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Probiotic Therapy for the Treatment and Prevention of Bacterial Vaginosis

by

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Abstract

Bacterial vaginosis (BV) is a common presenting complaint in healthcare, and patients often experience recurrences at a frustrating rate. The mainstay of current treatment is antibiotic therapy, either via oral or vaginal route. When recurrences occur, stronger antibiotics are often employed. This current method of treatment does not address an underlying component that impacts infection recurrence and rate of recurrence- the patient's baseline vaginal microbiome and the healthy bacteria that support it. To determine the efficacy and safety of probiotic therapy for the treatment of bacterial vaginosis, a literature review was completed using databases PubMed and Embase. The results of this literature review confirm that probiotic therapy is safe for the treatment of BV, and more effective than antibiotics alone. There are a variety of different probiotic bacterial strains and concentrations utilized for either monotherapy to treat BV or in conjunction with antibiotic therapy. Of the bacterial strains analyzed, those that were most studied were *L. crispatus* and *L.rhamnosus.* Strains that showed the strongest efficacy regarding reducing recurrence of bacterial vaginosis were *L. rhamnosus, L. fermentum,* and *L. planterum.* Both oral route and vaginal route of probiotic treatment were shown to be effective. Vaginal route showed a faster impact on microbiome, but also a faster rate of recurrence than oral probiotic use. Further research is needed regarding identifying all possible bacterial strains that are beneficial in treating/preventing BV, differing combinations of strains for increasing efficacy, along with identifying the necessary concentrations of these strains.

Key Words: vaginal diseases, vaginal infections, vaginitis, vaginal discharge, vaginal microbiome, probiotics

Introduction

Bacterial vaginosis (BV) infection is the most common vaginal condition in women ages fourteen to forty-four (Koumans et al. 2007). BV is an imbalance of good and bad bacteria in the vagina that can lead to patient symptoms of itching, burning, and foul odor (Mayo Clinic, 2023). This imbalance can also place women at increased risk for other issues, including sexually transmitted infections, pelvic inflammatory disease, and complications with pregnancy (Mayo Clinic, 2023). BV is often diagnosed by Nugent score, Amsel criteria, patient symptoms, or a combination of these. Amsel criteria is defined as the presence of adherent white discharge, vaginal pH greater than 4.5, an amine odor after application of KOH to the vaginal sample, and the presence of "clue cells." Meeting three or four of these criteria qualifies as BV (Amsel et al., 1983). Nugent score is determined via visualization of a gram-stained vaginal sample smear under a microscope. The smear is given a score of 0 to 4 points for the different morphotypes (shapes) of bacteria present in the sample- large, gram-positive rods (*Lactobacillus* species morphotypes), small gram-negative to variable rods (*Gardnerella vaginalis* and *Bacteriodes* species morphotypes) and curved gram-negative rods (*Mobiluncus* species morphotypes). A score of 7 or greater indicates BV, while a score of 4-6 is considered indeterminate, and a score of less than 3 is considered normal (Nugent et al., 1991).

Human microbiome research, along with how probiotics can be utilized for treatment of conditions with underlying bacterial dysbiosis has been an area of exponential growth over the past five decades (Puebla-Barrigan & Reid, 2019). The definition of probiotic was determined in the late 1990s by the World Health Organization (WHO) and the United Nations Food and Agriculture Organization was "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (FAO/WHO, 2001).

Statement of the Problem

Women who are diagnosed with a BV infection seem predisposed to developing recurrent issues with BV infections. According to Bradshaw et al. (2006), within the first year after antibiotic treatment with either metronidazole or clindamycin for BV 50-80% of women have a recurrence. Current methods of treatment for BV focus solely on treatment with antibiotics to eradicate harmful bacterial overgrowth. While initially effective, this method does not address the need for the vaginal microbiome to be re-established with healthy levels of bacteria, namely *Lactobacillus* species that keep the vaginal mucosa pH low and prevent infection reoccurrence. This leads to a shorter time to relapse than would be expected.

One of the biggest areas of probiotic research strives to determine which bacterial strains are best to return the microbiome to a balanced state. Strains of *Lactobacillus* have been shown to be the main bacterial species in the vaginal microbiome and serve to protect the host from infections, but not all *Lactobacillus* strains are created equal when judged on ability to colonize vaginal mucosa and maintain a healthy vaginal flora. Along with debate about strain efficacy, duration of treatment and mode of delivery (oral pill vs. vaginal suppository) are also areas of current research.

Research Question

In reproductive-age women with bacterial vaginosis (BV), is probiotic therapy a safe and effective treatment for preventing recurrence?

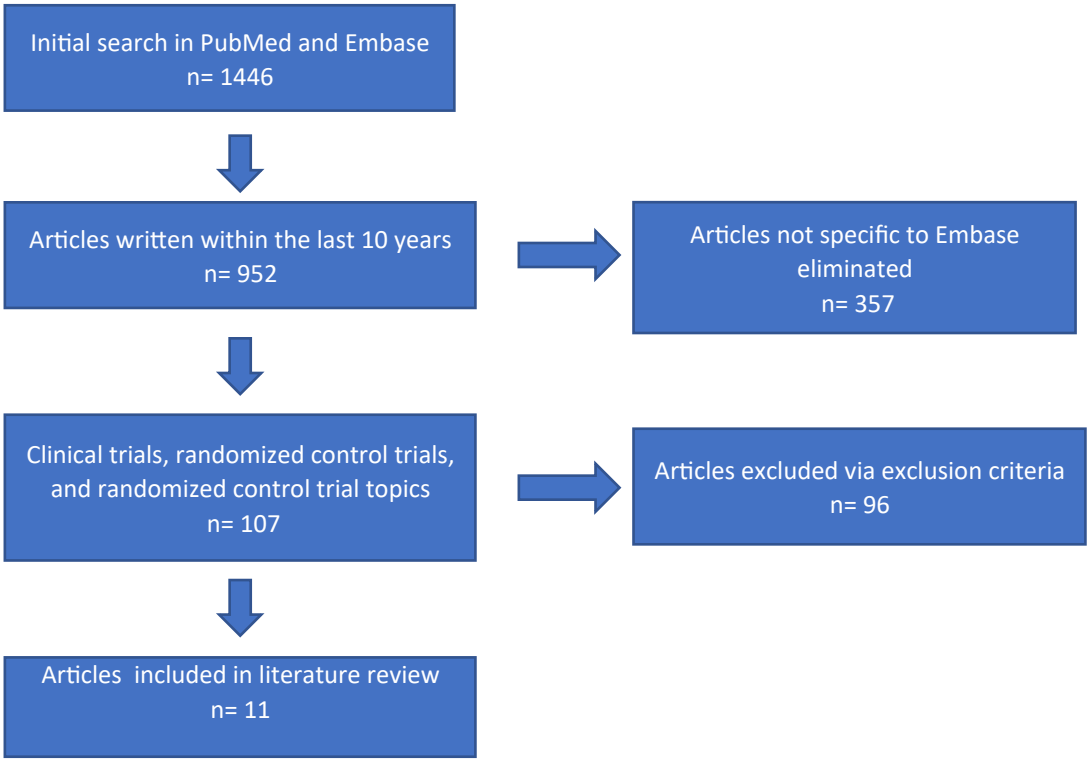
Methods

A literature review was performed using electronic search databases PubMed and Embase. Both keywords and mesh terms were used to define a set of literature discussing the use of probiotics to treat bacterial vaginosis. Keywords and mesh terms utilized in PubMed were

