

BMJ Open

BMJ Open is committed to open peer review. As part of this commitment we make the peer review history of every article we publish publicly available.

When an article is published we post the peer reviewers' comments and the authors' responses online. We also post the versions of the paper that were used during peer review. These are the versions that the peer review comments apply to.

The versions of the paper that follow are the versions that were submitted during the peer review process. They are not the versions of record or the final published versions. They should not be cited or distributed as the published version of this manuscript.

BMJ Open is an open access journal and the full, final, typeset and author-corrected version of record of the manuscript is available on our site with no access controls, subscription charges or pay-per-view fees (<http://bmjopen.bmj.com>).

If you have any questions on BMJ Open's open peer review process please email info.bmjopen@bmj.com

BMJ Open

Vaginal Probiotic Adherence and Acceptability in High-Risk Rwandan Women Participating in a Pilot Randomised Controlled Trial: A Mixed-Methods Approach

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2019-031819
Article Type:	Original research
Date Submitted by the Author:	20-May-2019
Complete List of Authors:	Verwijs, Marijn; Institute of Infection and Global Health, University of Liverpool, Agaba, Stephen; Rinda Ubuzima Umulisa, Marie ; Rinda Ubuzima Uwineza, Mireille; Rinda Ubuzima Nivoliez, Adrien; Biose, Lievens, Elke; Winlove van de Wijgert, Janneke H.H.M.; University of Liverpool, Institute of Infection and Global Health; Universitair Medisch Centrum Utrecht, Julius Center for Health Sciences and Primary Care
Keywords:	bacterial vaginosis, vaginal probiotic, adherence, acceptability, Africa

SCHOLARONE™
Manuscripts



I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our [licence](#).

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which [Creative Commons](#) licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

**Vaginal Probiotic Adherence and Acceptability in
High-Risk Rwandan Women Participating in a Pilot Randomised Controlled Trial:
A Mixed-Methods Approach**

Authors: Marijn C. Verwijs (MD)¹, Stephen K. Agaba (MD)², Marie-Michele Umulisa (MA)²,
Mireille Uwineza (BA)², Adrien Nivoliez (PhD)³, Elke Lievens (PhD)⁴, Janneke H.H.M. van de
Wijgert (MD PhD MPH)^{1,5}.

Author affiliations

¹Institute of Infection and Global Health, University of Liverpool, Liverpool, United Kingdom.

²Rinda Ubuzima, Kigali, Rwanda.

³Biose, Aurillac, France.

⁴Winlove Probiotics, Amsterdam, The Netherlands.

⁵Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht
University, Utrecht, The Netherlands.

Correspondence to

Professor Janneke van de Wijgert, MD PhD MPH

Department of Clinical Infection, Microbiology and Immunology

Institute of Infection and Global Health, University of Liverpool

Ronald Ross Building, 8 West Derby Street, Liverpool L69 7BE, United Kingdom

Email: j.vandewijgert@liverpool.ac.uk

ORCID: 0000-0003-2728-4560

Word counts:

Word count abstract: 300 words (max. 300 words).

Word count main text: 3,479 words (max. 4000 words).

Number of tables and figures: 5 (max. 5).

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

29 Number of supplementary tables and figures: 3.

For peer review only

1
2
3 30 **ABSTRACT**
4

5 31 **Objectives** Bacterial vaginosis (BV) recurrence is common. We evaluated the adherence and
6
7 32 acceptability of intermittent use of two vaginal probiotics and one antibiotic to prevent recurrence.
8

9 33 **Design** Repeated adherence and acceptability assessments using mixed methods within a pilot
10
11 34 randomised controlled trial.
12

13 35 **Setting** Research clinic in Kigali, Rwanda.
14

15 36 **Participants** High-risk Rwandan women (n=68) with BV and/or trichomoniasis.
16

17 37 **Interventions** Women were randomised to four groups (n=17 each) after completing metronidazole
18
19 38 treatment: behavioural counselling only, or behavioural counselling plus two-month intermittent use
20
21 39 of oral metronidazole, Ecologic Femi+ (EF+) vaginal capsule, or Gynophilus LP (GynLP) vaginal
22
23 40 tablet.
24
25

26 41 **Outcome measures** Adherence and acceptability data from randomised women were collected in
27
28 42 structured face-to-face interviews, semi-structured focus group discussions and in-depth interviews,
29
30 43 daily diaries, and counting of used/unused study products. Randomised women and women attending
31
32 44 recruitment sessions (n=131) were surveyed about vaginal infection knowledge.
33
34

35 45 **Results** Most women (93%) were sex workers. At baseline, they were unfamiliar with BV, and had
36
37 46 never used probiotics. All probiotic users reported that insertion became easier over time.
38
39 47 Triangulated adherence data showed that 100% of EF+ users and 88.2% of GynLP users used $\geq 80\%$
40
41 48 of required doses. Younger age, asking many questions at enrolment, having menses, and reporting
42
43 49 urogenital symptoms showed non-significant trends towards a lower perfect adherence likelihood.
44
45 50 Qualitative data suggested that women believed that the probiotics reduced BV recurrence, but that
46
47 51 partners were sometimes unsupportive of study participation. Self-reported vaginal washing practices
48
49 52 decreased during follow-up, but sexual risk behaviours did not. Most women (80%) with an
50
51 53 uncircumcised steady partner discussed penile hygiene with him, but many women found this
52
53 54 difficult, especially with male clients.
54

55 55 **Conclusions** High-risk women require education about vaginal infections. Vaginal probiotic
56
57 56 acceptability and adherence were high in this cohort. Our results can be used to inform future product
58
59 57 development and to fine-tune counselling messages in prevention programs.
60

1
2
3 58 **Trial registration** ClinicalTrials.gov (NCT02459665).
4

5 59 **Keywords (5)** bacterial vaginosis, vaginal probiotic, adherence, acceptability, Africa.
6
7 60

8
9 61 **ARTICLE SUMMARY**

10
11 62 **Strengths and limitations of this study**

- 12
13
14 63 • We conducted this research in the context of a pilot randomised controlled trial, and statistical
15
16 64 power was therefore limited.
17
18 65 • We triangulated different sources of adherence data to maximise accuracy, and used a mixed-
19
20 66 methods approach to evaluate acceptability.
21
22 67 • We could not directly compare experiences with, and opinions about, the two different vaginal
23
24 68 probiotics because each woman used only one product and qualitative data depth was suboptimal.
25
26 69 • Social desirability bias may have affected some of the results.
27
28
29 70 • The results of this study may not be generalizable to women at lower risk of HIV/STIs.
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

71 INTRODUCTION

72 Bacterial vaginosis (BV) is a vaginal condition in which fastidious anaerobes such as *Gardnerella*
73 *vaginalis* increase while beneficial, lactic acid-producing lactobacilli decrease.[1] Often
74 asymptomatic, it is associated with increased risks of sexually transmitted infections (STIs) and HIV
75 transmission, pelvic inflammatory disease, and adverse pregnancy outcomes.[2–5] Although BV is
76 treatable with antibiotics, the risk of recurrence is high.[6,7] The prevalence of BV varies among
77 regions and ethnic groups but is highest in sub-Saharan Africa, where it is estimated at 30-50%.[8]
78
79 Vaginally-administered probiotics containing lactobacilli are considered a promising new strategy to
80 restore a lactobacilli-dominated vaginal microbiota during and/or after antibiotic treatment, or to
81 prevent BV.[9] While some probiotics have been available on the market for several years, clinical
82 trials to support beneficial effects have only recently been initiated for most products.[10–13]
83 Acceptability is an important component of these trials, to maximise future uptake and adherence of
84 vaginal probiotics should they be proven efficacious. The acceptability of a novel vaginal product
85 depends on factors such as the characteristics of the population studied, characteristics of and
86 experiences with the product, types of sexual relationships and partner support, and community
87 perceptions.[14,15]

88
89 We conducted a clinical trial of intermittent use of two vaginal probiotics and oral metronidazole to
90 prevent BV recurrence in Rwandan women who had been treated for BV and/or *Trichomonas*
91 *vaginalis* (TV). We used qualitative and quantitative research methods to assess adherence and
92 acceptability with vaginal probiotic use. We triangulated various sources of adherence data to obtain
93 adherence estimates per woman for each period of intermittent product use in between study visits,
94 and determined correlates of adherence.

96 METHODS

97 The pilot clinical trial took place from June 2015 to February 2016 at the Rinda Ubuzima research
98 clinic in Kigali, Rwanda. Women who had been successfully treated for BV/TV with a seven-day

1
2
3 99 course of oral metronidazole (Tricozole, Laboratory & Allied Ltd, Nairobi, Kenya) were randomised
4
5 100 to four intervention groups (n=17 each) to prevent BV recurrence: behavioural counselling only
6
7 101 (controls), or behavioural counselling plus intermittent use of two different vaginal probiotics or oral
8
9 102 metronidazole for two months. Women were seen at screening, enrolment (product use initiation, if
10
11 103 applicable), Day 7, Month 1, Month 2 (product use cessation, if applicable), and Month 6. Product
12
13 104 efficacies were not known during the trial, and preliminary efficacy results are reported
14
15
16 105 elsewhere.[16] The behavioural counselling focussed on safer sex practices, cessation of vaginal
17
18 106 practices, and increasing male penile hygiene to prevent BV.
19
20 107

21 22 108 **Study population**

23
24 109 Women aged 18-45 at risk of HIV/STIs (defined as having had more than one sex partner and/or
25
26 110 having been treated for an STI and/or BV in the last 12 months) were eligible for enrolment if they
27
28 111 were confirmed HIV-negative, non-pregnant, diagnosed with BV and/or TV, and cured after seven-
29
30 112 day oral metronidazole treatment. Other clinical exclusion criteria were applied but were rare.[16]
31
32 113 Women were recruited by study staff with the assistance of Community Mobilisers who had strong
33
34 114 ties with local high-risk women (particularly sex workers).
35
36 115

37 38 39 116 **Study products and dosing**

40
41 117 Ecologic Femi+ (EF+; Winclove Probiotics, Amsterdam, Netherlands) is a vaginal capsule containing
42
43 118 lyophilised lactic acid-producing bacteria. EF+ was used once per day for five days followed by thrice
44
45 119 weekly, for two months. Gynophilus LP (GynLP; Biose, Aurillac, France) is a tablet containing the
46
47 120 *Lactobacillus rhamnosus* Lcr35 strain. The tablet disintegrates in the vagina and forms a gel that
48
49 121 slowly releases the probiotic bacteria. GynLP was used once every four days for two months. The first
50
51 122 dose was inserted at the clinic under direct observation of a clinician, and remaining doses were self-
52
53 123 administered at home. Women were asked not to cleanse or insert other products into the vagina after
54
55 124 probiotic insertion to allow the probiotics to dissolve. They were also told that they were allowed to
56
57 125 cease probiotic use during menses, but were encouraged to continue. Intermittent metronidazole use
58
59 126 was chosen as a positive control intervention because studies conducted in the U.S. and Kenya have

1
2
3 127 shown a 30-40% reduction in BV recurrence.[17,18] Metronidazole users took 500 mg generic oral
4
5 128 metronidazole (Laboratory & Allied ltd, Nairobi, Kenya) twice weekly for two months. Participants
6
7 129 and clinicians were not blinded.
8

9 130

11 131 **Acceptability, adherence, behavioural, and vaginal infection knowledge assessments**

13 132 Acceptability was assessed at the enrolment visit prior to product use initiation and at the Month 2
14
15 133 visit after the full two months of use. Adherence was assessed during the intervention period, at the
16
17 134 Day 7, Month 1, and Month 2 visits. Sexual and other behaviours were assessed at all study visits.
18
19 135 Participants were interviewed face-to-face in Kinyarwanda by a trained study nurse using structured
20
21 136 questionnaires with multiple-choice questions, questions requiring a number or date, and an adherence
22
23 137 self-rating scale (from 0-10). In between visits, participants used pictorial diary cards (online
24
25 138 supplementary material figure 1) to record daily episodes of product use, vaginal sex, condom use,
26
27 139 and vaginal practices. Those using study products returned the product packaging and unused
28
29 140 products (if applicable) to their clinic visits, where they were counted by study staff. Any
30
31 141 discrepancies between data sources were discussed with participants, and consensus, triangulated
32
33 142 assessments of adherence were recorded on the questionnaires. Additionally, 131 women were
34
35 143 interviewed about their knowledge of vaginal infections (such as BV and STIs) using a structured
36
37 144 questionnaire during recruitment sessions (n=61; regardless of eligibility) and at enrolment visits
38
39 145 (n=70; this included the 68 randomised women, and two women who attended enrolment visits but
40
41 146 turned out to be ineligible). Women were interviewed before being counselled at study visits or before
42
43 147 receiving information at recruitment sessions. This questionnaire contained multiple-choice and open-
44
45 148 ended questions. Responses to the open-ended questions were categorised and discussed by two
46
47 149 different researchers until consensus about the answer categories was reached.
48
49

50 150

51
52
53 151 Four semi-structured focus group discussions (FGDs) with 7-11 participants per group (total n=38),
54
55 152 and four semi-structured individual in-depth interviews (IDIs) were held with enrolled participants,
56
57 153 about their experiences with and opinions of the products, sexual behaviour, and vaginal practices.
58
59 154 Women randomised to the behavioural counselling only group were not included in the FGDs and
60

1
2
3 155 IDIs. All had completed their product use period. The interviews were unlinked anonymous, and
4
5 156 women used pseudonyms to enable them to talk freely despite the fact that the discussions and
6
7 157 interviews were taped. All interviews took place between November 2015 and March 2016, were held
8
9 158 in Kinyarwanda, recorded on tape, transcribed verbatim, and translated into English.

159

160 **Data analysis**

161 Questionnaire data were analysed using Stata 13 (StataCorp, College Station, TX, USA). The
162 proportion of women with $\geq 80\%$ / $\geq 90\%$ / 100% adherence in the probiotic groups were compared by
163 Fisher's exact tests. Changes in self-reported vaginal practices and sexual behaviours over time were
164 tested using McNemar's test for binary outcomes, and Wilcoxon's signed-rank test for continuous
165 outcomes. To study associations of participant characteristics with triangulated adherence, we used
166 bivariable mixed effects models, with perfect adherence (defined as having used all doses as
167 instructed) per interval between study visits during the intervention period as the outcome, participant
168 identification numbers as the random effect, and one participant characteristic at the time as the fixed
169 effect. We could not determine correlates of acceptability due to limited variation in the acceptability
170 data (reported acceptability was high throughout the trial).

171

172 The FGD and IDI transcripts were read and discussed by three researchers (MV, MU, and JvdW). The
173 Chief Investigator (JvdW) decided that data saturation had been met when the fourth FGD and the
174 fourth IDI transcript had become available in March 2016. The transcripts were then coded using
175 NVivo 10.0 (QSR International, Melbourne, Australia) by one single researcher (MV). The
176 discussions and interviews were semi-structured, with themes and associated codes prepared a priori,
177 as well as new elements that emerged from the data. The codes were derived from an acceptability
178 framework that has been used in studies of vaginal products for contraception or HIV
179 prevention.[14,15,19] Components of the framework include study population characteristics, product
180 attributes, sexual encounter and relational attributes, and the contextual environment (e.g. community
181 perceptions of product use).

182

1
2
3 **183 Ethical statement**
4

5 184 All participants provided written consent for study participation, and separate consent for participation
6
7 185 in FGDs/IDIs. All non-married participants aged 18-20 also required parental/guardian consent per
8
9 186 Rwandan law at the time of the study. The participants received 3 GBP per visit (in local currency) as
10
11 187 a reimbursement for time and transport costs. Care was taken to protect participant privacy and
12
13 188 confidentiality. The study was sponsored by the University of Liverpool, approved by the Rwanda
14
15 189 National Ethics Committee and the University of Liverpool Research Ethics Subcommittee for
16
17 190 Physical Interventions, and registered on ClinicalTrials.gov (NCT02459665).
18
19

20 191

21
22 **192 Participant and public involvement**
23

24 193 A subset of the enrolled participants were invited to comment on study design and experiences with
25
26 194 the interventions during the FGDs/IDIs. Participants were not invited to develop outcomes, interpret
27
28 195 the results, or to contribute to the writing or editing of this document for readability or accuracy. The
29
30 196 preliminary results of this study were discussed with 32 stakeholders during a workshop held at the
31
32 197 Ministry of Health in Kigali, Rwanda, in December 2017. These stakeholders included representatives
33
34 198 of the Ministry of Health, the National University of Rwanda, the National Ethics Committee, local
35
36 199 hospitals and clinics, and local non-governmental and women's organisations.
37
38

39 200

40
41 **201 RESULTS**
42

43 **202 Baseline characteristics**
44

45 203 We screened 176 women: bacterial STI prevalence was 31.3% and BV prevalence by Gram stain
46
47 204 Nugent scoring was 47.9%. All 68 randomised women were at risk of STI/HIV transmission, with
48
49 205 93.1% reporting having exchanged sex for money and/or goods in the previous month (figure 1,
50
51 206 online supplementary material table 1). We collected 29.93 person-years of data. Four women
52
53 207 withdrew their informed consent during the study (for reasons unrelated to study product
54
55 208 acceptability). None were lost to follow-up.
56
57

58 209

59
60 210

211 Adherence

212 Triangulated adherence was high: 100% of EF+ users and 88.2% of GynLP users used $\geq 80\%$ of
213 required doses (Fisher's exact $p=0.103$; table 1), and these percentages were 88.2% and 68.8% for
214 $\geq 90\%$ ($p=0.225$), and 58.8% and 50% for 100% of required doses ($p=0.732$), respectively. In
215 comparison, these percentages were 88.2%, 82.4%, and 70.6%, respectively, for oral metronidazole
216 users. Reported reasons of non-adherence to vaginal probiotics during face-to-face interviews were
217 'simply forgetting' ($n=9$), experiencing side-effects ($n=2$), menses ($n=2$), and being away from home
218 and having left products at home ($n=1$). Additional reasons for missing doses mentioned during
219 FGDs/IDIs were being drunk ($n=2$) and being confused about the dosing schedule ($n=2$). Only one
220 woman in the metronidazole arm reported missing doses due to experiencing side-effects. Most
221 women in FGDs reported using all doses as instructed and finding it easy to adhere, and thought that
222 the diary cards served as a useful reminder to use the products.

223 **Table 1: Adherence to study interventions**
224

Adherence to study products	Metronidazole (n=17)	EF+ (n=17)	GynLP (n=16)
Adherence Enr–D7, median % (IQR)	100 (100–100)	100 (100–100)	100 (100–100)
Adherence D7–M1, median % (IQR)	100 (100–100)	100 (100–100)	100 (91.7–100)
Adherence M1–M2, median % (IQR)	100 (100–100)	100 (100–100)	100 (92.3–100)
Overall adherence Enr–M2, median % (IQR)	100 (96.3–100)	100 (100–100)	98.3 (89.3–100)
Overall adherence Enr–M2 n (%)			
- Perfect*	12 (70.6)	10 (58.8)	8 (50.0)
- Adherence ≥90%	14 (82.4)	15 (88.2)	11 (68.8)
- Adherence ≥80%	15 (88.2)	17 (100)	13 (81.3)
Number of times menses Enr–M2 n (%)†			
- Never	7 (41.2)	4 (23.5)	2 (12.5)
- Once	6 (35.3)	5 (29.4)	4 (25.0)
- Twice	4 (23.5)	8 (47.1)	10 (62.5)
Did not use product during menses at least once n (%)			
- Yes	4 (23.5)	3 (17.6)	5 (31.3)
- NA (never had menses)	7 (41.2)	4 (23.5)	2 (12.5)
Self-reported reasons for non-adherence‡	Metronidazole	EF+	GynLP
D7: Self-reported reasons why not able to use all doses as instructed n (%)§			
- Simply forgot	0	2 (11.8)	0
- Product had side effects	0	0	1 (6.7)¶
M1: Self-reported reasons why not able to use all doses as instructed n (%)§			
- Simply forgot	1 (6.3)	1 (5.9)	1 (6.3)
- Product had side effects	1 (6.3)	0	1 (6.3)‡‡
- Did not like product for another reason	1 (6.3)	0	0
- Other	1 (6.3)**	1 (5.9)††	2 (12.5)§§
M2: Self-reported reasons why not able to use all doses as instructed n (%)§			
- Simply forgot	1 (6.3)	2 (11.8)	3 (18.8)
- Travelled and forgot to take product	1 (6.3)	0	1 (6.25)
- Other	0	1 (5.9)¶¶	1 (6.3)
D7: Participant thinks she used product correctly most of the time n (%)	17 (100)	16 (94.1)	14 (93.3)
M1: Participant thinks she used product correctly most of the time n (%)	13 (86.7)	17 (100)	11 (68.8)
M2: Participant thinks she used product correctly most of the time n (%)	15 (93.7)	16 (94.1)	14 (87.5)

225 *Defined as 100% of the prescribed doses used at the prescribed times after nurse review of the participant's diary card and returned used
226 packaging and unused product.
227 †Number of times menses in the control group: never 2 (11.8%), once 3 (17.8%), twice 11 (64.7%), and thrice 1 (5.9%).
228 ‡Numbers of participants per randomisation group may vary slightly due to loss to follow-up. Participants with ≥90% adherence not shown.
229 §Multiple answers possible.
230 ¶Participant reported vulval itching and burning when passing urine.
231 ||Participant reported mild gastritis and wanting to withdraw from the study anyway.
232 **Participant reported receiving oral metronidazole therapy for 7 days due to infection.
233 ††Participant reported having menses twice in one month; decided to use less of her product until the next study visit.
234 ‡‡Participant reported genital itching, genital burning, and pain during sex.
235 §§One participant reported missing the D7 study visit and therefore running out of supplies. Another participant reported not to have used
236 the study product during menses (which she was allowed to do).
237 ¶¶Participant reported being drunk and therefore forgetting to take the study product.
238 ||||Participant reported taking the study product correctly but that the product came out during menses
239 D7, Day 7 visit; EF+, Ecologic Femi+; Enr, enrolment visit; GynLP, Gynophilus LP; IQR, inter-quartile range; M1/2, Month 1/2 visit; NA,
240 not applicable.

1
2
3 241 **Acceptability**

4
5 242 *Ease-of-use*

6
7 243 No participants reported having heard about probiotics before study participation. After product use,
8
9 244 all vaginal probiotic users reported feeling very comfortable with insertion and that insertion became
10
11 245 easier over time. All but one woman reported inserting while lying down (online supplementary
12
13 246 material table 2).

14
15
16 247

17
18 248 *Bodily changes and product perception*

19
20 249 During FGDs, several women using either vaginal probiotic reported the product (partially) “*coming*
21
22 250 *out*” during the first few uses, but that this decreased after having gained experience. Many EF+ and
23
24 251 GynLP users reported an increase in vaginal wetness, which was considered a positive attribute by
25
26 252 most. Some women reported increased libido. For example, one EF+ user said: “*I felt a great desire to*
27
28 253 *[have] sex again and again.*” In contrast, one metronidazole user reported a decrease in libido. Most
29
30 254 women believed that the vaginal probiotics decreased the recurrence of symptomatic BV (our
31
32 255 preliminary efficacy data suggest that BV incidence had in fact decreased),[16] and a few believed
33
34 256 that they also prevented STI acquisition (the trial had insufficient statistical power to assess this).

35
36
37 257

38
39 258 *Support*

40
41 259 One social harm related to vaginal probiotic use was reported: a GynLP user was verbally harassed by
42
43 260 her partner and her sister because of her study participation, and opted to withdraw her informed
44
45 261 consent. Reports of partner, family, and community support during the FGDs/IDIs were mixed: some
46
47 262 women reported problems with loved ones. Negative reactions from male partners were more often
48
49 263 based on suspicions about study participation than the products themselves. One EF+ user said: “*He*
50
51 264 *[her partner] did not accept that. He asked me to go together with him to the clinic [a local health*
52
53 265 *centre] and check if I am not HIV-positive.*” Another participant using metronidazole mentioned
54
55 266 wanting to join the study to her husband, who forbade her to participate. However, she decided to join
56
57 267 anyway: “*he did not know that I was using the study product, because he had refused me to join [the]*
58
59
60

1
2
3 268 *study before... I used them [the study products] without informing him.*" All sex workers except one
4
5 269 stated that they had not discussed study participation with male clients.
6

7 270
8

9 271 *Worries and concerns*

10
11 272 In the FGDs, one woman reported hearing rumours prior to enrolling that vaginal products "*can*
12
13 273 *damage the uterus or cause tumours in the womb.*" However, most participants thought that vaginal
14
15 274 probiotics would be acceptable to Rwandan women. One GynLP user argued: "*They [already] give us*
16
17 275 *vaginal pills*", by which she meant vaginal medications for yeast infections. Some women were
18
19 276 concerned about future product availability and pricing. They hoped that probiotics would be
20
21 277 distributed cheaply through the Rwandan *Mutuelle* public health insurance because they would
22
23 278 otherwise be inaccessible to many women. One metronidazole user was concerned about a limited
24
25 279 applicability of probiotics because BV is not diagnosed by laboratory testing in Rwanda: "*They do not*
26
27 280 *have adequate medical instruments to test diseases, you tell the physician how [...] you feel and by*
28
29 281 *guessing the disease, he gives you at least four medications, saying that you may have trichomonas,*
30
31 282 *you may have syphilis, you may have gonorrhoea [she refers to syndromic management.[20,21]] At*
32
33 283 *health centre-level they do not have medical equipment to test diseases, meaning that they will not*
34
35 284 *know who to give that [probiotic/antibiotic maintenance therapy] medication.*"
36
37
38

39 285

40 41 286 **Vaginal practices and sexual risk-taking**

42
43 287 At enrolment, 49.3% of the women reported to never use products inside the vagina, and at Month 6,
44
45 288 this increased to 81.5% (OR 5.2, 95% CI 1.96-17.34; table 2). During FGDs, some women understood
46
47 289 that vaginal washing practices may increase the risk of vaginal infection, but others did not. A
48
49 290 participant stated: "*You get them [i.e., vaginal diseases] anyway... whether you wash or not*". In one
50
51 291 FGD, 10 of 11 participants stated having ceased vaginal practices thanks to the study counselling. It
52
53 292 should be noted that in contrast to many other African populations, Rwandan women use vaginal
54
55 293 practices to increase rather than reduce vaginal lubrication. Women mentioned the use of herbs
56
57 294 (*umushishiro*), Vaseline, and oils for this purpose. Self-reported sexual risk taking by face-to-face
58
59 295 interview did not change over time, except for a significant reduction in reported numbers of sex
60

1
2
3 296 partners in the previous month at Month 6 compared to enrolment. No women in FGDs/IDIs
4
5 297 mentioned adopting safer sex practices (such as consistent condom use) in response to the counselling
6
7 298 messages. During face-to-face interviews at the Month 2 visit, 12 of 15 women (80%) who had an
8
9 299 uncircumcised main sex partner reported asking him to regularly clean his penis in the future (online
10
11 300 supplementary material table 2). While most women in FGDs understood that using condoms and
12
13 301 improved penile hygiene could reduce BV rates, some mentioned that they found it difficult to discuss
14
15 302 these topics with male partners. One participant stated that this is especially difficult being a sex
16
17 303 worker: *“a man gives you his own money and you start educating him to wash!”* However, another
18
19 304 sex worker reported refusing sex with uncircumcised clients: *“you leave him, because he has a lot [of]*
20
21 305 *germs”*. Several women reported discussing circumcision with their partners; one participant reported
22
23 306 telling her husband: *“It is better that you do circumcision because it is a good thing... you would get a*
24
25 307 *chance of not contracting diseases.”*
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

308 **Table 2: Changes in reported vaginal cleansing practices and (sexual) behaviour between the**
 309 **enrolment and the M6 visit.**

310

Self-reported sociodemographic characteristics	Enr (n=71)	M6 (n=65)	OR (95% CI)*
			P value*
Reports using no products inside the vagina (other than for managing menses; all participants) n (%)	35 (49.3)	53 (81.5)	5.2 (1.96–17.34) <0.001
Reports using no products inside the vagina (other than for managing menses; controls and metronidazole users only)† n (%)	15 (44.1)	27 (79.4)	13.0 (1.95–552.5) 0.002
Reports using water only n (%)	23 (32.4)	10 (15.4)	0.37 (0.13–0.92) 0.029
Reports using water and soap n (%)	3 (4.2)	2 (3.1)	0.67 (0.06–5.82) 1.00
Reports using paper, cloth or cotton wool n (%)	9 (12.7)	0 (0)	0.13 (0.00–0.93)‡ 0.008
Reports using traditional herbs, stones, powders as vaginal cleansing practice n (%)	1 (1.4)	1 (1.5)	1.00 (0.01–78.5)‡ 1.00
Mean weekly frequency of vaginal practices (95% CI)	2.15 (0.97–3.34)	0.64 (0.18–1.11)	NA 0.328
Median number of sex partners in last month at baseline or per month during follow-up period (IQR)	5 (3–16)	2 (1–4)	NA <0.001
Any condom use reported in past two weeks (Enr) or since last study visit (M6), versus none n (%)	64 (90.1)	60 (92.3)	1.67 (0.32–10.7) 0.727
Reports exchanging sex for money/goods in past month (Enr) or since last study visit (M6) n (%)	65 (91.5)	58 (89.2)	0.80 (0.16–3.72) 1.00

311 *McNemar's OR and p-value for binary variables and Wilcoxon signed-rank test p-value for continuous variables, comparing the response at M6
 312 with the response at Enr. ORs with 95% CI were also calculated for binary pre-post data.

313 †N=34.

314 ‡To enable calculation of effect measures, a zero value was replaced by 1.

315 CI, confidence interval; Enr, enrolment visit; IQR, inter-quartile range; M6, Month 6 visit; NA, not applicable; OR, odds ratio.

316 Correlates of adherence

317 In bivariable mixed effects models including the probiotic groups only, no participant characteristics
 318 were significantly associated with perfect adherence (table 3). However, non-significant trends were
 319 observed. Younger age, asking many questions at enrolment, having menses during the previous study
 320 interval, and reporting urogenital symptoms were associated with a lower likelihood of perfect
 321 adherence. When including oral metronidazole users, menses was significantly associated with a
 322 lower likelihood of perfect adherence (p=0.008). There were no significant associations between
 323 randomisation group and perfect adherence.

324 **Table 3: Participant characteristics associated with perfect adherence**

Participant characteristics	EF+ and GynLP users		EF+, GynLP and oral metronidazole users	
	OR (95% CI)	P value	OR (95% CI)	P value
Randomisation group: GynLP versus EF+	0.68 (0.22–2.11)	0.505	ND	ND
Randomisation group: - EF+ versus metronidazole - GynLP versus metronidazole	ND	ND	0.53 (0.15–1.81) 0.36 (0.11–1.23)	0.308 0.103
Age in years: ≥30 years versus <30	2.66 (0.90–7.82)	0.076	1.60 (0.61–4.15)	0.336
Marital status: - Married versus never married - Divorced versus never married - Widowed versus never married	0.97 (0.14–6.58) 1.18 (0.29–4.79) ND	0.976 0.912 0.991	1.17 (0.20–6.99) 1.39 (0.42–4.57) ND	0.865 0.586 0.990
At least some schooling versus no schooling	1.20 (0.59–2.45)	0.619	0.80 (0.22–2.95)	0.740
Number of sex partners last month: five or more versus four or less.	0.58 (0.18–1.83)	0.351	0.49 (0.17–1.37)	0.173
Exchanged sex for money/goods past month	ND	0.990	ND	0.986
Nurse reported participant asked questions at Enr - Yes, many versus none - Yes, a few versus none	0.19 (0.02–1.52) 0.83 (0.24–2.83)	0.116 0.761	0.15 (0.02–1.19) 0.83 (0.27–2.57)	0.072 0.744
Had menses during study visit interval	0.41 (0.14–1.20)	0.104	0.26 (0.09–0.70)	0.008
Reported alcohol consumption during study: - Once or twice per week versus never - More than twice per week versus never	0.54 (0.14–2.12) 0.92 (0.18–4.81)	0.373 0.920	0.34 (0.11–1.08) 0.81 (0.19–3.49)	0.068 0.774
Reported at least one urogenital symptom during study interval versus none	0.11 (0.01–1.56)	0.103	0.30 (0.04–2.16)	0.231
Reported at least one adverse event during study visit interval (excluding urogenital symptoms) versus none	0.43 (0.10–1.83)	0.253	0.55 (0.15–2.05)	0.371

326 Sociodemographic characteristics associated with perfect adherence in bivariable mixed effects models, in the enrolment–D7, D7–M1, and
 327 M1–M2 study visit intervals.
 328 CI, confidence interval; D7, Day 7 visit; EF+, Ecologic Femi+; Enr, enrolment visit; GynLP, Gynophilus LP; M1, Month 1 visit; M2, Month
 329 2 visit; ND, non-determinable; OR, odds ratio.

