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Complete List of Authors:	Kusemererwa, Sylvia; MRC/UVRI and LSHTM Uganda Research Unit Ruzagira, Eugene; MRC/UVRI and LSHTM Uganda Research Unit; London School of Hygiene & Tropical Medicine Faculty of Epidemiology and Population Health Kabarambi, Anita; International Centre for Child Health and Development Onyango, Martin; MRC/UVRI and LSHTM Uganda Research Unit Abaasa, Andrew; MRC/UVRI and LSHTM Uganda Research Unit, Statistics & Data Science; London School of Hygiene & Tropical Medicine, Infectious Disease Epidemiology
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Effect of intravaginal practices on incidence of sexually transmitted infections and bacterial vaginosis among women enrolled in the Dapivirine vaginal ring trial (The Ring Study) in southwestern Uganda

Authors: Sylvia Kusemererwa[1*], Eugene Ruzagira[1,3], Martin Onyango[1], Anita Kabarambi[4], Andrew Abaasa[2,3]

Institution

[1]Department of viral pathogen epidemiology and interventions, Medical Research Council/ Uganda Virus Research Institute and London School of Hygiene and Tropical Medicine, Uganda Research Unit, Entebbe, Uganda

[2]Department of statistics, Medical Research Council/ Uganda Virus Research Institute and London School of Hygiene and Tropical Medicine, Uganda Research Unit, Entebbe, Uganda

[3] Department of infectious diseases epidemiology, London School of Hygiene & Tropical Medicine, London, UK

[4] Department of research, International Center for Child Health and Development, Masaka, Uganda

Correspondence to: Sylvia Kusemererwa, [1]Medical Research Council/Uganda Virus Research Institute (MRC/UVRI) and London School of Hygiene and Tropical Medicine (LSHTM), Uganda Research Unit, Plot 51-59, Nakiwogo Road, P.O Box 49 Entebbe, Uganda. Phone: 256 (0) 417 704000, or 256 (0) 70229100. Sylvia.Kusemererwa@mrcuganda.org or kansykarus@gmail.com

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Abstract

Objectives: We assessed the effect of intravaginal practices (IVP) on the incidence of sexually transmitted infections (STIs) and bacterial vaginosis (BV) among women using the dapivirine vaginal ring (DVR) or placebo vaginal ring in southwestern Uganda.

Methods: Women at risk of HIV infection were recruited into The Ring Study that evaluated the safety and efficacy of the DVR between 2013 and 2016. At baseline, a behavioral questionnaire was administered to obtain information on sexual activity and IVP (exposure) defined as; insertion inside the vagina of any items aimed at cleaning the vagina for any reason before, during or after sex other than practices to manage menses. Each participant self-inserted the DVR/placebo and replaced it every 4weeks for 2 years. Outcomes were diagnosis of STIs i.e., *Chlamydia trachomatis* (CT), *Neisseria gonorrhea* (NG), *Trichomonas vaginalis* (TV), HIV and BV. The incidence rate of STI/BV was estimated, overall, by IVP and trial arm in single-event-per-subject and multiple-event-per-subject analyses.

Results: Of the 197 women enrolled, 66 (33.5%) were <25 years of age. Overall, 93 (47.2%) practiced at least one form of IVP. During follow up, 172 (87.3%) women were diagnosed with an STI/BV at least once. Majority had TV (73.6%, n=145*)*. Overall rate of STI/BV was 51.9/100 person-years, 95% confidence interval (CI): 44.7-60.3 [IVP: Yes, 51.0 (40.8-63.8) vs. No, 52.6 (43.0-64.4)]. IVP were not statistically significantly associated with rate of individual STIs/BV. Similar results were observed when the analyses were conducted separately for each trial arm.

Conclusions: IVP had no effect on the risk of STIs/BV in The Ring Study.

Registration no: NCT01539226

Key words: Intravaginal practices, sexually transmitted infection, Bacterial vaginosis,

Dapivirine, Vaginal Ring, Uganda

Study strengths and limitations

The strengths of this study is that we were able to investigate multiple STIs and BV in the same population. The study also had the ability to collect recurrent data on genital conditions which

allowed for conduct of both single-event-per-subject and multiple-event-per-subject analyses. The limitations of the study included a small sample size and confirmatory tests that were not done to confirm absence of an STI/BV prior to DVR insertion.

INTRODUCTION

Sexually transmitted infections (STIs) remain a health challenge globally. In 2018, WHO estimated that nearly one million people become infected every day with a curable STI caused by: *Chlamydia trachomatis* (CT), *Neisseria gonorrhea* (NG), or *Trichomonas vaginalis* (TV)[1]. STIs disproportionately affect low-income and middle-income countries, with 90% of the new infections occurring in these countries[2]. Women in sub-Saharan Africa are majorly affected by STIs, with those at high risk of HIV infection also having high STI burden [3 4]. Bacterial vaginosis (BV), a common vaginal condition has also been associated with increased risk of STIs and HIV infection[5]. High rates (37% to 68%) of BV have been reported among women in Southern and East Africa[6].

The high prevalence and increased risk of acquisition of STIs/BV has been associated with intravaginal practices[7]. The latter include various behaviors that women use to manage their sexual life and health[8]. These practices are often used for genital hygiene and to make women sexually desirable [9 10]. Products used have been associated with increased vaginal pH resulting in overgrowth of organisms related to BV, which has also been associated with increased risk of HIV acquisition[11 12]. Practices which increase a woman's susceptibility to HIV could reduce the effectiveness of vaginal microbicides[13 14].

The monthly dapivirine vaginal ring (DVR), a female controlled HIV prevention tool, was found to reduce the risk of HIV acquisition by approximately 30% in two phase 3 trials[15 16]. With continued and consistent use, risk reduction was even greater (62%) in an open-label extension trial[17]. However, data are limited on the effect of intravaginal practices on vaginal flora and risk of STIs/BV among women using the DVR. We assessed the effect of vaginal practices on the incidence of STIs/BV among women using the DVR or placebo vaginal ring in southwestern Uganda.

METHODS

Study design

This was a secondary analysis using data collected in the The Ring Study, a phase 3 microbicide trial[15].

Study setting and population

Details of the Ring Study have been described elsewhere [15]. Briefly, The Ring Study was a multicenter microbicide trial, that evaluated the safety and efficacy of the DVR between 2013 and 2016 in Uganda and South Africa. The study was sponsored by the International Partnership for Microbicides. In Uganda, the study was conducted by the Medical Research Council (MRC)/Uganda Virus Research Institute (UVRI) and London School of Hygiene and Tropical Medicine (LSHTM) Uganda Research Unit in Masaka, southwestern Uganda. The site recruited women at risk of HIV infection from towns along the trans-African highway and the shores of Lake Victoria. Details of the recruitment procedures and study population have been described elsewhere [18].

Study procedures

Participants consented for screening and enrolment on two separate occasions. At the first visit (screening), data on demographics, inclusion and exclusion criteria were obtained. A physical and genital examination was done. All potential participants were provided with HIV/STI risk-reduction counselling and HIV pre- and post-test counselling. At the second visit (enrolment), women who met all inclusion criteria and no exclusion criteria, had a normal pelvic examination, and negative HIV rapid tests were enrolled into the trial. Eligible women were randomised in a 2:1 ratio to either the DVR arm or placebo arm. At the enrolment visit, an interviewer-administered behavioral questionnaire was used to obtain baseline information on sexual activity and vaginal practices. At 4 weeks post-enrolment and every 24 weeks thereafter for the next 2 years, follow-up data on vaginal practices were collected using an interviewer-administered behavioral questionnaire. Participants self-inserted the vaginal rings every 4 weeks for up to 104 weeks.

Measurement of exposure [intravaginal practices (IVP)]: IVP was defined as insertion inside the vagina of any items aimed at cleaning the vagina for any reason before, during or after sex other than practices to manage menses. Items included: materials such as paper, cloth, or cotton

wool; water only; water and soap; fingers to clean or insert something. Women were asked if they were inserting or using any of the aforementioned items inside the vagina to clean their vagina either as a general cleaning/hygiene practice before or after sex or to prepare the vagina for sex in the past, at baseline and every 24 weeks.

Measurement of outcome (diagnosis of STIs/BV): Cervico-vaginal samples were collected at the first screening visit and every 12 weeks (3 months) for 2 years. Samples were tested for TV (OSOM® Trichomonas Rapid test- Sekisui Diagnostics, LLC, USA) and CT/NG (Cobas® Amplicor CT/NG -PCR test, Roche Diagnostic Systems, Branchburg, New Jersey). Vaginal samples were also collected for assessment of vaginal flora (using Nugent's score) and vaginal fluid pH at the enrolment visit (prior to ring insertion) and every 12 weeks. Samples with a score of ≥7 were classified as BV present.

Participants were tested for HIV at the screening and enrolment visit. Serial rapid HIV antibody tests were done using Alere Determine™ HIV-1/2 (Alere, Medical co., Ltd, Matsuhidai, Matsudo-shi, Chiba, Japan) followed by OraQuick- ADVANCE® Rapid HIV-1/2 Test (OraQuick-OraSure Technologies Inc, Pennsylvania) to confirm a positive Determine result and Uni- Gold™ HIV (Trinity Biotech, Ireland) as the tie breaker. At the screening/enrolment visits, a participant was confirmed to have HIV infection if they tested positive on at least two rapid HIV antibody tests. Post-enrolment, HIV testing was done serially as described above. However, for participants who tested positive or discordant on two rapid HIV antibody tests, a confirmatory test using Western Blot (J. Mitra and Co.Pvt. ltd, India) was done as previously described[15]. Blood samples were collected and stored every 4 weeks. Stored samples for participants with confirmed HIV infection were retrospectively tested for HIV using the HIV RNA PCR test.

Statistical analysis

Data analyses were performed in STATA version 15.0 (Stata Corp, College Station, TX, USA). Participants' characteristics were summarized using frequencies and percentages overall and by IVP status. We determined the proportion of participants that were positive for a given STI/BV as the number who tested positive for an STI/BV at least once during the study divided by the total number tested. We determined the effect of IVP on the incidence of each STI/BV, by estimating

the rate of STI/BV overall and stratified by IVP status. We used two approaches for measuring the rate of STI/BV; (a) a single-event-per-subject and (b) a multiple-event-per-subject. The incidence rate of a given STI/BV was estimated as the number of participants who tested positive for STI/BV divided by the person-time (years) at risk (pyr) expressed as per 100 pyr. PYR were estimated as a sum of the time from enrolment into the trial (those negative at baseline) to the date of trial completion or censoring (stopped trial visits or ring use). Participants that tested positive for STI/BV at baseline were given treatment and started to contribute person-time after completing the course of treatment. We further adjusted the effect of IVP on rate of STI/BV for age and baseline STI/BV status by fitting Poison regression models. In the analysis, we used Poisson regression model with time-varying covariates, allowing for intergroup correlation (because women had multiple records) by using robust standard errors.

Compliance with Ethical Standards

Approval was obtained from the Uganda Virus Research Institute Research Ethics Committee (Ref#-GC/127/13/03/33), the Uganda National Council of Science and Technology (Ref#-HS1362) and the National Drug Authority (Ref #-166/ESR/NDA/DID-07/2013). Written informed consent was obtained from each woman before any study procedures were performed. Women who tested HIV-positive were referred to an HIV care provider of their choice. Treatment was provided to those who tested positive for STIs/BV according to the Centers for Disease Control and Prevention (CDC) STD Treatment guidelines 2010[19].

Patient and Public Involvement Statement

Communities where the study was conducted were involved from the inception of the study. Community gate keepers: Local Council leaders, political leaders, village health teams, community based and faith based organizations were informed of the study via various engagement meetings prior to study start. The site Community Advisory Board members were engaged to review study documents, consent and other study literacy documents and confirm translations to the local language. Volunteer recruitment and retention was supported by local leaders. Results were disseminated to study participants, CAB and community members through

community meetings upon study completion and presentations at national and international meetings, seminars and conferences.

RESULTS

Socio-demographic characteristics

Of the 197 women enrolled (67% on the DVR arm) in the trial, 66 (33.5%) were less than 25 years of age, 82 (41.6%) were married and very few [24 (12.2%)] had secondary school education. Majority (82.2%, n=162) of the women reported having a main partner but only 75 (46.3%) lived with this partner (Table 1). About a half (50.1%, n=100) of the women tested positive for an STI/ BV at baseline.

Table 1: Baseline characteristics of 197 women enrolled in The Ring Study in southwestern Uganda

Variable	All	Intravaginal practices		p-value
		No	Yes	
	n (%)	n (%)	n (%)	
Overall	197	104 (52.8)	93 (47.2)	
Trial arm				0.835
Dapivirine vaginal ring	132 (67.0)	69 (52.3)	63 (47.7)	
Placebo	65 (33.0)	35 (53.8)	30 (46.2)	
Age (years)				0.275
18-24	66 (33.5)	30 (45.4)	36 (54.6)	
25-34	98 (49.8)	57 (58.2)	41 (41.8)	
35+	33 (16.7)	17 (51.5)	16 (48.5)	
Education level				0.276
Incomplete primary school	68 (34.5)	37 (54.4)	31 (45.6)	
Complete primary school	105 (53.3)	58 (55.2)	47 (44.8)	
Secondary school*	24 (12.2)	9 (37.5)	15 (62.5)	
Marital status				**0.020**
Single and never married	90 (45.7)	38 (42.2)	52 (57.8)	
Single but previously married	25 (12.7)	14 (56.0)	11 (44.0)	
Married	82 (41.6)	52 (63.4)	30 (36.6)	
Has main partner				0.194
No	35 (17.8)	15 (42.9)	20 (57.1)	
Yes	162 (82.2)	89 (54.9)	73 (45.1)	
Duration lived with main partner (years)				0.690
<1	35 (21.6)	17 (48.6)	18 (51.4)	
1-2	34 (21.0)	19 (55.9)	15 (44.1)	
3+	93 (57.4)	53 (57.0)	40 (43.0)	

Lived with (main) partner in the past year				0.171
All the time	48 (37.8)	38 (61.3)	24 (38.7)	
Some of the time	14 (11.0)	7 (36.8)	12 (63.2)	
No	65 (51.2)	44 (54.3)	37 (45.7)	
Currently lives with main partner				0.376
No	87 (53.7)	45 (51.7)	42 (48.3)	
Yes	75 (46.3)	44 (58.7)	31 (41.3)	
Baseline STI/BV status				0.528
Negative	97 (49.2)	49 (50.5)	48 (49.5)	
Positive	100 (50.8)	55 (55.0)	45 (45.0)	

n=Number; *Includes one woman who had greater than secondary education

IVP

Ninety-three (47.2%) women reported at least one form of IVP. The commonly used substances to clean the vagina included: soap (n=73, 78.5%), cloth (n=5, 5.4%), detergent (n=3, 3.2%) and others (honey, herbs, perfume n=6, 6.5%). Reported IVP were more common among single and never married women compared to single but previously married or currently married women (p=0.020) (Table 1).

Proportion and rate of STIs/BV

A total of 172 (87.3%) women were diagnosed with an STI at least once during follow up, with an overall incidence rate of 51.9 per 100 pyr. The most common STI was TV (73.6%, n=145/197 diagnosed at least once) with a rate of 92.7 per 100 pyr and 88.9 per 100 pyr in the single-event-per-subject and multiple-events-per-subject analysis respectively (Table 2).

