Does the effect of periodic presumptive treatment with oral metronidazole and fluconazole on the incidence of vaginal infections and *Lactobacillus* colonization depend on baseline vaginal infection status?

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Abstract

Does the effect of periodic presumptive treatment with oral metronidazole and fluconazole on the incidence of vaginal infections and *Lactobacillus* colonization depend on baseline vaginal infection status?

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Background: Vaginal infections are common, and associated with adverse health outcomes. Periodic presumptive treatment (PPT) is an effective intervention for preventing these infections. The PPT effect could be improved if it were restricted to women most likely to benefit from it.

Methods: We conducted a subgroup analysis of data from a PPT trial in Mombasa, Kenya using Andersen-Gill proportional hazards models with robust variance estimates to assess the effect of the intervention among participants with a baseline vaginal infection versus those without.

Results: Of 302 women eligible for analysis, 137 (45%) had one or more vaginal infections at baseline, while 165 (55%) had none. In the subgroup with a baseline vaginal infection, the intervention reduced the risk of incident BV [hazard ratio (HR)=0.55, 95% confidence interval (CI): 0.41-0.76] and increased vaginal colonization with any *Lactobacillus* species (HR=1.61, 95% CI: 1.01-2.56) as well as H_2O_2-producing *Lactobacillus* species (HR=1.85, 95% CI: 1.00-3.45) compared to placebo. In the subgroup without a baseline vaginal infection, the effect of the

intervention to reduce incident BV (HR=0.71, 95% CI: 0.47-1.09) and increase vaginal colonization with any *Lactobacillus* species (HR=1.32, 95% CI: 0.85-2.04) as well as H_2O_2-producing *Lactobacillus* species (HR=1.48, 95% CI: 0.73-3.01), was less strong and not statistically significant.

Conclusion: In this PPT trial, the effect of the intervention was driven primarily by participants with a baseline vaginal infection. Restricting PPT to women who are most likely to benefit from it may improve the effect of the intervention.

DEDICATION

To Nelius, my love and Abby, my little princess

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BACKGROUND

Among women of child-bearing age, vaginal infections including bacterial vaginosis (BV), vulvovaginal candidiasis (VVC) and *Trichomonas vaginalis* are a common public health problem (1-3). These infections have been associated with an increased risk of adverse health outcomes such as preterm delivery and chorioamnionitis (4), pelvic inflammatory disease (5) and other sexually transmitted infections (STIs) (6-15). Perhaps most importantly, in terms of long-term consequences, BV has been associated with a 60% increase in the relative risk of HIV-1 acquisition (13, 16). Vulvovaginal candidiasis and *Trichomonas vaginalis* have also been associated with a 1.5 to 2-fold increased risk of HIV-1 acquisition (13, 14, 17-19).

Interventions that reduce the incidence of vaginal infections could potentially reduce women's risk of STIs, including HIV-1. One such intervention is periodic presumptive treatment (PPT) which involves intermittently providing asymptomatic women with antimicrobial agents effective against micro-organisms causing these infections, with the aim of treating and potentially preventing the infections. To test this strategy, we conducted a randomized controlled trial of monthly oral PPT using a directly-observed regimen of 2g metronidazole and 150mg fluconazole versus matching placebos among women in Mombasa, Kenya (20). Compared to placebo, the intervention reduced the risk of incident BV by Gram stain [Nugent's] criteria (21) (HR=0.55, 95% CI: 0.49–0.63) and increased vaginal colonization with H_2O_2-producing *Lactobacillus* species (HR=1.63, 95% CI: 1.16–2.27).

Another randomized trial conducted in the US followed women who had been successfully treated for symptomatic BV (22). Participants were assigned to suppressive therapy consisting of bi-weekly topical 0.75% metronidazole vaginal gel versus matching placebo. Compared to placebo, suppressive therapy with metronidazole gel reduced recurrence of BV by clinical [Amsel's] criteria (23) [Relative Risk (RR)=0.43, 95% CI: 0.25-0.73]. In both trials however, the effect of the interventions did not persist after they were stopped (22, 24).

