Monthly Antibiotic Chemoprophylaxis and Incidence of Sexually Transmitted Infections and HIV-1 Infection in Kenyan Sex Workers: A Randomized Controlled Trial

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Sexually transmitted infections (STIs) are important cofactors in the human immunodeficiency virus type 1 (HIV-1)/AIDS pandemic. In HIV-infected individuals, not only may symptomatic and asymptomatic STIs enhance sexual transmission of HIV-1 by increasing virus shedding from the genital tract, but at the same time HIV-1 infection itself increases susceptibility to STIs. There is also considerable evidence that STIs may increase HIV-1 susceptibility in uninfected individuals, although differentiating cause from effect is more difficult in this situation.

Prevention or control of STIs as a strategy for preventing HIV-1 transmission has met with mixed success. Improved syndromic management of STIs reduced HIV-1 incidence in communities in Mwanza, Tanzania, but in Uganda neither a similar strategy nor antibiotic mass-treatment of whole communities had an impact on HIV-1 incidence.

Context Sexually transmitted infections (STIs) are common in female sex workers (FSWs) and may enhance susceptibility to infection with human immunodeficiency virus type 1 (HIV-1).

Objective To examine regular antibiotic prophylaxis in FSWs as a strategy for reducing the incidence of bacterial STIs and HIV-1.

Design, Setting, and Participants Randomized, double-blind, placebo-controlled trial conducted between 1998-2002 among FSWs in an urban slum area of Nairobi, Kenya. Of 890 FSWs screened, 466 who were seronegative for HIV-1 infection were enrolled and randomly assigned to receive azithromycin (n=230) or placebo (n=236). Groups were well matched at baseline for sexual risk taking and STI rates.

Intervention Monthly oral administration of 1 g of azithromycin or identical placebo, as directly observed therapy. All participants were provided with free condoms, risk-reduction counseling, and STI case management.

Main Outcome Measures The primary study end point was incidence of HIV-1 infection. Secondary end points were the incidence of STIs due to Neisseria gonorrhoeae, Chlamydia trachomatis, Trichomonas vaginalis, Treponema pallidum, and Haemophilus ducreyi, as well as bacterial vaginosis. Analysis of herpes simplex virus type 2 (HSV-2) infection was performed post hoc.

Results Seventy-three percent of participants (n=341) were followed up for 2 or more years or until they reached an administrative trial end point. Incidence of HIV-1 did not differ between treatment and placebo groups (4% [19 cases per 473 person-years of follow-up] vs 3.2% [16 cases per 495 person-years of follow-up] rate ratio [RR], 1.2; 95% CI, 0.6-2.5). Incident HIV-1 infection was associated with preceding infection with N gonorrhoeae (rate ratio [RR], 4.9; 95% CI, 1.7-14.3) or C trachomatis (RR, 3.0; 95% CI, 1.1-8.9). There was a reduced incidence in the treatment group of infection with N gonorrhoeae (RR, 0.46; 95% CI, 0.31-0.68), C trachomatis (RR, 0.38; 95% CI, 0.26-0.57), and T vaginalis (RR, 0.56; 95% CI, 0.40-0.78). The sero-prevalence of HSV-2 infection at enrollment was 72.7%, and HSV-2 infection at baseline was independently associated with HIV-1 acquisition (RR, 6.3; 95% CI, 1.5-27.1).

Conclusions Despite an association between bacterial STIs and acquisition of HIV-1 infection, the addition of monthly azithromycin prophylaxis to established HIV-1 risk reduction strategies substantially reduced the incidence of STIs but did not reduce the incidence of HIV-1. Prevalent HSV-2 infection may have been an important cofactor in acquisition of HIV-1.

JAMA. 2004;291:2555-2562

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incidence. Factors contributing to the lack of efficacy in the Uganda trials may have included the greater effectiveness of continuously available STI treatment services and the reduction in spread of HIV-1 during primary infection due to counseling given at the time of STI therapy. Another important factor may be that the Tanzanian study was performed early in the epidemic, when community prevalence of HIV-1 was below 5%. The Ugandan studies, by contrast, were performed later, in communities with much higher prevalences of HIV-1 (range, 10%-16%). Curable STIs may play a lesser role in HIV-1 transmission in the context of a “mature” epidemic, because most transmission occurs in the context of stable partnerships, reducing the potential impact of STI prevention and treatment. Interventions based on prevention or control of STIs may therefore be more effective in communities in the early stages of an epidemic or in subgroups at high risk of STIs.

