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Randomized trial of multi-strain *Lactobacillus crispatus* vaginal live biotherapeutic products after antibiotic therapy for bacterial vaginosis: study protocol for VIBRANT (vaginal lIve biotherapeutic RANdomized trial)

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ABSTRACT

Background: Globally, approximately 30 % of women have bacterial vaginosis (BV). Antibiotic treatment is frequently followed by recurrence, likely due to lack of colonization with beneficial lactobacilli.

Methods: This is a Phase 1, randomized, placebo-controlled trial of vaginal live biotherapeutic products (LBP) after antibiotic treatment for BV to establish *Lactobacillus* colonization. The LBP are vaginal tablets containing 6 *L. crispatus* strains (LC106) or 15 *L. crispatus* strains (LC115), at 2 x 10^9 colony forming units (CFU) per dose. Participants with BV in the United States and South Africa will receive seven days of oral metronidazole twice daily and will be randomized 1:1:1:1:1 to: seven days placebo; seven days LC106; three days LC106/four days placebo; seven days LC106 starting day 3 of the metronidazole course; or seven days LC115. Safety will be assessed by the number and percentage of ≥ Grade 2 related adverse events during or after product use. The primary outcome is LBP colonization defined as relative abundance ≥5 % of any LBP strain or ≥10 % of a combination of LBP strains by metagenomic sequencing any time in the 5 weeks after randomization. A generalized linear model will measure the association between treatment group and colonization, adjusting for site.

Conclusions: This study seeks to establish proof of concept for a multi-strain LBP to promote vaginal *L. crispatus* colonization in two geographically distinct populations.

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1. Background and introduction

Vaginal communities dominated by *Lactobacillus* are associated with lower risk for preterm birth, HIV acquisition, human papillomavirus (HPV) persistence and cervical dysplasia [1–6]. A diverse vaginal community with low abundance of lactobacilli is present in a quarter of women worldwide [7]. Clinically, this is known as bacterial vaginosis (BV) and can be associated with a vaginal discharge, odor and irritation. Although antibiotic treatment of BV reduces the absolute burden of BV-associated bacteria, one-month cure rates range from 40 to 70 %, demonstrating rapid and frequent recurrence [8–12]. Treatment strategies for BV have not changed in over 40 years[13,14].

After antibiotic treatment the vaginal microbiome is frequently dominated by *L. iners*, a *Lactobacillus* species that is associated with lower community stability and less protection against adverse outcomes compared to *L. crispatus* [15–17]. One proposed solution to reduce BV recurrence is to induce vaginal colonization with the more beneficial species. Products containing live bacteria can be probiotics, which are regulated as dietary supplements or cosmetics, or live biotherapeutic products (LBP), which are regulated as medications [18–20]. In a Phase 2a trial, a single-strain *L. crispatus* LBP (LACTIN-V) colonized 50 % of treated women after 5 daily doses, but 18 days later colonization dropped to 44 % [20]. In the Phase 2b trial, after 5 daily doses and then twice weekly dosing for 11 weeks, 79 % of women were colonized, but this dropped to 48 % after an additional 12 weeks with no treatment [18]. Clinically, women treated with LACTIN-V had a significantly lower risk for recurrent BV at both 12 and 24 weeks. These results suggest that 1) vaginal inoculation with *L. crispatus* can decrease recurrent clinical BV, and 2) inoculating a single strain of *L. crispatus* might be insufficient to establish durable colonization in many women.

In stable *L. crispatus*-dominant communities, the metagenome of *L. crispatus* typically includes more genes than can be accounted for in a single isolate [21]. This suggests that multiple strains of *L. crispatus* are necessary to ensure stable colonization and could explain the lower colonization with LACTIN-V after cessation of product use. We hypothesize that a multi-strain *L. crispatus* LBP will be more successful at establishing durable colonization and dominance.

This Phase 1, first-in-human trial will enroll women with BV in South Africa and the United States and will include a placebo control arm. The rational for including people with the condition of interest is that we would be unable to show a difference between arms if we enrolled healthy people who already have *Lactobacillus* dominance. We will include a placebo arm because after antibiotic treatment some people do establish *L. crispatus* colonization, so the placebo group will allow us to identify the added benefit of the intervention.

2. Methods/design

2.1. Study Setting

The study sites are the CAPRISA Vulindlela Clinical Research Site in rural Kwa-Zulu Natal province of South Africa (ZA site) and Massachusetts General Hospital (MGH) in Boston, Massachusetts, United States of America (US site).