To produce a stronger and more sustained PPT effect, several strategies have been proposed. The first involves administering the intervention frequently or for an extended duration of time (25). The second involves vaginal or a combination of oral and vaginal as opposed to just oral delivery of the intervention (22, 26). Intravaginal delivery of metronidazole and topical azole preparations is similarly effective as oral therapy (27-30). While a number of studies have suggested that majority of women prefer oral treatment (31-33), an intravaginal route for frequent or extended administration of PPT could be better tolerated due to reduced systemic absorption.

A third strategy is based on the hypothesis that the efficacy of a PPT intervention might be higher within the subgroup of women with a prevalent vaginal infection since these women have been shown to be more likely to experience recurrence (22, 25, 34). For BV, up to 30% of recurrences will occur within three months of receiving treatment (35). As a result, women with prevalent BV may be particularly likely to benefit from a PPT intervention. Conversely, some women have persistently normal vaginal microbiota, so they are unlikely to benefit from an intervention designed to prevent vaginal infections (36, 37).

With this background, we conducted a subgroup analysis of data from our PPT trial, with the aim of testing the hypothesis that the efficacy of the intervention would be higher in the subgroup of participants with a baseline vaginal infection compared to those without.

METHODS

Study population and procedures

Detailed methods of the trial have been published (20). Briefly, participants were recruited from a cohort of women in a prospective study of risk factors for HIV-1 and STIs in Mombasa, Kenya. These women reported exchanging sex for cash or in-kind payment. We enrolled HIV-1 negative, non-pregnant women between 18-45 years old. These women were randomized to receive monthly PPT consisting of a directly-observed oral regimen of 2g metronidazole plus 150mg fluconazole or matching placebos for both drugs. They were asked to return to the clinic monthly for 12 months. Ethical approval was obtained from the review committees of the University of Nairobi and the University of Washington. All participants provided written informed consent.

At enrollment and each monthly follow up visit, participants completed face-to-face interviews using a standardized questionnaire to ascertain information on medical, reproductive and sexual history. A physical examination was performed and included a pelvic speculum examination with collection of genital specimens for diagnosis of genital infections. Blood was collected to diagnose infection with HIV-1, and urine was collected for β-HCG pregnancy testing. Each month, the participants were provided with counseling and free condoms to reduce their risk of STIs, including HIV-1. Participants with genital infections were treated according to local guidelines. Women who acquired HIV-1 infection were provided with a comprehensive care package including antiretroviral therapy (ART) according to Kenyan national guidelines.

Laboratory methods

Screening for HIV-1 was performed using enzyme linked immunosorbent assay (ELISA) (Detect-HIV 1/2; BioChem ImmunoSystems, Montreal, Canada), and positive results were confirmed using a second ELISA (Recombigen; Cambridge Biotech, Worcester, MA, USA or Vironostika; Biomeriux). Vaginal trichomoniasis and candidiasis were diagnosed by microscopic identification of motile trichomonads and yeast elements, respectively, on a vaginal wet mount at 40X magnification. In addition, culture for *Trichomonas vaginalis* was performed in modified Diamond's media.

Diagnosis of BV was performed by microscopic examination of Gram-stained vaginal secretions [Nugent score] (21). *Neisseria gonorrhoeae* was diagnosed by culture of cervical secretions on modified Thayer-Martin agar. Non-specific cervicitis was defined as the presence of ≥30 polymorphonuclear leucocytes per high-power field on Gram-stained cervical secretions. Herpes simplex virus (HSV) type-2 testing was performed on serum using an ELISA-based method (HerpesSelect-2, Focus Technologies, Cypress, California, USA). As we have done previously (12), we considered samples to be positive for HSV-2 if they had an index value of ≥1.1, according to the manufacturer's instructions.