330 331 **Vaginal infection knowledge**

332 Almost all participants reported having heard of ‘diseases of the vagina’ and STIs before, but only
 333 4.6% knew what bacteria were (table 4). The STIs most often spontaneously named (in numerical
 334 order) were HIV, gonorrhoea, and syphilis; only one participant reported having heard of BV. After
 335 having received an explanation about what BV is, only one woman reported ever having been
 336 diagnosed with BV. Most participants could name at least one cause or potential consequence of
 337 vaginal infections. Consequences wrongfully attributed to vaginal infections were death (4.6% of
 338 women), infant malformations (3.9%), and cervical cancer/tumours (3.1%).

339 **Table 4: Vaginal infection knowledge**
 340

	Recruitment (n=61)	Enrolment (n=70)	Total (n=131)
Median age (IQR)	32 (27–35)*	31 (27–35)	31 (27–35)
Has heard of diseases of the vagina before n (%)	60 (98.4)	70 (100)	130 (99.2)
Reports knowing what bacteria are before study n (%)	5 (8.2)	1 (1.4)	6 (4.6)
Reports having heard about STIs before study n (%)	61 (100)	70 (100)	131 (100)
If yes, spontaneously named, without probing† n (%)			
- HIV	58 (95.1)	65 (92.9)	123 (93.9)
- Gonorrhoea	58 (95.1)	65 (92.9)	123 (93.9)
- Syphilis	44 (72.1)	59 (84.3)	103 (78.7)
- Trichomoniasis	38 (62.3)	48 (68.6)	86 (65.7)
- Hepatitis	3 (4.9)	3 (4.3)	6 (4.6)
- Yeast infection	0	3 (4.3)	3 (2.3)
- BV	0	2 (2.9)	2 (1.5)
- Urinary tract infection	1 (1.6)	1 (1.4)	2 (1.5)
- Chlamydia	0	1 (1.4)	1 (0.8)
- Herpes	0	1 (1.4)	1 (0.8)
- HPV / cervical cancer	1 (1.6)	0	1 (0.8)
Reports having heard about BV before this study n (%)	1 (1.6)	0	1 (0.8)
Spontaneously reported reasons why women get vaginal disease, without probing† n (%)			
- Poor toilet hygiene	37 (60.7)	40 (57.1)	77 (58.8)
- Multiple sex partners	28 (45.9)	36 (51.4)	64 (48.9)
- After sex	25 (41.0)	30 (43.0)	55 (42.0)
- Dirty underwear	19 (31.2)	35 (50.0)	54 (41.2)
- Poor vaginal hygiene	26 (42.6)	22 (31.4)	48 (36.6)
- Poor penile hygiene of male partner(s)	4 (6.6)	17 (24.3)	21 (16.0)
- Traditional vaginal practices and washing	3 (4.9)	12 (17.1)	15 (11.5)
- New sex partner	6 (9.8)	3 (4.3)	9 (6.9)
- Use of contraception	1 (1.6)	3 (4.3)	4 (3.1)
- (Improper) use of sanitary pads or tampons	1 (1.6)	3 (4.3)	4 (3.1)
- Other	3 (4.9)‡	1 (1.4)§	4 (3.1)
- Cannot name any reasons	1 (1.6)	0	1 (0.8)
Spontaneously reported negative consequences of vaginal disease being named, without probing† n (%)			
- Foul smell from the vagina	30 (49.2)	39 (56.5)	69 (53.1)
- Difficulty getting pregnant	18 (29.5)	33 (47.8)	51 (39.2)
- Miscarriage	16 (26.2)	33 (47.8)	49 (37.7)
- Abnormal vaginal discharge	12 (19.7)	28 (40.6)	40 (30.8)
- Baby born too early	16 (26.2)	22 (31.9)	38 (29.2)
- Severe infection / fever of the woman	7 (11.5)	7 (10.1)	14 (10.8)
- Infection / fever of the newborn baby	5 (8.2)	3 (4.4)	8 (6.2)
- Itching	4 (6.6)	4 (5.8)	8 (6.2)
- Other consequences to the baby	3 (4.9)	3 (4.4)	6 (4.6)
- Cervical cancer or tumours	2 (3.3)	3 (4.4)	5 (3.9)
- Death	4 (6.6)	0	4 (3.1)
- HIV/STIs	1 (1.6)	3 (4.4)	4 (3.1)
- Pain during intercourse	0	3 (4.4)	3 (2.3)
- Cannot name any consequence	17 (27.9)	19 (27.5)	36 (27.7)

*One missing value.

†Open-ended question. Totals may be more than 100%.

‡Participants report: "If you are infected with STIs", sharing underwear, and unprotected sex.

§Participant reports: vaginal medicine.

BV, bacterial vaginosis; HPV, human papilloma virus; IQR, interquartile range; STI, sexually transmitted infection.

DISCUSSION

Several studies of different vaginal probiotics have been conducted, some of them in sub-Saharan Africa.[10–13] However, none reported in-depth acceptability and adherence data. Our study suggests high vaginal probiotic acceptability and adherence in high-risk Rwandan women. We found no statistically significant correlates of perfect adherence, partially due to limited statistical power, but younger age, asking many questions about product use at enrolment, current menses, and reporting urogenital symptoms showed trends towards a lower likelihood of perfect adherence. Vaginal probiotics are currently unavailable on the market in most African countries, and it is important to study acceptability in different target populations to inform product development and future marketing strategies.

We could not evaluate the impact of self-reported acceptability aspects on adherence because almost all women reported very high acceptability in face-to-face interviews throughout the trial. Such interviews are known to suffer from social desirability bias. However, women seemed to speak freely in the FGDs, and those data indicate that they did not have major issues with product attributes or insertion. However, some women reported difficulties due to lack of male partner support. The reported increase in vaginal wetness after probiotic insertion was not considered problematic, as lubrication during sex is preferred by most Rwandan men and women.[22] This might be different in other countries where dry sex is preferred.[23] We did find a non-significant lower adherence to GynLP compared to EF+, which might be explained by differences in formulation: GynLP forms a gel in the vagina whereas EF+ capsules merely release lyophilised bacteria. Previous research indicated high adherence to GynLP.[24] Unfortunately, the impact of these formulation differences was insufficiently probed during the FGDs. Participants indicated that they found the diary cards helpful in reminding them to use their products, and we believe that self-monitoring tools might indeed be helpful in maximising adherence.[25]

Our data suggest that counselling was partially effective in changing behaviours that increase BV risk. Significantly more women reported not engaging in vaginal practices at the end of the study, and most

1
2
3 374 women with uncircumcised steady male partners reported having discussed penile hygiene with them.
4
5 375 However, many women mentioned in FGDs that they found it difficult to discuss condom use and
6
7 376 penile hygiene with male partners, especially clients. Women reduced their sexual risks only to a
8
9 377 limited extent during follow-up, reporting a reduction in numbers of sex partners but no differences in
10
11 378 engaging in sex work and condom use in face-to-face interviews. While these results are encouraging,
12
13 379 it is difficult to assess to what extent they were influenced by social desirability bias.
14
15

16 380

17
18 381 Our survey with women at recruitment sessions and enrolment visits showed that high-risk Rwandan
19
20 382 women had heard of several STIs, but were generally unaware of BV, its causes and potential
21
22 383 consequences, and what they can do to prevent it. Experiences with HIV show that public health
23
24 384 interventions can only succeed if health care professionals and the public have sufficient knowledge
25
26 385 of causes and consequences of disease.[26–28] High-risk Rwandan women (and health care
27
28 386 professionals) should therefore be educated about BV.
29

30 387

31 388 **Limitations**

32
33
34 389 Our study had limited statistical power, and social desirability bias may have affected some of our
35
36 390 results, as is often the case in studies of this nature. Additionally, it should be noted that product
37
38 391 efficacy, availability and cost are important determinants of acceptability, and were not evaluated in
39
40 392 our study, although preliminary efficacy results in this study were promising.[16] We could not
41
42 393 directly compare experiences with, and opinions about, the two different vaginal probiotics because
43
44 394 each woman used only one product and qualitative data depth was suboptimal. In the FGDs/IDIs, it
45
46 395 was sometimes difficult to ascertain whether participants were referring to personal experiences, or to
47
48 396 wider community perceptions. Strengths of our study include the use of a mixed-methods approach
49
50 397 and triangulated adherence data.
51

52 398

53 399 **CONCLUSIONS**

54
55
56 400 The prevention of BV recurrence will likely have to include several components to be successful,
57
58 401 such as improved diagnostics, treatments, and prophylactic products (for example probiotics), but also
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

402 improved information, education, and counselling messages targeted to at-risk women and their
403 partners. The results of this study can be used to inform future product development, and to fine-tune
404 counselling messages in future trials.

For peer review only

1
2
3 405 **Acknowledgments** We thank the study participants, the Rinda Ubuzima team and other colleagues in
4
5 406 Rwanda and the United Kingdom who contributed, the funders of this study, as well as the Trial
6
7 407 Steering Committee for trial oversight. We thank Biose and Winlove for the donation of study
8
9 408 products.
10

11 409
12
13 410 **Author contributions** JvdW obtained the research funding and wrote the study protocol and data
14
15 411 collection documents. AN, EL, SA, and JvdW were members of the Trial Steering Committee. SA,
16
17 412 MU, MMU, and JvdW collected the primary data. MU and MMU performed the FGDs and IDIs. MV
18
19 413 and JvdW developed the analytical approach and performed the statistical analyses. MV and JvdW
20
21 414 wrote the manuscript. All authors commented on and approved the final manuscript.
22
23

24 415
25
26 416 **Funding** This work was funded by the DFID/MRC/Wellcome Trust Joint Global Health Trials
27
28 417 Scheme as a Development Project (grant reference MR/M017443/1; grant title: “Preparing for a
29
30 418 clinical trial of interventions to maintain normal vaginal microbiota for preventing adverse
31
32 419 reproductive health outcomes in Africa”). Vaginal probiotics for use in the trial were donated free of
33
34 420 charge by Winlove Probiotics (Amsterdam, The Netherlands) and Biose (formerly Probionov;
35
36 421 Aurillac, France). The findings and conclusions in this paper are those of the authors and do not
37
38 422 necessarily represent the views of the authors’ institutions or companies, or the funder. None of the
39
40 423 authors were paid to write this article. The corresponding author had full access to the data and had
41
42 424 final responsibility for the decision to submit for publication.
43
44

45 425
46
47 426 **Competing interests** AN is employed by Biose (owner of trial product GynLP) and EL by Winlove
48
49 427 Probiotics BV (owner of trial product EF+). AN has financial and/or intellectual investments in
50
51 428 competing products. The other authors report no competing interests.
52

53 429
54
55 430 **Patient consent** All participants provided written informed consent for study participation, and
56
57 431 separate written informed consent for participation in FGDs/IDIs.
58
59
60

1
2
3 432 **Ethics approval** The study was sponsored by the University of Liverpool, approved by the Rwanda
4
5 433 National Ethics Committee and the University of Liverpool Research Ethics Subcommittee for
6
7 434 Physical Interventions, and registered on ClinicalTrials.gov (NCT02459665).
8

9 435
10
11 436 **Data sharing statement** The data supporting the findings of this publication are retained by the
12
13 437 corresponding author (JvdW) and will not be made openly accessible due to privacy concerns. Fully
14
15 438 anonymised data can be made available by written request to j.vandewijgert@liverpool.ac.uk after
16
17 439 assurance that the intended data usage is compliant with relevant ethical approvals and privacy will be
18
19 440 maintained.
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 441 **Figure footnotes**
4

5 442

6
7 443 **Figure 1: Flowchart of the study**

8 444 *Totals to 110 reasons among 102 women because there could be more than one reason per woman.

9 445 †Reasons: outside of metronidazole treatment window (n=5), enrolment target already met (n=4), has a mental disorder (n=1), did not complete
10 446 screening procedures and was subsequently lost to follow-up (n=1), withdrew consent during the screening visit because she thought the
11 447 reimbursement was too low (n=1).

12 448 ‡Reasons: moved away from Kigali (n=2), lost interest because symptoms resolved (n=1), and was verbally harassed by partner and sister
13 449 about study participation (n=1).

14 450

15 451 Acceptability assessments were made at enrolment and at the M2 visit. Adherence assessments were made using self-rated assessments,
16 452 pictorial diary cards, and returned packaging at the D7, M1, and M2 visits (after which product use was ceased). The vaginal infection
17 453 knowledge survey was held at recruitment sessions in the community and at the enrolment visit. Changes in sexual risk-taking and vaginal
18 454 practices were assessed at each follow-up visits and compared to answers given during the enrol visit. All these themes were discussed during
19 455 the eight FGDs and IDIs.

20 456

21 457 BV, bacterial vaginosis; D7, day 7 visit; FGD, focus group discussion; IDI, in-depth interview; M1/2/6, month 1/2/6 visit; RU, Rinda
22 458 Ubuzima; TV, *Trichomonas vaginalis*.

459 **References**

- 460 1 van de Wijgert JHHM, Borgdorff H, Verhelst R, *et al.* The vaginal microbiota: what have
461 we learned after a decade of molecular characterization? *PLOS ONE* 2014;**9**:e105998.
462 doi:10.1371/journal.pone.0105998
- 463 2 van de Wijgert JHHM, Morrison CS, Cornelisse PGA, *et al.* Bacterial vaginosis and
464 vaginal yeast, but not vaginal cleansing, increase HIV-1 acquisition in African women. *J*
465 *Acquir Immune Defic Syndr* 1999 2008;**48**:203–10. doi:10.1097/QAI.0b013e3181743936
- 466 3 van de Wijgert JHHM. The vaginal microbiome and sexually transmitted infections are
467 interlinked: consequences for treatment and prevention. *PLOS Med* 2017;**14**:e1002478.
468 doi:10.1371/journal.pmed.1002478
- 469 4 Li J, McCormick J, Bocking A, *et al.* Importance of vaginal microbes in reproductive
470 health. *Reprod Sci* 2012;**19**:235–42. doi:10.1177/1933719111418379
- 471 5 Nelson DB, Hanlon AL, Wu G, *et al.* First trimester levels of BV-associated bacteria and
472 risk of miscarriage among women early in pregnancy. *Matern Child Health J*
473 2015;**19**:2682–7. doi:10.1007/s10995-015-1790-2
- 474 6 Hay P. Recurrent bacterial vaginosis. *Curr Opin Infect Dis* 2009;**22**:82–6.
475 doi:10.1097/QCO.0b013e32832180c6
- 476 7 Verstraelen H, Verhelst R. Bacterial vaginosis: an update on diagnosis and treatment.
477 *Expert Rev Anti Infect Ther* 2009;**7**:1109–24. doi:10.1586/eri.09.87
- 478 8 Torrone EA, Morrison CS, Chen P-L, *et al.* Prevalence of sexually transmitted infections
479 and bacterial vaginosis among women in sub-Saharan Africa: An individual participant
480 data meta-analysis of 18 HIV prevention studies. *PLOS Med* 2018;**15**:e1002511.
481 doi:10.1371/journal.pmed.1002511
- 482 9 Bradshaw CS, Brotman RM. Making inroads into improving treatment of bacterial
483 vaginosis – striving for long-term cure. *BMC Infect Dis* 2015;**15**:292.
484 doi:10.1186/s12879-015-1027-4
- 485 10 Anukam KC, Osazuwa E, Osemene GI, *et al.* Clinical study comparing probiotic
486 *Lactobacillus* GR-1 and RC-14 with metronidazole vaginal gel to treat symptomatic
487 bacterial vaginosis. *Microbes Infect* 2006;**8**:2772–6. doi:10.1016/j.micinf.2006.08.008
- 488 11 Ling Z, Liu X, Chen W, *et al.* The restoration of the vaginal microbiota after treatment
489 for bacterial vaginosis with metronidazole or probiotics. *Microb Ecol* 2013;**65**:773–80.
490 doi:10.1007/s00248-012-0154-3
- 491 12 Ngugi BM, Hemmerling A, Bukusi EA, *et al.* Effects of bacterial vaginosis-associated
492 bacteria and sexual intercourse on vaginal colonization with the probiotic *Lactobacillus*
493 *crispatus* CTV-05. *Sex Transm Dis* 2011;**38**:1020–7.
494 doi:10.1097/OLQ.0b013e3182267ac4
- 495 13 Bradshaw CS, Pirota M, De Guingand D, *et al.* Efficacy of oral metronidazole with
496 vaginal clindamycin or vaginal probiotic for bacterial vaginosis: randomised placebo-

- 1
2
3 497 controlled double-blind trial. *PLoS ONE* 2012;**7**:e34540.
4 498 doi:10.1371/journal.pone.0034540
5
6
7 499 14 van der Straten A, Montgomery ET, Cheng H, *et al.* High acceptability of a vaginal ring
8 500 intended as a microbicide delivery method for HIV prevention in African women. *AIDS*
9 501 *Behav* 2012;**16**:1775–86. doi:10.1007/s10461-012-0215-0
10
11 502 15 Merckatz RB, Plagianos M, Hoskin E, *et al.* Acceptability of the nesterone®/ethinyl
12 503 estradiol contraceptive vaginal ring: development of a model; implications for
13 504 introduction. *Contraception* 2014;**90**:514–21. doi:10.1016/j.contraception.2014.05.015
14
15 505 16 van de Wijgert JHHM, Verwijs MC, Agaba SK, *et al.* Intermittent use of vaginal
16 506 probiotics or oral metronidazole to prevent bacterial vaginosis recurrence: safety and
17 507 preliminary efficacy by Nugent scoring and 16S rRNA gene sequencing. 2018.
18 508 Conference talk presented at the Keystone Symposium "Role of the genital tract
19 509 microbiome in sexual and reproductive health", Cape Town, South Africa.
20
21
22 510 17 Sobel JD, Ferris D, Schwebke J, *et al.* Suppressive antibacterial therapy with 0.75%
23 511 metronidazole vaginal gel to prevent recurrent bacterial vaginosis. *Am J Obstet Gynecol*
24 512 2006;**194**:1283–9. doi:10.1016/j.ajog.2005.11.041
25
26 513 18 McClelland RS, Richardson BA, Hassan WM, *et al.* Improvement of vaginal health for
27 514 Kenyan women at risk for acquisition of Human Immunodeficiency Virus type 1: results
28 515 of a randomized trial. *J Infect Dis* 2008;**197**:1361–8. doi:10.1086/587490
29
30
31 516 19 Kestelyn E, Nuil JIV, Umulisa MM, *et al.* High acceptability of a contraceptive vaginal
32 517 ring among women in Kigali, Rwanda. *PLOS ONE* 2018;**13**:e0199096.
33 518 doi:10.1371/journal.pone.0199096
34
35 519 20 World Health Organization. Guidelines for the management of sexually transmitted
36 520 infections. 2003.<http://applications.emro.who.int/aiecf/web79.pdf> (accessed 12 Mar
37 521 2019).
38
39
40 522 21 Binagwaho A, editor. *National guidelines for prevention and management of HIV, STIs*
41 523 *& other blood borne infections*. Republic of Rwanda - Ministry of Health 2013.
42 524 https://aidsfree.usaid.gov/sites/default/files/hts_policy_rwanda.pdf (accessed 12 Mar
43 525 2019).
44
45 526 22 Veldhuijzen N, Nyinawabega J, Umulisa M, *et al.* Preparing for microbicide trials in
46 527 Rwanda: focus group discussions with Rwandan women and men. *Cult Health Sex*
47 528 2006;**8**:395–406. doi:10.1080/13691050600859302
48
49
50 529 23 Low N, Chersich MF, Schmidlin K, *et al.* Intravaginal practices, bacterial vaginosis, and
51 530 HIV infection in women: individual participant data meta-analysis. *PLOS Med*
52 531 2011;**8**:e1000416. doi:10.1371/journal.pmed.1000416
53
54 532 24 Dausset C, Patrier S, Gajer P, *et al.* Comparative phase I randomized open-label pilot
55 533 clinical trial of Gynophilus® (Lcr regenerans®) immediate release capsules versus slow
56 534 release muco-adhesive tablets. *Eur J Clin Microbiol Infect Dis* 2018;**37**:1869–80.
57 535 doi:10.1007/s10096-018-3321-8
58
59
60

- 1
2
3 536 25 Safren SA, W. Otto M, Worth JL, *et al.* Two strategies to increase adherence to HIV
4 537 antiretroviral medication: Life-Steps and medication monitoring. *Behav Res Ther*
5 538 2001;**39**:1151–62. doi:10.1016/S0005-7967(00)00091-7
6
7
8 539 26 Glick P, Sahn DE. Changes in HIV/AIDS knowledge and testing behavior in Africa: how
9 540 much and for whom? *J Popul Econ* 2007;**20**:383–422. doi:10.1007/s00148-006-0085-8
10
11 541 27 Peltzer K, Matseke G, Mzolo T, *et al.* Determinants of knowledge of HIV status in South
12 542 Africa: results from a population-based HIV survey. *BMC Public Health* 2009;**9**:174.
13 543 doi:10.1186/1471-2458-9-174
14
15 544 28 Fay H, Baral SD, Trapence G, *et al.* Stigma, health care access, and HIV knowledge
16 545 among men who have sex with men in Malawi, Namibia, and Botswana. *AIDS Behav*
17 546 2011;**15**:1088–97. doi:10.1007/s10461-010-9861-2
18
19
20 547
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

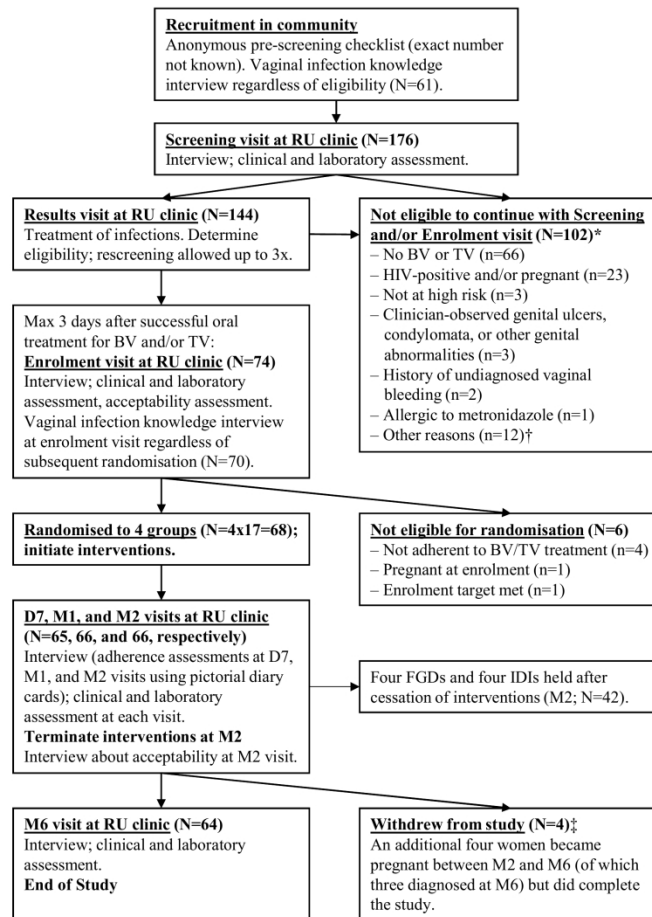


Figure 1: Flowchart of the study

*Totals to 110 reasons among 102 women because there could be more than one reason per woman.

†Reasons: outside of metronidazole treatment window (n=5), enrolment target already met (n=4), has a mental disorder (n=1), did not complete screening procedures and was subsequently lost to follow-up (n=1), withdrew consent during the screening visit because she thought the reimbursement was too low (n=1).

‡Reasons: moved away from Kigali (n=2), lost interest because symptoms resolved (n=1), and was verbally harassed by partner and sister about study participation (n=1).

Acceptability assessments were made at enrolment and at the M2 visit. Adherence assessments were made using self-rated assessments, pictorial diary cards, and returned packaging at the D7, M1, and M2 visits (after which product use was ceased). The vaginal infection knowledge survey was held at recruitment sessions in the community and at the enrolment visit. Changes in sexual risk-taking and vaginal practices were assessed at each follow-up visits and compared to answers given during the enrol visit. All these themes

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60












were discussed during the eight FGDs and IDIs.

BV, bacterial vaginosis; D7, day 7 visit; FGD, focus group discussion; IDI, in-depth interview; M1/2/6, month 1/2/6 visit; RU, Rinda Ubuzima; TV, *Trichomonas vaginalis*.

215x279mm (300 x 300 DPI)

BMJ Open: first published as 10.1136/bmjopen-2019-031819 on 19 May 2020. Downloaded from <http://bmjopen.bmj.com/> on April 20, 2024 by guest. Protected by copyright.

Supplementary Figure 1: Pictorial diary card

Date/Month	Descriptions	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Indicate each time you used study product								
	Used study product							
 Indicate each sex act 								
	Sex with condom							
	Sex without condom							
 Indicate each time you washed/inserted something inside the vagina other than study product 								
 By washing inside, we mean inserting an entire finger inside the vaginal canal 								
	Washed inside vagina with water only							
	Washed inside vagina with soap and water							
	Inserted something else (herbs, powders, etc.)							
Indicate each day of menstrual bleeding								
	Had menstrual bleeding							

The picture provided is the English translation of the pictorial card; participants received a version in Kinyarwanda.

Supplementary Table 1: Baseline characteristics of enrolled population

	Controls (n=17)	Metronidazole (n=17)	EF+ (n=17)	GynLP (n=17)
Median age (IQR)	29 (24–36)	30 (27–34)	33 (28–35)	30 (27–35)
Marital status n (%)				
- Never married	16 (94.1)	11 (64.7)	10 (58.8)	13 (76.5)
- Married	1 (5.9)	1 (5.9)	2 (11.8)	1 (5.9)
- Divorced	0	5 (29.4)	4 (23.5)	3 (17.6)
- Widowed	0	0	1 (5.9)	0
Education level n (%)				
- No schooling	5 (29.4)	3 (17.6)	3 (17.6)	3 (17.7)
- Primary school not completed	7 (41.2)	7 (41.2)	13 (76.5)	4 (23.5)
- Primary school completed	4 (23.5)	5 (29.4)	1 (5.9)	7 (41.2)
- At least some secondary school	1 (5.9)	2 (11.8)	0	3 (17.7)
Median number of sex partners last month (IQR)	5 (3–20)	5 (2–10)	3 (2–15)	3 (2–20)
Exchanged sex for money/goods past month n (%)	17 (100)	14 (82.4)	15 (88.2)	17 (100)
At least one laboratory-confirmed STI* n (%)	8 (47.1)	8 (47.1)	4 (23.5)	9 (52.9)
Median weekly frequency of washing body (IQR)	7 (7–7)	7 (7–7)	7 (7–7)	7 (4–7)
Ever washing the genitalia n (%)				
- Yes, outside only	12 (70.7)	14 (82.4)	15 (88.3)	14 (82.3)
- Yes, both inside and outside	5 (29.4)	3 (17.6)	2 (11.7)	3 (17.7)
- Yes, inside only	0	0	0	0
If reports washing inside, median weekly frequency (IQR)	14 (7–16)	14 (14–14)	11 (7–14)	7 (3–12)

*Chlamydia, gonorrhoea, and/or syphilis.

EF+, Ecologic Femi+; Enr, enrolment visit; GynLP, Gynophilus LP; IQR, inter-quartile range; M2, Month 2 visit; STI, sexually transmitted disease.

Supplementary Table 2: Acceptability of interventions

Acceptability of study products at Enr	Controls (n=17)	Metronidazole (n=17)	EF+ (n=17)	GynLP (n=17)
Nurse reports having explained intervention to participant in detail n (%)	17 (100)	17 (100)	17 (100)	17 (100)
Nurse reports participant asked questions n (%)*				
- Yes, a few	6 (35.3)	2 (11.8)	11 (64.7)	11 (64.7)
- Yes, many	0	0	0	2 (11.8)
First dose applied† under supervision n (%)	NA	17 (100)	17 (100)	17 (100)
Median number of attempts participant made until successful application (IQR)	NA	NA	1 (1–1)	1 (1–1)
Participant seemed comfortable with the insertion after these attempts, according to study nurse n (%)	NA	NA		
- Yes, very			17 (100)	16 (94.1)
- Yes, somewhat			0	1 (5.9)
Acceptability of study products at M2				
Self-reported usual time of insertion n (%)				
- Before going to sleep	NA	NA	17 (100)	15 (100)‡
- After bathing in the morning			0	0
Level of comfort with vaginal insertion after 2 months of use, self-reported n (%)				
- Very comfortable	NA	NA	17 (100)	15 (100)‡
- Somewhat comfortable			0	0
Reported insertion becoming easier over time n (%)	NA	NA	17 (100)	15 (100)‡
Reported manner of insertion§ n (%)				
- While lying down	NA	NA	17 (100)	14 (93.3)‡
- While squatting			1 (5.9)	1 (6.7)
Acceptability of penile hygiene intervention at M2				
Reports having told main sex partner to regularly clean the penis, including underneath the foreskin n (%)¶				
- Yes	3 (17.7)	3 (18.8)	3 (17.6)	3 (18.8)
- No, because he is circumcised	10 (58.8)	9 (56.2)	6 (35.3)	5 (31.3)
- No, other reason	1 (5.9)	0	1 (5.9)	1 (6.3)
If yes, response by the main partner n (%)				
- He said that he would do so in the future	2 (66.7)	1 (33.3)	1 (33.3)	1 (33.3)
- He said that he already does this	1 (33.3)	1 (33.3)	0	1 (33.3)
- He said that he is not interested	0	1 (33.3)	2 (66.7)	1 (33.3)

*One missing value.

†Whether oral insertion (oral metronidazole group) or vaginal insertion (Ecologic Femi+ and Gynophilus LP groups).

‡N=15 due to participants withdrawing informed consent.












§Multiple answers possible; hence totals can be more than 100%.

¶Women with no main sex partner not included.

||N=3 in all four groups.

EF+, Ecologic Femi+; Enr, enrolment visit; GynLP, Gynophilus LP; IQR, inter-quartile range; M2, Month 2 visit; NA, Not applicable.

Supplementary Figure 1: Pictorial diary card

Date/Month	Descriptions	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Indicate each time you used study product								
	Used study product							
 Indicate each sex act 								
	Sex with condom							
	Sex without condom							
 Indicate each time you washed/inserted something inside the vagina other than study product 								
 By washing inside, we mean inserting an entire finger inside the vaginal canal 								
	Washed inside vagina with water only							
	Washed inside vagina with soap and water							
	Inserted something else (herbs, powders, etc.)							
Indicate each day of menstrual bleeding								
	Had menstrual bleeding							

The picture provided is the English translation of the pictorial card; participants received a version in Kinyarwanda.

Supplementary Table 1: Baseline characteristics of enrolled population

	Controls (n=17)	Metronidazole (n=17)	EF+ (n=17)	GynLP (n=17)
Median age (IQR)	29 (24–36)	30 (27–34)	33 (28–35)	30 (27–35)
Marital status n (%)				
- Never married	16 (94.1)	11 (64.7)	10 (58.8)	13 (76.5)
- Married	1 (5.9)	1 (5.9)	2 (11.8)	1 (5.9)
- Divorced	0	5 (29.4)	4 (23.5)	3 (17.6)
- Widowed	0	0	1 (5.9)	0
Education level n (%)				
- No schooling	5 (29.4)	3 (17.6)	3 (17.6)	3 (17.7)
- Primary school not completed	7 (41.2)	7 (41.2)	13 (76.5)	4 (23.5)
- Primary school completed	4 (23.5)	5 (29.4)	1 (5.9)	7 (41.2)
- At least some secondary school	1 (5.9)	2 (11.8)	0	3 (17.7)
Median number of sex partners last month (IQR)	5 (3–20)	5 (2–10)	3 (2–15)	3 (2–20)
Exchanged sex for money/goods past month n (%)	17 (100)	14 (82.4)	15 (88.2)	17 (100)
At least one laboratory-confirmed STI* n (%)	8 (47.1)	8 (47.1)	4 (23.5)	9 (52.9)
Median weekly frequency of washing body (IQR)	7 (7–7)	7 (7–7)	7 (7–7)	7 (4–7)
Ever washing the genitalia n (%)				
- Yes, outside only	12 (70.7)	14 (82.4)	15 (88.3)	14 (82.3)
- Yes, both inside and outside	5 (29.4)	3 (17.6)	2 (11.7)	3 (17.7)
- Yes, inside only	0	0	0	0
If reports washing inside, median weekly frequency (IQR)	14 (7–16)	14 (14–14)	11 (7–14)	7 (3–12)

*Chlamydia, gonorrhoea, and/or syphilis.