Effect of IVP on rate of STIs/BV

In the single-event-per-subject analysis, STI and BV rates were not associated with reported IVP (Table 2). However, in the multiple-events-per subject analysis, the rate of CT was statistically significantly lower among women who reported IVP versus those who did not (p=0.030). After adjusting for participant characteristics, overall, in both the single-event and multiple-event per subject analyses, IVP was not associated with any significant change in STI/BV rates (Table 3). There was also no effect of IVP on STI/BV rates in the different trial arms.

Table 2: Effect of intravaginal practices on rate of sexually transmitted infection/Bacterial vaginosis among women in the Ring Study in southwestern Uganda

Sexually transmitted infection/condition	Single-event-per-subject analysis					
	Overall	Intravaginal practices				
		Yes	No			
	Rate/100 PYR (95% CI)	Rate/100 PYR (95% CI)	Rate/100 PYR (95% CI)	p-value	uRR (95% CI)	aRR (95% CI)
HIV	5.8 (3.0-11.1)	7.0 (2.9-17.0)	4.7 (1.8-12.4)	0.278		
Trichomonas vaginalis	92.7 (78.7-109.0)	97.4 (76.9-123.3)	88.7 (70.9-111.1)	0.288	1.10 (0.79-1.52)	1.10 (0.67-1.63)
Neisseria gonorrhea	23.4 (18.3-29.9)	22.8 (15.9-32.9)	23.8 (17.0-33.4)	0.435	0.96 (0.58-1.57)	0.99 (0.56-1.79)
Chlamydia trachomatis	28.4 (22.7-35.6)	27.9 (20.2-38.8)	28.9 (21.2-39.4)	0.446	0.97 (0.62-1.52)	083 (0.50-1.38)
Bacterial vaginosis	14.1 (10.6-18.6)	13.4 (8.9-20.4)	14.7 (10.1-21.4)	0.383	0. 92 (0.52-1.61)	1.13 (0.53-2.37)
Multiple-events-per-subject analysis						
Trichomonas vaginalis	88.9 (79.6-99.5)	90.2 (76.8-106.0)	87.9 (75.3-102.5)	0.406	1.03 (0.77-1.37)	1.07 (0.80-1.43)
Neisseria gonorrhea	28.4 (23.3-34.6)	28.7 (21.5-38.1)	28.2 (21.5-37.0)	0.935	1.02 (0.61-1.69)	0.99 (0.59-1.67)
Chlamydia trachomatis	43.6 (37.2-51.1)	36.6 (28.4-47.1)	49.9 (40.7-61.2)	0.030	0.73 (0.45-1.20)	0.64 (0.38-1.05)
Bacterial vaginosis	18.8 (11.1-31.7)	12.1 (4.5-32.2)	24.1 (13.0-44.8)	0.124	0.51 (0.13-1.95)	0.48 (0.16-1.47)

PYR = person-years at risk; CI = Confidence interval; uRR = unadjusted rate ratio; aRR = adjusted rate ratio; ¶Adjusted for age, trial arm, STI/BV at baseline

Table 3: Effect of intravaginal practices on rate of sexually transmitted infection/Bacterial vaginosis stratified by trial arm among women in the Ring Study in southwestern Uganda

Single-event-per-subject analysis						
Sexually transmitted infection/condition	**Overall**	**Trial arm**				
		DVR	**Placebo**			
	IVP use	**Rate/100 PYR (95% CI)**	**Rate/100 PYR (95% CI)**	**p-value**	**aRR (DVR) (95% CI)**	**aRR (Placebo) (95% CI)**
HIV	Yes	7.0 (2.9-17.0)	4.7 (1.8-12.4)	0.278		
Trichomonas vaginalis	Yes	105.8 (79.7-140.4)	82.4 (53.7-126.4)	0.171	1.34 (0.78-2.31)	0.59 (0.28-1.26)
Neisseria gonorrhea	Yes	20.6 (13.0-32.6)	28.0 (15.5-50.5)	0.211	0.73 (0.35-1.53)	1.88 (0.49-7.20)
Chlamydia trachomatis	Yes	29.7 (20.2-43.6)	24.3 (13.1-45.2)	0.305	1.09 (0.60-2.00)	0.37 (0.11-1.36)
Bacterial vaginosis	Yes	12.6 (7.5-21.3)	15.2 (7.6-30.3)	0.332	0.74 (0.26-2.09)	1.51 (0.35-6.52)
Multiple-events-per-subject analysis						
Trichomonas vaginalis	Yes	94.4 (77.9-114.2)	81.5 (60.5-109.9)	0.213	1.12 (0.80-1.56)	0.76 (0.44-1.30)
Neisseria gonorrhea	Yes	25.2 (17.4-36.4)	36.0 (23.0-56.5)	0.116	0.74 (0.42-1.30)	2.24 (0.61-8.31)
Chlamydia trachomatis	Yes	40.4 (30.2-54.2)	28.4 (17.1-47.2)	0.126	0.75 (0.43-1.29)	0.33 (0.14-0.78)
Bacterial vaginosis	Yes	12.5 (4.0-38.7)	11.1 (1.6-78.7)	0.491	0.64 (0.11-3.71)	0.39 (0.10-2.72)

PYR = person-years at risk; CI = Confidence interval; uRR = unadjusted rate ratio; aRR = adjusted rate ratio; ¶Adjusted for age, and STI/BV at baseline

DISCUSSION

Our study aimed to assess the effect of IVP on the incidence of STIs and BV among women using the DVR or placebo. Overall, we found that nearly one in every two women practiced at least one form of IVP. IVP have been reported to be high in African women with proportions of between 30-50%, and even higher among women at risk of acquiring HIV[20 21].

Overall, we found no association between IVP and incidence of STI (including HIV) or BV. IVP have been associated with change in the vaginal flora, resulting in an increase majorly of BV, that increases one's susceptibility to acquiring other STIs like CT and NG[5 6]. Most of the reported associations have been between IVP and BV and HIV acquisition [12]. However, studies done in Africa have had conflicting results. Whereas one systematic review found that IVP increased the risk of vaginal infections (BV, TV, and vulvovaginal candidiasis) [22], two others found no association between IVP and TV [9 23] or BV[23]. One study in South Africa found that IVP was associated with increased risk of HIV infection but not other STIs[24].

We also found no association between IVP and incidence of STI (including HIV) or BV when the analyses were conducted separately for each trial arm. The DVR has been associated with minimal changes in the vaginal microbiota that were likely not clinically significant [25]. It has generally been found to be well tolerated in adult as well as adolescent girls and young women[26 27]. Various studies on microbicides have reported that their use may result in little or no difference in the risk of acquiring STIs like CT, NG or TV[28].

The strengths of this study included the opportunity to investigate multiple STIs and BV in the same population and the ability to collect recurrent data on genital conditions which allowed for conduct of both single-event-per-subject and multiple-event-per-subject analyses. The multiple-event-per-subject analysis models the total rate of events over the entire follow-up period and has more power for detecting associations compared to the single-event-per-subject analysis [29].

A limitation of the study was the small sample size which may have impacted the study power and consequently the ability to detect any differences in STI/BV rates between women who reported IVP and those who did not. Another limitation was that no confirmatory tests were done to confirm absence of an STI/BV prior to DVR insertion. However, duration of treatment and absence of symptoms and signs were used as a proxy for lack of an STI/BV.

In conclusion, we found a high prevalence of IVP and incidence of STIs/BV among women enrolled in the Ring Study in Uganda. IVP did not statistically significantly impact STI/BV rates. An analysis with a bigger sample size could be helpful to better understand whether there is a link between use of the DVR, IVP, and incidence of STIs/BV.

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Author Contributions:

SK, AA, ER designed the study and wrote the initial manuscript draft, and AA did the data analysis. KA and MO conducted the study while SK directed the work. KA and MO contributed to the writing and editing of the manuscript. All authors contributed to the interpretation of the results and critically commented and provided revisions to the manuscript. All authors approved the final version of the manuscript. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

Data availability statement: Data are available on reasonable request.

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STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	5
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5, 6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5, 6
Bias	9	Describe any efforts to address potential sources of bias	6, 7
Study size	10	Explain how the study size was arrived at	5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6, 7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6, 7
		(*b*) Describe any methods used to examine subgroups and interactions	NA
		(*c*) Explain how missing data were addressed	NA
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	NA
		(*e*) Describe any sensitivity analyses	6, 7

Continued on next page

Results			**Page**
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8, 9
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8, 9
		(b) Indicate number of participants with missing data for each variable of interest	NA
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	8, 9, 10, 11
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			
Key results	18	Summarise key results with reference to study objectives	12
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	13
Generalisability	21	Discuss the generalisability (external validity) of the study results	13
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	13

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Effect of intravaginal practices on incidence of sexually transmitted infections and bacterial vaginosis among women enrolled in the Dapivirine vaginal ring trial (The Ring Study) in southwestern Uganda, a retrospective secondary analysis.

Authors: Sylvia Kusemererwa[1*], Eugene Ruzagira[1,3], Martin Onyango[1], Anita Kabarambi[4], Andrew Abaasa[2,3]

Institution

[1]Department of viral pathogen epidemiology and interventions, Medical Research Council/ Uganda Virus Research Institute and London School of Hygiene and Tropical Medicine, Uganda Research Unit, Entebbe, Uganda

[2]Department of statistics, Medical Research Council/ Uganda Virus Research Institute and London School of Hygiene and Tropical Medicine, Uganda Research Unit, Entebbe, Uganda

[3] Department of infectious diseases epidemiology, London School of Hygiene & Tropical Medicine, London, UK

[4] Department of research, International Center for Child Health and Development, Masaka, Uganda

Correspondence to: Sylvia Kusemererwa, [1]Medical Research Council/Uganda Virus Research Institute (MRC/UVRI) and London School of Hygiene and Tropical Medicine (LSHTM), Uganda Research Unit, Plot 51-59, Nakiwogo Road, P.O Box 49 Entebbe, Uganda. Phone: 256 (0) 417 704000, or 256 (0) 70229100. Sylvia.Kusemererwa@mrcuganda.org or kansykarus@gmail.com

Effect of intravaginal practices on incidence of sexually transmitted infections and bacterial vaginosis among women enrolled in the Dapivirine vaginal ring trial (The Ring Study) in southwestern Uganda, a retrospective secondary analysis.

Abstract

Objectives: We assessed the effect of intravaginal practices (IVP) on the incidence of sexually transmitted infections (STIs) and bacterial vaginosis (BV) among women using the dapivirine vaginal ring (DVR) or placebo vaginal ring in southwestern Uganda.

Methods: This was a retrospective secondary analysis of data collected from women at risk of HIV infection in The Ring Study. The Ring Study evaluated the safety and efficacy of the DVR between 2013 and 2016. At baseline, a behavioral questionnaire was administered to obtain information on sexual activity and IVP (exposure) defined as; insertion inside the vagina of any items aimed at cleaning the vagina for any reason before, during or after sex other than practices to manage menses. Each participant self-inserted the DVR/placebo and replaced it every 4weeks for 2 years. Outcomes were diagnosis of STIs i.e., *Chlamydia trachomatis* (CT), *Neisseria gonorrhea* (NG), *Trichomonas vaginalis* (TV), HIV and BV. The incidence rate of STI/BV was estimated, overall, by IVP and trial arm in single-event-per-participant and multiple-event-per-participant analyses.

Results: Of the 197 women enrolled, 66 (33.5%) were <25 years of age. Overall, 93 (47.2%) practiced at least one form of IVP. During follow up, 172 (87.3%) women were diagnosed with an STI/BV at least once. Majority had TV (73.6%, n=145*)*. Overall rate of STI/BV was 51.9/100 person-years, 95% confidence interval (CI): 44.7-60.3 [IVP: Yes, 51.0 (40.8-63.8) vs. No, 52.6 (43.0-64.4)]. IVP were not statistically significantly associated with rate of individual STIs/BV. Similar results were observed when the analyses were conducted separately for each trial arm.

Conclusions: IVP had no effect on the risk of STIs/BV in The Ring Study.

Key words: Intravaginal practices, sexually transmitted infection, Bacterial vaginosis, Dapivirine, Vaginal Ring, Uganda

Study strengths and limitations

- The study was able to investigate multiple STIs and BV in the same population.
- The longitudinal nature of the study allowed for both single-event-per-participant and multiple-event-per-participant analyses.
- Extensive analyses were to some extent limited by a small sample size.
- Tests to confirm absence of STI/BV prior to DVR insertion were not done.

INTRODUCTION

Sexually transmitted infections (STIs) remain a health challenge globally. In 2018, WHO estimated that nearly one million people become infected every day with a curable STI caused by: *Chlamydia trachomatis* (CT), *Neisseria gonorrhea* (NG), Syphilis or *Trichomonas vaginalis* (TV) [1]. STIs disproportionately affect low-and-middle-income countries, with 90% of the new infections occurring in these countries [2]. Women in sub-Saharan Africa have a high prevalence of STIs, particularly those at high risk of HIV infection also having a high STI burden [3, 4]. Bacterial vaginosis (BV), a common vaginal condition has also been associated with increased risk of STIs and HIV infection [5]. High rates (37% to 68%) of BV have been reported among women in Southern and East Africa [6].

BV results from variation in normal vaginal flora attributed to reduction in the prevalence of Lactobacilli (dominant species in healthy vaginal environment) and an increase in the concentration of pathogenic organisms: *G. vaginalis*, *Bacteroides (Prevotella)* species, *Mobiluncus* species, and *Mycoplasma hominis* [7, 8]. Increasing evidence shows that vaginal microbiota may play a role in mediating susceptibility to STIs. Vaginal Lactobacilli utilize several actions to protect against colonization by genital pathogens [9]. These include production of lactic acid that supports the maintenance of a lower vaginal pH, which may prevent pathogen growth [10], and exposure to hydrogen peroxide that has been shown to decrease activity of BV and other genital tract organisms [11, 12].

The high prevalence and increased risk of acquisition of STIs/BV and HIV has been associated with vaginal practices [13]. These practices include various behaviors used to maintain health, wellness, and enhance sexual pleasure [14-16]. The World Health Organization (WHO) has suggested seven classifications for vaginal practices: external washing, intravaginal cleansing, external application, intravaginal insertion, oral ingestion, vaginal streaming or smoking and anatomical modification [17]. On the other hand, intravaginal practices (IVPs) refer to both intravaginal cleansing (cleaning or washing inside the vagina with fingers or substances like soap to remove fluids), and intravaginal insertion (placing something inside the vagina, like powders, creams, herbs, or tissue) [18, 19].

Soaps, detergents and antiseptics used to cleanse inside the vagina can cause chemical damage and increase vaginal pH resulting in overgrowth of BV related organisms, which has also been associated with increased risk of HIV acquisition [19, 20]. Other products have also been reported to cause genital lesions, and swellings, creating favorable conditions for the transmission of STIs, including HIV [17]. Items like cloth commonly used in some communities to clean the vagina repeatedly might also act as fomites, carrying TV organisms [21]. TV has also been associated with increased risk of HIV acquisition [22, 23]. These practices which increase a woman's susceptibility to HIV could reduce the effectiveness of vaginal microbicides [24, 25].