vaginal diseases, vaginal infections, vaginitis, vaginal discharge, vaginal microbiome, and probiotics. This search yielded 489 articles. These were further sifted by placing a 10-year filter (341 articles), selecting only clinical trials and randomized control trials (54), and eliminating articles that met exclusion criteria. Exclusion criteria for this literary review included studies looking specifically at the prepuberty and pregnant populations. Studies were excluded if they involved only infections other than BV (yeast, trichomonas, HIV etc.). Studies that involved non-human subjects, co-application of another agent (e.g. lactoferrin), were not available in English, did not publish their entire data set, studied the outcome on fertility, or researched for clinical cure instead of prevention/remission were also excluded. A similar search was conducted using Embase and the keywords vagina disease and probiotic agent, which yielded 957 results, which reduced to 611 results with a 10-year filter applied, then to 149 results looking specifically at clinical trials, randomized control trials, and randomized control trial topics. These 149 results were filtered for articles specific to Embase which gave 53 articles. After the same exclusion criteria were applied as listed above, zero articles remained. The following figure (figure 1) summarizes the search flow through PubMed and Embase for articles selected for this literature review.

Figure 1

Summary of Research Flow



Literature Review

Orally Consumed Probiotic Monotherapy to Treat Bacterial Vaginosis and Maintain Remission

Mändar et al. (2023) conducted a randomized double-blind placebo controlled two-arm parallel study with 89 participants aged 18-50 years with bacterial vaginosis. Bacterial vaginosis was defined as current complaints plus Amsel criteria. These patients were treated with either

vaginal or oral probiotic therapy that contained two strains of *Lactobacillus crispatus* (DSM32717 and DSM32720) with $3x10^{10}$ cfu per capsule. There were 28 participants in the oral probiotic group, 31 participants in the vaginal probiotic group, and 30 participants in the placebo group. The participants consumed (either vaginally or orally) one capsule per day for three months (20 days per month during their intermenstrual period).

The study's main objective was to evaluate this novel probiotic's efficacy in both oral and vaginal route of administration vs. placebo in reduction of symptoms defined by questionnaire, blood analysis, and vaginal samples. Patients with BV experienced a significant reduction in signs and symptoms with oral probiotic therapy with Nugent score reductions from 7.6 (1.4) to 5.3 (2.7). A reduction in BV symptoms including abnormal discharge and unpleasant smell was also observed via self-assessment in the patients utilizing oral probiotics but was just below significance threshold. This study was effective in demonstrating that oral administration of the novel probiotic is effective in symptom reduction and clinical evaluation of BV. (Mändar et al., (2023).

A strength identified by this study was using strains of lactobacilli that are native to the vaginal tract and were shown to be safe and tolerable in a previous study conducted by the investigators. Weaknesses of the study included that there was trial “trend toward fatigue” according to Mändar et al. (2023) and women started using the capsules less regularly toward the end of the study. Because of this, the investigators showed the intermediate (four week) effects in the studies tables and figures.

Pino et al. (2021) conducted a study investigating the use of *acticaseibacillus rhamnosus* TOM 22.8 strain in women with vaginal dysbiosis to determine efficacy in returning the vaginal flora to a eubiotic state. The study included 30 women who were 18-45 years old and had at least

one vaginal sign or symptom of BV (leukorrhea, burning, itching, or subjective discomfort), at least three Amsel criteria positive, and a Nugent score >7. Participants were placed into one of three groups- group A (vaginally treated), group B (orally treated), or group C (no treatment- wait and see approach). Each group had 10 participants. Each participant was instructed to take one capsule (either orally or vaginally) per day for 10 consecutive days. Each capsule contained 10 log cfu/g of *Lacticaseibacillus rhamnosus* TOM 22.8. All women had three appointments- at baseline (zero days), 10 days after treatment, and 30 days after the end of either oral or vaginal administration.

During the study women kept a personal record of any adverse reactions or use of any other treatments. If they experienced worsening symptoms, they were immediately excluded and provided with conventional therapy. At each scheduled appointment, the participants were vaginally swabbed, and clinical criteria were evaluated (Pino et al., 2021).

Women who were treated with the oral probiotic therapy on average increased their vaginal lactobacilli count from 3.85 +/- 0.22 log cfu/mL from baseline to 8.23 +/- 0.12 log cfu/mL thirty days after treatment ($p<0.05$) and had Nugent scores decrease from 7-10 (100%) at baseline to 0-3 (80%) and 4-6 (20%) at thirty days post-treatment ($p<0.05$) (Pino et al., 2021). The women in the control (wait and see) group averaged 4.02 +/- 0.71 log cfu/mL lactobacilli at baseline to 3.87 +/- 0.89 log cfu/mL lactobacilli at 30 days, although this didn't reach statistical significance ($p=0.7138$). The control group had very little reduction in Nugent score, from 7-9 (100%) at baseline to 7-9 (90%) and 4-6 (10%) at 30 days, although this also didn't reach statistical significance ($p=0.355$). Orally treated groups also saw a significant reduction in Amsel score ($p<0.05$), and a decrease in pathogenic bacteria.

This study conducted by Pino et al. in 2021 showed that *Lacticaseibacillus rhamnosus*

TOM 22.8 can reduce pathogenic bacteria and increase vaginal lactobacilli counts through oral administration. Strengths of this study include assessing both clinical (Amsel) and microbiological (Nugent score and pathogen count vs. Lactobacilli count) criteria. Weaknesses of this study are its small sample size, short duration, and absence of a conventionally treated control group.

A randomized, double-blind, multicentric, placebo-controlled study conducted by Vujic et al. (2013) investigated the use of orally administered probiotics containing *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 (Lactogyn) in comparison to placebo in women diagnosed with BV for efficacy. Women 18 years and older diagnosed with a vaginal infection (BV, trichomoniasis, candidiasis, or a combination of these) were included in the study. For the purposes of this literature review, the data from participants who had only candidiasis or only trichomoniasis were excluded from analysis. BV was diagnosed by at least three of four Amsel criteria or 7-10 Nugent score, candidiasis or trichomoniasis confirmed via routine laboratory procedures. Women were divided into the placebo group (n=149) or probiotic group (n=395). The probiotic group received capsules that contained $>10^9$ CFU *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 and the placebo group received identical capsules that did not contain probiotics. Each group took two capsules by mouth per day for six weeks. If a participant had trichomoniasis infection they were additionally given a single dose of 2g of metronidazole. Follow-up visits were at six and 12 weeks. Vaginal samples were taken and evaluated using the procedure during study inclusion.

