited, so we could not assess BV by clinical criteria.[69] However, vaginal pH during the pelvic examination and the presence of clue cells on saline microscopy (2 components of the clinical criteria for BV) were assessed.

Since participants could contribute multiple infections to the analysis, we used generalized estimating equations models with a logit link, independent correlation structure and robust standard errors to compare demographic, behavioral and clinical characteristics by HIV status. This method accounts for clustering due to multiple observations per participant. We also used this method to assess factors that may be associated with persistent TV infection, including HIV status. Potential confounding factors assessed at the time of diagnosis or assessment of cure that differed substantially between HIV-seropositive and HIV-seronegative participants ($p < 0.10$) were considered for inclusion in multivariable models using a process of forward stepwise logistic regression. Covariates were retained in the adjusted model if they changed the coefficient for the association between HIV-serostatus and persistent TV by ≥10%. Unprotected sex in the last week and calendar year of diagnosis were included *a priori* in the adjusted model as important potential confounding factors. Analyses were conducted using Stata version 11.0 (StataCorp, Inc., College Station, TX). All statistical tests were assessed using a 2-sided alpha of 0.05.

3.3 Results

Between February 1993 and December 2010, we observed 957 episodes of TV where any treatment was dispensed within 14 days of diagnosis. Of those, there were 616 episodes where the participant returned for an assessment of cure within 60 days. Alternative metronidazole dosing regimens were dispensed for 46 of these 616 episodes, leaving 570 infections contributed by 360 participants for inclusion in the analysis. The median number of infections was the same in both HIV-seropositive and seronegative women (median [interquartile range] = 1 [1-2]).

Demographic, behavioral, and clinical characteristics at TV diagnosis by HIV status are presented in Table 1. Participants that were HIV-seropositive at the time of TV diagnosis were slightly older, less likely to report sex in the past week, more likely to report vaginal washing in

the past week, more likely to use injectable hormonal contraception, and less likely to have cervicitis compared to HIV-seronegative participants. Self-reported symptoms of genital tract infections were not evaluated at enrollment into the cohort. Therefore, data on the presence of self-reported vaginal itching and abnormal vaginal discharge at TV diagnosis were only available for 349 infections. Vaginal itching, abnormal vaginal discharge, or both were reported concurrently with 75 (21%) TV infections and did not differ by HIV status (54/245 [22%] HIV-seropositive versus 21/104 [20%] seronegative; p=0.7). Among participants with concurrent BV and TV infection, symptoms were reported at 33 of 138 (24%) TV infections. Mean time from diagnosis to assessment of cure was identical by HIV status (33 days ± standard deviation [SD] 8; p=0.5). Behavioral and clinical factors evaluated at the assessment of cure were similar to those reported at diagnosis (Table 2.) At the assessment of cure, HIV-seropositive participants were less likely to report sex in the past week, more likely to report vaginal washing, and more likely to have cervicitis compared to HIV-seronegative participants.

We observed 42 (15%; 95% confidence interval [CI] 11%, 20%) persistent TV infections among HIV-seropositive participants versus 35 (12%; 95% CI 9%, 16%) among HIV-seronegative participants (odds ratio [OR] = 1.27; 95% CI 0.75, 2.12). Mean time from diagnosis to assessment of cure was similar among participants with persistent TV compared to participants with parasitologic cure (persistent TV infection = 34 days ± SD 8 versus cured TV infection = 33 days ± SD 9; p-value=0.4). Demographic and behavioral characteristics were not associated with persistent TV (Table 3). Among women with BV at TV diagnosis, there were 44 (17%) persistent TV infections at the assessment of cure compared to 33 (10%) persistent TV infections among women without BV (OR=1.81; 95% CI 1.12, 2.92). This association was essentially the same after adjustment for HIV status, calendar year, unprotected sex in the past week, vaginal washing in the past week, yeast at the assessment of cure and cervicitis at diagnosis (adjusted OR=1.82; 95% CI 1.11, 2.99). Similarly, the presence of clue cells on saline wet preparation at the time of TV diagnosis was associated with an increased likelihood

of persistent TV compared to women with no clue cells (37 [18%] versus 40 [11%], respectively; OR=1.74; 95% CI 1.08, 2.80). However, there was no association between baseline vaginal pH >4.5 and risk of persistent TV infection (data not shown).