Female sex workers (FSWs) constitute an important vulnerable group in the acquisition and transmission of both HIV-1 infection and STIs but may be excluded from household-based community studies of STI control. It has therefore been suggested that interventions for control of STIs should target these women specifically. Studies in Kenya have shown that certain FSW cohorts have an annual HIV incidence of 16% to 19% and a high incidence of cervicitis due to infection with Neisseria gonorrhoeae and Chlamydia trachomatis. This may be partly attributed to low levels of condom use and poor access to STI counseling and treatment services. We hypothesized that these high rates of bacterial STI and HIV-1 infection would make FSWs an ideal population in which to test antibiotic prophylaxis of common genital tract infections as an HIV-1 prevention strategy. Since the use of prophylactic antibiotics by FSWs has been associated with increased sexual risk taking, we elected to test this intervention in a blinded fashion.

**METHODS**

The study objective was to examine the effect of monthly antibiotic prophylaxis on the incidence of STIs and HIV-1 infection in a cohort of FSWs. The study design and methods are summarized below and have been presented in detail previously.

**Study Population and Design**

Self-identified FSWs were recruited from Kisumu, a urban slum area of Kisumu, Kenya. Inclusion criteria for the trial were (1) negative HIV-1 serology results at baseline; (2) current engagement in sex work, ie, reporting having received money or gifts in exchange for sex over the past month; (3) age 18 years or older; (4) expected residence in Nairobi for at least 2 years; and (5) no history of adverse drug reaction to macrolide antibiotics. Although only HIV-seronegative women were eligible for this trial of HIV-1 prevention, all FSWs who provided written informed consent for HIV-1 counseling and testing were provided with medical and counseling services for the duration of the trial. Approval for the trial was obtained from institutional review boards at the Kenyatta National Hospital (Nairobi, Kenya) and the University of Manitoba (Winnipeg, Manitoba). Clinic staff assigned study numbers consecutively at enrollment; neither study staff nor the participants were aware of group assignment. The primary outcome measure was incidence of HIV-1 infection, and secondary outcome measures were the rates of bacterial STIs (infection with N gonorrhoeae, C trachomatis, Trichomonas vaginalis, Treponema pallidum, and Haemophilus ducreyi; bacterial vaginosis).

**Study Procedures**

The study drug was administered as directly observed therapy, from numbered containers that were packaged, labeled, and shipped by the Misericordia General Hospital pharmacy (Winnipeg, Manitoba). Clinic staff assigned study numbers consecutively at enrollment; neither study staff nor the participants were aware of group assignment. The primary outcome measure was incidence of HIV-1 infection, and secondary outcome measures were the rates of bacterial STIs (infection with N gonorrhoeae, C trachomatis, Trichomonas vaginalis, Treponema pallidum, and Haemophilus ducreyi; bacterial vaginosis).

A total of 466 FSWs seronegative for HIV-1 infection were enrolled. Recruitment was based on a series of community visits, assisted by an established network of sex worker peer educators. All study participants were provided with state-of-the-art HIV prevention services, which included peer and clinic counseling on reducing high-risk behavior for HIV-1 infection, the provision of free male condoms, the prompt treatment of any symptomatic STI, and screening and therapy of asymptomatic STIs every 6 months. Two standardized risk-reduction counseling sessions of 1 hour were provided to all women at enrollment, with subsequent peer and clinic-based counseling given upon request. This counseling followed a previously described model, with the addition of information on the need for consistent condom use with regular partners or boyfriends. To compensate women for travel expenses and lost revenue, participants were reimbursed a sum of 100 KSh (US $1.65) per study visit, an amount that increased to 150 KSh (US $2.48) over the course of the study.

The study was designed as a randomized, double-blinded, placebo-controlled trial. Women were block randomized in groups of 50 to receive 1 g of oral azithromycin per month or an identical placebo (both provided by Pfizer Canada Inc, Montreal, Quebec). The study drug was administered as directly observed therapy, from numbered containers that were packaged, labeled, and shipped by the Misericordia General Hospital pharmacy (Winnipeg, Manitoba). Clinic staff assigned study numbers consecutively at enrollment; neither study staff nor the participants were aware of group assignment. The primary outcome measure was incidence of HIV-1 infection, and secondary outcome measures were the rates of bacterial STIs (infection with N gonorrhoeae, C trachomatis, Trichomonas vaginalis, Treponema pallidum, and Haemophilus ducreyi; bacterial vaginosis).

**Study Procedures**

The study drug was administered monthly in the clinic, and a simultaneous urine specimen was collected and stored at –20°C. After study termination, all urine specimens were tested for STIs using N gonorrhoeae and C trachomatis polymerase chain reaction (PCR) assays. A detailed behavioral questionnaire was administered at enrollment and then every 3 months to collect information on numbers of clients, condom use (on a semiquantitative scale ranging from 0 [never use] to 5 [always use]), and types of sexual activity (anal sex, sex during menses). Blood was collected for HIV-1 enzyme-linked immunosorbent assay every 3 months. All women underwent a full physical examination and STI diagnostic testing prior to enrollment and every 6 months thereafter, with cervical