2.2. Patient and public involvement

In ZA, the CAPRISA community program will inform and educate local residents to mobilize input for the research process. Community research support groups play an active role as advocates for their constituents' best interests. At MGH, this role is served by the Community Access, Recruitment and Engagement group, which helps researchers develop communication about research studies, and identify concerns about the research being conducted among the studied populations. The recruitment teams at both sites will raise awareness of clinical trial opportunities and educate potential participants regarding eligibility, screening and enrolment. After enrolment, study staff will make every reasonable effort to ensure retention by collecting adequate locator information for follow-up tracking, visit reminders and retention activities.

2.3. Study design

This is a Phase I randomized placebo-controlled trial of two novel vaginally delivered LBPs.

2.4. Study population

We aim to enrol pre-menopausal, non-pregnant individuals ages 18–40 years, with BV diagnosed by an Amsel score of 3 or 4, and a Nugent score of 7–10.

2.5. Study schema

Participants who meet eligibility criteria (Table 1) will be randomized to one of five study arms in a 1:1:1:1:1 ratio (Fig. 1).

2.5.1. Primary outcomes

- Safety: Defined by the number of related Grade 2 or higher adverse events (AEs) at any point during or after use of study product.
- Colonization by LBP strains: Defined as the presence in metagenomic sequencing results of any LBP strain at $\geq$ 5 % relative abundance, or a combination of LBP strains at $\geq$ 10 % at any visits in the 5 weeks after initiation of antibiotic treatment.

2.5.2. Secondary outcomes

- Presence of L. *crispatus* dominant vaginal microbiome at any visit, defined as $>$ 50 % relative abundance in metagenomic sequencing.
- Kinetics of colonization with LBP strains (*i.e.*, duration, maximal relative abundance, number of strains that colonize per participant)
- Recurrence of BV by Nugent score and/or Amsel criteria
- Acceptability of the study product

2.5.3. Exploratory outcomes

- Genetic, cellular and proteomic associations with colonization and changes in vaginal microbiota

2.5.4. Interventions

Participants in all arms will receive oral metronidazole twice daily, dose per local standard of care (400 mg in ZA and 500 mg in the US).

The study product, LC106, LC115 or placebo (manufactured by Biose Industrie, France), is a 1-g tablet that is placed vaginally using an applicator. LC106 includes 6 strains of *L. crispatus* and LC115 includes 15 strains. These strains were originally obtained from women in the US and ZA who had a *L. crispatus* dominant community at multiple time points in a row over several months. The placebo is similar in appearance to the LBPs and includes only inactive ingredients. Participants

who experience an adverse reaction to the product will be asked to stop use if any doses remain.

All participants will be on continuous combined oral contraceptives or medroxyprogesterone acetate to suppress menses and reduce the possibility of disruption of product colonization in the vagina because of bleeding or use of menstrual protection products.

Concomitant medications: During participation in the trial participants will be asked not to use any other products in the vagina. If a participant is diagnosed with vulvovaginal candidiasis, they will be treated with oral fluconazole.

2.6. Sample size and power calculations

Sample size was calculated based on the colonization outcome. The strains included in the LBPs were derived from women in the US and ZA, thus a few people in the placebo group (n = 1/10) may have one of the strains. Our goal is to have sufficient power within each site to detect a difference between any individual active arm and the placebo arm. We plan to perform an analysis including data from both sites, however we hypothesize that site could be an effect modifier and so want to have power to perform a stratified analysis by site.

With 10 participants per arm at each site, and assuming that 10 % of those in the placebo arm and 70 % in the active arms will be colonized at or before the 5-week timepoint, we will have 82.3 % power to detect a difference of 60 % in the colonization proportion between an active arm and the placebo arm within site using a one-sided Fisher's Exact test with a significance threshold of 0.05. The comparison between active arms will be underpowered, but by utilizing the longitudinal data describing the kinetics of colonization we will be able to assess the relative merits of each dosing strategy.

To ensure biological integrity and homogeneity of the samples collected from participants, we will replace participants (*i.e.*, enroll an additional participant in that study arm and site with the goal of having 10 people per arm per site for a per protocol analysis) who meet the following criteria.

