**Lactobacilli-containing vaginal probiotics to cure or prevent bacterial or fungal vaginal dysbiosis: a systematic review and recommendations for future trial designs**

Janneke H.H.M. van de Wijgert1,2 and Marijn C. Verwijs1

**Affiliations:**

1. Institute of Infection and Global Health, University of Liverpool, Ronald Ross Building, 8 West Derby Street, Liverpool L69 7BE, UK
2. Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht University, Stratenum Huispost nr STR 6.131, PO Box 85 500, 3508 GA Utrecht, The Netherlands

**Correspondence to:**

Professor Janneke van de Wijgert, MD PhD MPH

Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht University, Stratenum Huispost nr STR 6.131, PO Box 85 500, 3508 GA Utrecht, The Netherlands

ORCID: 0000-0003-2728-4560

E-mail: [j.vandewijgert@liverpool.ac.uk](mailto:j.vandewijgert@liverpool.ac.uk)

**Running title:** Systematic review of vaginal probiotics

**Word counts:**

Abstract: 250 words (max 250)

Text: words 3,500 (max 3,500)

Tables/Figures: 5

**Abstract**

**Background:** Vaginal probiotics claiming to cure and/or prevent bacterial and/or fungal vaginal dysbiosis are available on the market but did, until recently, not have to be approved as drugs for human use.

**Objectives:** We evaluated the impact of vaginal probiotics on bacterial vaginosis (BV) and vulvovaginal candidiasis (VVC) cure and/or recurrence, as well as vaginal microbiota (VMB) composition and vaginal detection of probiotic strains.

**Search strategy:** We performed a systematic literature search in MEDLINE and Embase up to 15 January 2019.

**Selection criteria:** There were no restrictions in probiotic strains/formulations, study populations, and designs. BV had to be diagnosed by Nugent or Ison-Hay Gram stain scoring, VVC by culture, wet mount, or PCR, and VMB composition/detection by molecular techniques.

**Data collection and analysis:** The authors independently extracted data.

**Main results:** All 22 vaginal probiotics evaluated in the 34 eligible studies contained *Lactobacillus* strains, and some contained additional active ingredients. The probiotics hold promise for BV cure and prevention, but much less so for VVC cure and prevention. No major safety concerns were reported in any of the studies. Vaginal detection of probiotic strains never lasted long beyond the dosing period, suggesting that they did not colonise the vagina. However, findings are not definitive because heterogeneity was high and the quality of most studies suboptimal.

**Conclusions:** Availability of vaginal probiotics for vaginal health indications will likely decline in 2020 because of regulatory changes. We urge the field to invest in clinical evidence-based product development, and to conduct future trials more rigorously.

**Funding:** None.

**Prospero registration:** CRD42017075717.

**Keywords:** Probiotics, live biotherapeutic products, lactobacilli, bacterial vaginosis, vulvovaginal candidiasis, vaginal microbiota.

**Tweetable abstract:** Lactobacilli-containing vaginal probiotics hold promise for bacterial vaginosis cure and prevention, but not for vulvovaginal candidiasis.

**Introduction**

Understanding of the vaginal microbiota (VMB) has advanced significantly in the last two decades due to the increased availability of next generation sequencing.1 Optimal VMBs are dominated by lactobacilli, which do not cause immune activation or inflammation, and are not associated with adverse outcomes such as HIV acquisition and preterm birth.2 Of the five most common lactobacilli in the vaginal niche (*Lactobacillus crispatus*, *L. iners*, *L. gasseri*, *L. jensenii*, and *L. vaginalis*), *L. crispatus*-domination is most efficient at keeping bacterial vaginosis (BV)-associated anaerobes at bay. *L. crispatus* produces more lactic acid than the other lactobacilli, and produces several other antimicrobial compounds.3

BV is the most common bacterial vaginal dysbiosis, and is usually characterised by a highly diverse community of anaerobes including *Gardnerella vaginalis*, but sometimes by *G. vaginalis*-domination.1 *G. vaginali*s is thought to play an important role in biofilm formation, and *G. vaginalis*-domination may indicate the presence of a biofilm.4 Vaginal dysbiosis by bacterial pathobionts with a higher pathogenicity potential than BV-anaerobes (such as streptococci, staphylococci, enterococci, and Enterobacteriaceae) is much less common, but clinically relevant.2 Almost all cases of fungal vaginal dysbiosis are caused by *Candida* species, predominantly *C. albicans*.2 In contrast to BV-anaerobes, bacterial pathobionts and *Candida* species often co-exist with lactobacilli in the VMB, perhaps because they can tolerate high concentrations of lactic acid.5