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swabs obtained for *N gonorrhoeae* and *C trachomatis* PCR assays (Amplicor PCR Diagnostics, Roche Diagnostics, Montreal, Quebec), and for *N gonorrhoeae* culture. *Trichomonas vaginalis* culture was performed using the In Pouch TV culture (Biomed Diagnostics, San Jose, Calif), a Gram stain was performed, and blood was drawn for rapid plasma reagin testing for syphilis. Bacterial vaginosis was defined as a Nugent score of 7 to 10, and lactobacillus colonization and candidiasis were defined as the finding of any lactobacilli or yeast, respectively, on the Gram-stained specimen. Any genital tract infections identified were treated as a “late” dose. An STI was defined as a “late” dose. An STI was defined as the finding of any lactobacillus colonization and candidiasis occurrence between enrollment and the first 3-monthly follow-up visit was assumed to be due to HIV-1 infection acquired prior to enrollment and was not recorded as a study end point. An STI was defined as incident when the preceding test for that STI had been negative, and as incident syphilis when an RPR titer increased from less than 1:8 to 1:8 or greater. Statistical analysis was performed using SPSS 11 (SPSS Inc, Chicago, Ill); *P* < .05 was used to determine statistical significance. Analysis was based on intention to treat, with all outcomes analyzed in relation to the original randomization assignment (azithromycin or placebo). Statistical analysis included all data available from all participants, in either group, up to the time of loss to follow-up or discontinuation of treatment, for whatever reason.

**Sample Size and Statistical Analyses**

Based on previous studies of sex worker cohorts in Kenya, we estimated that the annual HIV-1 incidence would be 1.5%. In the Mwanza trial, improved management of bacterial STIs in a population with relatively low rates of STIs reduced incidence of HIV-1 infection by 40%. We hypothesized that bacterial STIs would be more frequent in sex workers and therefore would be responsible for a greater population-attributable fraction of HIV-1 infections. Therefore, we estimated that prevention of bacterial STIs would reduce HIV-1 incidence in a cohort of FSWs by 50%. With a β error of .20, a 2-sided α error of .05, a 2-year follow-up period, and an anticipated loss to follow-up of 30% at most, 170 women were required per study group. Incidence of HIV-1 infection and STI was calculated as number per 100 person-years. Poisson regression was used to calculate rate ratios (RRs) and 95% confidence intervals (CIs) for comparison of STI incidence rates between study groups. Time to HIV-1 seroconversion was analyzed using Kaplan-Meier survival analysis and Cox regression with time-dependent variables. Seroconversion occurring between enrollment and the first 3-monthly follow-up visit was assumed to be due to HIV-1 infection acquired prior to enrollment and was not recorded as a study end point. An STI was defined as incident when the preceding test for that STI had been negative, and as incident syphilis when an RPR titer increased from less than 1:8 to 1:8 or greater. Statistical analysis was performed using SPSS 11 (SPSS Inc, Chicago, Ill); *P* < .05 was used to determine statistical significance. Analysis was based on intention to treat, with all outcomes analyzed in relation to the original randomization assignment (azithromycin or placebo). Statistical analysis included all data available from all participants, in either group, up to the time of loss to follow-up or discontinuation of treatment, for whatever reason.

**RESULTS**

**Study Enrollment and Baseline Characteristics of Participants**

Recruitment began in May 1998, and after 2 years the data and safety monitoring board recommended that both enrollment and participant follow-up be extended due to a lower than expected incidence of HIV-1 infection. Recruitment therefore continued until the end of January 2002; blinded follow-up continued until the end of July 2002.

The trial profile is shown in Figure 1. A total of 890 self-identified FSWs underwent HIV-1 counseling and testing and were screened and treated (if necessary) for prevalent genital tract infections: 251 (28.2%) were seropositive for HIV-1, and 173 (19.4%) were seronegative but declined enrollment. The remaining 466 women were randomized to receive monthly azithromycin (*n* = 230) or placebo (*n* = 236). Baseline characteristics, including risk-taking behavior for HIV-1 and prevalence of genital tract infections, were similar in the 2 groups. However, women seropositive for HIV-1 reported a younger age at first sex, used condoms less frequently, were more likely to drink alcohol every day, and were more likely to have bacterial vaginosis or *T vaginalis* infection. Compared with seronegative FSWs enrolled in the study, those who were seronegative and declined enrollment or who were not eligible for enrollment had lower numbers of sexual partners and a lower prevalence of *C trachomatis* infection and a higher prevalence of infection with *N gonorrhoeae* and *T vaginalis* (Table 1). Fe-
male sex workers randomly allocated to treatment and placebo groups were generally well matched (Table 1).