- Loss to follow-up or withdrawal before starting study product
- Loss to follow-up or withdrawal during study product use, with no follow up
- Bleeding: 3 days of heavy bleeding (*i.e.*, soaking one pad at least once in a day) during study product dosing, or more than 5 days in a row of heavy bleeding during follow up
- Need for antibiotic treatment for any reason after their initial course of metronidazole and before their Visit 6.
- High grade pap smear (HSIL, ASCUS-H, AGUS) at screening visit (V0).

Therefore with 10 people per arm per site, with 5 arms, and an estimated 10 potential replacements per site, the study aims to enrol a maximum of 60 participants per site (120 across both sites).

Randomization/Blinding process: Participants will be randomized to one of five treatment arms in a 1:1:1:1:1 ratio using block randomization with a fixed block size of 5. This design ensures equal representation across arms within each block and minimized potential imbalance if the study ends prematurely. An unblinded statistician who will not be part of the analysis team will generate the randomization list, which will be provided to Biose Industrie (France), the manufacturer of the study product, who will label study product, and ship to the study sites. The packaging for the study products is identical, allowing masking of staff and participants. Trial personnel enrolling the participants will be unaware of the treatment-group assignment, except for Arm 4, which starts study product during the metronidazole course. When dispensing product, the blinded site pharmacist will select the next identical-appearing box of study product in sequential order and distribute to masked study personnel. Laboratory staff analysing samples will all be masked to treatment allocation (even for Arm 4).

If a participant has a serious adverse event that is felt to be related to study product, and if that participant is on one of the blinded arms, the

Table 1
Trial eligibility criteria.

Trial inclusion and exclusion criteria
Inclusionary criteria
• Willing and able to provide written informed consent
• Pre-menopausal individuals, 18–40 years old
• BV by Amsel criteria (at least 3 of 4 criteria must be present)
• Nugent score: ≥7
• HIV uninfected
• Not pregnant
• Using depot medroxyprogesterone acetate or continuous combined oral contraceptives
• Willing and able to attend study visits and comply with study procedures
Exclusionary criteria
• History of clinically significant vaginal, cervical, or uterine disease
• Prior hysterectomy
• Diagnosed with cervicovaginal infection (inclusive of *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, or *Trichomonas vaginalis*) within the 30 days prior or at the enrolment visit.
• Use of antibiotics in the past 30 days
• Syphilis
• Vulvovaginal candidiasis diagnosed on brightfield microscopy of vaginal fluid
• Allergy to or contraindication to use of oral metronidazole
• Currently participating in another study of an investigational product (excluding COVID-19 vaccine studies)
• Use of long-acting systemic investigational product within the past year
• Subject taking any of the following medications currently or in the past 30 days: systemic steroids, interleukins, systemic interferons or systemic chemotherapy
• History of coronary artery disease, myocardial infarction, chronic obstructive pulmonary disease, chronic renal failure, decompensated cirrhosis.
• Use of an intrauterine device
• Use of oral or vaginal probiotics, prebiotics or synbiotics within past 30 days.
• Active SARS-CoV-2 infection
• Vaginal cleansing practices in the past 30 days
• Menopause: surgical; or absence of periods not due to hormonal contraception and in the setting of prior chemotherapy
• Use of testosterone
• Systolic blood pressure >180 mmHg or diastolic blood pressure >110 mmHg at screening or enrollment
• Hemoglobin <9 g/dL
• Less than 2 weeks since COVID vaccination or booster
• Either breastfeeding/lactating or pregnant within 8 weeks prior to study entry
• Any other condition or situation that in the opinion of the investigator will compromise ability to participate in the study.

allocation can be revealed to the study staff and participant by the unblinded statistician at the data coordinating center.

2.6.1. Study procedures and data collection

Screening visit (V0): Participants will provide written informed consent in their preferred language. Participants will complete a questionnaire to assess understanding of the consent form and ensure they comprehend critical information about the study. The informed consent process will be conducted prior to the start of any study related procedures, in accordance with the International Conference on Harmonisation Good Clinical Practice guidelines. Consent forms at each site request permission for storage of samples and data for future analyses related to reproductive health.

After providing consent, participants will self-collect vaginal swabs to test for BV [22,23]. If eligible by Amsel criteria, participants will complete additional screening procedures including pregnancy test, physical and pelvic exam. Participants will have blood type and blood count measured and will be tested for the following sexually transmitted infections (STIs); *Neisseria gonorrhoea*, *Chlamydia trachomatis*, *Trichomonas vaginalis*, *Mycoplasma genitalium,* herpes simplex virus 1 and 2, human papillomavirus (HPV), syphilis, and HIV. A pap smear will be performed. A medical history will be obtained, and participants will be asked to complete questionnaires about demographic information, genitourinary symptoms, sexual behavior and dietary habits. Eligibility will be assessed according to Table 1. Eligible participants will be scheduled for a randomization visit within 30 days of screening.

