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Menstrual cups to reduce bacterial vaginosis and STIs through reduced harmful sexual and menstrual practices among economically vulnerable women: protocol of a single arm trial in western Kenya

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Abstract

Background In western Kenya, menstrual hygiene management (MHM) is a pervasive problem. Challenges are compounded for economically constrained women who continue to engage in sex during menses and resort to practices such as vaginal insertion of tissue and cotton to maintain dryness during sex. These practices can be harmful to the vaginal microbiome (VMB) and can lead to high rates of sexually transmitted infections (STIs) and HIV. This study will evaluate whether menstrual cups that can be worn during intercourse may be beneficial to the VMB and help prevent Bacterial vaginosis (BV) and STI acquisition among these economically vulnerable women.

Methods In this single-arm trial among economically vulnerable women in semi-urban western Kenya, we will evaluate the preliminary efficacy of menstrual cups on non-optimal VMB, BV, and STIs, and investigate safety, acceptability, and implementation needs. Through peer referral we aim to recruit 402 menstruating women aged 15–35 who exchange sex for money or basic needs. Women who are pregnant, have delivered in the past six months, or use an intrauterine device (IUD) will not be eligible. Participants will be seen every six months for 24 months and be asked about their sexual and MHM practices, with samples collected to assess BV and VMB. At baseline, 12-, and 24-month visits, additional samples will be collected to measure HIV and STIs (*C. trachomatis*, *N. gonorrhoeae*, and *T. vaginalis*). HSV-2 status will be assessed at baseline. Intervention provision will consist of one reusable disc-shaped menstrual cup per participant and a group-based training within four weeks of the 12-month visit, followed by monthly telephone surveys for the first three months to assess cup use, adverse events, and provide any assistance. Primary analyses of preliminary efficacy will compare probabilities of optimal VMB, BV, and STIs in the pre-intervention period to the post-intervention period. Primary safety analyses will compare occurrence of menstrual toxic shock syndrome and cervicovaginal laceration.

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Discussion If demonstrated safe and effective, this multipurpose reproductive health intervention will offer a dignified solution for the menstrual hygiene needs of women who engage in sex for livelihood and reduce their occurrence of non-optimal VMB, BV, and STIs.

Trial registration Clinicaltrials.gov NCT05666778 (28th December, 2022); Pan African Clinical Trials Registry 202,305,912,778,108 (25th May, 2023).

Keywords Sexual and reproductive health, Vaginal microbiome, BV, STIs, Menstruation, Menstrual health and hygiene, Menstrual disc, Menstrual cup, Clinical trial

Background

Economically vulnerable women face a disproportionate burden of sexually transmitted infections (STIs) and HIV [1–3]. Six in seven new HIV infections in sub-Saharan Africa are in adolescent girls and young women, and HIV risk is up to 30 times higher for women who engage in sex for economic livelihood than those in the general population [3–6]. Bacterial vaginosis (BV) affects 20–50% of women in sub-Saharan Africa [7], and doubles the risk of HIV acquisition and transmission [8–10], increases risk of adverse pregnancy outcomes [11–15], and increases risk of *Trichomonas vaginalis* (TV) [16, 17], herpes simplex virus-2 (HSV-2) [18], *Neisseria gonorrhoeae* (NG), *Chlamydia trachomatis* (CT) [19], and human papillomavirus (HPV) [20]. HIV and BV are syndemic with STIs, with high prevalences in Kenya [21–23], and higher still among women who engage in transactional sex [1, 24]. This study will be based in western Kenya, where the HIV prevalence is 12% among women in the general population and 29% among women who exchange sex for livelihood [25]. HSV-2 prevalence in the area is high at 27% among 15–19 year-old females to 73% among 20–24 year-old [26]. Multipurpose interventions that can effectively address multiple contributing factors to STIs and HIV risk are critically needed in these populations.

