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

*BV* bacterial vaginosis, *PY* person-years at risk, *RCT* randomised controlled trial, *VMB* vaginal microbiota.

Main results of studies with BV cure and/or recurrence (by Nugent or Ison-Hay scoring of Gram stains) and/or molecular VMB composition endpoints: 14 results in 13 studies.

| **Reference** | **Probiotic** | **Study design** | **Results** |
| --- | --- | --- | --- |
| ***Articles with a medium overall risk of bias*** | | | |
| Larsson18 | EcoVag (*L. gasseri* EB01-DSM 14869 + *L. rhamnosus* Lbp PB01-DSM 14870) | RCT: placebo control | 10 days of EcoVag after clindamycin did not improve BV cure (by Ison-Hay; 32/50 cured) compared to placebo (37/50 cured) but significantly reduced the cumulative incidence of BV/intermediate microbiota within 4 menstrual cycles when used for 10 days after each menses (13/37 versus 21/39 incident cases, respectively). |
| Petricevic19 | Gynophilus (*L. casei rhamnosus* Lcr35) | RCT: no-intervention control | 7 days of Gynophilus after clindamycin resulted in significantly improved BV cure (by Nugent 0-3) compared to women receiving clindamycin only (69/83 versus 31/88, respectively) assessed 5 weeks after cessation of the intervention period. |
| Mastro-  marino20 | Florisia *(L. brevis* CD2 + *L. salivarius* subsp. s*alicinius* FV2 + *L. plantarum* FV9) | RCT: placebo control | 7 days of Florisia (without any antibiotic) was significantly more efficacious in curing BV (15/18 Nugent 0-3, 3/18 Nugent 4-6) than placebo (2/16 Nugent 4-6; 14/16 persistent Nugent 7-10) and was associated with significantly lower BV (Nugent 7-10) cumulative incidence in the 2 weeks after Florisia cessation (7/18 and 13/16, respectively). |
| Bradhaw21 | Gynoflor *(L. acidophilus* KS400 + 0.03mg oestriol) | RCT: placebo and clindamycin cream controls | 12 days of Gynoflor after oral metronidazole treatment for BV (Nugent 7-10) resulted in a borderline (p=0.13) lower BV recurrence (9/133) at Month 1 compared to 12 days of placebo (13/135) but higher compared to 7 days of vaginal clindamycin (5/140). At Month 6, BV recurrence was comparable between the three arms (37/133, 36/135, and 42/140, respectively). |
| van de  Wijgert10 | Ecologic Femi+ (*B. bifidum* W28 + *L. acidophilus* W70 + *L. helveticus* W74 + *L. brevis* W63 + *L. plantarum* W21 + *L. salivarius* W24) | RCT: no-intervention and oral metronidazole controls | Intermittent Ecologic Femi+ use after oral metronidazole BV treatment resulted in significantly lower BV (Nugent 7-10) recurrence than no-intervention (incidence 3.58/PY versus 10.18/PY, respectively), and significantly reduced BV-anaerobes concentration by sequencing, during the 2 months of use, but the effect disappeared by 4 months after cessation of use. |
| Van de Wijgert10 | Gynophilus LP (*L. rhamnosus* Lcr35 *regenerans*) | RCT: no-intervention and oral metronidazole controls | Intermittent Gynophilus LP use after oral metronidazole BV treatment resulted in a non-significant lower BV (Nugent 7-10) recurrence than controls (incidence 5.36/PY), and non-significantly reduced BV-anaerobes concentration by sequencing, during the 2 months of use, but the effect disappeared by 4 months after cessation of use. |
| ***Articles with a high overall risk of bias*** | | | |