Randomization visit (V1): Participants will be randomized to one of 5 study arms and will be given 7 days of oral metronidazole. If the participant is in Arm 4, they will also be given study product. Participants will practice inserting a placebo study product to ensure they are comfortable with the process. They will also collect vaginal swabs, a disposable menstrual cup, have blood drawn and a pelvic exam, as well as complete questionnaires (see Table 2).

Home Procedures: For the five weeks between the randomization visit (V1) and follow up visit (V6), participants will be asked to complete a daily diary and self-collect vaginal swabs daily for a maximum of 35 swabs. During the week of study product use participants are asked to collect the swab before placing the dose of study product. At each visit they will be provided with swabs and storage tubes containing a non-toxic nucleic acid stabilizing buffer. Participants will be instructed to insert a dry swab (FLOQswab®, COPAN, Murrieta, CA) 4–5 cm into the vagina, move the swab in a circle 2 times, then place the swab into the provided cryovial containing 1.0 mL stabilizing buffer (Microbiome MDG buffer, Maryland Genomics, Baltimore, MD), twirl it 3 times, and then breakoff/cut the swab at its breakpoint, cap the cryovial and store at room temperature. They will be asked to return used and missed/unused swabs at their upcoming visit. The daily diary collects information about sexual activity, product use, and vaginal bleeding.

2.6.2. Visit procedures

After the randomization visit, participants will return weekly for 5 weeks (V2-V6), then at 7 (V7), 9 (V8) and 12 weeks (V9) for in-person visits (Fig. 1). At each visit, they will complete some or all of the sample collection procedures outlined below and will answer questionnaires (Table 2).

Blood Draw: A trained study staff member will perform venipuncture to collect blood for laboratory tests and research.

Urine Sample: Participants will be asked to provide urine for a pregnancy test and urinalysis.

Pelvic Exam: The pelvic exam conducted by a study clinician will include inspection of the external genitalia, placement of a speculum to visualize the vaginal mucosa and cervix, and a bimanual exam to assess uterine and adnexal tenderness. A Pap test will be performed at screening, and swabs for *N. gonorrhoea, C. trachomatis, T. vaginalis*, *M. genitalium*, and HPV collected at V0, V6, V9.

Cytobrush: During the pelvic exam, the study clinician will introduce a cytobrush (Puritan, Sterile Cytology Brush, Guilford ME) into the cervical os until the bristles are no longer visible and rotate it 2 times. The cytobrush will be transported to the laboratory on wet ice for flow cytometry analysis of immune cells.

Self-collected vaginal swabs (in clinic): At in-person visits, participants will be asked to self-collect 6 flocked vaginal swabs (Puritan PurFlock Ultra, Guilford ME) for research and 2 polyester-tipped swabs (Puritan Polyester Tipped Applicator, Guilford ME) for assessment of pH, Nugent score and Amsel criteria. Participants will insert 2 vaginal swabs at a time 4–5 cm into the vagina and move them in a circle 2 times. The vaginal pH will be assessed using Hydrion (pH 4.5–8.0) pH strips, and pH interpreted according to the manufacturer-provided chart. Research swabs will be placed in cryovials/tubes containing the same stabilizing buffer as home swabs (n = 1), 20 % glycerol + thioglycolate solution (n = 3) or no liquid (n = 2) for further research assays and stored at −80 °C.