Studies are investigating improved products for menstrual hygiene management (MHM) as possible multipurpose interventions to manage menstruation and to also improve girls' and women's sexual and reproductive health [27–31]. Accessibility of hygienic products remains a pervasive problem across low- and middle-income countries (LMIC), with detrimental effects for women's reproductive health [32, 33]. In one study in western Kenya, 25% of women reported using traditional materials (e.g., rags, cloths, cotton wool, grass, tissue, and newspaper) for MHM [34]. Commercial disposable MHM products are expensive and often unavailable in rural and peri-urban areas in LMIC [35]. Poor disposal facilities and inadequate environments for cleaning and drying reusable products can increase infection risk and leave women and girls with few dignified options to manage their menses [33, 35–37]. These challenges are compounded for women who engage in sex for their livelihood who continue having sex during menses, and often resort to using sub-optimal absorbents intravaginally (i.e., tissue, cotton wool, mattress stuffing, sponges) to hide their bleeding [38]. These practices and increased intravaginal washing during menses have been associated with self-reported vaginal discharge, chronic infections, pain during intercourse, and bad odours [38, 39]. Studies have also found these practices lead to higher reproductive tract harms, and increases in BV and HIV infections [40, 41]. McKinnon and colleagues found that among 1,640 females engaging in sex for livelihood in Nairobi, Kenya, sex during menses was common (40%), and HIV acquisition was 6-fold higher (adjusted Hazard Ratio = 6.19) for those who engaged in sex during menses [42]. Hygienic MHM solutions for this population are urgently needed.

Menstrual cups have been evaluated as cost-effective, reusable products to tackle some of these challenges [29, 30, 43, 44]. These bell- or disc-shaped receptacles are inserted vaginally to collect menstrual flow, and for women needing to work while menstruating, they have a number of notable advantages: they are a discrete option for menstrual blood collection (i.e., undetectable to others), are durable and can last up to 10 years, can reduce odour, do not require extensive cleaning or open-air drying, and some can be used during sex [45]. Some users and manufacturers refer to menstrual cups that can be worn during sex as menstrual discs. A meta-analysis of 15 studies found that 95.6% of women continued using their menstrual cups post study, and reported positive effects on participants' lives, decreased stress concerning leakage and staining, and improved mobility [45]. Moreover, evidence is building that menstrual cups may provide biological protection to the female reproductive tract, leading to reductions in BV, HSV-2, and STIs [43, 44]. Our prior study among secondary schoolgirls in western Kenya, found that among those given a menstrual cup relative to girls in the control group using their usual MHM products, there was a 24% reduced odds of BV, a 37% increased odds of having an optimal *Lactobacillus crispatus* dominated vaginal microbiome (VMB), and a higher relative abundance of *L. crispatus* [44].

In this study, we hypothesize that provision of soft disc-shaped menstrual cups that can be worn during sex will result in reduced occurrence of non-optimal VMB, BV,

and STIs, independent of sexual practices, in economically vulnerable women engaging in sex for livelihood in western Kenya.

Methods

Hypothesis and specific aims

This study seeks to evaluate the preliminary efficacy of menstrual cups that can be worn during sex on BV, non-optimal VMB, and STIs of women engaged in sex for livelihood in western Kenya, to assess the cups' safety profile, and to understand implementation needs. This study hypothesizes that among women who rely on sex for livelihood, provision of menstrual cups that can be worn during sex will result in reduced occurrence of BV, non-optimal VMB, and STIs, independent of sexual practices.

Specifically, the study aims are:

- Aim 1: To evaluate the preliminary efficacy of menstrual cups on BV, VMB composition, and the incidence of STIs.
- Aim 2: To conduct integrated surveillance on cup safety endpoints, pharmacovigilance for cup contamination, and the associated water, sanitation, and hygiene facilities.
- Aim 3: To conduct qualitative study (a) for integrative analysis of biological and behavioural data, and (b) to identify constructs for successful menstrual health and hygiene (MHH) program implementation.

Study design

This trial will employ a single-arm cross-over interventional design. The study will enrol 402 women aged 15–35 in semi-urban western Kenya and follow them for two years. The first 12 months will constitute the control/pre-intervention phase, while the 12–24 month interval will form the intervention phase. While a randomized controlled trial (RCT) design is ideal for assessing and confirming efficacy, this design was chosen through consultation with community-based organizations working with the target population which advised an RCT would be unethical, as providing menstrual cups to some women and not others would be perceived as unfair, given their high unmet MHM needs. In this context, an RCT approach could lead to spillover effects (e.g., via sharing or taking menstrual cups) or unwillingness to complete study visits among those randomized to control. Moreover, because menstrual cups that can be worn during sex may address two potential mechanisms ([1] unhygienic MHM in general, and [2] unsafe vaginal practices to continue sex during menses), it is unclear what the appropriate control would be, as other menstrual products like pads or tampons would not address women's use of unsafe vaginal practices to continue sex during menses.