**Follow-up of Study Participants**

Women were encouraged to remain in the trial for at least 2 years. Since trial follow-up was extended beyond this time, after 2 years participants were free to choose to continue in their randomization group or to exit the trial and continue to attend the clinic as needed for medical reasons. Study staff and participants remained double-blinded throughout the study. Duration of follow-up was similar in the 2 groups (treatment group: median, 801 days; range, 0-1607 days; placebo group: median, 764 days; range, 0-1524 days) (P=.70). Overall, 341 (73.1%) participants (169 in the treatment group and 172 in the placebo group) were followed up for 2 or more years or until they reached an administrative end point (defined as seroconversion [n=35], death [n=3], severe adverse event requiring discontinuation [n=5], followed up for 2 years or until trial termination [n=298]). One hundred twenty-five participants stopped the trial prematurely (treatment group, n=61; placebo group, n=64) for the following reasons: bad health precluded attendance (treatment, n=1; placebo, n=0); moved away (treatment, n=21; placebo, n=27); stopped sex work (treatment, n=5; placebo, n=5); lost interest (treatment, n=34; placebo, n=32). Of the 125 women who discontinued early, 75 (60%) attended at least one 3-month clinic visit for repeat HIV-1 serologic testing, and no follow-up serology results were available for the remaining 50 participants (10.7% [treatment: 25/230 [10.9%]; placebo: 25/236 [10.6%]]).

There were 17 pregnant women at enrollment. After excluding them, we have reported pregnancy data on 430 women in the follow-up period, 210 in the treatment group and 220 in the placebo group. The pregnancy rate was 30.0% (63/210) in the treatment group and 30.9% (68/220) in the placebo group (P=.84). There were a total of 9966 scheduled drug administration follow-up visits, and attendance was classified as on time (ie, within 2 weeks of scheduled date) for 9149 visits (91.8%). At only 5 visits was drug not administered by directly observed therapy (3 in treatment group, 2 in the placebo group).

**Effect of Azithromycin on HIV-1 Incidence**

There were a total of 35 incident HIV-1 infections during the study period, 19 (4%) in the treatment group and 16 (per 495 person-years of follow-up) (3.2%) in the placebo group. There was no difference between study groups in the risk of HIV-1 infection (RR, 1.2; 95% CI, 0.6-2.5; P=.50) by Kaplan-Meier analysis (FIGURE 2). The HIV-1 incidence was 4.0 per 100 person-years in the treatment group and 3.2 per 100 person-years in the placebo group.

**Effect of Azithromycin on Incidence and Prevalence of Bacterial STIs**

There was a significant reduction in the incidence of STIs in the treatment group (TABLE 2), including laboratory-confirmed infection with N gonorrhoeae (RR, 0.46; 95% CI, 0.31-0.68), C trachomatis (RR, 0.38; 95% CI, 0.26-0.57), and T vaginalis (RR, 0.56; 95% CI, 0.40-0.79). Most of these STIs (73.2% of N gonorrhoeae, 83.7% of C trachomatis, and 81.0% of T vaginalis infections) were asymptomatic. Azithromycin also reduced the incidence of symptomatic infection with N gonorrhoeae (RR, 0.24; 95% CI, 0.08-0.71) and C trachomatis (RR, 0.15; 95% CI, 0.04-0.68) but not with T vaginalis (RR, 0.66; 95% CI, 0.31-1.40) (P=.30). No difference was observed in the incidence of bacterial vaginosis (RR, 0.91; 95% CI, 0.77-1.10) or syphilis (RR, 1.02; 95% CI, 0.54-1.95), or in the prevalence of colonization by candida (RR, 1.16; 95% CI, 0.87-1.56) or lactobacillus species (RR, 1.04; 95% CI, 0.92-1.17). Ulcerative STIs were very uncommon in both study groups, with only 12 incident cases of genital ulcer disease recorded during the