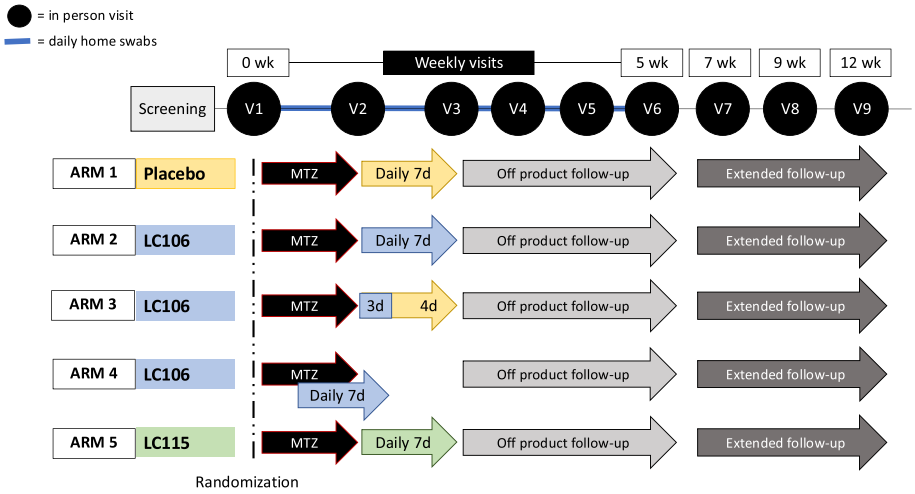

Fig. 1. Study schema showing dosing regimen and distribution of participants across the five treatment arms. All participants receive oral metronidazole (MTZ) between visit 1 and visit 2. Between visit 1 and visit 6 participants collect daily vaginal swabs at home. **Arm 1: Placebo.** Vaginal placebo tablet daily for 7 days after the completion of metronidazole. **Arm 2: LC106.** Vaginal 6-strain LBP tablet daily for 7 days after the completion of metronidazole. **Arm 3: Short course LC106.** Vaginal 6-strain LBP tablet daily for 3 days, then 4 days of placebo, following completion of metronidazole. **Arm 4: Crossover LC106.** Vaginal 6-strain LBP tablet daily for 7 days starting on day 3 of metronidazole treatment. **Arm 5: LC115.** Vaginal 15-strain LBP tablet daily for 7 days after the completion of metronidazole. (d = days, wk = week).

Table 2
Schedule of samples and questionnaires.

Visit #	V0	V1	V2	V3	V4	V5	V6	V7	V8	V9
Blood Draw	**X**	**X**	**X**				**X**			**X**
HIV/HSV/Syphilis[a]	X						X			X
Complete blood count/Blood Type	X									
Research		X	X				X			X
Urine Sample	**X**	**X**	**X**							
Pregnancy test	X	X	X							
Urinalysis	X	X	X				X			X
Pelvic Exam	**X**	**X**	**X**				**X**			**X**
GC/CT/TV/MG[b]	X						X			X
Cytobrush		X	X				X			X
Self-Collected Vaginal Swabs	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Self-collected disposable menstrual cup	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Rectal Swab	**X**	**X**	**X**				**X**			**X**
Daily home swabs and diary		**X**	**X**	**X**	**X**	**X**				
Questionnaires	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Baseline Questionnaire (demographics, medical history, obstetrics, gynecologic and sexual history, symptoms)	X									
Follow up Questionnaire (symptoms, sexual behavior, vaginal product use)		X	X	X	X	X	X	X	X	X
Product Acceptability Assessment				X						
Dietary Questionnaire	X						X			X

[a] HIV = Human Immunodeficiency virus antigen/antibody test, HSV = herpes simplex virus antibody test, Syphillis = Treponema pallidum antibody test.
[b] *GC* = *Neisseria gonorrhoea*, CT = *Chlamydia trachomatis*, TV = *Trichomonas vaginalis*, MG = *Mycoplasma genitalium*.

Disposable menstrual cup vaginal secretion collection: Disposable menstrual cups (Softdisc, The Flex Company, CA) will be inserted into the vagina for 20–60 min and removed by the participant. If a participant is unable to insert or remove the menstrual cup, the study clinician will aid the participant. The menstrual cup will be placed in a sterile container for transport to the laboratory on wet ice for further processing to support exploratory analyses of proteins in genital fluid. Aliquoted samples will be stored at −80 °C.

Rectal swabs: The study clinician or participant will insert a dry foam swab [Sterile Foam Tipped applicator, Puritan, Guildford, ME] through the anal sphincter until the swab head is no longer visible and rotate it slowly two times. The swab will be placed in a cryovial and stored at −80 °C for analysis of the gut microbiome.

Questionnaires: At follow-up visits, participants will be asked to complete questionnaires about genitourinary symptoms, sexual behavior, vaginal product use, medication use and adherence to study procedures. Participants will also report contraceptive use at these visits.