EF+, Ecologic Femi+; Enr, enrolment visit; GynLP, Gynophilus LP; IQR, inter-quartile range; M2, Month 2 visit; STI, sexually transmitted disease.

Supplementary Table 2: Acceptability of interventions

Acceptability of study products at Enr	Controls (n=17)	Metronidazole (n=17)	EF+ (n=17)	GynLP (n=17)
Nurse reports having explained intervention to participant in detail n (%)	17 (100)	17 (100)	17 (100)	17 (100)
Nurse reports participant asked questions n (%)*				
- Yes, a few	6 (35.3)	2 (11.8)	11 (64.7)	11 (64.7)
- Yes, many	0	0	0	2 (11.8)
First dose applied† under supervision n (%)	NA	17 (100)	17 (100)	17 (100)
Median number of attempts participant made until successful application (IQR)	NA	NA	1 (1–1)	1 (1–1)
Participant seemed comfortable with the insertion after these attempts, according to study nurse n (%)	NA	NA		
- Yes, very			17 (100)	16 (94.1)
- Yes, somewhat			0	1 (5.9)
Acceptability of study products at M2				
Self-reported usual time of insertion n (%)				
- Before going to sleep	NA	NA	17 (100)	15 (100)‡
- After bathing in the morning			0	0
Level of comfort with vaginal insertion after 2 months of use, self-reported n (%)				
- Very comfortable	NA	NA	17 (100)	15 (100)‡
- Somewhat comfortable			0	0
Reported insertion becoming easier over time n (%)	NA	NA	17 (100)	15 (100)‡
Reported manner of insertion§ n (%)				
- While lying down	NA	NA	17 (100)	14 (93.3)‡
- While squatting			1 (5.9)	1 (6.7)
Acceptability of penile hygiene intervention at M2				
Reports having told main sex partner to regularly clean the penis, including underneath the foreskin n (%)¶				
- Yes	3 (17.7)	3 (18.8)	3 (17.6)	3 (18.8)
- No, because he is circumcised	10 (58.8)	9 (56.2)	6 (35.3)	5 (31.3)
- No, other reason	1 (5.9)	0	1 (5.9)	1 (6.3)
If yes, response by the main partner n (%)				
- He said that he would do so in the future	2 (66.7)	1 (33.3)	1 (33.3)	1 (33.3)
- He said that he already does this	1 (33.3)	1 (33.3)	0	1 (33.3)
- He said that he is not interested	0	1 (33.3)	2 (66.7)	1 (33.3)

*One missing value.

†Whether oral insertion (oral metronidazole group) or vaginal insertion (Ecologic Femi+ and Gynophilus LP groups).

‡N=15 due to participants withdrawing informed consent.

§Multiple answers possible; hence totals can be more than 100%.

¶Women with no main sex partner not included.

||N=3 in all four groups.

EF+, Ecologic Femi+; Enr, enrolment visit; GynLP, Gynophilus LP; IQR, inter-quartile range; M2, Month 2 visit; NA, Not applicable.

STROBE Statement—Checklist of items that should be included in reports of *cohort studies*

	Item No	Recommendation	Reported on page #
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1
		(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	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	4,5
		(b) For matched studies, give matching criteria and number of exposed and unexposed	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	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	6
Bias	9	Describe any efforts to address potential sources of bias	6
Study size	10	Explain how the study size was arrived at	NA (Primary outcomes paper)
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6
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	6,7
		(c) Explain how missing data were addressed	NA (indicated in tables if missing)
		(d) If applicable, explain how loss to follow-up was addressed	NA
		(e) Describe any sensitivity analyses	NA
Results			
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	Page 8, Figure 1
		(b) Give reasons for non-participation at each stage	8, Figure 1
		(c) Consider use of a flow diagram	Figure 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8
		(b) Indicate number of participants with missing data for each variable of	8 (missing

		interest	data in footnotes in table, if applicable)
		(c) Summarise follow-up time (eg, average and total amount)	8
Outcome data	15*	Report numbers of outcome events or summary measures over time	9
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	Table 1
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Page 12 and beyond
Discussion			
Key results	18	Summarise key results with reference to study objectives	Page 18
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	Page 19
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Page 18, 19
Generalisability	21	Discuss the generalisability (external validity) of the study results	Page 18, 19
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	Page 21

*Give information separately for exposed and unexposed groups.

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 <http://www.strobe-statement.org>.

Vaginal Probiotic Adherence and Acceptability in

High-Risk Rwandan Women Participating in a Pilot Randomised Controlled Trial:

A Mixed-Methods Approach

Authors: Marijn C. Verwijs (MD)¹, Stephen K. Agaba (MD)², Marie-Michele Umulisa (MA)², Mireille Uwineza (BA)², Adrien Nivoliez (PhD)³, Elke Lievens (PhD)⁴, Janneke H.H.M. van de Wijgert (MD PhD MPH)^{1,5}.

Author affiliations

¹Institute of Infection and Global Health, University of Liverpool, Liverpool, United Kingdom.

²Rinda Ubuzima, Kigali, Rwanda.

³Biose, Aurillac, France.

⁴Winlove Probiotics, Amsterdam, The Netherlands.

⁵Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht University, Utrecht, The Netherlands.

Correspondence to

Professor Janneke van de Wijgert, MD PhD MPH

Department of Clinical Infection, Microbiology and Immunology

Institute of Infection and Global Health, University of Liverpool

Ronald Ross Building, 8 West Derby Street, Liverpool L69 7BE, United Kingdom

Email: j.vandewijgert@liverpool.ac.uk

ORCID: 0000-0003-2728-4560

Word counts:

Word count abstract: 300 words (max. 300 words).

Word count main text: 3,104 479 words (max. 4000 words).

Number of tables and figures: 5 (max. 5).

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

29 Number of supplementary tables and figures: ~~23~~.

For peer review only

1
2
3 30 **ABSTRACT**
4

5 31 **Objectives** ~~The recurrence of~~ Bacterial vaginosis (BV) recurrence is common. We evaluated the
6
7 32 adherence and acceptability of intermittent use of two vaginal probiotics and one antibiotic to prevent
8
9 33 recurrence.
10

11 34 **Design** Repeated adherence and acceptability assessments using mixed methods within a pilot
12
13 35 randomised controlled trial.
14

15 36 **Setting** Research clinic in Kigali, Rwanda.
16

17 37 **Participants** High-risk Rwandan women (n=68) with BV and/or trichomoniasis.
18

19 38 **Interventions** Women were randomised to four groups (n=17 each) after completing metronidazole
20
21 39 treatment: behavioural counselling only, or behavioural counselling plus two-month intermittent use
22
23 40 of oral metronidazole, Ecologic Femi+ (EF+) vaginal capsule, or Gynophilus LP (GynLP) vaginal
24
25 41 tablet ~~for two months~~.
26
27

28 42 **Outcome measures** Adherence and acceptability data from ~~the~~ randomised women were collected in
29
30 43 structured face-to-face interviews, semi-structured focus group discussions and in-depth interviews,
31
32 44 daily diaries, and counting of used/unused study products. Randomised Wwomen and women
33
34 45 attending recruitment sessions (n=131) were surveyed about ~~their~~ vaginal infection knowledge.
35
36

37 46 **Results** Most ~~randomised~~ women (93%) were sex workers. At baseline, they were unfamiliar with
38
39 47 BV, and had never used probiotics. All ~~vaginal~~ probiotic users reported that insertion became easier
40
41 48 over time. Triangulated adherence data showed that 100% of EF+ users and 88.2% of GynLP users
42
43 49 used $\geq 80\%$ of required doses. Younger age, asking many questions at enrolment, having menses, and
44
45 50 reporting urogenital symptoms showed non-significant trends towards a lower perfect adherence
46
47 51 likelihood. Qualitative data suggested that women believed that the probiotics reduced BV recurrence,
48
49 52 but that partners were sometimes unsupportive of study participation. Self-reported vaginal washing
50
51 53 practices decreased during follow-up, but sexual risk behaviours did not. Most women (80%) with an
52
53 54 uncircumcised steady partner discussed penile hygiene with him, but many women found this
54
55 55 difficult, especially with male clients.
56
57
58
59
60

1
2
3 56 **Conclusions** High-risk women require education about vaginal infections. Vaginal probiotic
4
5 57 acceptability and adherence were high in this cohort. Our results can be used to inform future product
6
7 58 development and to fine-tune counselling messages in prevention programs.
8
9

10 59

11 60 **Trial registration** ClinicalTrials.gov (NCT02459665).12 61 **Keywords (5)** bacterial vaginosis, vaginal probiotic, adherence, acceptability, Africa.
13
14
15
16 6217
18 63 **ARTICLE SUMMARY**19
20 64 **Strengths and limitations of this study**

- 21
22 65 • We conducted this research in the context of a ~~tightly controlled and conducted pilot~~ randomised
23
24 66 ~~controlled trial, and statistical power was therefore limited. However, our statistical power was~~
25
26 67 ~~limited.~~
27
28
29 68 • We triangulated different sources of adherence data to maximise accuracy, and used a mixed-
30
31 69 methods approach to evaluate acceptability. ~~However,~~
32
33 70 • ~~We could not directly compare experiences with, and opinions about, the two different vaginal~~
34
35 71 ~~probiotics because each woman used only one product and qualitative data depth was suboptimal.~~
36
37 72 • ~~social~~ Social desirability bias may have affected some of the results.
38
39 73 • ~~We enrolled women at high risk of HIV in Kigali, Rwanda, the majority of whom were female~~
40
41 74 ~~sex workers.~~ The results of this study may not be generalizable to ~~other groups of~~ women at lower
42
43 75 risk of HIV/STIs.
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

76 INTRODUCTION

77 Bacterial vaginosis (BV) is a vaginal condition in which fastidious anaerobes such as *Gardnerella*
78 *vaginalis* increase while beneficial, lactic acid-producing lactobacilli decrease.[1] Often
79 asymptomatic, it is associated with increased risks of sexually transmitted infections (STIs) and HIV
80 transmission, pelvic inflammatory disease, and adverse pregnancy outcomes.[2–5] Although BV is
81 treatable with antibiotics, the risk of recurrence is high.[6,7] ~~BV~~The prevalence of BV varies among
82 regions and ethnic groups but is highest in sub-Saharan Africa, where it is estimated at 30-50%.[8]
83
84 Vaginally-administered probiotics containing lactobacilli are considered a promising new strategy to
85 restore a lactobacilli-dominated vaginal microbiota during and/or after antibiotic treatment, or to
86 prevent BV.[9] While some probiotics have been available on the market for several years, clinical
87 trials to support beneficial effects have only recently been initiated for most products.[10–13]
88 Acceptability is an important component of these trials, to maximise future uptake and adherence of
89 vaginal probiotics should they be proven efficacious. The acceptability of a novel vaginal product
90 depends on factors such as the characteristics of the population studied, characteristics of and
91 experiences with the product, types of sexual relationships and partner support, and community
92 perceptions.[14,15]

93
94 We conducted a clinical trial of intermittent use of two vaginal probiotics and oral metronidazole to
95 prevent BV recurrence in Rwandan women who had been treated for BV and/or *Trichomonas*
96 *vaginalis* (TV). We used qualitative and quantitative research methods to assess adherence and
97 acceptability with vaginal probiotic use. We triangulated various sources of adherence data to obtain
98 adherence estimates per woman for each period of intermittent product use in between study visits,
99 and determined correlates of adherence.

101 METHODS

102 The pilot clinical trial took place from June 2015 to February 2016 at the Rinda Ubuzima research
103 clinic in Kigali, Rwanda. Women who had been successfully treated for BV/TV with a seven-day

1
2
3 104 course of oral metronidazole (Tricozole, Laboratory & Allied Ltd, Nairobi, Kenya) were randomised
4
5 105 to four intervention groups (n=17 each) to prevent BV recurrence: bBehavioural counselling only
6
7 106 (controls), or behavioural counselling plus intermittent use of two different vaginal probiotics or oral
8
9 107 metronidazole for two months. Women were seen at screening, enrolment (product use initiation, if
10
11 108 applicable), Day 7, Month 1, Month 2 (product use cessation, if applicable), and Month 6. Product
12
13 109 efficacies were not known during the trial, and preliminary efficacy results are reported
14
15
16 110 elsewhere.[16] The behavioural counselling focussed on safer sex practices, cessation of vaginal
17
18 111 practices, and increasing male penile hygiene to prevent BV.
19
20 112

21 22 113 **Study population**

23
24 114 Women aged 18-45 at risk of HIV/STIs (defined as having had more than one sex partner and/or
25
26 115 having been treated for an STI and/or BV in the last 12 months) were eligible for enrolment if they
27
28 116 were confirmed HIV-negative, non-pregnant, diagnosed with BV and/or TV, and cured after seven-
29
30 117 day oral metronidazole treatment. Other clinical exclusion criteria were applied but were rare. [16]
31
32 118 Women were recruited by study staff with the assistance of Community Mobilisers who had strong
33
34 119 ties with local high-risk women (particularly sex workers).
35
36 120

37 38 39 121 **Study products and dosing**

40
41 122 Ecologic Femi+ (EF+; Winclove Probiotics, Amsterdam, Netherlands) is a vaginal capsule containing
42
43 123 lyophilised lactic acid-producing bacteria. EF+ was used once per day for five days followed by thrice
44
45 124 weekly, for two months. Gynophilus LP (GynLP; Biose, Aurillac, France) is a tablet containing the
46
47 125 *Lactobacillus rhamnosus* Lcr35 strain. The tablet disintegrates in the vagina and forms a gel that
48
49 126 slowly releases the probiotic bacteria. GynLP was used once every four days for two months. The first
50
51 127 dose was inserted at the clinic under direct observation of a clinician, and remaining doses were self-
52
53 128 administered at home. Women were asked not to cleanse or insert other products into the vagina after
54
55 129 probiotic insertion to allow the probiotics to dissolve. They were also told that they were allowed to
56
57
58 130 cease probiotic use during menses, but were encouraged to continue. Intermittent metronidazole use
59
60 131 was chosen as a positive control intervention because studies conducted in the U.S. and Kenya have

1
2
3 132 shown a 30-40% reduction in BV recurrence.^[17,18] Metronidazole users took 500 mg generic oral
4
5 133 metronidazole (Laboratory & Allied Ltd, Nairobi, Kenya) twice weekly for two months. Participants
6
7 134 and clinicians were not blinded.
8

9 135

11 136 **Adherence and Acceptability, adherence, behavioural, and vaginal infection knowledge**
12
13
14 137 **assessments**

15
16 138 Acceptability was assessed at the enrolment visit prior to product use initiation and at the Month 2
17
18 139 visit after the full two months of use. Adherence was assessed during the intervention period, at the
19
20 140 Day 7, Month 1, and Month 2 visits. Sexual and other behaviours were assessed at all study visits.
21
22 141 Participants were interviewed face-to-face in Kinyarwanda by a trained study nurse using structured
23
24 142 questionnaires with multiple-choice questions, questions requiring a number or date, and an adherence
25
26 143 self-rating scale (from 0-10). In between visits, participants used pictorial diary cards (online
27
28 144 supplementary material figure 1) to record daily episodes of product use, vaginal sex, condom use,
29
30 145 and vaginal practices. Those using study products returned the product packaging and unused
31
32 146 products (if applicable) to their clinic visits, where they were counted by study staff. At each visit,
33
34 147 trained study nurses interviewed participants in Kinyarwanda using a structured questionnaire
35
36 148 (including a self-rating adherence scale from 0-10), and reviewed daily diaries and returned
37
38 149 packaging. Any discrepancies between data sources were discussed with participants, and consensus,
39
40 150 triangulated assessments of adherence were recorded on the questionnaires. Additionally, 131 women
41
42 151 were interviewed about their knowledge of vaginal infections (such as BV and STIs) using a
43
44 152 structured questionnaire during recruitment sessions (n=61; regardless of eligibility) and at enrolment
45
46 153 visits (n=70; this included the 68 randomised women, and two women who attended enrolment visits
47
48 154 but turned out to be ineligible). Women were interviewed before being counselled at study visits or
49
50 155 before receiving information at recruitment sessions. This questionnaire contained multiple-choice
51
52 156 and open-ended questions. Responses to the open-ended questions were categorised and discussed by
53
54 157 two different researchers until consensus about the answer categories was reached.
55
56
57
58 158

1
2
3 159 Four semi-structured focus group discussions (FGDs) with 7-11 participants per group (total n=38),
4
5 160 and four semi-structured individual in-depth interviews (IDIs) were held with enrolled participants,
6
7 161 about their experiences with and opinions of the products, ~~The acceptability of and adherence to the~~
8
9 162 ~~products were also discussed, as well as changes in~~ sexual behaviour, and vaginal practices. Women
10
11 163 randomised to the behavioural counselling only group were not included in the FGDs and IDIs. All
12
13 164 had completed their product use period. The interviews were unlinked anonymous, and women used
14
15 165 pseudonyms to enable them to talk freely despite the fact that the discussions and interviews were
16
17 166 taped. All interviews took place between November 2015 and March 2016, were held in
18
19 167 Kinyarwanda, recorded on tape, transcribed verbatim, and translated into English.
20
21
22 168

169 **Data analysis**

26 170 ~~Quantitative-uestionnaire~~ data were analysed using Stata 13 (StataCorp, College Station, TX, USA).
27
28 171 The proportion of women with $\geq 80\%$ / $\geq 90\%$ / 100% adherence in the probiotic groups were compared
29
30 172 by Fisher's exact tests. Changes in self-reported vaginal practices and sexual behaviours over time
31
32 173 were tested using McNemar's test for binary outcomes, and Wilcoxon's signed-rank test for
33
34 174 continuous~~;~~ outcomes. To study associations of participant characteristics with triangulated adherence,
35
36 175 we used bivariable mixed effects models, with perfect adherence (defined as having used all doses as
37
38 176 instructed) per interval between study visits during the intervention period as the outcome, participant
39
40 177 identification numbers as the random effect, and one participant characteristic at the time as the fixed
41
42 178 effect. We could not determine correlates of acceptability due to limited variation in the acceptability
43
44 179 data (reported acceptability was high throughout the trial).
45
46 180

49 181 The ~~eight~~ FGDs and IDI transcripts were read and discussed by three researchers (MV, MU, and
50
51 182 JvdW). The Chief Investigator (JvdW) decided that data saturation had been met when the fourth
52
53 183 FGD and the fourth IDI transcript had become available in March 2016. The transcripts were then
54
55 184 coded using NVivo 10.0 (QSR International, Melbourne, Australia) by one single researcher (MV).
56
57 185 The discussions and interviews were semi-structured, with themes and associated codes prepared a
58
59 186 priori, as well as new elements that emerged from the data. The codes were derived from an

1
2
3 187 acceptability framework that has been used in studies of vaginal products for contraception or HIV
4
5 188 prevention.[14,15,19] Components of the framework include study population characteristics, product
6
7 189 attributes, sexual encounter and relational attributes, and the contextual environment (e.g. community
8
9 190 perceptions of product use).
10

11
12 191

13 14 192 **Ethical statement**

15
16 193 All participants provided written consent for study participation, and separate consent for participation
17
18 194 in FGDs/IDIs. All non-married participants aged 18-20 also required parental/guardian consent per
19
20 195 Rwandan law at the time of the study. The participants received 3 GBP per visit (in local currency) as
21
22 196 a reimbursement for time and transport costs. Care was taken to protect participant privacy and
23
24 197 confidentiality. The study was sponsored by the University of Liverpool, approved by the Rwanda
25
26 198 National Ethics Committee and the University of Liverpool Research Ethics Subcommittee for
27
28 199 Physical Interventions, and registered on ClinicalTrials.gov (NCT02459665).
29

30
31 200

32 33 201 **Participant and public involvement**

34
35 202 A subset of the enrolled participants were invited to comment on study design and experiences with
36
37 203 the interventions during the FGDs/IDIs. Participants were not invited to develop outcomes, interpret
38
39 204 the results, or to contribute to the writing or editing of this document for readability or accuracy. The
40
41 205 preliminary results of this study were discussed with 32 stakeholders during a workshop held at the
42
43 206 Ministry of Health in Kigali, Rwanda, in December 2017. These stakeholders included representatives
44
45 207 of the Ministry of Health, the National University of Rwanda, the National Ethics Committee, local
46
47 208 hospitals and clinics, and local non-governmental and women's organisations.
48

49
50 209

51 52 210 **RESULTS**

53 54 211 **Baseline characteristics**

55
56 212 We screened 176 women: bacterial STI prevalence was 31.3% and BV prevalence by Gram stain
57
58 213 Nugent scoring was 47.9%. All 68 randomised women were at risk of STI/HIV transmission, with
59
60 214 93.1% reporting having exchanged sex for money and/or goods in the previous month (figure 1,

1
2
3 215 online supplementary material table 1). We collected 29.93 person-years of data. Four women
4
5 216 withdrew their informed consent during the study (for reasons unrelated to study product
6
7 217 acceptability). None were lost to follow-up.
8
9
10 218

11 219 **Adherence**

12
13 220 Triangulated adherence was high: 100% of EF+ users and 88.2% of GynLP users used $\geq 80\%$ of
14
15 221 required doses (Fisher's exact $p=0.103$; table 1), and these percentages were 88.2% and 68.8% for
16
17 222 $\geq 90\%$ ($p=0.225$), and 58.8% and 50% for 100% of required doses ($p=0.732$), respectively. In
18
19 223 comparison, these percentages were 88.2%, 82.4%, and 70.6%, respectively, for oral metronidazole
20
21 224 users. Reported reasons of non-adherence to vaginal probiotics during face-to-face interviews were
22
23 225 'simply forgetting' ($n=9$), experiencing side-effects ($n=2$), menses ($n=2$), and being away from home
24
25 226 and having left products at home ($n=1$). Additional reasons for missing doses mentioned during
26
27 227 FGDs/IDIs were being drunk ($n=2$) and being confused about the dosing schedule ($n=2$). Only one
28
29 228 woman in the metronidazole arm reported missing doses due to experiencing side-effects. Most
30
31
32
33 229 women in FGDs reported using all doses as instructed and finding it easy to adhere, and thought that
34
35 230 the diary cards served as a useful reminder to use the products.
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

231 **Table 1: Adherence to study interventions**

232

Adherence to study products	Metronidazole (n=17)	EF+ (n=17)	GynLP (n=16)
Adherence Enr–D7, median % (IQR)	100 (100–100)	100 (100–100)	100 (100–100)
Adherence D7–M1, median % (IQR)	100 (100–100)	100 (100–100)	100 (91.7–100)
Adherence M1–M2, median % (IQR)	100 (100–100)	100 (100–100)	100 (92.3–100)
Overall adherence Enr–M2, median % (IQR)	100 (96.3–100)	100 (100–100)	98.3 (89.3–100)
Overall adherence Enr–M2 n (%)			
- Perfect*	12 (70.6)	10 (58.8)	8 (50.0)
- Adherence ≥90%	14 (82.4)	15 (88.2)	11 (68.8)
- Adherence ≥80%	15 (88.2)	17 (100)	13 (81.3)
Number of times menses Enr–M2 n (%)†			
- Never	7 (41.2)	4 (23.5)	2 (12.5)
- Once	6 (35.3)	5 (29.4)	4 (25.0)
- Twice	4 (23.5)	8 (47.1)	10 (62.5)
Did not use product during menses at least once n (%)			
- Yes	4 (23.5)	3 (17.6)	5 (31.3)
- NA (never had menses)	7 (41.2)	4 (23.5)	2 (12.5)
Self-reported reasons for non-adherence‡	Metronidazole	EF+	GynLP
D7: Self-reported reasons why not able to use all doses as instructed n (%)§			
- Simply forgot	0	2 (11.8)	0
- Product had side effects	0	0	1 (6.7)¶
M1: Self-reported reasons why not able to use all doses as instructed n (%)§			
- Simply forgot	1 (6.3)	1 (5.9)	1 (6.3)
- Product had side effects	1 (6.3)	0	1 (6.3)‡‡
- Did not like product for another reason	1 (6.3)	0	0
- Other	1 (6.3)**	1 (5.9)††	2 (12.5)§§
M2: Self-reported reasons why not able to use all doses as instructed n (%)§			
- Simply forgot	1 (6.3)	2 (11.8)	3 (18.8)
- Travelled and forgot to take product	1 (6.3)	0	1 (6.25)
- Other	0	1 (5.9)¶¶	1 (6.3)
D7: Participant thinks she used product correctly most of the time n (%)	17 (100)	16 (94.1)	14 (93.3)
M1: Participant thinks she used product correctly most of the time n (%)	13 (86.7)	17 (100)	11 (68.8)
M2: Participant thinks she used product correctly most of the time n (%)	15 (93.7)	16 (94.1)	14 (87.5)

233 *Defined as 100% of the prescribed doses used at the prescribed times after nurse review of the participant's diary card and returned used
 234 packaging and unused product.
 235 †Number of times menses in the control group: never 2 (11.8%), once 3 (17.8%), twice 11 (64.7%), and thrice 1 (5.9%).
 236 ‡Numbers of participants per randomisation group may vary slightly due to loss to follow-up. Participants with ≥90% adherence not shown.
 237 §Multiple answers possible.
 238 ¶Participant reported vulval itching and burning when passing urine.
 239 ||Participant reported mild gastritis and wanting to withdraw from the study anyway.
 240 **Participant reported receiving oral metronidazole therapy for 7 days due to infection.
 241 ††Participant reported having menses twice in one month; decided to use less of her product until the next study visit.
 242 ‡‡Participant reported genital itching, genital burning, and pain during sex.
 243 §§One participant reported missing the D7 study visit and therefore running out of supplies. Another participant reported not to have used
 244 the study product during menses (which she was allowed to do).
 245 ¶¶Participant reported being drunk and therefore forgetting to take the study product.
 246 ||||Participant reported taking the study product correctly but that the product came out during menses
 247 D7, Day 7 visit; EF+, Ecologic Femi+; Enr, enrolment visit; GynLP, Gynophilus LP; IQR, inter-quartile range; M1/2, Month 1/2 visit; NA,
 248 not applicable.

249 **Acceptability**

250 *Ease-of-use*

251 No participants reported having heard about probiotics before study participation. After product use,
252 all vaginal probiotic users reported feeling very comfortable with insertion and that insertion became
253 easier over time. All but one woman reported inserting while lying down (online supplementary
254 material table 2).

255

256 *Bodily changes and product perception*

257 During FGDs, ~~some~~ several women using either vaginal probiotic reported the product (partially)
258 “*coming out*” during the first few uses, but that this decreased after having gained experience. Many
259 ~~women~~ EF+ and GynLP users reported an increase in vaginal wetness, which was considered a
260 positive attribute by most. Some women reported increased libido. For example, one EF+ user said: “*I*
261 *felt a great desire to [have] sex again and again.*” In contrast, o ~~One~~ metronidazole user reported a
262 decrease in libido. Most women believed that the vaginal probiotics decreased the recurrence of
263 symptomatic BV (our preliminary efficacy data suggest that they did BV incidence had in fact
264 decreased), [16] and a few believed that they also prevented STI transmission-acquisition (the trial had
265 insufficient statistical power to assess this).

266

267 *Support*

268 One social harm related to vaginal probiotic use was reported: a GynLP user participant was verbally
269 harassed by her partner and her sister because of her study participation, and opted to withdraw her
270 informed consent. Reports of partner, family, and community support during the FGDs/IDIs were
271 mixed: some women reported problems with loved ones. Negative reactions from male partners were
272 more often based on suspicions about study participation than the products themselves. One ~~woman~~
273 EF+ user said: “*He [her partner] did not accept that. He asked me to go together with him to the clinic*
274 *[a local health centre] and check if I am not HIV-positive.*” Another participant using metronidazole
275 mentioned wanting to join the study to her husband, who forbade her to participate. However, she
276 decided to join anyway: “*he did not know that I was using the study product, because he had refused*

277 *me to join [the] study before... I used them [the study products] without informing him.” All sex*
278 *workers except one stated that they had not discussed study participation with male clients.*

279

280 *Worries and concerns*

281 In the FGDs, one woman reported hearing rumours prior to enrolling that vaginal products “*can*
282 *damage the uterus or cause tumours in the womb.*” However, most participants thought that vaginal
283 probiotics would be acceptable to Rwandan women. One GynLP user argued: “*They [already] give us*
284 *vaginal pills*”, by which she meant vaginal medications for yeast infections. Some women were
285 concerned about future product availability and pricing. They hoped that probiotics would be
286 distributed cheaply through the Rwandan *Mutuelle* public health insurance because they would
287 otherwise be inaccessible to many women. One participant-metronidazole user was concerned about a
288 limited applicability of probiotics because BV is not diagnosed by laboratory testing in Rwanda:
289 “*They do not have adequate medical instruments to test diseases, you tell the physician how [...] you*
290 *feel and by guessing the disease, he gives you at least four medications, saying that you may have*
291 *trichomonas, you may have syphilis, you may have gonorrhoea* [she refers to syndromic
292 management.[20,21]] *At health centre-level they do not have medical equipment to test diseases,*
293 *meaning that they will not know who to give that [probiotic/antibiotic maintenance therapy]*
294 *medication.*”

295

296 **Vaginal practices and sexual risk-taking**

297 At enrolment, 49.3% of the women reported to never use products inside the vagina, and at Month 6,
298 this increased to 81.5% (OR 5.2, 95% CI 1.96-17.34; table 2). During FGDs, some women understood
299 that vaginal washing practices may increase the risk of vaginal infection, but others did not. A
300 participant stated: “*You get them [i.e., vaginal diseases] anyway... whether you wash or not*”. In one
301 FGD, 10 of 11 participants stated having ceased vaginal practices thanks to the study counselling. It
302 should be noted that in contrast to many other African populations, Rwandan women use vaginal
303 practices to increase rather than reduce vaginal lubrication. Women mentioned the use of herbs
304 (*umushishiro*), Vaseline, and oils for this purpose. Self-reported sexual risk taking by face-to-face

1
2
3 305 interview did not change over time, except for a significant reduction in reported numbers of sex
4
5 306 partners in the previous month at Month 6 compared to enrolment. No women in FGDs/IDIs
6
7 307 mentioned adopting safer sex practices (such as consistent condom use) in response to the counselling
8
9 308 messages. During face-to-face interviews at the Month 2 visit, 12 of 15 women (80%) who had an
10
11 309 uncircumcised main sex partner reported asking him to regularly clean his penis in the future (online
12
13 310 supplementary material table 2). While most women in FGDs understood that using condoms and
14
15 311 improved penile hygiene could reduce BV rates, some mentioned that they found it difficult to discuss
16
17 312 these topics with male partners. One participant stated that this is especially difficult being a sex
18
19 313 worker: “*a man gives you his own money and you start educating him to wash!*” However, another
20
21 314 sex worker reported refusing sex with uncircumcised clients: “*you leave him, because he has a lot [of]*
22
23 315 *germs*”. Several women reported discussing circumcision with their partners; one participant reported
24
25 316 telling her husband: “*It is better that you do circumcision because it is a good thing... you would get a*
26
27 317 *chance of not contracting diseases.*”
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

318 **Table 2: Changes in reported vaginal cleansing practices and (sexual) behaviour between the**
 319 **enrolment and the M6 visit.**

320

Self-reported sociodemographic characteristics	Enr (n=71)	M6 (n=65)	OR (95% CI)*
			P value*
Reports using no products inside the vagina (other than for managing menses; all participants) n (%)	35 (49.3)	53 (81.5)	5.2 (1.96–17.34) <0.001
Reports using no products inside the vagina (other than for managing menses; controls and metronidazole users only)† n (%)	15 (44.1)	27 (79.4)	13.0 (1.95–552.5) 0.002
Reports using water only n (%)	23 (32.4)	10 (15.4)	0.37 (0.13–0.92) 0.029
Reports using water and soap n (%)	3 (4.2)	2 (3.1)	0.67 (0.06–5.82) 1.00
Reports using paper, cloth or cotton wool n (%)	9 (12.7)	0 (0)	0.13 (0.00–0.93)‡ 0.008
Reports using traditional herbs, stones, powders as vaginal cleansing practice n (%)	1 (1.4)	1 (1.5)	1.00 (0.01–78.5)‡ 1.00
Mean weekly frequency of vaginal practices (95% CI)	2.15 (0.97–3.34)	0.64 (0.18–1.11)	NA 0.328
Median number of sex partners in last month at baseline or per month during follow-up period (IQR)	5 (3–16)	2 (1–4)	NA <0.001
Any condom use reported in past two weeks (Enr) or since last study visit (M6), versus none n (%)	64 (90.1)	60 (92.3)	1.67 (0.32–10.7) 0.727
Reports exchanging sex for money/goods in past month (Enr) or since last study visit (M6) n (%)	65 (91.5)	58 (89.2)	0.80 (0.16–3.72) 1.00

321 *McNemar's OR and p-value for binary variables and Wilcoxon signed-rank test p-value for continuous variables, comparing the response at M6
 322 with the response at Enr. ORs with 95% CI were also calculated for binary pre-post data.

