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

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Abstract

Background—This analysis compared the frequency of persistent *Trichomonas vaginalis* (TV) among HIV-seropositive and HIV-seronegative women.

Methods—Data were obtained from women enrolled in an open cohort study of sex workers in Kenya. Participants were examined monthly, and those diagnosed with TV by saline microscopy were treated with single-dose 2g oral metronidazole. All women on antiretroviral therapy (ART) used nevirapine-based regimens. Generalized estimating equations with a logit link were used to compare the frequency of persistent TV (defined as the presence of motile trichomonads by saline microscopy at the next exam visit within 60 days) by HIV status.

Results—Three-hundred and sixty participants contributed 570 infections to the analysis (282 HIV-seropositive and 288 HIV-seronegative). There were 42 (15%) persistent infections among HIV-seropositive participants versus 35 (12%) among HIV-seronegative participants (adjusted odds ratio [aOR]=1.14; 95% confidence interval [CI] (0.70, 1.87)). Persistent TV was highest among HIV-seropositive women using ART (21/64 [33%]) compared to HIV-seropositive women not using ART (21/217 [10%]). Concurrent bacterial vaginosis (BV) at TV diagnosis was associated with an increased likelihood of persistent TV (aOR=1.90; 95% CI 1.16, 3.09).

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POTENTIAL CONFLICTS OF INTEREST

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Conclusions—The frequency of persistent TV infection following treatment with single-dose 2g oral metronidazole was similar by HIV status. Alternative regimens, including multi-day antibiotic treatment, may be necessary to improve cure rates for women using nevirapine-based ART and women with TV and concurrent BV.

Keywords

Trichomonas vaginalis; metronidazole; efficacy; persistence; HIV infection; nevirapine; antiretroviral therapy

INTRODUCTION

Trichomonas vaginalis (TV) is a sexually transmitted protozoan that accounts for more than half of all curable sexually transmitted infections (STIs) worldwide [1]. The prevalence of TV is highest among reproductive-aged women [1, 2] and is associated with pelvic inflammatory disease, adverse pregnancy outcomes, and increased risk of HIV acquisition [3]. Trichomoniasis is also frequently diagnosed among HIV-seropositive women [4–8] and has been associated with increased genital HIV RNA shedding [5, 9]. Treatment of TV infection can decrease genital HIV shedding [9, 10], potentially reducing the risk of transmission to HIV-uninfected partners [11].

The Centers for Disease Control and Prevention recommends single-dose 2g oral metronidazole or tinidazole for treatment of TV infection [12]. 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 18.3% of HIV-seropositive women with TV were still positive 1 month after treatment with 2g single-dose metronidazole compared to 8% of HIV-seronegative women ($p=0.01$) [6]. 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 [13]. 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 remained positive for TV following treatment with single-dose 2g oral metronidazole.

METHODS

Study population and procedures

Data were obtained from women enrolled in an open cohort study of sex workers in Mombasa, Kenya between February 1993 and December 2010. Detailed methods for the cohort have been described [14]. 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 women were also invited to enroll. The study received approval from the institutional review boards at 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 monthly follow-up visits, a standardized face-to-face interview was conducted to collect information on medical and sexual history. A physical examination was performed, including speculum pelvic examination with collection of genital secretions for diagnosis of genital tract infections and assessment of vaginal pH. Participants who reported

symptoms of a genital tract infection were treated syndromically according to Kenyan National Guidelines [15]. 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 were offered a routine package of HIV care at no cost. In addition, women who met Kenyan National Guidelines for initiation of antiretroviral therapy (ART) were offered ART in our clinic beginning in March, 2004 [16]. Participants on ART used regimens consisting of nevirapine, lamivudine, and stavudine or zidovudine. All participants were given a follow-up appointment to receive laboratory test results one week after their visit. At that time, additional treatment was provided 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 [17]. *Lactobacillus* culture was performed on Rogosa agar [18], and sub-culture of *Lactobacillus* isolates on tetramethylbenzidine agar containing horseradish peroxidase was performed to evaluate hydrogen peroxide production [19]. 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). Endocervical secretions were cultured on modified Thayer–Martin media for *Neisseria gonorrhoeae*. 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 *Chlamydia 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 [bioMérieux, Marcy l’Etoile, France]). CD4 cell counts were assessed by Coulter (Cytosphere, Haileah, USA), Zymune (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 of initial diagnosis. 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.