Statistical methods

Demographic, behavioral and laboratory characteristics of the participants at trial enrollment were summarized, stratifying on the presence of one or more baseline vaginal infections (BV, VVC and *Trichomonas vaginalis*). We calculated median and interquartile range (IQR) for continuous variables as well as counts and percentages (%) for categorical variables. To determine whether our stratification resulted in unequal distribution of baseline

characteristics within the study arms, we compared these characteristics among women in the intervention versus those in the placebo arm using Pearson's chi-squared test for categorical data and Wilcoxon rank-sum test for continuous data.

Primary outcomes included the incidence per 100 person-years of BV, VVC, *Trichomonas vaginalis* infection and vaginal colonization with any *Lactobacillus* species as well as H_2O_2-producing *Lactobacillus* species. We compared the rates of each outcome by study arm using Andersen-Gill proportional hazards models with robust variance estimates to allow for multiple events per participant (38). Hazard ratios represent the ratio of the incidence of each outcome in the intervention arm compared to the placebo arm. For each outcome, Andersen-Gill proportional hazards models were built that included an interaction term between study arm and baseline vaginal infection status. A test of interaction was conducted by evaluating the statistical significance of the beta coefficient associated with the interaction term (Wald Test). All statistical tests were evaluated using a 2-sided α-value of 0.05. Statistical analyses were conducted using Stata version 12 (StataCorp Inc., College Station, TX, USA).

RESULTS

Three hundred and seventy eight women were screened between May 2003 and November 2005 (Figure 1). Of these, 310 were enrolled. Seven women did not return for further follow up. One woman was found to be HIV-1 seropositive at the enrollment visit based on HIV-1 RNA testing of her baseline sample, and was excluded from the analysis. Among the 302 women eligible for this subgroup analysis, 165 (55%) had no vaginal infection at the baseline visit while 137 (45%) had one or more vaginal infections at baseline. Of these 137 women, 105 (77%) had BV, 32 (23%) had VVC and 6 (4%) had *Trichomonas vaginalis* infection.

Of those participants with a baseline vaginal infection, 62 (45%) were randomized to the intervention arm and 75 (55%) to the placebo arm. Among those without a baseline vaginal infection, 89 (54%) were randomized to the intervention arm and 76 (46%) to the placebo arm. The median number of follow up visits among women with a baseline vaginal infection was 12 (IQR) 8-12) and 12 (IQR 11-12) in the intervention and placebo arms, respectively. Among women without a baseline vaginal infection, the median number of follow up visits was 12 (IQR 9-12) in the intervention arm and 12 (IQR 11-12) in the placebo arm.

Baseline characteristics of the subgroups are summarized in Table 1. Participants with a baseline vaginal infection had a median age of 28 (IQR 24-31) while those without a baseline infection had a median age of 30 (IQR 25-35). The majority of participants reported not having a secondary education and were widowed or separated. Participants in both subgroups also reported a median of 1 sex partner and 1 sex act in the week prior to enrolment. Most participants reported using a condom at all sex acts in the past week. Within each subgroup, study arms were generally well-balanced with respect to baseline characteristics. In the subgroup with a baseline vaginal infection, there was a small but statistically significant difference in the proportion of participants who reported at least one drink per week in the intervention arm (90%) compared to the placebo arm (75%). This difference is likely due to chance, given the number of statistical comparison tests we conducted.

The majority of BV episodes occurred among participants with a baseline vaginal infection (Table 2). The incidence of BV among these women (422 episodes per 100 person-years) was more than twice that among women without a baseline vaginal infection (169 episodes per 100 person-years). In the subgroup of women with a baseline vaginal infection, the

intervention reduced the risk of BV by almost half compared to women receiving the placebo (HR=0.55, 95% CI: 0.41-0.76). The magnitude of the effect of the intervention to reduce episodes of BV in participants without a baseline vaginal infection was lower and not statistically significant (HR=0.71, 95% CI: 0.47-1.09). The effect of the intervention on overall vaginal *Lactobacillus* species colonization was higher among participants with a baseline vaginal infection (HR=1.61, 95% CI: 1.01-2.56) compared to those without (HR=1.32, 95% CI: 0.85-2.04). Similarly, women with a baseline vaginal infection had higher rates of vaginal colonization with H_2O_2-producing *Lactobacillus* species (HR=1.85, 95% CI: 1.00-3.45) compared to those without (HR=1.48, 95% CI: 0.73-3.01).