| Eriksson22 | *L. gasseri* + *L. casei* var *rhamnosus* + *L. fermentum* (impregnated tampons) | RCT: placebo control | Use of lactobacilli-impregnated tampons for 5 or more days during the first and second menses after clindamycin treatment was not efficacious in reducing BV recurrence (by Nugent 4-10) by the end of the two menstrual cycles (41/91) compared to placebo tampons (34/96). |
| Anukam23 | RC-14/GR-1 *(L. fermentum* RC-14 + *L. rhamnosus* GR-1) | RCT: metronidazole gel control | 5 days of GR-1+RC-14 capsules (without any antibiotic) was significantly more efficacious in curing BV (12/20 Nugent 0-3) than 5 days of metronidazole gel (6/20) when assessed directly after the intervention, and also in reducing BV recurrence (Nugent 7-10) up to 25 days post-intervention (2/17 versus 9/18, respectively). |
| Hemalatha24 | Florisia (*L. brevis* CD2 + *L. salivarius* subsp. *salicinius* + *L. plantarum*) | RCT: pH lowering tablet control | 8 days of Florisia (without any antibiotic) was not efficacious in curing BV (by Nugent; 36/75 cured) compared to pH-lowering tablet (29/73 cured). |
| Ling25 | *L. delbrueckii* subsp. *lactis* DM8909 | RCT: metronidazole control | 7 days of unnamed *L.* *delbrueckii*-containing probiotic (without any antibiotic) was equally effective as vaginal metronidazole gel in curing BV (assessed 5 days after treatment completion; 22/25 and 25/30 cured, respectively) and was associated with a significantly lower recurrence 30 days after treatment completion than metronidazole gel (0/25 versus 5/30 recurrences). The molecular VMB data also suggest benefits of using the probiotic. |
| Bisanz26 | RC-14/GR-1 (*L. reuteri* RC-14 + *L. rhamnosus* GR-1) | Randomised controlled cross-over trial: placebo control | In women with Nugent 4-6, 3 days of GR-1/RC-14 use (without any antibiotic) did not result in more women with Nugent score improvement compared to placebo use (2/10 and 0/10 improved one day after use, and 2/9 and 1/8 improved 8 days after use). However, the *Lactobacillus* relative abundance was significantly increased, and *Atopobium* relative abundance significantly decreased, one day after use. |
| Verdenelli27 | SYNBIO gin (*L. rhamnosus* IMC 501 + *L. paracasei* IMC 502) | Pre-/post-intervention study | In women with Nugent 0-6, 7 days of SYNBIO gin (without any antibiotic) resulted in a significantly higher proportion of women with Nugent 0-3 directly after therapy (28/35 versus 21/35 at baseline), and this persisted for 21 days after cessation of therapy (28/35 still had Nugent 0-3; insufficient data for McNemar testing). SYNBIO gin use also resulted in a significant increase in *Lactobacillus* concentration (p<0.01). |
| Bohbot28 | Physioflor (*L. crispatus* IP 174178) | RCT: placebo control | Physioflor for 14 days immediately after metronidazole therapy, plus 14 days in three subsequent menstrual cycles, resulted in significantly lower BV cumulative incidence (Nugent 7-10), and time to BV recurrence, compared to placebo (8/39 and 16/39 had at least one recurrence, respectively). However, the effects disappeared 84 days after product cessation (p=0.922, absolute numbers not given). |
| Rapisarda29 | *L. acidophilus* LA14 | Pre-/post-intervention study | In women with Nugent 4-10, 14 days of *L. acidophilus* LA14 use (without any antibiotic) resulted in 46/60 women cured (Nugent 0-3) at the end of therapy, and this persisted for 28 days after product cessation (50/60). |