At baseline and every six months participants will be surveyed to document their health and sexual and menstrual practices, and vaginal swabs will be taken for VMB and BV assessments; STIs and HIV will be evaluated annually. After their 12-month visit, women will be trained on safe menstrual cup use and care and be provided one reusable menstrual cup that can be worn during sex. In the first three months following cup provision, participants will be telephoned monthly to determine cup uptake and troubleshoot any issues encountered.

In addition to the participant assessments, both during the control phase and during the intervention phase, physical aspects of sanitation infrastructure at work venues utilized by participants will be assessed by observation to understand critical components of their MHM environment and to identify important confounders and mediators to the interventions' effect. Lastly, using an implementation science framework, we will evaluate the implementation context and identify factors that optimize program uptake in this population, involving a multiplicity of stakeholders that provide social, medical, and financial support.

Eligibility and participant recruitment

Women aged 15–35 years who exchange sex for money or other items of value, who are menstruating (i.e., post menarche and premenopausal), and not currently pregnant or having delivered within six months are eligible. We will exclude women with intrauterine devices (IUDs) even if they are still menstruating, as risk of IUD expulsion is not insignificant and would possibly require trimming the IUD string.

Inclusion criteria

- Adolescent girls and women aged 15 to 35 years.
- Menstruation in the past two months.
- Having exchanged sex for money or items of value within the past two months.
- Living and/or working in the Kisumu area.
- Consent to participate.

Exclusion criteria

- Outside of eligible age range (< 15 years or > 35 years).
- Last menstrual period more than two months ago.
- Current pregnancy (as determined by urine hCG test).
- Pregnancy within the past six months.
- Presently having an IUD.

- Not living or working in study area.
- Refused consent.

Women will be recruited via Peers from Kisumu-based organizations (e.g., Keeping Alive Society's Hope and Kisumu Sex Worker Alliance) that provide support to women who engage in sex for livelihood, either as a dependency or to supplement income. At these organizations, women are provided HIV counselling and testing services, legal and peer support, and health referrals. Female Peers will provide potential participants with a brief study overview. Interested women will be given appointments to come to the study clinic for eligibility screening within four weeks. If eligible, women who choose to participate will provide written informed consent [see Additional file 2] and be enrolled into the study.

Study population and site

This study will be conducted in Kisumu, western Kenya. The city is located approximately 200 miles west from the capital Nairobi and is predominantly inhabited by the Luo ethnic group. Kisumu County is home to an estimated 1,155,600 individuals, with ~600,000 residing in the larger metropolitan area of Kisumu City [46]; approximately half live in informal settlements [47]. While sex for economic livelihood is criminalized in Kenya, studies estimate there are over 530 active hotspots and 4,000 women engaged in sex for livelihood in and around Kisumu City [48–50]. The HIV prevalence among these women in Kenya is disproportionately higher than in the general population at 29.3% (vs. 4.5%), with higher incidence rates seen among those in western Kenya relative to other parts of the country [51].

Trial intervention and assignment

The menstrual cup is a medical-grade silicone bell or disc-shaped container that is inserted into the vagina to collect menstrual flow [45]. It can be used continuously throughout a menstrual period, requiring emptying at regular intervals, and lasts up to 10 years. Cleaning by boiling is recommended prior to and at the end of each cycle. There are both firm and soft cups: soft cups with no tail are wearable during sex and sit close to the cervix and are often referred to as "discs". The soft cup used in this trial has a capacity of 47.5mL and is wearable without emptying for up to 12 h. All participants enrolled into the study will receive a menstrual cup during the intervention period. Participants will be trained on the female reproductive health system, basic hygiene, and menstrual cup safe use and care. Participants will be instructed on methods of correct insertion, and discuss how to manage discomfort, prevent leakage, empty the cup, and clean and store the cup between uses. Participants will also be advised on what to do if the cup is dropped or damaged, and whether and how to talk about the cup to different types of sex partners, using messaging from the qualitative study.