These findings were similar when we conducted a sensitivity analysis stratifying on baseline BV status (Table 3). The majority of BV episodes that occurred during follow up were among women with BV at the baseline visit. The incidence of BV in this subgroup (461 episodes per 100 person-years) was more than twice that in the subgroup without BV at the baseline visit (189 episodes per 100 person-years). Among participants with BV at the baseline visit, the intervention reduced the risk of BV by almost half compared to placebo (HR=0.56, 95% CI: 0.40-0.78). Interestingly, even women without BV at the baseline visit had a lower risk of BV during follow up compared to those using placebo (HR=0.63, 95% CI: 0.43-0.91), although the magnitude of this effect was smaller than in women with BV at enrolment. Finally, the effect of the intervention to increase overall vaginal *Lactobacillus* species colonization was higher among participants with BV at baseline (HR=2.15, 95% CI: 1.26-3.65) compared to those without (HR=1.23, 95% CI: 0.83-1.81). Similarly, women with BV at baseline had a higher risk for vaginal colonization with H_2O_2-producing *Lactobacillus* species (HR=3.45, 95% CI: 1.58-7.55)

compared to those without (HR=1.25, 95% CI: 0.69-2.28). The tests of interaction for each outcome were not statistically significant (Table 4).

DISCUSSION

In this subgroup analysis of data from a randomized controlled trial of monthly oral PPT, we observed fewer episodes of BV among women in the intervention arm compared to those in the placebo, within each subgroup. However, the magnitude of the reduction of BV was higher among women with a baseline vaginal infection compared to those without. Similarly, the increase in vaginal *Lactobacillus* colonization was higher among participants who had a vaginal infection at the baseline visit.

The PPT intervention administered in this trial consisted of a monthly oral, directly-observed regimen of 2g metronidazole and 150 mg fluconazole. Both oral and vaginal delivery of 5-nitroimidazole derivatives for treatment of BV has been associated with cure rates between 80 and 86% (26). However, BV recurrence after treatment is common, with rates of up to 58% at 12 months (35). Additionally, long-term intermittent use of fluconazole has been associated with up to 90% protection against recurrent VVC but the effect is lost when the intermittent treatment is discontinued (25).

While recurrences of BV and VVC are common among women diagnosed with these infections, there is also a group of women who have persistently normal vaginal microbiota. Data from Rakai, Uganda showed that among women with a prior normal Nugent score, 76% still had a normal score at the next weekly visit (37). A secondary analysis of data from our PPT trial also suggested that some participants had a persistently normal vaginal environment during the entire duration of the trial (36). These data suggest that a proportion of women are able to

independently maintain a normal vaginal microbiota. Such women would not benefit from a PPT intervention designed to prevent vaginal infections.

In this subgroup analysis, we observed that the incidence of BV among women with a baseline vaginal infection was more than twice that among those who did not have a baseline vaginal infection. Additionally, we found that the effect of the PPT intervention in reducing episodes of BV and increasing vaginal *Lactobacillus* colonization in the trial as a whole was driven by those participants who had a vaginal infection at baseline. Importantly, among women with a baseline vaginal infection, the absolute reduction in BV incidence (228 episodes per 100 person-years) was greater than what we saw among women in the trial as a whole (127 episodes per 100 person-years). Even though these resulted in similar hazard ratios, the subgroup with the larger magnitude change would have a greater power for showing differences in adverse health outcomes associated with BV, including other STIs. These findings are consistent with the hypothesis that a PPT intervention could be more beneficial when restricted to women with a prevalent vaginal infection.

This study has several strengths. Data were collected in the course of a randomized controlled trial where participants were randomly allocated to study arms. As such, the study design controlled for both known and unknown potential sources of bias. Additionally, while we stratified on baseline vaginal infection status, it remains likely that we retained the benefits of randomization. Our trial also had a high rate of participant retention and regularly measured biological outcomes, enabling a more precise assessment of the vaginal environment. Finally, to diagnose BV, we used Nugent's criteria which were evaluated by highly experienced technologists who were also blinded to treatment allocation. We have previously used these

criteria for studies showing an increased risk of HIV-1 associated with BV (13) which made this an appropriate outcome for this intervention.