3.4 Discussion

In this analysis of HIV-seropositive and seronegative Kenyan women with trichomoniasis, nearly 1 in 7 women had persistent TV following treatment with single-dose 2g oral metronidazole. The prevalence of persistent TV did not differ by HIV status. Women with TV and concurrent BV were more likely to have persistent TV at the assessment of cure compared to women without BV. We observed a similar magnitude of risk for persistent TV associated with having clue cells on microscopy at TV diagnosis compared to participants with no clue cells. To our knowledge, this is the largest study to date to examine the effect of single-dose 2g oral metronidazole on TV infection by HIV status.

In randomized trials of treatment for TV infection conducted among HIV-seronegative women, approximately 5% to 10% of women treated with single-dose 2g oral metronidazole failed to achieve parasitologic cure.[66,93] The overall prevalence of persistent TV in our study (13.5%), which may represent treatment failure or early re-infection, was slightly higher than what was observed in randomized trials. However, it was similar to levels observed in cohort studies conducted in the US (9.7%) and South Africa (13.3%) that compared rates of TV persistence by HIV status, both of which used culture for TV detection.[35,36] Although the US study reported an overall difference in the proportion of participants who were TV positive one month after treatment with single-dose 2g metronidazole, they observed similar proportions of probable treatment failure among HIV-seropositive and seronegative women who denied a history of possible re-exposure (10.0% versus 7.3%, respectively; p=0.44).[36]

We observed a higher rate of persistent TV infection among women with concurrent BV at TV diagnosis. Our findings are consistent with observations from a study of HIV-seropositive

women enrolled in a randomized trial of TV treatment. In the trial, participants with concurrent BV and TV infection at enrollment that were randomized to the single-dose 2g metronidazole arm had a 4.16-fold increased risk of being TV positive at the test of cure visit (6-12 days after completion of treatment) compared to women without BV by Gram stain (23.8% versus 5.7%, respectively; 95% CI 1.02 to 16.89).[94] Treatment outcomes were similar in women with TV infection in the presence and absence of BV if they were randomized to receive twice daily 500mg metronidazole for 7 days (8.0% versus 7.5%, respectively; relative risk [RR]=1.07; 95% CI 0.28, 4.04). Taken together, these findings suggest that concurrent BV may reduce efficacy of single-dose metronidazole for TV infection in both HIV-seropositive and seronegative women. The biological mechanism for this finding requires further investigation.

Consistent with other studies, the majority TV infections in this study were asymptomatic.[4,95] While current guidelines recommend treatment with multi-day oral metronidazole (500mg twice daily for 7 days) for women with TV infection and symptomatic BV, women with TV infection concurrent with asymptomatic BV are frequently treated with single-dose metronidazole.[66] Given the reduced efficacy of single-dose metronidazole among women with concurrent asymptomatic BV and TV infection, this sub-group may benefit from initial treatment with longer courses of metronidazole or from administration of drugs with longer half-lives.

Adherence to treatment with multi-day regimens is always a concern, since incremental improvements in effectiveness with multi-day regimens may be lost due to lower levels of adherence. However, results from a recently completed effectiveness trial conducted among HIV-seropositive women with TV infection are reassuring. The authors reported that use of twice daily 500mg oral metronidazole for 7 days was more effective than single-dose metronidazole for TV treatment (8.5% TV+ versus 16.8% TV+ at the test of cure, respectively; RR=0.50, 95% CI 0.25, 1.00).[96] In addition, very high levels of adherence were observed in both the multi-day (95%) and single-dose (98%) arms.