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**Table 1. Baseline Characteristics of Study Participants**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Treatment Group (n = 230)</th>
<th>Placebo Group (n = 236)</th>
<th>Seronegative, Not Enrolled (n = 173)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD), y</td>
<td>29.1 (7.8)</td>
<td>28.1 (7.7)</td>
<td>28.5 (7.6)</td>
</tr>
<tr>
<td>Age at first sex, mean (SD), y</td>
<td>16.3 (2.5)</td>
<td>16.0 (2.5)</td>
<td>16.2 (2.4)</td>
</tr>
<tr>
<td>Duration of sex work, mean (SD), y</td>
<td>5.6 (4.7)</td>
<td>4.8 (4.3)</td>
<td>4.7 (3.8)</td>
</tr>
<tr>
<td>No. of sex partners/wk, mean (SD)</td>
<td>16.3 (11.8)</td>
<td>14.6 (10.4)</td>
<td>13.2 (10.6)*</td>
</tr>
<tr>
<td>Amount charged for sex, mean (SD), Ksh†</td>
<td>127.5 (156.7)</td>
<td>145.9 (170.8)</td>
<td>135 (202.2)</td>
</tr>
<tr>
<td>Condom use, mean (SD)‡</td>
<td>2.4 (1.7)</td>
<td>2.4 (1.8)</td>
<td>2.3 (1.9)</td>
</tr>
<tr>
<td>Regular male partner, No. (%)</td>
<td>106 (46.3)</td>
<td>120 (51.1)</td>
<td>76 (43.9)</td>
</tr>
<tr>
<td>Hormonal contraception, No. (%)</td>
<td>90 (39.1)</td>
<td>92 (39.0)</td>
<td>59 (34.1)</td>
</tr>
<tr>
<td>Practice vaginal douching, No. (%)</td>
<td>158 (68.7)</td>
<td>165 (69.9)</td>
<td>115 (66.4)</td>
</tr>
<tr>
<td>Ever practice sex during menses, No. (%)</td>
<td>43 (18.8)</td>
<td>48 (20.3)</td>
<td>33 (24.6)</td>
</tr>
<tr>
<td>Ever practice anal sex, No. (%)</td>
<td>37 (16.1)</td>
<td>34 (14.4)</td>
<td>24 (14.0)</td>
</tr>
<tr>
<td>Ever use intravenous drugs, No. (%)</td>
<td>8 (3.5)</td>
<td>11 (4.7)</td>
<td>5 (2.9)</td>
</tr>
<tr>
<td>Drink alcohol daily, No. (%)</td>
<td>101 (43.9)</td>
<td>121 (51.3)</td>
<td>94 (55.0)</td>
</tr>
<tr>
<td>Work out of own home, No. (%)</td>
<td>168 (73.0)§</td>
<td>143 (60.6)</td>
<td>116 (67.1)</td>
</tr>
<tr>
<td>Gonorreal infection at enrollment, No. (%)</td>
<td>23 (10.0)</td>
<td>22 (9.3)</td>
<td>15 (9.5)</td>
</tr>
<tr>
<td>Chlamydial infection at enrollment, No. (%)</td>
<td>22 (9.6)</td>
<td>21 (9.0)</td>
<td>13 (7.7)*</td>
</tr>
<tr>
<td>Trichomonas infection at enrollment, No. (%)</td>
<td>26 (11.4)</td>
<td>27 (11.8)</td>
<td>31 (18.5)*</td>
</tr>
<tr>
<td>Bacterial vaginosis at enrollment, No. (%)</td>
<td>113 (52.8)</td>
<td>110 (49.1)</td>
<td>80 (53.2)</td>
</tr>
<tr>
<td>Syphilis at enrollment, No. (%)</td>
<td>10 (4.4)</td>
<td>9 (3.8)</td>
<td>10 (5.8)</td>
</tr>
<tr>
<td>HSV-2 seropositive at enrollment, No. (%)</td>
<td>161 (74.9)</td>
<td>161 (70.6)</td>
<td>NA</td>
</tr>
</tbody>
</table>

Abbreviations: HSV-2, herpes simplex virus type 2; NA, not applicable.
*P<.05 for enrolled vs nonenrolled female sex workers among those seronegative for HIV-1 infection at screening.
†Reported on a semiquantitative scale (range, 0 [never use] to 5 [always use]).
‡Reported on a semiquantitative scale (range, 0 [never use] to 5 [always use]).
§Weisskopf gonorrhea infection detected by either cervical polymerase chain reaction or bacterial culture.
study (+ in the treatment group, 8 in the placebo group). All genital ulcers were culture-negative for H ducreyi and were clinically compatible with recurrent HSV-2 infection.

The duration of laboratory-confirmed STIs was examined in both groups using results of monthly N gonorrhoeae and C trachomatis urine PCR assays. The duration of N gonorrhoeae infections, reported as consecutive monthly visits presenting with infection, was shorter in the azithromycin group than in the placebo group (mean monthly visits, 1.05 [SD, 0.22] vs 1.38 [SD, 0.78], respectively; P = .04), as was the duration of C trachomatis infections (mean monthly visits, 1.11 [SD, 0.42] vs 1.84 [SD, 1.26]; P = .001). Using urine PCR, we examined the incidence of N gonorrhoeae or C trachomatis infection among women more than 2 weeks late for their monthly drug administration visits. Incident STIs were more common at visits classified as late than at those classified as on time. The association between late visits and an STI was strongest for women randomized to receive azithromycin (24.4% of STIs; odds ratio, 3.6; 95% CI, 1.8-7.5), but an association was also observed in the placebo group (12.4% of STIs; odds ratio, 1.9; 95% CI, 1.1-3.4). Therefore, although the association of STIs with late clinic attendance might have reflected a loss of azithromycin protection in the treatment group, behavioral factors were also involved.