This analysis satisfied a majority of the Subgroup Analysis of Trials Is Rarely Easy (SATIRE) criteria for assessing credibility of subgroup effects in randomized clinical trials (39, 40). First, we evaluated subgroup effects based on data from within the trial suggesting that some participants may have been unlikely to benefit from the intervention because they had a lower risk of developing BV during follow up. Second, we stratified participants based on vaginal infection status at enrollment. This approach would help to mitigate potentially unmeasured sources of bias. Third, we correctly anticipated the direction of the subgroup effect. We found that the majority of BV episodes occurred in the subgroup of women with a baseline vaginal infection. In fact, the effect of the intervention in this sub-population actually drove the overall effect seen among women in the trial as a whole.

Fourth, the hypothesized subgroup effect is biologically plausible, given previous findings from the Rakai studies and from this PPT trial which found that women with a prevalent vaginal infection are more likely to have recurrence after treatment. Fifth, we limited this secondary analysis to testing a single subgroup effect. Within the subgroup of women with a baseline vaginal infection, the intervention reduced episodes of BV and increased vaginal *Lactobacillus* colonization. The consistency of findings across these related outcomes provides support for the subgroup findings. Finally, the magnitude of the subgroup effect among women with a baseline vaginal infection was large and statistically significant.

The tests of interaction in this subgroup analysis were not statistically significant suggesting that the apparent subgroup effect could be as a result of chance. However, due to our

stratification, we had small sample sizes within each subgroup. As a result, we had limited statistical power to test for interaction. Future studies evaluating this subgroup effect would ideally pre-specify the hypothesis and calculate a sample size that would provide adequate statistical power to detect an effect.

The results of this subgroup analysis should be interpreted in the context of several limitations. We used the presence of a baseline vaginal infection as a marker of women who are more likely to develop future vaginal infections. While it is not possible to predict with precision which women will develop vaginal infections, available data suggest that if women with a prevalent vaginal infection are treated and followed up, they are likely to have recurrence of these infections compared to those without. Additionally, the generalizability of our findings may be limited by behavioral characteristics that are unique to this population of high-risk women, including differences in level of sexual activity, condom usage as well as intravaginal practices. However, the effect of PPT in populations that differ according to these behavioral characteristics has not been evaluated.

In conclusion, the results of this subgroup analysis add to the existing data and will be useful in planning future trials of the efficacy of PPT. This is the first study in sub-Saharan Africa looking at the effect of PPT within a subgroup of women in which the intervention could potentially be more beneficial. Since women with a prevalent vaginal infection are more likely to have a recurrence after treatment, our findings suggest that restricting delivery of a PPT to these women may provide better clinical benefit. Finally, these data are important for comparison with ongoing trials of topical PPT, in which participants are selected on the basis of baseline vaginal infection status.

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TABLES & FIGURES

Figure 1: Participant flow through the study according to baseline vaginal infection status