This increased VMB knowledge has led to a renewed interest in using exogenous lactobacilli (probiotics or live biotherapeutic products)6 to optimise the VMB as part of curative or preventive vaginal dysbiosis interventions. There are multiple reasons for this: antibiotic BV treatment (with oral or vaginal metronidazole or clindamycin) and antifungal treatment of vulvovaginal candidiasis (VVC; with oral or vaginal –azoles) result in suboptimal cure fractions and high recurrence rates;7 BV biofilms are not penetrated sufficiently by antibiotics;4 and prolonged use increases the probability of side effects and drug resistance. Possible mechanisms by which probiotic strains might exert positive effects include niche occupation so that other bacteria cannot expand and biofilms cannot be established, increased local production or release of lactic acid and other antimicrobial compounds, modulation of local cervicovaginal mucosal immune responses, and/or inhibition of *C. albicans* hyphae formation.3,8,9

We performed a systematic review to examine the impact of vaginally-applied lactobacilli-containing probiotics on the VMB in clinical studies. We also offer recommendations for future trial designs based on this review and our own experiences.10

**Methods**

The systematic review focussed on BV and VVC cure and/or recurrence as defined by conventional diagnostic methods, as well as VMB composition and vaginal detection of probiotic strains as assessed by molecular methods. It was conducted according to the PRISMA 200911 guidelines and registered on PROSPERO (CRD42017075717).12 Patients or the public were not involved in this review and a core outcome set was not available. We searched MEDLINE13 and Embase14 up to 15 January 2019 (search terms in Appendix S1), and also included a trial completed by our group that had been fully analysed, and presented at an international conference, but not yet published, on that date.10 No filters were used except for ‘English language’. Duplicates were manually removed. Two researchers independently screened titles and abstracts (with JvdW as tiebreaker) and additional articles were identified by screening the reference lists of selected articles, relevant reviews, and expert opinion pieces. The corresponding author of a conference abstract was contacted but this did not yield sufficient information for inclusion. We included studies of any vaginally-applied probiotic performed in sexually active women regardless of menopausal, pregnancy, or vaginal dysbiosis status. We excluded two studies that assessed yoghurt because the bacteria present in the yoghurt were not specified. All study designs and probiotic use schedules (with or without additional oral or vaginal antibiotic or antifungal treatment) were allowed. BV had to be diagnosed by Nugent15 or Ison-Hay16 Gram stain scoring or the bacterial VMB comprehensibly assessed by molecular methods. Studies using Amsel criteria, bacterial culture, other forms of Gram stain scoring (*Lactobacillus* counts only), rapid tests, or participant-reported symptoms and/or clinician-observed signs only, or with unclear diagnostic procedures, were excluded. VVC had to be diagnosed by wet mount, culture, or *Candida* polymerase chain reaction (PCR). Studies using participant-reported symptoms and/or clinician-observed signs only or with unclear diagnostic procedures were excluded.

Both authors extracted data from all selected articles and discussed their findings. Each article was assessed for risk of bias as described in the Cochrane Handbook for Systematic Reviews of Interventions.17 We also determined an overall risk of bias per study. Articles for which all potential biases (selection, performance, detection/ ascertainment, attrition, reporting, and ‘other’ bias, with the latter including confounding) were assessed as low risk were judged as ‘overall low risk of bias’, those with a maximum of two high or unclear risks of bias as ‘overall medium risk of bias’, and those with three or more high or unclear risks of bias as ‘overall high risk of bias’. Articles were not excluded based on risk of bias assessments. Risk of bias plots were made using RevMan 5.3.5 (The Cochrane Collaboration, Copenhagen, Denmark). If no statistical testing was reported, but absolute numbers of cases per exposure group were reported, we performed Chi-squared testing (cross-sectional comparisons between groups) or McNemar’s testing (pre-post within-arm comparisons) using Stata 13 (StataCorp, College Station, USA). The full data extraction database can be requested from the corresponding author by email.

**Results**

The search identified 2,949 unique articles but only 34 studies were eligible (flow diagram in Figure 1).10,18–50 Thirteen studies reported 14 results on BV and/or molecular VMB composition (Table 1),10,18–29 12 studies reported 12 results on VVC (Table 2),29–41 and 14 studies reported 15 results on vaginal probiotic strain detection using molecular techniques (Table S1).10,26,27,30,39,42–50 The selected studies evaluated 22 different vaginal probiotics (Table S2). Gynoflor (*L. acidophilus* KS400 plus 30 μg oestriol; three studies with 359 participants),32,33,39,47 Lactin-V (*L. crispatus* CTV-05; four studies with 132 participants),30,31,46,56–58 and *L. reuteri* RC-14 with *L. rhamnosus* GR-1 (four studies with 47 participants)23,26,42,44 were most studied. The majority of probiotics contained 108-1010 colony-forming units per dose, and all contained lactobacilli as an active ingredient. Additional active ingredients included oestriol (Gynoflor), *Streptococcus thermophilus* (Lactagyn, Femilac), *Bifidobacterium bifidum* (Ecologic Femi+), *Pediococcus acidilactici* (Ellen capsules), and acidifying agents (Kramegin, Florisia, ActiCand 30, and one unnamed probiotic).