**Determinants of Incident HIV-1 Infection**

Enrollment was associated with major increases in use of condoms (the proportion using condoms with all clients increased from <20% to >50% within 1 month) and decreases in numbers of clients (from >16/wk to <6/wk within 6 months), as previously reported. There were no differences between treatment and placebo groups in reduced high-risk behavior during follow-up. At the last recorded follow-up visit, the mean number of weekly clients was 3.0 (SD, 4.4) vs 3.5 (SD, 5.4) (P = .30), and condom use with all clients was reported by 48% (105/219) vs 50% (113/225) (P = .60) of sex workers in the treatment and placebo groups, respectively. Self-reported client numbers and condom use were combined into an estimated number of weekly unprotected sex contacts to examine behavioral associations of HIV-1 seroconversion using Cox regression with time-dependent covariates. There was a significant association between HIV-1 seroconversion and the estimated mean number of weekly unprotected sex contacts during the year of seroconversion (per-partner RR, 1.19; 95% CI, 1.03-1.35).

Table 2. Incidence of Genital Tract Infections, by Study Group

<table>
<thead>
<tr>
<th>Infection</th>
<th>Treatment Group</th>
<th>Placebo Group</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial vaginosis</td>
<td>53.0 (247)</td>
<td>57.4 (292)</td>
<td>.40</td>
</tr>
<tr>
<td>Trichomonas vaginalis (culture)</td>
<td>11.3 (52)</td>
<td>20.4 (101)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Neisseria gonorrhoeae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine PCR†</td>
<td>4.7 (21)</td>
<td>12.7 (61)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Cervical PCR†</td>
<td>2.6 (12)</td>
<td>5.7 (28)</td>
<td>.02</td>
</tr>
<tr>
<td>Chlamydia trachomatis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine PCR†</td>
<td>6.3 (28)</td>
<td>14.5 (70)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Cervical PCR†</td>
<td>1.1 (5)</td>
<td>6.5 (32)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Syphilis†</td>
<td>3.9 (18)</td>
<td>3.8 (19)</td>
<td>&gt;.99</td>
</tr>
<tr>
<td>Genital ulcer disease</td>
<td>0.9 (4)</td>
<td>1.6 (8)</td>
<td>.30</td>
</tr>
</tbody>
</table>

Abbreviation: PCR, polymerase chain reaction.

*Urine collected monthly at time of directly observed drug administration.
†Performed every 6 months or to diagnose symptomatic sexually transmitted infections.
‡Defined as increase in rapid plasma reagin from <1:8 to ≥1:8.
the presence of a genital tract infection over the preceding 3 months. There was an association between HIV-1 seroconversion and infection with either *N gonorrhoeae* (RR, 4.9; 95% CI, 1.7-14.3) or *C trachomatis* (RR, 3.0; 95% CI, 1.1-8.9) during this time. No association was found between seroconversion and a preceding *T vaginalis* infection (RR, 0.7; 95% CI, 0.2-5.0), vaginal candidiasis (RR, 0.7; 95% CI, 0.1-5.1), or bacterial vaginosis (RR, 1.2; 95% CI, 0.2-3.3). The association between seroconversion and a recent urine PCR assay result positive for either *N gonorrhoeae* or *C trachomatis* (overall RR, 4.5; 95% CI, 2.0-10.1) was strongest in the azithromycin group (RR, 9.3; 95% CI, 2.9-29.7), with a similar trend in the placebo group (RR, 2.9; 95% CI, 0.8-9.2).

Since STIs and HIV-1 infection may be correlated due to measured or unmeasured behavioral and/or biological factors that enhance risk of acquisition of both infections, the association between bacterial STIs and subsequent HIV-1 seroconversion could be due to confounding by common risk factors. To adjust for this, the Cox analysis was rerun to examine the association between incident HSV-2 infection and subsequent HIV-1 acquisition was then examined in a multivariable model that included the covariates of study group, HSV-2 serostatus at baseline, age, and estimated number of weekly unprotected sexual contacts. Among the 322 women with HSV-2 infection, 33 acquired HIV-1 infection; 2 women acquired HIV-1 infection among the 121 without HSV-2 infection. Prevalent HSV-2 infection was significantly associated with subsequent HIV-1 infection (RR, 5.8; 95% CI, 1.4-24.0) in univariate analysis, and this association was strengthened slightly in multivariable analysis using Cox regression with fixed covariates (RR, 6.3; 95% CI, 1.5-27.1). As only 2 HIV-1 seroconversions occurred among women who were HSV-2 seronegative at baseline, it was not possible to examine the association between incident HSV-2 infection and HIV-1 seroconversion.