The primary outcome of this study was the rate of restitution of normal vaginal microbiota after the ix-week follow-up. At this time, restitution of normal vaginal microbiota occurred in 40 (26.85%) of the placebo group vs. 243 (61.52%) of the probiotic group

(p=<0.001) (Vujic et al., 2013). At the additional 12-week follow-up, normal vaginal microbiota was still maintained by over half of the women in the probiotic group (51.14%) vs. one-fifth (20.81%) of women in the placebo group (p<0.001). High counts of *Lactobacillus* (>10^5 CFU/mL) were recovered from 81.5% of the probiotic group women, but only 28.9% of the women in the placebo group, showing that restitution of vagina microbiota highly coincided (p=<0.001) with the presence of lactobacilli in vaginal swabs on follow-up visits. These findings confirm the benefit of oral probiotics in vaginitis. They also support the main outcome of the study- the associated between positive post-treatment *Lactobacillus sp.* culture and those achieving a restored vaginal microbiota.

Strengths of this study conducted by Vujic et al. 2013 include a large sample size and the use of multicenter. Another important strength is the wide population of recruitment and the effects of treatment in a broad range of clinical settings that is useful for future use. A limitation of the study was the short follow-up period of six- and 12-weeks post-baseline, which was utilized to prevent dispersal of study subjects, along with analyzing participants who had BV mixed with another infection (candidiasis or trichomoniasis).

Orally Consumed Probiotic Therapy in Conjunction with Initial Antibiotic Therapy to Treat Bacterial Vaginosis and Maintain Remission

Reznichenko et al. (2020) conducted a randomized, parallel group prospective placebo-controlled study investigating the use of oral lactobacilli treatment after BV cure using metronidazole utilizing three strains of lactobacilli. The study included 166 women ages 18-45 who had diagnosed BV that was treated with oral metronidazole tablets (500 mg 2 times daily for seven days). Within 48 hours of metronidazole treatment, the women started either the lactobacilli treatment (5.4 billion total dose of a combination of *Lactobacillus crispatus* 60%,

Lactobacillus brevis 20%, and *Lactobacillus acidophilus* 20%) or a placebo supplement. There were 82 participants included in the verum group and 84 participants in the placebo group. Each participant took either the probiotic or placebo pill two times daily for the first seven days, then one time each day for the next eight to 120 days continuously after a meal.

Participants received a phone call visit on day 59 and day 119 of the study to assess symptoms and side effects, and had an initial office visit, then office visits during week 8 and week 16 to discuss BV symptoms, have a gynecologic examination, and test vaginal discharge for Amsel criteria and Nugent score. A recurrence of BV was defined as three of four Amsel criteria and one or a combination of typical symptoms self-reported of BV (vaginal discharge or vaginal odor) (Reznichenko et al., 2020).

The primary objective of the study was to assess the percent of recurrences of BV in the verum vs. placebo groups during the 16-week intervention. Secondary outcomes of the study were Nugent scores at each office visit (baseline, interim, and final) and the BV rate recurrence. The study found that BV recurred in 15 (18.3%) of the verum group and 27 (32.1%) of the placebo group ($p= .014$). The mean time to recurrence of BV was 97.3 (26.7) days in the verum group and 74.7 (27.7) days for the women in the placebo group (Reznichenko et al., 2020). Both groups tolerated treatment well. These results indicate that a 16-week treatment of lactobacilli (in the indicated ratio and strains) started immediately after BV cure using metronidazole can significantly decrease BV recurrence and helped participants remain BV free for longer.

Strengths listed by the study conducted by Reznichenko et al., 2020 include a high total dose of Lactobacilli (686 billion) and in its randomized double-blind controlled prospective parallel group design that included 7 clinical centers and a predefined sample size. The significant limitation discussed the use of a less strict criteria to diagnose BV compared to others.

This study considered three of four Amsel criteria to be positive for BV, whereas the FDA issued recent guidelines that recommend BV be diagnosed when all four of four Amsel criteria are present. Upon reanalyzing, the investigators found all women included in the study to have 4 of 4 criteria present and still qualify as having BV under the new FDA guidelines. Another limitation to this study is the lack of microbiological end points.

Heczko et al. (2015) conducted a multicenter, randomized, double-blind, placebo controlled parallel-group study comparing the use of prOVag probiotic simultaneously with the standard BV treatment for reducing the recurrence rate of BV relapses versus the standard treatment alone. A total of 241 women of 18–50-year-old women of European descent who menstruated regularly and had histories including recurrent BV. These participants were divided into the oral probiotic group (n=118) or placebo group (n=123).

Participants were eligible if they had three of four Amsel criteria. They were then given a standard treatment at visit one for BV (500 mg oral metronidazole twice daily for seven days), then used either the probiotic or placebo twice daily for 10 days. The tested probiotic (prOVag) contained a mixture of *L. gasseri* 57C, *L. fermentum* 57A, and *L. planeterum* 57B for a total of > 10^8 CFU. At visit two (14 days after the probiotic or placebo treatment finished) participants were tested via vaginal swab and symptoms. If symptoms and *G. Vaginalis* were still present via swab, participants were treated with oral clindamycin taken with probiotic or placebo twice daily for 10 days. If no infection was found at visit two, participants continued with probiotic or placebo once daily for 10 days during their peri-menstrual period (days 18-22 of the menstrual cycle) for the next three months. If participants who were treated with clindamycin at visit two were found to be treated successfully at visit two b, they continued using either probiotics or placebo in the above manner for the next three months as well. All participants had

scheduled follow-ups three through five within seven days after completion of each menstrual period where vaginal swabs were taken and symptoms reported (Heczko et al., 2015).

A recurrence of BV at visit three through five was the study endpoint. There was no significant difference in adverse events between probiotic and placebo groups. Relapse rate based on clinical symptoms didn't differ between the two groups, but average time to relapse did. Time to relapse on average was 47.3 (SD=26.98) days in the placebo group and 71.4 (SD=37.51) days in the prOVag group (Heczko et al., 2015). This interval is statistically significantly different between the two groups (p=0.013). Relapse rate based on microbiological criteria ($>10^5$ CFU per swab of any bacterial species potentially related to BV at visits three through five) occurred in 38 placebo participants (47%) and 33 prOVag participants (45.2%) but didn't quite qualify as statistically significant (p=0.087). Fewer participants had microbiologically confirmed BV at visit five than those in the placebo group (p=0.046). Total *Lactobacillus* vaginal counts increased on average one menstrual cycle sooner (visit three) in the prOVag group than in the placebo group (visit four).

