ORIGINAL ARTICLE

Intermediate vaginal flora is associated with HIV prevalence as strongly as bacterial vaginosis in a cross-sectional study of participants screened for a randomised controlled trial

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ABSTRACT

Objective The authors analysed data from female sex workers screened prior to participation in a microbicide trial to examine the association between prevalent vaginal flora abnormalities and HIV infection, with special emphasis on the role of the intermediate vaginal flora (IVF) in this association.

Methods Data from the Kampala, Cotonou, Chennai and Mudhol/Jamkhandi sites were analysed. Participants were interviewed and provided blood for HIV and syphilis antibody testing, genital samples for the diagnosis of vaginal flora abnormalities (using Nugent score) and other reproductive tract infections. Log-binomial regression was used to estimate the HIV prevalence ratio (PR) in relation to IVF and bacterial vaginosis (BV).

Results Among 1367 women, BV, IVF and HIV prevalences were 47.6% (95% CI=45.0% to 50.3%), 19.2% (95% CI=17.1% to 21.2%) and 27.0% (95% CI=24.6% to 29.3%), respectively. In multivariate analysis, adjusting for study site, age, years of education, occupation, female sterilisation, oral sex, past history of sexually transmitted infection, gonorrhoea and candidiasis, IVF was significantly associated with HIV infection with a PR similar to that of BV (adjusted PR=1.56 (95% CI=1.22 to 1.98) and 1.48 (95% CI=1.20 to 1.84), respectively).

Conclusions Though the cross-sectional design of the study precludes directional interpretation of the findings, the data do suggest that IVF may be as important as BV in HIV acquisition. The authors recommend prospective research to better understand the association between IVF and HIV acquisition.

INTRODUCTION

Bacterial vaginosis (BV) is the most common cause of vaginal infection in women of reproductive age and is more prevalent in developing than in developed countries.[1] Prevalence of BV is particularly high in female sex workers (FSWs), up to 70% in some reports.[2] The aetiological agent of BV is still controversial, but the current consensus is that of a polymicrobial infection.[1] Typically, BV is clinically diagnosed by the Amsel's criteria[3] and biologically by Nugent's method.[4] The latter is the current gold standard for BV diagnosis and is based on a standardised scoring system grading vaginal flora as normal (Nugent score (NS) =0–3), intermediate (NS=4–6) and BV (NS=7–10). This method has been validated and proved to have both high sensitivity and specificity as well as good inter-observer agreement.[4]

The association between BV and HIV infection was first reported in 1995.[5] Since that time, there has been accumulating evidence suggesting that BV is an important risk factor for HIV infection.[6–13] However, most studies that investigated this association classified BV into two categories (BV vs non-BV),[5–7 11 12 14] and as such, little attention has been paid to the role of the intermediate vaginal flora (IVF) in this association. The few studies that have examined this association using the three-level categorisation of BV (with the normal flora category as reference) have found discrepant results. Some of these studies found a statistically significant increase of HIV risk with BV but not with IVF.[15 16] However, in other studies, this increase was significant for both BV and IVF,[10 17 18] but the extent to which these two levels of risk were different was not assessed. From a public health viewpoint, it is critical to understand the risk associated with both IVF and BV for maximal HIV prevention. A recent meta-analysis of individual-level patient data, including 10 previous prospective studies conducted in six Eastern and Southern African countries, reclassified data on vaginal flora abnormalities from two (BV vs non-BV) to three categories with the primary objective of analysing the relationship between intra-vaginal practices and HIV acquisition.[19] This study found that both IVF and BV were significantly associated with an increased risk of HIV with respective adjusted HRs of 1.41 (95% CI=1.12 to 1.79) and 1.53 (95% CI=1.24 to 1.89) when considering vaginal flora status as assessed at the visit before HIV seroconversion.

The present study used data from the screening visits of the CONRAD Cellulose Sulphate trial[20] to examine the association between HIV, IVF and BV.

