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# Early Repeated Infections with *Trichomonas vaginalis* among HIV-Positive and HIV-Negative Women

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## Abstract

**Background—**The purpose of the study was to examine whether early repeated infections due to *Trichomonas vaginalis* among human immunuodeficiency virus (HIV)–positive and HIV-negative women are reinfections, new infections, or cases of treatment failure.

**Methods—**Women attending an HIV outpatient clinic and a family planning clinic in New Orleans, Louisiana, who had culture results positive for *T. vaginalis* were treated with 2 g of metronidazole under directly observed therapy. At 1 month, detailed sexual exposure and sexual partner treatment information was collected. Isolates from women who had clinical resistance (i.e., who tested positive for a third time after treatment at a higher dose) were tested for metronidazole susceptibility in vitro.

**Results—**Of 60 HIV-positive women with trichomoniasis, 11 (18.3%) were *T. vaginalis* positive 1 month after treatment. The 11 recurrences were classified as 3 probable reinfections (27%), 2 probable infections from a new sexual partner (18%), and 6 probable treatment failures (55%); 2 of the 6 patients who experienced probable treatment failure had isolates with mild resistance to metronidazole. Of 301 HIV-negative women, 24 (8.0%) were *T. vaginalis* positive 1 month after treatment. The 24 recurrences were classified as 2 probable reinfections (8%) and 22 probable treatment failures (92%); of the 22 patients who experienced probable treatment failure, 2 had strains with moderate resistance to metronidazole, and 1 had a strain with mild resistance to metronidazole.

**Conclusion—**HIV-positive women were more likely to have sexual re-exposure than were HIV-negative women, although the rate of treatment failure was similar in both groups. High rates of treatment failure among both HIV-positive and HIV-negative women indicate that a 2-g dose of metronidazole may not be adequate for treatment of some women and that rescreening should be considered.



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*Trichomonas vaginalis* is a sexually transmitted infection that is associated with significant morbidity, especially among pregnant women [1]. In addition to preterm delivery and other adverse birth outcomes, *T. vaginalis* is associated with vaginitis, cervicitis, urethritis, and pelvic inflammatory disease [1, 2]. *T. vaginalis* infection also increases vaginal shedding of HIV and is associated with increased susceptibility to HIV infection and higher HIV transmission rates [3, 4]. The US prevalence of *T. vaginalis* infection among women of reproductive age is estimated to be 3.1%, and risk factors include non-Hispanic black race/ethnicity, being born in the United States, having a greater number of lifetime sexual partners, increasing age, lower educational level, poverty, and vaginal douching [5].

The Centers for Disease Control and Prevention recommend the treatment of *T. vaginalis* with use of either 2 g of metronidazole or 2 g of tinidazole in a single oral dose or, as an alternative, 500 mg of metronidazole administered orally twice daily for 7 days [6]. In randomized clinical trials, these recommended metronidazole regimens have resulted in cure rates of 90%–95%, and the recommended tinidazole regimen has resulted in cure rates of 86%–100% [7]. Metronidazole resistance has been documented in 2.5%–9.6% of clinical isolates [8–10] and is defined as aerobic minimal lethal concentrations (MLC) of ≥50 $\mu$g/mL. Most resistant strains are found to have MLCs of 50–100 $\mu$g/mL, which is considered to be mild resistance [10], and infections due to these strains can generally be cured with either a higher dose of metronidazole or tinidazole therapy.

Reinfection by an untreated sexual partner is a potential source of recurrence. Although the treatment of partners is recommended, expedited partner treatment (i.e., the provision of medicine to the index patient for use by her partner) has not been recommended for *T. vaginalis* infection [11]. There also are no recommendations for rescreening of asymptomatic persons [6].

Rates of repeated infection have been found to be 5%–8% among women [11–13] and up to 30% among HIV-infected women [2, 12, 14, 15]. At present, the mechanism by which these repeated infections occur is not fully understood. Moreover, the reason why HIV-positive women, compared with HIV-negative women, tend to experience higher rates of repeat infection also needs further exploration [2, 15]. This has particular public health significance because of the burden of *T. vaginalis* infection on HIV-positive and HIV-negative women and its association with exacerbation of HIV transmission [16–18]. A better understanding of the nature of repeat infections can assist in developing interventions to prevent them. Repeated infections could be caused by a lack of treatment adherence by the index patient, reinfection by an untreated sexual partner, infection by a new sexual partner, or treatment failure [19].