The monthly dapivirine vaginal ring (DVR) microbicide, a female controlled HIV prevention tool, was found to reduce the risk of HIV acquisition by approximately 30% in two phase 3 trials [26, 27]. With continued and consistent use, the risk of HIV acquisition was even lower (62%) in an open-label extension trial [28]. However, data are limited on the effect of IVP on vaginal flora and risk of STIs/BV among women using the DVR. We assessed the effect of IVP on the incidence of STIs including HIV, and BV among women using the DVR or placebo vaginal ring in southwestern Uganda.

METHODS

Study design

This was a retrospective secondary analysis using data collected in The Ring Study, a phase 3 microbicide trial [26].

Study setting and population

Details of the Ring Study (Registration number: NCT01539226) have been described elsewhere [26]. Briefly, The Ring Study was a multicenter microbicide trial, that evaluated the safety and efficacy of the DVR between 2013 and 2016 in Uganda and South Africa. The study was sponsored by the International Partnership for Microbicides. In Uganda, the study was conducted by the Medical Research Council (MRC)/Uganda Virus Research Institute (UVRI) and London School of Hygiene and Tropical Medicine (LSHTM) Uganda Research Unit in Masaka, southwestern Uganda. The site recruited women at high risk of HIV infection from towns along the trans-African highway and the shores of Lake Victoria. Details of the recruitment procedures

and study population have been described elsewhere [29]. Briefly, the research site enrolled 197 women (18-45years of age) at high risk of HIV infection. Women were identified from sex work hotspots (bars, restaurants, hair salons, small shops, and other small-scale businesses). High risk was defined by presence of any two of the following: i) history of STIs in the past three months; ii) self-reported condom less sex with multiple sex partners or a new partner in the past three months; and iii) use of recreational drugs (marijuana, alcohol) in the past three months. Of the women enrolled, two in five were working in bars and restaurants and nearly a third had small scale businesses. A woman was included in the main study if they were not pregnant, not breastfeeding, asymptomatic for genital infections and tested HIV negative at the time of enrolment. Those diagnosed with any clinically significant curable STI, were initiated on treatment and only enrolled after completing a full course of treatment.

Study procedures

Participants consented for screening and enrolment on two separate occasions. At the first visit (screening), data on demographics, inclusion and exclusion criteria were obtained. A physical and genital examination was done. All potential participants were provided with HIV/STI risk-reduction counselling and HIV pre- and post-test counselling. At the second visit (enrolment), women who met all inclusion criteria and no exclusion criteria, had a normal pelvic examination, and negative HIV rapid tests were enrolled into the trial. Eligible women were randomised in a 2:1 ratio to either the DVR arm or placebo arm. At the enrolment visit, an interviewer-administered behavioral questionnaire was used to obtain baseline information on sexual activity and vaginal practices. At 4 weeks post-enrolment and every 24 weeks thereafter for the next 2 years, follow-up data on vaginal practices were collected using an interviewer-administered behavioral questionnaire. Participants self-inserted a vaginal ring every 4 weeks for up to 104 weeks.

Measurement of exposure [intravaginal practices (IVP)]: IVP was defined as insertion inside the vagina of any items aimed at cleaning the vagina for any reason before, during or after sex other than practices to manage menses. Items included: materials such as paper, cloth, or cotton wool; water only; water and soap; fingers to clean or insert something. Women were asked if they were inserting or using any of the aforementioned items inside the vagina to clean their vagina

either as a general cleaning/hygiene practice before or after sex or to prepare the vagina for sex in the past, at baseline and every 24 weeks.

Measurement of outcome (diagnosis of STIs/BV): Cervico-vaginal swabs were collected at the first screening visit and every 12 weeks (3 months) for 2 years. The swabs were tested for TV (OSOM® Trichomonas Rapid test- Sekisui Diagnostics, LLC, USA) and CT/NG (Cobas® Amplicor CT/NG -PCR test, Roche Diagnostic Systems, Branchburg, New Jersey). Vaginal fluid samples were also collected by trained clinicians using sterile swabs for assessment of vaginal flora (using Nugent's score) and vaginal fluid pH at the enrolment visit (prior to ring insertion) and every 12 weeks. Samples with a score of ≥7 were classified as BV present. Each slide was scored by trained laboratory technologists. For internal quality control, a single batch of slides were re-examined by an independent reader weekly. Discrepant results were resolved by expert consensus. External quality control was assured using College of American Pathologists Vaginitis screen, vaginal gram stain-VS2 as part of the site standard operating procedures.

Participants were tested for HIV at the screening and enrolment visit using whole blood samples collected by venipuncture. Serial rapid HIV antibody tests were done using Alere Determine™ HIV-1/2 (Alere, Medical co., Ltd, Matsuhidai, Matsudo-shi, Chiba, Japan) followed by OraQuick- ADVANCE® Rapid HIV-1/2 Test (OraQuick-OraSure Technologies Inc, Pennsylvania) to confirm a positive Determine result and Uni- Gold™ HIV (Trinity Biotech, Ireland) as the tie breaker. At the screening/enrolment visits, a participant was confirmed to have HIV infection if they tested positive on at least two rapid HIV antibody tests. Post-enrolment, HIV testing was done serially as described above. However, for participants who tested positive or discordant on two rapid HIV antibody tests, a confirmatory test on stored plasma was done using Western Blot (J. Mitra and Co.Pvt. ltd, India) as previously described. Blood samples were collected, and plasma stored every 4 weeks. Stored plasma samples for participants with confirmed HIV infection were retrospectively tested at a central laboratory in South Africa (Bioanalytical Research Corporation) for HIV ribonucleic acid (RNA) copies (viral load) using the polymerase-chain-reaction (PCR) assay [26].

Statistical analysis

Data analyses were performed in Stata version 15.0 (Stata Corp, College Station, TX, USA). Participants' baseline characteristics were summarized using frequencies and percentages overall and by IVP status. We determined the proportion of participants that were positive for a given STI/BV (event) as the number who tested positive for an STI/BV at least once during the study divided by the total number tested. We determined the effect of IVP on the incidence of each STI/BV, by estimating the rate of STI/BV overall and stratified by IVP status. We used two approaches for measuring the rate of STI/BV; (a) a single-event-per-participant (allowing for one event per participant-first STI/BV event) and (b) a multiple-event-per-participant (allowing for two or more STI/BV events for the same participant) since these are recurrent events. The incidence rate of a given STI/BV was estimated as the number of participants who tested positive for STI/BV divided by the person-time (years) at risk (pyr) expressed as per 100 pyr. PYR were estimated as a sum of the time from enrolment into the trial (those negative at baseline) to the date of trial completion or censoring (stopped trial visits or ring use). Participants that tested positive for STI/BV at baseline were given treatment and started to contribute person-time after completing the course of treatment. We further adjusted the effect of IVP on rate of STI/BV for age and baseline STI/BV status by fitting Poisson regression models. In the analysis, we used Poisson regression model with time-varying covariates, allowing for intergroup correlation (because women had multiple records) by using robust standard errors. For HIV, we estimated the rate of HIV infection as number of HIV positive cases divided by the total person years at risk expressed as per 100 person years at risk in a single-event-per-participant survival analysis. Person time at risk were calculated as sum of the time from enrolment to the last HIV seronegative date or to the estimated date of HIV infection. The HIV infection date was estimated as a random date between the last HIV seronegative and the first HIV+ result date in a multiple imputation.

Compliance with Ethical Standards

Approval was obtained from the Uganda Virus Research Institute Research Ethics Committee (Ref#-GC/127/13/03/33), the Uganda National Council of Science and Technology (Ref#-HS1362) and the National Drug Authority (Ref #-166/ESR/NDA/DID-07/2013). Written informed consent was obtained from each woman before any study procedures were performed. Women who tested HIV-positive were referred to an HIV care provider of their choice. Treatment was

provided to those who tested positive for STIs/BV according to the Centers for Disease Control and Prevention (CDC) STD Treatment guidelines 2010 [30].

Patient and Public Involvement Statement

Communities where the study was conducted were involved from the inception of the study. Community gate keepers: Local Council leaders, political leaders, village health teams, community based and faith-based organizations were informed of the study via various engagement meetings prior to study start. The site Community Advisory Board (CAB) members were engaged to review study documents, consent and other study literacy documents and confirm translations to the local language. CAB members were compensated for all the activities that they engaged in. Volunteer recruitment and retention was supported by local leaders. Results were disseminated to study participants, the CAB, and community members through community meetings upon study completion and presentations at national and international meetings, seminars and conferences. All stakeholders were compensated for the time spent during engagement meetings.

RESULTS

Baseline socio-demographic characteristics

In total, 197 women enrolled, 67% on the DVR trial arm. Of those, 66 (33.5%) were less than 25 years of age, 82 (41.6%) were married and very few 24 (12.2%) had secondary school education. Majority, 162 (82.2%) reported having a main partner but only 75 (46.3%) lived with this partner (Table 1). About a half, 100 (50.8%) tested positive for an STI/ BV at baseline. Compared with those who had one episode of STI/BV, participants with two or more episodes were likely to be single and never married (47.8% vs. 35.6%), not currently living with a main partner (56.5% vs. 37.5%, p=0.033) and inconsistently living with the main partner in the past year (69.6% vs. 41.7%; p=0.001) but otherwise similar in regard to other participant characteristics.

Table 1: Baseline socio-demographic characteristics of 197 women enrolled in The Ring Study in southwestern Uganda between 2013 and 2016

Variable	All	Intravaginal practices		p-value
		No	Yes	
	n (%)	n (%)	n (%)	
Overall	197	104 (52.8)	93 (47.2)	
Trial arm				0.835
Dapivirine vaginal ring	132 (67.0)	69 (52.3)	63 (47.7)	
Placebo	65 (33.0)	35 (53.8)	30 (46.2)	
Age (years)				0.275
18-24	66 (33.5)	30 (45.4)	36 (54.6)	
25-34	98 (49.8)	57 (58.2)	41 (41.8)	
35+	33 (16.7)	17 (51.5)	16 (48.5)	
Education level				0.276
Incomplete primary school	68 (34.5)	37 (54.4)	31 (45.6)	
Complete primary school	105 (53.3)	58 (55.2)	47 (44.8)	
Secondary school*	24 (12.2)	9 (37.5)	15 (62.5)	
Marital status				0.020
Single and never married	90 (45.7)	38 (42.2)	52 (57.8)	
Single but previously married	25 (12.7)	14 (56.0)	11 (44.0)	
Married	82 (41.6)	52 (63.4)	30 (36.6)	
Number of lifetime sex partners				0.002
Median (IQR)	6 (4-12)	5 (3-9)	8 (5-20)	
Has main partner				0.194
No	35 (17.8)	15 (42.9)	20 (57.1)	
Yes	162 (82.2)	89 (54.9)	73 (45.1)	
Duration lived with main partner (years)				0.690
<1	35 (21.6)	17 (48.6)	18 (51.4)	
1-2	34 (21.0)	19 (55.9)	15 (44.1)	
3+	93 (57.4)	53 (57.0)	40 (43.0)	
Lived with (main) partner in the past year				0.171
All the time	48 (37.8)	38 (61.3)	24 (38.7)	
Some of the time	14 (11.0)	7 (36.8)	12 (63.2)	
No	65 (51.2)	44 (54.3)	37 (45.7)	
Currently lives with main partner				0.376
No	87 (53.7)	45 (51.7)	42 (48.3)	
Yes	75 (46.3)	44 (58.7)	31 (41.3)	
Baseline STI/BV status				
TV				
Negative	121 (61.4)	61 (50.4)	60 (49.6)	0.399
Positive	76 (38.6)	43 (56.6)	33 (43.4)	
NG				
Negative	173 (87.8)	92 (53.2)	81 (46.8)	0.770
Positive	24 (12.2)	12 (50.0)	12 (50.0)	
CT				
Negative	177 (89.8)	97 (54.8)	80 (45.2)	0.093
Positive	20 (10.2)	7 (35.0)	13 (65.0)	

BV				
	Negative	191 (96.9)	102 (53.4)	89 (46.6)	0.332
	Positive	6 (3.1)	2 (33.3)	4 (66.7)	

n=Number; *Includes one woman who had greater than secondary education, TV- *Trichomonas vaginalis,* NG-*Neisseria gonorrhea*, CT-*Chlamydia trachomatis*, BV-Bacterial vaginosis

Proportion of women reporting intravaginal practices (IVP)

Ninety-three (47.2%) women reported at least one form of IVP. The commonly used substances to clean the vagina included: soap (n=76, 81.7%), cloth (n=5, 5.4%), and others (honey, herbs, perfume n=12, 12.9%). Reported IVP were more common among single and never married women compared to single but previously married or currently married women (p=0.020) and among those with more sex partners (p=0.002) (Table 1).

Proportion and rate of STIs/BV

A total of 172 (87.3%) women were diagnosed with an STI/BV at least once during follow up, with an overall incidence rate of 51.9 per 100 pyrs. The overall incidence rate for HIV was 5.8 per 100 pyrs. The most common STI was TV (73.6%, n=145/197 diagnosed at least once) with a rate of 92.7 per 100 pyrs and 88.9 per 100 pyrs in the single-event-per-participant and multiple-events-per-participant analysis respectively (Table 2).

Effect of IVP on rate of STIs/BV

In the single-event-per-participant analysis, STI/BV and HIV rates were not associated with reported IVP (Table 2). However, in the multiple-events-per participant analysis, the rate of CT was statistically significantly lower among women who reported IVP versus those who did not (p=0.030). After adjusting for participant baseline characteristics, overall, in both the single-event and multiple-event per participant analyses, IVP was not associated with any significant change in STI/BV or HIV rates (Table 3). There was no statistically significant effect of IVP use on the rates of STIs/BV and HIV among women using the DVR compared to placebo.