MATERIALS AND METHODS

Settings, subjects and procedures

Detailed methods for the randomised trial are presented elsewhere.[20] Five sites located in Durban (South Africa), Kampala (Uganda), Cotonou (Benin), Chennai and Mudhol/Jamkhandi (India)

participated in the trial. Due to some changes that occurred in the BV diagnosis procedures at the Durban site, the present analysis was restricted to participants screened at the other four sites. Women were recruited at the Mulago Hospital (Makerere University) in Kampala, Uganda; at a community clinic and a sexually transmitted infections (STIs) clinic in Cotonou, Benin; at the YRG Care Center in Chennai, India, and in clinics in Mudhol and Jamkhandi in Karnataka State, India. The study was approved by ethics committees of each collaborating centre.

All participants were aged 18 years or older and provided written informed consent. At the screening visit, trained health providers administered a questionnaire, which captured information on participants' socio-demographics, sexual behaviours, current contraceptive method, intra-vaginal cleansing practices (product and reason) and history of STIs. A gynaecological examination was conducted including speculum and bimanual pelvic examination and vaginal pH measurement. Two endocervical swabs (for gonorrhoea and chlamydia testing) and one high vaginal swab (for BV, trichomoniasis and candidiasis testing) were collected. Venous blood was taken for syphilis and HIV antibody testing and urine for pregnancy testing.

Vaginal flora abnormalities were assessed by scoring a gram-stained smear according to Nugent *et al*.[4] Trichomoniasis and candidiasis were diagnosed microscopically on wet mount. The endocervical swabs were tested with the Strand Displacement Amplification technique, SDA BD ProbeTec ET CT/NG (Becton Dickinson, Sparks, Nevada, USA).

HIV antibody testing was performed according to national HIV testing guidelines. In general, two rapid assays were used (in series or in parallel) and a third as a tie-breaker in case of discordant results.

For syphilis diagnosis, Rapid Plasma Reagin test was performed and reactive samples were then further analysed using a treponemal antibody-specific test. Samples reactive by both Rapid Plasma Reagin and the treponemal antibody-specific test were considered positive for syphilis.

The brand of pregnancy test varied among the study sites, but all used rapid assays. All the above-mentioned tests were performed according to the manufacturer's instructions.

Statistical methods

Continuous variables were described by medians and IQR. Proportions were computed for categorical variables.

We used two types of vaginal flora abnormality categorisation in the analysis: (1) two-level categorisation (BV (NS=7–10) vs non-BV (NS=0–6, referent)) and (2) three-level categorisation (normal (NS=0–3, referent), IVF (NS=4–6) and BV (NS=7–10)). Prevalences of BV, IVF and HIV and respective 95% CIs were computed.

Factors reported in the literature as potential HIV risk factors were selected for the regression analyses. Bivariate analyses of each potential risk factor and HIV, controlling for study site, were done first. Variables with p values <0.20 in bivariate analyses were included in a multivariate model along with variables identified in previous studies as potential confounding variables (regardless of p value). We attempted to fit the multivariate model using backward selection of covariates with an a priori specified rule that covariates which resulted in >10% change in the prevalence ratio (PR) would be retained. However, no variable by its removal resulted in a >10% change, therefore covariates with a p value <0.05 were retained in the final model. The significance level for testing associations was set at 0.05. All analyses were performed using log-binomial regression and effect estimates are reported as PRs. All statistical tests were two tailed. Data were analysed with SAS V.9.1 (SAS Institute Inc.).

RESULTS

From July 2005 to January 2007, 1491 women were screened at the four study sites. BV data were available for 1367 women and all were included in the present analysis. The characteristics of the 124 excluded women did not differ significantly from those of women included in the analysis (data not shown). The socio-demographic and behavioural characteristics of the women included are summarised in table 1.