*BV and molecular VMB composition outcomes*

Of the 13 studies with BV and/or molecular VMB composition as outcome, five were judged to have medium, and eight high, overall risk of bias (Figure 2). Most studies (11/13) were randomised controlled trials, including six with placebo controls,18,20–22,26,28 two with ‘no intervention’ controls,10,19 four with metronidazole or clindamycin controls,10,21,23,25 and one with a vaginal pH lowering tablet control24 (two trials included both placebo/‘no intervention’ and antibiotic controls10,21) (Table 1). Five clinical trials were judged medium risk10,18–21 and the other six high risk.22–29 The two remaining studies (both judged high risk) were pre-post intervention studies in women receiving a vaginal probiotic without an antibiotic.27,29

Nine of the 14 main results reported in the 13 studies were on BV cure or post-treatment Nugent score improvement (2/9 after initial antibiotic treatment and 7/9 without antibiotic use), nine on BV recurrence (6/9 and 3/9, respectively), and four on BV cure and recurrence (Table 1). Most studies showed beneficial effects of vaginal probiotic interventions for both BV cure and recurrence, and the distribution of results did not differ significantly between studies judged to be of medium or high risk. Of the seven randomised controlled trials that evaluated BV cure, four showed a significant increase,19,20,23,25 one a non-significant increase,26 and two no increase in BV cure fraction compared to controls when assessed immediately to five weeks after probiotic use cessation.18,24 One of the two studies with negative BV cure results went on to show significantly reduced BV recurrence after continued use during the next four menstrual cycles,18 and the other one used a pH-lowering tablet as the control product (the cure fractions were less than 50% in both groups).24 The two pre-post intervention studies enrolled women with a range of baseline Nugent scores and showed significantly higher proportions of women with Nugent 0-3 immediately after probiotic use cessation, which lasted up to 21-28 days post-treatment.27,29 Of the nine BV recurrence results in eight randomised controlled trials, six were significant reductions,10,18,20,23,25,28 two non-significant reductions,10,21 and one no reduction.22 The one study that showed no reduction in BV recurrence included women who used the vaginal probiotic during menses only.22 All except one of the other studies avoided probiotic use during menses, and in the one study that did not, women used probiotics in-between and during menses.10 The two medium risk randomised controlled trials that compared vaginal probiotic use after initial antibiotic treatment to placebo or no-intervention controls as well as prolonged antibiotic use controls both showed that prolonged antibiotic use was more efficacious in reducing BV recurrence than probiotic use.10,21

*VVC outcomes*

Of the 12 studies with VVC as outcome, one was judged to have medium, and 11 high, overall risk of bias (Figure 2). Six of 12 studies were pre-post intervention studies,29,31,33,35,37,38,41 two were non-randomised cohort studies with parallel comparison groups (one with a placebo group36 and one comparing fluconazole plus probiotic to fluconazole only39), and four were randomised controlled trials (two with a placebo group,30,32 one with a ‘no intervention’ group,40 and one comparing itraconazole plus probiotic to itraconazole only34) (Table 2). The one medium risk study was a randomised placebo-controlled trial.30

Four of the 12 probiotic studies with a VVC outcome reported on VVC cure (all without any antifungal use), eight on VVC recurrence or incidence (2/8 with concurrent antifungal use, 2/8 after antifungal use, 2/8 after antibiotic treatment for another indication and no antifungal use, and 2/8 without any antifungal or antibiotic use), and two on both (Table 2). The four studies that reported on VVC cure were pre-post intervention studies in women who received probiotic treatment in the absence of antifungal treatment.29,33,35,41 They reported cure fractions ranging from 57-100% after therapy durations ranging from six days to two months, but VVC recurrence beyond one month after treatment cessation was not assessed. The four studies that assessed VVC recurrence after combined probiotic/antifungal treatment showed mixed results: two studies that compared antifungal plus probiotic use with antifungal use alone showed no difference in VVC recurrence between the groups,34,39 and two studies that compared probiotic after antifungal use with either placebo after antifungal use36 or antifungal use alone40 showed significantly lower VVC recurrence. One of the two studies that assessed VVC incidence after antibiotic use for a non-VVC indication showed reduced VVC incidence31 and the other one did not.32 Finally, the two studies in women who used a vaginal probiotic in the absence of antifungal or antibiotic therapy showed similar VVC incidence in probiotic and placebo users,30 or no difference in proportions of women with VVC before and after probiotic use.37,38