323 †N=34.

324 ‡To enable calculation of effect measures, a zero value was replaced by 1.

325 CI, confidence interval; Enr, enrolment visit; IQR, inter-quartile range; M6, Month 6 visit; NA, not applicable; OR, odds ratio.

326 Correlates of adherence

327 In bivariable mixed effects models including the probiotic groups only, no participant characteristics
 328 were significantly associated with perfect adherence (table 3). However, non-significant trends were
 329 observed. Younger age, asking many questions at enrolment, having menses during the previous study
 330 interval, and reporting urogenital symptoms were associated with a lower likelihood of perfect
 331 adherence. When including oral metronidazole users, menses was significantly associated with a
 332 lower likelihood of perfect adherence (p=0.008). There were no significant associations between
 333 randomisation group and perfect adherence.

334 **Table 3: Participant characteristics associated with perfect adherence**

Participant characteristics	EF+ and GynLP users		EF+, GynLP and oral metronidazole users	
	OR (95% CI)	P value	OR (95% CI)	P value
Randomisation group: GynLP versus EF+	0.68 (0.22–2.11)	0.505	ND	ND
Randomisation group: - EF+ versus metronidazole - GynLP versus metronidazole	ND	ND	0.53 (0.15–1.81) 0.36 (0.11–1.23)	0.308 0.103
Age in years: ≥30 years versus <30	2.66 (0.90–7.82)	0.076	1.60 (0.61–4.15)	0.336
Marital status: - Married versus never married - Divorced versus never married - Widowed versus never married	0.97 (0.14–6.58) 1.18 (0.29–4.79) ND	0.976 0.912 0.991	1.17 (0.20–6.99) 1.39 (0.42–4.57) ND	0.865 0.586 0.990
At least some schooling versus no schooling	1.20 (0.59–2.45)	0.619	0.80 (0.22–2.95)	0.740
Number of sex partners last month: five or more versus four or less.	0.58 (0.18–1.83)	0.351	0.49 (0.17–1.37)	0.173
Exchanged sex for money/goods past month	ND	0.990	ND	0.986
Nurse reported participant asked questions at Enr - Yes, many versus none - Yes, a few versus none	0.19 (0.02–1.52) 0.83 (0.24–2.83)	0.116 0.761	0.15 (0.02–1.19) 0.83 (0.27–2.57)	0.072 0.744
Had menses during study visit interval	0.41 (0.14–1.20)	0.104	0.26 (0.09–0.70)	0.008
Reported alcohol consumption during study: - Once or twice per week versus never - More than twice per week versus never	0.54 (0.14–2.12) 0.92 (0.18–4.81)	0.373 0.920	0.34 (0.11–1.08) 0.81 (0.19–3.49)	0.068 0.774
Reported at least one urogenital symptom during study interval versus none	0.11 (0.01–1.56)	0.103	0.30 (0.04–2.16)	0.231
Reported at least one adverse event during study visit interval (excluding urogenital symptoms) versus none	0.43 (0.10–1.83)	0.253	0.55 (0.15–2.05)	0.371

336 Sociodemographic characteristics associated with perfect adherence in bivariable mixed effects models, in the enrolment–D7, D7–M1, and
 337 M1–M2 study visit intervals.
 338 CI, confidence interval; D7, Day 7 visit; EF+, Ecologic Femi+; Enr, enrolment visit; GynLP, Gynophilus LP; M1, Month 1 visit; M2, Month
 339 2 visit; ND, non-determinable; OR, odds ratio.

340 341 **Vaginal infection knowledge**

342 Almost all participants reported having heard of ‘diseases of the vagina’ and STIs before, but only
 343 4.6% knew what bacteria were (table 4). The STIs most often spontaneously named (in numerical
 344 order) were HIV, gonorrhoea, and syphilis; only one participant reported having heard of BV. After
 345 having received an explanation about what BV is, only one woman reported ever having been
 346 diagnosed with BV. Most participants could name at least one cause or potential consequence of
 347 vaginal infections. Consequences wrongfully attributed to vaginal infections were death (4.6% of
 348 women), infant malformations (3.9%), and cervical cancer/tumours (3.1%).

349 **Table 4: Vaginal infection kKnowledge of BV and other STIs**
 350

	Recruitment (n=61)	Enrolment (n=70)	Total (n=131)
Median age (IQR)	32 (27–35)*	31 (27–35)	31 (27–35)
Has heard of diseases of the vagina before n (%)	60 (98.4)	70 (100)	130 (99.2)
Reports knowing what bacteria are before study n (%)	5 (8.2)	1 (1.4)	6 (4.6)
Reports having heard about STIs before study n (%)	61 (100)	70 (100)	131 (100)
If yes, spontaneously named, without probing† n (%)			
- HIV	58 (95.1)	65 (92.9)	123 (93.9)
- Gonorrhoea	58 (95.1)	65 (92.9)	123 (93.9)
- Syphilis	44 (72.1)	59 (84.3)	103 (78.7)
- Trichomoniasis	38 (62.3)	48 (68.6)	86 (65.7)
- Hepatitis	3 (4.9)	3 (4.3)	6 (4.6)
- Yeast infection	0	3 (4.3)	3 (2.3)
- BV	0	2 (2.9)	2 (1.5)
- Urinary tract infection	1 (1.6)	1 (1.4)	2 (1.5)
- Chlamydia	0	1 (1.4)	1 (0.8)
- Herpes	0	1 (1.4)	1 (0.8)
- HPV / cervical cancer	1 (1.6)	0	1 (0.8)
Reports having heard about BV before this study n (%)	1 (1.6)	0	1 (0.8)
Spontaneously reported reasons why women get vaginal disease, without probing† n (%)			
- Poor toilet hygiene	37 (60.7)	40 (57.1)	77 (58.8)
- Multiple sex partners	28 (45.9)	36 (51.4)	64 (48.9)
- After sex	25 (41.0)	30 (43.0)	55 (42.0)
- Dirty underwear	19 (31.2)	35 (50.0)	54 (41.2)
- Poor vaginal hygiene	26 (42.6)	22 (31.4)	48 (36.6)
- Poor penile hygiene of male partner(s)	4 (6.6)	17 (24.3)	21 (16.0)
- Traditional vaginal practices and washing	3 (4.9)	12 (17.1)	15 (11.5)
- New sex partner	6 (9.8)	3 (4.3)	9 (6.9)
- Use of contraception	1 (1.6)	3 (4.3)	4 (3.1)
- (Improper) use of sanitary pads or tampons	1 (1.6)	3 (4.3)	4 (3.1)
- Other	3 (4.9)‡	1 (1.4)§	4 (3.1)
- Cannot name any reasons	1 (1.6)	0	1 (0.8)
Spontaneously reported negative consequences of vaginal disease being named, without probing† n (%)			
- Foul smell from the vagina	30 (49.2)	39 (56.5)	69 (53.1)
- Difficulty getting pregnant	18 (29.5)	33 (47.8)	51 (39.2)
- Miscarriage	16 (26.2)	33 (47.8)	49 (37.7)
- Abnormal vaginal discharge	12 (19.7)	28 (40.6)	40 (30.8)
- Baby born too early	16 (26.2)	22 (31.9)	38 (29.2)
- Severe infection / fever of the woman	7 (11.5)	7 (10.1)	14 (10.8)
- Infection / fever of the newborn baby	5 (8.2)	3 (4.4)	8 (6.2)
- Itching	4 (6.6)	4 (5.8)	8 (6.2)
- Other consequences to the baby	3 (4.9)	3 (4.4)	6 (4.6)
- Cervical cancer or tumours	2 (3.3)	3 (4.4)	5 (3.9)
- Death	4 (6.6)	0	4 (3.1)
- HIV/STIs	1 (1.6)	3 (4.4)	4 (3.1)
- Pain during intercourse	0	3 (4.4)	3 (2.3)
- Cannot name any consequence	17 (27.9)	19 (27.5)	36 (27.7)

*One missing value.

†Open-ended question. Totals may be more than 100%.

‡Participants report: "If you are infected with STIs", sharing underwear, and unprotected sex.

§Participant reports: vaginal medicine.

BV, bacterial vaginosis; HPV, human papilloma virus; IQR, interquartile range; STI, sexually transmitted infection.

DISCUSSION

Several studies of different vaginal probiotics have been conducted, some of them in sub-Saharan Africa.[10–13] However, none reported in-depth acceptability and adherence data. Our study suggests high vaginal probiotic acceptability and adherence in high-risk Rwandan women. We found no statistically significant correlates of perfect adherence, partially due to limited statistical power, but younger age, asking many questions about product use at enrolment, current menses, and reporting urogenital symptoms showed trends towards a lower likelihood of perfect adherence. Vaginal probiotics are currently unavailable on the market in most African countries, and it is important to study acceptability in different target populations to inform product development and future marketing strategies.

We could not evaluate the impact of self-reported acceptability aspects on adherence because almost all women reported very high acceptability in face-to-face interviews throughout the trial. Such interviews are known to suffer from social desirability bias. However, women seemed to speak freely in the FGDs, and those data indicate that they did not have major issues with product attributes or insertion. However, some women reported difficulties due to lack of male partner support. The reported increase in vaginal wetness after probiotic insertion was not considered problematic, as lubrication during sex is preferred by most Rwandan men and women.[22] This might be different in other countries where dry sex is preferred.[23] We did find a non-significant lower adherence to GynLP compared to EF+, which might be explained by differences in formulation: GynLP forms a gel in the vagina whereas EF+ capsules merely release lyophilised bacteria. Previous research indicated high adherence to GynLP.[24] Unfortunately, the impact of these formulation differences was insufficiently probed during the FGDs. Participants indicated that they found the diary cards helpful in reminding them to use their products, and we believe that self-monitoring tools might indeed be helpful in maximising adherence.[25]

Our data suggest that counselling was partially effective in changing behaviours that increase BV risk. Significantly more women reported not engaging in vaginal practices at the end of the study, and most

1
2
3 384 women with uncircumcised steady male partners reported having discussed penile hygiene with them.
4
5 385 However, many women mentioned in FGDs that they found it difficult to discuss condom use and
6
7 386 penile hygiene with male partners, especially clients. Women reduced their sexual risks only to a
8
9 387 limited extent during follow-up, reporting a reduction in numbers of sex partners but no differences in
10
11 388 engaging in sex work and condom use in face-to-face interviews. While these results are encouraging,
12
13 389 it is difficult to assess to what extent they were influenced by social desirability bias.
14
15

16 390

17
18 391 Our survey with women at recruitment sessions and enrolment visits showed that high-risk Rwandan
19
20 392 women had heard of several STIs, but were generally unaware of BV, its causes and potential
21
22 393 consequences, and what they can do to prevent it. Experiences with HIV show that public health
23
24 394 interventions can only succeed if health care professionals and the public have sufficient knowledge
25
26 395 of causes and consequences of disease.[26–28] High-risk Rwandan women (and health care
27
28 396 professionals) should therefore be educated about BV.
29

30 397

31 398 **Limitations**

32
33
34 399 Our study had limited statistical power, and social desirability bias may have affected some of our
35
36 400 results, as is often the case in studies of this nature. Additionally, it should be noted that product
37
38 401 efficacy, availability and cost are important determinants of acceptability, and were not evaluated in
39
40 402 our study, although preliminary efficacy results in this study were promising.[16] We could not
41
42 403 directly compare experiences with, and opinions about, the two different vaginal probiotics because
43
44 404 each woman used only one product and qualitative data depth was suboptimal. In the FGDs/IDIs, it
45
46 405 was sometimes difficult to ascertain whether participants were referring to personal experiences, or to
47
48 406 wider community perceptions. Strengths of our study include the use of a mixed-methods approach
49
50 407 and triangulated adherence data.
51

52 408

53 409 **CONCLUSIONS**

54
55
56 410 The prevention of BV recurrence will likely have to include several components to be successful,
57
58 411 such as improved diagnostics, treatments, and prophylactic products (for example probiotics), but also
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

412 improved information, education, and counselling messages targeted to at-risk women and their
413 partners. The results of this study can be used to inform future product development, and to fine-tune
414 counselling messages in future trials.

For peer review only

1
2
3 415 **Acknowledgments** We thank the study participants, the Rinda Ubuzima team and other colleagues in
4
5 416 Rwanda and the United Kingdom who contributed, the funders of this study, as well as the Trial
6
7 417 Steering Committee for trial oversight. We thank Biose and Winlove for the donation of study
8
9 418 products.
10

11 419
12
13 420 **Author contributions** JvdW obtained the research funding and wrote the study protocol and data
14
15 421 collection documents. AN, EL, SA, and JvdW were members of the Trial Steering Committee. SA,
16
17 422 MU, MMU, and JvdW collected the primary data. MU and MMU performed the FGDs and IDIs. MV
18
19 423 and JvdW developed the analytical approach and performed the statistical analyses. MV and JvdW
20
21 424 wrote the manuscript. All authors commented on and approved the final manuscript.
22
23 425

24
25 426 **Funding** This work was funded by the DFID/MRC/Wellcome Trust Joint Global Health Trials
26
27 427 Scheme as a Development Project (grant reference MR/M017443/1; grant title: “Preparing for a
28
29 428 clinical trial of interventions to maintain normal vaginal microbiota for preventing adverse
30
31 429 reproductive health outcomes in Africa”). Vaginal probiotics for use in the trial were donated free of
32
33 430 charge by Winlove Probiotics (Amsterdam, The Netherlands) and Biose (formerly Probionov;
34
35 431 Aurillac, France). The findings and conclusions in this paper are those of the authors and do not
36
37 432 necessarily represent the views of the authors’ institutions or companies, or the funder. None of the
38
39 433 authors were paid to write this article. The corresponding author had full access to the data and had
40
41 434 final responsibility for the decision to submit for publication.
42
43 435

44
45 436 **Competing interests** AN is employed by Biose (owner of trial product GynLP) and EL by Winlove
46
47 437 Probiotics BV (owner of trial product EF+). AN has financial and/or intellectual investments in
48
49 438 competing products. The other authors report no competing interests.
50
51 439

52
53 440 **Patient consent** All participants provided written informed consent for study participation, and
54
55 441 separate written informed consent for participation in FGDs/IDIs.
56
57
58
59
60

1
2
3 442 **Ethics approval** The study was sponsored by the University of Liverpool, approved by the Rwanda
4
5 443 National Ethics Committee and the University of Liverpool Research Ethics Subcommittee for
6
7 444 Physical Interventions, and registered on ClinicalTrials.gov (NCT02459665).
8
9
10 445

11 446 **Data sharing statement** The data supporting the findings of this publication are retained by the
12
13 447 corresponding author (JvdW) and will not be made openly accessible due to privacy concerns. Fully
14
15 448 anonymised data can be made available by written request to j.vandewijgert@liverpool.ac.uk after
16
17 449 assurance that the intended data usage is compliant with relevant ethical approvals and privacy will be
18
19
20 450 maintained.
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 451 **Figure footnotes**
4

5 452

6
7 453 **Figure 1: Flowchart of the study**

8 454 *Totals to 110 reasons among 102 women because there could be more than one reason per woman.

9 455 †Reasons: outside of metronidazole treatment window (n=5), enrolment target already met (n=4), has a mental disorder (n=1), did not complete
10 456 screening procedures and was subsequently lost to follow-up (n=1), withdrew consent during the screening visit because she thought the
11 457 reimbursement was too low (n=1).

12 458 ‡Reasons: moved away from Kigali (n=2), lost interest because symptoms resolved (n=1), and was verbally harassed by partner and sister
13 459 about study participation (n=1).

14 460

15 461 Acceptability assessments were made at enrolment and at the M2 visit. Adherence assessments were made using self-rated assessments,
16 462 pictorial diary cards, and returned packaging at the D7, M1, and M2 visits (after which product use was ceased). The vaginal infection
17 463 knowledge survey was held at recruitment sessions in the community and at the enrolment visit. Changes in sexual risk-taking and vaginal
18 464 practices were assessed at each follow-up visits and compared to answers given during the enrol visit. All this themes were discussed during
19 465 the eight FGDs and IDIs.

20 466

21 467 BV, bacterial vaginosis; D7, day 7 visit; FGD, focus group discussion; IDI, in-depth interview; M1/2/6, month 1/2/6 visit; RU, Rinda
22 468 Ubuzima; TV, *Trichomonas vaginalis*.

469 **References**

- 470 1 van de Wijgert JHHM, Borgdorff H, Verhelst R, *et al.* The vaginal microbiota: what have
471 we learned after a decade of molecular characterization? *PLOS ONE* 2014;**9**:e105998.
472 doi:10.1371/journal.pone.0105998
- 473 2 van de Wijgert JHHM, Morrison CS, Cornelisse PGA, *et al.* Bacterial vaginosis and
474 vaginal yeast, but not vaginal cleansing, increase HIV-1 acquisition in African women. *J*
475 *Acquir Immune Defic Syndr* 1999 2008;**48**:203–10. doi:10.1097/QAI.0b013e3181743936
- 476 3 van de Wijgert JHHM. The vaginal microbiome and sexually transmitted infections are
477 interlinked: consequences for treatment and prevention. *PLOS Med* 2017;**14**:e1002478.
478 doi:10.1371/journal.pmed.1002478
- 479 4 Li J, McCormick J, Bocking A, *et al.* Importance of vaginal microbes in reproductive
480 health. *Reprod Sci* 2012;**19**:235–42. doi:10.1177/1933719111418379
- 481 5 Nelson DB, Hanlon AL, Wu G, *et al.* First trimester levels of BV-associated bacteria and
482 risk of miscarriage among women early in pregnancy. *Matern Child Health J*
483 2015;**19**:2682–7. doi:10.1007/s10995-015-1790-2
- 484 6 Hay P. Recurrent bacterial vaginosis. *Curr Opin Infect Dis* 2009;**22**:82–6.
485 doi:10.1097/QCO.0b013e32832180c6
- 486 7 Verstraelen H, Verhelst R. Bacterial vaginosis: an update on diagnosis and treatment.
487 *Expert Rev Anti Infect Ther* 2009;**7**:1109–24. doi:10.1586/eri.09.87
- 488 8 Torrone EA, Morrison CS, Chen P-L, *et al.* Prevalence of sexually transmitted infections
489 and bacterial vaginosis among women in sub-Saharan Africa: An individual participant
490 data meta-analysis of 18 HIV prevention studies. *PLOS Med* 2018;**15**:e1002511.
491 doi:10.1371/journal.pmed.1002511
- 492 9 Bradshaw CS, Brotman RM. Making inroads into improving treatment of bacterial
493 vaginosis – striving for long-term cure. *BMC Infect Dis* 2015;**15**:292.
494 doi:10.1186/s12879-015-1027-4
- 495 10 Anukam KC, Osazuwa E, Osemene GI, *et al.* Clinical study comparing probiotic
496 *Lactobacillus* GR-1 and RC-14 with metronidazole vaginal gel to treat symptomatic
497 bacterial vaginosis. *Microbes Infect* 2006;**8**:2772–6. doi:10.1016/j.micinf.2006.08.008
- 498 11 Ling Z, Liu X, Chen W, *et al.* The restoration of the vaginal microbiota after treatment
499 for bacterial vaginosis with metronidazole or probiotics. *Microb Ecol* 2013;**65**:773–80.
500 doi:10.1007/s00248-012-0154-3
- 501 12 Ngugi BM, Hemmerling A, Bukusi EA, *et al.* Effects of bacterial vaginosis-associated
502 bacteria and sexual intercourse on vaginal colonization with the probiotic *Lactobacillus*
503 *crispatus* CTV-05. *Sex Transm Dis* 2011;**38**:1020–7.
504 doi:10.1097/OLQ.0b013e3182267ac4
- 505 13 Bradshaw CS, Pirota M, De Guingand D, *et al.* Efficacy of oral metronidazole with
506 vaginal clindamycin or vaginal probiotic for bacterial vaginosis: randomised placebo-

- 1
2
3 507 controlled double-blind trial. *PLoS ONE* 2012;**7**:e34540.
4 508 doi:10.1371/journal.pone.0034540
5
6
7 509 14 van der Straten A, Montgomery ET, Cheng H, *et al.* High acceptability of a vaginal ring
8 510 intended as a microbicide delivery method for HIV prevention in African women. *AIDS*
9 511 *Behav* 2012;**16**:1775–86. doi:10.1007/s10461-012-0215-0
10
11 512 15 Merkatz RB, Plagianos M, Hoskin E, *et al.* Acceptability of the nectorone®/ethinyl
12 513 estradiol contraceptive vaginal ring: development of a model; implications for
13 514 introduction. *Contraception* 2014;**90**:514–21. doi:10.1016/j.contraception.2014.05.015
14
15 515 16 van de Wijgert JHHM, Verwijs MC, Agaba SK, *et al.* Intermittent use of vaginal
16 516 probiotics or oral metronidazole to prevent bacterial vaginosis recurrence: safety and
17 517 preliminary efficacy by Nugent scoring and 16S rRNA gene sequencing. 2018.
18 518 Conference talk presented at the Keystone Symposium "Role of the genital tract
19 519 microbiome in sexual and reproductive health", Cape Town, South Africa.
20
21
22 520 17 Sobel JD, Ferris D, Schwebke J, *et al.* Suppressive antibacterial therapy with 0.75%
23 521 metronidazole vaginal gel to prevent recurrent bacterial vaginosis. *Am J Obstet Gynecol*
24 522 2006;**194**:1283–9. doi:10.1016/j.ajog.2005.11.041
25
26 523 18 McClelland RS, Richardson BA, Hassan WM, *et al.* Improvement of vaginal health for
27 524 Kenyan women at risk for acquisition of Human Immunodeficiency Virus type 1: results
28 525 of a randomized trial. *J Infect Dis* 2008;**197**:1361–8. doi:10.1086/587490
29
30
31 526 19 Kestelyn E, Nuil JIV, Umulisa MM, *et al.* High acceptability of a contraceptive vaginal
32 527 ring among women in Kigali, Rwanda. *PLOS ONE* 2018;**13**:e0199096.
33 528 doi:10.1371/journal.pone.0199096
34
35 529 20 World Health Organization. Guidelines for the management of sexually transmitted
36 530 infections. 2003.<http://applications.emro.who.int/aiecf/web79.pdf> (accessed 12 Mar
37 531 2019).
38
39
40 532 21 Binagwaho A, editor. *National guidelines for prevention and management of HIV, STIs*
41 533 *& other blood borne infections*. Republic of Rwanda - Ministry of Health 2013.
42 534 https://aidsfree.usaid.gov/sites/default/files/hts_policy_rwanda.pdf (accessed 12 Mar
43 535 2019).
44
45 536 22 Veldhuijzen N, Nyinawabega J, Umulisa M, *et al.* Preparing for microbicide trials in
46 537 Rwanda: focus group discussions with Rwandan women and men. *Cult Health Sex*
47 538 2006;**8**:395–406. doi:10.1080/13691050600859302
48
49
50 539 23 Low N, Chersich MF, Schmidlin K, *et al.* Intravaginal practices, bacterial vaginosis, and
51 540 HIV infection in women: individual participant data meta-analysis. *PLoS Med*
52 541 2011;**8**:e1000416. doi:10.1371/journal.pmed.1000416
53
54 542 24 Dausset C, Patrier S, Gajer P, *et al.* Comparative phase I randomized open-label pilot
55 543 clinical trial of Gynophilus® (Lcr regenerans®) immediate release capsules versus slow
56 544 release muco-adhesive tablets. *Eur J Clin Microbiol Infect Dis* 2018;**37**:1869–80.
57 545 doi:10.1007/s10096-018-3321-8
58
59
60

- 1
2
3 546 25 Safren SA, W. Otto M, Worth JL, *et al.* Two strategies to increase adherence to HIV
4 547 antiretroviral medication: Life-Steps and medication monitoring. *Behav Res Ther*
5 548 2001;**39**:1151–62. doi:10.1016/S0005-7967(00)00091-7
6
7
8 549 26 Glick P, Sahn DE. Changes in HIV/AIDS knowledge and testing behavior in Africa: how
9 550 much and for whom? *J Popul Econ* 2007;**20**:383–422. doi:10.1007/s00148-006-0085-8
10
11 551 27 Peltzer K, Matseke G, Mzolo T, *et al.* Determinants of knowledge of HIV status in South
12 552 Africa: results from a population-based HIV survey. *BMC Public Health* 2009;**9**:174.
13 553 doi:10.1186/1471-2458-9-174
14
15 554 28 Fay H, Baral SD, Trapence G, *et al.* Stigma, health care access, and HIV knowledge
16 555 among men who have sex with men in Malawi, Namibia, and Botswana. *AIDS Behav*
17 556 2011;**15**:1088–97. doi:10.1007/s10461-010-9861-2
18
19
20 557
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

BMJ Open

Vaginal Probiotic Adherence and Acceptability in Rwandan Women with High Sexual Risk Participating in a Pilot Randomised Controlled Trial: A Mixed-Methods Approach

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2019-031819.R1
Article Type:	Original research
Date Submitted by the Author:	23-Jan-2020
Complete List of Authors:	Verwijs, Marijn; Institute of Infection and Global Health, University of Liverpool, Agaba, Stephen; Rinda Ubuzima Umulisa, Marie ; Rinda Ubuzima Uwineza, Mireille; Rinda Ubuzima Nivoliez, Adrien; Biose, Lievens, Elke; Winlove van de Wijgert, Janneke H.H.M.; University of Liverpool, Institute of Infection and Global Health; Universitair Medisch Centrum Utrecht, Julius Center for Health Sciences and Primary Care
Primary Subject Heading:	Sexual health
Secondary Subject Heading:	Sexual health, Obstetrics and gynaecology, Public health, Qualitative research
Keywords:	bacterial vaginosis, vaginal probiotic, adherence, acceptability, Africa

SCHOLARONE™
Manuscripts



I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our [licence](#).

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which [Creative Commons](#) licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

1
2
3 **1 Vaginal Probiotic Adherence and Acceptability in Rwandan Women with High Sexual Risk**
4
5 **2 Participating in a Pilot Randomised Controlled Trial: A Mixed-Methods Approach**
6
7
8
9

10 **4** Authors: Marijn C. Verwijs (MD)¹, Stephen K. Agaba (MD)², Marie-Michele Umulisa (MA)²,
11 Mireille Uwineza (BA)², Adrien Nivoliez (PhD)³, Elke Lievens (PhD)⁴, Janneke H.H.M. van de
12 Wiggert (MD PhD MPH)^{1,5}.
13
14
15

16
17
18 **8 Author affiliations**

19
20 ¹Institute of Infection and Global Health, University of Liverpool, Liverpool, United Kingdom.

21
22 ²Rinda Ubuzima, Kigali, Rwanda.

23
24 ³Biose, Aurillac, France.

25
26 ⁴Winlove Probiotics, Amsterdam, The Netherlands.

27
28 ⁵Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht
29 University, Utrecht, The Netherlands.
30
31
32

33
34
35 **16 Correspondence to**

36
37 Professor Janneke van de Wiggert, MD PhD MPH

38
39 Department of Clinical Infection, Microbiology and Immunology

40
41 Institute of Infection and Global Health, University of Liverpool

42
43 Ronald Ross Building, 8 West Derby Street, Liverpool L69 7BE, United Kingdom

44
45 Email: j.vandewijgert@liverpool.ac.uk

46
47 ORCID: 0000-0003-2728-4560
48
49
50

51
52 **24 Word counts:**

53
54 Word count abstract: 300 words (max. 300 words).

55
56 Word count main text: 3,911 words (max. 4000 words).

57
58 Number of tables and figures: 5 (max. 5).

59
60 Number of supplementary tables and figures: 3.

1
2
3 29 **ABSTRACT**
4

5 30 **Objectives** To evaluate adherence and acceptability of intermittent vaginal probiotic or antibiotic use
6
7 31 to prevent bacterial vaginosis (BV) recurrence.
8

9 32 **Design** Repeated adherence and acceptability assessments using mixed methods within a pilot
10
11 33 randomised controlled trial.
12

13 34 **Setting** Research clinic in Kigali, Rwanda.
14

15 35 **Participants** Rwandan women with high sexual risk.
16

17 36 **Interventions** Women diagnosed with BV and/or trichomoniasis were randomised to four groups
18
19 37 (n=17 each) after completing metronidazole treatment: behavioural counselling only, or behavioural
20
21 38 counselling plus two-month intermittent use of oral metronidazole, Ecologic Femi+ (EF+) vaginal
22
23 39 capsule, or Gynophilus LP (GynLP) vaginal tablet.
24
25

26 40 **Outcome measures** Adherence and acceptability were assessed by structured face-to-face interviews,
27
28 41 semi-structured focus group discussions and in-depth interviews, daily diaries, and counting of
29
30 42 used/unused study products in randomised women (n=68). Vaginal infection knowledge was assessed
31
32 43 by structured face-to-face interviews in randomised women and women attending recruitment
33
34 44 sessions (n=131).
35
36

37 45 **Results** Most women (93%) were sex workers, 99.2% were unfamiliar with BV, and none had ever
38
39 46 used probiotics. All probiotic users (n=32) reported that insertion became easier over time.
40
41 47 Triangulated adherence data showed that 17/17 EF+ users and 13/16 GynLP users used $\geq 80\%$ of
42
43 48 required doses (Fisher's exact $p=0.103$). Younger age ($p=0.076$), asking many questions at enrolment
44
45 49 ($p=0.116$), having menses ($p=0.104$), and reporting urogenital symptoms ($p=0.103$) were non-
46
47 50 significantly associated with lower perfect adherence. Women believed that the probiotics reduced
48
49 51 BV recurrence, but reported that partners were sometimes unsupportive of study participation. Self-
50
51 52 reported vaginal washing practices decreased during follow-up, but sexual risk behaviours did not.
52
53 53 Most women (12/15) with an uncircumcised steady partner discussed penile hygiene with him, but
54
55 54 many women found this difficult, especially with male clients.
56
57
58
59
60

1
2
3 55 **Conclusions** High-risk women require education about vaginal infections. Vaginal probiotic
4
5 56 acceptability and adherence were high in this cohort. Our results can be used to inform future product
6
7 57 development and to fine-tune counselling messages in prevention programs.
8

9 58 **Trial registration** ClinicalTrials.gov (NCT02459665).

