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The Effectiveness of *Lactobacillus crispatus* Probiotics as Prophylactic Therapy for Bacterial Vaginosis Recurrence: A Systematic Review and Meta-analysis

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ABSTRACT

Background: Bacterial vaginosis (BV) is one the main causes of fluor albus in women which is found in 15 – 50% of all reproductive age in the world. Regimen therapy for bacterial vaginosis consists of antibiotics, but the recurrence rates remain high. Probiotics appear to have an effect on treating or preventing bacterial vaginosis recurrence. One of the probiotics is Lactobacillus crispatus. **Methods:** The result of online searching on Pubmed-MEDLINE, Scopus, EBSCOhost, Cambridge Core, ProQuest, Cochrane library, ClinicalTrials.gov, and Google Scholar databases had found 4 relevant articles included in this systematic review (n = 516 subjects), from which 3 articles were used in meta-analysis (n = 350 subjects). **Results:** The meta-analysis of effectiveness of *L. crispatus* probiotics intervention group compared to the placebo group showed a homogeneity in data (Q=2.170, df=2; p=0.338, Tau2=0.026). Meta-analysis showed the value of Q statistic was z value = -2.683; (p=0.007) and overall risk ratio is 0.694 (95% CI= 0.532 to 0.906) with p=0.007. This demonstrated that the group with *L. crispatus* probiotics had 0.694 times lower risk in having bacterial vaginosis recurrence compared to the placebo group. The test for overall effect found the time to recurrence of placebo group was shorter compared to probiotics group with overall mean time to recurrence between probiotics group and placebo group was -0.960±0.20 (Z value= -4.730; p<0.001). Systematic review showed the administration of probiotic L. crispatus prevented recurrence and prolonged the occurence of BV recurrence with a good safety profile. **Conclusion:** Systematic review and meta-analysis results show that Lactobacillus crispatus probiotics administration after antibiotics therapy can significantly lower the incidence of bacterial vaginosis recurrence and prolong the time to recurrence.

Keywords: bacterial vaginosis, probiotics, *Lactobacillus crispatus*

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Background

Bacterial vaginosis is one of the main cause of *fluor albus* in women which is found in 15 – 50% of all reproductive age in the world.[1,2] Prevalence and distribution are varied all around the world. Bacterial vaginosis prevalence globally is 29,2%[3] and is 32% in Indonesian women. [4] Bacterial vaginosis is the result of vaginal dysbiosis due to anaerobic bacterial growth combined with the reduction of *Lactobacillus* population in vagina. This condition is a risk factor for preterm birth, chorioamnionitis, neonatal infection, and other reproductive disorders. On the other hand, bacterial vaginosis elevates the risk of having sexual transmitted infections, such as *Human immunodeficiency virus* (HIV) infection. [5]

The diagnosis of bacterial vaginosis is based on several diagnostic tests. The most common used methods are Amsel criteria and Nugent score. According to Amsel (1983), women are diagnosed as having BV if they present three of the following criteria, homogenous, thin, white or greyish leucorrhoea; a vaginal pH > 4,5; presence of clue cells (+) by microscopic examination (at least 20% of all wet mount samples); and whiff test (+) which is when amine test shows positive results of rotten fish odour after potassium hydroxide 10% addition of vaginal discharge. Nugent score on microscopic examination is considered as the gold standard in diagnosing BV. The score is determined from *Lactobacillus, Gardnerella,* and other bacterial morphotypes counts in high power field. The range of the score is 0 -10 and clinical diagnosis can be confirmed as BV if the score is 7 – 10. Diagnostic criteria for BV whether it is according to Amsel (3 out of 4 criteria) or to Nugent score (7 – 10) for women are infected for the first time, is the same. [6,7]

Bacterial vaginosis therapeutic regimen currently consists of antibiotics such as metronidazole or clindamycin given either orally or intravaginally. [1] Several studies showed even after treated with antibiotics, the recurrence rates are quite high, 57 - 90% in 1 – 3 months and 34 – 51% in 1 year. [8] Bacterial vaginosis recurrence may cause emotional, sexual, and social disturbances, also is a big burden in health sector economically. Hence, BV recurrence prevention needs novel regimen as a therapy. [2] Several clinical studies showed probiotics administration could reduce BV symptoms and prevent its recurrence. [5]

Probiotics are live microorganisms which give health benefits if consumed in proper dose. [9] A large number of studies proved the effectiveness of probiotic supplementation in treating or preventing vaginal infections, such as bacterial vaginosis, vulvovaginal candidiasis, and gonorrhea. [10,11] Most probiotic bacteria belong to the genus of *Lactobacillus.* [1] Several *Lactobacillus* strains and species such as *L. rhamnosus* GR-1, *L. reuteri* RC-14, *L. acidophilus*, *L. brevis*, *L. plantarum*, *L. gasseri*, *L. crispatus*, *L. Fermentum* have been known as vaginal probiotics to prevent or treat BV. [12]