2.6.3. Data collection and management

Trial data will be recorded on case report forms (CRFs) according to protocol requirements. At a site level, all data collected on CRFs will undergo 2 sets of quality control reviews to ensure that the data is error free, and CRFs will be scanned into the trial's database (DFdiscover) within 5 days of the visit. Participant files at each site will be stored securely to maintain confidentiality. All data and samples will only be identified by a participant id number, with no patient identifying information. The CAPRISA Data Management Department will serve as the Data Coordinating Center (DCC) and will send weekly queries about missing or incomplete data to the respective trial sites for resolution.

An external monitor will conduct intermittent site visits to review compliance with the protocol, and will review source documents to ensure correct completion of study procedures.

2.6.4. Recruitment, retention and adherence

At both sites the study will be advertised widely using locally appropriate modalities. Key opinion leaders in the community will be asked to disseminate information about the study.

To ensure participant retention, study staff will maintain continuous contact with enrolled participants throughout the study via email and/or phone by sending study visit reminders a few days before the visit. Participants are provided with appointment cards and/or electronic calendar invitations to integrate their upcoming visits into their personal schedules. Participants will also receive reimbursement for travel costs, and a stipend for their time and effort to participate in the study.

Participants will receive daily diaries to help track when they take antibiotics, insert study product and collect daily swabs. At the US site, participants will receive a daily email twice a day prompting them to complete their electronic daily diary, take study product, and collect their daily vaginal swabs. At the ZA site, the participants receive a check-in midweek call to remind them to complete their daily home procedures.

Adherence to antibiotic treatment will be assessed by questionnaire, and by asking participants to return their pill bottle with any unused pills. Participants are asked to return both used and unused study product applicators and tablets. Applicators will be stained with FD7C Blue Dye #1 to assess whether the applicator was used [24,25].

2.6.5. Safety monitoring

A Protocol Safety Team (PST) will oversee clinical safety during the conduct of the study and will 1) provide blinded clinical oversight of participant safety; 2) undertake regular internal reviews of participant safety data; 3) receive and review ad-hoc clinical queries or safety concerns from clinical staff as they arise; 4) provide guidance to sites regarding clinical management of AEs; 5) make decisions to withhold study product temporarily or permanently for any safety concerns. The PST will be comprised of the two site principal investigators (PIs), site project managers and site clinicians who are approved and delegated to work on the study. The study PIs will lead the team and consult with an independent clinician on safety concerns that arise when needed. No major safety concerns are expected in this trial; therefore, the PST will receive and review safety reports monthly and review meetings will occur ad hoc. Given the small trial size, the short duration of intervention and the expected safety of the intervention, a data monitoring committee was not required by the regulatory bodies.

2.6.6. Statistical analysis

Baseline data will be presented using descriptive statistics; continuous variables will be presented using mean and standard deviation or median and interquartile range (IQR) and categorical variables using counts and percentages. The frequency of missing values per variable will be reported as a footnote to tables. Missing data will be summarized descriptively by group for each outcome variable. Missing data will be assumed missing at random and will not be imputed, unless otherwise stated.

2.7. Primary outcomes

The safety analysis will be conducted overall and by site using the safety population: all randomized participants meeting inclusion/exclusion criteria, who received at least one LBP dose and had any post-LBP data available, analyzed according to the treatment which they received. AEs will be graded per the DAIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 2.0, November 2014 and Addendum 1 (Female Genital Grading Table for Use in Microbicide Studies). Summaries by site and arm will show the frequency and percentage of participants experiencing AEs within each MedDRA system organ class (SOC), the median duration and proportion resolved at end of study. Moreover, number and percentages of participants experiencing each specific AE will be tabulated by severity and relationship to study product. A complete listing of adverse experiences for each participant will provide details including severity, relationship to study product, onset, duration and outcome.

We will compare the cumulative frequency of AEs and the percentage of participants experiencing AEs between each active LBP arm and the placebo arm. Each AE will be counted only once according to its worst severity level. The primary safety outcome is the number of related Grade 2 or higher adverse events (AEs) at any point during or after use of study product.

The colonization analysis will be conducted using a modified intent to treat (mITT) population: all randomized participants who met inclusion/exclusion criteria, used study product and returned for at least one follow up visit after study product use prior to 5 weeks. A sensitivity analysis will be performed with the per protocol population: all randomized people who met inclusion/exclusion criteria, did not meet replacement criteria, completed >80 % of study product doses (by self-report and/or applicator staining), and had at least one follow up visit prior to week 7, analyzed in the groups to which they were assigned. Colonization will be defined using metagenomic sequencing detection of any one of the LBP strains at $\geq$ 5 % relative abundance, or any combination of the strains at $\geq$ 10 %. The proportion of participants colonized with LBP *L. crispatus* strains at least once at or before 5 weeks will be estimated, together with 95 % confidence intervals for each treatment group. For those participants with no samples indicating colonization at or before 5 weeks, regardless of whether some weekly samples are missing, it will be assumed that colonization did not occur. A generalized linear model, adjusting for site, will be used to measure the association between treatment group and colonization.