10 59 **Keywords (5)** bacterial vaginosis, vaginal probiotic, adherence, acceptability, Africa.
11
12
13
14 60

15 61 **ARTICLE SUMMARY**

16 62 **Strengths and limitations of this study**

- 17 63 • We conducted this research in the context of a pilot randomised controlled trial, and statistical
18 64 power was therefore limited.
 - 19 65 • We triangulated different sources of adherence data to maximise accuracy, and used a mixed-
20 66 methods approach to evaluate acceptability.
 - 21 67 • We could not directly compare experiences with, and opinions about, the two different vaginal
22 68 probiotics because each woman used only one product and qualitative data depth was suboptimal.
 - 23 69 • Social desirability bias may have affected some of the results.
 - 24 70 • The results of this study may not be generalizable to women at lower risk of sexually transmitted
25 71 or urogenital infections.
- 26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

72 INTRODUCTION

73 Bacterial vaginosis (BV) is a vaginal condition in which fastidious anaerobes such as *Gardnerella*
74 *vaginalis* increase while beneficial, lactic acid-producing lactobacilli decrease.[1] Often
75 asymptomatic, it is associated with increased risks of sexually transmitted infections (STIs) and HIV
76 acquisition, pelvic inflammatory disease, and adverse pregnancy outcomes.[2–5] Although BV is
77 treatable with antibiotics, the risk of recurrence is high.[6,7] The prevalence of BV varies among
78 regions and ethnic groups but is highest in sub-Saharan Africa, where it is estimated at 30-50%.[8]
79
80 Vaginally-administered probiotics containing lactobacilli are considered a promising new strategy to
81 restore a lactobacilli-dominated vaginal microbiota during and/or after antibiotic treatment, or to
82 prevent BV.[9] While some probiotics have been available on the market for several years, clinical
83 trials to support beneficial effects have only recently been initiated for most products.[10–13] Future
84 uptake and adherence of a vaginal probiotic, once proven efficacious, is determined to a large extent
85 by its acceptability in target populations. The acceptability, in turn, depends on factors such as
86 characteristics of the target population, characteristics of and experiences with the product, types of
87 sexual relationships and partner support, and community perceptions.[14,15]
88
89 We conducted a clinical trial of intermittent use of two vaginal probiotics and oral metronidazole to
90 prevent BV recurrence in Rwandan women who had been treated for BV and/or *Trichomonas*
91 *vaginalis* (TV). We used qualitative and quantitative research methods to assess adherence and
92 acceptability with vaginal probiotic use. We triangulated various sources of adherence data to obtain
93 adherence estimates per woman for each period of intermittent product use in between study visits,
94 and determined correlates of adherence.

96 METHODS

97 The pilot clinical trial took place from June 2015 to February 2016 at the Rinda Ubuzima research
98 clinic in Kigali, Rwanda. The trial was a pilot trial with a modest sample size at the request of the
99 funder. Women who had been successfully treated for BV/TV with a seven-day course of oral

1
2
3 100 metronidazole (Tricozole, Laboratory & Allied Ltd, Nairobi, Kenya) were randomised to four
4
5 101 intervention groups (n=17 each) to prevent BV recurrence: behavioural counselling only (controls), or
6
7 102 behavioural counselling plus intermittent use of two different vaginal probiotics or oral metronidazole
8
9 103 for two months. The behavioural counselling included counselling on safer sex, vaginal hygiene
10
11 104 (including discouragement of intravaginal washing), and penile hygiene (i.e. encouragement of
12
13 105 cleansing the penis, including underneath the foreskin), because these behaviours are known to reduce
14
15 106 BV recurrence risk somewhat.[6,16] We counselled all women in all randomisation groups because
16
17 107 we considered it unethical to withhold this information from women at risk. Women were seen at
18
19 108 screening, enrolment (product use initiation, if applicable), Day 7, Month 1, Month 2 (product use
20
21 109 cessation, if applicable), and Month 6. Product efficacies were not known during the trial, and the
22
23 110 efficacy results of the pilot trial are reported elsewhere.[17] Briefly, the vaginal probiotics did
24
25 111 improve the vaginal environment (increased lactobacilli and reduced BV-associated bacteria)
26
27 112 compared to counselling only, but not as much as oral metronidazole did.
28
29
30
31
32

33 114 **Study population**

34
35 115 Women aged 18-45 at risk of HIV/STIs (defined as having had more than one sex partner and/or
36
37 116 having been treated for an STI and/or BV in the last 12 months) were eligible for enrolment if they
38
39 117 were confirmed HIV-negative, non-pregnant, diagnosed with BV and/or TV, and cured after seven-
40
41 118 day oral metronidazole treatment. Other clinical exclusion criteria were applied but were rare.[17]
42
43 119 Women were recruited by study staff with the assistance of Community Mobilisers who had strong
44
45 120 ties with local high-risk women (particularly sex workers).
46
47
48
49

50 122 **Study products and dosing**

51
52 123 Ecologic Femi+ (EF+; Winclove Probiotics, Amsterdam, Netherlands) is a vaginal capsule containing
53
54 124 lyophilised lactic acid-producing bacteria. EF+ was used once per day for five days followed by thrice
55
56 125 weekly, for two months. Gynophilus LP (GynLP; Biose, Aurillac, France) is a tablet containing the
57
58 126 *Lactobacillus rhamnosus* Lcr35 strain. The tablet disintegrates in the vagina and forms a gel that
59
60 127 slowly releases the probiotic bacteria. GynLP was used once every four days for two months. The first

1
2
3 128 dose was inserted at the clinic under direct observation of a clinician, and remaining doses were self-
4
5 129 administered at home. Women were asked not to cleanse or insert other products into the vagina after
6
7 130 probiotic insertion to allow the probiotics to dissolve. They were also told that they were allowed to
8
9 131 cease probiotic use during menses, but were encouraged to continue. Intermittent metronidazole use
10
11 132 was chosen as a positive control intervention because studies conducted in the U.S. and Kenya have
12
13 133 shown a 30-40% reduction in BV recurrence.[18,19] Metronidazole users took 500 mg generic oral
14
15 134 metronidazole (Laboratory & Allied Ltd, Nairobi, Kenya) twice weekly for two months. The rationale
16
17 135 for selecting these study products and their dosing schedules can be found in the manuscript
18
19 136 describing the efficacy results of the pilot trial.[17] Participants and clinicians were not blinded.
20
21
22
23

137

138 **Acceptability, adherence, behavioural, and vaginal infection knowledge assessments**

139 Acceptability was assessed at the enrolment visit prior to product use initiation and at the Month 2
140 visit after the full two months of use. Adherence was assessed during the intervention period, at the
141 Day 7, Month 1, and Month 2 visits. Sexual and other behaviours were assessed at all study visits.
142 Participants were interviewed face-to-face in Kinyarwanda by a trained study nurse using structured
143 questionnaires with multiple-choice questions, questions requiring a number or date, and an adherence
144 self-rating scale (from 0-10). In between visits, participants used pictorial diary cards (online
145 supplementary material figure 1) to record daily episodes of product use, vaginal sex, condom use,
146 and vaginal practices. Those using study products returned the product packaging and unused
147 products (if applicable) to their clinic visits, where they were counted by study staff. Any
148 discrepancies between data sources were discussed with participants and the consensus assessments
149 were recorded on the questionnaires. The adherence data based on the self-rating scale, the diary card,
150 and the returned product packaging were triangulated by the data analyst at the data analysis stage.
151 Additionally, 131 women were interviewed about their knowledge of vaginal infections (such as BV
152 and STIs) using a structured questionnaire during recruitment sessions (n=61; regardless of eligibility)
153 and at enrolment visits (n=70; this included the 68 randomised women, and two women who attended
154 enrolment visits but turned out to be ineligible; figure 1). Women were interviewed before being
155 counselled at study visits or before receiving information at recruitment sessions. This questionnaire

1
2
3 156 contained multiple-choice and open-ended questions. Responses to the open-ended questions were
4
5 157 categorised and discussed by two different researchers until consensus about the answer categories
6
7 158 was reached.
8
9 159
10
11 160 Four semi-structured focus group discussions (FGDs) with 7-11 participants per group (total n=38),
12
13 161 and semi-structured individual in-depth interviews (IDIs) with four additional participants, were held.
14
15 162 The main themes of these FGDs and IDIs were experiences with and opinions of the study products,
16
17 163 sexual behaviour, and vaginal practices. Women randomised to the behavioural counselling only
18
19 164 group were not approached for the FGDs and IDIs, but all other randomised participants who had
20
21 165 completed their product use period were approached until data saturation had been achieved. The
22
23 166 interviews were unlinked anonymous, and women used pseudonyms to enable them to talk freely
24
25 167 despite the fact that the discussions and interviews were taped. All interviews took place between
26
27 168 November 2015 and March 2016, were held in Kinyarwanda, recorded on tape, transcribed verbatim,
28
29 169 and translated into English. The FGD and IDI transcripts were read and discussed by three researchers
30
31 170 (MV, MU, and JvdW) at regular intervals. The Chief Investigator (JvdW) decided that data saturation
32
33 171 had been met when the fourth FGD and the fourth IDI transcript had become available in March 2016.
34
35
36
37
38

39 173 **Data analysis**

40
41 174 The primary outcomes of this study were acceptability and triangulated adherence in women
42
43 175 randomised to study product use. Secondary outcomes included vaginal infection knowledge of the
44
45 176 target population more broadly, and behavioural changes (of the behaviours included in the
46
47 177 counselling messages) in all randomised women. Questionnaire data were analysed using Stata 13
48
49 178 (StataCorp, College Station, TX, USA). The proportion of women with $\geq 80\%$ / $\geq 90\%$ /100% adherence
50
51 179 in the probiotic groups were compared by Fisher's exact tests. Changes in self-reported vaginal
52
53 180 practices and sexual behaviours over time were tested using McNemar's test for binary outcomes, and
54
55 181 Wilcoxon's signed-rank test for continuous outcomes. To study associations of participant
56
57 182 characteristics with triangulated adherence, we used bivariable mixed effects models, with perfect
58
59 183 adherence (defined as having used all doses as instructed) per interval between study visits during the
60

1
2
3 184 intervention period as the outcome, participant identification numbers as the random effect, and one
4
5 185 participant characteristic at the time as the fixed effect. We could not determine correlates of
6
7 186 acceptability due to limited variation in the acceptability data (reported acceptability was high
8
9 187 throughout the trial).

10
11 188
12
13 189 The FGD and IDI transcripts were coded using NVivo 10.0 (QSR International, Melbourne,
14
15 190 Australia) by one single researcher (MV). The discussions and interviews were semi-structured, with
16
17 191 the above-mentioned themes and associated codes prepared a priori, as well as new elements that
18
19 192 emerged from the data. The codes were derived from an acceptability framework that has been used in
20
21 193 studies of vaginal products for contraception or HIV prevention.[14,15,20] Components of the
22
23 194 framework include study population characteristics, product attributes, sexual encounter and relational
24
25 195 attributes, and the contextual environment (e.g. community perceptions of product use).

26
27
28 196

197 **Ethical statement**

198 All participants provided written consent for study participation, and separate consent for participation
199 in FGDs/IDIs. All non-married participants aged 18-20 also required parental/guardian consent per
200 Rwandan law at the time of the study. The participants received 3 GBP per visit (in local currency) as
201 a reimbursement for time and transport costs. Care was taken to protect participant privacy and
202 confidentiality. The study was sponsored by the University of Liverpool, approved by the Rwanda
203 National Ethics Committee and the University of Liverpool Research Ethics Subcommittee for
204 Physical Interventions, and registered on ClinicalTrials.gov (NCT02459665).

205

206 **Participant and public involvement**

207 As part of the FGDs/IDIs, a subset of the enrolled participants were invited to comment on study
208 design and experiences with the interventions. Participants were not invited to develop outcomes,
209 interpret the results, or to contribute to the writing or editing of this document for readability or
210 accuracy. The preliminary results of this study were discussed with 32 stakeholders during a
211 workshop held at the Ministry of Health in Kigali, Rwanda, in December 2017. These stakeholders

1
2
3 212 included representatives of the Ministry of Health, the National University of Rwanda, the National
4
5 213 Ethics Committee, local hospitals and clinics, and local non-governmental and women's
6
7 214 organisations.
8
9
10 215

11 216 **RESULTS**

12 217 **Baseline characteristics**

13
14
15 218 We screened 176 women: bacterial STI prevalence was 31.3% and BV prevalence by Gram stain
16
17 219 Nugent scoring was 47.9%. All 68 randomised women were treated for BV and/or TV prior to
18
19 220 randomisation and at risk of HIV/STIs, with 93.1% reporting having exchanged sex for money and/or
20
21 221 goods in the previous month (figure 1, online supplementary material table 1). We collected 29.93
22
23 222 person-years of data. Four women withdrew their informed consent during the study (for reasons
24
25 223 unrelated to study product acceptability). None were lost to follow-up.
26
27
28 224

29 225 **Adherence**

30
31 226 Triangulated adherence was high: 17/17 (100%) of EF+ users and 13/16 (81.3%) of GynLP users used
32
33 227 $\geq 80\%$ of required doses (Fisher's exact $p=0.103$; table 1), and these percentages were 15/17 (88.2%)
34
35 228 and 11/16 (68.8%) for $\geq 90\%$ ($p=0.225$), and 10/17 (58.8%) and 8/16 (50%) for 100% of required
36
37 229 doses ($p=0.732$), respectively. In comparison, these percentages were 15/17 (88.2%), 14/17 (82.4%),
38
39 230 and 12/17 (70.6%), respectively, for oral metronidazole users. Reported reasons of non-adherence to
40
41 231 vaginal probiotics during face-to-face interviews were 'simply forgetting' ($n=9$), experiencing side-
42
43 232 effects ($n=2$), menses ($n=2$), and being away from home and having left products at home ($n=1$).
44
45 233 Additional reasons for missing doses mentioned during FGDs/IDIs were being drunk ($n=2$) and being
46
47 234 confused about the dosing schedule ($n=2$). Only one woman in the metronidazole arm reported
48
49 235 missing doses due to experiencing side-effects. Most women in FGDs reported using all doses as
50
51 236 instructed and finding it easy to adhere, and thought that the diary cards served as a useful reminder to
52
53 237 use the products.
54
55
56 238
57
58 239

240 **Table 1: Adherence to study interventions**

Adherence to study products	Metronidazole (n=17)	EF+ (n=17)	GynLP (n=16)
Adherence Enr–D7, median % (IQR)	100 (100–100)	100 (100–100)	100 (100–100)
Adherence D7–M1, median % (IQR)	100 (100–100)	100 (100–100)	100 (91.7–100)
Adherence M1–M2, median % (IQR)	100 (100–100)	100 (100–100)	100 (92.3–100)
Overall adherence Enr–M2, median % (IQR)	100 (96.3–100)	100 (100–100)	98.3 (89.3–100)
Overall adherence Enr–M2 n (%)			
- Perfect*	12 (70.6)	10 (58.8)	8 (50.0)
- Adherence ≥90%	14 (82.4)	15 (88.2)	11 (68.8)
- Adherence ≥80%	15 (88.2)	17 (100)	13 (81.3)
Number of times menses Enr–M2 n (%)†			
- Never	7 (41.2)	4 (23.5)	2 (12.5)
- Once	6 (35.3)	5 (29.4)	4 (25.0)
- Twice	4 (23.5)	8 (47.1)	10 (62.5)
Did not use product during menses at least once n (%)			
- Yes	4 (23.5)	3 (17.6)	5 (31.3)
- NA (never had menses)	7 (41.2)	4 (23.5)	2 (12.5)
Self-reported reasons for non-adherence‡	Metronidazole	EF+	GynLP
D7: Self-reported reasons why not able to use all doses as instructed n (%)§			
- Simply forgot	0	2 (11.8)	0
- Product had side effects	0	0	1 (6.7)¶
M1: Self-reported reasons why not able to use all doses as instructed n (%)§			
- Simply forgot	1 (6.3)	1 (5.9)	1 (6.3)
- Product had side effects	1 (6.3)	0	1 (6.3)‡‡
- Did not like product for another reason	1 (6.3)	0	0
- Other	1 (6.3)**	1 (5.9)††	2 (12.5)§§
M2: Self-reported reasons why not able to use all doses as instructed n (%)§			
- Simply forgot	1 (6.3)	2 (11.8)	3 (18.8)
- Travelled and forgot to take product	1 (6.3)	0	1 (6.25)
- Other	0	1 (5.9)¶¶	1 (6.3)
D7: Participant thinks she used product correctly most of the time n (%)	17 (100)	16 (94.1)	14 (93.3)
M1: Participant thinks she used product correctly most of the time n (%)	13 (86.7)	17 (100)	11 (68.8)
M2: Participant thinks she used product correctly most of the time n (%)	15 (93.7)	16 (94.1)	14 (87.5)

*Defined as 100% of the prescribed doses used at the prescribed times after nurse review of the participant's diary card and returned used packaging and unused product.

†Number of times menses in the control group: never 2 (11.8%), once 3 (17.8%), twice 11 (64.7%), and thrice 1 (5.9%).

‡Numbers of participants per randomisation group may vary slightly due to loss to follow-up. Participants with ≥90% adherence not shown.

§Multiple answers possible.

¶Participant reported vulval itching and burning when passing urine.

||Participant reported mild gastritis and wanting to withdraw from the study anyway.

**Participant reported receiving oral metronidazole therapy for 7 days due to infection.

††Participant reported having menses twice in one month; decided to use less of her product until the next study visit.

‡‡Participant reported genital itching, genital burning, and pain during sex.

§§One participant reported missing the D7 study visit and therefore running out of supplies. Another participant reported not to have used the study product during menses (which she was allowed to do).

¶¶Participant reported being drunk and therefore forgetting to take the study product.

||||Participant reported taking the study product correctly but that the product came out during menses

D7, Day 7 visit; EF+, Ecologic Femi+; Enr, enrolment visit; GynLP, Gynophilus LP; IQR, inter-quartile range; M1/2, Month 1/2 visit; NA, not applicable.

1
2
3 259 **Acceptability**

4
5 260 *Ease-of-use*

6
7 261 No participants reported having heard about probiotics before study participation. After product use,
8
9 262 all vaginal probiotic users reported feeling very comfortable with insertion and that insertion became
10
11 263 easier over time. All but one woman reported inserting while lying down (online supplementary
12
13 264 material table 2).
14

15 265

16
17 266 *Bodily changes and product perception*

18
19 267 During FGDs, several women using either vaginal probiotic reported the product (partially) “*coming*
20
21 268 *out*” during the first few uses, but that this decreased after having gained experience. Many EF+ and
22
23 269 GynLP users reported an increase in vaginal wetness, which was considered a positive attribute by
24
25 270 most. Some women reported increased libido. For example, one EF+ user said: “*I felt a great desire to*
26
27 271 *[have] sex again and again.*” In contrast, one metronidazole user reported a decrease in libido. Most
28
29 272 women believed that the vaginal probiotics decreased the recurrence of symptomatic BV (our
30
31 273 preliminary efficacy data suggest that BV incidence had in fact decreased),^[17] and a few believed
32
33 274 that they also prevented STI acquisition (the trial had insufficient statistical power to assess this).
34
35 275

36 276

37
38 276 *Support*

39
40 277 One social harm related to vaginal probiotic use was reported: a GynLP user was verbally harassed by
41
42 278 her partner and her sister because of her study participation, and opted to withdraw her informed
43
44 279 consent. Reports of partner, family, and community support during the FGDs/IDIs were mixed: some
45
46 280 women reported problems with loved ones. Negative reactions from male partners were more often
47
48 281 based on suspicions about study participation than the products themselves. One EF+ user said: “*He*
49
50 282 *[her partner] did not accept that. He asked me to go together with him to the clinic [a local health*
51
52 283 *centre] and check if I am not HIV-positive.*” Another participant using metronidazole mentioned
53
54 284 wanting to join the study to her husband, who forbade her to participate. However, she decided to join
55
56 285 anyway: “*he did not know that I was using the study product, because he had refused me to join [the]*
57
58
59
60

1
2
3 286 *study before... I used them [the study products] without informing him.*" All sex workers except one
4
5 287 stated that they had not discussed study participation with male clients.
6

7 288
8

9 289 *Worries and concerns*

10
11 290 In the FGDs, one woman reported hearing rumours prior to enrolling that vaginal products "*can*
12
13 291 *damage the uterus or cause tumours in the womb.*" However, most participants thought that vaginal
14
15 292 probiotics would be acceptable to Rwandan women. One GynLP user argued: "*They [already] give us*
16
17 293 *vaginal pills*", by which she meant vaginal medications for yeast infections. Some women were
18
19 294 concerned about future product availability and pricing. They hoped that probiotics would be
20
21 295 distributed cheaply through the Rwandan *Mutuelle* public health insurance because they would
22
23 296 otherwise be inaccessible to many women. One metronidazole user was concerned about a limited
24
25 297 applicability of probiotics because BV is not diagnosed by laboratory testing in Rwanda: "*They do not*
26
27 298 *have adequate medical instruments to test diseases, you tell the physician how [...] you feel and by*
28
29 299 *guessing the disease, he gives you at least four medications, saying that you may have trichomonas,*
30
31 300 *you may have syphilis, you may have gonorrhoea [she refers to syndromic management.[21,22]] At*
32
33 301 *health centre-level they do not have medical equipment to test diseases, meaning that they will not*
34
35 302 *know who to give that [probiotic/antibiotic maintenance therapy] medication.*"
36
37
38
39
40

41 304 **Vaginal practices and sexual risk-taking**

42
43 305 At enrolment, 35/71 (49.3%) of the women reported to never use products inside the vagina, and at
44
45 306 Month 6, this increased to 53/65 (81.5%) (OR 5.2, 95% CI 1.96-17.34; table 2). During FGDs, some
46
47 307 women understood that vaginal washing practices may increase the risk of vaginal infection, but
48
49 308 others did not. A participant stated: "*You get them [i.e., vaginal diseases] anyway... whether you wash*
50
51 309 *or not*". In one FGD, 10 of 11 participants (90.9%) stated having ceased vaginal practices thanks to
52
53 310 the study counselling. It should be noted that in contrast to many other African populations, Rwandan
54
55 311 women use vaginal practices to increase rather than reduce vaginal lubrication. Women mentioned the
56
57 312 use of herbs (*umushishiro*), Vaseline, and oils for this purpose. Self-reported sexual risk taking by
58
59 313 face-to-face interview did not change over time, except for a significant reduction in reported numbers
60

1
2
3 314 of sex partners in the previous month at Month 6 compared to enrolment. No women in FGDs/IDIs
4
5 315 mentioned adopting safer sex practices (such as consistent condom use) in response to the counselling
6
7 316 messages. During face-to-face interviews at the Month 2 visit, 12 of 15 women (80%) who had an
8
9 317 uncircumcised main sex partner reported asking him to regularly clean his penis in the future (online
10
11 318 supplementary material table 2). While most women in FGDs understood that using condoms and
12
13 319 improved penile hygiene could reduce BV rates (as shown in [6,16]), some mentioned that they found
14
15 320 it difficult to discuss these topics with male partners. One participant stated that this is especially
16
17 321 difficult being a sex worker: *“a man gives you his own money and you start educating him to wash!”*
18
19 322 However, another sex worker reported refusing sex with uncircumcised clients: *“you leave him,*
20
21 323 *because he has a lot [of] germs”*. Several women reported discussing circumcision with their partners;
22
23 324 one participant reported telling her husband: *“It is better that you do circumcision because it is a good*
24
25 325 *thing... you would get a chance of not contracting diseases.”*
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

326 **Table 2: Changes in reported vaginal cleansing practices and (sexual) behaviour between the**
 327 **enrolment and the M6 visit.**

Self-reported sociodemographic characteristics	Enr (n=71)	M6 (n=65)	OR (95% CI)*
			P value*
Reports using no products inside the vagina (other than for managing menses; all participants) n (%)	35 (49.3)	53 (81.5)	5.2 (1.96–17.34) <0.001
Reports using no products inside the vagina (other than for managing menses; controls and metronidazole users only)† n (%)	15 (44.1)	27 (79.4)	13.0 (1.95–552.5) 0.002
Reports using water only n (%)	23 (32.4)	10 (15.4)	0.37 (0.13–0.92) 0.029
Reports using water and soap n (%)	3 (4.2)	2 (3.1)	0.67 (0.06–5.82) 1.00
Reports using paper, cloth or cotton wool n (%)	9 (12.7)	0 (0)	0.13 (0.00–0.93)‡ 0.008
Reports using traditional herbs, stones, powders as vaginal cleansing practice n (%)	1 (1.4)	1 (1.5)	1.00 (0.01–78.5)‡ 1.00
Mean weekly frequency of vaginal practices (95% CI)	2.15 (0.97–3.34)	0.64 (0.18–1.11)	NA 0.328
Median number of sex partners in last month at baseline or per month during follow-up period (IQR)	5 (3–16)	2 (1–4)	NA <0.001
Any condom use reported in past two weeks (Enr) or since last study visit (M6), versus no condom use reported n (%)	64 (90.1)	60 (92.3)	1.67 (0.32–10.7) 0.727
Reports exchanging sex for money/goods in past month (Enr) or since last study visit (M6) n (%)	65 (91.5)	58 (89.2)	0.80 (0.16–3.72) 1.00

329 *McNemar's OR and p-value for binary variables and Wilcoxon signed-rank test p-value for continuous variables, comparing the response at M6
 330 with the response at Enr. ORs with 95% CI were also calculated for binary pre-post data.

331 †N=34.

332 ‡To enable calculation of effect measures, a zero value was replaced by 1.

333 CI, confidence interval; Enr, enrolment visit; IQR, inter-quartile range; M6, Month 6 visit; NA, not applicable; OR, odds ratio.

334

335 **Correlates of adherence**

336 In bivariable mixed effects models including the probiotic groups only, no participant characteristics
 337 were significantly associated with perfect adherence (table 3). However, non-significant trends were
 338 observed. Younger age ($p=0.076$), asking many questions at enrolment (compared to a few questions
 339 or no questions; structurally judged by a study nurse; $p=0.116$), having menses during the previous
 340 study interval ($p=0.104$), and reporting urogenital symptoms ($p=0.103$) were associated with a lower
 341 likelihood of perfect adherence. When including oral metronidazole users, menses was significantly
 342 associated with a lower likelihood of perfect adherence ($p=0.008$). There were no significant
 343 associations between randomisation group and perfect adherence.

344

345

346 **Table 3: Participant characteristics associated with perfect adherence**

Participant characteristics	EF+ and GynLP users		EF+, GynLP and oral metronidazole users	
	OR (95% CI)	P value	OR (95% CI)	P value
Randomisation group: GynLP versus EF+	0.68 (0.22–2.11)	0.505	ND	ND
Randomisation group: - EF+ versus metronidazole - GynLP versus metronidazole	ND	ND	0.53 (0.15–1.81) 0.36 (0.11–1.23)	0.308 0.103
Age in years: ≥ 30 years versus < 30	2.66 (0.90–7.82)	0.076	1.60 (0.61–4.15)	0.336
Marital status: - Married versus never married - Divorced versus never married - Widowed versus never married	0.97 (0.14–6.58) 1.18 (0.29–4.79) ND	0.976 0.912 0.991	1.17 (0.20–6.99) 1.39 (0.42–4.57) ND	0.865 0.586 0.990
At least some schooling versus no schooling	1.20 (0.59–2.45)	0.619	0.80 (0.22–2.95)	0.740
Number of sex partners last month: five or more versus four or less.	0.58 (0.18–1.83)	0.351	0.49 (0.17–1.37)	0.173
Exchanged sex for money/goods past month	ND	0.990	ND	0.986
Nurse reported participant asked questions at Enr - Yes, many versus none - Yes, a few versus none	0.19 (0.02–1.52) 0.83 (0.24–2.83)	0.116 0.761	0.15 (0.02–1.19) 0.83 (0.27–2.57)	0.072 0.744
Had menses during study visit interval	0.41 (0.14–1.20)	0.104	0.26 (0.09–0.70)	0.008
Reported alcohol consumption during study: - Once or twice per week versus never - More than twice per week versus never	0.54 (0.14–2.12) 0.92 (0.18–4.81)	0.373 0.920	0.34 (0.11–1.08) 0.81 (0.19–3.49)	0.068 0.774
Reported at least one urogenital symptom during study interval versus none	0.11 (0.01–1.56)	0.103	0.30 (0.04–2.16)	0.231
Reported at least one adverse event during study visit interval (excluding urogenital symptoms) versus none	0.43 (0.10–1.83)	0.253	0.55 (0.15–2.05)	0.371

347 Sociodemographic characteristics associated with perfect adherence in bivariable mixed effects models, in the enrolment–D7, D7–M1, and
 348 M1–M2 study visit intervals.
 349 CI, confidence interval; D7, Day 7 visit; EF+, Ecologic Femi+; Enr, enrolment visit; GynLP, Gynophilus LP; M1, Month 1 visit; M2, Month
 350 2 visit; ND, non-determinable; OR, odds ratio.

352 **Vaginal infection knowledge**

353 Almost all participants reported having heard of ‘diseases of the vagina’ and STIs before, but only
 354 6/131 (4.6%) knew what bacteria were (table 4). The STIs most often spontaneously named (in
 355 numerical order) were HIV, gonorrhoea, and syphilis; only one participant reported having heard of
 356 BV. After having received an explanation about what BV is, only one of 131 woman reported ever
 357 having been diagnosed with BV. Most participants could name at least one cause or potential
 358 consequence of vaginal infections. Consequences wrongfully attributed to vaginal infections were
 359 cervical cancer/tumours (5/131; 3.8%), consequences to the infant such as being born with BV or
 360 congenital malformations (6/131; 4.6%), and death (4/131; 3.1%).

361 **Table 4: Vaginal infection knowledge**
362

	Recruitment (n=61)	Enrolment (n=70)	Total (n=131)
Median age (IQR)	32 (27–35)*	31 (27–35)	31 (27–35)
Has heard of diseases of the vagina before n (%)	60 (98.4)	70 (100)	130 (99.2)
Reports knowing what bacteria are before study n (%)	5 (8.2)	1 (1.4)	6 (4.6)
Reports having heard about STIs before study n (%)	61 (100)	70 (100)	131 (100)
If yes, spontaneously named, without probing† n (%)			
- HIV	58 (95.1)	65 (92.9)	123 (93.9)
- Gonorrhoea	58 (95.1)	65 (92.9)	123 (93.9)
- Syphilis	44 (72.1)	59 (84.3)	103 (78.7)
- Trichomoniasis	38 (62.3)	48 (68.6)	86 (65.7)
- Hepatitis	3 (4.9)	3 (4.3)	6 (4.6)
- Yeast infection	0	3 (4.3)	3 (2.3)
- BV	0	2 (2.9)	2 (1.5)
- Urinary tract infection	1 (1.6)	1 (1.4)	2 (1.5)
- Chlamydia	0	1 (1.4)	1 (0.8)
- Herpes	0	1 (1.4)	1 (0.8)
- HPV / cervical cancer	1 (1.6)	0	1 (0.8)
Reports having heard about BV before this study n (%)	1 (1.6)	0	1 (0.8)
Spontaneously reported reasons why women get vaginal disease, without probing† n (%)			
- Poor toilet hygiene	37 (60.7)	40 (57.1)	77 (58.8)
- Multiple sex partners	28 (45.9)	36 (51.4)	64 (48.9)
- After sex	25 (41.0)	30 (43.0)	55 (42.0)
- Dirty underwear	19 (31.2)	35 (50.0)	54 (41.2)
- Poor vaginal hygiene	26 (42.6)	22 (31.4)	48 (36.6)
- Poor penile hygiene of male partner(s)	4 (6.6)	17 (24.3)	21 (16.0)
- Traditional vaginal practices and washing	3 (4.9)	12 (17.1)	15 (11.5)
- New sex partner	6 (9.8)	3 (4.3)	9 (6.9)
- Use of contraception	1 (1.6)	3 (4.3)	4 (3.1)
- (Improper) use of sanitary pads or tampons	1 (1.6)	3 (4.3)	4 (3.1)
- Other	3 (4.9)‡	1 (1.4)§	4 (3.1)
- Cannot name any reasons	1 (1.6)	0	1 (0.8)
Spontaneously reported negative consequences of vaginal disease being named, without probing† n (%)			
- Foul smell from the vagina	30 (49.2)	39 (56.5)	69 (53.1)
- Difficulty getting pregnant	18 (29.5)	33 (47.8)	51 (39.2)
- Miscarriage	16 (26.2)	33 (47.8)	49 (37.7)
- Abnormal vaginal discharge	12 (19.7)	28 (40.6)	40 (30.8)
- Baby born too early	16 (26.2)	22 (31.9)	38 (29.2)
- Severe infection / fever of the woman	7 (11.5)	7 (10.1)	14 (10.8)
- Infection / fever of the newborn baby	5 (8.2)	3 (4.4)	8 (6.1)
- Itching	4 (6.6)	4 (5.8)	8 (6.1)
- Other consequences to the baby: being born with BV, congenital malformations, and others.	3 (4.9)	3 (4.4)	6 (4.6)
- Cervical cancer or tumours	2 (3.3)	3 (4.4)	5 (3.8)
- Death	4 (6.6)	0	4 (3.1)
- HIV/STIs	1 (1.6)	3 (4.4)	4 (3.1)
- Pain during intercourse	0	3 (4.4)	3 (2.3)
- Cannot name any consequence	17 (27.9)	19 (27.5)	36 (27.7)

*One missing value.