*Vaginal detection outcomes*

Fourteen studies assessed vaginal detection of probiotic strains (with one study reporting results for two probiotics) using a variety of molecular techniques on culture isolates (10/14) or on DNA extracted from vaginal swabs (4/14; Table S1). Probiotic strains were detected in 56-100% of probiotic users, or 20-39% of collected swabs, during or directly after the intervention period. The timing between last probiotic insertion and vaginal sample collection for detection measurement was never reported, and was the most clear in a study by Dausset et al that evaluated two, three, or five times per week dosing and assessed probiotic strain concentrations by quantitative PCR every day.50 This study showed rapid probiotic strain concentration increases and decreases after each administration. The 11 studies that assessed probiotic strain detection more than a week after cessation of use showed that detection always decreased within weeks; mean probiotic strain survival in the vagina cannot be determined more precisely because most studies included only one post-use assessment ranging from 14 days to six months after cessation.10,26,27,39,42,44–46,49 None of the three studies that assessed the association between vaginal detection and self-reported adherence found such an association,10,39,47 but one study showed higher probiotic strain concentrations in women who ever had any strain detected during the intervention period compared to women who did not.10 This sequencing study also highlighted the high inter-individual variability in vaginal probiotic strain detection and concentration, as well as overall VMB composition. Two *L. crispatus* CTV-05 studies reported that the probiotic strain was less often detected in women who had had sexual intercourse during the product use period (with or without condoms),45,48 and four studies found that detection was less likely in women who had autologous lactobacilli prior to probiotic use (*L. crispatus* in the case of three *L. crispatus* CTV-05 studies and any lactobacilli in the case of an EcoVag study).39,43,45,48

*Quality assessment*

We judged overall risk of bias to be high for 26 studies,medium for eight studies, and low for no studies (Figure 2). The majority of efficacy studies were likely underpowered and the majority of descriptive vaginal detection studies had low precision, with 9/14 studies assessing fewer than 20 women per probiotic. None of the studies compared responders to non-responders in a comprehensive manner, partly due to sample size limitations. Only 12 studies reported adherence data, and only two of those included adherence measures other than self-report.10,47 The exact timing between last probiotic insertion and vaginal sample collection for outcome assessment was never reported. Most BV/molecular VMB studies (11/13), but only 4/12 VVC studies, were randomised controlled trials and participants and clinicians in 16/24 trials were not blinded. Outcome ascertainment rigour and quantification improved over time with the increased availability of molecular technologies (Figure 2). However, microbiological inclusion criteria often differed from microbiological outcome assessments, and it was usually unclear whether these assessments were blinded. Insufficient consideration of potential confounding factors, such as hormonal contraception use, pregnancy, menses, vaginal practices, sexual behaviour, any antimicrobial use that was not part of the study design, and presence of sexually transmitted infections, was also common.

**Discussion**

*Main findings and interpretations*

The systematic review results suggest that lactobacilli-containing vaginally applied probiotics hold promise for BV cure and prevention, but much less so for VVC cure and prevention. Four of the six medium risk randomised controlled trials with BV endpoints showed significant benefits, and the other two showed non-significant benefits. No major safety concerns were reported in any of the studies. In addition, we found consistent evidence that vaginal detection of probiotic strains does not last long beyond the dosing period, suggesting that none of the evaluated probiotic strains colonise the vagina. However, these findings are not definitive because heterogeneity was high and the quality of most studies suboptimal. Furthermore, publication bias is likely to be high, although we did not formally assess this due to high heterogeneity.

We opted to evaluate all vaginally applied probiotics for bacterial and fungal vaginal dysbiosis indications because no single product-indication combination had been sufficiently evaluated to allow for a meaningful synthesis of results. While all identified probiotics contained lactobacilli, it is not yet clear which *Lactobacillus* strains are the most promising, if any. The probiotics included in this review therefore varied in terms of *Lactobacillus* strains, other active ingredients, excipients, application methods, and doses. We could not identify any one product that seemed to outperform the others in any of the clinical indications or vaginal detection. Most of the probiotic strains originated from the gut or from traditional fermented foods, and have been marketed for decades by non-pharmaceutical companies, even before clinical evidence became available.6 In recent years, the regulatory landscape for vaginal probiotics has changed considerably. When health claims are made, the US Food and Drug Administration has required human drug approval since 2016, and the European Medicines Authority will follow suit per 1 May 2020. As far as we know, only one of the products included in this review is being developed as a drug for human use: Lactin-V. It contains a *L. crispatus* strain (CTV-05) that was isolated from a healthy woman.43 While the results of the five vaginal detection and safety trials conducted with *L. crispatus* CTV-05 to date warranted continued development,30,43,45,47,48,51 we did not find early indications that this strain is likely to outperform other *Lactobacillus* strains. A Lactin-V efficacy trial for BV recurrence is currently ongoing.52

