

Bacterial vaginosis modifies the association between hormonal contraception and HIV acquisition

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Objective: To examine bacterial vaginosis as an effect modifier for the association between hormonal contraception and incident HIV infection.

Design: Serodiscordant couples enrolled in an open longitudinal cohort in Lusaka, Zambia from 1994 to 2012. This analysis was restricted to couples with an HIV-positive man enrolled between 1994 and 2002 when a quarterly genital tract examination and HIV testing was performed.

Methods: Multivariate Cox models evaluated the association between contraceptive method and HIV-acquisition, stratified by time-varying bacterial vaginosis status.

Results: Among 564 couples contributing 1137.2 couple-years of observation, bacterial vaginosis was detected at 15.5% of study visits. Twenty-two of 106 seroconversions occurred during intervals after bacterial vaginosis was detected [12 on no method/nonhormonal method (nonhormonal contraception), two on injectables, eight on oral contraceptive pills (OCPs)]. Unadjusted seroincidence rates per 100 couple-years for nonhormonal contraception, injectable, and OCP users, respectively, during intervals with bacterial vaginosis were 8.3, 20.8, and 31.0 and during intervals without bacterial vaginosis were 8.2, 9.7, and 12.3. In the bacterial vaginosis-positive model, there was a significant increase in incident HIV among those using injectables (adjusted hazard ratio, aHR 6.55, 95% CI 1.14–37.77) and OCPs (aHR 5.20, 95% CI 1.68–16.06) compared with nonhormonal contraception. Hormonal contraception did not increase the hazard of HIV acquisition in bacterial vaginosis-negative models. These findings persisted in sensitivity analyses whenever all covariates from the nonstratified model previously published were included, whenever other genital tract findings were excluded from the model and with the addition of condom-less sex and sperm on wet-prep.

Conclusion: Future research should consider a potential interaction with bacterial vaginosis whenever evaluating the impact of hormonal contraception on HIV acquisition.

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Introduction

Hormonal contraception is widely used globally for the prevention of unintended pregnancy. However, some have postulated that hormonal contraception may increase susceptibility to HIV infection [1–3]. Although overall data on the association between combined hormonal contraceptive pills and HIV acquisition are limited, there appears to be no increased risk in women with use of these methods [3]. With injectable contraceptive use, particularly depot medroxyprogesterone acetate (DMPA), recent meta-analyses suggest a 20–70% increased risk of HIV acquisition [3,4]. Importantly, not all studies have demonstrated a consistent link between use of DMPA and HIV risk in women, with several well designed studies finding no statistical association [5–7]. The World Health Organization (WHO) recognizes this important knowledge gap and encourages research to clarify the association and explore mechanisms associated with hormonal contraceptives that may underlie an increase in HIV transmission risk [8,9].

Several local factors within the vagina may increase HIV transmission risk and mediate the association between hormonal contraception and HIV acquisition [10]. One potential factor is bacterial vaginosis, a condition of a polymicrobial vaginal flora with an elevated vaginal pH and divergence from a healthy lactobacilli-dominant vaginal flora [10,11]. There is a growing body of literature supporting the role of the microbiota in altering local and systemic immune function [12–14]. Several studies have found that HIV acquisition increases in the setting of bacterial vaginosis and nonlactobacilli-dominant flora [15–18]. Although there is growing evidence of this direct association between vaginal microbiota and HIV acquisition, it is unclear if vaginal microbiota may be implicated in the noted association between hormonal contraception and HIV risk.

Most studies suggest that combined estrogen–progestin and progestin-only hormonal contraception does not increase the presence of bacterial vaginosis and some methods may even be protective against bacterial vaginosis [19–21]. In a recent meta-analysis, all studies reviewed showed either a statistically significant decrease or no significant difference in the incidence of bacterial vaginosis in hormonal contraceptive users whenever compared with nonhormonal contraceptive users. Whenever including the three highest quality studies, they found a 10–20% reduction in the incidence of bacterial vaginosis in combined oral contraceptive users and an 18–30% reduction in DMPA users versus nonhormonal method users [22]. Another meta-analysis including 55 studies reported an approximate 25% reduction in bacterial vaginosis in hormonal contraceptive users compared with nonusers, with this pattern similar for both combined estrogen–progestin and progestin-only methods [23]. These data suggest that alterations in the vaginal microflora

with contraceptive use are not part of the mechanisms for the observed increased HIV acquisition risk with hormonal contraception.