Approximately 70% of the 1367 women were recruited at the two African sites and the remaining at the two Indian sites. The median age was 28 years (IQR=23–35), and the median number of years of education was 6 (IQR=2–8). The median for reported numbers of sexual partners in the previous 3 months was 80 (IQR=25–270). The median numbers of sexual acts per week and in the week prior to screening were 12 (IQR=5–25)

Table 1 Characteristics of 1367 female sex workers screened at two African and two Indian sites prior to enrolment in a microbicide trial

Characteristics	N (%) or median (IQR)
Sites	
Kampala	516 (37.7)
Chennai	355 (26.0)
Cotonou	447 (32.7)
Mudhol/Jamkhandi	49 (3.6)
Age in years	28 (23–35)
Completed years of school	6 (2–8)
Cohabiting with a man	288 (21.1)
Having an occupation other than sex work	906 (66.3)
Currently used contraceptive	
None	31 (2.3)
Hormonal	221 (16.2)
Intra-uterine device	11 (0.8)
Female sterilisation	294 (21.5)
Condom	809 (59.2)
Number of sexual partners in the past 3 months	80 (25–270)
Average number of sexual acts per week	12 (5–25)
Number of sexual acts in the past 7 days	8 (4–20)
Condom use at the last sexual act*	1061 (77.7)
Oral sex in the past 30 days	114 (8.3)
Anal sex in the past 30 days	30 (2.2)
Past history of STIs	615 (45.0)
Recent history (<6 months) of STIs†	362 (27.0)
Intra-vaginal cleansing	1340 (98.0)
Vaginal pH	5 (5–6)
Irregular menstrual cycles‡	331 (24.4)
Current STIs and reproductive tract infections	
HIV	369 (27.0)
Neisseria gonorrhoeae§	111 (8.1)
Chlamydia trachomatis§	80 (5.9)
Syphilis§	82 (6.0)
Trichomonas vaginalis	92 (6.7)
Vaginal candida colonisation	421 (30.8)
Vaginal flora abnormalities	
Bacterial vaginosis (NS=7–10)	651 (47.6)
Intermediate vaginal flora (NS=4–6)	262 (19.2)
Normal vaginal flora (NS=0–3)	454 (33.2)

*Missing data for one woman.
†Missing data for 11 women.
‡Missing data for 13 women.
§Missing data for three women.
NS, Nugent score; STIs, sexually transmitted infections.

Table 2 Association between HIV and socio-demographic, behavioural and medical factors among 1367 female sex workers recruited at two African and two Indian sites: bivariate analysis (controlling for site for each factor)