This study conducted by Heczko et al. 2015 showed that the use of prOVag significantly contributes to the maintenance and prolongation of treatment outcomes of BV and targeted antibiotics for BV. The strength of the study was its large sample size. A weakness of the study identified by the investigators as a competing interest is that the company that manufactures the prOVag probiotic paid for this study to be conducted.

Laue et al. (2018) conducted a parallel armed, double-blind, placebo-controlled, randomized clinical trial investigating a *Lactobacillus*-containing yogurt drink and its effects on BV. The study included 36 women who were divided into the probiotic group (n=18) and the placebo group (n=18) who had all been diagnosed with BV by having at least three of four

Amsel criteria. All women were treated with the standard BV treatment of oral metronidazole 500mg twice daily for seven days. Participants began either consuming the probiotic yogurt or the placebo yogurt on the same day as metronidazole treatment and agreed to abstain from ingesting any other form of fermented dairy product. Dosage was two yogurt drinks (125g each) per day, one in the morning and one in the evening. The probiotic yogurt contained starter culture of *S. thermophilus* and *L. delbrueckii* along with living strains of *L. crispatus, L. gasseri, L. rhamnosus,* and *L. jensenii* with a minimum concentration of $1x10^7$ CFU/mL. The placebo group received chemically acidified milk without the probiotic strains. The participants consumed the yogurt for 4 weeks. During the study, each participant attended three visits (day zero, 14, and 31) where they were given the next allotment of yogurt and interviewed for symptoms. All participants also had a visit on day 28 with a gynecologist who took a vaginal swab to test for Amsel criteria and Nugent score.

The primary objective of this study was to compare the effect of orally administered yogurt containing the above four probiotic strains versus that of a placebo in the rate of recovery of BV after standard metronidazole treatment via Amsel criteria and Nugent score. A total of 33 women were available to analyze at the conclusion of the study- 16 in the probiotic group and 17 in the placebo group. At the conclusion of the four weeks of treatment, 17/17 (100%) of the women in the probiotic yogurt group were BV-free, vs. 11/17 (64.7%) of the placebo group were BV-free (p=0.018) via Amsel criteria. Via Nugent score, at the end of the study 16/16 (100%) of the probiotic group were BV-free, whereas only 13/17 (76.5%) of the placebo group were BV-free (p=0.013) (Laue et al., 2018). After the four-week intervention, the Amsel criteria decreased by –3.41 +/- 0.71 in the probiotic group versus decreasing by –1.94 +/- 1.95 in the placebo group (p=0.037). Nugent score decreased by –4.65 +/- 2.85 in the probiotic group and decreased by –

2.82 +/- 3.59 in the placebo group (p=0.158).

This study conducted by Laue et al. 2018 concluded that consuming yogurt containing the above strains improves the recovery rate and symptoms of BV. The study did not explicitly list strengths and weaknesses. A weakness of this study would be its relatively small sample size. It is also not able to conclude that the other probiotics present in the yogurt drink *(S. thermophilus* and *L. delbrueckii)* colonized the vagina of the women in the study.

Vaginal Suppository Probiotic Monotherapy to Treat Bacterial Vaginosis and Maintain Remission

Previously analyzed within theme one regarding oral probiotic administration, Mändar et al. (2023) also studied the impact of vaginally inserted probiotics. The study design, inclusion and exclusion criteria, probiotic strains, and concentrations are discussed in theme one. This study found that patients with BV experienced a significant reduction in signs and symptoms with vaginal probiotic therapy with Nugent score reductions from 7.6 (1.8) to 5.3 (3.6). A statistically significant reduction in BV symptoms including abnormal discharge (p=0.002) and unpleasant smell (p=0.001) was also observed via self-assessment in the patients utilizing vaginal probiotics. This study was effective in demonstrating that vaginal administration of the novel probiotic is effective in symptom reduction and clinical evaluation of BV. (Mändar et al. (2023).

As noted above, a strength of this study was using strains of Lactobacilli that are native to the vaginal tract and were shown to be safe and tolerable in a previous study conducted by the investigators. Weaknesses of the study included that there was trial “trend toward fatigue” and women started use the capsules less regularly toward the end of the study. Because of this, the investigators showed the intermediate (four week) effects in the studies tables and figures

(Mändar et al., 2023).

Previously analyzed within theme one regarding oral probiotic administration, Pino et al. (2021) also studied the impact of vaginally inserted probiotics. The study design, inclusion and exclusion criteria, probiotic strains, and concentrations are discussed in theme one. Women in the vaginal administration group averaged a change of 2.95 +/- 0.25 log cfu/mL lactobacilli at baseline to 5.90 +/- 0.11 log cfu/mL ($p=0.0001$) after 30 days and reduction in Nugent score from 7-10 (100%) to 0-3 (100%) ($p<0.05$). The women in the control (wait and see) group averaged 4.02 +/- 0.71 log cfu/mL lactobacilli at baseline to 3.87 +/- 0.89 log cfu/mL lactobacilli at 30 days, although this didn't reach statistical significance ($p=0.714$). The control group had very little reduction in Nugent score, from 7-9 (100%) at baseline to 7-9 (90%) and 4-6 (10%) at 30 days, although this also didn't reach statistical significance ($p=0.355$). Vaginally treated groups also saw a significant reduction in Amsel criteria ($p<0.05$), and a decrease in pathogenic bacteria.

This study showed that *Lacticaseibacillus rhamnosus* TOM 22.8 can reduce pathogenic bacteria and increase vaginal lactobacilli counts through vaginal administration. As noted above, strengths of this study include assessing both clinical (Amsel criteria) and microbiological (Nugent score and pathogen count vs. lactobacilli count) criteria. Weaknesses of this study are its small sample size, short duration, and absence of a conventionally treated control group (Pino et al., 2012

Vicariotto et al. (2014) conducted a placebo-controlled, pilot trial that included 35 women between 18 and 50 years to determine if slow-release vaginal tablets containing *L. fermentum LF15* and *L. plantarum LP01* reduce the prevalence of BV. Women included in the study had BV diagnosed by at least three of four Amsel criteria and Nugent score 7-10. Subjects

were divided into a probiotic group (n=24) and a placebo group (n=11). The probiotic group received vaginal tablets containing *L. fermentum LF15* and *L. plantarum LP01* (400 million live cells of each strain per dose), along with tara gum, arabinogalactan, and fructooligosaccharides. The placebo pills contained the same ingredients but instead of probiotics they contained microcrystalline cellulose and anhydrous calcium hydrogen phosphate.

