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A cross-sectional study of bacterial vaginosis, intravaginal practices and HIV genital shedding; implications for HIV transmission and women’s health

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ABSTRACT

Objectives: Bacterial vaginosis (BV) is associated with an increased risk of HIV transmission, and intravaginal practices (IVP) are an important risk factor for developing BV. The relationship between IVP, BV and HIV lower genital shedding, responsible for HIV transmission, has not been examined in women receiving antiretrovirals in Zambia.

Design: Cross-sectional study.

Setting: Community Health Center in Lusaka, Zambia.

Participants and methods: Participants were HIV-infected women receiving antiretroviral therapy and engaging in IVP (n=128). Participants completed audio computer-administered self-interviews to assess IVP and underwent a vaginal examination. BV was diagnosed using Nugent criteria. HIV-1 lower genital shedding was assessed by measuring HIV-1 RNA in cervicovaginal lavages.

Results: Most women engaged in IVP daily (114, 89.0%) and 81 (63.3%) of the participants had BV. HIV-1 genital shedding was detected in 18 (14.2%) participants. BV was associated with daily use of IVP (prevalence ratio, PR=4.58, CI 1.26 to 16.64, p=0.02) and weekly use of traditional medicines for IVP (PR=1.33, CI 1.05 to 1.68, p=0.02). The only factor associated with HIV-1 lower genital shedding was plasma viraemia (PR=4.61, CI 2.02 to 10.54, p<0.001). Neither IVP nor BV were associated with HIV shedding.

Conclusions: Despite the frequency of IVP and high prevalence of BV, plasma viraemia was the primary factor associated with HIV lower genital shedding. These findings support early initiation of antiretrovirals as an HIV prevention tool. Given adverse health outcomes associated with BV, the association between frequent IVP and BV, and the powerful local norms and traditions encouraging IVP, there is a need for studies assessing culturally tailored interventions to decrease BV in high-prevalence settings.

INTRODUCTION

Bacterial vaginosis (BV) is the most common genital infection in women and occurs in up to 50% of women with HIV infection.1–3 BV is characterised by a vaginal discharge with fishy odour, a shift in vaginal pH, loss of vaginal lactobacilli and increase in Gram-negative anaerobic bacteria. BV has important associations with adverse health outcomes such as an increased risk of preterm delivery, spontaneous abortion and complications of gynaecological surgeries.1–4 BV is also associated with an increased risk of acquisition and transmission of sexually transmitted infections (STI), and HIV, to sexual partners and new borns.5–10

The aetiology of BV is controversial and remains largely unknown. Epidemiological and clinical studies have found associations of BV with intravaginal cleansing, new sexual partners, multiple sex partners and unprotected vaginal intercourse; but it has also been suggested that BV may be caused by a sexually transmitted pathogen.11–13 BV,
Lower genital tract HIV shedding and HIV transmission occurs primarily in the presence of plasma viremia, but reproductive tract infections such as BV could also facilitate lower genital HIV shedding and HIV transmission. As most studies assessing HIV genital shedding have included women with detectable plasma viremia, there is a need to evaluate HIV genital shedding as a potential marker of HIV transmission in women on suppressive antiretroviral therapy (ART).

Zambia is a sub-Saharan country with high rates of HIV infection. In Zambia, BV are widely used and rates of BV are high. Little research in this country has been conducted to explore the relationship between IVP, BV and HIV lower genital shedding. The objective of this study is to describe types, frequencies and reasons for IVP, and to examine the relationship between IVP, BV and HIV shedding among women on ART, as a first step to understand how IVP and BV may increase HIV transmission. We hypothesised that frequent IVP would be associated with BV, and both IVP and BV would be associated with HIV lower genital shedding in women on ART.

METHODS

Participants were provided with information about the study and assured of confidentiality of information and study records. Voluntary signed informed consent was obtained from all participants prior to participating in the study.

Study design

This study is a cross-sectional study. Participants completed audio computer-administered self-interview (ACASI) questionnaires assessing demographic and sexual risk factors, as well as frequency and types of IVP, and reasons for engaging in IVP; they also underwent vaginal examinations to assess BV and HIV lower genital shedding. Data presented are part of a larger study developing a culturally tailored intervention to decrease IVP and BV among HIV-1-infected women in Lusaka. Data presented in this cross-sectional analysis were obtained at baseline and prior to any participant receiving any study-related intervention. The study coordinator described IVP (intravaginal cleansing and intravaginal insertion) to the participants prior to completing the IVP questionnaire, and emphasised that IVP did not refer to external vaginal practices. Participants were recruited from May 2013 to February 2014. Study activities took place at a community health centre in urban Lusaka, Zambia. All audio and consent materials were provided in participants' preferred local languages (English, Bemba, Nyanja).