### Prevalent HSV-2 Infection and HIV-1

Analysis of HSV-2 infection was not a predefined primary or secondary study end point, since our major aim was to examine the impact of bacterial STIs and their treatment/prevention on incidence of HIV-1 infection. Analysis of HSV-2 infection was performed post hoc using cryopreserved plasma samples for 95.1% of FSWs (443/466), including all 35 women who acquired HIV-1 during the study. The baseline prevalence of HSV-2 infection in the cohort was 72.7% (322/443) and did not differ between the treatment and placebo groups (74.9% vs 70.6%, *P*=.30) (Table 1). The association between prevalent HSV-2 infection and subsequent HIV-1 acquisition was then examined in a multivariable model that included the covariates of study group, HSV-2 serostatus at baseline, age, and estimated number of weekly unprotected sexual contacts. Among the 322 women with HSV-2 infection, 33 acquired HIV-1 infection; 2 women acquired HIV-1 infection among the 121 without HSV-2 infection. Prevalent HSV-2 infection was significantly associated with subsequent HIV-1 infection (RR, 5.8; 95% CI, 1.4-24.0) in univariate analysis, and this association was strengthened slightly in multivariable analysis using Cox regression with fixed covariates (RR, 6.3; 95% CI, 1.5-27.1). As only 2 HIV-1 seroconversions occurred among women who were HSV-2 seronegative at baseline, it was not possible to examine the association between incident HSV-2 infection and HIV-1 seroconversion.

### Study Deaths and Potential Adverse Effects of Study Drug

There were 3 deaths during the study, 1 in the treatment group and 2 in the placebo group. All deaths were due to trauma, and the study drug was not believed to have contributed to any death. Five women withdrew from the study due to adverse events, all related to severe epigastric pain; 2 of these required hospitalization. Of these severe adverse events, 3 occurred in the treatment group and 2 in the placebo group. Forty additional women (47 visits) had adverse events believed to be possibly or probably related to the study drug (22 in the treatment group, 18 in the placebo group), including epigastric pain, vomiting, hyperacidity, and diarrhea. All resolved with symptomatic treatment. Overall, there was no significant difference in rates of death or adverse events between the treatment and placebo groups.

### COMMENT

This study randomly allocated 466 HIV-1-seronegative FSWs to receive either monthly azithromycin for bacterial STI prophylaxis, or an identical placebo. No effect on HIV-1 incidence was demonstrated, despite substantial reductions in the treatment group of the incidence of bacterial STIs. The study was powered to demonstrate a 50% reduction in HIV-1 incidence over 2 years, based on a 15% annual incidence of HIV-1, with 170 women per group and 30% loss to follow-up. There was a lower than expected incidence of HIV-1 infection in the study cohort, so both the sample size and duration of follow-up were increased while maintaining study blinding. Despite these measures, 33 primary outcomes (incident HIV-1 infections) were observed compared with the 54 expected in our power calculations, and the 95% CI for the effect of azithromycin ranged from a 40% reduction in risk of HIV-1 infection to a 150% increase in risk. Thus, although minor reductions in incidence of HIV-1 infection cannot be ruled out, our results do allow us to definitively rule out the hypothesized 50% protective effect as well as the 40% reduction in HIV-1 incidence observed in the Mwanza trial.9

Incident HIV-1 infection was strongly associated with infection with both *N gonorrhoeae* and *C trachomatis* over the preceding 3 months, confirming the association between HIV-1 and a prior STI. However, the observed failure of monthly antibiotic prophylaxis to protect against HIV-1 acquisition was not due to a failure to prevent bacterial STIs. Bacterial STIs were common, with incidence rates in the placebo group of PCR-confirmed infection by *N gonorrhoeae* and *C trachomatis* of 13 per 100 person-years and 15 per 100 person-years, respectively. Azithromycin administration was associated with substantial decreases in both the incidence
and period prevalence of these STIs as well as of infection with *T vaginalis*.

There are several plausible explanations for these seemingly contradictory findings. First, the high level of care provided to all study participants may have reduced our power to detect a treatment effect. Symptomatic genital infections were promptly treated in both study groups, and asymptomatic STIs were screened and treated every 6 months. This baseline level of care was well above the standard of care for this region, and this may have reduced the fraction of seroconversions potentially attributable to STIs. In addition, we observed a substantial increase in reported condom use after peer- and clinic-based counseling and a decrease in reported client numbers. These changes were each associated with reduced STI rates, suggesting that they were real rather than the result of reporting bias. Therefore, although providing STI prevention services that are above the prevailing standard of care clearly provides an important benefit to study participants, future clinical trials will need to consider the potential impact of such interventions on study end point rates and statistical power. That said, both the baseline prevalence and the reduction in incidence of *N gonorrhoeae* and *C trachomatis* infection in this cohort were substantial, making it unlikely that the trial missed a significant intervention effect on this basis. However, these STI preventive services may explain the low rates of ulcerative STIs observed in the cohort. Participant follow-up can also be problematic in highly mobile groups such as FSWs, but in this study we exceeded our predefined criteria for successful follow-up, which compared favorably with another recent large trial in a multicenter cohort of African FSWs.