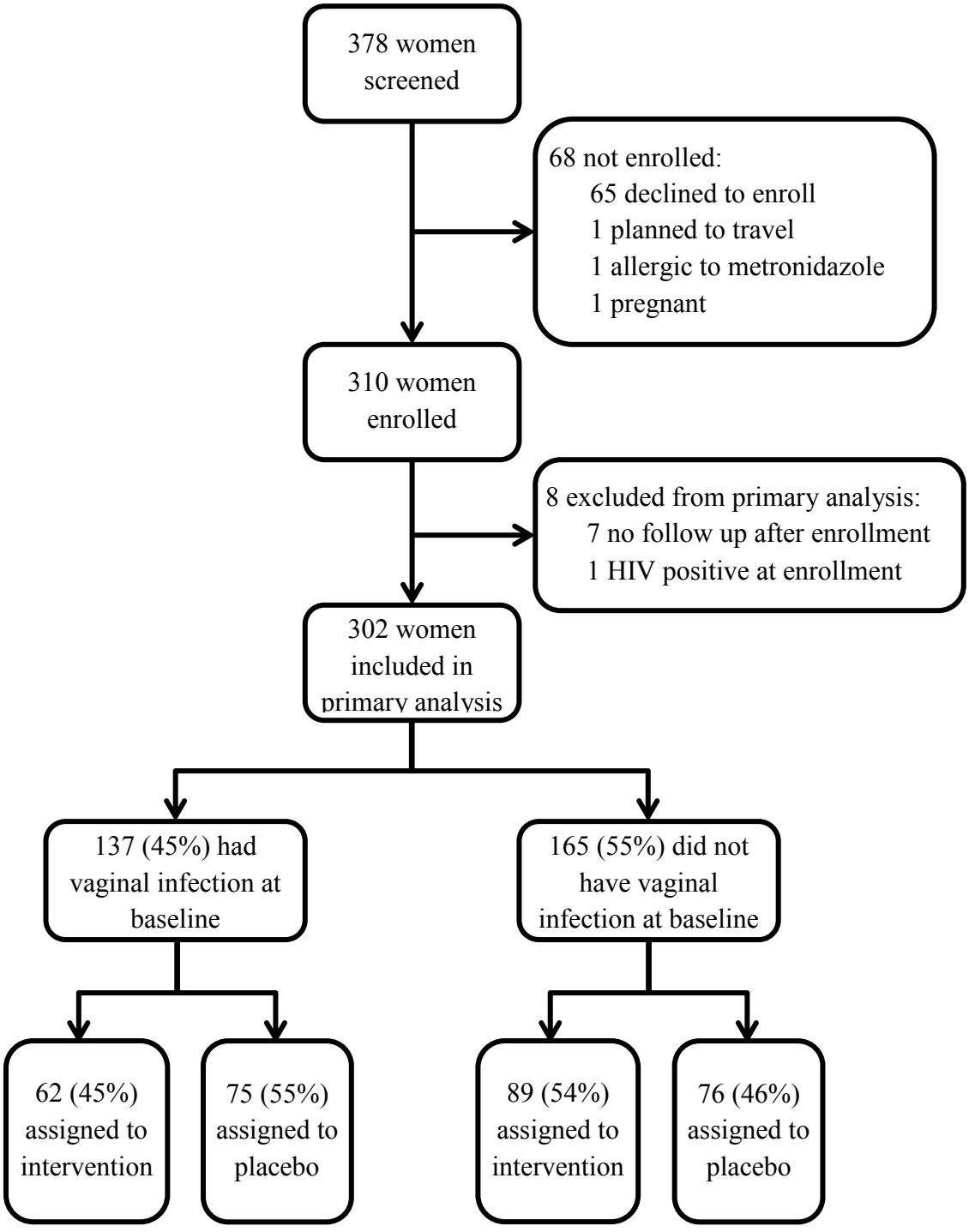

Table 1: Characteristics of participants at trial enrollment according to baseline vaginal infection status[1]

	Vaginal infection present (n=137)			**Vaginal infection absent (n=165)**		
	Intervention Arm (n=62)	Placebo Arm (n=75)	p-value[2]	Intervention Arm (n=89)	Placebo Arm (n=76)	p-value[2]
Age (years)	28 (24-30)	28 (24-34)	0.31	30 (23-34)	31 (26-37)	0.21
Years of education	8 (6-10)	8 (7-10)	0.40	8 (7-11)	8 (6-11)	0.92
Parity	1 (1-2)	1 (1-3)	0.55	2 (1-3)	1 (1-2)	0.28
Marital status			0.57			0.68
Never married	29 (47)	31 (41)		34 (38)	34 (45)	
Currently married	2 (3)	1 (1)		1 (1)	1 (1)	
Widowed/divorced	31 (50)	43 (57)		54 (61)	41 (54)	
Duration of sex work (years)	1 (0-3)	1 (0-3)	0.65	2 (0-5)	1 (0-5)	0.62
Age of first sex (years)	17 (16-18)	17 (15-18)	0.15	17 (15-19)	17 (16-19)	0.38
Place of work			0.43			0.34
Bar	37 (60)	54 (71)		62 (70)	62 (82)	
Nightclub	16 (26)	14 (20)		22 (25)	11 (15)	
Home-based	4 (7)	2 (3)		1 (1)	1 (1)	
Other	5 (8)	5 (7)		4 (5)	2 (3)	
Alcohol & substance use						
Drinks alcohol	56 (90)	56 (75)	0.02	68 (77)	59 (78)	0.96
Smokes cigarettes	12 (19)	9 (12)	0.22	15 (17)	9 (12)	0.36
Chews miraa	8 (13)	10 (13)	0.94	12 (14)	6 (8)	0.24
Uses marijuana	1 (2)	1 (1)	0.88	1 (1)	-	0.35
Uses injection drugs	-	-	-	-	-	-