In order to develop questionnaires addressing IVP, focus groups, key informant interviews and introductory meetings with the clinic staff and local Community Advisory Boards were conducted at the community health centre; results have previously been described. Participants of the focus groups, key informants and members of the advisory boards, did not participate in the study. Participants were self-referred after hearing about the study from enrolled participants or clinic staff, and presented documentation of HIV-1 infection and treatment at the time of enrolment. Participants were HIV-1-infected women receiving antiretroviral medications and engaging in IVP in the month prior to enrolment, older than 18 years of age, sexually active, engaging in vaginal intercourse with men, not pregnant, not on contraceptive medications and not using an intrauterine device, and living in the Lusaka metropolitan area. The women underwent study assessment during the proliferative phase of the menstrual cycle (7–14 days after the first day of their menses). As IVP are widely used by Zambian women, all participants enrolled in the study had engaged in some type of IVP in the month prior to enrolment.

Participants underwent a 10 mL blood draw and vaginal examination. Genital samples collected were a vaginal swab collected from the mid-vaginal wall and cervicovaginal lavage (CVL). The vaginal swab was placed on a microscopy slide and analysed by Gram stain. Local clinic laboratory staff received training prior to reading the slides and the reading was performed by the same trained laboratory technician. In case of an unclear reading, the slide was reviewed by a second technician and the chief of the laboratory until an agreement was achieved. A didactic training session was conducted by the principal investigator at the laboratory site using a computer-based presentation and printed images. The principal investigator performed quality control by reviewing 20% of the slides at the clinic laboratory. CVL was performed by instilling 10 cc of sterile saline solution into the vaginal and cervical areas. Fluid was collected by aspiration after 60 s. Blood and CVL samples were transported on ice to the laboratory for analysis. Analysis of RNA-1 viral load in blood and CVL was performed in fresh samples on arrival to the laboratory and within 2 h of collection.

Demographics and sexual risk factors

The demographic and sexual risk factors questionnaire was adapted by questionnaires previously used in similar settings by our group and included age, marital status,
income, educational level, current partner HIV status, number of partners, sexual modalities (oral and anal sex), history of exchanging sex for money or gifts and use of condoms.

Medical history
Medical history was self-reported. The medical history questionnaire included information on date of HIV infection, most current CD4 cells per millilitre, history of prior STIs (chlamydia, gonorrhoea, syphilis, trichomoniasis, genital herpes and genital warts) and history of prior episodes of BV. We did not perform CD4 count testing and did not collect data on ART regimens.

Intravaginal practices
The IVP questionnaire assessed specific products used for IVP, frequency of IVP and reasons for engaging in IVP. Products used for IVP and reasons for engaging in IVP were identified in the focus groups and key informant interviews. Questions to assess product used and reason for use in the prior month were on a dichotomous scale, ‘yes’=1, ‘no’=0; and each product was assessed individually. Frequency of each product was scored using ‘daily’=1, ‘weekly’=2 and ‘monthly’=3, respectively. Products included: water alone, soap, a cloth rag or a sponge, herbs or flowers taken directly from the land, traditional medicines given by traditional healers, vinegar, salt, beer and yogurt. Reasons included: general hygiene, to get rid of a discharge, to remove blood after menses, to prevent pregnancy, to prevent STIs and to please sexual partner; responses to the reasons for engaging in IVP were not mutually exclusive. In order to assess frequency of IVP when different products were used, the number of women engaging in daily IVP was calculated by adding the number of women who answered yes to ‘daily use’ to any of the products; the number of women engaging in weekly IVP was calculated by adding the number of women who said yes to ‘weekly use’ and excluding those who endorsed daily IVP.

Genital examination
Detailed genital examination was performed in every participant by a qualified healthcare professional. Variables assessed included the presence of genital lesions (external, intravaginal or cervical), vaginal examination (vaginal discharge and characteristics), cervical examination (cervical discharge, ectopy, friability and tenderness), odour and pH.

Bacterial vaginosis
BV was diagnosed using Nugent criteria (normal vaginal flora=Nugent 0–3; intermediate vaginal flora=Nugent 4–6; BV=Nugent 7–10). Diagnosis of BV was made when the Nugent score was 7 or above.

Plasma viraemia and HIV-1 lower genital shedding
Plasma viraemia and HIV-1 genital shedding were assessed by measuring plasma and CVL HIV-1 RNA viral load, respectively, using an Abbott m2000 RealTime PCR system. CVL was aliquoted into Eppendorf tubes and directly used for determining viral load. Lower limit of detection was 40 copies/mL. Testing was conducted at Centre for Infectious Diseases Research in Zambia (CIDRZ) laboratories. Validation studies were performed to determine CVL viral load measures.