†Open-ended question. Totals may be more than 100%.

‡Participants report: "If you are infected with STIs", sharing underwear, and unprotected sex.

§Participant reports: vaginal medicine.

BV, bacterial vaginosis; HPV, human papilloma virus; IQR, interquartile range; STI, sexually transmitted infection.

1
2
3 368 **DISCUSSION**
4

5 369 Several studies of different vaginal probiotics have been conducted, some of them in sub-Saharan
6
7 370 Africa.[10–13] However, none reported in-depth acceptability and adherence data. Our study suggests
8
9 371 high vaginal probiotic acceptability and adherence in high-risk Rwandan women. We found no
10
11 372 statistically significant correlates of perfect adherence, partially due to limited statistical power, but
12
13 373 younger age, asking many questions about product use at enrolment, current menses, and reporting
14
15 374 urogenital symptoms showed trends towards a lower likelihood of perfect adherence. Vaginal
16
17 375 probiotics are currently unavailable on the market in most African countries, and it is important to
18
19 376 study acceptability in different target populations to inform product development and future
20
21 377 marketing strategies. Adherence to metronidazole was comparable to, or slightly higher than,
22
23 378 adherence reported in previously conducted studies.[19,23]
24
25

26 379

27
28 380 We could not evaluate the impact of self-reported acceptability aspects on adherence because almost
29
30 381 all women reported very high acceptability in face-to-face interviews throughout the trial. Such
31
32 382 interviews are known to suffer from social desirability bias. However, women seemed to speak freely
33
34 383 in the FGDs, and those data indicate that they did not have major issues with product attributes or
35
36 384 insertion. However, some women reported difficulties due to lack of male partner support. The
37
38 385 reported increase in vaginal wetness after probiotic insertion was not considered problematic, as
39
40 386 lubrication during sex is preferred by most Rwandan men and women.[24] This might be different in
41
42 387 other countries where dry sex is preferred.[25] We did find a non-significant lower adherence to
43
44 388 GynLP compared to EF+, which might be explained by differences in formulation: GynLP forms a
45
46 389 gel in the vagina whereas EF+ capsules merely release lyophilised bacteria. Previous research
47
48 390 indicated high adherence to GynLP.[26] Unfortunately, the impact of these formulation differences
49
50 391 was insufficiently probed during the FGDs; the impact of product formulation on acceptability and
51
52 392 adherence should be investigated in future clinical trials. Participants indicated that they found the
53
54 393 diary cards helpful in reminding them to use their products, and we believe that self-monitoring tools
55
56 394 might indeed be helpful in maximising adherence and therefore recommend them for use in future
57
58 395 studies.[27]
59
60

1
2
3 396 Our data suggest that counselling was partially effective in changing behaviours that increase BV risk.
4
5 397 While these results are encouraging, it is difficult to assess to what extent they were influenced by
6
7 398 social desirability bias. Significantly more women reported not engaging in vaginal practices at the
8
9 399 end of the study, and most women with uncircumcised steady male partners reported having discussed
10
11 400 penile hygiene with them. However, many women mentioned in FGDs that they found it difficult to
12
13 401 discuss condom use and penile hygiene with male partners, especially clients. Women reduced their
14
15 402 sexual risks only to a limited extent during follow-up, reporting a reduction in numbers of sex partners
16
17 403 but no differences in engaging in sex work and condom use in face-to-face interviews. We did not ask
18
19 404 women to what extent they depended on sex work for subsistence. Women who only partially depend
20
21 405 on sex work may find it easier to negotiate with male partners.
22
23
24 406

25
26 407 Two probiotics-related themes that emerged from the stakeholders consultations that had not been
27
28 408 raised by the study participants were uncertainty about long-term side effects (women in the pilot trial
29
30 409 used the products for only two months) and whether probiotic bacteria (in this case lactobacilli) could
31
32 410 also be delivered orally instead of vaginally. We have since conducted a systematic review, which
33
34 411 showed that long-term safety of vaginal probiotics has not yet been evaluated.[28]
35
36
37 412

38
39 413 Our survey with women at recruitment sessions and enrolment visits showed that high-risk Rwandan
40
41 414 women had heard of several STIs, but were generally unaware of BV, its causes and potential
42
43 415 consequences, and what they can do to prevent it. Experiences with HIV show that public health
44
45 416 interventions can only succeed if health care professionals and the public have sufficient knowledge
46
47 417 of causes and consequences of disease.[29–31] High-risk Rwandan women (and health care
48
49 418 professionals) should therefore be educated about BV, and vaginal probiotics studies should include
50
51 419 counselling for all participants on vaginal diseases and how to prevent them.
52
53
54 420

55 421 **Limitations**

56
57 422 Our study had limited statistical power, and social desirability bias may have affected some of our
58
59 423 results, as is often the case in studies of this nature. Additionally, it should be noted that product
60

1
2
3 424 efficacy, availability and cost are important determinants of acceptability, and were not evaluated in
4
5 425 our study, although preliminary efficacy results in this study were promising.[17] We could not
6
7 426 directly compare experiences with, and opinions about, the two different vaginal probiotics because
8
9 427 each woman used only one product and qualitative data depth was suboptimal. In the FGDs/IDIs, it
10
11 428 was sometimes difficult to ascertain whether participants were referring to personal experiences, or to
12
13 429 wider community perceptions. Strengths of our study include the use of a mixed-methods approach
14
15
16 430 and triangulated adherence data.
17

18 431

19 432 **CONCLUSIONS**

20
21
22 433 The prevention of BV recurrence will likely have to include several components to be successful,
23
24 434 such as improved diagnostics, treatments, and prophylactic products (for example probiotics), but also
25
26 435 improved information, education, and counselling messages targeted to at-risk women and their
27
28 436 partners. The results of this study can be used to inform future product development, and to fine-tune
29
30 437 counselling messages in future trials.
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 438 **Acknowledgments** We thank the study participants, the Rinda Ubuzima team and other colleagues in
4
5 439 Rwanda and the United Kingdom who contributed, the funders of this study, as well as the Trial
6
7 440 Steering Committee for trial oversight. We thank Biose and Winlove for the donation of study
8
9 441 products.
10

11 442
12
13 443 **Author contributions** JvdW obtained the research funding and wrote the study protocol and data
14
15 444 collection documents. AN, EL, SA, and JvdW were members of the Trial Steering Committee. SA,
16
17 445 MU, MMU, and JvdW collected the primary data. MU and MMU performed the FGDs and IDIs. MV
18
19 446 and JvdW developed the analytical approach and performed the statistical analyses. MV and JvdW
20
21 447 wrote the manuscript. All authors commented on and approved the final manuscript.
22
23 448

24
25
26 449 **Funding** This work was funded by the DFID/MRC/Wellcome Trust Joint Global Health Trials
27
28 450 Scheme as a Development Project (grant reference MR/M017443/1; grant title: “Preparing for a
29
30 451 clinical trial of interventions to maintain normal vaginal microbiota for preventing adverse
31
32 452 reproductive health outcomes in Africa”). Vaginal probiotics for use in the trial were donated free of
33
34 453 charge by Winlove Probiotics (Amsterdam, The Netherlands) and Biose (formerly Probionov;
35
36 454 Aurillac, France). The findings and conclusions in this paper are those of the authors and do not
37
38 455 necessarily represent the views of the authors’ institutions or companies, or the funder. None of the
39
40 456 authors were paid to write this article. The corresponding author had full access to the data and had
41
42 457 final responsibility for the decision to submit for publication.
43
44 458

45
46
47 459 **Competing interests** AN is employed by Biose (owner of trial product GynLP) and EL by Winlove
48
49 460 Probiotics BV (owner of trial product EF+). AN has financial and/or intellectual investments in
50
51 461 competing products. The other authors report no competing interests.
52
53 462

54
55
56 463 **Patient consent** All participants provided written informed consent for study participation, and
57
58 464 separate written informed consent for participation in FGDs/IDIs.
59
60

1
2
3 465 **Ethics approval** The study was sponsored by the University of Liverpool, approved by the Rwanda
4
5 466 National Ethics Committee and the University of Liverpool Research Ethics Subcommittee for
6
7 467 Physical Interventions, and registered on ClinicalTrials.gov (NCT02459665).
8
9
10 468

11 469 **Data sharing statement** The data supporting the findings of this publication are retained by the
12
13 470 corresponding author (JvdW) and will not be made openly accessible due to privacy concerns. Fully
14
15 471 anonymised data can be made available by written request to j.vandewijgert@liverpool.ac.uk after
16
17 472 assurance that the intended data usage is compliant with relevant ethical approvals and privacy will be
18
19
20 473 maintained.
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 474 **Figure footnotes**
4

5 475

6
7 476 **Figure 1: Flowchart of the study**
8

9 477 *Totals to 110 reasons among 102 women because there could be more than one reason per woman.

10 478 †Reasons: outside of metronidazole treatment window (n=5), enrolment target already met (n=4), has a mental disorder (n=1), did not
11 479 complete screening procedures and was subsequently lost to follow-up (n=1), withdrew consent during the screening visit because she
12 480 thought the reimbursement was too low (n=1).

13 481 ‡Reasons: moved away from Kigali (n=2), lost interest because symptoms resolved (n=1), and was verbally harassed by partner and sister
14 482 about study participation (n=1).
15 483

16 484 Acceptability assessments were made at enrolment and at the M2 visit. Adherence assessments were made using self-rated assessments,
17 485 pictorial diary cards, and returned packaging at the D7, M1, and M2 visits (after which product use was ceased). The vaginal infection
18 486 knowledge survey was held at recruitment sessions in the community and at the enrolment visit. Changes in sexual risk-taking and vaginal
19 487 practices were assessed at each follow-up visits and compared to answers given during the enrolment visit. All of these themes were
20 488 discussed during the eight FGDs and IDIs.
21 489

22 490 BV, bacterial vaginosis; D7, day 7 visit; FGD, focus group discussion; IDI, in-depth interview; M1/2/6, month 1/2/6 visit; RU, Rinda
23 491 Ubuzima; TV, *Trichomonas vaginalis*.

492 **References**

- 493 1 van de Wijgert JHHM, Borgdorff H, Verhelst R, *et al.* The vaginal microbiota: what have
494 we learned after a decade of molecular characterization? *PLOS ONE* 2014;**9**:e105998.
495 doi:10.1371/journal.pone.0105998
- 496 2 van de Wijgert JHHM, Morrison CS, Cornelisse PGA, *et al.* Bacterial vaginosis and
497 vaginal yeast, but not vaginal cleansing, increase HIV-1 acquisition in African women. *J*
498 *Acquir Immune Defic Syndr* 1999 2008;**48**:203–10. doi:10.1097/QAI.0b013e3181743936
- 499 3 van de Wijgert JHHM. The vaginal microbiome and sexually transmitted infections are
500 interlinked: consequences for treatment and prevention. *PLOS Med* 2017;**14**:e1002478.
501 doi:10.1371/journal.pmed.1002478
- 502 4 Li J, McCormick J, Bocking A, *et al.* Importance of vaginal microbes in reproductive
503 health. *Reprod Sci* 2012;**19**:235–42. doi:10.1177/1933719111418379
- 504 5 Nelson DB, Hanlon AL, Wu G, *et al.* First trimester levels of BV-associated bacteria and
505 risk of miscarriage among women early in pregnancy. *Matern Child Health J*
506 2015;**19**:2682–7. doi:10.1007/s10995-015-1790-2
- 507 6 Hay P. Recurrent bacterial vaginosis. *Curr Opin Infect Dis* 2009;**22**:82–6.
508 doi:10.1097/QCO.0b013e32832180c6
- 509 7 Verstraelen H, Verhelst R. Bacterial vaginosis: an update on diagnosis and treatment.
510 *Expert Rev Anti Infect Ther* 2009;**7**:1109–24. doi:10.1586/eri.09.87
- 511 8 Torrone EA, Morrison CS, Chen P-L, *et al.* Prevalence of sexually transmitted infections
512 and bacterial vaginosis among women in sub-Saharan Africa: An individual participant
513 data meta-analysis of 18 HIV prevention studies. *PLOS Med* 2018;**15**:e1002511.
514 doi:10.1371/journal.pmed.1002511
- 515 9 Bradshaw CS, Brotman RM. Making inroads into improving treatment of bacterial
516 vaginosis – striving for long-term cure. *BMC Infect Dis* 2015;**15**:292.
517 doi:10.1186/s12879-015-1027-4
- 518 10 Anukam KC, Osazuwa E, Osemene GI, *et al.* Clinical study comparing probiotic
519 *Lactobacillus* GR-1 and RC-14 with metronidazole vaginal gel to treat symptomatic
520 bacterial vaginosis. *Microbes Infect* 2006;**8**:2772–6. doi:10.1016/j.micinf.2006.08.008
- 521 11 Ling Z, Liu X, Chen W, *et al.* The restoration of the vaginal microbiota after treatment
522 for bacterial vaginosis with metronidazole or probiotics. *Microb Ecol* 2013;**65**:773–80.
523 doi:10.1007/s00248-012-0154-3
- 524 12 Ngugi BM, Hemmerling A, Bukusi EA, *et al.* Effects of bacterial vaginosis-associated
525 bacteria and sexual intercourse on vaginal colonization with the probiotic *Lactobacillus*
526 *crispatus* CTV-05. *Sex Transm Dis* 2011;**38**:1020–7.
527 doi:10.1097/OLQ.0b013e3182267ac4
- 528 13 Bradshaw CS, Pirota M, De Guingand D, *et al.* Efficacy of oral metronidazole with
529 vaginal clindamycin or vaginal probiotic for bacterial vaginosis: randomised placebo-

- 1
2
3 530 controlled double-blind trial. *PLOS ONE* 2012;**7**:e34540.
4 531 doi:10.1371/journal.pone.0034540
5
6
7 532 14 van der Straten A, Montgomery ET, Cheng H, *et al.* High acceptability of a vaginal ring
8 533 intended as a microbicide delivery method for HIV prevention in African women. *AIDS*
9 534 *Behav* 2012;**16**:1775–86. doi:10.1007/s10461-012-0215-0
10
11 535 15 Merkatz RB, Plagianos M, Hoskin E, *et al.* Acceptability of the nectorone®/ethinyl
12 536 estradiol contraceptive vaginal ring: development of a model; implications for
13 537 introduction. *Contraception* 2014;**90**:514–21. doi:10.1016/j.contraception.2014.05.015
14
15 538 16 Liu CM, Hungate BA, Tobian AAR, *et al.* Penile microbiota and female partner bacterial
16 539 vaginosis in Rakai, Uganda. *mBio* 2015;**6**:e00589. doi:10.1128/mBio.00589-15
17
18
19 540 17 van de Wijgert J, Verwijs MC, Agaba SK, *et al.* Intermittent lactobacilli-containing
20 541 vaginal probiotic or metronidazole use to prevent bacterial vaginosis recurrence: safety
21 542 and preliminary efficacy by microscopy and sequencing. *MedRxiv* Published Online
22 543 First: 8 July 2019. doi:10.1101/19001156
23
24 544 18 Sobel JD, Ferris D, Schwebke J, *et al.* Suppressive antibacterial therapy with 0.75%
25 545 metronidazole vaginal gel to prevent recurrent bacterial vaginosis. *Am J Obstet Gynecol*
26 546 2006;**194**:1283–9. doi:10.1016/j.ajog.2005.11.041
27
28
29 547 19 McClelland RS, Richardson BA, Hassan WM, *et al.* Improvement of vaginal health for
30 548 Kenyan women at risk for acquisition of Human Immunodeficiency Virus type 1: results
31 549 of a randomized trial. *J Infect Dis* 2008;**197**:1361–8. doi:10.1086/587490
32
33 550 20 Kestelyn E, van Nuil JI, Umulisa MM, *et al.* High acceptability of a contraceptive vaginal
34 551 ring among women in Kigali, Rwanda. *PLOS ONE* 2018;**13**:e0199096.
35 552 doi:10.1371/journal.pone.0199096
36
37
38 553 21 World Health Organization. Guidelines for the management of sexually transmitted
39 554 infections. 2003.<http://applications.emro.who.int/aiecf/web79.pdf> (accessed 12 Mar
40 555 2019).
41
42 556 22 Binagwaho A, editor. *National guidelines for prevention and management of HIV, STIs*
43 557 *& other blood borne infections*. Republic of Rwanda - Ministry of Health 2013.
44 558 https://aidsfree.usaid.gov/sites/default/files/hts_policy_rwanda.pdf (accessed 12 Mar
45 559 2019).
46
47
48 560 23 McClelland RS, Balkus JE, Lee J, *et al.* Randomized trial of periodic presumptive
49 561 treatment with high-dose intravaginal metronidazole and miconazole to prevent vaginal
50 562 infections in HIV-negative women. *J Infect Dis* 2015;**211**:1875–82.
51 563 doi:10.1093/infdis/jiu818
52
53 564 24 Veldhuijzen N, Nyinawabega J, Umulisa M, *et al.* Preparing for microbicide trials in
54 565 Rwanda: focus group discussions with Rwandan women and men. *Cult Health Sex*
55 566 2006;**8**:395–406. doi:10.1080/13691050600859302
56
57
58 567 25 Low N, Chersich MF, Schmidlin K, *et al.* Intravaginal practices, bacterial vaginosis, and
59 568 HIV infection in women: individual participant data meta-analysis. *PLOS Med*
60 569 2011;**8**:e1000416. doi:10.1371/journal.pmed.1000416

- 1
2
3 570 26 Dausset C, Patrier S, Gajer P, *et al.* Comparative phase I randomized open-label pilot
4 571 clinical trial of Gynophilus® (Lcr regenerans®) immediate release capsules versus slow
5 572 release muco-adhesive tablets. *Eur J Clin Microbiol Infect Dis* 2018;**37**:1869–80.
6 573 doi:10.1007/s10096-018-3321-8
7
8
9 574 27 Safren SA, W. Otto M, Worth JL, *et al.* Two strategies to increase adherence to HIV
10 575 antiretroviral medication: Life-Steps and medication monitoring. *Behav Res Ther*
11 576 2001;**39**:1151–62. doi:10.1016/S0005-7967(00)00091-7
12
13 577 28 van de Wijgert JHHM, Verwijs MC. Lactobacilli-containing vaginal probiotics to cure or
14 578 prevent bacterial or fungal vaginal dysbiosis: a systematic review and recommendations
15 579 for future trial designs. *BJOG*;0. doi:10.1111/1471-0528.15870
16
17 580 29 Glick P, Sahn DE. Changes in HIV/AIDS knowledge and testing behavior in Africa: how
18 581 much and for whom? *J Popul Econ* 2007;**20**:383–422. doi:10.1007/s00148-006-0085-8
19
20 582 30 Peltzer K, Matseke G, Mzolo T, *et al.* Determinants of knowledge of HIV status in South
21 583 Africa: results from a population-based HIV survey. *BMC Public Health* 2009;**9**:174.
22 584 doi:10.1186/1471-2458-9-174
23
24 585 31 Fay H, Baral SD, Trapence G, *et al.* Stigma, health care access, and HIV knowledge
25 586 among men who have sex with men in Malawi, Namibia, and Botswana. *AIDS Behav*
26 587 2011;**15**:1088–97. doi:10.1007/s10461-010-9861-2
27
28
29
30 588
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

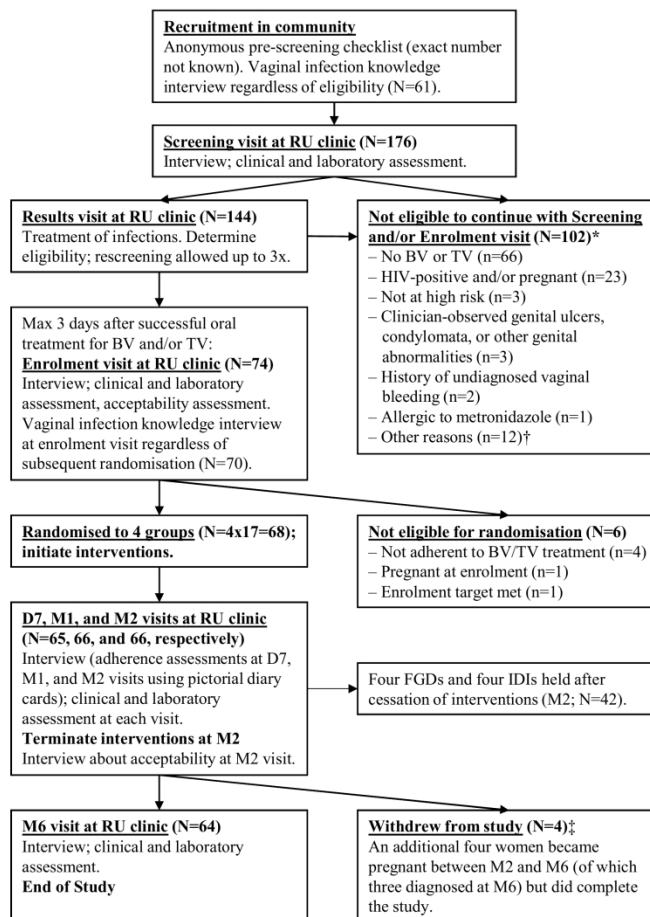


Figure 1: Flowchart of the study

*Totals to 110 reasons among 102 women because there could be more than one reason per woman.

†Reasons: outside of metronidazole treatment window (n=5), enrolment target already met (n=4), has a mental disorder (n=1), did not complete screening procedures and was subsequently lost to follow-up (n=1), withdrew consent during the screening visit because she thought the reimbursement was too low (n=1).

‡Reasons: moved away from Kigali (n=2), lost interest because symptoms resolved (n=1), and was verbally harassed by partner and sister about study participation (n=1).












Acceptability assessments were made at enrolment and at the M2 visit. Adherence assessments were made using self-rated assessments, pictorial diary cards, and returned packaging at the D7, M1, and M2 visits (after which product use was ceased). The vaginal infection knowledge survey was held at recruitment sessions in the community and at the enrolment visit. Changes in sexual risk-taking and vaginal practices were assessed at each follow-up visits and compared to answers given during the enrol visit. All these themes

1
2
3 were discussed during the eight FGDs and IDIs.
4

5 BV, bacterial vaginosis; D7, day 7 visit; FGD, focus group discussion; IDI, in-depth interview; M1/2/6,
6 month 1/2/6 visit; RU, Rinda Ubuzima; TV, *Trichomonas vaginalis*.

7
8 215x279mm (300 x 300 DPI)
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Supplementary Figure 1: Pictorial diary card

Date/Month	Descriptions	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Indicate each time you used study product								
	Used study product							
 Indicate each sex act 								
	Sex with condom							
	Sex without condom							
 Indicate each time you washed/inserted something inside the vagina other than study product 								
 By washing inside, we mean inserting an entire finger inside the vaginal canal 								
	Washed inside vagina with water only							
	Washed inside vagina with soap and water							
	Inserted something else (herbs, powders, etc.)							
Indicate each day of menstrual bleeding								
	Had menstrual bleeding							

The picture provided is the English translation of the pictorial card; participants received a version in Kinyarwanda.

Supplementary Table 1: Baseline characteristics of enrolled population

	Controls (n=17)	Metronidazole (n=17)	EF+ (n=17)	GynLP (n=17)
Median age (IQR)	29 (24–36)	30 (27–34)	33 (28–35)	30 (27–35)
Marital status n (%)				
- Never married	16 (94.1)	11 (64.7)	10 (58.8)	13 (76.5)
- Married	1 (5.9)	1 (5.9)	2 (11.8)	1 (5.9)
- Divorced	0	5 (29.4)	4 (23.5)	3 (17.6)
- Widowed	0	0	1 (5.9)	0
Education level n (%)				
- No schooling	5 (29.4)	3 (17.6)	3 (17.6)	3 (17.7)
- Primary school not completed	7 (41.2)	7 (41.2)	13 (76.5)	4 (23.5)
- Primary school completed	4 (23.5)	5 (29.4)	1 (5.9)	7 (41.2)
- At least some secondary school	1 (5.9)	2 (11.8)	0	3 (17.7)
Median number of sex partners last month (IQR)	5 (3–20)	5 (2–10)	3 (2–15)	3 (2–20)
Exchanged sex for money/goods past month n (%)	17 (100)	14 (82.4)	15 (88.2)	17 (100)
At least one laboratory-confirmed STI* n (%)	8 (47.1)	8 (47.1)	4 (23.5)	9 (52.9)
Median weekly frequency of washing body (IQR)	7 (7–7)	7 (7–7)	7 (7–7)	7 (4–7)
Ever washing the genitalia n (%)				
- Yes, outside only	12 (70.7)	14 (82.4)	15 (88.3)	14 (82.3)
- Yes, both inside and outside	5 (29.4)	3 (17.6)	2 (11.7)	3 (17.7)
- Yes, inside only	0	0	0	0
If reports washing inside, median weekly frequency (IQR)	14 (7–16)	14 (14–14)	11 (7–14)	7 (3–12)

*Chlamydia, gonorrhoea, and/or syphilis.

EF+, Ecologic Femi+; Enr, enrolment visit; GynLP, Gynophilus LP; IQR, inter-quartile range; M2, Month 2 visit; STI, sexually transmitted disease.

Supplementary Table 2: Acceptability of interventions

Acceptability of study products at Enr	Controls (n=17)	Metronidazole (n=17)	EF+ (n=17)	GynLP (n=17)
Nurse reports having explained intervention to participant in detail n (%)	17 (100)	17 (100)	17 (100)	17 (100)
Nurse reports participant asked questions n (%)*				
- Yes, a few	6 (35.3)	2 (11.8)	11 (64.7)	11 (64.7)
- Yes, many	0	0	0	2 (11.8)
First dose applied† under supervision n (%)	NA	17 (100)	17 (100)	17 (100)
Median number of attempts participant made until successful application (IQR)	NA	NA	1 (1–1)	1 (1–1)
Participant seemed comfortable with the insertion after these attempts, according to study nurse n (%)	NA	NA		
- Yes, very			17 (100)	16 (94.1)
- Yes, somewhat			0	1 (5.9)
Acceptability of study products at M2				
Self-reported usual time of insertion n (%)				
- Before going to sleep	NA	NA	17 (100)	15 (100)‡
- After bathing in the morning			0	0
Level of comfort with vaginal insertion after 2 months of use, self-reported n (%)				
- Very comfortable	NA	NA	17 (100)	15 (100)‡
- Somewhat comfortable			0	0
Reported insertion becoming easier over time n (%)	NA	NA	17 (100)	15 (100)‡
Reported manner of insertion§ n (%)				
- While lying down	NA	NA	17 (100)	14 (93.3)‡
- While squatting			1 (5.9)	1 (6.7)
Acceptability of penile hygiene intervention at M2				
Reports having told main sex partner to regularly clean the penis, including underneath the foreskin n (%)¶				
- Yes	3 (17.7)	3 (18.8)	3 (17.6)	3 (18.8)
- No, because he is circumcised	10 (58.8)	9 (56.2)	6 (35.3)	5 (31.3)
- No, other reason	1 (5.9)	0	1 (5.9)	1 (6.3)
If yes, response by the main partner n (%)				
- He said that he would do so in the future	2 (66.7)	1 (33.3)	1 (33.3)	1 (33.3)
- He said that he already does this	1 (33.3)	1 (33.3)	0	1 (33.3)
- He said that he is not interested	0	1 (33.3)	2 (66.7)	1 (33.3)

*One missing value.

†Whether oral insertion (oral metronidazole group) or vaginal insertion (Ecologic Femi+ and Gynophilus LP groups).

‡N=15 due to participants withdrawing informed consent.

§Multiple answers possible; hence totals can be more than 100%.

¶Women with no main sex partner not included.

||N=3 in all four groups.

EF+, Ecologic Femi+; Enr, enrolment visit; GynLP, Gynophilus LP; IQR, inter-quartile range; M2, Month 2 visit; NA, Not applicable.

STROBE Statement—Checklist of items that should be included in reports of *cohort studies*

	Item No	Recommendation	Reported on page #
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1
		(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	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	4,5
		(b) For matched studies, give matching criteria and number of exposed and unexposed	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	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	6
Bias	9	Describe any efforts to address potential sources of bias	6
Study size	10	Explain how the study size was arrived at	NA (Primary outcomes paper)
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6
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	6,7
		(c) Explain how missing data were addressed	NA (indicated in tables if missing)
		(d) If applicable, explain how loss to follow-up was addressed	NA
		(e) Describe any sensitivity analyses	NA
Results			
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	Page 8, Figure 1
		(b) Give reasons for non-participation at each stage	8, Figure 1
		(c) Consider use of a flow diagram	Figure 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8
		(b) Indicate number of participants with missing data for each variable of	8 (missing

		interest	data in footnotes in table, if applicable)
		(c) Summarise follow-up time (eg, average and total amount)	8
Outcome data	15*	Report numbers of outcome events or summary measures over time	9
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	Table 1
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Page 12 and beyond
Discussion			
Key results	18	Summarise key results with reference to study objectives	Page 18
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	Page 19
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Page 18, 19
Generalisability	21	Discuss the generalisability (external validity) of the study results	Page 18, 19
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	Page 21

*Give information separately for exposed and unexposed groups.

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 <http://www.strobe-statement.org>.

BMJ Open

Vaginal Probiotic Adherence and Acceptability in Rwandan Women with High Sexual Risk Participating in a Pilot Randomised Controlled Trial: A Mixed-Methods Approach

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2019-031819.R2
Article Type:	Original research
Date Submitted by the Author:	04-Mar-2020
Complete List of Authors:	Verwijs, Marijn; Institute of Infection and Global Health, University of Liverpool, Agaba, Stephen; Rinda Ubuzima Umulisa, Marie ; Rinda Ubuzima Uwineza, Mireille; Rinda Ubuzima Nivoliez, Adrien; Biose, Lievens, Elke; Winlove van de Wijgert, Janneke H.H.M.; University of Liverpool, Institute of Infection and Global Health; Universitair Medisch Centrum Utrecht, Julius Center for Health Sciences and Primary Care
Primary Subject Heading:	Sexual health
Secondary Subject Heading:	Sexual health, Obstetrics and gynaecology, Public health, Qualitative research
Keywords:	bacterial vaginosis, vaginal probiotic, adherence, acceptability, Africa

SCHOLARONE™
Manuscripts



I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our [licence](#).

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which [Creative Commons](#) licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

1
2
3 **1 Vaginal Probiotic Adherence and Acceptability in Rwandan Women with High Sexual Risk**
4
5 **2 Participating in a Pilot Randomised Controlled Trial: A Mixed-Methods Approach**
6
7
8
9

10 Authors: Marijn C. Verwijs (MD)¹, Stephen K. Agaba (MD)², Marie-Michele Umulisa (MA)²,
11 Mireille Uwineza (BA)², Adrien Nivoliez (PhD)³, Elke Lievens (PhD)⁴, Janneke H.H.M. van de
12 Wiggert (MD PhD MPH)^{1,5}.
13
14
15
16
17

18 **Author affiliations**

19
20 ¹Institute of Infection and Global Health, University of Liverpool, Liverpool, United Kingdom.

21
22 ²Rinda Ubuzima, Kigali, Rwanda.

23
24 ³Biose, Aurillac, France.

25
26 ⁴Winlove Probiotics, Amsterdam, The Netherlands.

27
28 ⁵Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht
29 University, Utrecht, The Netherlands.
30
31
32
33
34

35 **Correspondence to**

36
37 Professor Janneke van de Wiggert, MD PhD MPH

38
39 Department of Clinical Infection, Microbiology and Immunology

40
41 Institute of Infection and Global Health, University of Liverpool

42
43 Ronald Ross Building, 8 West Derby Street, Liverpool L69 7BE, United Kingdom

44
45 Email: j.vandewijgert@liverpool.ac.uk

46
47 ORCID: 0000-0003-2728-4560
48
49
50
51

52 **Word counts:**

53
54 Word count abstract: 300 words (max. 300 words).

55
56 Word count main text: 3,934 words (max. 4000 words).

57
58 Number of tables and figures: 5 (max. 5).

59
60 Number of supplementary tables and figures: 3.