Our analysis has a number of limitations that should be considered when interpreting the results. The primary limitation of our study was the use microscopy to detect TV. Though this test remains the most common method for TV diagnosis in clinical settings[97], it has considerably lower sensitivity compared to culture[98,99] and especially compared to nucleic acid amplification testing (NAAT).[99,100] It is very likely that a proportion of participants who appeared to achieve parasitologic cure were still infected with TV at concentrations too low to detect on microscopy. Further studies using NAAT detection will be helpful to determine whether the rate of low-level TV persistence differs by HIV status. In this study, assessment of cure was performed at the next examination within 60 days of diagnosis. This interval is similar to other studies.[36] Nonetheless, the longer the interval between treatment and assessment of cure, the more difficult it is to definitively determine whether participants who continued to test positive for TV were positive due to treatment failure or re-infection. Lastly, for some infections, partner treatment with single-dose 2g metronidazole was offered. However, in this cohort of women reporting transactional sex, partner treatment was not frequently accepted and data on partner treatment were not systematically collected.

In this analysis of Kenyan women infected with TV and treated with single-dose 2g oral metronidazole, there was a high level of TV persistence that did not differ by HIV status. Persistent TV infection was most common among women with concurrent BV by Gram stain or clue cells present on microscopy. The findings from this study are consistent with other prospective studies that report that TV persistence, whether due to treatment failure or early re-infection, is common following treatment with single-dose metronidazole.[34-36,39] Given the associations between TV infection and adverse reproductive health outcomes as well as HIV acquisition and transmission potential, the high proportion of women with persistent TV observed in this study is concerning. Our data suggest that single-dose metronidazole for TV infection may not be adequate, especially in certain sub-groups of women for which this treatment is recommended under current guidelines.

Table 3.1. Demographic, behavioral and clinical characteristics at the time of diagnosis with *T. vaginalis* infection by HIV-1 status*

	HIV-1-seropositive N=282	HIV-1-seronegative N=288	P-value**
Age (years)	36 ± 5	34 ± 7	0.01
Educational level (years)	7 ± 3	7 ± 3	0.3
Duration of sex work (years)	10 ± 11	8 ± 6	0.04
Sexual activity in the past week[1]	142 (50)	175 (63)	0.008
Sexual episodes in the past week[1]			
None	140 (50)	101 (37)	--
1 sex act	71 (25)	67 (24)	0.3
2 or more sex acts	71 (25)	108 (39)	0.002
Partners in the past week[1]			
None	140 (50)	101 (37)	--
1 sex partner	113 (40)	121 (44)	0.06
2 or more partners	29 (10)	54 (19)	0.006
Unprotected sex in the last week[1]	47 (17)	72 (25)	0.2
Hormonal contraceptive use[2]			
None	197 (70)	236 (82)	---
Oral contraceptive pills	20 (7)	22 (8)	0.8
Injectables	61 (22)	29 (10)	0.006
Norplant	4 (1)	1 (<1)	0.2
Vaginal washing in the last week[3]	269 (95)	244 (85)	0.001
Bacterial vaginosis[4]	124 (44)	129 (45)	0.8
Intermediate vaginal flora[5]	100 (35)	111 (39)	0.2
Clue cells present on wet prep	114 (40)	94 (33)	0.09
Yeast present on wet prep	29 (10)	23 (8)	0.4
Gonorrhea	8 (3)	8 (3)	1.0
Cervicitis[6]	14 (5)	37 (13)	0.001
CD4 count (cells/mm)[8]	370 ± 235	-- ---	--
On ART[7]	65 (23)	-- ---	--

N (%) or mean ± SD presented.

* N represents the number of TV infections contributed by 360 unique women. Eleven women contributed infections while HIV-1-seronegative and HIV-1-seropositive.

** P-value generated from models using generalized estimating equations with a logit link to account for multiple infections per woman.

[1] 12 infections among HIV-1-seronegative women missing sexual activity data at the time of infection.

[2] Compared to women not using any method of hormonal contraception (i.e. used nothing, condoms, diaphragm, tubal ligation, spermicide or had a hysterectomy).

[3] Vaginal washing data were available for 457 infections at the time of diagnosis. Missing values were imputed based on vaginal washing status at enrollment.

[4] Nugent score 7-10. Data missing for 1 HIV-seronegative infection.

[5] Nugent score 4-6. Data missing for 1 HIV-seronegative infection.

[6] Defined as the presence of an average of ≥30 polymorphonuclear leukocytes per high-power field on microscopic examination of Gram-stained cervical secretions (original magnification X100). Data missing for 1 HIV-seronegative infection.