Prior studies have evaluated the effect of herpes simplex virus (HSV) or genital tract ulcers as potential effect modifiers in the association between hormonal contraception and HIV acquisition in women [7,24]. However, no studies to date have evaluated whether bacterial vaginosis may modify this association. Our objective was to leverage a large HIV discordant couple cohort to explore the association between hormonal contraception and HIV acquisition in women, considering bacterial vaginosis as a potential effect modifier. Our previous evaluation of the cohort did not demonstrate a significant increase in incident HIV with oral contraceptive, injectable contraceptive or contraceptive implant use. We hypothesized that we would see a different association between hormonal contraception and incident HIV in the presence of bacterial vaginosis. This hypothesized interaction between two independent potential risk factors may explain some of the inconsistencies in the literature that cloud our interpretation of hormonal contraception as a potential HIV risk factor.

Methods

Study design, participants and ethics

The study is a secondary analysis of a longitudinal cohort of heterosexual HIV serodiscordant couples in which the man is HIV-positive and the woman HIV-negative (M+F–) in Lusaka, Zambia. Heterosexual married or cohabitating HIV serodiscordant couples were invited to enroll in an open cohort study between 1994 and 2012. The study recruitment [25,26], intervention design, uptake of contraception immediately after an educational intervention [27], impact of informed consent on knowledge and concerns about contraceptive methods [28], demographics of the cohort, rates of unintended pregnancy and impact of contraceptive method on unintended pregnancy [29], impact of the intervention on incident pregnancy [30], patterns of contraceptive use and discontinuation [31], impact of hormonal contraception on HIV acquisition risk [7], HIV transmission to partners [32], and disease progression [33] have been previously reported. The Institutional Review Boards at Emory University and the University of Zambia approved this study. Written informed consent was obtained from all participating couples.

Exposure of interest

Contraceptive method used since last visit [none/condoms only, oral contraceptive pills (OCPs), DMPA (150 mg intramuscular dosage), copper intrauterine device (IUD), contraceptive implant (Levonorgestrel implant: Norplant, Jadelle), or permanent methods (hysterectomy/tubal

ligation/vasectomy)) was recorded at baseline and 3-monthly follow-up visits. The majority of OCPs were combined pills containing both an estrogen and progestin, with progesterone-only pills being primarily prescribed to breastfeeding women until children were 6 months old or the minority of women with contraindications to estrogens. In our primary analysis, contraceptive methods were categorized as implant, injectable, or OCP versus nonhormonal (nonhormonal contraception), including none/condoms only or permanent methods. All methods were provided at the research site.

Outcome of interest

The primary outcome evaluated any incident HIV infection among women, either linked or unlinked to the cohabiting male partner. HIV testing using rapid serologic tests was conducted at intervals of 3 months [26].

Baseline covariates

At enrollment, baseline demographic data was collected including age of the man and woman, years cohabiting, monthly income, and woman's literacy in Nyanja. Other possible risk factors evaluated as covariates included number of previous pregnancies, number of sexual partners for the woman in the last year, and viral load (\log_{10} copies/ml) of the positive male partner.

Time-varying covariates

At scheduled 3-monthly (or client-initiated interim) follow-up visits, time-varying exposures of interest were collected including time from enrollment (0–3 months reflecting prior to couples voluntary counseling and testing (CVCT) versus >3 months, reflecting those receiving CVCT), pregnancy, self-reported sex without a condom with study partner in past 3 months, self-reported sex with a condom with study partner in past 3 months, sperm present on vaginal swab wet-prep, candida by wet-prep, vaginal discharge on exam, general vaginal inflammation on exam, sexually transmitted infection (STI; gonorrhea and/or chlamydia based on purulent endocervical discharge and/or trichomonas based on wet-prep), bilateral inguinal adenopathy (BIA) on exam, and active genital or perianal ulcers for woman in past 3 months (by self-report or examination finding).

Effect modification evaluation

The effect modifier of interest was a time-varying diagnosis of bacterial vaginosis. This was diagnosed by a wet-prep at baseline and at scheduled or client-initiated interim follow-up visits. Bacterial vaginosis was diagnosed with microscopy (wet-prep for clue cells, KOH prep for whiff test, and/or Gram stain) at a routine or interim visit.