Each subject inserted a vaginal tablet once daily for seven consecutive nights, then one tablet every three days for three weeks, then one tablet per week for the remaining 4 weeks. The study lasted a total of two months. Each subject was examined for Nugent score at enrollment (day 0), 28 days, and 56 days. Thirty-four women completed the study. At 28 days (one month), 22/24 (91.7%, $p<0.01$) women had a reduction of Nugent score in the probiotic group. Eight women (33.3%) reduced their Nugent score to 4-6 which places them in the intermediate category for BV. The remaining 14/22 (58.3%) had a Nugent score of 0-3 which indicates a restoration of the vaginal microbiota. At the conclusion of the study (day 56), four women in the probiotic group had a Nugent score of >7 indicating BV (16.7%, $p=0.065$) versus 9/10 (90%) of the women in the placebo group had a Nugent score of >7 indicating BV at the conclusion of treatment. Baseline average Nugent score in the probiotic group was 8.54 and in the placebo group was 7.90 ($p=0.137$). On day 28 and day 56 the mean Nugent score in the placebo group was still >7 versus 3.5 and 4.25 respectively in the probiotic group (Vicariotto et al., 2014).

This study conducted by Vicariotto et al. 2014 suggested that *L. fermentum LF15* and *L. plantarum LP01* can effectively counteract *Gardnerella* infections and improve BV symptoms. It is possible that some of this benefit is due to tara gum creating a mechanical barrier against *Gardnerella* adhesion of the vaginal mucosa. The placebo effect of this study was very low,

since no significant statistical differences were found after 28 days (p=0.619) or at the end of the study (p=0.823) which suggests that the improvement seen with the probiotic capsule is due to the probiotic strains and not the inert ingredients. No specific strengths or weaknesses were identified by this study, though a weakness could be the relatively small number of participants.

Vaginal Suppository Probiotic Therapy in Conjunction with Initial Antibiotic Therapy to Treat Bacterial Vaginosis and Maintain Remission

Bohbot et al. (2018) conducted a prospective, multi-center, double blind, randomized phase III trial with women who had diagnosed BV that had been cured using metronidazole investigating the efficacy and safety of vaginal suppository lactobacilli probiotic treatment to reduce BV recurrence. They selected 85 women (18 years or older, mean age 35.7 years) who currently had BV (three of four Amsel criteria) and had at least two episodes of BV in the previous year and had been treated with oral metronidazole (one g/day for seven days). There were 44 women randomly selected for the treatment group, and 41 women randomly selected for the placebo group.

Each group was treated for their current BV infection with metronidazole 500 mg orally twice daily for seven days. Participants were then screened to ensure cure with metronidazole therapy. They then were given either a placebo pill or a pill containing *Lactobacillus crispatus* IP 174178 (10^9 CFU per gram) to use vaginally once daily for 14 days for two menstrual cycles. On day 28 participants were contacted via telephone to ensure compliance. On day 56 participants were clinically examined. Participants were then given another course of treatment (either placebo or probiotic) for the next two menstrual cycles. On day 84 participants were contacted via telephone to ensure compliance. On day 112 participants were clinically examined for Amsel criteria and bacterial swabs were obtained. On day 156

women were clinically examined and bacterial swabs taken for the completion of the study (Bohbot et al., 2018).

The primary endpoint of this study was the number of patients in the treatment groups who presented with a bacterial or bacterial and symptomatic recurrence of BV infection at visit four on day 112. A secondary outcome was the time to recurrence of clinical BV. In the per protocol population, 16/37 participants (43.2%) of the placebo group presented with at least one BV recurrence versus 8/39 (20.5%) of the *L. crispatus* IP 174178 group (p=0.033). The time to first recurrence was shorter in the placebo group (median 2.93 +/- 0.17 months) than the *L. crispatus* IP 174178 group (3.75 +/- 0.16 months) (p=0.03) (Bohbot et al., 2018). This study suggests that *L. crispatus* IP 174178 is effective at preventing the recurrence of BV, with 59% of the placebo group not experiencing a BV recurrence during the study, while 79.5% of the *L. crispatus* IP 174178 group remained recurrence-free.

While no study-specific strengths were noted by Bohbot et al. 2018, many study limitations were. These include small sample size (58 were eligible for data inclusion), inclusion criteria that is too selective (two medically documented cases of BV within the past year), stopping recruitment at 167 participants in order to have time to analyze data, BV diagnosed with only three of four Amsel criteria, only assessing certain BV risk factors (smoking, douching, etc.) at inclusion and not throughout the study, and the fact that some participants used unauthorized antibiotics during the study.

Cohen et al. (2020) conducted a randomized, double-blind, placebo-controlled phase 2b trial to evaluate a strain of *Lactobacillus* (*Lactobacillus crispatus* CTV-05, also called Lactin-V) to prevent BV recurrence. The study included women ages 18-45 who were diagnosed with BV and had already completed a course of vaginal metronidazole gel. A diagnosis of bacterial

vaginosis was defined as at least three of four Amsel criteria, and a Nugent score of 4-10 via gram stain. Women who met these criteria were then treated with a five-day course of 0.75% metronidazole vaginal gel and returned to begin the study two days later.

A total of 228 women were included in the study, 152 in the Lactin-V group and 76 in the placebo group (2:1 ratio). Each group received either Lactin-V in a $2x10^9$ CFU dose or placebo to be inserted vaginally for four consecutive daily doses during week one, followed by twice-weekly doses for 10 weeks. Participants logged their administration of the treatment, along with symptoms, sexual activity, and menstruation details throughout the study. Follow-up visits were completed at four, eight, 12, and 24 weeks after the study started. At each of these appointments vaginal swabs were taken for Amsel criteria, Nugent score evaluation, and presence of *L. crispatus* by PCR (Cohen et al., 2020).

The primary outcome of this study was the percentage of women who had a recurrent bacterial vaginosis (at least three of four Amsel criteria and a Nugent score 4-10) at any follow up visit. Final data was available for 197 participants. Recurrence of BV by week 12 occurred in 46 (30%) of the Lactin-V group participants and in 34 (45%) of the placebo group participants (p=0.01). Of those participants who didn't have a recurrence by week 12, 13/106 (12%) of the Lactin-V group and 4/42 (9.5%) of the placebo group participants had recurrences by week 24. The presence of *L. crispatus* CTV-05 was detected in Lactin-V group participants 79-84% of the time in weeks 4-12 and 48% of participants at week 24. In the placebo group, *L. crispatus* CVT-05 was detected in 2-6% of participants during weeks 4-12 and in 2% of participants at week 24 (Cohen et al., 2020). Adverse effects of treatment did not differ significantly between the two treatment groups.

This study conducted by Cohen et al. 2020 found

that the use of Lactin-V (*L. Crispatus CVT-05*) after traditional treatment of BV with metronidazole vaginal gel resulted in a significantly lower recurrence incidence versus placebo at 12 weeks, and overall benefit appeared to persist through week 24. Strengths of this study include their large sample size, the use of two different methods to determine BV infection (Nugent score and Amsel criteria), and their determination that Lactin-V colonized the vaginal mucosa through PCR detection methods. The study did not identify any potential weaknesses.