Statistical analysis
IBM SPSS Statistics for Windows V.21 (SPSS, Armonk, New York, USA) and SAS V.9.3 (SAS Institute, Inc, Cary, North Carolina, USA) were used for analysis. A sample of 128 women was calculated using BV as the primary outcome. Descriptive analyses were performed to describe demographic, medical and sexual risk factors, and IVP, BV and HIV-1 shedding. The dependent variables included (1) having the diagnosis of BV and (2) having HIV-1 RNA detectable in the CVL (HIV shedding). Independent variables included demographics, medical and sexual risk factor characteristics, and IVP. Bivariate prevalence ratios (PR) were calculated using a cross-tabulation of results; significance was assessed using a 95% CI and p value was calculated using χ² or Fischer’s exact test. For statistically significant variables we then used a generalised linear model for a binary outcome with a log link to obtain the PR for a multivariable model. We report the PR and 95% CIs. A p value of <0.05 was considered to be significant. Missing data were handled on a variable by variable basis. If the variable was missing, the case was eliminated for that analysis only. We did not impute data.

RESULTS
Characteristics of study participants
One hundred and twenty-eight HIV-1-infected participants receiving ART and engaging in IVP were enrolled. Median age was 37.3 years (range 24–60). The majority of participants had low education and an HIV-infected partner; and over one-third reported having other sexual partners in the prior month. Rates of reported oral sex, anal sex, history of exchanging sex for money and condom use were low. Most women had HIV infection for over 5 years and almost half self-reported having more than 500 CD4 T cells/mL. Sociodemographic characteristics, risk behaviours and medical characteristics of the study population are illustrated in table 1.

Genital examination
Genital examination revealed no lesions in over 90% of participants. One participant had genital warts and one participant had ulcerations in the vagina. Vaginal secretions had a normal appearance in about 80% of participants and although participants did not report vaginal discharge, almost 20% of participants had a malodorous off-white/greyish thin discharge coating the vaginal walls. Cervical examination was normal in 120 participants (94.5%); 6 participants had cervical ectopy and 2...
**Table 1** Demographic, risk factors, medical history and IVP