The mixed results of trials with VVC indications are not surprising to us given that molecular studies have shown that *Candida* species often co-exist with lactobacilli in the VMB, and epidemiological studies have shown negative associations between BV and VVC, including increased VVC incidence after BV treatment.5 In fact, some BV intervention trials assessed VVC incidence as a safety endpoint.10,53 While the use of exogenous lactobacilli to cure or prevent BV is much more in line with the molecular and epidemiological evidence to date, it is not clear what the best dosing strategies would be: as a main treatment in the absence of antibiotic treatment, as an adjuvant treatment to antibiotic treatment, as a maintenance therapy to prevent incident or recurrent BV, or a combination of these. This review could not provide definitive answers to these questions because none of the studies directly compared different treatment strategies with the same probiotic. However, the adjuvant and maintenance therapy studies were of higher quality, and more convincing, than the studies that used a vaginal probiotic as a stand-alone BV treatment. Only two studies directly compared antibiotic plus probiotic use with prolonged antibiotic use and they both found that the latter was more efficacious in reducing BV recurrence. This would not be a reason to discontinue vaginal probiotic development because most would agree that prolonged or frequent antibiotic use is not desirable given risks of side effects and antimicrobial resistance.

None of the articles explained why specific doses (about 108 colony-forming units per insertion for most products) and dosing frequency and duration were chosen. We suspect that many of these depended on marketing approvals dating from before the American and European drug regulatory approval restrictions (as was the case in our study),10 and are not necessarily based on clinical evidence. While probiotic strains may not have to be present in high concentrations to exert beneficial effects, the vaginal detection findings of this review beg the question whether doses and/or dosing frequency/duration should be increased. Some studies suggested that baseline VMB composition and sexual behaviour during probiotic use may influence efficacy,10,39,43,45,48 suggesting that stratified, or even personalised, VMB modulation may be required in the most difficult cases of persistent or recurrent BV.

*Strengths and limitations*

The quality of most studies was suboptimal. Some of the traditional epidemiological biases (such as insufficient statistical power, and lack of randomisation, blinding, appropriate controls, and adjustment for confounding) could have been avoided, but others are unique to vaginal probiotic trials and are methodologically challenging. The latter tended to improve over time, with the increasing availability of molecular methods. An important limitation of all studies was that the time duration between last probiotic insertion and vaginal sample collection for endpoint assessment and adherence were not known with sufficient precision, and were not taken into account in analyses. This is particularly problematic in the context of BV endpoints by Nugent or Ison-Hay scoring, because those scores heavily depend on counting bacteria with a *Lactobacillus* morphotype (Gram-positive rod) under a microscope. The scoring cannot differentiate between probiotic lactobacilli and autologous lactobacilli, and is prone to inflated counts if the sample is taken soon after insertion. Only the two most recent studies managed to partially address this problem. Dausset et al used daily sampling in the context of intermittent dosing, as well as quantitative PCR to differentiate between probiotic and autologous *Lactobacillus* strains.10,50 Our study employed 16S rRNA gene quantification and sequencing, which enabled us to not only quantify probiotic and autologous *Lactobacillus* strains, but also all BV-associated anaerobes combined.10 Reduction of the overall BV-anaerobes concentration over time, in addition to the increased lactobacilli concentration over time, provided convincing evidence for the beneficial effects of intermittent probiotic use. Our study also showed that host responses to vaginal probiotic treatment are highly variable, which calls the appropriateness of cross-sectional and pre-post assessments, as well as the use of group means/medians, into question.

Moving forward, we recommend that future trials incorporate quantitative molecular methods that differentiate between probiotic and autologous *Lactobacillus* strains; assess efficacy and safety endpoints regularly before, during, and after cessation of probiotic use; standardise and optimise the time duration between insertion and sample collection; improve statistical power to allow for direct comparisons between responders and non-responders; take all known confounders and adherence into account; and avoid generic epidemiological biases as much as possible (Table 3). Ideally, future trials would also quantify BV-anaerobes, and assess the impact on biofilm formation, *Candida* species, and bacterial pathobionts other than BV-anaerobes.

**Conclusion**

Vaginal probiotics hold promise for BV cure and prevention. We expect that the availability of vaginal probiotics for these indications will decline in 2020 because of regulatory changes. We therefore urge the field to invest in clinical evidence-based product development, and to conduct future clinical trials more rigorously.

**Acknowledgments**

The authors thank medical student Connie Rees for assistance with the search and data extraction.

**Disclosures of Interests**

The authors conducted a clinical trial with Ecologic Femi+ (Winclove Probiotics, Amsterdam, Netherlands) and Gynophilus LP (Biose, Aurillac, France). This trial was funded by the UK Medical Research Council and the University of Liverpool, but the two companies donated their products for use in the trial free of charge. The authors have no financial or intellectual investments in these products, and do not report any other competing interests.

**Contribution to Authorship**

JvdW conceived and planned the systematic reviewed, and JvdW and MCV performed the review and analyses, and wrote the manuscript, together.