Procedures

Participants' main study activities

Eligible participants who provide written informed consent will complete an interviewer-administered questionnaire via an Android tablet and answer individual questions on sociodemographics, MHM practices, sexual practices, and psychosocial health. During the intervention phase, participants will also be asked questions on menstrual cup use and acceptability. After completing the survey, a clinician will obtain participants' medical history, including medical and surgical history, medication use and known allergies, and any genitourinary symptoms using a standardized form. At baseline, 12- and 24-month visits a standardized medical examination will be conducted that includes a bimanual and speculum examination and physical examination to obtain vital signs, anthropometrics, review of systems (oral, head and neck, lymphatics, chest, abdomen, and skin), and detailed genital and pelvic examination. Four vaginal swab specimens will be collected for BV, VMB, CT/NG, and TV after insertion of the speculum and before bimanual examination. During the 6- and 18-month visits, participants will self-collect two vaginal swabs for the BV and VMB assessment unless they opt for a clinician to collect the specimen. After appropriate counselling, women who agree to self-collect vaginal swabs will be asked to wash their hands, and to then squat slightly and spread the labia with one hand while using the other hand to insert the swab 1–2 inches into the vagina. They will twirl the swab for 20 s [52], with the clinician assisting with time keeping by setting a digital timer. After collecting the sample, the participant will hand the swab to the clinician who will insert it immediately into the sterile sample collection tube. She will then proceed with collecting the second sample. For any participants reporting genitourinary symptoms during these 6- and 18-month visits, the same standardized genital examination conducted at baseline and annual visits will be advised by the study clinician.

Participants with signs or symptoms of STI will be provided appropriate pharmacologic therapy for free. If not treated at the initial point of care, women who test positive for BV, TV, CT, or NG will be asked to return to the clinic to receive their results, treatment, and counselling. Participants with complicated STI (e.g., pelvic inflammatory disease (PID) potentially requiring inpatient treatment) will be referred to the adjacent sub-County Hospital (i.e., 2 min walk) or nearby Provincial Hospital (~7 to 10 min drive) and transportation will be provided for emergent presentation.

Participants who give consent to HIV testing will receive individual, confidential HIV testing and counselling (HTC) following the Kenya Ministry of Health HTC Guidelines at baseline, 12-, and 24-month visits. Trained HTC counsellors will guide participants, conduct a rapid diagnostic test using a finger-prick blood sample [53], and provide results confidentially. Any participants who test positive for HIV will be referred and linked to comprehensive HIV care by the HTC counsellor.

During the intervention phase, participants will be asked to bring their menstrual cup to their study visit to be swabbed for assessment of the menstrual cup microbiome. Women will be asked about any problems with cup use.

Safety monitoring will be conducted to assess the relationship between the menstrual cup and the occurrence of any adverse events (AEs) or serious adverse events (SAEs). All events will be triaged and details on the nature of the event, date of onset, severity, corrective therapies given, and outcome will be collected. Clinical judgment will be used to assess causality (i.e., the likelihood that the event was potentially caused by use of the menstrual cup) and consider alternative causes, such as natural history of any underlying diseases, concomitant therapy, other risk factors, and the temporal relationship of the event to menstrual cup use. SAEs related to menstrual cups are defined as menstrual toxic shock syndrome with menstrual cup in situ or cervicovaginal laceration Grade 2 (i.e., moderate) or higher with the menstrual cup in situ, presenting within 48 h. Grade 2 laceration is defined as "large disruption extending through the mucosa or large superficial disruptions, hospitalization not indicated" [54]. Each potentially related SAE, as determined by the local PI, will be presented to the trial's Data and Safety Monitoring Board (DSMB) within 24 h via email, and evaluated by the DSMB within 72 h for final determination [see Additional file 1].

Halting Rule If SAEs related to menstrual cup use exceed 1%, the trial will be temporarily halted until consideration and recommendation by the DSMB and sponsor. If the DSMB determines that an SAE is not related to menstrual cup use it will not contribute to the 1% threshold.

Qualitative study

In addition to the main study visits, separate focus group discussions (FGDs) will be organized with participants, male clients, members of organizations and stakeholders who support women engaged in sex for economic livelihood (including non-governmental organizations, community-based organizations, county level Ministry of Health officials, providers of reproductive health services, and donors), and owners or employees of work places and affiliated structures. FGDs will include ~ 6–8 members of each group, conducted separately (e.g. FGDs on client perceptions will be composed of 6–8 male clients), be moderated by a female study staff member, take approximately 1.5 h, and be conducted in English, DhoLuo, or Kiswahili depending on participant preference. These will be audio-recorded with consent, and translated and transcribed verbatim for analysis.