| Study population number, % N=128 | Number and % among participants with BV n=81 | Number and % among participants without BV n=47 | Unadjusted PR (95% CI) for having bacterial vaginosis n=18 | Number and % among participants with HIV shedding n=188 | Number and % among participants without HIV shedding n=108 | Unadjusted PR (95% CI) for having HIV shedding |
|---------------------------------|-----------------------------------------------|-----------------------------------------------|---------------------------------------------------------|---------------------------------------------------------|---------------------------------------------------------|---------------------------------------------------------|
| **Demographics**                |                                               |                                               |                                                         |                                                         |                                                         |                                                         |
| **Age <37 years**               |                                               |                                               |                                                         |                                                         |                                                         |                                                         |
| Yes 62, 48.4                    | 37, 45.7                                      | 25, 53.2                                      | 0.85 (0.68 to 1.16)                                      | 7, 38.9                                                 | 58, 53.7                                                 | 1.67 (0.69 to 4.04)                                      |
| No 66, 51.6                     | 44, 54.3                                      | 22, 46.8                                      | 11, 61.1                                                | 50, 46.3                                                 |                                                         |                                                         |
| **HIV positive partner; n=111** |                                               |                                               |                                                         |                                                         |                                                         |                                                         |
| Yes 86, 77.5                    | 54, 78.3                                      | 32, 76.2                                      | 1.04 (0.73 to 1.49)                                      | 4, 25                                                   | 20, 21.5                                                 | 0.84 (0.30 to 2.38)                                      |
| No 25, 22.5                     | 15, 21.7                                      | 10, 23.8                                      | 12, 75                                                  | 73, 78.5                                                 |                                                         |                                                         |
| **Primary education or less**   |                                               |                                               |                                                         |                                                         |                                                         |                                                         |
| Yes 58, 45.3                    | 39, 48.1                                      | 19, 40.4                                      | 1.12 (0.86 to 1.45)                                      | 12, 66.7                                                 | 58, 53.7                                                 | 0.62 (0.25 to 1.56)                                      |
| No 70, 54.7                     | 42, 51.9                                      | 28, 59.6                                      | 6, 33.3                                                  | 50, 46.3                                                 |                                                         |                                                         |
| **Sexual Risk factors**         |                                               |                                               |                                                         |                                                         |                                                         |                                                         |
| **More than one sex partner**   |                                               |                                               |                                                         |                                                         |                                                         |                                                         |
| Yes 44, 34.4                    | 25, 30.9                                      | 19, 40.4                                      | 0.85 (0.63 to 1.14)                                      | 11, 66.1                                                 | 71, 65.7                                                 | 1.18 (0.49 to 2.84)                                      |
| No 84, 65.6                     | 56, 69.1                                      | 28, 59.6                                      | 7, 36.3                                                  | 37, 34.3                                                 |                                                         |                                                         |
| **Oral sex in the prior month n=94** |                                               |                                               |                                                         |                                                         |                                                         |                                                         |
| Yes 16, 17.0                    | 10, 15.4                                      | 6, 20.7                                       | 0.88 (0.59 to 1.33)                                      | 11, 84.6                                                 | 65, 82.3                                                 | 0.86 (0.21 to 3.52)                                      |
| No 78, 83.0                     | 55, 84.6                                      | 23, 79.3                                      | 2, 15.4                                                  | 14, 17.7                                                 |                                                         |                                                         |
| **Anal sex in the prior month; n=94** |                                               |                                               |                                                         |                                                         |                                                         |                                                         |
| Yes 4, 4.25                     | 1, 1.5                                        | 3, 10.3                                       | 0.35 (0.06 to 1.92)                                      | 11, 84.6                                                 | 77, 97.5                                                 | 4.00 (1.29 to 12.32)                                     |
| No 90, 95.7                     | 64, 98.5                                      | 29, 89.7                                      | 2, 15.4                                                  | 2, 2.5                                                   |                                                         |                                                         |
| **Exchange sex money/gifts**    |                                               |                                               |                                                         |                                                         |                                                         |                                                         |
| Yes 13, 10.1                    | 5, 6.2                                        | 8, 17.0                                       | 0.58 (0.28 to 1.17)                                      | 15, 83.3                                                 | 98, 90.7                                                 | 1.73 (0.57 to 5.21)                                      |
| No 115, 89.8                    | 76, 93.8                                      | 39, 83.0                                      | 3, 16.7                                                  | 10, 9.3                                                  |                                                         |                                                         |
| **100% condom use; n=94**       |                                               |                                               |                                                         |                                                         |                                                         |                                                         |
| Yes 4, 4.3                      | 4, 6.2                                        | 0, 0                                           | 12, 92.3                                                 | 79, 96.2                                                 | 1.83 (0.31 to 10.83)                                     |                                                         |
| No 90, 95.7                     | 61, 93.8                                      | 29, 100                                       | 1.4 (1.28 to 1.70)                                       | 1, 7.7                                                   | 3, 3.8                                                   |                                                         |
| **Medical history**             |                                               |                                               |                                                         |                                                         |                                                         |                                                         |
| **More than 5 years with HIV**  |                                               |                                               |                                                         |                                                         |                                                         |                                                         |
| Yes 87, 68.0                    | 56, 69.1                                      | 31, 66.0                                      | 1.05 (0.78 to 1.41)                                      | 7, 38.9                                                 | 33, 30.6                                                 | 0.73 (0.30 to 1.74)                                      |
| No 41, 32.0                     | 25, 30.9                                      | 16, 34.0                                      | 11, 61.1                                                 | 75, 69.4                                                 |                                                         |                                                         |
| **CD4 T cells >500 cells/mL; n=106** |                                               |                                               |                                                         |                                                         |                                                         |                                                         |
| Yes 50, 47.2                    | 31, 62.0                                      | 25, 43.2                                      | 1.12 (0.81 to 1.54)                                      | 5, 38.5                                                 | 50, 54.9                                                 | 1.79 (0.62 to 5.12)                                      |
| No 56, 52.8                     | 31, 55.4                                      | 25, 56.8                                      | 8, 61.5                                                  | 41, 45.1                                                 |                                                         |                                                         |
| **History of STIs**             |                                               |                                               |                                                         |                                                         |                                                         |                                                         |
| Yes 25, 19.5                    | 15, 18.5                                      | 10, 21.3                                      | 0.93 (0.65 to 1.33)                                      | 14, 77.8                                                 | 87, 80.6                                                 | 1.15 (0.41 to 3.20)                                      |
| No 103, 80.5                    | 66, 81.5                                      | 37, 78.7                                      | 4, 22.2                                                  | 21, 19.4                                                 |                                                         |                                                         |
| **Plasma viraemia n=126**       |                                               |                                               |                                                         |                                                         |                                                         |                                                         |
| Yes 26, 20.6                    | 16, 20.0                                      | 10, 21.7                                      | 0.96 (0.68 to 1.34)                                      | 8, 44.4                                                 | 16, 15.1                                                 | 4.72 (2.06 to 10.73)*                                    |
| No 700, 79.4                    | 64, 80.0                                      | 36, 78.3                                      | 10, 55.6                                                 | 90, 84.9                                                 |                                                         |                                                         |