Longitudinal data collection

Participants were provided with free outpatient care and the full range of contraceptive methods at the research clinic. Data collection varied by type and frequency of data collected over 17 years of follow-up. From 1994 to

2002, both partners were seen every 3 months and underwent physical exams including rapid plasma reagin (RPR) screening for syphilis, genital exams, and wet-prep with repeat HIV testing of the HIV- partner. After 2002, physical exams and wet-prep diagnoses were performed at baseline and thereafter only if signs and symptoms of infections were present. Given this change in approach to only evaluating symptomatic individuals, we restricted this analysis to 1994–2002.

Data analysis

Analyses were conducted with SAS v9.3 (Cary, North Carolina, USA). Our analytic approach was informed by recommendations for a rigorous and consistent analysis of the association of hormonal contraception and HIV acquisition described by Polis *et al.* [34]. This approach was used previously in our evaluation with this cohort and demonstrated no statistically significant association between hormonal contraception and HIV acquisition in women without consideration of effect modification with bacterial vaginosis [7]. All analyses were stratified and separately analyzed for intervals with bacterial vaginosis and intervals without bacterial vaginosis.

Couple-years of follow-up were calculated from enrollment until the couple was censored. Couples were censored whenever either partner died, the couples separated, the positive partner started anti-retroviral therapy (ART), or if either partner was lost to follow-up. HIV incidence rates for each contraceptive method type were compared with the nonhormonal contraception reference group using univariate Cox models. These rates with corresponding 95% confidence intervals (95% CI) were calculated as the number of incident infections per couple-year of follow-up, stratified by whether a woman had a bacterial vaginosis infection noted at the visit prior to that study interval.

Baseline and time-varying data were described by HIV acquisition status using counts and percentages for categorical data or means and standard deviations for continuous data. These descriptive analyses were calculated across all study intervals and were stratified by bacterial vaginosis status.

Bivariate associations between baseline and time-varying covariates and outcome infection of interest were evaluated via unadjusted Cox models to generate crude hazard ratios and 95% CIs. Additional bivariate associations between these baseline and time-varying covariate were compared for the combined outcome groups (seroconverters and nonconverters) by bacterial vaginosis status.

Multivariate Cox models estimated the total effect of time-varying contraceptive method type on time to outcome infection. Covariates significantly ($P < 0.05$) associated with the exposure and outcome of interest were considered as potential confounders. Variable multicollinearity was assessed (condition indices of 0.30

and variance decomposition proportions of 0.05 as cutoff criteria); if any two variables were found to be collinear, the variable with the weakest association with the outcome was removed. The proportional hazards assumption using Schoenfeld residuals and graphical methods [plots of log $[-\log(\text{survival probability})]$ versus log (time)] was confirmed for time-independent covariates. Adjusted hazard ratios (aHRs) and 95% CIs are presented for covariates in the final multivariate models. For each model, contraceptive method was forced into the final multivariable models. Breslow–Day test was used to determine the significance of an interaction by time-varying bacterial vaginosis status.

Sensitivity analysis

We ran a sensitivity analysis including all the variables included in a nonbacterial vaginosis-stratified model, we previously published on from this cohort [7], given potential for unidentified confounding in the smaller stratified samples. Similarly, we ran an additional analysis including all covariates in the stratified models if found to be a confounder (associated with exposure and outcome at <0.05) in either one or both of the strata. We also ran the analysis including variables in the prior cohort evaluation [7] adding as covariates sperm on wet-prep and self-reported unprotected sex. We ran an analysis excluding the genital tract findings (infections and ulcerations) as confounders as bacterial vaginosis was highly correlated with several of these findings. Further, we also ran the previously published model, excluding the genital tract findings.

Results

Baseline demographics and rates for seroconversion

Among the 564 couples, a total of 106 women seroconverted over 1137.2 couple-years of observation (Table 1). Bacterial vaginosis was detected at 648 of 4183

study visits (15.5%). Twenty-two of the seroconversions occurred during intervals where bacterial vaginosis was detected at the visit prior to the seroconversion and 84 during intervals without bacterial vaginosis detected. Among the 4183 visits, implants were used at 17 visits (0.4%), injectable methods at 373 visits (8.9%), OCPs at 568 visits (13.6%) and nonhormonal methods at 3225 visits [77.1%, with copper IUD at 40 visits (0.9%), tubal ligation or vasectomy at 42 visits (1.0%) and no method or only condoms at 3143 visits (75.1%)].