	Vaginal infection present (n=137)			Vaginal infection absent (n=165)		
	Intervention Arm (n=62)	Placebo Arm (n=75)	p-value[2]	Intervention Arm (n=89)	Placebo Arm (n=76)	p-value[2]
Sexual risk practices						
Sexual episodes in the past week	1 (1-2)	1 (0-2)	0.71	1 (0-2)	1 (0-2)	0.99
Reported sexual intercourse in the past week	48 (77)	54 (72)	0.47	51 (57)	46 (61)	0.68
100% condom use[3]	35 (73)	35 (65)	0.37	32 (63)	32 (70)	0.74
Sexual partners in the past week	1 (1-1)	1 (0-1)	0.67	1 (0-1)	1 (0-1)	0.95
Reported deep kissing in the past week	9 (15)	8 (11)	0.50	4 (5)	7 (9)	0.23
Compensation for sex			0.68			0.33
Living expenses paid	21 (34)	28 (37)		32 (36)	33 (43)	
Cash	40 (66)	46 (62)		57 (64)	43 (57)	
Cash amount (Ksh)	500 (200-1000)	500 (300-1000)	0.92	500 (200-1000)	500 (200-1000)	0.16
Intravaginal practices						
Vaginal washing past week			0.56			0.65
None	1 (2)	4 (5)		6 (7)	7 (9)	
Water alone	14 (23)	20 (26)		25 (28)	25 (33)	
Soap	46 (74)	49 (65)		56 (64)	44 (58)	
Other	1 (2)	2 (3)		1 (1)	-	
Contraceptive method/device			0.69			0.29
None	40 (65)	46 (61)		59 (66)	47 (62)	
Condoms only	-	1 (1)		1 (1)	-	
OCP	5 (8)	3 (4)		7 (8)	6 (8)	
Depo-Provera	12 (19)	18 (24)		16 (18)	21 (28)	
IUD	3 (5)	2 (3)		-	-	
BTL	1 (2)	4 (5)		4 (5)	-	

	Vaginal infection present (n=137)			Vaginal infection absent (n=165)		
	Intervention Arm (n=62)	Placebo Arm (n=75)	p-value[2]	Intervention Arm (n=89)	Placebo Arm (n=76)	p-value[2]
Norplant	1 (2)	1 (1)		2 (2)	2 (3)	
Genital infections						
HSV-2 seropositive	52 (84)	68 (91)	0.23	76 (88)	62 (82)	0.23
Neisseria gonorrhoeae	1 (2)	-	0.27	-	-	-
Non-specific cervicitis[4]	-	-		-	2 (3)	0.12
Bacterial vaginosis	48 (77)	57 (76)	0.85	-	-	-
Vulvovaginal candidiasis	14 (23)	18 (24)	0.85	-	-	-
Trichomonas vaginalis	4 (7)	2 (3)	0.27	-	-	-
Lactobacillus colonization						
Any lactobacillus	16 (26)	23 (31)	0.53	82 (92)	63 (83)	0.07
H_2O_2-prod. lactobacillus	1 (33)	-	0.36	6 (60)	2 (50)	0.73