[7] HIV-1-seropositive women only. CD4 counts available for 260 infections within 6 months pre or post infection.

Table 3.2. Behavioral and clinical characteristics reported at the assessment of cure by HIV-1 status*

	HIV-1-seropositive N=282		HIV-1-seronegative N=288		P-value**
Sexual activity in the past week	141	(50)	168	(58)	0.08
Sexual episodes in the past week					
None	141	(50)	120	(42)	--
1 sex act	65	(23)	65	(23)	0.5
2 or more sex acts	76	(27)	103	(36)	0.04
Partners in the past week					
None	141	(50)	120	(42)	--
1 partner	112	(40)	124	(43)	0.2
2 or more partners	29	(10)	44	(15)	0.09
Unprotected sex in the past week[1]	43	(15)	64	(22)	0.06
Vaginal washing in the past week	268	(95)	240	(83)	0.001
Bacterial vaginoisis[2]	117	(42)	120	(42)	1.00
Clue cells detected on wet prep	113	(40)	103	(36)	0.3
Yeast present on wet prep	38	(14)	24	(8)	0.05
Gonorrhea	9	(3)	11	(4)	0.7
Cervicitis[4]	5	(2)	30	(10)	0.002

N (%) presented.

* N represents the number of TV infections contributed by 360 unique women. Eleven women contributed infections while HIV-1-seronegative and HIV-1-seropositive.

** P-value generated from models using generalized estimating equations with a logit link to account for multiple infections per woman.

[1] Unprotected sex vs. 100% condom use or no sex.

[2] Nugent score 7-10.

[3] Defined as the presence of an average of ≥30 polymorphonuclear leukocytes per high-power field on microscopic examination of Gram-stained cervical secretions (original magnification X100).

Table 3.3. Associations of demographic, behavioral and clinical characteristics with persistent *T. vaginalis**

	Persistent TV N=77	Parasitologic cure N=493	Unadjusted OR (95% CI)	Adjusted** OR (95% CI)
HIV-1-seropositive	42 (55)	240 (49)	1.27 (0.75, 2.12)	1.26 (0.75, 2.12)
Age at diagnosis (years)	35 ± 6	35 ± 7	1.00 (0.96, 1.03)	
Educational level (years)	7 ± 3	7 ± 3	0.97 (0.90, 1.04)	
Duration of sex work (years)	9 ± 6	9 ± 9	1.00 (0.97, 1.02)	
Sexual activity in the past week	39 (51)	270 (55)	0.85 (0.53, 1.36)	
Number of sexual episodes in the past week				
None	38 (49)	223 (45)	-- --	
1 sex act	19 (25)	111 (23)	1.00 (0.54, 1.86)	
2 or more sex acts	20 (26)	159 (32)	0.74 (0.41, 1.34)	
Number of partners in the last week				
None	38 (49)	223 (45)	-- --	
1 partner	29 (38)	207 (42)	0.82 (0.49, 1.38)	
2 or more sex partners	10 (13)	63 (13)	0.93 (0.44, 1.96)	
Unprotected sex in the last week[1]	11 (14)	96 (19)	0.69 (0.34, 1.41)	0.68 (0.32, 1.42)
Hormonal contraceptive use				
None	61 (79)	372 (75)	-- --	
Oral contraceptive pills	4 (5)	38 (8)	0.64 (0.24, 1.73)	
DMPA	12 (16)	78 (16)	0.94 (0.43, 2.05)	
Hormonal contraception[2]	16 (21)	121 (25)	0.81 (0.42, 1.55)	
Vaginal washing in the last week	66 (86)	442 (90)	0.70 (0.29, 1.69)	0.67 (0.27, 1.67)
Bacterial vaginosis at diagnosis	44 (57)	209 (43)	1.81 (1.12, 2.92)	1.82 (1.11, 2.99)
Bacterial vaginosis at assessment of cure	36 (47)	201 (41)	1.28 (0.81, 2.02)	
Yeast present on wet prep at diagnosis	8 (10)	44 (9)	1.18 (0.52, 2.67)	
Yeast present on wet prep at assessment of cure	4 (5)	58 (12)	0.41 (0.14, 1.17)	0.41 (0.13, 1.26)
Gonorrhea at diagnosis	1 (1)	15 (3)	0.42 (0.05, 3.29)	
Gonorrhea at assessment of cure	1 (1)	19 (4)	0.33 (0.04, 2.51)	
Cervicitis at diagnosis	3 (4)	48 (10)	0.38 (0.12, 1.19)	0.43 (0.14, 1.35)
Cervicitis at assessment of cure	6 (7)	30 (6)	1.07 (0.41, 2.82)	

N (%) or mean ± SD presented.