The purpose of the study was to examine whether repeated infections due to *T. vaginalis* among HIV-positive and HIV-negative women who received 2 g of metronidazole under directly observed therapy were attributable to probable reinfection, probable infection by a new sexual partner, or treatment failure. The rate of metronidazole resistance among isolates obtained from patients who experienced treatment failure was also examined.

## METHODS

### Study design

These are secondary analyses of data from 2 separate cohorts. Data for the HIV-negative women came from a randomized, controlled trial of expedited sexual partner treatment for *T. vaginalis* infection among HIV-negative women. This study was conducted from September 2001 through December 2003. Methods used in this study have been published elsewhere

[20]. In brief, the study included women who attended a public family planning clinic who were ≥16 years old, denied being HIV positive, were scheduled to undergo a pelvic examination, were not in the first trimester of pregnancy, and agreed to participate in the randomized trial of sexual partner treatment. Because it was a study of sexual partner treatment, women had to name at least 1 sexual partner to be included in the study.

The second study involved a cohort of HIV-infected women who attended an outpatient clinic and who were ≥18 years of age, not pregnant, and scheduled to undergo a pelvic examination. The goal of this study was to examine HIV vaginal viral shedding among women with and women without *T. vaginalis* infection. Detailed study methods have been described elsewhere [21]. Women's HIV status was confirmed from documentation in their medical records, and women did not need to name a sexual partner to be included.

### Diagnosis of *T. vaginalis* infection, treatment, and follow-up

Women in both studies were tested via wet mount microscopy by their provider and had a confirmatory culture performed using InPouch medium (Biomed Diagnostics). Cultures were incubated at 37°C and were read by trained technicians 3 times over a 72-h period. Women who had negative test results via wet mount but had positive culture results were called back to the clinics and offered the opportunity to enroll in the study. Women who had symptomatic bacterial vaginosis were excluded from both studies.

Treatment for the index infection in both studies was a single 2-g dose of metronidazole under direct observation from the study staff. Because of differences in the study designs, 33% of the HIV-negative women [20] and 100% of the HIV-positive women [21] received expedited sexual partner treatment (i.e., they were provided with metronidazole to give to their partners). All expedited sexual partner treatment was provided in containers with childproof caps and was accompanied by patient instructions that included a number that the partner could call with questions regarding the treatment.

In both studies, women were asked to return 1 month after treatment (with a 2–8-week window) for a second interview and retesting for *T. vaginalis* infection. At baseline and follow-up, women underwent a computer-assisted self-administered interview. This interview elicited detailed sexual partner information regarding demographic characteristics, sexual and condom behaviors, and treatment status. Women who had positive test results at 1 month were given a higher dose of metronidazole (i.e., 500 mg administered twice per day for 7 or 10 days) and were asked to return 2 weeks later for a third *T. vaginalis* culture. If the woman was still *T. vaginalis* positive after 2 courses of metronidazole and denied unprotected sex with an untreated partner, she was considered to have clinically resistant infection, and her specimen was prepared and sent to the Centers for Disease Control and Prevention (Atlanta, GA) for in vitro susceptibility testing.

### Definitions of outcomes

Based on self-reported sexual behavior with all sexual partners during the follow-up period, 3 categories of outcome were determined: probable reinfection (for patients with unprotected sexual exposure to an untreated sexual partner), probable infection by a new sexual partner (for patients with no sexual exposure to the original partner but unprotected sex with a new partner), and probable treatment failure (for patients with positive results of retesting after 2 courses of metronidazole and no sexual exposure).