Continued
## Table 1 Continued

| Study population number, % | Number and % among participants with BV n=81 | Number and % among participants without BV n=47 | Unadjusted PR (95% CI) for having bacterial vaginosis | Number and % among participants with HIV shedding n=18 | Number and % among participants without HIV shedding n=108 | Unadjusted PR (95% CI) for having HIV shedding |
|---------------------------|---------------------------------------------|-----------------------------------------------|------------------------------------------------------|-----------------------------------------------|--------------------------------------------------------|----------------------------------|
| BV                        |                                             |                                               |                                                     |                                               |                                                        |                                  |
| Yes                       | 81, 63.3                                    |                                               | 5, 27.8                                              | 40, 37.0                                     |                                                        | 1.44 (0.55 to 3.79)                |
| No                        | 47, 36.7                                    |                                               | 13, 72.2                                             | 68, 63.0                                     |                                                        |                                  |
| HIV shedding; n=126       |                                             |                                               |                                                     |                                               |                                                        |                                  |
| Yes                       | 18, 14.3                                    |                                               | 1.14 (0.83, 1.58)                                    |                                               |                                                        |                                  |
| No                        | 108, 85.7                                   |                                               |                                                      |                                               |                                                        |                                  |
| Intravaginal practices    |                                             |                                               |                                                     |                                               |                                                        |                                  |
| Daily use of any IVP      |                                             |                                               |                                                     |                                               |                                                        |                                  |
| Yes                       | 114, 89.1                                   | 79, 97.5                                      | 35, 74.5                                             | 1, 5.6                                       | 12, 11.1                                               | 1.95 (0.28 to 13.5)                |
| No                        | 14, 10.9                                    | 2, 2.5                                       | 12, 25.5                                             | 17, 94.4                                     | 96, 88.9                                               |                                  |
| Daily use of water        |                                             |                                               |                                                     |                                               |                                                        |                                  |
| Yes                       | 111, 86.7                                   | 76, 93.8                                      | 35, 74.5                                             | 16, 88.9                                     | 94, 87.0                                               | 1.16 (0.29 to 4.59)                |
| No                        | 17, 13.3                                    | 5, 6.2                                       | 12, 25.5                                             | 2, 11.1                                      | 14, 13.0                                               |                                  |
| Daily use of soap         |                                             |                                               |                                                     |                                               |                                                        |                                  |
| Yes                       | 83, 64.8                                    | 63, 77.8                                      | 20, 40.6                                             | 11, 61.1                                     | 71, 65.7                                               | 0.84 (0.35 to 2.02)                |
| No                        | 45, 35.3                                    | 18, 22.2                                      | 27, 57.4                                             | 37, 34.3                                     |                                                        |                                  |
| Daily use of cloth/rag/sponge |                                   |                                               |                                                     |                                               |                                                        |                                  |
| Yes                       | 48, 37.5                                    | 41, 50.5                                      | 7, 14.9                                              | 6, 33.3                                       | 41, 38.9                                               | 0.81 (0.32 to 2.02)                |
| No                        | 80, 62.5                                    | 40, 49.4                                      | 40, 85.1                                             | 12, 66.7                                     | 66, 61.1                                               |                                  |
| Daily use of herbs        |                                             |                                               |                                                     |                                               |                                                        |                                  |
| Yes                       | 16, 12.5                                    | 11, 13.6                                      | 5, 10.6                                              | 1, 5.6                                       | 15, 13.9                                               | 0.40 (0.05 to 2.83)                |
| No                        | 112, 87.5                                   | 70, 84.4                                      | 42, 89.4                                             | 17, 94.4                                     | 93, 86.1                                               |                                  |
| Daily use of traditional medicines |                                       |                                               |                                                     |                                               |                                                        |                                  |
| Yes                       | 13, 10.2                                    | 11, 13.6                                      | 2, 4.3                                               | 2, 11.1                                       | 11, 10.2                                               | 1.08 (0.28 to 4.20)                |
| No                        | 115, 89.8                                   | 70, 86.4                                      | 45, 95.7                                             | 16, 88.9                                     | 97, 89.8                                               |                                  |
| Weekly use of any IVP     |                                             |                                               |                                                     |                                               |                                                        |                                  |
| Yes                       | 5, 3.9                                      | 0, 0                                          | 5, 10.6                                              | 3, 2.8                                       | 17, 94.4                                               | 1.79 (0.31 to 10.36)              |
| No                        | 123, 96.1                                   | 81, 100.0                                     | 42, 89.4                                             | 105, 97.2                                    |                                                        |                                  |
| Weekly use of cloth/rag/sponge |                                       |                                               |                                                     |                                               |                                                        |                                  |
| Yes                       | 12, 9.4                                     | 10, 83.3                                      | 1.36 (1.01 to 1.82)                                   | 4, 22.2                                       | 8, 7.4                                                  | 3.64 (0.85 to 15.61)              |
| No                        | 116, 90.6                                   | 71, 61.2                                      | 14, 77.8                                             | 100, 92.6                                    |                                                        |                                  |
| Weekly use of herbs       |                                             |                                               |                                                     |                                               |                                                        |                                  |
| Yes                       | 5, 3.9                                      | 4, 4.9                                        | 1, 2.1                                               | 1, 5.6                                       | 4, 3.7                                                  | 1.42 (0.23 to 8.67)               |
| No                        | 123, 96.1                                   | 77, 95.1                                      | 46, 97.9                                             | 104, 96.3                                    |                                                        |                                  |
| Weekly use of traditional medicines |                                       |                                               |                                                     |                                               |                                                        |                                  |
| Yes                       | 12, 9.4                                     | 11, 13.6                                      | 1, 2.1                                               | 2, 1.1                                       | 10, 9.3                                                  | 1.18 (0.31 to 4.55)              |
| No                        | 116, 90.6                                   | 70, 84.6                                      | 46, 97.9                                             | 98, 90.7                                     |                                                        |                                  |