Water, sanitation, and hygiene (WASH) assessments

During the control phase and again during the intervention phase, the physical aspects of the WASH facilities available to women at venues of transactional sex will be assessed, as an essential component of their MHM [55, 56]. We will assess the facilities' structure (e.g., stable slab, locking doors, functional lighting), cleanliness, and privacy, and the availability of water and soap, using a closed ended and standardized checklist (adapted from [57]).

Storing and processing biospecimen samples

The majority of specimens will be processed in Kisumu, though amplicon sequencing will take place at the Rush University Genomics and Microbiome Core Facility in Chicago, USA. All specimens will be logged and stored on site at the Universities of Nairobi, Illinois and Manitoba Research and Training Centre (UNIM) laboratory. A laboratory technician will ensure adequacy of sample preparation (e.g., checking smears for sufficiency), and that laboratory requisitions are correctly completed while clinicians and participants are all still present, in case of a need to repeat samples or other clarifications. Conditions of storage and processing will follow manufacturers' directions for specific assays. Specimen shipping will use a courier that maintains cold-chain transport.

Vaginal swabs will be immediately put into a sterile sample collection tube post collection. The laboratory technician will ensure that tubes are appropriately pre-labelled with study number and date of collection, prior to sample collection events. The first swab will be used for vaginal microbiome characterization. Additionally, for Aim 2 study on the menstrual cup microbiome (detailed below), during the intervention phase a female research assistant or clinician will swab the entire interior and exterior of the participant's menstrual cup for 30 s. Menstrual cup surfaces will be sampled using a flocculated swab pre-moistened with sterile buffer solution. While twirling continuously, the swab will be rubbed over the entire surface of the inside and outside of the menstrual cup, including attention to the rim. These microbiome swabs and cup specimens will be preserved using OMNIgene Vaginal microbial collection and stabilization kits (DNA Genotek), which maintain nucleic acid integrity at frozen to ambient temperatures (-20 °C to 30 °C) for up to 30 days. Specimens will be stored at

−80 °C at UNIM laboratory until shipment to the Rush University Genomics and Microbiome Core Facility (GMCF) in Chicago, USA. The genital microbiome will be profiled via 16s gene amplicon sequencing. Genomic DNA extracted from the specimens will be characterized using high-throughput amplicon sequencing of the near full-length microbial 16 S ribosomal RNA (rRNA) genes (e.g [58]). We will include DNA extraction and blank Polymerase Chain Reaction (PCR) controls. Sequencing will be performed on a PacBio Revio sequencer, implementing Kinnex chemistry. For microbial community structure analysis, sequences will be processed through an annotation pipeline implemented within the software package QIIME2 [59]. Reads will be processed to identify amplicon sequence variants (ASVs) generated by DADA2, and each ASV will be taxonomically classified using different approaches, including vSpeciateDB [60], VSEARCH [61] and a Naive Bayes classifier [62] with SILVA 138 [63], Refseq+RDP [64, 65], and GTDB r202 [66, 67] reference databases. Vaginal community state types will be assigned following a nearest centroid classification method [68]. A biological observation matrix (BIOM; [69]) will be generated at the lowest taxonomic level identifiable and used for alpha and beta diversity analyses.

The second vaginal swab will be used for Gram stain and morphological analysis to assess BV according to Nugent's criteria [70]. The Nugent score results will inform the treatment decision and will be used as outcome for statistical analyses. A 25% simple random sample of duplicate slide preparations will be read by a second laboratory technician for validation of morphological analysis. Slides that are discrepant to a degree that leads to different categorization of BV will require both technicians to read the slides together and come to consensus.

The third swab will assess NG and CT infections via GeneXpert qualitative real-time PCR test [71, 72]. Lastly, the fourth swab will be used to detect the presence of TV using rapid immunochromatographic assay (OSOM, by Seksui Diagnostics [73]). Serum specimens will be tested for HSV-2 immunoglobin G (IgG) antibody using Kalon IgG test [74]. Rapid screening tests will be used to test for HIV in accordance with the current Kenyan Ministry of Health Guidelines. While the algorithm is periodically updated and specific tests are changed based on Ministry of Health negotiations with donor agencies and purchasing, the current algorithm is sequential rapid tests (Determine, confirmation with First response HIV-1-2-0 Card, repeated, with a PCR tie break with ELISA 3rd Generation Bio-Rad).