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 these practices at enrollment and

follow-up [20]. We assumed an effect window of 85 days for hormonal contraceptive use [21]. Data on the whiff test to detect amines were limited. Therefore, BV by clinical criteria could not be assessed [22]. 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.

Participants could contribute multiple infections to the analysis; therefore, generalized estimating equations models with a logit link, independent correlation structure and robust standard errors were used to compare demographic, behavioral and clinical characteristics by HIV status. This method accounts for clustering due to multiple observations per participant. This method was also used to assess factors that may be associated with persistent TV infection, including HIV status. Potential confounding factors assessed at diagnosis or assessment of cure that differed substantially by HIV status ($p < 0.10$) were considered for inclusion in a multivariable model 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 was included *a priori* in the adjusted model as an important potential confounding factor. Participants were categorized as having unprotected sex if they reported one or more sex acts in the past week and had less than 100% condom use. Participants were enrolled in a relatively equal distribution across the study period. Therefore, in order to account for possible temporal trends in STI incidence and access to antiretroviral therapy, calendar year of diagnosis was also included in the adjusted model. 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.

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%] HIV-seronegative; $p=0.7$). Among participants with concurrent BV and TV infection, symptoms were reported at 33/138 (24%) TV infections. Mean time from diagnosis to assessment of cure was identical by HIV status (33 days $\pm$ 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 $\pm$ SD 8 versus parasitologic cure = 33 days $\pm$ SD 9; p -value=0.4). Demographic and behavioral characteristics were not associated with persistent TV (Table 3). TV persistence was highest among HIV-seropositive participants using ART (21/64 [33%]) compared to HIV-seropositive participants not using ART (21/217 [10%]) and HIV-seronegative participants (35/288 [12%]). Compared to HIV-seronegative participants, HIV-seropositive women not using ART had no increased risk of persistent TV (aOR=0.82, 95% CI 0.44, 1.52), whereas participants using ART had a 2.91-fold increased risk of persistent infection at the assessment of cure (95% CI 1.91, 7.27) (Table 4). Among HIV-seropositive participants, those using ART had a 2.88-fold increased risk of persistent TV compared to participants not using ART (95% CI 1.32, 6.30). Sexual behaviors at TV diagnosis and the assessment of cure did not differ between HIV-seropositive participants on ART versus those not on ART (data not shown).

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 similar after adjustment for HIV status, calendar year, and unprotected sex in the past week (aOR=1.90; 95% CI 1.16, 3.09). The presence of clue cells on saline wet preparation at the time of TV diagnosis was also associated with an increased likelihood of persistent TV compared to participants with no clue cells (37 [18%] versus 40 [11%], respectively; OR=1.74; 95% CI 1.08, 2.80).

DISCUSSION

In this analysis of HIV-seropositive and seronegative 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. HIV-seropositive women using nevirapine-based ART had a 1 in 3 chance of persistent infection, which was much higher than the rate of persistence observed in other participants. In addition, 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 and the first study to report an association between ART use and an increased risk of persistent TV.

Few studies have evaluated the association between antiretroviral use and persistent TV. Conducted over a decade ago, these studies primarily included women using protease inhibitors (PI) and reported no association between PI use and recurrent or re-infection with TV [7, 8]. In our study, all participants on ART used nevirapine-containing regimens and were highly adherent, with a median of 100% adherence. The strong association between use of nevirapine-based ART and increased risk of persistent TV is a novel finding that suggests an interaction between metronidazole and nevirapine. Several reports of drug interactions resulting in decreased efficacy of metronidazole have been documented [23, 24]. Metronidazole and nevirapine are both metabolized in the liver by enzymes in the cytochrome P450 system [25]. Nevirapine is a known cytochrome P450 inducer [26] and could increase clearance of metronidazole and its active metabolites through up-regulation of this system, therefore reducing the effectiveness of metronidazole. Higher doses and longer durations of metronidazole treatment may be required to achieve cure among women

with TV using nevirapine-based ART. The pharmacokinetic relationship between nevirapine use and metronidazole metabolism requires further exploration.