**Details of Ethics Approval**

Not applicable.

**Funding**

None.

**References**

1. van de Wijgert JHHM, Borgdorff H, Verhelst R, Crucitti T, Francis S, Verstraelen H, et al. The vaginal microbiota: what have we learned after a decade of molecular characterization? PLoS One 2014;9:e105998.

2. van de Wijgert JHHM, Jespers V. The global health impact of vaginal dysbiosis. Res Microbiology 2017;168:859–64.

3. Tachedjian G, Aldunate M, Bradshaw CS, Cone RA. The role of lactic acid production by probiotic *Lactobacillus* species in vaginal health. Res Microbiology 2017;168:782–92.

4. Muzny CA, Schwebke JR. Biofilms: an underappreciated mechanism of treatment failure and recurrence in vaginal infections. Clin Infect Dis 2015;61:601–6.

5. van de Wijgert JHHM. The vaginal microbiome and sexually transmitted infections are interlinked: consequences for treatment and prevention. PLoS Med 2017;14(12):e1002478.

6. O’Toole PW, Marchesi JR, Hill C. Next-generation probiotics: the spectrum from probiotics to live biotherapeutics. Nat Microbiol 2017;2:17057.

7. Bradshaw CS, Brotman RM. Making inroads into improving treatment of bacterial vaginosis – striving for long-term cure. BMC Infect Dis 2015;15:292.

8. Petrova MI, Imholz NCE, Verhoeven TLA, Balzarini J, Van Damme EJM, Schols D, et al. Lectin-like molecules of *Lactobacillus rhamnosus* GG inhibit pathogenic *Escherichia coli* and *Salmonella* biofilm formation. PLoS One 2016;11: e0161337.

9. Allonsius CN, Vandenheuvel D, Oerlemans EFM, Petrova MI, Donders GGG, Cos P, et al. Inhibition of *Candida albicans* morphogenesis by chitinase from *Lactobacillus rhamnosus* GG. Sci Rep 2019;9:2900.

10. van de Wijgert JHHM, Verwijs MC, Agaba SK, Bronowski C, Mwambarangwe L, Uwineza M, et al. Intermittent lactobacilli-containing vaginal probiotic or oral metronidazole use to prevent bacterial vaginosis recurrence: safety and preliminary efficacy by microscopy and sequencing. MedRxiv 2019; doi.org/10.1101/19001156.

11. Moher D, Liberati A, Tetzlaff J, Altman DG, Group TP. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med 2009;6:e1000097.

12. Prospero - International prospective register of systematic reviews [Internet]. Available from: https://www.crd.york.ac.uk/PROSPERO/display\_record.php?RecordID=75717. Accessed 18 July 2018.

13. PubMed - NCBI [Internet]. Available from: https://www.ncbi.nlm.nih.gov/pubmed/. Accessed 17 July 2018.

14. Embase | Elsevier [Internet]. Available from: https://www.elsevier.com/solutions/embase-biomedical-research. Accessed 17 July 2018.

15. Nugent RP, Krohn MA, Hillier SL. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. J Clin Microbiol 1991;29:297–301.

16. Ison C, Hay P. Validation of a simplified grading of Gram stained vaginal smears for use in genitourinary medicine clinics. Sex Transm Infect 2002;78:413–5.

17. Cochrane handbook for systematic reviews of interventions version 5.1.0 [Internet]. The Cochrane Collaboration; 2011. Available from: http://handbook-5-1.cochrane.org/. Accessed 11 July 2018.

18. Larsson P-G, Stray-Pedersen B, Ryttig KR, Larsen S. Human lactobacilli as supplementation of clindamycin to patients with bacterial vaginosis reduce the recurrence rate; a 6-month, double-blind, randomized, placebo-controlled study. BMC Women’s Health 2008;8:3.

19. Petricevic L, Witt A. The role of *Lactobacillus casei rhamnosus* Lcr35 in restoring the normal vaginal flora after antibiotic treatment of bacterial vaginosis. BJOG 2008;115:1369–74.

20. Mastromarino P, Macchia S, Meggiorini L, Trinchieri V, Mosca L, Perluigi M, et al. Effectiveness of *Lactobacillus*-containing vaginal tablets in the treatment of symptomatic bacterial vaginosis. Clin Microbiol Infect 2009;15:67–74.

21. Bradshaw CS, Pirotta M, De Guingand D, Hocking JS, Morton AN, Garland SM, et al. Efficacy of oral metronidazole with vaginal clindamycin or vaginal probiotic for bacterial vaginosis: randomised placebo-controlled double-blind trial. PLoS One 2012;7:e34540.

22. Eriksson K, Carlsson B, Forsum U, Larsson P-G. A double-blind treatment study of bacterial vaginosis with normal vaginal lactobacilli after an open treatment with vaginal clindamycin ovules. Acta Derm-Venereol 2005;85:42–6.