Association between demographic, risk factors, medical history, IVP and the presence of BV and genital HIV shedding. Sexual risk factors were assessed in the month prior to enrolment, except exchanging sex for money, which was assessed per lifetime. Bivariate analysis was performed using PR with 95% CI. χ² or Fischer's exact test was also used to detect significance at the threshold of p<0.05.

*Bold typeface indicates significance at the level of p<0.05.

BV, bacterial vaginosis; IVP, intravaginal practices; PR, prevalence ratios; STIs, sexually transmitted infections.
presented ulcerations. Normal vaginal pH (4.5 or less) occurred in only 6.7% of the participants; almost 80% of women had a vaginal pH over 5. Cervical discharge and cervical tenderness was present in one participant (data not shown in table).

Bacterial vaginosis
Thirty participants (23.5%) had normal vaginal flora (Nugent 0–3), 18 (14%) had intermediate vaginal flora (Nugent 4–6) and 81 (63.3%) had BV.

HIV-1 viral load in plasma and cervicovaginal samples
Twenty-six of 128 women (20.3%) had detectable HIV-1 in plasma, 100 (78.1%) had undetectable plasma viraemia and 2 had missing values. The median plasma viral load among those with a detectable level was 3.80 log \(_{10}\) copies/mL (IQR=2.13). Eighteen of 128 women (14.2%) had detectable HIV-1 in cervicovaginal samples. The median CVL viral load among those with a detectable level was 2.82 log \(_{10}\) copies/mL (IQR=1.42).

Of the 190 women with undetectable HIV-1 in plasma, 8 (8%) had HIV-1 lower genital shedding. The median CVL level in the eight women with undetectable plasma viral load and HIV-1 genital shedding was 2.32 log \(_{10}\) copies/mL (IQR=1.42) (not shown in table). Of these eight women, all engaged in daily IVP and seven had BV.

Intravaginal practices
Women reported engaging in IVP at a young age (median=15 years, range 8–41 years). Most women had used IVP in the prior 2 days (105, 82%) and used IVP daily (114, 89%). Five women (3.9%) reported using IVP only weekly and seven (5.5%) used IVP monthly. Among different products used in the prior month, water had been used by 118 (92.2%) women, soap by 88 (68.7%), a cloth, rag or sponge by 68 (53.1%), traditional medicines by 34 (26.5%), and herbs and flowers from outdoors by 24 (18.7%) women. Other products were vinegar (4, 3.1%), salt (11, 8.6%), beer (8, 6.3%) and lemon (10, 7.8%).

Different products were used for different reasons: water and soap were used for general hygiene (111, 86.7% and 84, 65.6% of women respectively), followed by ‘to get rid of a discharge or odour’ (36, 28.1% for water and 36, 28.1% for soap); a cloth (or rag or sponge) was used for general hygiene (61, 47.7% of women) followed by ‘to please sexual partner’ (33, 25.8%); traditional medicines were used for general hygiene (24, 18.7%) and to please sexual partners (24, 18.7%). Herbs and flowers collected from outside were used for general hygiene (23, 17.9%) and to please sexual partners (13, 10.1%). Participant’s responses regarding the use of IVP are reported in table 2.