Table 2: Effect of intravaginal practices on rate of sexually transmitted infection/Bacterial vaginosis among women in the Ring Study in southwestern Uganda

Sexually transmitted infection/condition	Single-event-per-participant analysis					
	Overall	Intravaginal practices				
		Yes	No			
	Rate/100 PYR (95% CI)	Rate/100 PYR (95% CI)	Rate/100 PYR (95% CI)	p-value	uRR (95% CI)	aRR (95% CI)
HIV	5.8 (3.0-11.1)	7.0 (2.9-17.0)	4.7 (1.8-12.4)	0.278		
Trichomonas vaginalis	92.7 (78.7-109.0)	97.4 (76.9-123.3)	88.7 (70.9-111.1)	0.288	1.10 (0.79-1.52)	1.10 (0.67-1.63)
Neisseria gonorrhea	23.4 (18.3-29.9)	22.8 (15.9-32.9)	23.8 (17.0-33.4)	0.435	0.96 (0.58-1.57)	0.99 (0.56-1.79)
Chlamydia trachomatis	28.4 (22.7-35.6)	27.9 (20.2-38.8)	28.9 (21.2-39.4)	0.446	0.97 (0.62-1.52)	083 (0.50-1.38)
Bacterial vaginosis	14.1 (10.6-18.6)	13.4 (8.9-20.4)	14.7 (10.1-21.4)	0.383	0. 92 (0.52-1.61)	1.13 (0.53-2.37)
Multiple-events-per-participant analysis						
Trichomonas vaginalis	88.9 (79.6-99.5)	90.2 (76.8-106.0)	87.9 (75.3-102.5)	0.406	1.03 (0.77-1.37)	1.07 (0.80-1.43)
Neisseria gonorrhea	28.4 (23.3-34.6)	28.7 (21.5-38.1)	28.2 (21.5-37.0)	0.935	1.02 (0.61-1.69)	0.99 (0.59-1.67)
Chlamydia trachomatis	43.6 (37.2-51.1)	36.6 (28.4-47.1)	49.9 (40.7-61.2)	0.030	0.73 (0.45-1.20)	0.64 (0.38-1.05)
Bacterial vaginosis	18.8 (11.1-31.7)	12.1 (4.5-32.2)	24.1 (13.0-44.8)	0.124	0.51 (0.13-1.95)	0.48 (0.16-1.47)

PYR = person-years at risk; CI = Confidence interval; uRR = unadjusted rate ratio; aRR = adjusted rate ratio; ¶Adjusted for age, trial arm, STI/BV at baseline

Table 3: Effect of intravaginal practices on rate of sexually transmitted infection/Bacterial vaginosis stratified by trial arm among women in the Ring Study in southwestern Uganda

Single-event-per-participant analysis						
Sexually transmitted infection/condition	**Overall**	**Trial arm**				
		DVR	**Placebo**			
	IVP	**Rate/100 PYR (95% CI)**	**Rate/100 PYR (95% CI)**	**p-value**	**aRR (DVR) (95% CI)**	**aRR (Placebo) (95% CI)**
HIV	Yes	5.6 (2.5-12.4)	6.1 (2.0-18.9)	0.439		
Trichomonas vaginalis	Yes	105.8 (79.7-140.4)	82.4 (53.7-126.4)	0.171	1.34 (0.78-2.31)	0.59 (0.28-1.26)
Neisseria gonorrhea	Yes	20.6 (13.0-32.6)	28.0 (15.5-50.5)	0.211	0.73 (0.35-1.53)	1.88 (0.49-7.20)
Chlamydia trachomatis	Yes	29.7 (20.2-43.6)	24.3 (13.1-45.2)	0.305	1.09 (0.60-2.00)	0.37 (0.11-1.36)
Bacterial vaginosis	Yes	12.6 (7.5-21.3)	15.2 (7.6-30.3)	0.332	0.74 (0.26-2.09)	1.51 (0.35-6.52)
Multiple-events-per-participant analysis						
Trichomonas vaginalis	Yes	94.4 (77.9-114.2)	81.5 (60.5-109.9)	0.213	1.12 (0.80-1.56)	0.76 (0.44-1.30)
Neisseria gonorrhea	Yes	25.2 (17.4-36.4)	36.0 (23.0-56.5)	0.116	0.74 (0.42-1.30)	2.24 (0.61-8.31)
Chlamydia trachomatis	Yes	40.4 (30.2-54.2)	28.4 (17.1-47.2)	0.126	0.75 (0.43-1.29)	0.33 (0.14-0.78)
Bacterial vaginosis	Yes	12.5 (4.0-38.7)	11.1 (1.6-78.7)	0.491	0.64 (0.11-3.71)	0.39 (0.10-2.72)

PYR = person-years at risk; CI = Confidence interval; uRR = unadjusted rate ratio; aRR = adjusted rate ratio; ¶Adjusted for age, and STI/BV at baseline

DISCUSSION

Our study aimed to assess the effect of IVP on the incidence of STIs including HIV, and BV among women using the DVR or placebo. Overall, we found that nearly one in every two women practiced at least one form of IVP. IVP is reported to be high in African women with proportions of between 30-50%, and even higher among women at high risk of acquiring HIV [31, 32]. Generally, IVP in this population are driven by cultural and social norms as well as the need for personal hygiene in relation to sexual health and relationships [33]. Women recruited in our study were those engaging in transactional sex and are thus expected to present themselves to their male partners in a fresh vaginal state [34, 35]. The frequency of IVP amongst women involved in transactional sex may be influenced by the need to remain clean/fresh coupled with worries about HIV infection. Prior studies in Uganda and Tanzania showed that a higher frequency of sex was associated with more frequent engagement with IVP [25, 36, 37].

The prevalence of STIs/BV in our study population was high confirming the fact that the women were at very high risk of HIV infection. More events were reported among single women and those who did not live with a main partner and those with more sex partners. Women at high risk of HIV infection engage in transactional sex and have multiple partners, that puts them at higher risk of acquiring STIs/BV. Those who were single also engaged more in IVP. Earlier studies showed that women engaging in high frequency of sex require to present themselves as clean to their male partners [14, 34]. IVP is generally practiced for hygiene purposes and sexuality [15, 16]. IVP has been associated with changes in vaginal flora and resulting BV. The latter is associated with increased susceptibility to STIs including HIV [17]. The interaction between BV and STIs including HIV has been well documented with each causing genital inflammation [38]. It is reported that organisms associated with BV may overgrow due to increased vaginal pH brought about by substances that may be used for IVP like soaps which most of the women in our study used. With increased vaginal pH, the protective Lactobacilli species are replaced by pathogenic organisms resulting in BV [19, 20]. The products used may cause genital lesions enabling the transmission of STIs [17].

We observed a high incidence of STIs in this population, with the commonest STI being TV. TV is reported to be prevalent among women engaging in transactional sex with high proportions reported globally (16%) and the African region contributing even higher proportions (23%) [39]. TV has also been associated with BV especially among women that use cloth for IVP as these act as fomites

[21]. Although the STI incidence was high, we did not see any significant difference in the incidence of STIs/BV among women using IVP compared to those who did not, except for CT that was lower. It is not clear and has not been documented that IVP reduces the risk of acquiring STIs/BV, though the reverse has been reported. IVP causes changes to vaginal flora resulting in increased risk of STIs/BV including HIV. A recent systematic review found that IVP increased the risk of vaginal infections (BV, TV, and vulvovaginal candidiasis) [40] while two others found no association between IVP and TV [15] or BV [41]. One study in South Africa found that IVP was associated with increased risk of HIV infection but not other STIs [42]. Organisms related with BV have been associated with the production of metabolites that are used by STIs likes CT as growth factors enabling their multiplication [43]. However, production of glycogen by genital epithelial cells, provides energy for Lactobacillus species to flourish [44]. These species may provide for protection against organisms like CT [45]. In vitro studies using substances that lower vaginal pH has resulted in reduction in susceptibility to CT organisms [44]. It is not clear if the products the women used in our study could have reduced vaginal pH and thus provided some protection against CT.

We also found that there was no statistically significant effect of the DVR microbicide or placebo on STI/BV including HIV rates among those using IVP. The DVR has been associated with minimal changes in the vaginal microbiota that were likely not clinically significant [46]. It has generally been found to be well tolerated in adult as well as adolescent girls and young women [47, 48]. Various studies have reported that use of microbicides results in little or no difference in the risk of acquiring STIs like CT, NG or TV [49]. In the Buffer Gel microbicide study for example, no significant changes in colonization with Lactobacillus species was reported [50].

The strengths of this study included the opportunity to investigate multiple STIs and BV in the same population and the ability to collect recurrent data on genital conditions which allowed for conduct of both single-event-per-participant and multiple-event-per-participant analyses. The multiple-event-per-participant analysis models the total rate of events over the entire follow-up period and has more power for detecting associations compared to the single-event-per-participant analysis [51].

A limitation of the study was the small sample size which may have impacted the study power and consequently the ability to detect any differences in STI/BV rates between women who reported IVP and those who did not. Another limitation was that no confirmatory tests were done to confirm absence

of an STI/BV prior to DVR insertion. However, duration of treatment and absence of symptoms and signs were used as a proxy for lack of an STI/BV.

In conclusion, we found a high prevalence of IVP and incidence of STIs/BV among women enrolled in the Ring Study in Uganda. IVP did not statistically significantly increase STI/BV rates. This implies that women who practice intravaginal cleansing/insertion could continue using these practices in the presence of the DVR microbicide. However, our results should be interpreted with caution because of the small sample size that could generate a hypothesis but not conclusively test it. An analysis with a bigger sample size could be helpful to better understand whether there is a link between use of the DVR, IVP, and incidence of STIs/BV.

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Author contributions

SK, AA, ER designed the study and wrote the initial manuscript draft, and AA did the data analysis. KA and MO conducted the study while SK directed the work. KA and MO contributed to the writing and editing of the manuscript. All authors contributed to the interpretation of the results and critically commented and provided revisions to the manuscript. All authors approved the final version of the

manuscript. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

Data availability statement: Data are available on reasonable request.

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STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4, 5
Objectives	3	State specific objectives, including any prespecified hypotheses	4, 5
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5, 6
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	5, 6
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6. 7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6. 7
Bias	9	Describe any efforts to address potential sources of bias	6, 7
Study size	10	Explain how the study size was arrived at	5, 6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6, 7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7, 8
		(*b*) Describe any methods used to examine subgroups and interactions	NA
		(*c*) Explain how missing data were addressed	NA
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	NA
		(*e*) Describe any sensitivity analyses	7. 8

Continued on next page

Results			**Page**
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	9, 10, 11
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9, 10, 11
		(b) Indicate number of participants with missing data for each variable of interest	NA
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	11, 12, 13
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	11
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			
Key results	18	Summarise key results with reference to study objectives	14, 15
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	15, 16
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	16
Generalisability	21	Discuss the generalisability (external validity) of the study results	16
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	16

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Associations between intravaginal practices and incidence of sexually transmitted infections and bacterial vaginosis among women enrolled in the Dapivirine vaginal ring trial (The Ring Study) in southwestern Uganda, a retrospective secondary analysis

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Associations between intravaginal practices and incidence of sexually transmitted infections and bacterial vaginosis among women enrolled in the Dapivirine vaginal ring trial (The Ring Study) in southwestern Uganda, a retrospective secondary analysis.

Authors: Sylvia Kusemererwa[1*], Eugene Ruzagira[1,3], Martin Onyango[1], Anita Kabarambi[4], Andrew Abaasa[2,3]

Institution

[1]Department of viral pathogen epidemiology and interventions, Medical Research Council/ Uganda Virus Research Institute and London School of Hygiene and Tropical Medicine, Uganda Research Unit, Entebbe, Uganda

[2]Department of statistics, Medical Research Council/ Uganda Virus Research Institute and London School of Hygiene and Tropical Medicine, Uganda Research Unit, Entebbe, Uganda

[3] Department of infectious diseases epidemiology, London School of Hygiene & Tropical Medicine, London, UK

[4] Department of research, International Center for Child Health and Development, Masaka, Uganda

Correspondence to: Sylvia Kusemererwa, [1]Medical Research Council/Uganda Virus Research Institute (MRC/UVRI) and London School of Hygiene and Tropical Medicine (LSHTM), Uganda Research Unit, Plot 51-59, Nakiwogo Road, P.O Box 49 Entebbe, Uganda. Phone: 256 (0) 417 704000, or 256 (0) 70229100. Sylvia.Kusemererwa@mrcuganda.org or kansykarus@gmail.com

Associations between intravaginal practices and incidence of sexually transmitted infections and bacterial vaginosis among women enrolled in the Dapivirine vaginal ring trial (The Ring Study) in southwestern Uganda, a retrospective secondary analysis.

Abstract

Objectives: We assessed associations between intravaginal practices (IVP) and the incidence of sexually transmitted infections (STIs) and bacterial vaginosis (BV) among women using the dapivirine vaginal ring (DVR) or placebo vaginal ring in southwestern Uganda.

Methods: This was a retrospective secondary analysis of data collected from women at risk of HIV infection recruited into The Ring Study. The latter evaluated the safety and efficacy of the DVR between 2013 and 2016. At baseline, a behavioral questionnaire was administered to obtain information on sexual activity and IVP (exposure) defined as; insertion inside the vagina of any items aimed at cleaning the vagina for any reason before, during or after sex other than practices to manage menses. Each participant self-inserted the DVR/placebo and replaced it every 4weeks for 2 years. Outcomes were diagnosis of STIs i.e., *Chlamydia trachomatis* (CT), *Neisseria gonorrhea* (NG), *Trichomonas vaginalis* (TV), HIV and BV. The incidence rate of STI/BV was estimated, overall, by IVP and trial arm in single-event-per-participant and multiple-event-per-participant analyses.

Results: Of the 197 women enrolled, 66 (33.5%) were <25 years of age. Overall, 93 (47.2%) practiced at least one form of IVP. During follow up, 172 (87.3%) women were diagnosed with an STI/BV at least once. Majority had TV (73.6%, n=145). Overall rate of STI/BV was 51.9/100 person-years, 95% confidence interval (CI): 44.7-60.3 [IVP: Yes, 51.0 (40.8-63.8) vs. No, 52.6 (43.0-64.4)]. IVP were not statistically significantly associated with rate of individual STIs/BV. Similar results were observed when the analyses were conducted separately for each trial arm.

Conclusions: IVP was not associated with risk of STIs/BV in The Ring Study.

Key words: Intravaginal practices, sexually transmitted infection, Bacterial vaginosis,

Dapivirine, Vaginal Ring, Uganda

Study strengths and limitations

- The study was able to investigate multiple STIs and BV in the same population.
- The longitudinal nature of the study allowed for both single-event-per-participant and multiple-event-per-participant analyses.
- Extensive analysis was to some extent limited by a small sample size.
- Confirmatory tests were not done to confirm absence of an STI/BV prior to DVR insertion.

INTRODUCTION

Sexually transmitted infections (STIs) remain a health challenge globally. In 2018, WHO estimated that nearly one million people become infected every day with a curable STI caused by: *Chlamydia trachomatis* (CT), *Neisseria gonorrhea* (NG), Syphilis or *Trichomonas vaginalis* (TV).[1] STIs disproportionately affect low-and-middle-income countries, with 90% of the new infections occurring in these countries.[2] Women in sub-Saharan Africa have a high prevalence of STIs, particularly those at high risk of HIV infection also having a high STI burden.[3, 4] Bacterial vaginosis (BV), a common vaginal condition has also been associated with increased risk of STIs and HIV infection. [5] High rates (37% to 68%) of BV have been reported among women at high risk of HIV acquisition in Southern and East Africa. [6]

BV results from variation in normal vaginal flora attributed to reduction in the prevalence of Lactobacilli (dominant species in healthy vaginal environment) and an increase in the concentration of pathogenic organisms: *G. vaginalis*, *Bacteroides (Prevotella)* species, *Mobiluncus* species, and *Mycoplasma hominis*. [7, 8] Increasing evidence shows that vaginal microbiota may play a role in mediating susceptibility to STIs. Vaginal Lactobacilli utilize a number of actions to protect against colonization by genital pathogens. [9] This can be through: production of lactic acid that supports the maintenance of a lower vaginal pH, which may prevent pathogen growth, [10] exposure to hydrogen peroxide that has also been shown to decrease activity of BV and other genital tract organisms. [11, 12]

The high prevalence and increased risk of acquisition of STIs/BV and HIV has been associated with vaginal practices.[13] These practices include various behaviors used to maintain health, wellness, and enhance sexual pleasure.[14-16] The World Health Organization (WHO) has suggested seven classifications for vaginal practices: external washing, intravaginal cleansing, external application, intravaginal insertion, oral ingestion, vaginal streaming or smoking and anatomical modification.[17] On the other hand intravaginal practices (IVPs) refer to both intravaginal cleansing (cleaning or washing inside the vagina with fingers or substances like soap to remove fluids), and intravaginal insertion (placing something inside the vagina, like powders, creams, herbs, or tissue).[18, 19]

Soaps, detergents and antiseptics used to cleanse inside the vagina can cause chemical damage and increase vaginal pH resulting in overgrowth of BV related organisms, which has also been associated with increased risk of HIV acquisition.[19, 20] Other products have also been reported to cause genital lesions, and swellings, creating favorable conditions for the transmission of STIs, including HIV.[17] Items like cloth commonly used in some communities to clean the vagina repeatedly might also act as fomites, carrying TV organisms. [21] TV has also been associated with increased risk of HIV acquisition. [22, 23] These practices which increase a woman's susceptibility to HIV could reduce the effectiveness of vaginal microbicides. [24, 25]

The monthly dapivirine vaginal ring (DVR) microbicide, a female controlled HIV prevention tool, was found to reduce the risk of HIV acquisition by approximately 30% in two phase 3 trials. [26, 27] With continued and consistent use, the risk of HIV acquisition was even lower (62%) in an open-label extension trial. [28] However, data are limited on the effect of intravaginal practices on vaginal flora and risk of STIs/BV among women using the DVR. We assessed associations between intravaginal practices and the incidence of STIs including HIV, and BV among women using the DVR or placebo vaginal ring in southwestern Uganda.