We also 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) [27]. 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 [2, 28]. 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 [12]. 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. Notably, clue cells may also be a useful marker of women with increased risk for TV persistence, and this test may be more readily available in a wide variety of clinical settings.

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) [29]. 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. First, only wet mount microscopy was used to detect TV. Culture for TV became available during the time that this study was conducted; however, it was not added because of cost. While saline microscopy remains the most common method for TV diagnosis in clinical settings, it has considerably lower sensitivity compared to culture and especially compared to nucleic acid amplification testing (NAAT) [30]. 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. A second limitation was the fact that the assessment of cure was performed up to 60 days following the initial diagnosis. This interval is similar to other studies [6]. 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 reinfection. A third limitation relates to the generalizability of the findings to other antiretroviral regimens. Because all participants included in the analysis used nevirapine-based ART, we were unable to assess the effect of

other ART regimens on TV persistence. Lastly, it is possible that a proportion of persistent infection were due to re-infection from an untreated partner. 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 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 HIV-seropositive women using nevirapine-based ART and 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 [5, 6, 8, 13]. 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. Higher doses and longer durations of metronidazole or tinidazole treatment that provide increased and sustained drug levels should be evaluated as an approach to improve cure rates among women using nevirapine-based ART or women with concurrent BV.

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Table 1

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

	HIV-seropositive N=282	HIV-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
Number of sexual episodes in the past week ¹			
1 sex act	71 (25)	67 (24)	0.3
2 or more sex acts	71 (25)	108 (39)	0.002
Number of partners in the past week ¹			
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			
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) ⁷	370 ± 235	-- ---	--
On ART	65 (23)	-- ---	--

N (%) or mean ± SD presented.

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

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

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

² Compared to participants 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-seropositive participants only. CD4 counts available for 260 infections within 6 months pre or post infection.

Table 2Behavioral and clinical characteristics reported at the assessment of cure by HIV status^{*}

	HIV-seropositive N=282	HIV-seronegative N=288	P-value ^{**}
Sexual activity in the past week	141 (50)	168 (58)	0.08
Number of sexual episodes in the past week			
1 sex act	65 (23)	65 (23)	0.5
2 or more sex acts	76 (27)	103 (36)	0.04
Number of partners in the past week			
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 vaginosis 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 ³	5 (2)	30 (10)	0.002

N (%) presented.

^{*} N represents the number of TV infections contributed by 360 unique participants. Eleven participants contributed infections while HIV-seronegative and HIV-seropositive.

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

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

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-seropositive	42 (55)	240 (49)	1.27 (0.75, 2.12)	1.14 (0.70, 1.87)
Age (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				
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 past week				
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 [†]	11 (14)	96 (19)	0.69 (0.34, 1.41)	0.64 (0.30, 1.36)
Hormonal contraceptive use				
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)	
Bacterial vaginosis at diagnosis	44 (57)	209 (43)	1.81 (1.12, 2.92)	1.90 (1.16, 3.09)
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)	
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)	
Cervicitis at assessment of cure	6 (7)	30 (6)	1.07 (0.41, 2.82)	

N (%) or mean ± SD presented.

* N represents the number TV infections contributed by 360 unique participants. Eleven participants contributed infections while HIV-seronegative and HIV-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 were associated with HIV status ($p < 0.10$) and either produced a 10% in coefficient or 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)

Table 4Associations of antiretroviral therapy use, HIV status and persistent *T. vaginalis**

	Persistent TV*	Unadjusted OR (95% CI)	Adjusted** OR (95% CI)
HIV-seronegative	35/288 (12%)	1.00 (Ref)	1.00 (Ref)
HIV-seropositive and <i>not</i> using ART	21/217 (10%)	0.77 (0.42, 1.41)	0.82 (0.44, 1.52)
HIV-seropositive and using ART***	21/64 (33%)	3.53 (1.96, 6.37)	2.91 (1.16, 7.27)

* Numerator is the number of persistent TV infections. Denominator is the number of TV infections in that stratum.

** Model adjusted for trimethoprim/sulfamethoxazole use, unprotected sex and calendar year.

*** One HIV-seropositive participant on an ART regimen that did not include nevirapine was excluded from this analysis. Median adherence to ART at TV diagnosis was 100% based on pill count (Interquartile range: 96–100%).