1
2
3 29 **ABSTRACT**
4

5 30 **Objectives** To evaluate adherence and acceptability of intermittent vaginal probiotic or antibiotic use
6
7 31 to prevent bacterial vaginosis (BV) recurrence.
8

9 32 **Design** Repeated adherence and acceptability assessments using mixed methods within a pilot
10
11 33 randomised controlled trial.
12

13 34 **Setting** Research clinic in Kigali, Rwanda.
14

15 35 **Participants** Rwandan women with high sexual risk.
16

17 36 **Interventions** Women diagnosed with BV and/or trichomoniasis were randomised to four groups
18
19 37 (n=17 each) after completing metronidazole treatment: behavioural counselling only, or behavioural
20
21 38 counselling plus two-month intermittent use of oral metronidazole, Ecologic Femi+ (EF+) vaginal
22
23 39 capsule, or Gynophilus LP (GynLP) vaginal tablet.
24

25 40 **Outcome measures** Adherence and acceptability were assessed by structured face-to-face interviews,
26
27 41 semi-structured focus group discussions and in-depth interviews, daily diaries, and counting of
28
29 42 used/unused study products in randomised women (n=68). Vaginal infection knowledge was assessed
30
31 43 by structured face-to-face interviews in randomised women and women attending recruitment
32
33 44 sessions (n=131).
34

35 45 **Results** Most women (93%) were sex workers, 99.2% were unfamiliar with BV, and none had ever
36
37 46 used probiotics. All probiotic users (n=32) reported that insertion became easier over time.
38
39 47 Triangulated adherence data showed that 17/17 EF+ users and 13/16 GynLP users used $\geq 80\%$ of
40
41 48 required doses (Fisher's exact $p=0.103$). Younger age ($p=0.076$), asking many questions at enrolment
42
43 49 ($p=0.116$), having menses ($p=0.104$), and reporting urogenital symptoms ($p=0.103$) were non-
44
45 50 significantly associated with lower perfect adherence. Women believed that the probiotics reduced
46
47 51 BV recurrence, but reported that partners were sometimes unsupportive of study participation. Self-
48
49 52 reported vaginal washing practices decreased during follow-up, but sexual risk behaviours did not.
50
51 53 Most women (12/15) with an uncircumcised steady partner discussed penile hygiene with him, but
52
53 54 many women found this difficult, especially with male clients.
54
55
56
57
58
59
60

1
2
3 55 **Conclusions** High-risk women require education about vaginal infections. Vaginal probiotic
4
5 56 acceptability and adherence were high in this cohort. Our results can be used to inform future product
6
7 57 development and to fine-tune counselling messages in prevention programs.
8

9 58 **Trial registration** ClinicalTrials.gov (NCT02459665).

10 59 **Keywords (5)** bacterial vaginosis, vaginal probiotic, adherence, acceptability, Africa.
11
12
13
14 60

15 61 **ARTICLE SUMMARY**

16 62 **Strengths and limitations of this study**

- 17 63 • We conducted this research in the context of a pilot randomised controlled trial, and statistical
18 64 power was therefore limited.
- 19 65 • We triangulated different sources of adherence data to maximise accuracy, and used a mixed-
20 66 methods approach to evaluate acceptability.
- 21 67 • We could not directly compare experiences with, and opinions about, the two different vaginal
22 68 probiotics because each woman used only one product and qualitative data depth was suboptimal.
- 23 69 • Social desirability bias may have affected some of the results.
- 24 70 • The results of this study may not be generalizable to women at lower risk of sexually transmitted
25 71 or urogenital infections.

72 INTRODUCTION

73 Bacterial vaginosis (BV) is a vaginal condition in which fastidious anaerobes such as *Gardnerella*
74 *vaginalis* increase while beneficial, lactic acid-producing lactobacilli decrease.[1] Often
75 asymptomatic, it is associated with increased risks of sexually transmitted infections (STIs) and HIV
76 acquisition, pelvic inflammatory disease, and adverse pregnancy outcomes.[2–5] Although BV is
77 treatable with antibiotics, the risk of recurrence is high.[6,7] The prevalence of BV varies among
78 regions and ethnic groups but is highest in sub-Saharan Africa, where it is estimated at 30-50%.[8]
79
80 Vaginally-administered probiotics containing lactobacilli are considered a promising new strategy to
81 restore a lactobacilli-dominated vaginal microbiota during and/or after antibiotic treatment, or to
82 prevent BV.[9] While some probiotics have been available on the market for several years, clinical
83 trials to support beneficial effects have only recently been initiated for most products.[10–13] Future
84 uptake and adherence of a vaginal probiotic, once proven efficacious, is determined to a large extent
85 by its acceptability in target populations. The acceptability, in turn, depends on factors such as
86 characteristics of the target population, characteristics of and experiences with the product, types of
87 sexual relationships and partner support, and community perceptions.[14,15]
88
89 We conducted a clinical trial of intermittent use of two vaginal probiotics and oral metronidazole to
90 prevent BV recurrence in Rwandan women who had been treated for BV and/or *Trichomonas*
91 *vaginalis* (TV). We used qualitative and quantitative research methods to assess adherence and
92 acceptability with vaginal probiotic use. We triangulated various sources of adherence data to obtain
93 adherence estimates per woman for each period of intermittent product use in between study visits,
94 and determined correlates of adherence.

96 METHODS

97 The pilot clinical trial took place from June 2015 to February 2016 at the Rinda Ubuzima research
98 clinic in Kigali, Rwanda. The trial was a pilot trial with a modest sample size at the request of the
99 funder. Women who had been successfully treated for BV/TV with a seven-day course of oral

1
2
3 100 metronidazole (Tricozole, Laboratory & Allied Ltd, Nairobi, Kenya) were randomised to four
4
5 101 intervention groups (n=17 each) to prevent BV recurrence: behavioural counselling only (controls), or
6
7 102 behavioural counselling plus intermittent use of two different vaginal probiotics or oral metronidazole
8
9 103 for two months. The behavioural counselling included counselling on safer sex, vaginal hygiene
10
11 104 (including discouragement of intravaginal washing), and penile hygiene (i.e. encouragement of
12
13 105 cleansing the penis, including underneath the foreskin), because these behaviours are known to reduce
14
15 106 BV recurrence risk somewhat.[6,16] We counselled all women in all randomisation groups because
16
17 107 we considered it unethical to withhold this information from women at risk. Women were seen at
18
19 108 screening, enrolment (product use initiation, if applicable), Day 7, Month 1, Month 2 (product use
20
21 109 cessation, if applicable), and Month 6. Product efficacies were not known during the trial, and the
22
23 110 efficacy results of the pilot trial are reported elsewhere.[17] Briefly, the vaginal probiotics did
24
25 111 improve the vaginal environment (increased lactobacilli and reduced BV-associated bacteria)
26
27 112 compared to counselling only, but not as much as oral metronidazole did.
28
29
30
31
32

33 114 **Study population**

34
35 115 Women aged 18-45 at risk of HIV/STIs (defined as having had more than one sex partner and/or
36
37 116 having been treated for an STI and/or BV in the last 12 months) were eligible for enrolment if they
38
39 117 were confirmed HIV-negative, non-pregnant, diagnosed with BV and/or TV, and cured after seven-
40
41 118 day oral metronidazole treatment. Other clinical exclusion criteria were applied but were rare.[17]
42
43 119 Women were recruited by study staff with the assistance of Community Mobilisers who had strong
44
45 120 ties with local high-risk women (particularly sex workers).
46
47
48
49

50 122 **Study products and dosing**

51
52 123 Ecologic Femi+ (EF+; Winclove Probiotics, Amsterdam, Netherlands) is a vaginal capsule containing
53
54 124 lyophilised lactic acid-producing bacteria. EF+ was used once per day for five days followed by thrice
55
56 125 weekly, for two months. Gynophilus LP (GynLP; Biose, Aurillac, France) is a tablet containing the
57
58 126 *Lactobacillus rhamnosus* Lcr35 strain. The tablet disintegrates in the vagina and forms a gel that
59
60 127 slowly releases the probiotic bacteria. GynLP was used once every four days for two months. The first

1
2
3 128 dose was inserted at the clinic under direct observation of a clinician, and remaining doses were self-
4
5 129 administered at home. Women were asked not to cleanse or insert other products into the vagina after
6
7 130 probiotic insertion to allow the probiotics to dissolve. They were also told that they were allowed to
8
9 131 cease probiotic use during menses, but were encouraged to continue. Intermittent metronidazole use
10
11 132 was chosen as a positive control intervention because studies conducted in the U.S. and Kenya have
12
13 133 shown a 30-40% reduction in BV recurrence.[18,19] Metronidazole users took 500 mg generic oral
14
15 134 metronidazole (Laboratory & Allied Ltd, Nairobi, Kenya) twice weekly for two months. The rationale
16
17 135 for selecting these study products and their dosing schedules can be found in the manuscript
18
19 136 describing the efficacy results of the pilot trial.[17] Participants and clinicians were not blinded.
20
21
22
23

137

138 **Acceptability, adherence, behavioural, and vaginal infection knowledge assessments**

139 Acceptability was assessed at the enrolment visit prior to product use initiation and at the Month 2
140 visit after the full two months of use. Adherence was assessed during the intervention period, at the
141 Day 7, Month 1, and Month 2 visits. Sexual and other behaviours were assessed at all study visits.
142 Participants were interviewed face-to-face in Kinyarwanda by a trained study nurse using structured
143 questionnaires with multiple-choice questions, questions requiring a number or date, and an adherence
144 self-rating scale (from 0-10). In between visits, participants used pictorial diary cards (online
145 supplementary material figure 1) to record daily episodes of product use, vaginal sex, condom use,
146 and vaginal practices. Those using study products returned the product packaging and unused
147 products (if applicable) to their clinic visits, where they were counted by study staff. Any
148 discrepancies between data sources were discussed with participants and the consensus assessments
149 were recorded on the questionnaires. The adherence data based on the self-rating scale, the diary card,
150 and the returned product packaging were triangulated by the data analyst at the data analysis stage.
151 Additionally, 131 women were interviewed about their knowledge of vaginal infections (such as BV
152 and STIs) using a structured questionnaire during recruitment sessions (n=61; regardless of eligibility)
153 and at enrolment visits (n=70; this included the 68 randomised women, and two women who attended
154 enrolment visits but turned out to be ineligible; figure 1). Women were interviewed before being
155 counselled at study visits or before receiving information at recruitment sessions. This questionnaire

1
2
3 156 contained multiple-choice and open-ended questions. Responses to the open-ended questions were
4
5 157 categorised and discussed by two different researchers until consensus about the answer categories
6
7 158 was reached.
8

9
10 159
11 160 Four semi-structured focus group discussions (FGDs) with 7-11 participants per group (total n=38),
12
13 161 and semi-structured individual in-depth interviews (IDIs) with four additional participants, were held.
14
15 162 The main themes of these FGDs and IDIs were experiences with and opinions of the study products,
16
17 163 sexual behaviour, and vaginal practices. Women randomised to the behavioural counselling only
18
19 164 group were not approached for the FGDs and IDIs, but all other randomised participants who had
20
21 165 completed their product use period were approached until data saturation had been achieved. The
22
23 166 interviews were unlinked anonymous, and women used pseudonyms to enable them to talk freely
24
25 167 despite the fact that the discussions and interviews were taped. All interviews took place between
26
27 168 November 2015 and March 2016, were held in Kinyarwanda, recorded on tape, transcribed verbatim,
28
29 169 and translated into English. The FGD and IDI transcripts were read and discussed by three researchers
30
31 170 (MV, MU, and JvdW) at regular intervals. The Chief Investigator (JvdW) decided that data saturation
32
33 171 had been met when the fourth FGD and the fourth IDI transcript had become available in March 2016.
34
35
36
37
38

39 173 **Data analysis**

40
41 174 The primary outcomes of this study were acceptability and triangulated adherence in women
42
43 175 randomised to study product use. Secondary outcomes included vaginal infection knowledge of the
44
45 176 target population more broadly, and behavioural changes (of the behaviours included in the
46
47 177 counselling messages) in all randomised women. Questionnaire data were analysed using Stata 13
48
49 178 (StataCorp, College Station, TX, USA). The proportion of women with $\geq 80\%$ / $\geq 90\%$ / 100% adherence
50
51 179 in the probiotic groups were compared by Fisher's exact tests. Changes in self-reported vaginal
52
53 180 practices and sexual behaviours over time were tested using McNemar's test for binary outcomes, and
54
55 181 Wilcoxon's signed-rank test for continuous outcomes. To study associations of participant
56
57 182 characteristics with triangulated adherence, we used bivariable mixed effects models, with perfect
58
59 183 adherence (defined as having used all doses as instructed) per interval between study visits during the
60

1
2
3 184 intervention period as the outcome, participant identification numbers as the random effect, and one
4
5 185 participant characteristic at the time as the fixed effect. We could not determine correlates of
6
7 186 acceptability due to limited variation in the acceptability data (reported acceptability was high
8
9 187 throughout the trial).
10

11
12 188

13
14 189 The FGD and IDI transcripts were coded using NVivo 10.0 (QSR International, Melbourne,
15
16 190 Australia) by one single researcher (MV). The discussions and interviews were semi-structured, with
17
18 191 the above-mentioned themes and associated codes prepared a priori, as well as new elements that
19
20 192 emerged from the data. The codes were derived from an acceptability framework that has been used in
21
22 193 studies of vaginal products for contraception or HIV prevention.[14,15,20] Components of the
23
24 194 framework include study population characteristics, product attributes, sexual encounter and relational
25
26 195 attributes, and the contextual environment (e.g. community perceptions of product use).
27

28
29 196

30 197 **Ethical statement**

31
32 198 All participants provided written consent for study participation, and separate consent for participation
33
34 199 in FGDs/IDIs. All non-married participants aged 18-20 also required parental/guardian consent per
35
36 200 Rwandan law at the time of the study. The participants received 3 GBP per visit (in local currency) as
37
38 201 a reimbursement for time and transport costs. Care was taken to protect participant privacy and
39
40 202 confidentiality. The study was sponsored by the University of Liverpool, approved by the Rwanda
41
42 203 National Ethics Committee and the University of Liverpool Research Ethics Subcommittee for
43
44 204 Physical Interventions, and registered on ClinicalTrials.gov (NCT02459665).
45
46

47 205

48 49 206 **Participant and public involvement**

50
51 207 As part of the FGDs/IDIs, a subset of the enrolled participants were invited to comment on study
52
53 208 design and experiences with the interventions. Participants were not invited to develop outcomes,
54
55 209 interpret the results, or to contribute to the writing or editing of this document for readability or
56
57 210 accuracy. The preliminary results of this study were discussed with 32 stakeholders during a
58
59 211 workshop held at the Ministry of Health in Kigali, Rwanda, in December 2017. These stakeholders
60

1
2
3 212 included representatives of the Ministry of Health, the National University of Rwanda, the National
4
5 213 Ethics Committee, local hospitals and clinics, and local non-governmental and women's
6
7 214 organisations.
8

9 215

11 216 **RESULTS**

13 217 **Baseline characteristics**

15 218 We screened 176 women: bacterial STI prevalence was 31.3% and BV prevalence by Gram stain
16
17 219 Nugent scoring was 47.9%. All 68 randomised women were treated for BV and/or TV prior to
18
19 220 randomisation and at risk of HIV/STIs, with 93.1% reporting having exchanged sex for money and/or
20
21 221 goods in the previous month (figure 1, online supplementary material table 1). We collected 29.93
22
23 222 person-years of data. Four women withdrew their informed consent during the study (for reasons
24
25 223 unrelated to study product acceptability). None were lost to follow-up.
26
27 224

29 225 **Adherence**

31 226 Triangulated adherence was high: 17/17 (100%) of EF+ users and 13/16 (81.3%) of GynLP users used
32
33 227 $\geq 80\%$ of required doses (Fisher's exact $p=0.103$; table 1), and these percentages were 15/17 (88.2%)
34
35 228 and 11/16 (68.8%) for $\geq 90\%$ ($p=0.225$), and 10/17 (58.8%) and 8/16 (50%) for 100% of required
36
37 229 doses ($p=0.732$), respectively. In comparison, these percentages were 15/17 (88.2%), 14/17 (82.4%),
38
39 230 and 12/17 (70.6%), respectively, for oral metronidazole users. Reported reasons of non-adherence to
40
41 231 vaginal probiotics during face-to-face interviews were 'simply forgetting' ($n=9$), experiencing side-
42
43 232 effects ($n=2$), menses ($n=2$), and being away from home and having left products at home ($n=1$).
44
45 233 Additional reasons for missing doses mentioned during FGDs/IDIs were being drunk ($n=2$) and being
46
47 234 confused about the dosing schedule ($n=2$). Only one woman in the metronidazole arm reported
48
49 235 missing doses due to experiencing side-effects. Most women in FGDs reported using all doses as
50
51 236 instructed and finding it easy to adhere, and thought that the diary cards served as a useful reminder to
52
53 237 use the products.
54
55 238

56 239

240 **Table 1: Adherence to study interventions**

Adherence to study products	Metronidazole (n=17)	EF+ (n=17)	GynLP (n=16)
Adherence Enr–D7, median % (IQR)	100 (100–100)	100 (100–100)	100 (100–100)
Adherence D7–M1, median % (IQR)	100 (100–100)	100 (100–100)	100 (91.7–100)
Adherence M1–M2, median % (IQR)	100 (100–100)	100 (100–100)	100 (92.3–100)
Overall adherence Enr–M2, median % (IQR)	100 (96.3–100)	100 (100–100)	98.3 (89.3–100)
Overall adherence Enr–M2 n (%)			
- Perfect*	12 (70.6)	10 (58.8)	8 (50.0)
- Adherence ≥90%	14 (82.4)	15 (88.2)	11 (68.8)
- Adherence ≥80%	15 (88.2)	17 (100)	13 (81.3)
Number of times menses Enr–M2 n (%)†			
- Never	7 (41.2)	4 (23.5)	2 (12.5)
- Once	6 (35.3)	5 (29.4)	4 (25.0)
- Twice	4 (23.5)	8 (47.1)	10 (62.5)
Did not use product during menses at least once n (%)			
- Yes	4 (23.5)	3 (17.6)	5 (31.3)
- NA (never had menses)	7 (41.2)	4 (23.5)	2 (12.5)
Self-reported reasons for non-adherence‡	Metronidazole	EF+	GynLP
D7: Self-reported reasons why not able to use all doses as instructed n (%)§			
- Simply forgot	0	2 (11.8)	0
- Product had side effects	0	0	1 (6.7)¶
M1: Self-reported reasons why not able to use all doses as instructed n (%)§			
- Simply forgot	1 (6.3)	1 (5.9)	1 (6.3)
- Product had side effects	1 (6.3)	0	1 (6.3)‡‡
- Did not like product for another reason	1 (6.3)	0	0
- Other	1 (6.3)**	1 (5.9)††	2 (12.5)§§
M2: Self-reported reasons why not able to use all doses as instructed n (%)§			
- Simply forgot	1 (6.3)	2 (11.8)	3 (18.8)
- Travelled and forgot to take product	1 (6.3)	0	1 (6.25)
- Other	0	1 (5.9)¶¶	1 (6.3)
D7: Participant thinks she used product correctly most of the time n (%)	17 (100)	16 (94.1)	14 (93.3)
M1: Participant thinks she used product correctly most of the time n (%)	13 (86.7)	17 (100)	11 (68.8)
M2: Participant thinks she used product correctly most of the time n (%)	15 (93.7)	16 (94.1)	14 (87.5)

*Defined as 100% of the prescribed doses used at the prescribed times after nurse review of the participant's diary card and returned used packaging and unused product.

†Number of times menses in the control group: never 2 (11.8%), once 3 (17.8%), twice 11 (64.7%), and thrice 1 (5.9%).

‡Numbers of participants per randomisation group may vary slightly due to loss to follow-up. Participants with ≥90% adherence not shown.

§Multiple answers possible.

¶Participant reported vulval itching and burning when passing urine.

||Participant reported mild gastritis and wanting to withdraw from the study anyway.

**Participant reported receiving oral metronidazole therapy for 7 days due to infection.

††Participant reported having menses twice in one month; decided to use less of her product until the next study visit.

‡‡Participant reported genital itching, genital burning, and pain during sex.

§§One participant reported missing the D7 study visit and therefore running out of supplies. Another participant reported not to have used the study product during menses (which she was allowed to do).

¶¶Participant reported being drunk and therefore forgetting to take the study product.

||||Participant reported taking the study product correctly but that the product came out during menses

D7, Day 7 visit; EF+, Ecologic Femi+; Enr, enrolment visit; GynLP, Gynophilus LP; IQR, inter-quartile range; M1/2, Month 1/2 visit; NA, not applicable.

259 **Acceptability**

260 *Ease-of-use*

261 No participants reported having heard about probiotics before study participation. After product use,
262 all vaginal probiotic users reported feeling very comfortable with insertion and that insertion became
263 easier over time. All but one woman reported inserting while lying down (online supplementary
264 material table 2).

265

266 *Bodily changes and product perception*

267 During FGDs, several women using either vaginal probiotic reported the product (partially) “*coming*
268 *out*” during the first few uses, but that this decreased after having gained experience. Many EF+ and
269 GynLP users reported an increase in vaginal wetness, which was considered a positive attribute by
270 most. Some women reported increased libido. For example, one EF+ user said: “*I felt a great desire to*
271 *[have] sex again and again.*” In contrast, one metronidazole user reported a decrease in libido. Most
272 women believed that the vaginal probiotics decreased the recurrence of symptomatic BV (our
273 preliminary efficacy data suggest that BV incidence had in fact decreased),^[17] and a few believed
274 that they also prevented STI acquisition (the trial had insufficient statistical power to assess this).

275

276 *Support*

277 One social harm related to vaginal probiotic use was reported: a GynLP user was verbally harassed by
278 her partner and her sister because of her study participation, and opted to withdraw her informed
279 consent. Reports of partner, family, and community support during the FGDs/IDIs were mixed: some
280 women reported problems with loved ones. Negative reactions from male partners were more often
281 based on suspicions about study participation than the products themselves. One EF+ user said: “*He*
282 *[her partner] did not accept that. He asked me to go together with him to the clinic [a local health*
283 *centre] and check if I am not HIV-positive.*” Another participant using metronidazole mentioned
284 wanting to join the study to her husband, who forbade her to participate. However, she decided to join
285 anyway: “*he did not know that I was using the study product, because he had refused me to join [the]*

1
2
3 286 *study before... I used them [the study products] without informing him.*" All sex workers except one
4
5 287 stated that they had not discussed study participation with male clients.
6

7 288
8

9 289 *Worries and concerns*

10
11 290 In the FGDs, one woman reported hearing rumours prior to enrolling that vaginal products "*can*
12
13 291 *damage the uterus or cause tumours in the womb.*" However, most participants thought that vaginal
14
15 292 probiotics would be acceptable to Rwandan women. One GynLP user argued: "*They [already] give us*
16
17 293 *vaginal pills*", by which she meant vaginal medications for yeast infections. We did not ask women
18
19 294 explicitly whether they would be willing to pay for the products, but some women mentioned
20
21 295 spontaneously in FGDs that they were concerned about future product availability and pricing. They
22
23 296 hoped that probiotics would be distributed cheaply through the Rwandan *Mutuelle* public health
24
25 297 insurance because they would otherwise be inaccessible to many women. One metronidazole user was
26
27 298 concerned about a limited applicability of probiotics because BV is not diagnosed by laboratory
28
29 299 testing in Rwanda: "*They do not have adequate medical instruments to test diseases, you tell the*
30
31 300 *physician how [...] you feel and by guessing the disease, he gives you at least four medications,*
32
33 301 *saying that you may have trichomonas, you may have syphilis, you may have gonorrhoea* [she refers
34
35 302 to syndromic management.[21,22]] *At health centre-level they do not have medical equipment to test*
36
37 303 *diseases, meaning that they will not know who to give that* [probiotic/antibiotic maintenance therapy]
38
39 304 *medication.*"
40
41
42
43
44

45 306 **Vaginal practices and sexual risk-taking**

46
47 307 At enrolment, 35/71 (49.3%) of the women reported to never use products inside the vagina, and at
48
49 308 Month 6, this increased to 53/65 (81.5%) (OR 5.2, 95% CI 1.96-17.34; table 2). During FGDs, some
50
51 309 women understood that vaginal washing practices may increase the risk of vaginal infection, but
52
53 310 others did not. A participant stated: "*You get them [i.e., vaginal diseases] anyway... whether you wash*
54
55 311 *or not*". In one FGD, 10 of 11 participants (90.9%) stated having ceased vaginal practices thanks to
56
57 312 the study counselling. It should be noted that in contrast to many other African populations, Rwandan
58
59 313 women use vaginal practices to increase rather than reduce vaginal lubrication. Women mentioned the
60

1
2
3 314 use of herbs (*umushishiro*), Vaseline, and oils for this purpose. Self-reported sexual risk taking by
4
5 315 face-to-face interview did not change over time, except for a significant reduction in reported numbers
6
7 316 of sex partners in the previous month at Month 6 compared to enrolment. No women in FGDs/IDIs
8
9 317 mentioned adopting safer sex practices (such as consistent condom use) in response to the counselling
10
11 318 messages. During face-to-face interviews at the Month 2 visit, 12 of 15 women (80%) who had an
12
13 319 uncircumcised main sex partner reported asking him to regularly clean his penis in the future (online
14
15 320 supplementary material table 2). While most women in FGDs understood that using condoms and
16
17 321 improved penile hygiene could reduce BV rates (as shown in [6,16]), some mentioned that they found
18
19 322 it difficult to discuss these topics with male partners. One participant stated that this is especially
20
21 323 difficult being a sex worker: “*a man gives you his own money and you start educating him to wash!*”
22
23 324 However, another sex worker reported refusing sex with uncircumcised clients: “*you leave him,*
24
25 325 *because he has a lot [of] germs*”. Several women reported discussing circumcision with their partners;
26
27 326 one participant reported telling her husband: “*It is better that you do circumcision because it is a good*
28
29 327 *thing... you would get a chance of not contracting diseases.*”
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

328 **Table 2: Changes in reported vaginal cleansing practices and (sexual) behaviour between the**
 329 **enrolment and the M6 visit.**

330

Self-reported sociodemographic characteristics	Enr (n=71)	M6 (n=65)	OR (95% CI)*
			P value*
Reports using no products inside the vagina (other than for managing menses; all participants) n (%)	35 (49.3)	53 (81.5)	5.2 (1.96–17.34) <0.001
Reports using no products inside the vagina (other than for managing menses; controls and metronidazole users only)† n (%)	15 (44.1)	27 (79.4)	13.0 (1.95–552.5) 0.002
Reports using water only n (%)	23 (32.4)	10 (15.4)	0.37 (0.13–0.92) 0.029
Reports using water and soap n (%)	3 (4.2)	2 (3.1)	0.67 (0.06–5.82) 1.00
Reports using paper, cloth or cotton wool n (%)	9 (12.7)	0 (0)	0.13 (0.00–0.93)‡ 0.008
Reports using traditional herbs, stones, powders as vaginal cleansing practice n (%)	1 (1.4)	1 (1.5)	1.00 (0.01–78.5)‡ 1.00
Mean weekly frequency of vaginal practices (95% CI)	2.15 (0.97–3.34)	0.64 (0.18–1.11)	NA 0.328
Median number of sex partners in last month at baseline or per month during follow-up period (IQR)	5 (3–16)	2 (1–4)	NA <0.001
Any condom use reported in past two weeks (Enr) or since last study visit (M6), versus no condom use reported n (%)	64 (90.1)	60 (92.3)	1.67 (0.32–10.7) 0.727
Reports exchanging sex for money/goods in past month (Enr) or since last study visit (M6) n (%)	65 (91.5)	58 (89.2)	0.80 (0.16–3.72) 1.00

331 *McNemar's OR and p-value for binary variables and Wilcoxon signed-rank test p-value for continuous variables, comparing the response at M6
 332 with the response at Enr. ORs with 95% CI were also calculated for binary pre-post data.

333 †N=34.

334 ‡To enable calculation of effect measures, a zero value was replaced by 1.

335 CI, confidence interval; Enr, enrolment visit; IQR, inter-quartile range; M6, Month 6 visit; NA, not applicable; OR, odds ratio.

336

337 **Correlates of adherence**

338 In bivariable mixed effects models including the probiotic groups only, no participant characteristics
 339 were significantly associated with perfect adherence (table 3). However, non-significant trends were
 340 observed. Younger age ($p=0.076$), asking many questions at enrolment (compared to a few questions
 341 or no questions; structurally judged by a study nurse; $p=0.116$), having menses during the previous
 342 study interval ($p=0.104$), and reporting urogenital symptoms ($p=0.103$) were associated with a lower
 343 likelihood of perfect adherence. When including oral metronidazole users, menses was significantly
 344 associated with a lower likelihood of perfect adherence ($p=0.008$). There were no significant
 345 associations between randomisation group and perfect adherence.

346

347

348 **Table 3: Participant characteristics associated with perfect adherence**

Participant characteristics	EF+ and GynLP users		EF+, GynLP and oral metronidazole users	
	OR (95% CI)	P value	OR (95% CI)	P value
Randomisation group: GynLP versus EF+	0.68 (0.22–2.11)	0.505	ND	ND
Randomisation group: - EF+ versus metronidazole - GynLP versus metronidazole	ND	ND	0.53 (0.15–1.81) 0.36 (0.11–1.23)	0.308 0.103
Age in years: ≥ 30 years versus < 30	2.66 (0.90–7.82)	0.076	1.60 (0.61–4.15)	0.336
Marital status: - Married versus never married - Divorced versus never married - Widowed versus never married	0.97 (0.14–6.58) 1.18 (0.29–4.79) ND	0.976 0.912 0.991	1.17 (0.20–6.99) 1.39 (0.42–4.57) ND	0.865 0.586 0.990
At least some schooling versus no schooling	1.20 (0.59–2.45)	0.619	0.80 (0.22–2.95)	0.740
Number of sex partners last month: five or more versus four or less.	0.58 (0.18–1.83)	0.351	0.49 (0.17–1.37)	0.173
Exchanged sex for money/goods past month	ND	0.990	ND	0.986
Nurse reported participant asked questions at Enr - Yes, many versus none - Yes, a few versus none	0.19 (0.02–1.52) 0.83 (0.24–2.83)	0.116 0.761	0.15 (0.02–1.19) 0.83 (0.27–2.57)	0.072 0.744
Had menses during study visit interval	0.41 (0.14–1.20)	0.104	0.26 (0.09–0.70)	0.008
Reported alcohol consumption during study: - Once or twice per week versus never - More than twice per week versus never	0.54 (0.14–2.12) 0.92 (0.18–4.81)	0.373 0.920	0.34 (0.11–1.08) 0.81 (0.19–3.49)	0.068 0.774
Reported at least one urogenital symptom during study interval versus none	0.11 (0.01–1.56)	0.103	0.30 (0.04–2.16)	0.231
Reported at least one adverse event during study visit interval (excluding urogenital symptoms) versus none	0.43 (0.10–1.83)	0.253	0.55 (0.15–2.05)	0.371

349 Sociodemographic characteristics associated with perfect adherence in bivariable mixed effects models, in the enrolment–D7, D7–M1, and
350 M1–M2 study visit intervals.
351 CI, confidence interval; D7, Day 7 visit; EF+, Ecologic Femi+; Enr, enrolment visit; GynLP, Gynophilus LP; M1, Month 1 visit; M2, Month
352 2 visit; ND, non-determinable; OR, odds ratio.

354 **Vaginal infection knowledge**

355 Almost all participants reported having heard of ‘diseases of the vagina’ and STIs before, but only
356 6/131 (4.6%) knew what bacteria were (table 4). The STIs most often spontaneously named (in
357 numerical order) were HIV, gonorrhoea, and syphilis; only one participant reported having heard of
358 BV. After having received an explanation about what BV is, only one of 131 woman reported ever
359 having been diagnosed with BV. Most participants could name at least one cause or potential
360 consequence of vaginal infections. Consequences wrongfully attributed to vaginal infections were
361 cervical cancer/tumours (5/131; 3.8%), consequences to the infant such as being born with BV or
362 congenital malformations (6/131; 4.6%), and death (4/131; 3.1%).