METHODS

Study design

This was a retrospective secondary analysis using data collected in The Ring Study, a phase 3 microbicide trial.[26]

Study setting and population

Details of the Ring Study (Registration number: NCT01539226) have been described elsewhere. [26] Briefly, The Ring Study was a multicenter microbicide trial, that evaluated the safety and efficacy of the DVR between 2013 and 2016 in Uganda and South Africa. The study was sponsored by the International Partnership for Microbicides. In Uganda, the study was conducted by the Medical Research Council (MRC)/Uganda Virus Research Institute (UVRI) and London School of Hygiene and Tropical Medicine (LSHTM) Uganda Research Unit in Masaka, southwestern Uganda. The site recruited women at high risk of HIV infection from towns along the trans-African highway and the shores of Lake Victoria. Details of the recruitment procedures

and study population have been described elsewhere.[29] Briefly, the research site enrolled 197 women (18-45years of age) at high risk of HIV infection. Women were identified from sex work hotspots (bars, restaurants, hair salons, small shops, and other small-scale businesses). High risk was defined by presence of any two of the following: i) history of STIs in the past three months; ii) self-reported condom less sex with multiple sex partners or a new partner in the past three months; and iii) use of recreational drugs (marijuana, alcohol) in the past three months. Of the women enrolled, two in five were working in bars and restaurants and nearly a third had small scale businesses. A woman was included in the main study if they were not pregnant, not breastfeeding, asymptomatic for genital infections and tested HIV negative at the time of enrolment. Those diagnosed with any clinically significant curable STI, were initiated on treatment for at least a week prior and enrolled after completing a full course of treatment.

Study procedures

Participants consented for screening and enrolment on two separate occasions. At the first visit (screening), data on demographics, inclusion and exclusion criteria were obtained. A physical and genital examination was done. All potential participants were provided with HIV/STI risk-reduction counselling and HIV pre- and post-test counselling. At the second visit (enrolment), women who met all inclusion criteria and no exclusion criteria, had a normal pelvic examination, and negative HIV rapid tests were enrolled into the trial. Eligible women were randomised in a 2:1 ratio to either the DVR arm or placebo arm. At the enrolment visit, an interviewer-administered behavioral questionnaire was used to obtain baseline information on sexual activity and vaginal practices. At 4 weeks post-enrolment and every 24 weeks thereafter for the next 2 years, follow-up data on vaginal practices were collected using an interviewer-administered behavioral questionnaire. The latter included questions to which participants replied by selecting from a variety of pre-specified responses and provided open-ended responses about their IVP. Participants self-inserted a vaginal ring every 4 weeks for up to 104 weeks.

Measurement of exposure [intravaginal practices (IVP)]: IVP was defined as insertion inside the vagina of any items aimed at cleaning the vagina for any reason before, during or after sex other than practices to manage menses. Items included: materials such as paper, cloth, or cotton

wool; water only; water and soap; fingers to clean or insert something. Women were asked if they were inserting or using any of the aforementioned items inside the vagina to clean their vagina either as a general cleaning/hygiene practice before or after sex or to prepare the vagina for sex in the past, at baseline and every 24 weeks.

Measurement of outcome (diagnosis of STIs/BV): Cervico-vaginal swabs were collected at the first screening visit and every 12 weeks (3 months) for 2 years. The swabs were tested for TV (OSOM® Trichomonas Rapid test- Sekisui Diagnostics, LLC, USA) and CT/NG (Cobas® Amplicor CT/NG -PCR test, Roche Diagnostic Systems, Branchburg, New Jersey). Vaginal fluid samples were also collected by trained clinicians using sterile swabs for assessment of vaginal flora (using Nugent's score) and vaginal fluid pH at the enrolment visit (prior to ring insertion) and every 12 weeks. Samples with a score of ≥7 were classified as BV present. Each slide was scored by trained laboratory technologists. For internal quality control, a single batch of slides were re-examined by an independent reader weekly. Discrepant results were resolved by expert consensus. External quality control was assured using College of American Pathologists Vaginitis screen, vaginal gram stain-VS2 as part of the site standard operating procedures.

Participants were tested for HIV at the screening and enrolment visit using whole blood samples collected by venipuncture. Serial rapid HIV antibody tests were done using Alere Determine™ HIV-1/2 (Alere, Medical co., Ltd, Matsuhidai, Matsudo-shi, Chiba, Japan) followed by OraQuick- ADVANCE® Rapid HIV-1/2 Test (OraQuick-OraSure Technologies Inc, Pennsylvania) to confirm a positive Determine result and Uni- Gold™ HIV (Trinity Biotech, Ireland) as the tie breaker. At the screening/enrolment visits, a participant was confirmed to have HIV infection if they tested positive on at least two rapid HIV antibody tests. Post-enrolment, HIV testing was done serially as described above. However, for participants who tested positive or discordant on two rapid HIV antibody tests, a confirmatory test on stored plasma was done using Western Blot (J. Mitra and Co.Pvt. ltd, India) as previously described. Blood samples were collected and plasma stored every 4 weeks. Stored plasma samples for participants with confirmed HIV infection were retrospectively tested at a central laboratory in South Africa (Bioanalytical Research Corporation) for HIV ribonucleic acid (RNA) copies (viral load) using the polymerase-chain-reaction (PCR) assay. [26]

Statistical analysis

Data analyses were performed in Stata version 15.0 (Stata Corp, College Station, TX, USA). Participants' baseline characteristics were summarized using frequencies and percentages overall and by IVP status and compared between IVP users and none users using a chi-squared test. We determined the proportion of participants that were positive for a given STI/BV (event) as the number who tested positive for an STI/BV at least once during the study divided by the total number tested. We determined associations between IVP and incidence of each STI/BV, by estimating the rate of STI/BV overall and stratified by IVP status. We used two approaches for measuring the rate of STI/BV; (a) a single-event-per-participant (allowing for one event per participant-first STI/BV event) and (b) a multiple-event-per-participant (allowing for two or more STI/BV events for the same participant) since these are recurrent events. The incidence rate of a given STI/BV was estimated as the number of participants who tested positive for STI/BV divided by the person-time (years) at risk (pyr) expressed as per 100 pyr. PYR were estimated as a sum of the time from enrolment into the trial (those negative at baseline) to the date of trial completion or censoring (trial end, end of ring use and their first event for a given outcome). Participants that tested positive for STI/BV at baseline were given treatment and started to contribute person-time after completing the course of treatment. Similar approach was followed for those that got infected during follow up though the person time was segmented to allow for multiple entry and exit from the analysis following treatment. We further adjusted the effect of IVP on rate of STI/BV for age and baseline STI/BV status by fitting Poisson regression models. In the analysis, we used Poisson regression model with time-varying covariates, allowing for intergroup correlation (because women had multiple records) by using cluster robust standard errors. For HIV, we estimated the rate of HIV infection as number of HIV positive cases divided by the total person years at risk expressed as per 100 person years at risk in a single-event-per-participant survival analysis. Person time at risk were calculated as sum of the time from enrolment to the last HIV seronegative date (for those that remained negative throughout the trial) or to the estimated date of HIV infection. The HIV infection date was estimated as a multiple imputation random date between the last HIV seronegative and the first HIV+ result date.

Compliance with Ethical Standards

Approval was obtained from the Uganda Virus Research Institute Research Ethics Committee (Ref#-GC/127/13/03/33), the Uganda National Council of Science and Technology (Ref#-HS1362) and the National Drug Authority (Ref #-166/ESR/NDA/DID-07/2013). Written informed consent was obtained from each woman before any study procedures were performed. Women who tested HIV-positive were referred to an HIV care provider of their choice. Treatment was provided to those who tested positive for STIs/BV according to the Centers for Disease Control and Prevention (CDC) STD Treatment guidelines 2010. [30]

Patient and Public Involvement Statement

Communities where the study was conducted were involved from the inception of the study. Community gate keepers: Local Council leaders, political leaders, village health teams, community based and faith based organizations were informed of the study via various engagement meetings prior to study start. The site Community Advisory Board (CAB) members were engaged to review study documents, consent and other study literacy documents and confirm translations to the local language. The CAB was compensated for all the activities they were involved in. Volunteer recruitment and retention was supported by local leaders. Results were disseminated to study participants, CAB and community members through community meetings upon study completion and presentations at national and international meetings, seminars and conferences. All stakeholders were compensated for the time spent during engagement meetings.

RESULTS

Baseline socio-demographic characteristics

In total, 197 women enrolled, 67% on the DVR trial arm. Of those, 66 (33.5%) were less than 25 years of age, 82 (41.6%) were married and very few 24 (12.2%) had secondary school education. Majority, 162 (82.2%) reported having a main partner but only 75 (46.3%) lived with this partner (Table 1). About a half, 100 (50.8%) tested positive for an STI/ BV at baseline. Compared with

those who had one episode of STI/BV, participants with two or more episodes were likely to be single and never married (47.8% vs. 35.6%), not currently living with a main partner (56.5% vs. 37.5%, p=0.033) and inconsistently living with the main partner in the past year (69.6% vs. 41.7%; p=0.001) but otherwise similar in regard to other participant characteristics.

Table 1: Baseline socio-demographic characteristics of 197 women enrolled in The Ring Study in southwestern Uganda between 2013 and 2016

Variable	All	Intravaginal practices		p-value
		No	Yes	
	n (%)	n (%)	n (%)	
Overall	197	104 (52.8)	93 (47.2)	
Trial arm				0.835
Dapivirine vaginal ring	132 (67.0)	69 (66.4)	63 (67.7)	
Placebo	65 (33.0)	35 (33.6)	30 (32.3)	
Age (years)				0.275
18-24	66 (33.5)	30 (28.8)	36 (38.7)	
25-34	98 (49.8)	57 (54.8)	41 (44.1)	
35+	33 (16.7)	17 (16.4)	16 (17.2)	
Education level				0.276
Incomplete primary school	68 (34.5)	37 (35.6)	31 (33.3)	
Complete primary school	105 (53.3)	58 (55.8)	47 (50.5)	
Secondary school*	24 (12.2)	9 (8.6)	15 (16.1)	
Marital status				**0.020**
Single and never married	90 (45.7)	38 (36.5)	52 (55.9)	
Single but previously married	25 (12.7)	14 (13.5)	11 (11.8)	
Married	82 (41.6)	52 (50.0)	30 (32.3)	
Number of life time sex partners				**0.002**
Median (IQR)	6 (4-12)	5 (3-9)	8 (5-20)	
Has main partner				0.194
No	35 (17.8)	15 (14.4)	20 (21.5)	
Yes	162 (82.2)	89 (85.6)	73 (78.5)	
Duration lived with main partner (years)				0.690
<1	35 (21.6)	17 (19.1)	18 (24.7)	
1-2	34 (21.0)	19 (21.3)	15 (20.5)	
3+	93 (57.4)	53 (59.6)	40 (54.8)	
Lived with (main) partner in the past year				0.171
All the time	48 (37.8)	38 (42.7)	24 (32.9)	
Some of the time	14 (11.0)	7 (7.9)	12 (16.4)	
No	65 (51.2)	44 (49.4)	37 (50.7)	
Currently lives with main partner				0.376
No	87 (53.7)	45 (50.6)	42 (57.5)	
Yes	75 (46.3)	44 (49.4)	31 (42.5)	

Unprotected sex with multiple/new partner in the past 3 months				
No	38 (19.3)	18 (17.3)	20 (21.5)	0.456
Yes	159 (80.7)	86 (82.7)	73 (78.5)	
Drug/alcohol use in the past 3 months				
No	48 (24.4)	32 (30.8)	16 (17.2)	**0.027**
Yes	149 (75.6)	72 (69.2)	77 (82.8)	
Baseline STI/BV status				
TV				
Negative	121 (61.4)	61 (58.7)	60 (64.5)	0.399
Positive	76 (38.6)	43 (41.3)	33 (35.5)	
NG				
Negative	173 (87.8)	92 (88.5)	81 (87.1)	0.770
Positive	24 (12.2)	12 (11.5)	12 (12.9)	
CT				
Negative	177 (89.8)	97 (93.3)	80 (86.0)	0.093
Positive	20 (10.2)	7 (6.7)	13 (14.0)	
BV				
Negative	191 (96.9)	102 (53.4)	89 (46.6)	0.332
Positive	6 (3.1)	2 (33.3)	4 (66.7)	

n=Number; *Includes one woman who had greater than secondary education, TV- *Trichomonas vaginalis,* NG-*Neisseria gonorrhea*, CT-*Chlamydia trachomatis*, BV-Bacterial vaginosis

Proportion of women reporting intravaginal practices (IVP)

Ninety-three (47.2%) women reported at least one form of IVP. The commonly used substances to clean the vagina included: soap (n=76, 81.7%), cloth (n=5, 5.4%), and others (honey, herbs, perfume n=12, 12.9%). Reported IVP were more common among single and never married women compared to single but previously married or currently married women (p=0.020), among those with more sex partners (p=0.002) and those who used drugs/alcohol in the past 3 months (p= 0.027) (Table 1).

Proportion and rate of STIs/BV

A total of 172 (87.3%) women were diagnosed with an STI/BV at least once during follow up, with an overall single-event-per participant incidence rate of 51.9 per 100 pyrs. The overall incidence rate for HIV was 5.8 per 100 pyrs. The most common STI was TV (73.6%, n=145/197 diagnosed at least once) with a rate of 92.7 per 100 pyrs in the single-event-per-participant analysis (Table 2).

Associations between IVP and rate of STIs/BV

In the single-event-per-participant analysis, STI/BV and HIV rates were not associated with reported IVP (Table 2). However, in the multiple-events-per participant analysis, the rate of CT was statistically significantly lower among women who reported IVP versus those who did not (p=0.030). On stratification by trial arm, the rate of NG was higher in the DVR arm compared to the placebo arm in both the single and multiple-events-per participant analysis among women not using IVP (p=0.024 and p=0.007 respectively) (Table 3). After adjusting for participant baseline characteristics, overall, in the multiple-event per participant analyses, IVP was only associated with lower rates of CT among women in the placebo arm [adjusted rate ratio (aRR)=0.33, 95% CI 0.14-0.78] (Table 3).