Demographic, risk and IVP factors associated with BV
The association of the different demographic and sexual risk factors, medical history and IVP, and the BV outcome is shown in table 1. There was no evidence that demographic and sexual risk factors were associated with BV. Daily use of IVP with any product was associated with BV. Daily use of water, soap and cloth was associated with BV; there was a strong relationship between the use of water, soap and cloth, suggesting that they are used together (not shown). Weekly use of IVP was also associated with BV, in particular with the use of traditional medicines for IVP. Owing to the low number of women using weekly water or soap, the association of weekly use of these products and BV was not calculated. Vaginal examination with malodorous off-white or greyish thin discharge coating the vaginal walls and high pH (suggestive of BV) was not associated with the BV outcome (not shown).

Multivariable analysis with calculation of PR was performed adjusting for factors that showed a significant association in univariate analysis. Owing to the close relationship between daily use of the different components (cloth, soap and water), and to avoid collinearity, only daily use for IVP was included in the model. Weekly use of traditional medicines was included in the model since it was strongly associated with BV. In the multivariable model, daily use of IVP and weekly use of traditional medicines was associated with having BV (table 3).

Demographic, risk and IVP factors associated with HIV-1 genital shedding
The association of the different demographic, sexual risk factors, medical history and IVP, and the HIV-1 genital shedding outcome, is shown in table 1. The only variable associated with lower genital HIV shedding was plasma viraemia. Multivariable analysis adjusted for daily use of IVP, BV and plasma viraemia was performed, and plasma viraemia was the only variable associated with HIV-1 shedding (table 3). Exploratory analyses were performed in women with undetectable HIV-1 plasma viral load to examine the relationship of the demographic and sexual risk factors, and medical history, including BV and IVP, with HIV-1 genital shedding. There was no significant association with sociodemographics, risk factors, medical history and BV, with HIV-1 genital shedding in women with undetectable HIV-1 plasma viral load (results not shown).

DISCUSSION
This study sought to clarify the relationship between IVP, BV and HIV lower genital shedding among women engaging in IVP in Zambia, as a first step in understanding if IVP affects HIV transmission in women on ART. We found that daily use of IVP and weekly use of traditional medicines were associated with BV; the only factor associated with HIV genital shedding was HIV plasma viraemia. Neither IVP nor BV were associated with lower genital HIV shedding in this study.

Studies addressing IVP in women in sub-Saharan Africa have primarily involved the HIV-uninfected...
population and have shown a significant association of IVP with BV; and of both IVP and BV with HIV acquisition. In HIV-infected women, an association between BV and HIV lower genital shedding, and BV and in utero HIV transmission, has been described. This may suggest an increase in HIV transmission in women with BV, but this study further describes the relationship between IVP, BV and HIV shedding in HIV-infected women on ART.

HIV lower genital shedding is responsible for HIV transmission, and viral suppression and treatment as prevention is one of the pillars of HIV prevention programmes in developed and in developing countries. However, it has been proposed that local factors such as STIs or BV, may increase HIV transmission and lower genital shedding in studies including untreated HIV women and adjusted for plasma viraemia. Our study confirms that the primary factor associated with HIV genital shedding is plasma viraemia, and if viraemia is suppressed with the use of ART, transmission is likely to be suppressed. However, our study did not find an association between BV and HIV lower genital shedding as previously found by others and this is likely due to the low number of women with detectable plasma viraemia. Our results support effective ART as the main strategy to prevent HIV-1 female genital shedding, and subsequent HIV transmission.

In our study, we confirmed that rates of BV among women engaging in IVP in Zambia are very high, and women use IVP frequently for reasons of hygiene and sexuality. Prior studies in sub-Saharan Africa have also noted rates of BV being around 40–50%, and higher in