Recine et al. (2015) conducted a case-control study to investigate the use of *Lactobacillus rhamnosus* BMX 54 as an adjunctive treatment with metronidazole for women with BV. They selected 250 sexually active women 18-45 years old who were diagnosed with BV by having at least three of four Amsel criteria. Women were separated into group A (125 participants) and group B (125 participants). Both groups completed standard BV treatment of metronidazole 500mg orally twice a day for seven days. Group B then used vaginal tablets containing $>10^4$ *L. rhamnosus* BMX 54 once a day for 10 days, twice a week for 15 days, then once every five days for seven months. Participants were evaluated for follow-up at months two, six, and nine and evaluated by vaginal examinations where swabs were taken, and symptoms were assessed. Symptoms assessment was conducted on a subjective scale from zero for no symptoms, one for mild symptoms, two for moderate symptoms, and three for severe symptoms.

At the first follow up (two months), 99/125 (79.2%) of group A versus 113/125 (90.4%) of group B had a clinical remission of BV ($p=0.014$). Vaginal physiological flora was achieved 36 women in group A (25.4%) and 106 (74.6%) women in group B at the six-month follow-up. At the nine-month follow-up, 118 women (79.7%) maintained a physiological flora versus 20.3% (n=30) women in group B (antibiotics alone, $p<0.05$). Difference in mean vaginal pH value became significant at the six month follow up of

4.476 +/- 0.57 in group A and 4.344 +/- 0.38 in group B (p=0.034). This pH difference between the two groups became greater at the nine month follow up, with group A median pH of 5.052 +/- 0.54 and group B median pH of 4.228 +/- 0.33 ($p<0.05$). Patient symptom reporting showed a 92% improvement in symptoms for participants who used the probiotic vaginal tablets versus a 79% symptom improvement for participants who used metronidazole therapy alone after nine months ($p=<0.001$) (Recine et al., 2015).

This study conducted by Recine et al. 2015 suggests that vaginally applied *Rhamnosus* BMX 54 can give long-term protection against BV infection. Weaknesses of the study include the use of a single center. A strength of this study is the large sample size and the length of study (nine months).

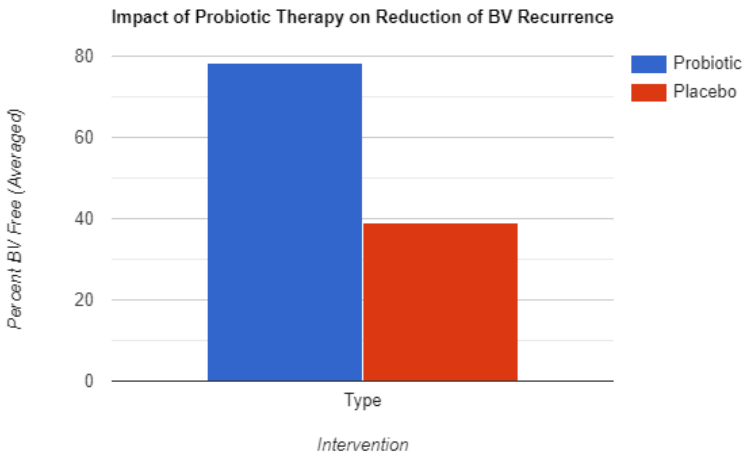
Discussion

There are many aspects of these studies that may influence overall efficacy in the treatment of BV. Some of these elements include the bacterial strains used, along with their relative concentrations. Other variables include the duration of probiotic treatment, the mode of delivery of the probiotic (oral vs. vaginal), and monotherapy of probiotics or probiotics in conjunction with initial antibiotic treatment. These different variables are important to remember when drawing overall conclusions from these studies and will be individually addressed here.

Overall, studies that analyzed BV recurrence using oral probiotic therapy (as either monotherapy or adjunctive to antibiotics) averaged a 27.9% decrease in BV recurrence (Laue et al., 2018; Reznichenko et al., 2020; Vujic et al. 2013). The fact that Vujic et al. 2013 included mixed infections (BV along with either trichomoniasis or candidiasis) makes their results less dependable when analyzing in terms of BV infection alone. Studies analyzing vaginal administration of probiotics (as either monotherapy or adjunctive to antibiotics) averaged a

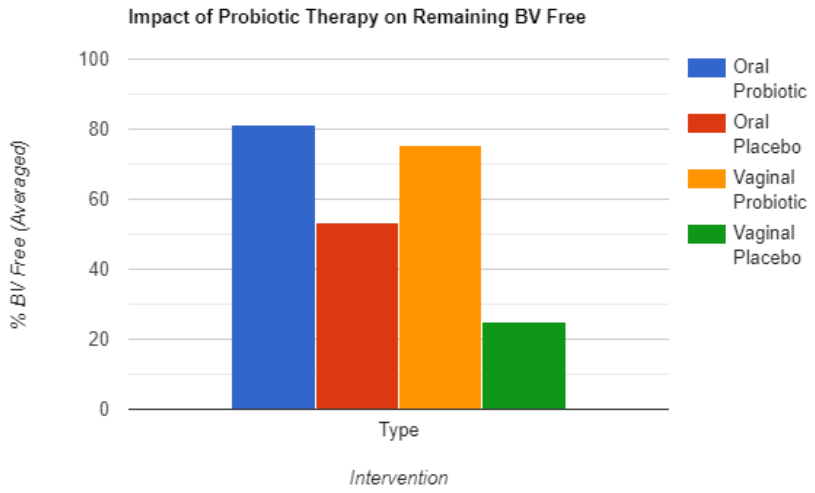
50.4% decrease in BV recurrence (Bohbot et al., 2018; Cohen et al., 2020; Recine et al., 2015; Vicariotto et al., 2014). Cohen et al. (2020) was the only study that utilized an antibiotic gel vehicle as opposed to a tablet form prior to probiotic treatment which may have influenced their results. Although vaginal administration showed a larger difference between probiotic and placebo recurrence (50.4%) at initial testing, oral administration still resulted in the largest percentage of participants remaining recurrence free at the end of the treatment period with 81.17% on average (Laue et al., 2018; Reznichenko et al., 2020; Vujic et al., 2013) compared to 75.55% with vaginal administration (Bohbot et al., 2018; Cohen et al., 2020; Recine et al., 2015; Vicariotto et al., 2018).

Figure 2



Note. Data from the following studies was included in the creation of this graph- Bohbot et al., 2018; Cohen et al., 2020; Laue et al., 2018; Recine et al., 2015; Reznichenko et al., 2020; Vicariotto et al., 2014; Vujic et al., 2013.

Figure 3



Note. Data from the following studies was included in the creation of this graph- oral probiotic monotherapy: Vujic et al., 2013. Oral probiotic adjunctive therapy: Laue et al., 2018; Reznichenko et al., 2020. Vaginal probiotic therapy: Vicariotto et al., 2014. Vaginal probiotic adjunctive therapy: Bohbot et al., 2018; Cohen et al., 2020; Recine et al., 2015.