Data management

Participants will use a study identification card during study visits. All data will be assigned a patient identifier and de-identified at the source to minimize risks of disclosure. Survey data will be collected on Open Data Kit on Android tablets. All STI and BV testing will be conducted in Kisumu at the UNIM laboratory. All laboratory information will be de-identified as specimens are only labelled with a study number, and laboratory personnel will be unable to link it with a particular study subject. Identifiers and all locator forms will be kept locked in Kenya at UNIM on hard copy records in a locked file cabinet in a locked office. All softcopy data will be directly stored on password-protected encrypted cloud servers with routine backup and only accessed by authorized individuals. Participant diagnosis and treatment forms will be manually recorded by a clinician-counsellor and entered into the study database for continuity of care at follow-up visits. Results will be disclosed to participants orally by a clinician-counsellor and provided in writing only at the written request of a participant.

Sample size considerations

Reports of vaginal and cervical lacerations or injury due to menstrual cup use are extremely rare; however, it is a priority to have power to detect SAEs with confidence. We require a sample of 402 women to detect SAEs with 0.5% probability of existence and 80–90% likelihood of occurrence, after accounting for 20% loss to follow-up (Required Sample Size to Detect a Problem in a Pilot, PASSv21). This sample will also provide ≥ 80% power (alpha = 0.05) to detect 20–25% reduction in BV during the intervention period compared to the historical control period (Single-State Phase II Clinical Trials [75], PASS v21). This was selected as an important and rational reduction in BV based on our findings of impact of menstrual cups in relation to BV among secondary school girls [76].

Outcomes

Primary outcome: Cumulative prevalence of BV (binary Gram stain Nugent score 7–10 vs. 0–6) in pre-intervention (baseline – 12 months) vs. post-intervention phase (12 months – 24 months).

Secondary outcomes:

- Aim 1: Comparison in pre-intervention vs. post-intervention phase of:
  - Cumulative incidence of STI (binary, composite of infection with CT, NG, and TV vs. no infection);
  - Occurrence of Community state type (CST) 1 dominated VMB (binary, CST-1 vs. other CST);

  - Continuous relative and absolute abundance of *L. crispatus*, *L. iners*, and *G. vaginalis*.

- Aim 2:

  - Adverse events (AE) and SAE: captured post-intervention distribution (via 1-, 2-, and 3-month post-intervention phone follow-ups, and at the 18- and 24-month in person visits). Those reporting pain during or after cup use will be asked to return to the clinic for a genitourinary exam, including speculum examination. Clinicians will record symptoms on a standardized closed form medical chart to record exam findings, which will include graphical mapping of vaginal and cervical findings. Other possible SAE and AE include: report of partner violence related to cup use, discomfort, and retained cups.
  - Relative abundance of putative pathogens (*E. coli*, *S. aureus*) detected in menstrual cup microbiomes;
  - CST of the cup microbiome;
  - WASH outcomes: "Acceptable" latrines will be defined as clean (no visible faeces or urine on floor), no strong/offensive smell, door and roof, no major holes in walls, stable floor slab. We will generate WASH scores ranging 0–3 based on the following observed data - [1] water available [2], soap available [3], acceptability of latrine - and compare across venues where women engage in sex for livelihood.

- Aim 3: Our qualitative outcomes will vary by time point (baseline, immediately post-intervention, and a year post-intervention), and relate to informing the intervention (baseline) or improving the intervention and context. In the first series, women's FGDs will assess implementation constructs related to anticipated intervention source, complexity, needs, resources and self-efficacy. Male FGDs will examine perspectives on menses, sex during menses, and thoughts on cup use during intercourse. These will support messaging during the training to help women know whether and how to discuss the menstrual cup with men. Subsequent outcomes will evaluate impact of the intervention on sexual practices, work conditions, well-being and safety in women, and men's observations and perceptions of menstrual cup use and impact on sexual practices and perceptions of health and safety. Finally, outcomes from post-intervention qualitative study with Ministry of Health officials, representatives from organizations that support women engaged in sex for livelihood, donors, and work places will assess constructs related to promotive settings (norms, expectations, availability of WASH and other resources), safety, and educational opportunities.