| Table 2 | Participant’s responses regarding IVP (N=128) |
|---------|-----------------------------------------------|
| **Participant’s Responses Regarding Intravaginal Practices** | |
| Age of IVP initiation in years, range | 15.8 years, 8–41 |
| N, % | |
| **Recency of IVP** | |
| In the last 2 days | 105, 82.0 |
| 2 days to 1 week | 5, 3.9 |
| 1–2 weeks | 2, 1.6 |
| 2 weeks to 1 month | 16, 12.5 |
| **Frequency of IVP (any product)** | |
| Daily | 114, 89 |
| Weekly | 7, 5.5 |
| Monthly | 7, 5.5 |
| **Products used for IVP in the prior month and frequency of use** | N=128 |
| Water | 118, 92.2 |
| Recency, in the last 2 days | 109, 85.1 |
| Frequency | |
| Daily | 111, 86.7 |
| Weekly | 2, 1.5 |
| Monthly | 5, 3.9 |
| Reason | |
| For general female hygiene | 111, 86.7 |
| To get rid of a discharge/odour | 36, 28.1 |
| To remove blood after menses | 30, 23.4 |
| To prevent pregnancy | 8, 6.3 |
| To prevent STIs | 1, 0.8 |
| To please sexual partner | 23, 17.9 |
| Soap | 88, 68.7 |
| Recency, in the last 2 days | 87, 67.9 |
| Frequency | |
| Daily | 83, 64.8 |
| Weekly | 2, 1.6 |
| Monthly | 3, 2.3 |
| Reason | |
| For general female hygiene | 84, 65.6 |
| To get rid of a discharge/odour | 36, 28.1 |
| To remove blood after menses | 29, 22.6 |
| To prevent pregnancy | 8, 6.2 |
| To prevent STIs | 0, 0 |
| To please sexual partner | 16, 12.5 |
| Cloth, rag or sponge | 68, 53.1 |
| Recency, in the last 2 days | 60, 46.8 |
| Frequency | |
| Daily | 48, 37.5 |
| Weekly | 12, 9.3 |
| Monthly | 8, 6.2 |
| Reason | |
| For general female hygiene | 61, 47.7 |
| To get rid of a discharge/odour | 23, 18 |
| To remove blood after menses | 18, 14.1 |
| To prevent pregnancy | 3, 2.3 |
| To prevent STIs | 0, 0 |
| To please sexual partner | 33, 25.8 |
| Traditional medicines | 34, 26.5 |
| Recency, in the last 2 days | 25, 19.5 |
| Frequency | |
| Daily | 13, 10.1 |
| Weekly | 12, 9.4 |
| Monthly | 9, 7.0 |
| **Table 2 Continued** | |
| **Reason** | **Frequency** |
| For general female hygiene | 24, 18.7 |
| To get rid of a discharge/odour | 5, 3.9 |
| To remove blood after menses | 6, 4.7 |
| To prevent pregnancy | 2, 1.6 |
| To prevent STIs | 0, 0 |
| To please sexual partner | 24, 18.7 |
| Herbs and flowers from outdoors | 24, 18.7 |
| Recency, in the last 2 days | 22, 17.1 |
| Frequency | |
| Daily | 16, 12.5 |
| Weekly | 5, 3.9 |
| Monthly | 3, 2.3 |
| Reason | |
| For general female hygiene | 23, 17.9 |
| To get rid of a discharge/odour | 5, 3.9 |
| To remove blood after menses | 4, 3.1 |
| To prevent pregnancy | 1, 0.8 |
| To prevent STIs | 0, 0 |
| To please sexual partner | 13, 10.1 |

Products listed are those that were used by more than 10% of the participants. Other products assessed and used by <10% of participants were vinegar, salt, beer and yogurt.

IVP, intravaginal practices; STIs, sexually transmitted infections.
women with HIV. Additionally, we confirmed that most cases of BV are asymptomatic. This is important to note as most BV cases would likely be missed in sub-Saharan settings where syndromic management is the norm. Since IVP are very prevalent in this population, finding a group of women not engaging in IVP appeared to be impossible, and we chose to evaluate if different frequency and products used for IVP were associated with BV. We found a strong relationship between daily use of IVP and BV. The aetiology of BV remains unknown and controversy exists regarding whether BV is caused by a sexually transmitted pathogen or not. While some studies suggest a strong association with BV, but, as previously reported, traditional medicines. Model of the outcome HIV shedding was adjusted for plasma viraemia, daily use of IVP and BV. BV, bacterial vaginosis; IVP, intravaginal practices; PR, prevalence ratio.

|                              | Adjusted PR (95% CI) for | p Value |
|------------------------------|--------------------------|---------|
| Plasma viraemia              | 4.61 (2.02 to 10.54)*    | 0.001*  |
| Daily use of IVP             | 1.29 (0.18 to 9.27)      | 0.803   |
| BV                           | 1.32 (0.51 to 3.38)      | 0.567   |

*Bold typeface indicates significance at the level of p<0.05. PR, 95% CI and p value. Model for the outcome BV was adjusted for having more than one sex partner, having history of exchanging sex for money, daily use of IVP and weekly use of traditional medicines. Model of the outcome HIV shedding was adjusted for plasma viraemia, daily use of IVP and BV. BV, bacterial vaginosis; IVP, intravaginal practices; PR, prevalence ratio.

In summary, this study addresses an extremely important area in the field of women’s health in sub-Saharan Africa, the relationship between IVP, BV and HIV shedding, and study outcomes highlight important reasons for IVP interventions. Our results indicate that effective ART remains the main strategy to prevent HIV female genital shedding and subsequent HIV transmission. These findings support early initiation of ART as an HIV prevention tool and support the need for additional studies to further assess causality in the associations between IVP and BV in ART-treated women, as well as for studies assessing culturally tailored interventions to decrease BV in high-prevalence settings.

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