### Laboratory methods for in vitro susceptibility testing

To prepare the *T. vaginalis*–positive culture samples for susceptibility testing, the following procedure was performed. After thoroughly mixing the medium with viable trichomonads in

the pouch using the "shoeshine" method (i.e., pulling the pouch up and down across the edge of a table and pressing gently against the table 3–4 times), the entire medium was removed with a sterile plastic transfer pipette and then transferred into a sterile conical tube. After transfer, the tube was spun at 1000 *g* for 2 min and the resulting supernatant was removed, taking care not to disturb the pellet. The pellet was then resuspended in 1 mL of fresh InPouch medium containing 5% dimethyl sulfoxide and was mixed well. The suspension of the organism was then transferred to a cryovial and was immediately frozen at −70°C. Isolates were sent to the Centers for Disease Control and Prevention and were expanded in Diamond culture medium prior to testing for metronidazole susceptibility.

Trichomonas isolates were tested for susceptibility to metronidazole according to the method of Meingassner and Thurner [22]. In brief, $10^4$ parasites were placed into wells of a round-bottom microtiter plate and were incubated with serial 2-fold dilutions (0.2–400 $\mu$g/mL) of metronidazole at 37°C. Assays were performed in triplicate, and standard metronidazole-resistant and metronidazole-susceptible isolates were included as controls in each assay. After 48 h of incubation, plates were examined using an inverted phase-contrast microscope. The MLC was defined as the lowest drug concentration at which no motile trichomonads were observed. Isolates with MLCs of 50–100 $\mu$g/mL are considered to have mild resistance, isolates with MLCs of 101–199 $\mu$g/mL are considered to have mild-to-moderate resistance, isolates with MLCs of 200–400 $\mu$g/mL are considered to have moderate resistance, and isolates with MLCs of >400 $\mu$g/mL are considered to have high resistance.

### Human subjects and statistics

Studies were approved by the institutional review boards of Tulane and Louisiana State Universities (New Orleans, LA), the Louisiana Office of Public Health and Charity Hospital (New Orleans, LA), and the Centers for Disease Control and Prevention. Women in both studies received a modest monetary incentive (e.g., $20–$30) to return for their follow-up study visit. $\chi^2$ and Fisher's exact tests were conducted using SPSS, version 14.0 (SPSS).

## RESULTS

### HIV-positive cohort

Of the 60 HIV-positive women examined, 20.3% were <30 years of age, 55 (91.7%) were black, 48 (79.3%) had a high school education or less, and 44 (73.3%) reported 1 sexual partner and 12 (20.0%) reported no sexual partners in the previous 4 weeks. Eighteen (30.0%) of the women had $CD4^+$ cell counts <200 cells/$mm^3$ [3, 18]. Four (6%) had undetectable plasma viral loads, 26 (32.2%) had detectable viral loads of up to 9999 copies/mL, and 30 (49.2%) had ≥10,000 copies/mL. More than one-half of the women (34 women; 56.7%) were receiving antiretroviral therapy, and of those, 28 (81.0%) reported taking all of their antiretroviral therapy as prescribed the day before the study visit. Index women reported that the majority of their sexual partners took their medication for *T. vaginalis* infection (41 women; 67.9%).

Of the 60 women, 28 (46.7%) had sex with at least 1 baseline partner, and 13 (21.7%) acquired a new partner (table 1). Unprotected sex with a baseline partner was reported by 12 (20.0%) of the study participants, and unprotected sex with a new partner was reported by 16.7%; thus, a total of 21 (35.0%) of the women reported having unprotected sex during the 1-month follow-up period.

Of 60 HIV-positive women with trichomoniasis, 11 (18.3%) were *T. vaginalis* positive 1 month after treatment (table 2), and 38 (63.6%) of the women who were positive 1 month

after treatment were symptomatic. Of the 11 patients with recurrent infection, 3 (27%) were classified as having cases of probable reinfection, 2 (18%) were classified as having cases of probable infection by a new sexual partner, and 6 (55%) were classified as having cases of probable treatment failure. Of the 6 patients who experienced probable treatment failure, 3 responded to higher doses of metronidazole. Isolates obtained from the remaining 3 individuals were tested for in vitro susceptibility; 2 isolates had mild resistance (MLC, 50–100 $\mu$g/mL), and the other isolate was found to be susceptible. Both women whose isolates had mild resistance were symptomatic for *T. vaginalis* infection.