[1]Continuous data summarized as median (IQR); Categorical data as counts (%)
[2]Pearson's Chi-square test used for categorical data and Wilcoxon rank-sum test for continuous data
[3]Calculated among those who reported sexual intercourse as the proportion of sexual episodes where a condom was used out of all sexual episodes reported in the past week
[4]Non-specific cervicitis defined as ≥30 PMNs on wet prep
OCPs: oral contraceptive pills, IUD: Intrauterine device, BTL: bilateral tubal ligation

Table 2: Episodes of vaginal infections and *Lactobacillus* species colonization per 100 person-years according to baseline vaginal infection status[1]

	Baseline vaginal infection present (n=137)				Baseline vaginal infection absent (n=165)			
	Intervention Arm (py=59)	Placebo Arm (py=76)	HR[2] (95% CI)	*P*	Intervention Arm (py=91)	Placebo Arm (py=76)	HR[2] (95% CI)	*P*
Bacterial vaginosis	294 (172)	522 (396)	0.55 (0.41-0.76)	<0.001	141 (129)	202 (154)	0.71 (0.47-1.09)	0.11
Vulvovaginal candidiasis	102 (60)	108 (82)	0.94 (0.59-1.51)	0.80	79 (72)	105 (80)	0.75 (0.46-1.23)	0.26
Trichomonas vaginalis	15 (9)	24 (18)	0.64 (0.24-1.71)	0.37	3 (3)	5 (4)	0.65 (0.15-2.85)	0.57
Any lactobacillus	135 (79)	84 (64)	1.61 (1.01-2.56)	0.04	140 (128)	109 (83)	1.32 (0.85-2.04)	0.22
H_2O_2-prod. lactobacillus	55 (32)	30 (23)	1.85 (1.00-3.45)	0.05	57 (52)	39 (30)	1.48 (0.73-3.01)	0.28

[1]Data presented as episodes of each outcome per 100 person-years (Total number of episodes)
[2]HR=hazard ratio: ratio of the risk of the outcome in the intervention arm compared to that in the placebo arm; py=person-years

Table 3: Episodes of vaginal infections and *Lactobacillus* species colonization per 100 person-years according to baseline BV status[1]

	Baseline BV present (n=105)				Baseline BV absent (n=197)			
	Intervention Arm (py=46)	Placebo Arm (py=57)	HR[2] (95% CI)	*P*	Intervention Arm (py=104)	Placebo Arm (py=96)	HR[2] (95% CI)	*P*
Bacterial vaginosis	323 (149)	572 (325)	0.56 (0.40-0.78)	0.001	146 (152)	235 (225)	0.63 (0.43-0.91)	0.01
Vulvovaginal candidiasis	93 (43)	83 (47)	1.14 (0.64-2.03)	0.65	86 (89)	120 (115)	0.72 (0.47-1.09)	0.12
Trichomonas vaginalis	13 (6)	25 (14)	0.52 (0.17-1.64)	0.27	6 (6)	8 (8)	0.70 (0.20-2.41)	0.58
Any lactobacillus	139 (64)	65 (37)	2.15 (1.26-3.65)	0.01	138 (143)	115 (110)	1.23 (0.83-1.81)	0.30
H_2O_2-producing	59 (27)	18 (10)	3.45 (1.58-7.55)	0.002	55 (57)	45 (43)	1.25 (0.69-2.28)	0.47

[1]Data presented as episodes of BV per 100 person-years (Total number of BV episodes)
[2]HR=hazard ratio: ratio of the risk of BV in the intervention arm compared to that in the placebo arm; py=person-years

Table 4: Wald statistic and p-values for test of interaction[1]

	Test statistic	p-value
Bacterial vaginosis	-1.62	0.11
Vulvovaginal candidiasis	0.93	0.36
Trichomonas vaginalis	-0.01	0.99
Any lactobacillus	0.94	0.35
H_2O_2-producing	0.62	0.54

[1]Test of interaction conducted by evaluating statistical significance of the beta coefficient associated with the interaction term (Wald Test)