2.8. Secondary outcomes

The secondary analysis for presence of an *L. crispatus* dominant vaginal microbial community will be performed as described above for the primary endpoint, considering *L. crispatus* dominance as >50 % relative abundance in metagenomic sequencing. As described above, the analysis will be conducted using the mITT populations to compare the proportion of participants with *L. crispatus* dominance between each treatment arm and placebo.

The kinetics of colonization with LBP strains (*i.e.*, duration, maximal relative abundance, number of strains that colonize per participant) will be described using summary statistics for both strain relative abundance in the metagenomics results, and strain-specific qPCR results.

Recurrence of BV by Nugent score will be assessed using the mITT population. Additionally, sensitivity analyses will also be conducted using the per protocol population. Recurrence of BV will be defined to have occurred in participants if their Nugent score was below 7 after antibiotic treatment and was 7 or above at any time point after that. Persistent BV will be defined as Nugent score 7 or above immediately after antibiotics (V2). Missing Nugent scores will be assumed to have occurred at random and a complete-case analysis will be performed. The proportion of participants with recurrent BV will be estimated, together with 95 % confidence intervals, by site and treatment group. A generalized linear model for binary data, adjusting for site, will be used to measure the association between treatment group and BV recurrence.

Acceptability of the study product will be described using responses to two questions: the proportion of participants who state that the study intervention was of benefit to them, and the proportion who say they would use the product again.

3. Ethics and dissemination of study findings

In South Africa, the University of KwaZulu-Natal's Biomedical Research Ethics Committee (BREC; reference BREC/00005620/2023) and the South African Health Products Regulatory Authority (SAHPRA; reference 20230615) have approved the conduct of the trial. In the United States of America, the Mass General Brigham Human Research Committee (MGBHRC; 2023P001035) and the Food and Drug Administration (FDA; IND 029629) have approved the conduct of the trial. Any protocol amendments will be submitted to the local ethics committee and the regulatory body, and once approved will be communicated to the study team via email and at study logistics meetings.

Findings arising from this trial will be disseminated through conference presentations, peer-reviewed publications and the clinical trial registries. Authorship for publications based on trial data will be determined according to the guidelines of the Vaginal Microbiome Research Consortium and will ensure representation from both study sites and teams.

CRediT authorship contribution statement

Callin Chetty: Writing – review & editing, Writing – original draft, Methodology. **Nomfuneko Mafunda:** Writing – review & editing, Writing – original draft, Methodology. **Anna-Ursula Happel:** Writing – review & editing, Methodology. **Anam Khan:** Writing – review & editing, Methodology. **Briah Cooley Demidkina:** Writing – review & editing, Methodology. **Nonhlanhla Yende-Zuma:** Writing – review & editing, Methodology. **Yusra Saidi:** Writing – review & editing, Methodology. **Asthu Mahabeer Polliah:** Writing – review & editing, Methodology. **Lara Lewis:** Writing – review & editing, Methodology. **Farzana Osman:** Writing – review & editing, Methodology. **Precious Radebe:** Writing – review & editing, Methodology. **Jo-Ann S. Passmore:** Writing – review & editing, Conceptualization. **Doug Kwon:** Writing – review & editing, Methodology, Conceptualization. **Jacques Ravel:** Writing – review & editing, Methodology, Conceptualization. **Sinaye Ngcapu:** Writing – review & editing, Methodology. **Lenine Liebenberg:** Writing – review & editing, Methodology. **Laura Symul:** Writing – review & editing, Methodology. **Susan Holmes:** Writing – review & editing, Methodology. **Caroline M. Mitchell:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Funding acquisition, Conceptualization. **Disebo Potloane:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Funding acquisition, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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The authors declare the following financial interests/personal relationships which may be considered as potential competing interests.

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Data availability

After completion of proposed analyses, sequences and de-identified metadata will be made available in publicly available data repositories as required for publication, and the remainder upon reasonable request, with appropriate human subjects approval. All code used to analyze trial results will be made publicly available.

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