### Family planning HIV-negative cohort

Of the 301 women in the HIV-negative cohort, 298 (99.0%) were black, 175 (58.1%) had a high school education or less, 222 (74.0%) were <30 years of age, 36 (12.1%) had >1 sexual partner in the 2 months before the baseline visit, and 301 (100%) denied being HIV positive. During the follow-up period, 138 (45.8%) said they had sex with a baseline partner, and 14 (4.7%) said they had sex with a newly acquired partner (table 1). The women reported that most (209; 69.3%) of their sexual partners took metronidazole. At the follow-up visit, 24 women (8.0%) had cultures positive for *T. vaginalis* (table 2). Of the 24 patients who experienced recurrence, 2 (8%) were classified as having cases of probable reinfection, and 22 (92%) were classified as having cases of probable treatment failure. Of the 22 patients with probable treatment failure, 17 responded to higher doses of metronidazole. The 5 remaining patients had isolates that were tested for in vitro susceptibility; 1 isolate was susceptible, 1 died in transit, and 2 had mild resistance (MLC, 50–100 $\mu$g/ mL) and 1 had moderate resistance to metronidazole (MLC, 200 $\mu$g/mL). Symptom information collection was started in July 2003 and was available for 49 of the women enrolled in the study. Of the 4 patients who experienced recurrent infection for whom symptom information was available, 1 (25%) exhibited symptoms at the follow-up visit.

### Comparisons between HIV-positive and HIV-negative women

When compared with the HIV-negative cohort, the women from the HIV-positive cohort were more likely to be older ($P$ =.001) and more likely to be nonblack ($P$ =.01; table 1). During the follow-up period, the HIV-positive women were more likely than the HIV-negative women to have had sex with a new partner ($P$ =.001), to have had unprotected sex with a new partner ($P$ =.001), and to have had vaginal sex without the use of a condom ($P$ =.01). They were less likely to report drinking alcohol ($P$ =.001) but were equally as likely to report having had multiple sexual partners ($P$ =.23), having had sex with their baseline partners ($P$ =.91), having had unprotected sex with their baseline partners ($P$ =.80), and that their baseline partners took metronidazole ($P$ =.86).

Repeated infections were more common among HIV-positive women than they were among HIV-negative women ($P$ =.01; table 2). HIV-positive women were more likely than HIV-negative women to be classified as having probable reinfection ($P$ =.03) or infection by a new sexual partner ($P$ =.03), but they were equally as likely to be classified as having experienced treatment failure ($P$ =.44) or to be infected with an isolate having resistance to metronidazole ($P$ =.19). The rate of probable treatment failure at the 1-month visit was high for both HIV-positive women (10.0%) and HIV-negative women (7.3%; table 2).

## DISCUSSION

In this study, we examined, using similar study designs, the nature of *T. vaginalis* recurrence rates according to self-reported sexual exposure among HIV-positive and HIV-negative women. Because all index patients received the 2-g single dose of metronidazole under

directly observed therapy, we were able to eliminate the confounder of treatment adherence by the index patients.

With respect to the most probable cause of reinfection, HIV-positive women were more likely to have been reinfected by an untreated baseline sexual partner or to have been infected by a newly acquired sexual partner, compared with HIV-negative women. These data corroborate our previous findings [23] and the findings of others [24] that HIV-positive women continue high-risk sexual behavior despite receiving a diagnosis of HIV infection and underscore the need to increase efforts to promote safer sex among these women.

Although there was a statistically nonsignificant trend toward a higher prevalence of drug resistance among isolates obtained from HIV-positive women, compared with among isolates obtained from HIV-negative women, the percentage of women with metronidazole-resistant *T. vaginalis* isolates in both groups was within the range that has been reported in the literature (2.5%–9.6%) [8–10, 25, 26]. If isolates obtained from all patients with repeat infections had been tested for in vitro susceptibility, a more precise understanding of the contribution of metronidazole resistance to the frequency of treatment failure could have been obtained. Larger studies could clarify whether a greater prevalence of drug-resistant *T. vaginalis* infection actually exists among HIV-positive women.