Factors	HIV prevalence by exposure level n_i/N_i (%)	PR (95% CI)	p Value
Site			
Kampala (Ref)	167/516 (32.4)	1.00 (—)	—
Mudhol/Jamkhandi	23/49 (46.9)	1.45 (1.05 to 2.00)	0.02
Cotonou	123/447 (27.5)	0.85 (0.70 to 1.03)	0.10
Chennai	56/355 (15.8)	0.49 (0.37 to 0.64)	<0.0001
Age in years (continuous)	—	1.02 (1.00 to 1.03)	0.004
Age (5-year categories)			
15—19 (Ref.)	10/66 (15.1)	1.00 (—)	—
20—24	100/380 (26.3)	1.86 (1.03 to 3.37)	0.04
25—29	109/357 (30.5)	2.26 (1.25 to 4.08)	0.01
30—34	68/221 (30.8)	2.72 (1.49 to 4.97)	0.001
35—60	82/343 (23.9)	2.33 (1.27 to 4.29)	0.01
p Trend	—	—	0.005
Years in school (continuous)	—	0.95 (0.92 to 0.97)	<0.0001
Years in school (in categories)			
0—6 (Ref.)	240/782 (30.7)	1.00 (—)	—
7—12	124/548 (22.6)	0.69 (0.57 to 0.83)	0.0001
13—17	5/37 (13.5)	0.38 (0.17 to 0.87)	0.02
p Trend	—	—	<0.0001
Cohabiting with a man			
Yes	51/288 (17.7)	0.78 (0.58 to 1.05)	0.11
No	318/1079 (29.5)	1.00 (—)	—
Having an occupation besides sex work			
Yes	243/906 (26.8)	0.75 (0.59 to 0.95)	0.02
No	126/461 (27.3)	1.00 (—)	—
Intra-vaginal cleansing			
Yes	357/1340 (26.6)	0.79 (0.51 to 1.22)	0.28
No	12/27 (44.4)	1.00 (—)	—
Products used for intra-vaginal cleansing			
No intra-vaginal cleansing (Ref.)	12/27 (44.4)	—	—
Water	238/892 (26.7)	0.77 (0.49 to 1.20)	0.24
Soap	100/345 (29.0)	0.82 (0.52 to 1.29)	0.39
Antiseptics	14/69 (20.3)	0.97 (0.49 to 1.90)	0.92
Soap and antiseptics	1/10 (10.0)	0.46 (0.07 to 3.16)	0.43
Others (baking soda, coca-cola, traditional)	4/24 (16.7)	0.45 (0.17 to 1.23)	0.12
Vaginal pH			
≤4.7 (Ref.)	43/234 (18.4)	1.00 (—)	—
>4.7	326/1133 (28.8)	1.25 (0.93 to 1.67)	0.14
Female sterilisation*			
Yes	43/294 (14.6)	0.57 (0.38 to 0.84)	0.005
No	326/1072 (30.4)	1.00 (—)	—
History of irregular menstrual cycles†			
Yes	103/331 (31.1)	1.24 (1.02 to 1.48)	0.03
No	261/1023 (25.5)	1.00 (—)	—
Number of sexual partners in the past 3 months			
3—99 (Ref.)	169/731 (23.1)	1.00 (—)	—
≥100	200/636 (31.4)	1.13 (0.92 to 1.40)	0.23
Average number of vaginal sexual acts per week			
3—19 (Ref.)	205/843 (24.3)	1.00 (—)	—
20—220	164/524 (31.3)	1.07 (0.88 to 1.31)	0.47
Number of vaginal sexual acts in the past 7 days			
≥10	175/630 (27.8)	0.87 (0.72 to 1.05)	0.14
<10 (Ref.)	194/737 (26.3)	1.00 (—)	—
Anal sex in the past 30 days			
Yes	5/30 (16.7)	0.74 (0.33 to 1.64)	0.46
No	364/1337 (27.2)	1.00 (—)	—
Oral sex in the past 30 days			
Yes	14/114 (12.3)	0.53 (0.32 to 0.88)	0.01
No	355/1253 (28.3)	1.00 (—)	—

Continued

Table 2 Continued

Factors	HIV prevalence by exposure level n_i/N_i (%)	PR (95% CI)	p Value
Condom use at the last sexual act*			
Yes	291/1061 (27.4)	1.01 (0.81 to 1.25)	0.95
No	78/305 (25.6)	1.00 (—)	—
Past history of STIs			
Yes	198/615 (32.2)	1.51 (1.27 to 1.81)	<0.0001
No	171/752 (22.7)	1.00 (—)	—
Recent history of STIs (<6 months)‡			
Yes	108/362 (29.8)	1.41 (1.15 to 1.73)	0.001
No	258/994 (26.0)	1.00 (—)	—
BV			
Present (NS=7—10)	192/651 (29.5)	1.18 (0.99—1.40)	0.07
Absent (NS=0—6)	177/716 (24.7)	1.00 (—)	—
Vaginal flora abnormalities			
BV (NS=7—10)	192/651 (29.5)	1.43 (1.15 to 1.77)	0.0012
Intermediate vaginal flora (NS=4—6)	84/262 (32.1)	1.58 (1.23 to 2.03)	0.0003
Normal vaginal flora (NS=0—3)	93/454 (20.5)	1.00 (—)	—
Syphilis§			
Present	29/82 (35.4)	1.45 (1.07 to 1.95)	0.01
Absent	339/1282 (26.4)	1.00 (—)	—
Neisseria gonorrhoeae§			
Present	52/111 (46.8)	1.68 (1.34 to 2.10)	<0.0001
Absent	316/1253 (25.2)	1.00 (—)	—
Chlamydia trachomatis§			
Present	23/80 (28.7)	0.97 (0.68 to 1.38)	0.86
Absent	345/1284 (26.9)	1.00 (—)	—
Trichomonas vaginalis			
Present	26/92 (28.3)	1.07 (0.76 to 1.49)	0.70
Absent	343/1275 (26.9)	1.00 (—)	—
Vaginal candida colonisation			
Present	118/421 (28.0)	1.10 (0.90 to 1.34)	0.37
Absent	251/946 (26.5)	1.00 (—)	—