Table 2: Associations between intravaginal practices and rate of sexually transmitted infection/Bacterial vaginosis among women in the Ring Study in southwestern Uganda

Sexually transmitted infection/condition	Single-event-per-participant analysis					
	Overall	Intravaginal practices				
		Yes	No			
	Rate/100 PYR (95% CI)	Rate/100 PYR (95% CI)	Rate/100 PYR (95% CI)	p-value*	uRR (95% CI)	aRR (95% CI)
HIV	5.8 (3.0-11.1)	7.0 (2.9-17.0)	4.7 (1.8-12.4)	0.278	1.51 (0.39-5.81)	1.29 (0.29-5.67
Trichomonas vaginalis	92.7 (78.7-109.0)	97.4 (76.9-123.3)	88.7 (70.9-111.1)	0.288	1.10 (0.79-1.52)	1.10 (0.67-1.63)
Neisseria gonorrhea	23.4 (18.3-29.9)	22.8 (15.9-32.9)	23.8 (17.0-33.4)	0.435	0.96 (0.58-1.57)	0.99 (0.56-1.79)
Chlamydia trachomatis	28.4 (22.7-35.6)	27.9 (20.2-38.8)	28.9 (21.2-39.4)	0.446	0.97 (0.62-1.52)	0.83 (0.50-1.38)
Bacterial vaginosis	14.1 (10.6-18.6)	13.4 (8.9-20.4)	14.7 (10.1-21.4)	0.383	0. 92 (0.52-1.61)	1.13 (0.53-2.37)
Multiple-events-per-participant analysis						
Trichomonas vaginalis	88.9 (79.6-99.5)	90.2 (76.8-106.0)	87.9 (75.3-102.5)	0.406	1.03 (0.77-1.37)	1.07 (0.80-1.43)
Neisseria gonorrhea	28.4 (23.3-34.6)	28.7 (21.5-38.1)	28.2 (21.5-37.0)	0.935	1.02 (0.61-1.69)	0.99 (0.59-1.67)
Chlamydia trachomatis	43.6 (37.2-51.1)	36.6 (28.4-47.1)	49.9 (40.7-61.2)	0.030	0.73 (0.45-1.20)	0.64 (0.38-1.05)
Bacterial vaginosis	18.8 (11.1-31.7)	12.1 (4.5-32.2)	24.1 (13.0-44.8)	0.124	0.51 (0.13-1.95)	0.48 (0.16-1.47)

PYR = person-years at risk; CI = Confidence interval; aRR = adjusted rate ratio; ¶Adjusted for age, trial arm, STI/BV at baseline, * Unadjusted p-value comparing the rate of each STI between IVP use and none use

Table 3: Associations between intravaginal practices and rate of sexually transmitted infection/Bacterial vaginosis stratified by trial arm among women in the Ring Study in southwestern Uganda

Sexually transmitted infection/condition	Overall	Trial arm				
		DVR	Placebo			
	IVP use	Rate/100 PYR (95% CI)	Rate/100 PYR (95% CI)	p-value*	aRR (DVR) (95% CI)	aRR (Placebo) (95% CI)
Single-event-per-participant analysis						
HIV	No	3.3 (0.8-12.9)	8.4 (2.1-33.8)	0.374	-	-
	Yes	8.8 (3.3-23.5)	3.9 (0.6-27.9)	0.518	-	-
Trichomonas vaginalis	No	77.5 (58.4-102.8)	118.3 (81.6-171.3)	0.081	1.00	1.00
	Yes	105.8 (79.7-140.4)	82.4 (53.7-126.4)	0.171	1.34 (0.78-2.31)	0.59 (0.28-1.26)
Neisseria gonorrhea	No	30.5 (21.1- 44.2)	11.8 (5.3-26.2)	0.024	1.0	
	Yes	20.6 (13.0-32.6)	28.0 (15.5-50.5)	0.211	0.73 (0.35-1.53)	1.88 (0.49-7.20)
Chlamydia trachomatis	No	28.5 (19.4-41.9)	29.5 (17.5-49.8)	0.911	1.00	1.00
	Yes	29.7 (20.2-43.6)	24.3 (13.1-45.2)	0.305	1.09 (0.60-2.00)	0.37 (0.11-1.36)
Bacterial vaginosis	No	13.7 (8.6-22.1)	16.5 (8.9-30.7)	0.636	1.00	1.00
	Yes	12.6 (7.5-21.3)	15.2 (7.6-30.3)	0.332	0.74 (0.26-2.09)	1.51 (0.35-6.52)
Multiple-events-per-participant analysis						
Trichomonas vaginalis	No	87.2 (72.2-105.3)	89.2 (68.3-116.5)	0.558	1.00	1.00
	Yes	94.4 (77.9-114.2)	81.5 (60.5-109.9)	0.213	1.12 (0.80-1.56)	0.76 (0.44-1.30)
Neisseria gonorrhea	No	34.7 (25.8-46.8)	14.9 (7.7-28.6)	0.007	1.00	1.00
	Yes	25.2 (17.4-36.4)	36.0 (23.0-56.5)	0.116	0.74 (0.42-1.30)	2.24 (0.61-8.31)
Chlamydia trachomatis	No	56.5 (44.7-71.5)	36.3 (24.0-55.2)	0.065	1.00	1.00
	Yes	40.4 (30.2-54.2)	28.4 (17.1-47.2)	0.126	0.75 (0.43-1.29)	0.33 (0.14-0.78)
Bacterial vaginosis	No	20.5 (9.2-45.7)	32.7 (12.3-87.0)	0.482	1.00	1.00
	Yes	12.5 (4.0-38.7)	11.1 (1.6-78.7)	0.491	0.64 (0.11-3.71)	0.39 (0.10-2.72)

PYR = person-years at risk; CI = Confidence interval; uRR = unadjusted rate ratio; aRR = adjusted rate ratio; ¶Adjusted for age, and STI/BV at baseline, * compares rates for IVP (yes or no) between DVR and placebo

DISCUSSION

Our study aimed to assess associations between IVP and incidence of STIs including HIV, and BV among women using the DVR or placebo. Overall, we found that nearly one in every two women practiced at least one form of IVP. IVP is reported to be high in African women with proportions of between 30-50%, and even higher among women at high risk of acquiring HIV.[31, 32] Generally, IVP in this population is driven by cultural and social norms as well as the need for personal hygiene in relation to sexual health and relationships.[33] Women recruited in our study were those engaging in transactional sex and are thus expected to present themselves to their male partners in a fresh vaginal state.[34, 35] The frequency of IVP amongst women involved in transactional sex may be influenced by the need to remain clean/fresh coupled with worries about HIV infection. Prior studies in Uganda and Tanzania showed that a higher frequency of sex was associated with more frequent engagement with IVP. [25, 36, 37]

The prevalence of STIs/BV in our study population was high confirming the fact that the women were at very high risk of HIV infection. More events were reported among single women and those who did not live with a main partner and those with more sex partners. Women at high risk of HIV infection engage in transactional sex and have multiple partners, that puts them at higher risk of acquiring STIs/BV. Those who were single also engaged more in IVP. Earlier studies showed that women engaging in high frequency of sex require to present themselves as clean to their male partners. [14, 34] IVP is generally practiced for hygiene purposes and sexuality. [15, 16] IVP has been associated with changes in vaginal flora and resulting BV. The latter is associated with increased susceptibility to STIs including HIV. [17] The interaction between BV and STIs including HIV has been well documented with each causing genital inflammation. [38] It is reported that organisms associated with BV may overgrow as a result of increased vaginal pH brought about by substances that may be used for IVP like soaps which most of the women in our study used. With increased pH, the protective Lactobacilli species are replaced by pathogenic organisms resulting in BV. [19, 20] The products used may cause genital lesions enabling the transmission of STIs.[17]

We observed a high incidence of STIs in this population, with the commonest STI being TV. TV is reported to be prevalent among women engaging in transactional sex with high proportions reported globally (16%) and the African region contributing even higher proportions (23%).[39] TV has also been associated with BV especially among women that use cloth for IVP as these act as fomites.[21]

Although the incidence of STIs was high, we did not see any significant difference in the rise in incidence of STIs/BV among women using IVP compared to those who did not, except for CT that was lower. It is not clear and has not been documented that IVP reduces the risk of acquiring STIs/BV, though the reverse has been reported. IVP causes changes to vaginal flora resulting in increased risk of STIs/BV including HIV. A recent systematic review found that IVP increased the risk of vaginal infections (BV, TV, and vulvovaginal candidiasis),[40] two others found no association between IVP and TV[15] or BV.[41] One study in South Africa found that IVP was associated with increased risk of HIV infection but not other STIs.[42] The fact that IVP results in BV, organisms related with BV have been associated with the production of metabolites that are used by STIs like CT as growth factors enabling their multiplication.[43] Incidence of CT has been reported to be high among women (5 per 100pyrs), especially among younger women (27.6 per 100 pyrs) in Kenya, but not associated with vaginal washing.[44]

We also found that women not using IVP, but using the DVR had higher rates of NG compared to those in the placebo arm. Women in this study generally had high rates of STIs. We have previously reported that rates of STIs decreased over time in the same cohort of women.[45] Apart from lower rates of CT among women using the placebo vaginal ring, there was no statistically significant association between the DVR microbicide and STI/BV including HIV rates among those using IVP. The DVR has been associated with minimal changes in the vaginal microbiota that were likely not clinically significant.[46] It has generally been found to be well tolerated in adult as well as adolescent girls and young women.[47, 48] Various studies on microbicides have reported that their use may result in little or no difference in the risk of acquiring STIs like CT, NG or TV.[49] In the Buffer Gel microbicide study for example, no significant changes in colonization with Lactobacillus species was reported.[50]

The strengths of this study included the opportunity to investigate multiple STIs and BV in the same population and the ability to collect recurrent data on genital conditions which allowed for conduct of both single-event-per-participant and multiple-event-per-participant analyses. The multiple-event-per-participant analysis models the total rate of events over the entire follow-up period and has more power for detecting associations compared to the single-event-per-participant analysis. [51] This analysis is also more clinically relevant because STI re-infection and BV recurrence are common.

A limitation of the study was the small sample size which may have impacted the study power and consequently the ability to detect any differences in STI/BV rates between women who reported IVP

and those who did not. Additionally, no confirmatory tests were done to confirm absence of an STI/BV prior to DVR insertion. However, duration of treatment and absence of symptoms and signs were used as a proxy for lack of an STI/BV. Furthermore, it was not possible to measure and evaluate any versus no IVP. This is challenging to do as different types of IVP and materials used affect the risk of HIV, STI and BV risk. This has also not been possible to measure in previous studies.

In conclusion, we found a high prevalence of IVP and incidence of STIs/BV among women enrolled in the Ring Study in Uganda. IVP did not statistically significantly increase STI/BV rates. Implying that women who practice intravaginal cleansing/insertion could continue using these practices in the presence of the microbicide. However, our results should be interpreted with caution because of a limitation in sample size that could generate a hypothesis and not conclusively test it. An analysis with a bigger sample size could be helpful to better understand whether there is a link between use of the DVR, IVP, and incidence of STIs/BV.

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Author Contributions:

SK, AA, ER designed the study and wrote the initial manuscript draft, and AA did the data analysis. KA and MO conducted the study while SK directed the work. KA and MO contributed to the writing

and editing of the manuscript. All authors contributed to the interpretation of the results and critically commented and provided revisions to the manuscript. All authors approved the final version of the manuscript. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

Data availability statement: Data are available on reasonable request.

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STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4, 5
Objectives	3	State specific objectives, including any prespecified hypotheses	4, 5
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5, 6
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	5, 6
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6, 7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6, 7
Bias	9	Describe any efforts to address potential sources of bias	6, 7
Study size	10	Explain how the study size was arrived at	5, 6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6, 7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7
		(*b*) Describe any methods used to examine subgroups and interactions	NA
		(*c*) Explain how missing data were addressed	NA
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	NA
		(*e*) Describe any sensitivity analyses	7

Continued on next page

Results			**Page**
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	9, 10, 11
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9, 10, 11
		(b) Indicate number of participants with missing data for each variable of interest	NA
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	11, 12, 13, 14
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	11, 12, 13, 14
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			
Key results	18	Summarise key results with reference to study objectives	15, 16
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	16,17
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	17
Generalisability	21	Discuss the generalisability (external validity) of the study results	17
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	17

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Associations between intravaginal practices and incidence of sexually transmitted infections and bacterial vaginosis among women enrolled in the Dapivirine vaginal ring trial (The Ring Study) in southwestern Uganda, a retrospective secondary analysis

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Associations between intravaginal practices and incidence of sexually transmitted infections and bacterial vaginosis among women enrolled in the Dapivirine vaginal ring trial (The Ring Study) in southwestern Uganda, a retrospective secondary analysis.

Authors: Sylvia Kusemererwa[1*], Eugene Ruzagira[1,3], Martin Onyango[1], Anita Kabarambi[4], Andrew Abaasa[2,3]

Institution

[1]Department of viral pathogen epidemiology and interventions, Medical Research Council/ Uganda Virus Research Institute and London School of Hygiene and Tropical Medicine, Uganda Research Unit, Entebbe, Uganda

[2]Department of statistics, Medical Research Council/ Uganda Virus Research Institute and London School of Hygiene and Tropical Medicine, Uganda Research Unit, Entebbe, Uganda

[3] Department of infectious diseases epidemiology, London School of Hygiene & Tropical Medicine, London, UK

[4] Department of research, International Center for Child Health and Development, Masaka, Uganda

Correspondence to: Sylvia Kusemererwa, [1]Medical Research Council/Uganda Virus Research Institute (MRC/UVRI) and London School of Hygiene and Tropical Medicine (LSHTM), Uganda Research Unit, Plot 51-59, Nakiwogo Road, P.O Box 49 Entebbe, Uganda. Phone: 256 (0) 417 704000, or 256 (0) 70229100. Sylvia.Kusemererwa@mrcuganda.org or kansykarus@gmail.com

Associations between intravaginal practices and incidence of sexually transmitted infections and bacterial vaginosis among women enrolled in the Dapivirine vaginal ring trial (The Ring Study) in southwestern Uganda, a retrospective secondary analysis.

Abstract

Objectives: We assessed associations between intravaginal practices (IVP) and the incidence of sexually transmitted infections (STIs) and bacterial vaginosis (BV) among women using the dapivirine vaginal ring (DVR) or placebo vaginal ring in southwestern Uganda.

Methods: This was a retrospective secondary analysis of data collected from women at risk of HIV infection recruited into The Ring Study. The latter evaluated the safety and efficacy of the DVR between 2013 and 2016. At baseline, a behavioral questionnaire was administered to obtain information on sexual activity and IVP (exposure) defined as; insertion inside the vagina of any items aimed at cleaning the vagina for any reason before, during or after sex other than practices to manage menses. Each participant self-inserted the DVR/placebo and replaced it every 4weeks for 2 years. Outcomes were diagnosis of STIs i.e., *Chlamydia trachomatis* (CT), *Neisseria gonorrhea* (NG), *Trichomonas vaginalis* (TV), HIV and BV. The incidence rate of STI/BV was estimated, overall, by IVP and trial arm in single-event-per-participant and multiple-event-per-participant analyses.