An alternative explanation for the failure to demonstrate a reduced HIV-1 incidence, despite dramatic reductions in STI rates, is that other causal pathways may account for the observed association between STIs and seroconversion. Such pathways could include increased viral shedding in male clients infected with HIV-1 and coinfected with STIs, a high prevalence of STIs among HIV-1–positive men, and enhanced host susceptibility to additional STIs after acute HIV-1 infection. If HIV-1–infected men are commonly coinfected with STIs, and these infections enhance HIV-1 shedding, then the acquisition of an STI by a FSW would be a surrogate marker for high-level HIV-1 exposure, and treatment of the woman’s STI would not reduce her risk of HIV-1 infection. Although these pathways will all result in the acquisition of an STI either coincident with or after HIV-1 infection, the STI may still be diagnosed first. This potential “diagnostic bias” is due to 2 factors. First, serologic testing for HIV-1 was performed every 3 months in this trial, while STIs were screened monthly in urine, which could bias to earlier diagnosis of STIs. In addition, there is a window between HIV-1 infection and seroconversion, so that an STI acquired coincident with or after HIV-1 may be diagnosed first. If this explains the association found between STIs and incident HIV-1 infection, then the major effect of STIs in facilitating HIV-1 transmission could be increased HIV-1 infectivity in a sex partner who is infected with both HIV-1 and an STI, so that reduction of STIs in HIV-1–infected individuals would be an important strategy for preventing sexual transmission. The feasibility of such an approach has recently been demonstrated in a South African mining community, where FSW presumptive treatment with monthly azithromycin reduced STI incidence in FSWs and STI prevalence in the miners themselves.

A final explanation for the lack of effect of STI reduction on HIV-1 incidence is that control of bacterial STIs in this setting is simply not an effective means of preventing HIV-1 infection. There is no doubt that STI prevention is a laudable outcome in and of itself, since untreated bacterial STIs may be associated with severe health outcomes such as ectopic pregnancy, sterility, and chronic pelvic pain. However, recent community-based trials examining prevention of bacterial STIs as a means to prevent HIV-1 have given conflicting results. The rationale underlying the current study was that an FSW population, in which both STIs and HIV-1 are very prevalent, would be an ideal one in which to address this issue. Although several other possible reasons for the negative trial outcome exist, as outlined above, a lack of efficacy of the strategy must be considered as a possible explanation.

To investigate a possible role of HSV-2 infection on HIV-1 acquisition, we performed a post hoc analysis of HSV-2 prevalence at enrollment and of subsequent HIV-1 incidence. As expected, HSV-2 infection was very common in this FSW cohort, with 73% of women infected at baseline. Prevalent HSV-2 infection was strongly associated with subsequent acquisition of HIV-1 in multivariable analysis, with 33 HIV-1 infections in the 322 HSV-2–infected FSWs (10.2%), and just 2 infections in the 121 HSV-2–uninfected women (1.7%; neither of these 2 cases had acquired incident HSV-2 infection prior to HIV-1 infection). It should be emphasized that our study was not designed to study the role of prevalent or incident HSV-2 infection in HIV-1 acquisition but was focused on prevention of bacterial STIs. We cannot prove that the association is causal; for example, unexamined biological factors may have increased participants’ susceptibility to both HSV-2 and HIV-1. Nonetheless, the findings confirm the association between HSV-2 infection and HIV-1 acquisition in 20–33 and provide a strong rationale for current trials of HSV-2 suppression as an HIV-1 prevention strategy in Africa. Unexpectedly, azithromycin was associated with a significant reduction in the incidence of genital infection with *T vaginalis*. There were no reported differences between study groups in risk-taking behavior, suggesting that the observed difference may have been due to an active effect of the antibiotic.
Azithromycin has demonstrated activity against other protozoa,22 including *Plasmodium*, *Cryptosporidium*, *Leishmania*, and *Toxoplasma*, and so it is perhaps not surprising to find a degree of activity against *Trichomonas*. However, whether this activity will be sufficient to treat established *Trichomonas* infection is an issue that will need to be addressed in future studies.

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**Funding/Support:** This study was supported by grants from the Rockefeller Foundation (2000 HE 025), the European Commission (DG VIII/B, contract No. 7-RPR-28), the Canadian Research Chair Program (Dr Kaul, Ontario HIV Treatment Network (Dr MacDonald, Career Scientist), the Canadian Institutes of Health Research (Dr Moses, Investigator Award), and the Canadian Infectious Disease Society (Dr Kaul, Bayer Young Investigator Award). The study drug and placebo were provided by Pfizer Inc.

**Role of the Sponsor:** None of the funding agencies had any role in the design, conduct, or interpretation of the study; the analysis or reporting of the data; or the preparation, review, or acceptance of the manuscript.

**Previous Presentation:** These data were presented in part at the Fifteenth Biennial Congress of the International Society for STD Research, July 27–30, 2003; Ottawa, Ontario.

**Acknowledgment:** We thank the members of the data and safety monitoring board, acknowledge the support of the Nairobi City Council, and give special thanks to all the Kibera study participants for their enthusiasm and support.

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