*Missing data for one woman.
†Missing data for 13 women.
‡Missing data for 11 women.
§Missing data for three women.
BV, bacterial vaginosis; NS, Nugent score; PR=prevalence ratio (adjusted for site); STIs, sexually transmitted infections.

and 8 (IQR=4—20), respectively. One thousand and sixty-one (77.7%) women self-reported condom use at the last sexual act. Almost all participants (98.0%) practiced intra-vaginal cleansing and 615 (45.0%) reported a history of at least one STI.

Three hundred sixty-nine women were diagnosed with HIV, 651 with BV and 262 with IVF, giving prevalences of 27.0% (95% CI=24.7% to 29.4%), 47.6% (95% CI=45.0% to 50.3%) and 19.2% (95% CI=17.1% to 21.2%), respectively. Trichomoniasis and candidiasis were present in 92 (6.7%) and 421 (30.8%) women, respectively. Cervical swabs were obtained from 1364 women among whom, 111 (8.1%) were positive for gonorrhoea and 80 (5.9%) for chlamydia. The prevalence of syphilis was 6.0% (82/1364).

In the analysis using dichotomous categorisation of BV while controlling for study site, there was a marginal positive association between BV and HIV with a PR=1.18 (95% CI=0.99 to 1.40) (table 2). In the analysis using three-level categorisation of BV, the association with HIV was significant with PR=1.58 (95% CI=1.23 to 2.03) and PR=1.43 (95% CI=1.15 to 1.77) for IVF and BV, respectively, versus normal flora.

Other factors positively and significantly associated with HIV included a past history of STIs, recent history of STIs, gonorrhoea, older age and irregular menstrual cycles. Those factors that were significantly, but negatively, associated with HIV included having an occupation besides sex work, oral sex in the past 30 days, a higher level of education and female sterilisation.

In multivariate analysis (table 3), BV classified in two categories was significantly associated with HIV (adjusted PR=1.21, 95% CI=1.03—1.44). This association was stronger when the three-level categorisation was used with PR=1.56 (95% CI=1.22 to 1.98) and PR=1.48 (95% CI=1.20 to 1.84), respectively, for IVF and BV. Other risk factors found to be significantly associated with HIV included study site, age, past history of STI, gonorrhoea and candidiasis for positive associations, and education, occupation, female sterilisation and oral sex for negative associations.

DISCUSSION

Among these high-risk women aged 18—60 years, recruited at two African and two Indian sites, we estimated BV and HIV prevalence at 47.6% and 27.0%, respectively. These figures confirmed the high prevalence of both infections among FSWs in sub-Saharan Africa and India.[2 14 21—24] In addition, 19.2% of women were found to have an IVF.

HIV prevalence was significantly higher in women with IVF compared with those with normal flora as evidenced by the PR of 1.56 (95% CI=1.22 to 1.98). This resulted in a substantially higher HIV PR when using women with normal vaginal flora as the referent versus using women without BV (combined normal flora and IVF) as the referent category. These results are consistent with some previous studies that also reported a significant association with IVF[10 17 19] but discrepant from

Table 3 Results from the multivariate analyses of the association between vaginal flora abnormalities and HIV infection among 1367 female sex workers recruited at two African and two Indian sites