Results: Of the 197 women enrolled, 66 (33.5%) were <25 years of age. Overall, 93 (47.2%) practiced at least one form of IVP. During follow up, 172 (87.3%) women were diagnosed with an STI/BV at least once. Majority had TV (73.6%, n=145). Overall rate of STI/BV was 51.9/100 person-years, 95% confidence interval (CI): 44.7-60.3 [IVP: Yes, 51.0 (40.8-63.8) vs. No, 52.6 (43.0-64.4)]. IVP were not statistically significantly associated with rate of individual STIs/BV. Similar results were observed when the analyses were conducted separately for each trial arm.

Conclusions: IVP was not associated with risk of STIs/BV in The Ring Study.

Key words: Intravaginal practices, sexually transmitted infection, Bacterial vaginosis,

Dapivirine, Vaginal Ring, Uganda

Study strengths and limitations

- The study was able to investigate multiple STIs and BV in the same population.
- The longitudinal nature of the study allowed for both single-event-per-participant and multiple-event-per-participant analyses.
- Extensive analysis was to some extent limited by a small sample size.
- Confirmatory tests were not done to confirm absence of an STI/BV prior to DVR insertion.

INTRODUCTION

Sexually transmitted infections (STIs) remain a health challenge globally. In 2018, WHO estimated that nearly one million people become infected every day with a curable STI caused by: *Chlamydia trachomatis* (CT), *Neisseria gonorrhea* (NG), Syphilis or *Trichomonas vaginalis* (TV).[1] STIs disproportionately affect low-and-middle-income countries, with 90% of the new infections occurring in these countries.[2] Women in sub-Saharan Africa have a high prevalence of STIs, particularly those at high risk of HIV infection also having a high STI burden.[3, 4] Bacterial vaginosis (BV), a common vaginal condition has also been associated with increased risk of STIs and HIV infection. [5] High rates (37% to 68%) of BV have been reported among women at high risk of HIV acquisition in Southern and East Africa. [6]

BV results from variation in normal vaginal flora attributed to reduction in the prevalence of Lactobacilli (dominant species in healthy vaginal environment) and an increase in the concentration of pathogenic organisms: *G. vaginalis*, *Bacteroides (Prevotella)* species, *Mobiluncus* species, and *Mycoplasma hominis*. [7, 8] Increasing evidence shows that vaginal microbiota may play a role in mediating susceptibility to STIs. Vaginal Lactobacilli utilize a number of actions to protect against colonization by genital pathogens. [9] This can be through: production of lactic acid that supports the maintenance of a lower vaginal pH, which may prevent pathogen growth, [10] exposure to hydrogen peroxide that has also been shown to decrease activity of BV and other genital tract organisms. [11, 12]

The high prevalence and increased risk of acquisition of STIs/BV and HIV has been associated with vaginal practices.[13] These practices include various behaviors used to maintain health, wellness, and enhance sexual pleasure.[14-16] The World Health Organization (WHO) has suggested seven classifications for vaginal practices: external washing, intravaginal cleansing, external application, intravaginal insertion, oral ingestion, vaginal streaming or smoking and anatomical modification.[17] On the other hand intravaginal practices (IVPs) refer to both intravaginal cleansing (cleaning or washing inside the vagina with fingers or substances like soap to remove fluids), and intravaginal insertion (placing something inside the vagina, like powders, creams, herbs, or tissue).[18, 19]

Soaps, detergents and antiseptics used to cleanse inside the vagina can cause chemical damage and increase vaginal pH resulting in overgrowth of BV related organisms, which has also been associated with increased risk of HIV acquisition.[19, 20] Other products have also been reported to cause genital lesions, and swellings, creating favorable conditions for the transmission of STIs, including HIV.[17] Items like cloth commonly used in some communities to clean the vagina repeatedly might also act as fomites, carrying TV organisms. [21] TV has also been associated with increased risk of HIV acquisition. [22, 23] These practices which increase a woman's susceptibility to HIV could reduce the effectiveness of vaginal microbicides. [24, 25]

The monthly dapivirine vaginal ring (DVR) microbicide, a female controlled HIV prevention tool, was found to reduce the risk of HIV acquisition by approximately 30% in two phase 3 trials. [26, 27] With continued and consistent use, the risk of HIV acquisition was even lower (62%) in an open-label extension trial. [28] However, data are limited on the effect of intravaginal practices on vaginal flora and risk of STIs/BV among women using the DVR. We assessed associations between intravaginal practices and the incidence of STIs including HIV, and BV among women using the DVR or placebo vaginal ring in southwestern Uganda.

METHODS

Study design

This was a retrospective secondary analysis using data collected in The Ring Study, a phase 3 microbicide trial. [26]

Study setting and population

Details of the Ring Study (Registration number: NCT01539226) have been described elsewhere. [26] Briefly, The Ring Study was a multicenter microbicide trial, that evaluated the safety and efficacy of the DVR between 2013 and 2016 in Uganda and South Africa. The study was sponsored by the International Partnership for Microbicides. In Uganda, the study was conducted by the Medical Research Council (MRC)/Uganda Virus Research Institute (UVRI) and London School of Hygiene and Tropical Medicine (LSHTM) Uganda Research Unit in Masaka, southwestern Uganda. The site recruited women at high risk of HIV infection from towns along the trans-African highway and the shores of Lake Victoria. Details of the recruitment procedures

and study population have been described elsewhere. [29] Briefly, the research site enrolled 197 women (18-45years of age) at high risk of HIV infection. Women were identified from sex work hotspots (bars, restaurants, hair salons, small shops, and other small-scale businesses). High risk was defined by presence of any two of the following: i) history of STIs in the past three months; ii) self-reported condom less sex with multiple sex partners or a new partner in the past three months; and iii) use of recreational drugs (marijuana, alcohol) in the past three months. Of the women enrolled, two in five were working in bars and restaurants and nearly a third had small scale businesses. A woman was included in the main study if they were not pregnant, not breastfeeding, asymptomatic for genital infections and tested HIV negative at the time of enrolment. Those diagnosed with any clinically significant curable STI, were initiated on treatment for at least a week prior and enrolled after completing a full course of treatment.

Study procedures

Participants consented for screening and enrolment on two separate occasions. At the first visit (screening), data on demographics, inclusion and exclusion criteria were obtained. A physical and genital examination was done. All potential participants were provided with HIV/STI risk-reduction counselling and HIV pre- and post-test counselling. At the second visit (enrolment), women who met all inclusion criteria and no exclusion criteria, had a normal pelvic examination, and negative HIV rapid tests were enrolled into the trial. Eligible women were randomised in a 2:1 ratio to either the DVR arm or placebo arm. At the enrolment visit, an interviewer-administered behavioral questionnaire was used to obtain baseline information on sexual activity and vaginal practices. At 4 weeks post-enrolment and every 24 weeks thereafter for the next 2 years, follow-up data on vaginal practices were collected using an interviewer-administered behavioral questionnaire. The latter included questions to which participants replied by selecting from a variety of pre-specified responses and provided open-ended responses about their IVP. Participants self-inserted a vaginal ring every 4 weeks for up to 104 weeks.

Measurement of exposure [intravaginal practices (IVP)]: IVP was defined as insertion inside the vagina of any items aimed at cleaning the vagina for any reason before, during or after sex other than practices to manage menses. Items included: materials such as paper, cloth, or cotton

wool; water only; water and soap; fingers to clean or insert something. Women were asked if they were inserting or using any of the aforementioned items inside the vagina to clean their vagina either as a general cleaning/hygiene practice before or after sex or to prepare the vagina for sex in the past, at baseline and every 24 weeks.

Measurement of outcome (diagnosis of STIs/BV): Cervico-vaginal swabs were collected at the first screening visit and every 12 weeks (3 months) for 2 years. The swabs were tested for TV (OSOM® Trichomonas Rapid test- Sekisui Diagnostics, LLC, USA) and CT/NG (Cobas® Amplicor CT/NG -PCR test, Roche Diagnostic Systems, Branchburg, New Jersey). Vaginal fluid samples were also collected by trained clinicians using sterile swabs for assessment of vaginal flora (using Nugent's score) and vaginal fluid pH at the enrolment visit (prior to ring insertion) and every 12 weeks. Samples with a score of ≥7 were classified as BV present. Each slide was scored by trained laboratory technologists. For internal quality control, a single batch of slides were re-examined by an independent reader weekly. Discrepant results were resolved by expert consensus. External quality control was assured using College of American Pathologists Vaginitis screen, vaginal gram stain-VS2 as part of the site standard operating procedures.

Participants were tested for HIV at the screening and enrolment visit using whole blood samples collected by venipuncture. Serial rapid HIV antibody tests were done using Alere Determine™ HIV-1/2 (Alere, Medical co., Ltd, Matsuhidai, Matsudo-shi, Chiba, Japan) followed by OraQuick- ADVANCE® Rapid HIV-1/2 Test (OraQuick-OraSure Technologies Inc, Pennsylvania) to confirm a positive Determine result and Uni- Gold™ HIV (Trinity Biotech, Ireland) as the tie breaker. At the screening/enrolment visits, a participant was confirmed to have HIV infection if they tested positive on at least two rapid HIV antibody tests. Post-enrolment, HIV testing was done serially as described above. However, for participants who tested positive or discordant on two rapid HIV antibody tests, a confirmatory test on stored plasma was done using Western Blot (J. Mitra and Co.Pvt. ltd, India) as previously described. Blood samples were collected and plasma stored every 4 weeks. Stored plasma samples for participants with confirmed HIV infection were retrospectively tested at a central laboratory in South Africa (Bioanalytical Research Corporation) for HIV ribonucleic acid (RNA) copies (viral load) using the polymerase-chain-reaction (PCR) assay. [26]

Statistical analysis

Data analyses were performed in Stata version 15.0 (Stata Corp, College Station, TX, USA). Participants' baseline characteristics were summarized using frequencies and percentages overall and by IVP status and compared between IVP users and none users using a chi-squared test. We determined the proportion of participants that were positive for a given STI/BV (event) as the number who tested positive for an STI/BV at least once during the study divided by the total number tested. We determined associations between IVP and incidence of each STI/BV, by estimating the rate of STI/BV overall and stratified by IVP status. We used two approaches for measuring the rate of STI/BV; (a) a single-event-per-participant (allowing for one event per participant-first STI/BV event) and (b) a multiple-event-per-participant (allowing for two or more STI/BV events for the same participant) since these are recurrent events. The incidence rate of a given STI/BV was estimated as the number of participants who tested positive for STI/BV divided by the person-time (years) at risk (pyr) expressed as per 100 pyr. PYR were estimated as a sum of the time from enrolment into the trial (those negative at baseline) to the date of trial completion or censoring (trial end, end of ring use and their first event for a given outcome). Participants that tested positive for STI/BV at baseline were given treatment and started to contribute person-time after completing the course of treatment. Similar approach was followed for those that got infected during follow up though the person time was segmented to allow for multiple entry and exit from the analysis following treatment. We further adjusted the effect of IVP on rate of STI/BV for age and baseline STI/BV status by fitting Poisson regression models. In the analysis, we used Poisson regression model with time-varying covariates, allowing for intergroup correlation (because women had multiple records) by using cluster robust standard errors. For HIV, we estimated the rate of HIV infection as number of HIV positive cases divided by the total person years at risk expressed as per 100 person years at risk in a single-event-per-participant survival analysis. Person time at risk were calculated as sum of the time from enrolment to the last HIV seronegative date (for those that remained negative throughout the trial) or to the estimated date of HIV infection. The HIV infection date was estimated as a multiple imputation random date between the last HIV seronegative and the first HIV+ result date.

Compliance with Ethical Standards

Approval was obtained from the Uganda Virus Research Institute Research Ethics Committee (Ref#-GC/127/13/03/33), the Uganda National Council of Science and Technology (Ref#-HS1362) and the National Drug Authority (Ref #-166/ESR/NDA/DID-07/2013). Written informed consent was obtained from each woman before any study procedures were performed. Women who tested HIV-positive were referred to an HIV care provider of their choice. Treatment was provided to those who tested positive for STIs/BV according to the Centers for Disease Control and Prevention (CDC) STD Treatment guidelines 2010. [30]

Patient and Public Involvement Statement

Communities where the study was conducted were involved from the inception of the study. Community gate keepers: Local Council leaders, political leaders, village health teams, community based and faith based organizations were informed of the study via various engagement meetings prior to study start. The site Community Advisory Board (CAB) members were engaged to review study documents, consent and other study literacy documents and confirm translations to the local language. The CAB was compensated for all the activities they were involved in. Volunteer recruitment and retention was supported by local leaders. Results were disseminated to study participants, CAB and community members through community meetings upon study completion and presentations at national and international meetings, seminars and conferences. All stakeholders were compensated for the time spent during engagement meetings.

RESULTS

Baseline socio-demographic characteristics

In total, 197 women enrolled, 67% on the DVR trial arm. Of those, 66 (33.5%) were less than 25 years of age, 82 (41.6%) were married and very few 24 (12.2%) had secondary school education. Majority, 162 (82.2%) reported having a main partner but only 75 (46.3%) lived with this partner (Table 1). About a half, 100 (50.8%) tested positive for an STI/ BV at baseline. Compared with

those who had one episode of STI/BV, participants with two or more episodes were likely to be single and never married (47.8% vs. 35.6%), not currently living with a main partner (56.5% vs. 37.5%, p=0.033) and inconsistently living with the main partner in the past year (69.6% vs. 41.7%; p=0.001) but otherwise similar in regard to other participant characteristics.

Table 1: Baseline socio-demographic characteristics of 197 women enrolled in The Ring Study in southwestern Uganda between 2013 and 2016

Variable	All	Intravaginal practices		p-value
		No	Yes	
	n (%)	n (%)	n (%)	
Overall	197	104 (52.8)	93 (47.2)	
Trial arm				0.835
Dapivirine vaginal ring	132 (67.0)	69 (66.4)	63 (67.7)	
Placebo	65 (33.0)	35 (33.6)	30 (32.3)	
Age (years)				0.275
18-24	66 (33.5)	30 (28.8)	36 (38.7)	
25-34	98 (49.8)	57 (54.8)	41 (44.1)	
35+	33 (16.7)	17 (16.4)	16 (17.2)	
Education level				0.276
Incomplete primary school	68 (34.5)	37 (35.6)	31 (33.3)	
Complete primary school	105 (53.3)	58 (55.8)	47 (50.5)	
Secondary school*	24 (12.2)	9 (8.6)	15 (16.1)	
Marital status				**0.020**
Single and never married	90 (45.7)	38 (36.5)	52 (55.9)	
Single but previously married	25 (12.7)	14 (13.5)	11 (11.8)	
Married	82 (41.6)	52 (50.0)	30 (32.3)	
Number of life time sex partners				**0.002**
Median (IQR)	6 (4-12)	5 (3-9)	8 (5-20)	
Has main partner				0.194
No	35 (17.8)	15 (14.4)	20 (21.5)	
Yes	162 (82.2)	89 (85.6)	73 (78.5)	
Duration lived with main partner (years)				0.690
<1	35 (21.6)	17 (19.1)	18 (24.7)	
1-2	34 (21.0)	19 (21.3)	15 (20.5)	
3+	93 (57.4)	53 (59.6)	40 (54.8)	
Lived with (main) partner in the past year				0.171
All the time	48 (37.8)	38 (42.7)	24 (32.9)	
Some of the time	14 (11.0)	7 (7.9)	12 (16.4)	
No	65 (51.2)	44 (49.4)	37 (50.7)	
Currently lives with main partner				0.376
No	87 (53.7)	45 (50.6)	42 (57.5)	
Yes	75 (46.3)	44 (49.4)	31 (42.5)	

Unprotected sex with multiple/new partner in the past 3 months				
No	38 (19.3)	18 (17.3)	20 (21.5)	0.456
Yes	159 (80.7)	86 (82.7)	73 (78.5)	
Drug/alcohol use in the past 3 months				
No	48 (24.4)	32 (30.8)	16 (17.2)	**0.027**
Yes	149 (75.6)	72 (69.2)	77 (82.8)	
Baseline STI/BV status				
TV				
Negative	121 (61.4)	61 (58.7)	60 (64.5)	0.399
Positive	76 (38.6)	43 (41.3)	33 (35.5)	
NG				
Negative	173 (87.8)	92 (88.5)	81 (87.1)	0.770
Positive	24 (12.2)	12 (11.5)	12 (12.9)	
CT				
Negative	177 (89.8)	97 (93.3)	80 (86.0)	0.093
Positive	20 (10.2)	7 (6.7)	13 (14.0)	
BV				
Negative	191 (96.9)	102 (53.4)	89 (46.6)	0.332
Positive	6 (3.1)	2 (33.3)	4 (66.7)	

n=Number; *Includes one woman who had greater than secondary education, TV- *Trichomonas vaginalis,* NG-*Neisseria gonorrhea*, CT-*Chlamydia trachomatis*, BV-Bacterial vaginosis

Proportion of women reporting intravaginal practices (IVP)

Ninety-three (47.2%) women reported at least one form of IVP. The commonly used substances to clean the vagina included: soap (n=76, 81.7%), cloth (n=5, 5.4%), and others (honey, herbs, perfume n=12, 12.9%). Reported IVP were more common among single and never married women compared to single but previously married or currently married women (p=0.020), among those with more sex partners (p=0.002) and those who used drugs/alcohol in the past 3 months (p= 0.027) (Table 1).