23. Anukam KC, Osazuwa E, Osemene GI, Ehigiagbe F, Bruce AW, Reid G. Clinical study comparing probiotic *Lactobacillus* GR-1 and RC-14 with metronidazole vaginal gel to treat symptomatic bacterial vaginosis. Microbes Infect 2006;8:2772–6.

24. Hemalatha R, Mastromarino P, Ramalaxmi BA, Balakrishna NV, Sesikeran B. Effectiveness of vaginal tablets containing lactobacilli versus pH tablets on vaginal health and inflammatory cytokines: a randomized, double-blind study. Eur J Clin Microbiol Infect Dis 2012;31:3097–105.

25. Ling Z, Liu X, Chen W, Luo Y, Yuan L, Xia Y, et al. The restoration of the vaginal microbiota after treatment for bacterial vaginosis with metronidazole or probiotics. Microb Ecol 2013;65:773–80.

26. Bisanz JE, Seney S, McMillan A, Vongsa R, Koenig D, Wong L, et al. A systems biology approach investigating the effect of probiotics on the vaginal microbiome and host responses in a double blind, placebo-controlled clinical trial of post-menopausal women. PLoS One 2014;9:e104511.

27. Verdenelli MC, Cecchini C, Coman MM, Silvi S, Orpianesi C, Coata G, et al. Impact of probiotic SYNBIO® administered by vaginal suppositories in promoting vaginal health of apparently healthy women. Curr Microbiol 2016;73:483–90.

28. Bohbot JM, Daraï E, Bretelle F, Brami G, Daniel C, Cardot JM. Efficacy and safety of vaginally administered lyophilized *Lactobacillus crispatus* IP 174178 in the prevention of bacterial vaginosis recurrence. J Gynecol Obstet Hum Reprod 2018;47:81–6.

29. Rapisarda AMC, Caldaci L, Valenti G, Brescia R, Sapia F, Sarpietro G, et al. Efficacy of vaginal preparation containing *Lactobacillus acidophilus*, lactic acid and deodorized garlic extract in treatment and prevention of symptomatic bacterial vaginitis: result from a single-arm pilot study. Ital J Gynaecol Obstet 2018;30:21–31.

30. Czaja CA, Stapleton AE, Yarova-Yarovaya Y, Stamm WE. Phase I trial of a *Lactobacillus crispatus* vaginal suppository for prevention of recurrent urinary tract infection in women. Infect Dis Obstet Gynecol 2007;2007:1–8.

31. Özmen S, Turhan NO, Seckin NC. Gardnerella-associated vaginitis: comparison of three treatment modalities. Turk J Med Sci 1998;28:171–3.

32. Pirotta M, Gunn J, Chondros P, Grover S, O’Malley P, Hurley S, et al. Effect of lactobacillus in preventing post-antibiotic vulvovaginal candidiasis: a randomised controlled trial. BMJ 2004;329:548.

33. Di Pierro F, Catacchio V, Candidi C, Zerbinati N, Alfonso R. Rhatany-based preparation in vulvovaginitis and vaginosis. Gazz Med Ital 2009;168:338–46.

34. Witt A, Kaufmann U, Bitschnau M, Tempfer C, Özbal A, Haytouglu E, et al. Monthly itraconazole versus classic homeopathy for the treatment of recurrent vulvovaginal candidiasis: a randomised trial: Monthly itraconazole versus classic homeopathy for treatment of RVVC. BJOG 2009;116:1499–505.

35. Vicariotto F, Del Piano M, Mogna L, Mogna G. Effectiveness of the association of 2 probiotic strains formulated in a slow release vaginal product, in women affected by vulvovaginal candidiasis: a pilot study. J Clin Gastroenterol 2012;46:S73–80.

36. De Seta F, Parazzini F, De Leo R, Banco R, Maso GP, De Santo D, et al. *Lactobacillus plantarum* P17630 for preventing *Candida* vaginitis recurrence: a retrospective comparative study. Eur J Obstet Gynecol Reprod Biol 2014;182:136–9.

37. Donders G, Neven P, Moegele M, Lintermans A, Bellen G, Prasauskas V, et al. Ultra-low-dose estriol and *Lactobacillus acidophilus* vaginal tablets (Gynoflor®) for vaginal atrophy in postmenopausal breast cancer patients on aromatase inhibitors: pharmacokinetic, safety, and efficacy phase I clinical study. Breast Cancer Res Treat 2014;145:371–9.

38. Donders G, Bellen G, Neven P, Grob P, Prasauskas V, Buchholz S, et al. Effect of ultra-low-dose estriol and lactobacilli vaginal tablets (Gynoflor®) on inflammatory and infectious markers of the vaginal ecosystem in postmenopausal women with breast cancer on aromatase inhibitors. Eur J Clin Microbiol Infect Dis 2015;34:2023–8.