Variables	HIV prevalence by exposure level, n_i/N_i (%)	APR (95% CI)	p Value
BV			
Present (NS=7–10)	192/651 (29.5)	1.21 (1.03 to 1.44)	0.02
Absent (NS=0–6)	177/716 (24.7)	1.00 (–)	–
Vaginal flora abnormalities			
Normal vaginal flora (NS=0–3)	93/454 (20.5)	1.00 (–)	–
IVF (NS=4–6)	84/262 (32.1)	1.56 (1.22 to 1.98)	0.0003
BV (NS=7–10)	192/651 (29.5)	1.48 (1.20 to 1.84)	0.0003
BV vs intermediate flora	–	0.95 (0.79 to 1.16)*	0.6347
Site			
Kampala (Ref.)	167/516 (32.4)	1.00 (–)	–
Mudhol/Jamkhandi	23/49 (46.9)	1.16 (0.79 to 1.68)	0.45
Cotonou	123/447 (27.5)	0.64 (0.50 to 0.81)	0.0002
Chennai	56/355 (15.8)	0.54 (0.37 to 0.80)	0.002
Past history of sexually transmitted infections			
Yes	198/615 (32.2)	1.43 (1.20 to 1.69)	<0.0001
No	171/752 (22.7)	1.00 (–)	–
Having an occupation besides sex work			
Yes	243/906 (26.8)	0.78 (0.62 to 0.98)	0.03
No	126/461 (27.3)	1.00 (–)	–
Female sterilisation†			
Yes	43/294 (14.6)	0.58 (0.39 to 0.86)	0.006
No	326/1072 (30.4)	1.00 (–)	–
Oral sex in the past 30 days			
Yes	14/114 (12.3)	0.57 (0.35 to 0.95)	0.03
No	355/1253 (28.3)	1.00 (–)	–
Age (years)			
15–19 (Ref.)	10/66 (15.1)	1.00 (–)	–
20–24	100/380 (26.3)	1.84 (1.02 to 3.32)	0.04
25–29	109/357 (30.5)	2.16 (1.20 to 3.89)	0.01
30–34	68/221 (30.8)	2.70 (1.49 to 4.90)	0.001
35–60	82/343 (23.9)	2.22 (1.21 to 4.05)	0.009
Years in school			
0–6 (Ref.)	240/782 (30.7)	1.00 (–)	–
7–12	124/548 (22.6)	0.75 (0.63 to 0.91)	0.003
13–17	5/37 (13.5)	0.47 (0.21 to 1.06)	0.07
Neisseria gonorrhoeae‡			
Positive	52/111 (46.8)	1.50 (1.21 to 1.85)	0.0002
Negative	316/1253 (25.2)	1.00 (–)	–
Vaginal candida colonisation			
Positive	118/421 (28.0)	1.27 (1.04 to 1.54)	0.02
Negative	251/946 (26.5)	1.00 (–)	–

The APR for BV versus non-BV and that for BV versus IVF were each obtained from a separate multivariate model, but including all other covariates presented in the present table.
*Incremental prevalence ratio (BV vs intermediate flora).
†One missing value.
‡Three missing values.
APR, adjusted prevalence ratio; BV, bacterial vaginosis; IVF, intermediate vaginal flora; NS, Nugent score.

other studies that did not find a significant difference between normal flora and IVF as to HIV risk.[15 16] Our findings suggest that using NS <7 as a reference to measure the increased risk of HIV related to BV could lead to underestimation. This is a biologically justified approach as it views IVF as a borderline stage of the same disease[25] and not a normal or 'non-disease' state. Indeed, both IVF and BV are likely to be determined by the same risk factors and it is theoretically reasonable to hypothesise that women with IVF convert more frequently between IVF and BV than between IVF and normal flora.