In the absence of testing the sexual partners for *T. vaginalis* infection, it is possible that there was some overlap between our categories of probable reinfection, probable new infection, and probable treatment failure, which were intended to be mutually exclusive. For example, some of those who were classified as having new infection may have experienced treatment failure if the new sexual partner was not infected with *T. vaginalis*, and some of those who were classified as having probable reinfection may also have experienced treatment failure if the baseline sexual partner was not infected. Up to one-half of *T. vaginalis* infections are asymptomatic, untreated infections can persist for a long duration [12], and most male sexual partners of infected women are infected [27]. One cohort study found incident cases of *T. vaginalis* among persons who denied sexual activity [28]. These persons could have had low parasite burden and had previously had false-negative test results. Future studies testing the sexual partners and taking advantage of newer techniques, such as rapid testing [29] and genotypic typing [30], and making use of PCR methods [31], which are known to be more sensitive than culture, would be beneficial for a more definitive classification.

It is also possible that women who claimed that they used condoms all of the time actually did not; in this case, patients who were classified as having experienced treatment failure could, in fact, have experienced reinfection. The use of the computer-assisted self-administered interview method has been shown to reduce misclassification errors caused by social desirability [32, 33]. We believe, therefore, that this source of potential bias was minimal.

It is also possible that some of the women at the family planning clinic were actually HIV positive, because we did not perform HIV testing at the time of the study visit. However, because the prevalence of HIV infection at the clinic is known to be <1% [34], and because the women denied being HIV positive, the potential for misclassification is believed to be very small.

The high rates of treatment failure at 1 month among both HIV-positive women (10.0%) and HIV-negative women (7.3%) are a cause for concern because of the reproductive health consequences and because of the link between *T. vaginalis* infection and HIV transmission. There was a high percentage of women with recurrent infection who were asymptomatic (36.4% of the HIV-positive women and 75% of the HIV-negative women). High rates of

treatment failure, coupled with asymptomatic infection, indicate that rescreening should be considered, and the optimal time for this rescreening merits further investigation. Finally, data from this study suggest that the 2-g dose of metronidazole is not adequate for the treatment of *T. vaginalis* infection in some women.

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## Table 1

Demographic characteristics of patients with *Trichomonas vaginalis* infection.

| Variable | Percentage of patients | | *P* |
|---|---|---|---|
| | Family planning clinic (*n* = 301) | HIV clinic (*n* = 60) | |
| Age <30 years | 73.4 | 20.3 | .001 |
| Black | 99.0 | 91.7 | .01 |
| Symptomatic at baseline | 61.8 | 41.7 | .01 |
| Alcohol consumption | 61.0 | 32.8 | .001 |
| No. of baseline sexual partners | | | |
| 0 | …[a] | 20.0 | .23 |
| 1 | 88.0 | 73.3 | |
| >1 | 12.0 | 6.7 | |
| Sex with baseline partner | 45.8 | 46.7 | .91 |
| Unprotected sex with baseline partner | 18.5 | 20.0 | .80 |
| Sex with new partner | 4.7 | 21.7 | .001 |
| Unprotected sex with new partner | 1.0 | 16.7 | .001 |
| Sexual contact without a condom during the follow-up period | 19.5 | 35.0 | .01 |
| Sexual contact without a condom before partner took medication | 6.7 | NM | … |
| Sexual partners took medication | 69.3[b] | 67.9[c] | .86 |

**NOTE.** *P* values were determined by Fisher's exact test. NM, not measured.

[a] Women had to have a sexual partner to be eligible for the study. HIV-positive patietns reported on partners within the previous 4 weeks, and HIV-negative patietns reported on partners within the previous 8 weeks.

[b] Of 326 sexual partners.

[c] Of 53 sexual partners.

## Table 2

No. (%) of patients with a second test result positive for *Trichomonas vaginalis* after treatment with metronidazole.

| | No. (%) of patients | | |
|---|---|---|---|
| **Variable** | **Family planning clinic (*n* = 301)** | **HIV clinic (*n* = 60)** | ***P*** |
| Second test result positive for *T. vaginalis* | 24 (8.0) | 11 (18.3) | .01 |
| Probable reinfection | 2 (0.7) | 3 (5.0) | .03 |
| Probable treatment failure | 22 (7.3) | 6 (10.0) | .44 |
| Probable infection from new sexual partner | 0 (0.0) | 2 (3.3) | .03 |
| Isolate resistant to metronidazole | 3 (1.0) | 2 (3.3) | .19 |

**NOTE.** *P* values were determined by Fisher's exact test.