Proportion and rate of STIs/BV

A total of 172 (87.3%) women were diagnosed with an STI/BV at least once during follow up, with an overall single-event-per participant incidence rate of 51.9 per 100 pyrs. The overall incidence rate for HIV was 5.8 per 100 pyrs. The most common STI was TV (73.6%, n=145/197 diagnosed at least once) with a rate of 92.7 per 100 pyrs in the single-event-per-participant analysis (Table 2).

Associations between IVP and rate of STIs/BV

In the single-event-per-participant analysis, STI/BV and HIV rates were not associated with reported IVP (Table 2). However, in the multiple-events-per participant analysis, the rate of CT was statistically significantly lower among women who reported IVP versus those who did not (p=0.030). On stratification by trial arm, the rate of NG was higher in the DVR arm compared to the placebo arm in both the single and multiple-events-per participant analysis among women not using IVP (p=0.024 and p=0.007 respectively) (Table 3). After adjusting for participant baseline characteristics, overall, in the multiple-event per participant analyses, IVP was only associated with lower rates of CT among women in the placebo arm [adjusted rate ratio (aRR)=0.33, 95% CI 0.14-0.78] (Table 3).

Table 2: Associations between intravaginal practices and rate of sexually transmitted infection/Bacterial vaginosis among women in the Ring Study in southwestern Uganda

Sexually transmitted infection/condition	Single-event-per-participant analysis					
	Overall	Intravaginal practices				
		Yes	No			
	Rate/100 PYR (95% CI)	Rate/100 PYR (95% CI)	Rate/100 PYR (95% CI)	p-value*	uRR (95% CI)	aRR (95% CI)
HIV	5.8 (3.0-11.1)	7.0 (2.9-17.0)	4.7 (1.8-12.4)	0.278	1.51 (0.39-5.81)	1.29 (0.29-5.67
Trichomonas vaginalis	92.7 (78.7-109.0)	97.4 (76.9-123.3)	88.7 (70.9-111.1)	0.288	1.10 (0.79-1.52)	1.10 (0.67-1.63)
Neisseria gonorrhea	23.4 (18.3-29.9)	22.8 (15.9-32.9)	23.8 (17.0-33.4)	0.435	0.96 (0.58-1.57)	0.99 (0.56-1.79)
Chlamydia trachomatis	28.4 (22.7-35.6)	27.9 (20.2-38.8)	28.9 (21.2-39.4)	0.446	0.97 (0.62-1.52)	0.83 (0.50-1.38)
Bacterial vaginosis	14.1 (10.6-18.6)	13.4 (8.9-20.4)	14.7 (10.1-21.4)	0.383	0. 92 (0.52-1.61)	1.13 (0.53-2.37)
Multiple-events-per-participant analysis						
Trichomonas vaginalis	88.9 (79.6-99.5)	90.2 (76.8-106.0)	87.9 (75.3-102.5)	0.406	1.03 (0.77-1.37)	1.07 (0.80-1.43)
Neisseria gonorrhea	28.4 (23.3-34.6)	28.7 (21.5-38.1)	28.2 (21.5-37.0)	0.935	1.02 (0.61-1.69)	0.99 (0.59-1.67)
Chlamydia trachomatis	43.6 (37.2-51.1)	36.6 (28.4-47.1)	49.9 (40.7-61.2)	0.030	0.73 (0.45-1.20)	0.64 (0.38-1.05)
Bacterial vaginosis	18.8 (11.1-31.7)	12.1 (4.5-32.2)	24.1 (13.0-44.8)	0.124	0.51 (0.13-1.95)	0.48 (0.16-1.47)

PYR = person-years at risk; CI = Confidence interval; aRR = adjusted rate ratio; ¶Adjusted for age, trial arm, STI/BV at baseline, * Unadjusted p-value comparing the rate of each STI between IVP use and none use

Table 3: Associations between intravaginal practices and rate of sexually transmitted infection/Bacterial vaginosis stratified by trial arm among women in the Ring Study in southwestern Uganda

Single-event-per-participant analysis						
Sexually transmitted infection/condition	**Overall**	**Trial arm**				
		DVR	**Placebo**			
	IVP use	**Rate/100 PYR (95% CI)**	**Rate/100 PYR (95% CI)**	**p-value***	**aRR (DVR) (95% CI)**	**aRR (Placebo) (95% CI)**
HIV	No	3.3 (0.8-12.9)	8.4 (2.1-33.8)	0.374	-	-
	Yes	8.8 (3.3-23.5)	3.9 (0.6-27.9)	0.518	-	-
Trichomonas vaginalis	No	77.5 (58.4-102.8)	118.3 (81.6-171.3)	0.081	1	1
	Yes	105.8 (79.7-140.4)	82.4 (53.7-126.4)	0.171	1.34 (0.78-2.31)	0.59 (0.28-1.26)
Neisseria gonorrhea	No	30.5 (21.1- 44.2)	11.8 (5.3-26.2)	0.024	1	1
	Yes	20.6 (13.0-32.6)	28.0 (15.5-50.5)	0.211	0.73 (0.35-1.53)	1.88 (0.49-7.20)
Chlamydia trachomatis	No	28.5 (19.4-41.9)	29.5 (17.5-49.8)	0.911	1	1
	Yes	29.7 (20.2-43.6)	24.3 (13.1-45.2)	0.305	1.09 (0.60-2.00)	0.37 (0.11-1.36)
Bacterial vaginosis	No	13.7 (8.6-22.1)	16.5 (8.9-30.7)	0.636	1	1
	Yes	12.6 (7.5-21.3)	15.2 (7.6-30.3)	0.332	0.74 (0.26-2.09)	1.51 (0.35-6.52)
Multiple-events-per-participant analysis						
Trichomonas vaginalis	No	87.2 (72.2-105.3)	89.2 (68.3-116.5)	0.558	1	1
	Yes	94.4 (77.9-114.2)	81.5 (60.5-109.9)	0.213	1.12 (0.80-1.56)	0.76 (0.44-1.30)
Neisseria gonorrhea	No	34.7 (25.8-46.8)	14.9 (7.7-28.6)	0.007	1	1
	Yes	25.2 (17.4-36.4)	36.0 (23.0-56.5)	0.116	0.74 (0.42-1.30)	2.24 (0.61-8.31)
Chlamydia trachomatis	No	56.5 (44.7-71.5)	36.3 (24.0-55.2)	0.065	1	1
	Yes	40.4 (30.2-54.2)	28.4 (17.1-47.2)	0.126	0.75 (0.43-1.29)	0.33 (0.14-0.78)
Bacterial vaginosis	No	20.5 (9.2-45.7)	32.7 (12.3-87.0)	0.482	1	1
	Yes	12.5 (4.0-38.7)	11.1 (1.6-78.7)	0.491	0.64 (0.11-3.71)	0.39 (0.10-2.72)

PYR = person-years at risk; CI = Confidence interval; uRR = unadjusted rate ratio; aRR = adjusted rate ratio; ¶Adjusted for age, and STI/BV at baseline, * compares rates for IVP (yes or no) between DVR and placebo

DISCUSSION

Our study aimed to assess associations between IVP and incidence of STIs including HIV, and BV among women using the DVR or placebo. Overall, we found that nearly one in every two women practiced at least one form of IVP. IVP is reported to be high in African women with proportions of between 30-50%, and even higher among women at high risk of acquiring HIV.[31, 32] Generally, IVP in this population is driven by cultural and social norms as well as the need for personal hygiene in relation to sexual health and relationships.[33] Women recruited in our study were those engaging in transactional sex and are thus expected to present themselves to their male partners in a fresh vaginal state.[34, 35] The frequency of IVP amongst women involved in transactional sex may be influenced by the need to remain clean/fresh coupled with worries about HIV infection. Prior studies in Uganda and Tanzania showed that a higher frequency of sex was associated with more frequent engagement with IVP. [25, 36, 37]

The prevalence of STIs/BV in our study population was high confirming the fact that the women were at very high risk of HIV infection. More events were reported among single women and those who did not live with a main partner and those with more sex partners. Women at high risk of HIV infection engage in transactional sex and have multiple partners, that puts them at higher risk of acquiring STIs/BV. Those who were single also engaged more in IVP. Earlier studies showed that women engaging in high frequency of sex require to present themselves as clean to their male partners. [14, 34] IVP is generally practiced for hygiene purposes and sexuality. [15, 16] IVP has been associated with changes in vaginal flora and resulting BV. The latter is associated with increased susceptibility to STIs including HIV. [17] The interaction between BV and STIs including HIV has been well documented with each causing genital inflammation. [38] It is reported that organisms associated with BV may overgrow as a result of increased vaginal pH brought about by substances that may be used for IVP like soaps which most of the women in our study used. With increased pH, the protective Lactobacilli species are replaced by pathogenic organisms resulting in BV. [19, 20] The products used may cause genital lesions enabling the transmission of STIs. [17]

We observed a high incidence of STIs in this population, with the commonest STI being TV. TV is reported to be prevalent among women engaging in transactional sex with high proportions reported globally (16%) and the African region contributing even higher proportions (23%).[39] TV has also been associated with BV especially among women that use cloth for IVP as these act as fomites.[21]

Although the incidence of STIs was high, we did not see any significant difference in the rise in incidence of STIs/BV among women using IVP compared to those who did not, except for CT that was lower. It is not clear and has not been documented that IVP reduces the risk of acquiring STIs/BV, though the reverse has been reported. IVP causes changes to vaginal flora resulting in increased risk of STIs/BV including HIV. A recent systematic review found that IVP increased the risk of vaginal infections (BV, TV, and vulvovaginal candidiasis),[40] two others found no association between IVP and TV[15] or BV.[41] One study in South Africa found that IVP was associated with increased risk of HIV infection but not other STIs.[42] The fact that IVP results in BV, organisms related with BV have been associated with the production of metabolites that are used by STIs like CT as growth factors enabling their multiplication.[43] Incidence of CT has been reported to be high among women (5 per 100pyrs), especially among younger women (27.6 per 100 pyrs) in Kenya, but not associated with vaginal washing.[44]

We also found that women not using IVP, but using the DVR had higher rates of NG compared to those in the placebo arm. Women in this study generally had high rates of STIs. We have previously reported that rates of STIs decreased over time in the same cohort of women. [45] Apart from lower rates of CT among women using the placebo vaginal ring, there was no statistically significant association between the DVR microbicide and STI/BV including HIV rates among those using IVP. The DVR has been associated with minimal changes in the vaginal microbiota that were likely not clinically significant.[46] It has generally been found to be well tolerated in adult as well as adolescent girls and young women.[47, 48] Various studies on microbicides have reported that their use may result in little or no difference in the risk of acquiring STIs like CT, NG or TV.[49] In the Buffer Gel microbicide study for example, no significant changes in colonization with Lactobacillus species was reported.[50]

The strengths of this study included the opportunity to investigate multiple STIs and BV in the same population and the ability to collect recurrent data on genital conditions which allowed for conduct of both single-event-per-participant and multiple-event-per-participant analyses. The multiple-event-per-participant analysis models the total rate of events over the entire follow-up period and has more power for detecting associations compared to the single-event-per-participant analysis. [51] This analysis is also more clinically relevant because STI re-infection and BV recurrence are common.

A limitation of the study was the small sample size which may have impacted the study power and consequently the ability to detect any differences in STI/BV rates between women who reported IVP

and those who did not. Additionally, no confirmatory tests were done to confirm absence of an STI/BV prior to DVR insertion. However, duration of treatment and absence of symptoms and signs were used as a proxy for lack of an STI/BV. Furthermore, it was not possible to measure and evaluate any versus no IVP. This is challenging to do as different types of IVP and materials used affect the risk of HIV, STI and BV risk. This has also not been possible to measure in previous studies.

In conclusion, we found a high prevalence of IVP and incidence of STIs/BV among women enrolled in the Ring Study in Uganda. IVP did not statistically significantly increase STI/BV rates. Implying that women who practice intravaginal cleansing/insertion could continue using these practices in the presence of the microbicide. However, our results should be interpreted with caution because of a limitation in sample size that could generate a hypothesis and not conclusively test it. An analysis with a bigger sample size could be helpful to better understand whether there is a link between use of the DVR, IVP, and incidence of STIs/BV.

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Author Contributions:

SK, AA, ER designed the study and wrote the initial manuscript draft, and AA did the data analysis. KA and MO conducted the study while SK directed the work. KA and MO contributed to the writing

and editing of the manuscript. All authors contributed to the interpretation of the results and critically commented and provided revisions to the manuscript. All authors approved the final version of the manuscript. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

Data availability statement: Data are available on reasonable request.

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STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4, 5
Objectives	3	State specific objectives, including any prespecified hypotheses	4, 5
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5, 6
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	5, 6
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6, 7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6, 7
Bias	9	Describe any efforts to address potential sources of bias	6, 7
Study size	10	Explain how the study size was arrived at	5, 6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6, 7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7
		(*b*) Describe any methods used to examine subgroups and interactions	NA
		(*c*) Explain how missing data were addressed	NA
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	NA
		(*e*) Describe any sensitivity analyses	7

Continued on next page

Results			**Page**
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	9, 10, 11
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9, 10, 11
		(b) Indicate number of participants with missing data for each variable of interest	NA
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	11, 12, 13, 14
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	11, 12, 13, 14
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			
Key results	18	Summarise key results with reference to study objectives	15, 16
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	16,17
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	17
Generalisability	21	Discuss the generalisability (external validity) of the study results	17
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	17

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.