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

*BV* bacterial vaginosis, *UTI* urinary tract infection, *VMB* vaginal microbiome, *VVC* vulvovaginal candidiasis.

Main results of the studies with VVC cure and/or incidence (by culture or wet mount) endpoints. None of the studies used *Candida* PCR: 12 results in 12 studies.

| **Reference** | **Probiotic** | **Study design** | **Results** |
| --- | --- | --- | --- |
| ***Articles with a medium overall risk of bias*** | | | |
| Czaja30 | *L. crispatus* CTV-05 (not Lactin-V) | RCT: placebo control | In women with a history of recurrent UTI but without current dysuria, 5 days of *L. crispatus* CTV-05 use resulted in a similar VVC cumulative incidence (4/15) compared to placebo use (2/15) up to 25 days after cessation of use. |
| ***Articles with a high overall risk of bias*** | | | |
| Özmen31 | Gynoflor (*L. acidophilus* KS400+ 0.03mg oestriol) | Pre-/post-intervention study in three separate, non-randomised groups | 12 days of Gynoflor with or without metronidazole, compared to metronidazole alone (to treat BV), resulted in significantly lower cumulative VVC incidence (3/96 and 2/97, versus 14/114, respectively) in the 10-13 days after cessation of use. |
| Pirotta32 | Femilac *(L. rhamnosus* + *L. delbrueckki* + *L. acidophilus* + *S. thermophilus*) | 2x2 factorial design RCT: placebo control | 10 days of Femilac during (6 days) and after (4 days) non-metronidazole antibiotic use, compared to placebo controls, was not effective in reducing VVC incidence within 18 days after cessation of Femilac use (17/59 versus 9/54, respectively). |
| Di Pierro33 | Kramegin *(L. acidophilus* + *Krameria triandra* plant extract + 15mg lactic acid) | Pre-/post-intervention study | 10 days of Kramegin (without antifungal) cured 75/75 women with acute VVC, and 20/30 women with recurrent VVC (both significant compared to baseline). Cure was assessed 7 days after last administration. |
| Witt34 | *L. gasseri* | RCT: probiotic + itraconazole versus itraconazole only | In women with acute VVC and a history of recurrent VVC, after induction with itraconazole twice weekly for one month, itraconazole (once per month) with *L. gasseri* (6 consecutive days per month) during 6 months resulted in a similar VVC recurrence rate as itraconazole (once per month) only (15/45 versus 12/31, respectively). Results were also similar 6 months after product cessation (6/25 versus 5/23, respectively). |
| Vicariotto35 | ActiCand *(L. fermentum* LF10 + *L. acidophilus* LA02) | Pre-/post-intervention study | In women with acute VVC and a history of recurrent VVC, ActiCand (without antifungal; 7 consecutive nights, followed by 3 nights per week for 3 weeks, and one night per week for 4 weeks) resulted in a significant reduction in VVC cases to 7/30 women after cessation of therapy at Day 56. |
| De Seta36 | Gyno-Canesflor (*L. plantarum* P17630) | Non-randomised prospective cohort study | In women with acute VVC but no history of recurrent VVC, 34 days of Gyno-Canesflor after clotrimazole treatment, compared to placebo use, resulted in a (non-significant, Fisher's p=0.095) lower cumulative VVC incidence (1/40 versus 5/40, respectively). |
| Donders37 & Donders38 | Gynoflor *(L. acidophilus* KS400 + 0.03mg oestriol) | Pre-/post-intervention study | A 84-day single-arm Gynoflor treatment scheme (once daily for 4 weeks followed by thrice weekly for 8 weeks) for atrophic vaginitis in postmenopausal women with breast cancer temporarily increased asymptomatic VVC prevalence after two weeks of use (from 2/14 to 7/16) but returned to baseline values after one (3/16), two (3/16), and three (3/13) months of use. |
| Pendharkar  - Trial II39 | EcoVag (*L. gasseri* DSM 14869 + *L. rhamnosus* DSM 14870) | Non-randomised prospective cohort study | In women with recurrent VVC, fluconazole (7 days in cycle 1 followed by once weekly in cycles 2+3, and biweekly in cycles 4-6) with EcoVag (10 days in cycle 2, and once weekly in cycles 2-6) did not significantly improve the VVC cure fraction 6 months after treatment cessation compared to the fluconazole regimen without EcoVag (8/9 versus 7/10, respectively) but almost all women in both groups were cured. |
| Kovachev40 | Lactagyn (*L. acidophilus, L. rhamnosus, S. thermophilus, L. delbrueckii* subsp. *bulgaricus*) | RCT: no-intervention control | In women with acute VVC, antifungal treatment (fluconazole and fenticonazole) followed by 10-day Lactagyn therapy resulted in lower VVC recurrence (10/209) at the final follow-up visit 25-30 days after product cessation than antifungal treatment alone (76/207). The authors report this as two non-significant pre/post results, but these are significant when the appropriate test (McNemar) is used, and when the two groups are compared directly. |
| Rapisarda29 | *L. acidophilus* LA14 | Pre-/post-intervention study | In women with Nugent 4-10 and 21/60 with acute VVC, 14 days of *L. acidophilus* LA14 without antifungals or antibiotics resulted in a significant reduction of VVC cases to 9/60 one day after cessation of use, and 6/60 4 weeks after cessation of use. Mean *Candida* culture counts also decreased significantly. |
| Murina41 | Estromineral Probiogel *(L. fermentum* LF10 + *L. plantarum* LP02) | Pre-/post-intervention study | Estromineral Probiogel (without antifungal) cured 51/82 women with acute VVC and 27/27 in women with recurrent VVC between 20-30 days after therapy initiation (both significant compared to baseline), but therapy longer than the intended 6 days was required in 57.3% and 63.0% women, respectively, due to persistence of symptoms. |