Our findings differed from those of most previous studies in that the strength of the association we found with IVF was similar to that with BV.[6 17 18 26] To our knowledge, only van de Wijgert *et al*,[10] in their study involving women from Zimbabwe and Uganda, found a higher association with IVF for the Uganda study site. The varying findings on the relationship between IVF and HIV across studies may be due to different biological characteristics of IVF, including the microbial community types, the duration of the intermediate status and the distribution of women regarding extreme scores of the category. Alternatively, considering the high frequency of intra-vaginal cleansing practice in some populations (as observed in our study), recent intra-vaginal cleansing could affect the gram stain scoring so that some women with BV may have been misclassified into the IVF category. If this were true, we would also expect an inflation of IVF cases; however, the prevalence of IVF in our study was not higher than those reported in previous studies. Also, in our study, gram-stained

slides with little or no epithelial cells and/or microorganisms or those with destroyed cells (because the swab was not properly rolled on the slide) were considered not suitable and thus not scored.

Indeed, the rapid fluctuation of the vaginal microbiota makes it difficult to disentangle the true difference, if any, in HIV risk associated with IVF versus BV, even in prospective studies. Brotman *et al*[27] recently found that women may naturally move between the three categories in as little as 3 days. Furthermore, half of the BV episodes diagnosed in this study lasted only for 3 days.[27] This observation suggests prospective studies assessing BV as risk factor for incident HIV should treat BV as a time-dependent variable.

Since our study was cross-sectional, the temporality of the associations we observed could not be established. The biological plausibility of the increased risk of HIV related to abnormal vaginal flora, however, has been well documented.[28–30] In addition, we were not able to collect data on some potential confounders such as herpes simplex virus type 2 infection and alcohol consumption, although we do not suspect that they could fully explain the observed association. Self-reported variables included in our multivariate model, such as condom use at the last intercourse, number of sexual partners in the last 3 months and past history of STIs may have been subject to social desirability and/or memory bias resulting in possible misclassification. The latter would have led to residual confounding of the association between BV, IVF and HIV. Our study involved women at high risk of HIV and other cofactors of HIV and may therefore not be generalisable to women from the general population.

On the other hand, the study presents several strengths. Most importantly, the study result revealed that the association between IVF and HIV was as strong as that between BV and HIV in this study population, highlighting the need to pay more attention to IVF in regard to HIV risk. In addition, the study included FSWs from geographically diverse areas including West Africa, Eastern Africa and India. Because screening for STIs was a routine study procedure, we were able to control for STIs in our analyses. In addition, as part of a phase III clinical trial, this study benefited from a large sample size, use of the current gold standard for BV diagnosis (NS) and research sites with well-trained research personnel and quality control procedures, which contributed to data accuracy and comparability across sites. Finally, as the outcome of interest (HIV) was highly prevalent in our study population, the use of log-binomial regression to measure the association should well approximate the RR (in comparison with logistic regression).

In summary, this study confirms the high prevalence of BV and HIV and the existence of a relationship between the two conditions among FSWs who are at high risk of acquiring and transmitting HIV, particularly in developing countries. In addition, the findings suggest that, compared with women with normal flora, those with IVF have a higher HIV prevalence similar to women with BV. There is a need for more prospective research, specifically designed to assess the causality of the association between vaginal flora abnormalities and HIV, with a special attention for the role of IVF in this association. This implies that the Nugent scoring system should be used (not Amsel's criteria) to enable delineation of the IVF and BV categories and that laboratory technicians should be adequately trained. Future studies may also focus on identifying specific (clinical or biological) features of IVF conducive to high risk for HIV and could develop an algorithm for selecting appropriate IVF cases for treatment.

Key messages

- There was a statistically significant association between IVF and prevalent HIV infection and this association was as strong as that between BV and HIV.
- Using two-level categorisation of BV (BV vs non-BV) to examine the effect of vaginal flora abnormalities on HIV prevalence underestimates this effect.
- There is a need for prospective studies designed to assess the causality of the association between vaginal flora abnormalities and HIV infection.

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Contributors All authors were involved in the parent multicentre microbicide clinical trial that generated the data. For the present manuscript, FAG and MA conducted the statistical analyses and interpreted results. FAG wrote the first draft of the manuscript and MA revised it before further revision by other co-authors. In addition, JD and MB edited the text. All authors revised and approved the present version of the manuscript.

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Notes