This data supports the idea that treating BV infections with probiotics (whether as monotherapy or adjunctive therapy with antibiotics) reduces BV recurrence more than placebo (Figure 2), but oral probiotic administration was still slightly superior in overall prevention of BV than vaginal administration over time (Figure 3). These averages contain more studies that used probiotic adjunctive therapy than probiotic monotherapy, which impacts overall conclusions that can be drawn.

Interestingly, the use of probiotic therapy alone without initial antibiotic therapy showed a higher success rate of reducing BV recurrence compared to the adjunctive use of antibiotics (54% vs. 35.6% averaged.) This data only included one oral (Vujic et al., 2013) and one vaginal (Vicariotto et al., 2018) probiotic-only study and five studies that analyzed probiotic use with

initial antibiotic treatment, so further research should be completed to determine the validity of this observation (Bohbot et al., 2018; Cohen et al., 2020; Laue et al., 2018; Recine et al., 2015; Reznichenko et al., 2020). Initial antibiotic treatment may reduce the concentration of probiotic needed to reduce BV recurrence. Vujic et al. (2013) used an average oral daily dose of 2 billion cfu of *L. rhamnosus* and *L. rhueteri* to reduce BV recurrence by 34.7% as monotherapy, while Laue et al. (2018) treated initially with antibiotics and only needed an average oral daily dose of 18.6 million cfu of *L. crispatus, L. gasseri, L. rhamnosus,* and *L. jensenii* to decrease BV recurrence by a very comparable 35.3%. This would suggest that by using an antibiotic for initial cure, less probiotic is necessary to reduce BV recurrence, though the specific strains utilized may also have contributed to these results. Both vaginal and orally administered probiotics were found to be safe in every study analyzed.

Every study analyzed showed that the addition of probiotics, either as an adjunctive to initial antibiotic therapy, or as a monotherapy, was effective regarding that study's endpoint. Efficacy was also proven via either the vaginal or oral route of administration. Endpoints included increase in lactobacilli bacteria, decrease in pH, decrease in Nugent score/Amsel criteria, decrease in recurrence, or increase in overall time to recurrence. Regardless of study endpoint, each of these is an indicator that the use of the probiotic therapy was effective in altering the vaginal microbiome flora in such a way that BV infection became less likely.

Bacterial vaginosis is a common presenting problem in healthcare. Many patients deal with recurrences at a rate higher than expected after having been treated with antibiotic therapy. The use of antibiotics disrupts the vaginal microbiome, killing off not only problematic bacteria (G*ardneralla vaginalis*) but also protective bacteria (strains of *Lactobacilli)*. *Lactobacillus* strains are positively correlated with vaginal health. *L. crispatus* and *L. rhamnosus*

are two of the most popular strains that researchers are studying to treat bacterial vaginosis, though data also strongly supports the use of *L. fermentum,* and *L. planterum.* Both oral and vaginal routes of administration are shown to be safe and more effective than placebo or antibiotic therapy alone to treat bacterial vaginosis.

Questions that remain unanswered include what is the ideal concentration and length of treatment with probiotics to gain maximum benefit in Nugent score reduction and thus reduction in BV occurrence? How do genetics, lifestyle, and nutrition impact the vaginal microbiome and its propensity to become imbalanced, and can probiotic therapy help to offset the increased risk of BV? Would there be benefit to treating with both oral and vaginal probiotics concurrently? What mechanical barrier impact do the inert ingredients within vaginal probiotic suppositories impose and how does this impact colonization of vaginal mucosa by either harmful or helpful bacteria?

Future studies researching probiotic use for vaginal bacterial infections should focus on how specific strains taken orally survive the GI tract, and how either vaginal or oral strains are able to colonize the vaginal mucosa prior to testing these strains with human participants. Studies should be longitudinal, following the same patients for years to discover how long-term probiotic therapy can impact vaginal microbiome. There is now new information that shows that a woman's eubiotic vaginal microbiome can be classified into one of many "community types" (Zhou et al., 2007). Using the newly discovered information regarding community types of vaginal microbiomes, research should look at how certain community-types impact propensity to BV infection and how treatment can be tailored to restoring an individual's vaginal microbiome. Research should also look at whether it is possible through probiotic therapy to change an individual's community type to one that is more protective against

BV infection. Strains that are studied should have been tested prior to ensure the orally administered strain can resist salivary enzymes and stomach acidity to colonize the intestinal tract and that orally or vaginally administered strain can colonize the vaginal epithelium.

Conclusion

Probiotic use (both vaginal and oral routes of administration) has been shown to be safe. It has also been shown to be effective in reducing Nugent score and increasing time to relapse of BV. Specific strains show more promise than others, specifically *L. crispatus* and *L rhamnosus.* Treating a patient with BV with antibiotics alone misses an opportunity to improve both patient outcomes and patient satisfaction. The use of antibiotics with adjunctive probiotic therapy is an effective and safe treatment option. Monotherapy with probiotics is also a treatment option that shows promise for those wishing to avoid antibiotic use. Vaginal administration shows high efficacy rates with lower concentrations needed (Recine et al., 2015; Vicariotto et al., 2014). The data from the studies analyzed is very promising for the treatment of BV with probiotic therapy alone or in conjunction with antibiotics.

Applicability to Clinical Practice

Repeat bacterial vaginosis infections are a common clinical problem. To date this issue has been treated with oral or vaginal antibiotic treatments, and when the patient ultimately has a relapse, a stronger antibiotic with a longer duration of treatment is selected. With the adverse effects of antibiotic use (not only on the patient but on antibiotic resistance at large) additional treatment modalities are needed to improve patient outcomes.

For clinical application, mode of delivery (oral or vaginal) may be left up to patient preference being that patient compliance greatly impacts efficacy. This is already a

concept practiced with antibiotic use for BV treatment, where a patient may choose to either use oral antibiotics or vaginally administered antibiotics. This same method of shared decision making could be employed when choosing a route of administration of probiotic therapy. Patients should be counseled on the benefits of probiotics in re-establishing and supporting the vaginal microbiome.

Although studies try to focus on objective data for research purposes, subjective data should not be ignored. It is not uncommon for patients to present with complaints of vaginal irritation that they assume is either a yeast infection or bacterial vaginosis, only to be tested and found to have neither infection present. These patients are still looking for relief from their symptoms. Being able to offer a safe option to try that can help restore their vaginal microbiota and reduce their subjective symptoms is very valuable.

As new technology emerges including the ability for a woman to have her vaginal microbiome sequenced via taking a vaginal swab at home and sending it to a laboratory in the mail (e.g. Evvy vaginal microbiota sequencing) we are entering a new era of knowledge regarding individual vaginal microbiomes and how we can personalize treatments for each patient. This individualization can include lessening or possibly eliminating the use of antibiotics in vaginal infections and utilizing probiotic therapy to reduce BV occurrence.