During intervals where bacterial vaginosis was detected, seroincidence rates per 100 couple-years were 8.3, 20.8, and 31.0 for nonhormonal, injectable, and OCP users, respectively. During intervals without bacterial vaginosis detected, seroincidence rates per 100-couple-years were 8.2, 9.7, and 12.3, for nonhormonal, injectable, and OCP users, respectively. No seroconversions occurred among implant users.

Bivariate analyses

Covariates significantly associated with bacterial vaginosis status included illiteracy to Nyanja (83 versus 76%), higher number of sex partners in the last year at baseline (1.09 versus 1.03), less injectable contraceptive usage (5 versus 10%), increased self-reported sex without a condom in the past 3 months (44 versus 38%), higher rates of sperm on wet-prep (24 versus 17%), increased vaginal discharge (14 versus 9%), increased STIs inflammation (15 versus 6%) and increased BIA in the woman (8 versus 7%; Table 2).

Among intervals with bacterial vaginosis, baseline, and time-varying covariates significantly associated with HIV incidence (nonseroconverting intervals versus seroconverting intervals) included younger age of the woman (27.46 versus 22.68), OCP use (14 versus 36%), being in the initial 3 months after enrollment in CVCT cohort (2 versus 23%), vaginal inflammation (3 versus 14%) and BIA on exam (8 versus 23%) in the past 3 months. Among

Table 1. Unadjusted seroincidence rates for linked and unlinked HIV seroconversion, stratified by time-varying bacterial vaginosis and contraceptive method use (N = 564 M+F– couples), 1994–2002.

Contraceptive method	Number of seroconversions	Couple-years of follow-up time	Seroincidence per 100 couple-years	95% CI	
Bacterial vaginosis = YES					
Nonhormonal contraception	12	145.4	8.3	4.5	14.0
OCPs	8	25.8	31.0	14.4	58.8
Injectables	2	9.6	20.8	3.5	68.8
Implant	0	1.3	0.0		
<i>Total</i>	22	182.1	12.1	7.8	18.0
Bacterial vaginosis = NO					
Nonhormonal contraception	61	746.9	8.2	6.3	10.4
OCPs	15	122.1	12.3	7.1	19.8
Injectables	8	82.8	9.7	4.5	18.4
Implant	0	3.2	0.0		
<i>Total</i>	84	955.0	8.8	7.1	10.8

Nonhormonal contraception, includes no method use, condom use, copper intrauterine device, and permanent method. CI, confidence interval; OCP, oral contraceptive pill.

Table 2. Descriptive analyses and P values from unadjusted associations (from Cox models) between covariates and time to linked and unlinked HIV seroconversion, stratified by time-varying bacterial vaginosis status (N = 564 M+F— couples), 1994–2002.