* N represents the number *T. vaginalis* infections contributed by 360 unique women. Eleven women contributed infections while HIV-1-seronegative and HIV-1-seropositive. Demographic characteristics and hormonal contraceptive use reported at diagnosis. All other characteristics reported at the assessment of cure unless specified otherwise.

** All variables were assessed for inclusion in the model. Variables that produced a >10% change in the coefficient or that had p-values <0.10 were included in the final model. Unprotected sex and calendar year were included in the model as *a priori* potential confounders.

[1] Unprotected sex vs. 100% condom use or no sex.

[2] Use of oral contraceptive pills, injectables or norplant compared to use of non-hormonal methods or no contraception (i.e. used nothing, condoms, diaphragm, tubal ligation, spermicide or had a hysterectomy

Conclusion

In these analyses of Kenyan women who reported engaging in transactional sex, vaginal infections were very common. As described in Chapter 1, periodic presumptive treatment with 2g single-dose metronidazole and 150mg fluconazole monthly for 12 months was effective at establishing and sustaining a healthy vaginal environment. However, the effect of PPT was not sustained in the 3-month interval following completion of the intervention (Chapter 2). In addition, a high prevalence of persistent TV was reported following treatment with single-dose 2g oral metronidazole, especially among women with concurrent BV (Chapter 3). The findings presented in Chapters 2 and 3 highlight the need for additional research to improve our understanding of the vaginal environment and new interventions for treatment and prevention of vaginal infections.

Historically, our understanding of the epidemiology of vaginal infections has been based on microscopy and culture. Recent advances in molecular technology have led to the development of novel diagnostic methods that may provide insights into the mechanisms of cure, persistence and recurrence of vaginal infections. For example, polymerase chain reaction (PCR) assays that detect bacterial genes such as the 16S ribosomal RNA gene (rRNA), allow for detection of both cultivation-resistant and cultivatable bacterial species. Broad range and species-specific quantitative PCR assays have been used to characterize the vaginal microbiota and have revealed several new species [76,101-109], some of which are strongly associated with persistent BV.[75] The use of these methodologies holds great promise for improving our understanding of the molecular epidemiology of BV, as well as the associations between BV, HIV-1 and STI acquisition, and adverse reproductive health outcomes.

There have also been recent advances in diagnostics for detection of TV. Nucleic acid amplification testing for detection of TV recently received approval from the US Food and Drug Administration.[110] Use of this highly sensitive assay will provide more precise estimates of the

effectiveness of current treatments for TV infection. In addition, the lower sensitivity of microscopy and culture can lead to difficulties in definitively determining whether women treated for TV infection who continue to test positive at a test of cure visit were positive due to treatment failure or re-infection. Several studies have reported the re-emergence of TV infection after successful treatment among women who reported no subsequent exposure, suggesting that women can continue to be infected at levels below the threshold of detection by microscopy and culture.[111,112] Future studies that utilize more sensitive molecular diagnostics will be important in clarifying the burden of TV persistence versus re-infection.

In summary, the findings from this dissertation suggest that current suppressive treatments do not sustain vaginal health following cessation of the intervention; while the efficacy of the most common treatment for TV infection appears to be reduced in certain sub-groups of women. Innovative treatment and prevention approaches are urgently needed to reduce the burden of disease caused by vaginal infections and their associated complications. The molecular methods described above could be used in combination with traditional microscopy and cultivation approaches to improve our overall understanding of the overall vaginal environment contribute to the development of new interventions. Research into the biological mechanisms of acquisition, cure, persistence and re-infection should continue to be a research priority in order to develop new interventions that effectively reduce the incidence and recurrence of vaginal infections.

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Curriculum Vitae

EDUCATION

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