363 **Table 4: Vaginal infection knowledge**
364

	Recruitment (n=61)	Enrolment (n=70)	Total (n=131)
Median age (IQR)	32 (27–35)*	31 (27–35)	31 (27–35)
Has heard of diseases of the vagina before n (%)	60 (98.4)	70 (100)	130 (99.2)
Reports knowing what bacteria are before study n (%)	5 (8.2)	1 (1.4)	6 (4.6)
Reports having heard about STIs before study n (%)	61 (100)	70 (100)	131 (100)
If yes, spontaneously named, without probing† n (%)			
- HIV	58 (95.1)	65 (92.9)	123 (93.9)
- Gonorrhoea	58 (95.1)	65 (92.9)	123 (93.9)
- Syphilis	44 (72.1)	59 (84.3)	103 (78.7)
- Trichomoniasis	38 (62.3)	48 (68.6)	86 (65.7)
- Hepatitis	3 (4.9)	3 (4.3)	6 (4.6)
- Yeast infection	0	3 (4.3)	3 (2.3)
- BV	0	2 (2.9)	2 (1.5)
- Urinary tract infection	1 (1.6)	1 (1.4)	2 (1.5)
- Chlamydia	0	1 (1.4)	1 (0.8)
- Herpes	0	1 (1.4)	1 (0.8)
- HPV / cervical cancer	1 (1.6)	0	1 (0.8)
Reports having heard about BV before this study n (%)	1 (1.6)	0	1 (0.8)
Spontaneously reported reasons why women get vaginal disease, without probing† n (%)			
- Poor toilet hygiene	37 (60.7)	40 (57.1)	77 (58.8)
- Multiple sex partners	28 (45.9)	36 (51.4)	64 (48.9)
- After sex	25 (41.0)	30 (43.0)	55 (42.0)
- Dirty underwear	19 (31.2)	35 (50.0)	54 (41.2)
- Poor vaginal hygiene	26 (42.6)	22 (31.4)	48 (36.6)
- Poor penile hygiene of male partner(s)	4 (6.6)	17 (24.3)	21 (16.0)
- Traditional vaginal practices and washing	3 (4.9)	12 (17.1)	15 (11.5)
- New sex partner	6 (9.8)	3 (4.3)	9 (6.9)
- Use of contraception	1 (1.6)	3 (4.3)	4 (3.1)
- (Improper) use of sanitary pads or tampons	1 (1.6)	3 (4.3)	4 (3.1)
- Other	3 (4.9)‡	1 (1.4)§	4 (3.1)
- Cannot name any reasons	1 (1.6)	0	1 (0.8)
Spontaneously reported negative consequences of vaginal disease being named, without probing† n (%)			
- Foul smell from the vagina	30 (49.2)	39 (56.5)	69 (53.1)
- Difficulty getting pregnant	18 (29.5)	33 (47.8)	51 (39.2)
- Miscarriage	16 (26.2)	33 (47.8)	49 (37.7)
- Abnormal vaginal discharge	12 (19.7)	28 (40.6)	40 (30.8)
- Baby born too early	16 (26.2)	22 (31.9)	38 (29.2)
- Severe infection / fever of the woman	7 (11.5)	7 (10.1)	14 (10.8)
- Infection / fever of the newborn baby	5 (8.2)	3 (4.4)	8 (6.1)
- Itching	4 (6.6)	4 (5.8)	8 (6.1)
- Other consequences to the baby: being born with BV, congenital malformations, and others.	3 (4.9)	3 (4.4)	6 (4.6)
- Cervical cancer or tumours	2 (3.3)	3 (4.4)	5 (3.8)
- Death	4 (6.6)	0	4 (3.1)
- HIV/STIs	1 (1.6)	3 (4.4)	4 (3.1)
- Pain during intercourse	0	3 (4.4)	3 (2.3)
- Cannot name any consequence	17 (27.9)	19 (27.5)	36 (27.7)

*One missing value.

†Open-ended question. Totals may be more than 100%.

‡Participants report: "If you are infected with STIs", sharing underwear, and unprotected sex.

§Participant reports: vaginal medicine.

BV, bacterial vaginosis; HPV, human papilloma virus; IQR, interquartile range; STI, sexually transmitted infection.

1
2
3 370 **DISCUSSION**
4

5 371 Several studies of different vaginal probiotics have been conducted, some of them in sub-Saharan
6
7 372 Africa.[10–13] However, none reported in-depth acceptability and adherence data. Our study suggests
8
9 373 high vaginal probiotic acceptability and adherence in high-risk Rwandan women. We found no
10
11 374 statistically significant correlates of perfect adherence, partially due to limited statistical power, but
12
13 375 younger age, asking many questions about product use at enrolment, current menses, and reporting
14
15 376 urogenital symptoms showed trends towards a lower likelihood of perfect adherence. Vaginal
16
17 377 probiotics are currently unavailable on the market in most African countries, and it is important to
18
19 378 study acceptability in different target populations to inform product development and future
20
21 379 marketing strategies. Adherence to metronidazole was comparable to, or slightly higher than,
22
23 380 adherence reported in previously conducted studies.[19,23]
24
25

26 381

27
28 382 We could not evaluate the impact of self-reported acceptability aspects on adherence because almost
29
30 383 all women reported very high acceptability in face-to-face interviews throughout the trial. Such
31
32 384 interviews are known to suffer from social desirability bias. However, women seemed to speak freely
33
34 385 in the FGDs, and those data indicate that they did not have major issues with product attributes or
35
36 386 insertion. However, some women reported difficulties due to lack of male partner support. The
37
38 387 reported increase in vaginal wetness after probiotic insertion was not considered problematic, as
39
40 388 lubrication during sex is preferred by most Rwandan men and women.[24] This might be different in
41
42 389 other countries where dry sex is preferred.[25] We did find a non-significant lower adherence to
43
44 390 GynLP compared to EF+, which might be explained by differences in formulation: GynLP forms a
45
46 391 gel in the vagina whereas EF+ capsules merely release lyophilised bacteria. Previous research
47
48 392 indicated high adherence to GynLP.[26] Unfortunately, the impact of these formulation differences
49
50 393 was insufficiently probed during the FGDs; the impact of product formulation on acceptability and
51
52 394 adherence should be investigated in future clinical trials. Participants indicated that they found the
53
54 395 diary cards helpful in reminding them to use their products, and we believe that self-monitoring tools
55
56 396 might indeed be helpful in maximising adherence and therefore recommend them for use in future
57
58 397 studies.[27]
59
60

1
2
3 398 Our data suggest that counselling was partially effective in changing behaviours that increase BV risk.
4
5 399 While these results are encouraging, it is difficult to assess to what extent they were influenced by
6
7 400 social desirability bias. Significantly more women reported not engaging in vaginal practices at the
8
9 401 end of the study, and most women with uncircumcised steady male partners reported having discussed
10
11 402 penile hygiene with them. However, many women mentioned in FGDs that they found it difficult to
12
13 403 discuss condom use and penile hygiene with male partners, especially clients. Women reduced their
14
15 404 sexual risks only to a limited extent during follow-up, reporting a reduction in numbers of sex partners
16
17 405 but no differences in engaging in sex work and condom use in face-to-face interviews. We did not ask
18
19 406 women to what extent they depended on sex work for subsistence. Women who only partially depend
20
21 407 on sex work may find it easier to negotiate with male partners.
22
23
24 408

26 409 Two probiotics-related themes that emerged from the stakeholders consultations that had not been
27
28 410 raised by the study participants were uncertainty about long-term side effects (women in the pilot trial
29
30 411 used the products for only two months) and whether probiotic bacteria (in this case lactobacilli) could
31
32 412 also be delivered orally instead of vaginally. We have since conducted a systematic review, which
33
34 413 showed that long-term safety of vaginal probiotics has not yet been evaluated.[28]
35
36
37 414

39 415 Our survey with women at recruitment sessions and enrolment visits showed that high-risk Rwandan
40
41 416 women had heard of several STIs, but were generally unaware of BV, its causes and potential
42
43 417 consequences, and what they can do to prevent it. Experiences with HIV show that public health
44
45 418 interventions can only succeed if health care professionals and the public have sufficient knowledge
46
47 419 of causes and consequences of disease.[29–31] High-risk Rwandan women (and health care
48
49 420 professionals) should therefore be educated about BV, and vaginal probiotics studies should include
50
51 421 counselling for all participants on vaginal diseases and how to prevent them.
52
53
54 422

56 423 **Limitations**

58 424 Our study had limited statistical power, and social desirability bias may have affected some of our
59
60 425 results, as is often the case in studies of this nature. Additionally, it should be noted that product

1
2
3 426 efficacy, availability and cost are important determinants of acceptability, and were not evaluated in
4
5 427 our study, although preliminary efficacy results in this study were promising.[17] We could not
6
7 428 directly compare experiences with, and opinions about, the two different vaginal probiotics because
8
9 429 each woman used only one product and qualitative data depth was suboptimal. In the FGDs/IDIs, it
10
11 430 was sometimes difficult to ascertain whether participants were referring to personal experiences, or to
12
13 431 wider community perceptions. Strengths of our study include the use of a mixed-methods approach
14
15 432 and triangulated adherence data.
16
17

18 433

19 434 **CONCLUSIONS**

20
21
22 435 The prevention of BV recurrence will likely have to include several components to be successful,
23
24 436 such as improved diagnostics, treatments, and prophylactic products (for example probiotics), but also
25
26 437 improved information, education, and counselling messages targeted to at-risk women and their
27
28 438 partners. The results of this study can be used to inform future product development, and to fine-tune
29
30 439 counselling messages in future trials.
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 440 **Acknowledgments** We thank the study participants, the Rinda Ubuzima team and other colleagues in
4
5 441 Rwanda and the United Kingdom who contributed, the funders of this study, as well as the Trial
6
7 442 Steering Committee for trial oversight. We thank Biose and Winlove for the donation of study
8
9 443 products.
10

11 444
12
13 445 **Author contributions** JvdW obtained the research funding and wrote the study protocol and data
14
15 446 collection documents. AN, EL, SA, and JvdW were members of the Trial Steering Committee. SA,
16
17 447 MU, MMU, and JvdW collected the primary data. MU and MMU performed the FGDs and IDIs. MV
18
19 448 and JvdW developed the analytical approach and performed the statistical analyses. MV and JvdW
20
21 449 wrote the manuscript. All authors commented on and approved the final manuscript.
22
23

24 450
25
26 451 **Funding** This work was funded by the DFID/MRC/Wellcome Trust Joint Global Health Trials
27
28 452 Scheme as a Development Project (grant reference MR/M017443/1; grant title: “Preparing for a
29
30 453 clinical trial of interventions to maintain normal vaginal microbiota for preventing adverse
31
32 454 reproductive health outcomes in Africa”). Vaginal probiotics for use in the trial were donated free of
33
34 455 charge by Winlove Probiotics (Amsterdam, The Netherlands) and Biose (formerly Probionov;
35
36 456 Aurillac, France). The findings and conclusions in this paper are those of the authors and do not
37
38 457 necessarily represent the views of the authors’ institutions or companies, or the funder. None of the
39
40 458 authors were paid to write this article. The corresponding author had full access to the data and had
41
42 459 final responsibility for the decision to submit for publication.
43
44

45 460
46
47 461 **Competing interests** AN is employed by Biose (owner of trial product GynLP) and EL by Winlove
48
49 462 Probiotics BV (owner of trial product EF+). AN has financial and/or intellectual investments in
50
51 463 competing products. The other authors report no competing interests.
52

53 464
54
55 465 **Patient consent** All participants provided written informed consent for study participation, and
56
57 466 separate written informed consent for participation in FGDs/IDIs.
58
59
60

1
2
3 467 **Ethics approval** The study was sponsored by the University of Liverpool, approved by the Rwanda
4
5 468 National Ethics Committee and the University of Liverpool Research Ethics Subcommittee for
6
7 469 Physical Interventions, and registered on ClinicalTrials.gov (NCT02459665).
8
9
10 470

11 471 **Data sharing statement** The data supporting the findings of this publication are retained by the
12
13 472 corresponding author (JvdW) and will not be made openly accessible due to privacy concerns. Fully
14
15 473 anonymised data can be made available by written request to j.vandewijgert@liverpool.ac.uk after
16
17 474 assurance that the intended data usage is compliant with relevant ethical approvals and privacy will be
18
19
20 475 maintained.
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 476 **Figure footnotes**
4
5 477

6
7 478 **Figure 1: Flowchart of the study**
8

9 479 *Totals to 110 reasons among 102 women because there could be more than one reason per woman.

10 480 †Reasons: outside of metronidazole treatment window (n=5), enrolment target already met (n=4), has a mental disorder (n=1), did not
11 481 complete screening procedures and was subsequently lost to follow-up (n=1), withdrew consent during the screening visit because she
12 482 thought the reimbursement was too low (n=1).

13 483 ‡Reasons: moved away from Kigali (n=2), lost interest because symptoms resolved (n=1), and was verbally harassed by partner and sister
14 484 about study participation (n=1).

15 485
16 486 Acceptability assessments were made at enrolment and at the M2 visit. Adherence assessments were made using self-rated assessments,
17 487 pictorial diary cards, and returned packaging at the D7, M1, and M2 visits (after which product use was ceased). The vaginal infection
18 488 knowledge survey was held at recruitment sessions in the community and at the enrolment visit. Changes in sexual risk-taking and vaginal
19 489 practices were assessed at each follow-up visits and compared to answers given during the enrolment visit. All of these themes were
20 490 discussed during the eight FGDs and IDIs.
21 491

22 492 BV, bacterial vaginosis; D7, day 7 visit; FGD, focus group discussion; IDI, in-depth interview; M1/2/6, month 1/2/6 visit; RU, Rinda
23 493 Ubuzima; TV, *Trichomonas vaginalis*.

494 **References**

- 495 1 van de Wijgert JHHM, Borgdorff H, Verhelst R, *et al.* The vaginal microbiota: what have
496 we learned after a decade of molecular characterization? *PLOS ONE* 2014;**9**:e105998.
497 doi:10.1371/journal.pone.0105998
- 498 2 van de Wijgert JHHM, Morrison CS, Cornelisse PGA, *et al.* Bacterial vaginosis and
499 vaginal yeast, but not vaginal cleansing, increase HIV-1 acquisition in African women. *J*
500 *Acquir Immune Defic Syndr* 1999 2008;**48**:203–10. doi:10.1097/QAI.0b013e3181743936
- 501 3 van de Wijgert JHHM. The vaginal microbiome and sexually transmitted infections are
502 interlinked: consequences for treatment and prevention. *PLOS Med* 2017;**14**:e1002478.
503 doi:10.1371/journal.pmed.1002478
- 504 4 Li J, McCormick J, Bocking A, *et al.* Importance of vaginal microbes in reproductive
505 health. *Reprod Sci* 2012;**19**:235–42. doi:10.1177/1933719111418379
- 506 5 Nelson DB, Hanlon AL, Wu G, *et al.* First trimester levels of BV-associated bacteria and
507 risk of miscarriage among women early in pregnancy. *Matern Child Health J*
508 2015;**19**:2682–7. doi:10.1007/s10995-015-1790-2
- 509 6 Hay P. Recurrent bacterial vaginosis. *Curr Opin Infect Dis* 2009;**22**:82–6.
510 doi:10.1097/QCO.0b013e32832180c6
- 511 7 Verstraelen H, Verhelst R. Bacterial vaginosis: an update on diagnosis and treatment.
512 *Expert Rev Anti Infect Ther* 2009;**7**:1109–24. doi:10.1586/eri.09.87
- 513 8 Torrone EA, Morrison CS, Chen P-L, *et al.* Prevalence of sexually transmitted infections
514 and bacterial vaginosis among women in sub-Saharan Africa: An individual participant
515 data meta-analysis of 18 HIV prevention studies. *PLOS Med* 2018;**15**:e1002511.
516 doi:10.1371/journal.pmed.1002511
- 517 9 Bradshaw CS, Brotman RM. Making inroads into improving treatment of bacterial
518 vaginosis – striving for long-term cure. *BMC Infect Dis* 2015;**15**:292.
519 doi:10.1186/s12879-015-1027-4
- 520 10 Anukam KC, Osazuwa E, Osemene GI, *et al.* Clinical study comparing probiotic
521 *Lactobacillus* GR-1 and RC-14 with metronidazole vaginal gel to treat symptomatic
522 bacterial vaginosis. *Microbes Infect* 2006;**8**:2772–6. doi:10.1016/j.micinf.2006.08.008
- 523 11 Ling Z, Liu X, Chen W, *et al.* The restoration of the vaginal microbiota after treatment
524 for bacterial vaginosis with metronidazole or probiotics. *Microb Ecol* 2013;**65**:773–80.
525 doi:10.1007/s00248-012-0154-3
- 526 12 Ngugi BM, Hemmerling A, Bukusi EA, *et al.* Effects of bacterial vaginosis-associated
527 bacteria and sexual intercourse on vaginal colonization with the probiotic *Lactobacillus*
528 *crispatus* CTV-05. *Sex Transm Dis* 2011;**38**:1020–7.
529 doi:10.1097/OLQ.0b013e3182267ac4
- 530 13 Bradshaw CS, Pirota M, De Guingand D, *et al.* Efficacy of oral metronidazole with
531 vaginal clindamycin or vaginal probiotic for bacterial vaginosis: randomised placebo-

- 1
2
3 532 controlled double-blind trial. *PLOS ONE* 2012;**7**:e34540.
4 533 doi:10.1371/journal.pone.0034540
5
6
7 534 14 van der Straten A, Montgomery ET, Cheng H, *et al.* High acceptability of a vaginal ring
8 535 intended as a microbicide delivery method for HIV prevention in African women. *AIDS*
9 536 *Behav* 2012;**16**:1775–86. doi:10.1007/s10461-012-0215-0
10
11 537 15 Merkatz RB, Plagianos M, Hoskin E, *et al.* Acceptability of the nectorone®/ethinyl
12 538 estradiol contraceptive vaginal ring: development of a model; implications for
13 539 introduction. *Contraception* 2014;**90**:514–21. doi:10.1016/j.contraception.2014.05.015
14
15 540 16 Liu CM, Hungate BA, Tobian AAR, *et al.* Penile microbiota and female partner bacterial
16 541 vaginosis in Rakai, Uganda. *mBio* 2015;**6**:e00589. doi:10.1128/mBio.00589-15
17
18 542 17 van de Wijgert J, Verwijs MC, Agaba SK, *et al.* Intermittent lactobacilli-containing
19 543 vaginal probiotic or metronidazole use to prevent bacterial vaginosis recurrence: safety
20 544 and preliminary efficacy by microscopy and sequencing. *MedRxiv* Published Online
21 545 First: 8 July 2019. doi:10.1101/19001156
22
23
24 546 18 Sobel JD, Ferris D, Schwebke J, *et al.* Suppressive antibacterial therapy with 0.75%
25 547 metronidazole vaginal gel to prevent recurrent bacterial vaginosis. *Am J Obstet Gynecol*
26 548 2006;**194**:1283–9. doi:10.1016/j.ajog.2005.11.041
27
28
29 549 19 McClelland RS, Richardson BA, Hassan WM, *et al.* Improvement of vaginal health for
30 550 Kenyan women at risk for acquisition of Human Immunodeficiency Virus type 1: results
31 551 of a randomized trial. *J Infect Dis* 2008;**197**:1361–8. doi:10.1086/587490
32
33 552 20 Kestelyn E, van Nuil JI, Umulisa MM, *et al.* High acceptability of a contraceptive vaginal
34 553 ring among women in Kigali, Rwanda. *PLOS ONE* 2018;**13**:e0199096.
35 554 doi:10.1371/journal.pone.0199096
36
37
38 555 21 World Health Organization. Guidelines for the management of sexually transmitted
39 556 infections. 2003.<http://applications.emro.who.int/aiecf/web79.pdf> (accessed 12 Mar
40 557 2019).
41
42 558 22 Binagwaho A, editor. *National guidelines for prevention and management of HIV, STIs*
43 559 *& other blood borne infections*. Republic of Rwanda - Ministry of Health 2013.
44 560 https://aidsfree.usaid.gov/sites/default/files/hts_policy_rwanda.pdf (accessed 12 Mar
45 561 2019).
46
47
48 562 23 McClelland RS, Balkus JE, Lee J, *et al.* Randomized trial of periodic presumptive
49 563 treatment with high-dose intravaginal metronidazole and miconazole to prevent vaginal
50 564 infections in HIV-negative women. *J Infect Dis* 2015;**211**:1875–82.
51 565 doi:10.1093/infdis/jiu818
52
53 566 24 Veldhuijzen N, Nyinawabega J, Umulisa M, *et al.* Preparing for microbicide trials in
54 567 Rwanda: focus group discussions with Rwandan women and men. *Cult Health Sex*
55 568 2006;**8**:395–406. doi:10.1080/13691050600859302
56
57
58 569 25 Low N, Chersich MF, Schmidlin K, *et al.* Intravaginal practices, bacterial vaginosis, and
59 570 HIV infection in women: individual participant data meta-analysis. *PLOS Med*
60 571 2011;**8**:e1000416. doi:10.1371/journal.pmed.1000416

- 1
2
3 572 26 Dausset C, Patrier S, Gajer P, *et al.* Comparative phase I randomized open-label pilot
4 573 clinical trial of Gynophilus® (Lcr regenerans®) immediate release capsules versus slow
5 574 release muco-adhesive tablets. *Eur J Clin Microbiol Infect Dis* 2018;**37**:1869–80.
6 575 doi:10.1007/s10096-018-3321-8
7
8
9 576 27 Safren SA, W. Otto M, Worth JL, *et al.* Two strategies to increase adherence to HIV
10 577 antiretroviral medication: Life-Steps and medication monitoring. *Behav Res Ther*
11 578 2001;**39**:1151–62. doi:10.1016/S0005-7967(00)00091-7
12
13 579 28 van de Wijgert JHHM, Verwijs MC. Lactobacilli-containing vaginal probiotics to cure or
14 580 prevent bacterial or fungal vaginal dysbiosis: a systematic review and recommendations
15 581 for future trial designs. *BJOG*;0. doi:10.1111/1471-0528.15870
16
17 582 29 Glick P, Sahn DE. Changes in HIV/AIDS knowledge and testing behavior in Africa: how
18 583 much and for whom? *J Popul Econ* 2007;**20**:383–422. doi:10.1007/s00148-006-0085-8
19
20 584 30 Peltzer K, Matseke G, Mzolo T, *et al.* Determinants of knowledge of HIV status in South
21 585 Africa: results from a population-based HIV survey. *BMC Public Health* 2009;**9**:174.
22 586 doi:10.1186/1471-2458-9-174
23
24 587 31 Fay H, Baral SD, Trapence G, *et al.* Stigma, health care access, and HIV knowledge
25 588 among men who have sex with men in Malawi, Namibia, and Botswana. *AIDS Behav*
26 589 2011;**15**:1088–97. doi:10.1007/s10461-010-9861-2
27
28
29
30 590
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

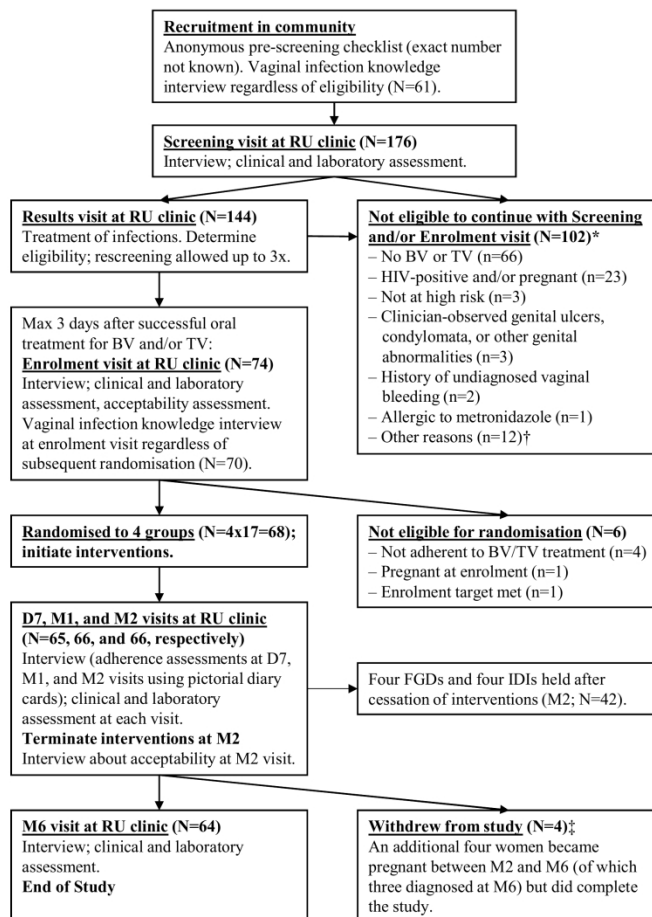


Figure 1: Flowchart of the study

*Totals to 110 reasons among 102 women because there could be more than one reason per woman.

†Reasons: outside of metronidazole treatment window (n=5), enrolment target already met (n=4), has a mental disorder (n=1), did not complete screening procedures and was subsequently lost to follow-up (n=1), withdrew consent during the screening visit because she thought the reimbursement was too low (n=1).

‡Reasons: moved away from Kigali (n=2), lost interest because symptoms resolved (n=1), and was verbally harassed by partner and sister about study participation (n=1).












Acceptability assessments were made at enrolment and at the M2 visit. Adherence assessments were made using self-rated assessments, pictorial diary cards, and returned packaging at the D7, M1, and M2 visits (after which product use was ceased). The vaginal infection knowledge survey was held at recruitment sessions in the community and at the enrolment visit. Changes in sexual risk-taking and vaginal practices were assessed at each follow-up visits and compared to answers given during the enrol visit. All these themes

1
2
3 were discussed during the eight FGDs and IDIs.
4

5 BV, bacterial vaginosis; D7, day 7 visit; FGD, focus group discussion; IDI, in-depth interview; M1/2/6,
6 month 1/2/6 visit; RU, Rinda Ubuzima; TV, *Trichomonas vaginalis*.

7
8 215x279mm (300 x 300 DPI)
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Supplementary Figure 1: Pictorial diary card

Date/Month	Descriptions	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Indicate each time you used study product								
	Used study product							
 Indicate each sex act 								
	Sex with condom							
	Sex without condom							
 Indicate each time you washed/inserted something inside the vagina other than study product 								
 By washing inside, we mean inserting an entire finger inside the vaginal canal 								
	Washed inside vagina with water only							
	Washed inside vagina with soap and water							
	Inserted something else (herbs, powders, etc.)							
Indicate each day of menstrual bleeding								
	Had menstrual bleeding							

The picture provided is the English translation of the pictorial card; participants received a version in Kinyarwanda.

Supplementary Table 1: Baseline characteristics of enrolled population

	Controls (n=17)	Metronidazole (n=17)	EF+ (n=17)	GynLP (n=17)
Median age (IQR)	29 (24–36)	30 (27–34)	33 (28–35)	30 (27–35)
Marital status n (%)				
- Never married	16 (94.1)	11 (64.7)	10 (58.8)	13 (76.5)
- Married	1 (5.9)	1 (5.9)	2 (11.8)	1 (5.9)
- Divorced	0	5 (29.4)	4 (23.5)	3 (17.6)
- Widowed	0	0	1 (5.9)	0
Education level n (%)				
- No schooling	5 (29.4)	3 (17.6)	3 (17.6)	3 (17.7)
- Primary school not completed	7 (41.2)	7 (41.2)	13 (76.5)	4 (23.5)
- Primary school completed	4 (23.5)	5 (29.4)	1 (5.9)	7 (41.2)
- At least some secondary school	1 (5.9)	2 (11.8)	0	3 (17.7)
Median number of sex partners last month (IQR)	5 (3–20)	5 (2–10)	3 (2–15)	3 (2–20)
Exchanged sex for money/goods past month n (%)	17 (100)	14 (82.4)	15 (88.2)	17 (100)
At least one laboratory-confirmed STI* n (%)	8 (47.1)	8 (47.1)	4 (23.5)	9 (52.9)
Median weekly frequency of washing body (IQR)	7 (7–7)	7 (7–7)	7 (7–7)	7 (4–7)
Ever washing the genitalia n (%)				
- Yes, outside only	12 (70.7)	14 (82.4)	15 (88.3)	14 (82.3)
- Yes, both inside and outside	5 (29.4)	3 (17.6)	2 (11.7)	3 (17.7)
- Yes, inside only	0	0	0	0
If reports washing inside, median weekly frequency (IQR)	14 (7–16)	14 (14–14)	11 (7–14)	7 (3–12)

*Chlamydia, gonorrhoea, and/or syphilis.

EF+, Ecologic Femi+; Enr, enrolment visit; GynLP, Gynophilus LP; IQR, inter-quartile range; M2, Month 2 visit; STI, sexually transmitted disease.

Supplementary Table 2: Acceptability of interventions

Acceptability of study products at Enr	Controls (n=17)	Metronidazole (n=17)	EF+ (n=17)	GynLP (n=17)
Nurse reports having explained intervention to participant in detail n (%)	17 (100)	17 (100)	17 (100)	17 (100)
Nurse reports participant asked questions n (%)*				
- Yes, a few	6 (35.3)	2 (11.8)	11 (64.7)	11 (64.7)
- Yes, many	0	0	0	2 (11.8)
First dose applied† under supervision n (%)	NA	17 (100)	17 (100)	17 (100)
Median number of attempts participant made until successful application (IQR)	NA	NA	1 (1–1)	1 (1–1)
Participant seemed comfortable with the insertion after these attempts, according to study nurse n (%)	NA	NA		
- Yes, very			17 (100)	16 (94.1)
- Yes, somewhat			0	1 (5.9)
Acceptability of study products at M2				
Self-reported usual time of insertion n (%)				
- Before going to sleep	NA	NA	17 (100)	15 (100)‡
- After bathing in the morning			0	0
Level of comfort with vaginal insertion after 2 months of use, self-reported n (%)				
- Very comfortable	NA	NA	17 (100)	15 (100)‡
- Somewhat comfortable			0	0
Reported insertion becoming easier over time n (%)	NA	NA	17 (100)	15 (100)‡
Reported manner of insertion§ n (%)				
- While lying down	NA	NA	17 (100)	14 (93.3)‡
- While squatting			1 (5.9)	1 (6.7)
Acceptability of penile hygiene intervention at M2				
Reports having told main sex partner to regularly clean the penis, including underneath the foreskin n (%)¶				
- Yes	3 (17.7)	3 (18.8)	3 (17.6)	3 (18.8)
- No, because he is circumcised	10 (58.8)	9 (56.2)	6 (35.3)	5 (31.3)
- No, other reason	1 (5.9)	0	1 (5.9)	1 (6.3)
If yes, response by the main partner n (%)				
- He said that he would do so in the future	2 (66.7)	1 (33.3)	1 (33.3)	1 (33.3)
- He said that he already does this	1 (33.3)	1 (33.3)	0	1 (33.3)
- He said that he is not interested	0	1 (33.3)	2 (66.7)	1 (33.3)

*One missing value.

†Whether oral insertion (oral metronidazole group) or vaginal insertion (Ecologic Femi+ and Gynophilus LP groups).

‡N=15 due to participants withdrawing informed consent.

§Multiple answers possible; hence totals can be more than 100%.

¶Women with no main sex partner not included.

||N=3 in all four groups.

EF+, Ecologic Femi+; Enr, enrolment visit; GynLP, Gynophilus LP; IQR, inter-quartile range; M2, Month 2 visit; NA, Not applicable.

STROBE Statement—Checklist of items that should be included in reports of *cohort studies*

	Item No	Recommendation	Reported on page #
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1
		(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	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	4,5
		(b) For matched studies, give matching criteria and number of exposed and unexposed	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	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	6
Bias	9	Describe any efforts to address potential sources of bias	6
Study size	10	Explain how the study size was arrived at	NA (Primary outcomes paper)
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6
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	6,7
		(c) Explain how missing data were addressed	NA (indicated in tables if missing)
		(d) If applicable, explain how loss to follow-up was addressed	NA
		(e) Describe any sensitivity analyses	NA
Results			
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	Page 8, Figure 1
		(b) Give reasons for non-participation at each stage	8, Figure 1
		(c) Consider use of a flow diagram	Figure 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8
		(b) Indicate number of participants with missing data for each variable of	8 (missing

		interest	data in footnotes in table, if applicable)
		(c) Summarise follow-up time (eg, average and total amount)	8
Outcome data	15*	Report numbers of outcome events or summary measures over time	9
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	Table 1
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Page 12 and beyond
Discussion			
Key results	18	Summarise key results with reference to study objectives	Page 18
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	Page 19
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Page 18, 19
Generalisability	21	Discuss the generalisability (external validity) of the study results	Page 18, 19
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	Page 21

*Give information separately for exposed and unexposed groups.

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 <http://www.strobe-statement.org>.