	Bacterial vaginosis = yes						Bacterial vaginosis = no						P value for bacterial vaginosis yes versus no										
	Total (n = 648 intervals)			Nonseroconversion interval (n = 626 intervals)			Linked and unlinked seroconversion interval (n = 22 intervals)			Total (n = 3535 intervals)				Nonseroconversion interval (n = 3451 intervals)			Linked and unlinked seroconversion interval (n = 84 intervals)						
	N	%		N	%		N	%		N	%			N	%		N	%		N	%		
Baseline covariates (n intervals, % intervals)																							
Man, age (mean, SD)	33.79	8.27		33.92	8.34		30.05	4.74		34.84	8.03		34.86	8.03		33.92	7.88		0.068	0.014		0.083	0.302
Woman, age (mean, SD)	27.30	7.92		27.46	7.97		22.68	4.64		27.83	7.38		27.87	7.40		26.35	6.61		0.118	0.118		0.323	0.083
Years cohabiting (mean, SD)	7.35	7.09		7.44	7.17		4.73	3.31		8.36	7.00		8.37	7.55		6.10	6.10		0.086	0.086		0.950	0.323
Monthly family income (mean, SD)	56.19	60.34		56.94	61.02		34.81	29.73		58.84	69.48		58.81	69.73		60.11	58.30		0.086	0.086		0.950	0.323
Woman reads Nyanja	107	17%		105	17%		2	9%		831	24%		815	24%		16	19%		0.332	0.332		0.331	0.332
Yes, easily	541	83%		521	83%		20	91%		2704	76%		2636	76%		68	81%		0.082	0.082		0.471	0.471
No/with difficulty	3.84	2.94		3.89	2.96		2.57	1.96		3.97	2.81		3.98	2.83		3.71	2.14		0.234	0.234		0.431	0.431
Number of previous pregnancies (mean, SD)	1.09	0.41		1.10	0.41		1.05	0.22		1.03	0.34		1.03	0.33		1.06	0.45		0.187	0.187		0.132	0.132
Number of sex partners in last year (mean, SD)	4.91	0.73		4.90	0.73		5.14	0.57		4.91	0.74		4.91	0.74		5.04	0.66		<0.001	<0.001		0.873	0.873
Log viral load of positive partner, log ₁₀ copies/ml (mean, SD) ^a																							
Time-varying covariates (N intervals, % interval)																							
Contraceptive method																							
Nonhormonal contraception	517	80%		505	81%		12	55%		2708	77%		2647	77%		61	73%		n/a	n/a		n/a	0.0157
Implant	2	0%		2	0%		0	0%		15	0%		15	0%		0	0%		0.004	0.004		0.312	0.312
Injectables	33	5%		31	5%		2	9%		340	10%		332	10%		8	10%		<0.001	<0.001		<0.001	<0.001
OCs	96	15%		88	14%		8	36%		472	13%		457	13%		15	18%		0.733	0.733		0.572	0.572
Study interval time from enrollment	18	3%		13	2%		5	23%		100	3%		88	3%		12	14%		0.126	0.126		0.312	0.312
0–3 months	630	97%		613	98%		17	77%		3435	97%		3363	97%		72	86%		0.917	0.917		0.081	0.081
>3 months																							
Pregnant during interval	76	12%		74	12%		2	9%		382	11%		370	11%		12	14%		0.044	0.044		0.664	0.664
Yes	566	88%		546	88%		20	91%		3107	89%		3035	89%		72	86%		0.068	0.068		0.081	0.081
No																							
Sex with study partner with a condom in past 3 months	494	76%		474	76%		20	91%		2884	82%		2818	82%		66	79%		0.462	0.462		0.169	0.169
Yes	154	24%		152	24%		2	9%		648	18%		630	18%		18	21%		0.044	0.044		0.664	0.664
No																							
Sex with study partner without a condom in past 3 months	288	44%		278	44%		10	45%		1335	38%		1293	38%		42	50%		0.044	0.044		0.664	0.664
Yes	360	56%		348	56%		12	55%		2197	62%		2155	63%		42	50%		0.068	0.068		0.081	0.081
No																							
Sperm present on wet-prep	122	24%		117	24%		5	31%		474	17%		459	16%		15	21%		0.958	0.958		0.211	0.211
Yes	377	76%		366	76%		11	69%		2390	83%		2333	84%		57	79%		0.462	0.462		0.169	0.169
No																							
Vaginal discharge	90	14%		86	14%		4	18%		311	9%		300	9%		11	13%		0.462	0.462		0.169	0.169
Yes	558	86%		540	86%		18	82%		3253	91%		3180	91%		73	87%		0.044	0.044		0.664	0.664
No																							
Vaginal inflammation on exam	21	3%		18	3%		3	14%		92	3%		89	3%		3	4%		0.044	0.044		0.664	0.664
Yes	616	97%		597	97%		19	86%		3324	97%		3243	97%		81	96%		0.068	0.068		0.081	0.081
No																							
Candida	129	20%		121	19%		8	36%		635	18%		614	18%		21	25%		0.068	0.068		0.081	0.081
Yes	519	80%		505	81%		14	64%		2865	82%		2802	82%		63	75%		0.368	0.368		0.004	0.004
STI	99	15%		95	15%		4	18%		202	6%		191	6%		11	13%		0.368	0.368		0.004	0.004
Yes	549	85%		531	85%		18	82%		3333	94%		3260	94%		73	87%		0.048	0.048		0.001	0.001
No																							
BIA of women	52	8%		47	8%		5	23%		254	7%		238	7%		16	19%		0.048	0.048		0.001	0.001
Yes	589	92%		572	92%		17	77%		3169	93%		3101	93%		68	81%		0.085	0.085		0.071	0.071
No																							
Genital ulceration in past three months	135	21%		127	20%		8	36%		730	21%		707	20%		23	27%		0.085	0.085		0.071	0.071
Yes	513	79%		499	80%		14	64%		2805	79%		2744	80%		61	73%		<0.001	<0.001		0.890	0.890
No																							