References

Amsel, R., Totten, P. A., Spiegel, C. A., Chen, K. C. S., Eschenbach, D. A., & Holmes, K. K. (1983). Nonspecific vaginitis. Diagnostic Criteria and microbial and Epidemiologic Associations. *The American Journal of Medicine*, *74*(1), 14–22. https://doi.org/10.1016/0002-9343(83)91137-3

Bohbot, J. M., Daraï, E., Bretelle, F., Brami, G., Daniel, C., & Cardot, J. M. (2018). Efficacy and safety of vaginally administered lyophilized *lactobacillus crispatus* IP 174178 in the prevention of bacterial vaginosis recurrence. *Journal of Gynecology Obstetrics and Human Reproduction*, *47*(2), 81–86. https://doi.org/10.1016/j.jogoh.2017.11.005

Bradshaw, C. S., Morton, A. N., Hocking, J., Garland, S. M., Morris, M. B., Moss, L. M., Horvath, L. B., Kuzevska, I., & Fairley, C. K. (2006). High recurrence rates of bacterial vaginosis over the course of 12 months after oral metronidazole therapy and factors associated with recurrence. *The Journal of Infectious Diseases*, *193*(11), 1478–1486. https://doi.org/10.1086/503780

Cohen, C. R., Wierzbicki, M. R., French, A. L., Morris, S., Newmann, S., Reno, H., Green, L., Miller, S., Powell, J., Parks, T., & Hemmerling, A. (2020). Randomized trial of lactin-V to prevent recurrence of bacterial vaginosis. *Obstetrical & Gynecological Survey*, *75*(10), 601–602. https://doi.org/10.1097/ogx.0000000000000845

Food and Agriculture Organization of the United Nations, Report of the joint FAO/WHO expert consultation on evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria, Córdoba, Argentina, 1-4 October 2001 (2001). Rome.

Heczko, P. B., Tomusiak, A., Adamski, P., Jakimiuk, A. J., Stefański, G., Mikołajczyk-Cichońska, A., Suda-Szczurek, M., & Strus, M. (2015). Supplementation of standard antibiotic therapy with oral probiotics for bacterial vaginosis and aerobic vaginitis: A randomised, double-blind, placebo-controlled trial. *BMC Women's Health*, *15*(1). https://doi.org/10.1186/s12905-015-0246-6

Koumans, E. H., Sternberg, M., Bruce, C., McQuillan, G., Kendrick, J., Sutton, M., & Markowitz, L. E. (2007). The prevalence of bacterial vaginosis in the United States, 2001–2004; associations with symptoms, sexual behaviors, and Reproductive Health. *Sexually Transmitted Diseases*, *34*(11), 864–869. https://doi.org/10.1097/olq.0b013e318074e565

Laue, C., Papazova, E., Liesegang, A., Pannenbeckers, A., Arendarski, P., Linnerth, B., Domig, K. J., Kneifel, W., Petricevic, L., & Schrezenmeir, J. (2018). Effect of a yoghurt drink containing *lactobacillus* strains on bacterial vaginosis in women – a double-blind, randomised, controlled clinical pilot trial. *Beneficial Microbes*, *9*(1), 35–50. https://doi.org/10.3920/bm2017.0018

Mändar, R., Sõerunurk, G., Štšepetova, J., Smidt, I., Rööp, T., Kõljalg, S., Saare, M., Ausmees, K., Le, D. D., Jaagura, M., Piiskop, S., Tamm, H., & Salumets, A. (2023). Impact of *lactobacillus crispatus*-containing oral and vaginal probiotics on Vaginal Health: A randomised double-blind placebo controlled clinical trial. *Beneficial Microbes*, *14*(2), 143–152. https://doi.org/10.3920/bm2022.0091

Mayo Foundation for Medical Education and Research. (2023, June 10). *Bacterial vaginosis*. Mayo Clinic. https://www.mayoclinic.org/diseases-conditions/bacterial-vaginosis/symptoms-causes/syc-20352279

Nugent, R. P., Krohn, M. A., & Hillier, S. L. (1991). Reliability of diagnosing bacterial vaginosis is improved by a standardized method of Gram Stain Interpretation. *Journal of Clinical Microbiology*, *29*(2), 297–301. https://doi.org/10.1128/jcm.29.2.297-301.1991

Pino, A., Rapisarda, A. M., Vitale, S. G., Cianci, S., Caggia, C., Randazzo, C. L., & Cianci, A. (2021). A clinical pilot study on the effect of the probiotic lacticaseibacillus rhamnosus Tom 22.8 strain in women with vaginal dysbiosis. *Scientific Reports*, *11*(1). https://doi.org/10.1038/s41598-021-81931-z

Puebla-Barragan, S., & Reid, G. (2019b). Forty-five-year evolution of probiotic therapy. *Microbial Cell*, *6*(4), 184–196. https://doi.org/10.15698/mic2019.04.673

Recine, N., Palma, E., Domenici, L., Giorgini, M., Imperiale, L., Sassu, C., Musella, A., Marchetti, C., Muzii, L., & Benedetti Panici, P. (2015). Restoring vaginal microbiota: Biological control of bacterial vaginosis. A prospective case–control study using *Lactobacillus* rhamnosus BMX 54 as adjuvant treatment against bacterial vaginosis. *Archives of Gynecology and Obstetrics*, *293*(1), 101–107. https://doi.org/10.1007/s00404-015-3810-2

Reznichenko, H., Henyk, N., Maliuk, V., Khyzhnyak, T., Tynna, Y., Filipiuk, I., Veresniuk, N., Zubrytska, L., Quintens, J., Richir, K., & Gerasymov, S. (2020). Oral intake of lactobacilli can be helpful in symptomatic bacterial vaginosis: A randomized clinical study. *Journal of Lower Genital Tract Disease*, *24*(3), 284–289. https://doi.org/10.1097/lgt.0000000000000518

Vicariotto, F., Mogna, L., & Del Piano, M. (2014). Effectiveness of the two microorganisms *lactobacillus fermentum LF15* and *lactobacillus plantarum LP01*, formulated in slow-release vaginal tablets, in women affected by bacterial vaginosis. *Journal of Clinical*

Gastroenterology, *48*(Supplement 1), S106–S112. https://doi.org/10.1097/mcg.0000000000000226

Vujic, G., Jajac Knez, A., Despot Stefanovic, V., & Kuzmic Vrbanovic, V. (2013). Efficacy of orally applied probiotic capsules for bacterial vaginosis and other vaginal infections: A double-blind, randomized, placebo-controlled study. *European Journal of Obstetrics & Gynecology and Reproductive Biology*, *168*(1), 75–79. https://doi.org/10.1016/j.ejogrb.2012.12.031

Zhou, X., Brown, C. J., Abdo, Z., Davis, C. C., Hansmann, M. A., Joyce, P., Foster, J. A., & Forney, L. J. (2007). Differences in the composition of vaginal microbial communities found in Healthy Caucasian and black women. *The ISME Journal*, *1*(2), 121–133. https://doi.org/10.1038/ismej.2007.12