39. Pendharkar S, Brandsborg E, Hammarström L, Marcotte H, Larsson P-G. Vaginal colonisation by probiotic lactobacilli and clinical outcome in women conventionally treated for bacterial vaginosis and yeast infection. BMC Infect Dis 2015;15:255.

40. Kovachev SM, Vatcheva-Dobrevska RS. Local probiotic therapy for vaginal *Candida albicans* infections. Probiot Antimicro Prot 2015;7:38–44.

41. Murina F, Vicariotto F, Di Francesco S. Thymol, eugenol and lactobacilli in a medical device for the treatment of bacterial vaginosis and vulvovaginal candidiasis. New Microbiol 2018;41:220–4.

42. Gardiner GE, Heinemann C, Bruce AW, Beuerman D, Reid G. Persistence of *Lactobacillus fermentum* RC-14 and *Lactobacillus rhamnosus* GR-1 but not *L. rhamnosus* GG in the human vagina as demonstrated by randomly amplified polymorphic DNA. Clin Vaccine Immunol 2002;9:92–6.

43. Antonio MAD, Hillier SL. DNA fingerprinting of *Lactobacillus crispatus* strain CTV-05 by repetitive element sequence-based PCR analysis in a pilot study of vaginal colonization. J Clin Microbiol 2003;41:1881–7.

44. Burton JP, Cadieux PA, Reid G. Improved understanding of the bacterial vaginal microbiota of women before and after probiotic instillation. Appl Environ Microbiol 2003;69:97–101.

45. Antonio MAD, Meyn LA, Murray PJ, Busse B, Hillier SL. Vaginal colonization by probiotic *Lactobacillus crispatus* CTV‐05 is decreased by sexual activity and endogenous lactobacilli. J Infect Dis 2009;199:1506–13.

46. Ehrström S, Daroczy K, Rylander E, Samuelsson C, Johannesson U, Anzén B, et al. Lactic acid bacteria colonization and clinical outcome after probiotic supplementation in conventionally treated bacterial vaginosis and vulvovaginal candidiasis. Microbes Infect 2010;12:691–9.

47. Hemmerling A, Harrison W, Schroeder A, Park J, Korn A, Shiboski S, et al. Phase 2a study assessing colonization efficiency, safety, and acceptability of *Lactobacillus crispatus* CTV-05 in women with bacterial vaginosis: Sex Transm Dis 2010;37:745–50.

48. Ngugi BM, Hemmerling A, Bukusi EA, Kikuvi G, Gikunju J, Shiboski S, et al. Effects of bacterial vaginosis-associated bacteria and sexual intercourse on vaginal colonization with the probiotic *Lactobacillus crispatus* CTV-05. Sex Transm Dis 2011;38:1020–7.

49. Tomusiak A, Strus M, Heczko P, Adamski P, Stefański G, Mikołajczyk-Cichońska A, et al. Efficacy and safety of a vaginal medicinal product containing three strains of probiotic bacteria: a multicenter, randomized, double-blind, and placebo-controlled trial. Drug Design Dev Ther 2015 Sep;9:5345–54.

50. Dausset C, Patrier S, Gajer P, Thoral C, Lenglet Y, Cardot J-M, et al. Comparative phase I randomized open-label pilot clinical trial of Gynophilus® (Lcr regenerans®) immediate release capsules versus slow release muco-adhesive tablets. Eur J Clin Microbiol Infect Dis. 2018;37:1869–80.

51. Stapleton AE, Au-Yeung M, Hooton TM, Fredricks DN, Roberts PL, Czaja CA, et al. Randomized, placebo-controlled Phase 2 trial of a *Lactobacillus crispatus* probiotic given intravaginally for prevention of recurrent urinary tract infection. Clin Infect Dis 2011;52:1212–7.

52. LACTIN-V study for recurrent bacterial vaginosis - ClinicalTrials.gov [Internet]. Available from: https://clinicaltrials.gov/ct2/show/NCT02766023. Accessed 23 April 2019.

53. McClelland RS, Richardson BA, Hassan WM, Chohan V, Lavreys L, Mandaliya K, et al. Improvement of vaginal health for Kenyan women at risk for acquisition of Human Immunodeficiency Virus type 1: results of a randomized trial. J Infect Dis 2008;197:1361–8.

**Tables, Figures, and Supplementary Materials Caption List**

**Table 1:** Summary of BV/VMB study results.

**Table 2:** Summary of VVC study results.

**Table 3:** Recommendations for vaginal probiotic efficacy evaluation

**Figure 1:** PRISMA flow diagram

**Figure 2:** Risk of bias assessments

**Appendix S1:** Search terms and filters

**Table S1:** Summary of vaginal probiotic strain detection results

**Table S2:** Product characteristics