Nonhormonal contraception includes no method use, condom use, copper intrauterine device, and permanent method. BIA, bilateral inguinal adenopathy; OCP, oral contraceptive pill; SD, standard deviation; STI, sexually transmitted infections such as gonorrhea and/or chlamydia based on purulent endocervical discharge and/or trichomonas based on wet-prep.
^aViral load collection began in 1999.

the intervals without bacterial vaginosis, being in the initial 3 months after enrollment in the CVCT cohort (3 versus 14%), having a STI inflammation (6 versus 13%) and BIA (7 versus 19%) in the past 3 months were associated with increased HIV incidence.

Contraceptive method at follow-up was significantly associated with seroconversion only among those with bacterial vaginosis, with an increase in seroconversion among those using OCPs compared with those using nonhormonal contraception.

Multivariate analyses

Hormonal contraceptive method was associated with incident HIV in the multivariable analysis in the time-varying bacterial vaginosis-positive models but not in the bacterial vaginosis-negative models, where bacterial vaginosis was a significant interaction term (Breslow-Day test for interaction by bacterial vaginosis: $P < 0.001$). In the bacterial vaginosis-positive model, when controlling for women's age (per year increase), vaginal inflammation in the past 3 months, and time interval since enrollment (0–3 versus 3 months), there was a significant increase in HIV acquisition among those using injectable contraception (aHR 6.55, 95% CI 1.14–37.77) and OCPs (aHR 5.20, 95% CI 1.68–16.06) compared with the nonhormonal contraception group. In the bacterial vaginosis-negative model, use of the implant, injectables, and OCPs did not have any increased hazards of HIV acquisition compared with the nonhormonal contraception group, whenever controlling for time interval since enrollment (0–3 months versus >3 months), STI in the past 3 months, and BIA in the past 3 months (Table 3).

Sensitivity analysis

Overall, the results from the sensitivity analyses led to similar conclusions. In the multivariate models limited to including all the covariates that were confounders in the nonstratified evaluation previously published, we found a similar significant increase in HIV acquisition with use of

injectable contraception (aHR 6.58, 95% CI 1.06–40.87) and OCPs (aHR 4.66, 95% CI 1.45–14.96) compared with nonhormonal contraception use for the bacterial vaginosis positive model. Similarly, whenever controlling for women's age, time interval since enrollment, vaginal inflammation, inflammatory STI, or BIA in last 3 months, the covariates that were identified as potential confounders in either or both strata, we found a similar increase in HIV acquisition with injectable (aHR 5.91, 95% CI 1.02–34.97) and OCP use (aHR 4.78, 95% CI 1.52–15.01). Whenever excluding other genital tract findings, there was a significant increase in HIV acquisition with OCP use compared with nonhormonal contraception use (aHR 5.7, 95% CI 1.9–17.2) in the bacterial vaginosis-positive model with the association among injectable users approaching significance (aHR 5.4, 95% CI 0.96–30.4, $P = 0.056$). Whenever sperm on wet-prep and condomless sex were added to the model, a similar increase in HIV acquisition was noted with injectable (aHR 9.2, 95% CI 1.3–62.5) and OCP use (aHR 7.6, 95% CI 1.9–31.0) compared with nonhormonal contraception use in the bacterial vaginosis models. Lastly, with all the confounders from the published analysis included and excluding the genital tract findings, there was a significant increase in HIV acquisition among injectable (aHR 5.9, 95% CI 1.02–33.9) and OCP (aHR 5.6, 95% CI 1.9–17.1) users compared with nonhormonal contraception users in the bacterial vaginosis-positive model. There was no association between use of hormonal contraception and HIV acquisition in the nonbacterial vaginosis models compared with nonhormonal contraception use in any of the sensitivity analyses.