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

*BV* bacterial vaginosis, *VMB* vaginal microbiota, *VVC* vulvovaginal candidiasis.

|  |  |
| --- | --- |
| **Topic** | **Recommendations** |
| Study design, populations, sample size | * Randomised placebo-controlled blinded trials are the preferred study design. Avoid pre-post studies without any parallel control groups. * Define the target population and recruitment strategy a priori. Avoid convenience samples. * Evaluate in women with and without urogenital symptoms, and in women at various levels of vaginal dysbiosis risk. * Conduct sample size calculations. Take into account that vaginal probiotic efficacy tends to have high inter-individual variability and that efficacy analyses should be controlled for known confounders such as hormonal status, menses, sexual behaviour, and presence of sexually transmitted infections. Allow for comparisons between responders and non-responders. |
| Interventions | * Consider dose per insertion, frequency of insertion, timing of insertion (also in relationship to timing of sample collection for efficacy evaluation), and how menses will be taken into account. * Provide clear use instructions, also in relation to vaginal hygiene practices and menses, and assess whether the participant understood them. Assess the participant’s ability to insert the probiotic correctly by direct observation. * Consider frequent sampling for outcome assessments. Participants could self-sample at home. * Assess adherence throughout the study, ideally not just by self-report. |
| Controls | * A parallel placebo control group is best, but if not possible, use no-intervention controls. * In addition, consider a positive control group of women using oral or vaginal metronidazole/clindamycin for BV or an antifungal for VVC. * Blind the laboratory technicians who conduct the outcome assessments, especially when blinding of the clinicians and participants is not possible. |
| Outcomes | * Assess outcomes prior to, during, and after cessation of probiotic use in all women at all time points regardless of symptomatology. Use the same outcome assessments to determine eligibility and/or cure of the initial BV/VVC prior to initiation of probiotic use (if applicable), at the start of probiotic use, and throughout follow-up. * Use (semi-)quantitative molecular methods to differentiate between probiotic lactobacilli, autologous lactobacilli, BV anaerobes, *Candida* species, and other organisms of interest. * In addition, use Nugent scoring and Amsel criteria to allow for comparisons with older studies and with current clinical practice. * Consider and record the timing between last probiotic insertion and sample collection for efficacy assessment. * Consider the vaginal microbiota holistically, including bacteria, yeasts, and sexually transmitted pathogens. * Always assess and report safety outcomes (adverse events). |
| Results reporting | * Report the name and manufacturer of the vaginal probiotic that was evaluated, as well as all active ingredients, excipients, and dosing information. Explain the rationale for selecting the product and be transparent about competing interests. * Report clearly how the probiotic was meant to be used: as a main therapy to cure BV or VVC, as an adjuvant therapy to an antimicrobial therapy, or as a maintenance therapy to prevent incidence or recurrence. * Report intent-to-treat and per-protocol statistical analysis results. |