Analysis

The final analysis of this trial will follow a Trial Statistical Analysis Plan, which will be finalized prior to the completion of data collection. The anticipated analysis is as follows:

Analysis of Aim 1

For the primary BV outcome and secondary STI outcome, a linear mixed model (LMM; observations nested in individual) with a binomial distribution, log link function, and fixed effect for intervention time will be used to analyse a vector of pre- and post-intervention measurements as the outcome. The LMM will be evaluated under restricted maximum likelihood estimation in R (version 3.6.1; R Foundation for Statistical Computing, Vienna, Austria). For both the primary and secondary analyses, pre-specified confounders and sub-group analyses will include age and baseline HIV, HSV-2, BV, and STI statuses. Time varying confounders will include unsafe menstrual practices during sex, hormonal contraceptive use, number of sexual partners in the past 30 days, and work venue WASH score. Per protocol analysis will rely on self-reported cup use during the study. To verify cup use, we will examine cups for colour change, odour, and other signs of use at each study visit. For the secondary VMB outcome (the intermediate variable), change will be assessed using both targeted and non-targeted analyses. For targeted analyses we will conduct growth curve mixed-effects models, using maximum likelihood fitting to examine the change in measures over time for dependent variables. Random effects for subjects will be included in the model, which treats subjects as the cluster in which measures are nested. For non-targeted analysis of VMB changes over time, we will employ stability selection for feature selection to identify specific taxa associated with menstrual cup use. We will conduct mixed-effect analysis for individual taxa of importance, with a random effect for individual and fixed effect for intervention and other covariates. Complementary to VMB analyses, we will also describe the change in alpha diversity measures over time.

Analysis of Aim 2

Analyses for Aim 2 are descriptive, comparing frequency distribution of occurrence and type of SAE and AE before and after initiation of menstrual cup use, and will make use of pairwise statistical approaches (before-and-after), such as McNemar's test (for paired categorical data),

paired t-test (for normally distributed continuous data), or Mann Whitney test (for paired, non-normally distributed continuous or count data).

We will compare *E. coli* and *S. aureus* prevalence and relative abundance (separately) by user characteristics using LMMs that accommodate different outcome structures: multinomial (CST), binomial (presence), and continuous (relative abundance). User characteristics will include: age, duration of use, living situation/crowding, genital and general hygiene, menstrual cup dropping-washing-storage, sex during menses. As a second analysis, we will assess to what extent the cup microbiome correlates to the user's VMB and whether any association changes over time by comparing VMB detection of *E. coli* and *S. aureus* to menstrual cup detection of *E. coli* and *S. aureus*, and VMB CST to menstrual cup microbiome CST and testing for interaction with time.

WASH conditions will be reported descriptively across study phases and controlled for in the Aim 1 analyses.

Analysis of Aim 3

FGD composition will be selected to match the age distribution of the cohort and work location (e.g., street, guest house). Transcripts (after translation and back translation) will be entered into NVivo (QSR International Ltd, Melbourne, Australia) then analysed using thematic content analysis. Inductive and deductive approaches will be used to reach Aim 3, whilst also enabling important new themes to emerge. Transcripts will be coded by two researchers separately using an iterative process whereby new codes are added to the original coding framework as they emerge and any text uncoded and reassigned as appropriate. Where disagreement over the interpretation of codes occurs both researchers will re-examine the codes and a third researcher will arbitrate if necessary. The coding framework will be revised to reflect agreed changes. The codes will be assigned under relevant themes and subthemes with a narrative written reflecting the assigned themes and illustrated with verbatim quotes.

Alternative analyses for the qualitative data will use Language Model tools like GPT-4 [77] or PaLM [78] to explore and mine the qualitative transcripts. Prompts tailored to each specific area of interest (e.g. MHM knowledge, practices, challenges, and contextual facilitators and barriers) will be constructed and their responses will be added to the model. The tool will be used to generate insights, summaries, or additional questions to help uncover participants narratives and emerging themes.