Discussion

We present results indicating bacterial vaginosis as a potent effect-modifier of the association between

Table 3. Multivariate models of hormonal contraception use and time to linked or unlinked HIV seroconversion, stratified by time-varying bacterial vaginosis status (N = 564 M+F couples), 1994–2002.

Number of outcomes modeled:	Bacterial vaginosis = yes			Bacterial vaginosis = no		
	22 (out of 22)			84 (out of 84)		
current contraceptive method at follow-up visit	aHR ^a	95% CI	P value (two-tail)	aHR ^b	95% CI	P value (two-tail)
Nonhormonal contraception	Ref			ref		
Implant	n/a			n/a		
Injectables	6.546	1.135–37.767	0.036	1.348	0.637–2.853	0.435
OCPs	5.196	1.681–16.062	0.004	1.360	0.764–2.422	0.296

Nonhormonal contraception includes no method use, condom use, copper intrauterine device, and permanent method. aHR, adjusted hazards ratio; CI, confidence interval; ref: reference; n/a: not applicable; OCP, oral contraceptive pill; SD, standard deviation.

^aControlling for woman's age (per year increase), vaginal inflammation of woman in past 3 months, and time interval since enrollment (0–3 versus >3 months).

^bControlling for inflammatory STI in the past 3 months, bilateral inguinal adenopathy in past 3 months, and time interval since enrollment (0–3 versus >3 months).

hormonal contraception and HIV acquisition that may have large global health implications. Although increasing evidence has supported a direct role for bacterial vaginosis in increasing HIV acquisition risk, our results suggest that this impact is amplified in the setting of hormonal contraception use, specifically injectables and OCPs, with increased hazard ratios greater than 5 for HIV acquisition in the setting of bacterial vaginosis for injectables and OCPs compared with nonhormonal contraception use. Though our numbers were small and confidence intervals large, these findings highlight the importance of acknowledging the role of the vaginal environment in HIV acquisition risk and evaluating the genital tract environment in future studies investigating the association between hormonal contraceptives and HIV risk.

Proposed mechanisms for the association between hormonal contraception and HIV have included alterations in the local genital tract immunologic milieu and the composition of key HIV target immune cells, as well as alterations in vaginal epithelial tight junctions and mucosal permeability [35–37]. Recent evidence has pointed towards the gut microbiota and the hormonal environment as working synergistically to influence the development of disease states such as obesity, diabetes, and certain cancers [38]. Although a significant amount of literature has been building to explore the importance of the gut microbiota, our current understanding of the significance of the vaginal microenvironment is relatively limited. Hormonal contraception may have a differential impact to amplify the effect of the microbiota on the vaginal epithelium and immune environment. Future studies are needed to elucidate the mechanism for this interaction.

Although epidemiologic studies have not previously explored this interaction, a recent study by Fichorova *et al.* [10] compliments our findings. Those results demonstrated that the immunomodulatory changes attributed to different hormonal contraceptives are dependent on the genital tract microenvironment. Specifically, they report an increase in RANTES (Regulated upon Activation, Normal T-cell Expressed, and Secreted), a chemokine that has been associated with increased HIV acquisition, among OCP users in the setting of bacterial vaginosis, a finding that is mechanistically consistent with our observations. Although the consensus from the literature suggests no association between OCP use and HIV risk [3], the reduction in bacterial vaginosis among OCP users and differences in OCP adherence may have diluted any significant results. In the context of the demonstrated effect-modification potential of bacterial vaginosis reported here, re-evaluation of those findings, if possible, is warranted.

The prevalence of bacterial vaginosis is variable based on the clinical setting and may vary by race and behavioral practices [39]. Although the prevalence may be

underestimated as many women are asymptomatic, the greatest burden of bacterial vaginosis is noted in sub-Saharan Africa [40]. Further, the sensitivity and specificity of clinical techniques are variable in the diagnosis of bacterial vaginosis. For example, using Amsel's criteria with a wet-prep, which can be done easily as a point-of-care approach, yet is only about 70% sensitive for bacterial vaginosis [41]. The presence of clue cells on wet-prep, is highly sensitive and specific for bacterial vaginosis [42]. Nugent scores from a Gram stain, currently considered the gold standard for diagnosis for bacterial vaginosis diagnosis, may have greater sensitivity, but can be subject to variability in interpretation. Utilizing more sensitive microbiome techniques, such as 16s gene rRNA sequencing, offers an opportunity to understand the microenvironment at the level of specific microbial species and define microbial diversity with more specific methodology. Although historically symptomatic bacterial vaginosis has been attributed to *Gardnerella vaginalis*, these newer technologies have identified other bacteria associated with dysbiotic vaginal microbial states including *Leptotrichia/Sneathia*, *Atopobium vaginae*, *Megasphaera sp.*, and members of *Clostridiales* sometimes referred to as bacterial vaginosis-associated bacteria (BVAB) [11,43–45]. Further, as recent studies have suggested that specific microbacteria in the vaginal environment may have a differential impact on the local immune environment and HIV risk [45], it is important to explore this interaction using these more sensitive techniques.