Discussion

We hypothesize that reusable menstrual cups that can be worn during sex will have beneficial impact on BV, VMB, and STIs for women who engage in sex for livelihood by [1] averting use of unhygienic items to manage menstrual bleeding that could promote vaginal dysbiosis, and [2] preventing risks stemming from unsafe sexual practices during menses. MHH is a pervasive yet overlooked concern for women relying on sex for livelihood, and their specific needs are unmet by current research and interventions developed for schoolgirls and women working in traditional workplaces. Unsafe practices undertaken to continue sex during menses have not been systematically characterized nor has their relation to the vaginal microbiome, BV, and STI risk. Rigorously quantifying this is necessary to interpret a potential beneficial effect of menstrual cups. Through this study we will be able to estimate the preliminary efficacy signal for any protective effect of menstrual cups on BV and STIs in a high-risk population of economically vulnerable women and will identify how potential benefits are achieved. Simultaneously, we will build an evidence-based implementation strategy for a menstrual cup MHM intervention for this population.

Dissemination efforts will include [1] a series of local dissemination meetings with the Ministry of Health, Community Advisory Boards, and community stakeholders; [2] beneficiary working group meetings to develop guidelines for MHH and sexual and reproductive health programme development; and [3] presentation of findings at national and international conferences and meetings. Other dissemination outputs will include publication of findings in peer-reviewed journals; and collaboration with international researchers, multinational organizations, and government agencies to inform policy formulation and future evaluation, quality assessments, and quality control of health promotion efforts in sub-Saharan Africa.

If positive, our trial will provide evidence that menstrual cups can be a multipurpose tool to improve MHH and reduce BV and STIs among economically vulnerable women. Given the lack of large-scale RCTs examining the effectiveness of menstrual cups in this population, evidence from this single-arm trial presents a key step to inform a larger multi-site trial. Coupled with biological outcome assessment, our implementation science framework applied to qualitative and quantitative assessment will support the successful implementation and scale up. Such a structured approach has not been previously applied in the development and assessment of MHM impact on VMB and STI/HIV risk.

Abbreviations

AE	Adverse event
BV	Bacterial vaginosis
CST	Community state type
CT	Chlamydia trachomatis
DNA	Deoxyribonucleic acid
DSMB	Data and Safety Monitoring Board
ELISA	Enzyme-linked immunosorbent assay
FGD	Focus group discussion

hCG	Human chorionic gonadotropin
HIV	Human immunodeficiency virus
HSV-2	Human simplex virus type 2
HTC	HIV testing and counselling
IgG	Immunoglobin G
IUD	Intrauterine device
LMIC	Low- and middle-income countries
LMM	Linear mixed model
MHH	Menstrual health and hygiene
MHM	Menstrual hygiene management
NG	Neisseria gonorrhoeae
PCR	Polymerase chain reaction
RCT	Randomised controlled trial
RNA	Ribonucleic acid
SAE	Serious adverse event
STI	Sexually transmitted infection
TV	Trichomonas vaginalis
UNIM	Universities of Nairobi, Illinois and Manitoba Research and Training Centre
VMB	Vaginal microbiome
WASH	Water, sanitation and hygiene

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12889-024-20491-z.

Supplementary Material 1: Supplementary Details from POWWeR Protocol

Supplementary Material 2: Informed Consent Form

Supplementary Material 3: SPIRIT Checklist

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Author contributions
SM conceived the study. SM, PPH, FO, SG, RB, LM, and AMvE wrote the grant. SM, PPH, and FO drafted the protocol. RB provided statistical guidance on the analysis plan. FO provided ministry and policy expertise, and PPH drafted trial governance and provided initial advisement for regulatory and ethical plans. All investigators contributed to the refinement of the study protocol and approved the final version. GZ drafted the manuscript. All authors read and approved the final manuscript prior to submission.

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Data availability
Not applicable, no datasets were generated or analysed for this article.

Declarations

Ethics approvals and consent to participate
This study and associated informed consent forms were approved by the institutional review boards (IRBs) of Jaramogi Oginga Odinga Teaching and Referral Hospital (ISERC/JOOTRH/657/22), Rush University Medical Center (RUMC, 22040505), and Liverpool School of Tropical Medicine (LSTM, 22–076). Research permission was granted by the Kenya National Commission for Science, Technology, and Innovation (NACOSTI/P/24/33000), and the Kenya Poisons and Pharmacy Board provided approval for trialling menstrual cups in Kenya (PPB/ECCT/23/07/01/2023(443). Any necessary protocol amendments must be approved by these IRBs and communicated to the sponsor.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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