The present study has several notable limitations. Our biggest limitation is the small number of women using contraception who seroconverted with bacterial vaginosis detected. Although even with this reduced power, we find a significant association; the stability of this finding weighs heavily on a small number of observations. Even during the years when we conducted routine examinations every 3 months, we may be missing episodes of bacterial vaginosis, because of the possible shifts between flora considered normal and flora considered intermediate or abnormal demonstrated in some studies [46]. This misclassification bias would primarily impact the association between contraception and HIV during the interval assessment without bacterial vaginosis detected. Additionally, we use several criteria in diagnosing bacterial vaginosis, which may increase our sensitivity while reducing the specificity of our bacterial vaginosis diagnoses. This could lead to misclassification. The generalizability of these results may be limited as this cohort consists of jointly tested and counseled HIV discordant couples who often adopt condom use following counseling with a corresponding reduction in transmission and seroconversion rates. Although we aimed to control for the impact of condom nonuse as a potential confounder, both sperm on wet-prep and self-reported unprotected sex have limitations in their ability to detect unprotected coital events, thus some degree of

unmeasured confounding is possible. Given these limitations, our results should be interpreted with caution and we encourage future studies to systematically evaluate bacterial vaginosis as a modifier in future studies.

Although this evaluation begins to elucidate some of the diversity noted in epidemiologic studies, many unanswered questions remain. It is unclear if the changes attributed to bacterial vaginosis that lead to increased acquisition risk and modification of the hormone contraception–HIV association are consistent among bacterial vaginosis–symptomatic compared with asymptomatic women. It is also unknown if treatment of bacterial vaginosis will alter these associations. For example, treatment of asymptomatic bacterial vaginosis during pregnancy does reduce the risk of preterm delivery associated with bacterial vaginosis [47]. As bacterial vaginosis is often recurrent, exploration of the role of suppressive or periodic presumptive treatment for bacterial vaginosis on biologic and clinical outcomes is warranted [48]. We must leverage newer sequencing approaches to effectively explore the role of individual taxa or microbial communities of these relationships utilizing rigorous epidemiologic methodology [49]. And lastly, we must explore alternative explanations for the findings we report. For example, is there something else about individuals who get bacterial vaginosis that predisposes them to having increased risk with hormonal contraceptive use, such as biologic or behavioral factors?

In conclusion, our findings suggest an association between HIV acquisition and HC use, specifically OCPs and injectables, among individuals with bacterial vaginosis. Current guidelines do not restrict the use of contraceptive methods for women at high-risk for HIV. The newest WHO recommendations specifically recommend that providers discuss the potential for increased acquisition with the use of DMPA [50]. An individual's contraceptive choice is influenced by many factors, and ultimately should remain with each woman in consultation with her provider. Data on alternative contraceptive options are critical for adequate counseling on relative risks for contraceptive use. Further, the vaginal microenvironment cannot be overlooked in the field of reproductive health and HIV, especially given recent data on the impact of *G. vaginalis* on antiretroviral drug concentrations used for preexposure prophylaxis effectiveness as well the possible increased risk of human papilloma virus (HPV) with nonlactobacillus dominant flora [51,52]. Future research is needed to further explore the interaction between bacterial vaginosis and hormonal contraception on HIV acquisition. If this interaction persists among other studies, identification of bacterial vaginosis may help tailor family planning discussions to appropriately counsel individuals on their risk. Further, periodic testing and treatment among asymptomatic women at high-risk for HIV and counseling

women on the symptoms of bacterial vaginosis may be important preventive strategies among reproductive aged women.

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Conflicts of interest

There are no conflicts of interest.

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