Lactobacillus crispatus is one of *Lactobacillus* species which is most often found in vagina, most active, stable, and protective. Various clinical studies have found the usage of single strain of *L. crispatus* or combination with other *Lactobacillus* species as probiotics. One of the single strain that has gone through studies was *L. crispatus* GAI 98332 as recurrence prevention against urinary tract infection (UTI), *L. crispatus* CTV-05 (LACTIN-V) and *L. crispatus* IP 174178 (Physioflor) as prevention against BV recurrence. [13] The combination of *L. crispatus* LMG S-29995, *L. brevis*, and *L. acidophilus* probiotics also has proven to be able prevent BV recurrence. [14] Miscellaneous studies with *L. crispatus* probiotics after antibiotics administration showed recurrence reduction in BV and prolonged time to recurrence. [5,13]

Material and Methods

Literature Search

The following databases were accessed until data analysis: Pubmed-MEDLINE, Scopus, EBSCO, Cambridge Core, ProQuest, Cochrane library, ClinicalTrials.gov, and Google Scholar.

The following MeSH terms were used for searching: ”Probiotic Lactobacillus crispatus” AND ” *Bacterial vaginosis*”. The literature search was performed by three reviewers independently using PRISMA flow diagram 2009. Every dispute in determining papers and data extraction was settled with consensus.

Inclusion criteria were clinical studies with randomization, women aged 18 – 50 years old, women with BV according to Amsel criteria (3 out of 4 criteria) dan with Nugent score of 7 to 10, already completed antibiotic therapy with oral or intravaginal metronidazole, and time to recurrence is fitting with Amsel criteria (3 out of 4) and Nugent score 7 to 10.

Exclusion criteria were case reports, case serials, editorial letters, systematical reviews, and literature reviews, articles written with languages other than Indonesian and English without any translation available, research subjects who were pregnant, breastfeeding, contracted other sexual transmitted infections, and with urinary tract infections.

Study Selection

Three reviewers conducted the study selection independently. Duplicated articles were removed. Title and abstract, as well as full-texts, were reviewed for eligibility using the predefined inclusion and exclusion criteria. Differences in opinion were resolved between all reviewers to reach a consensus.

Data Extraction

Data extraction was performed independently by three reviewers using The Cochrane Collaboration data collection form for RCTs only. Every dispute in determining papers and data extraction was settled with consensus.

Assessment of Risk of Bias

Risk of bias assessments were performed independently by three reviewers using The Cochrane Collaboration data collection form for RCTs only and The Cochrane Collaboration’s tool for assessing the risk of bias in randomized clinical trials.

Data Synthesis

Meta-analysis of difference in weighted mean was conducted using *The Cochrane systematic review software (Review Manager (RevMan) Version 5.4.1, 2020)*. If data were not available to enable pooling, a descriptive synthesis was performed.

Results

The search for research articles was conducted based on the 2009 Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) flowchart (Figure 1).

Study Characteristics

The majority of the studies were carried out in United States (n=2), followed by the France (n=1), and Ukraine (n=1). The total sample from those 4 studies was 516 subjects. Two studies had subjects of women aged 18 – 50 years old, and another one had women aged > 18 years old. All studies were performed on women with BV according to Amsel criteria (3 out of 4) and Nugent score 7 – 10, already completed antibiotic therapy with oral or intravaginal metronidazole before given *L. crispatus* probiotics, were not pregnant, breastfeeding, contracted with sexual transmitted infections, and urinary tract infections. Three out of four studies were comparing the usage of single strain *L. crispatus* probiotics with placebo, whereas another one used *L. crispatus* probiotics combined with other *Lactobacillus* strain orally to be compared with placebo. *Lactobacillus crispatus* probiotics therapy was given with various strains and doses. Reported research outcome from all four studies were BV recurrence rates and time to recurrence. Duration of intervention were from 19 – 120 days (16 weeks). Follow-up were conducted between 4 – 28 weeks. The characteristics of included studies are presented in Table 1.

Result of Qualitative Data Analysis (Systematic Review)

1. Hemmerling et al, 2010[15]

The authors conducted a phase 2a study assessing colonization efficiency, safety, tolerability, and acceptability of *Lactobacillus*

crispatus CTV-05 (Lactin-V) administered by a vaginal applicator. Twenty-four women with BV were randomized in a 3:1 ratio of active product to placebo. All women were between 18 and 50 years of age and premenopausal. All women diagnosed with BV using Amsel and Nugent criteria were then treated with a standard antibiotic, 0.75% topical metronidazole (MetroGel) for 5 consecutive days before enrollment. Participants used Lactin-V at 2 x 10^9 colony-forming units (cfu)/dose or placebo for 5 initial consecutive days, followed by a weekly application over 2 weeks (days 12 and 19). They returned for follow-up on Days 10 and 28.

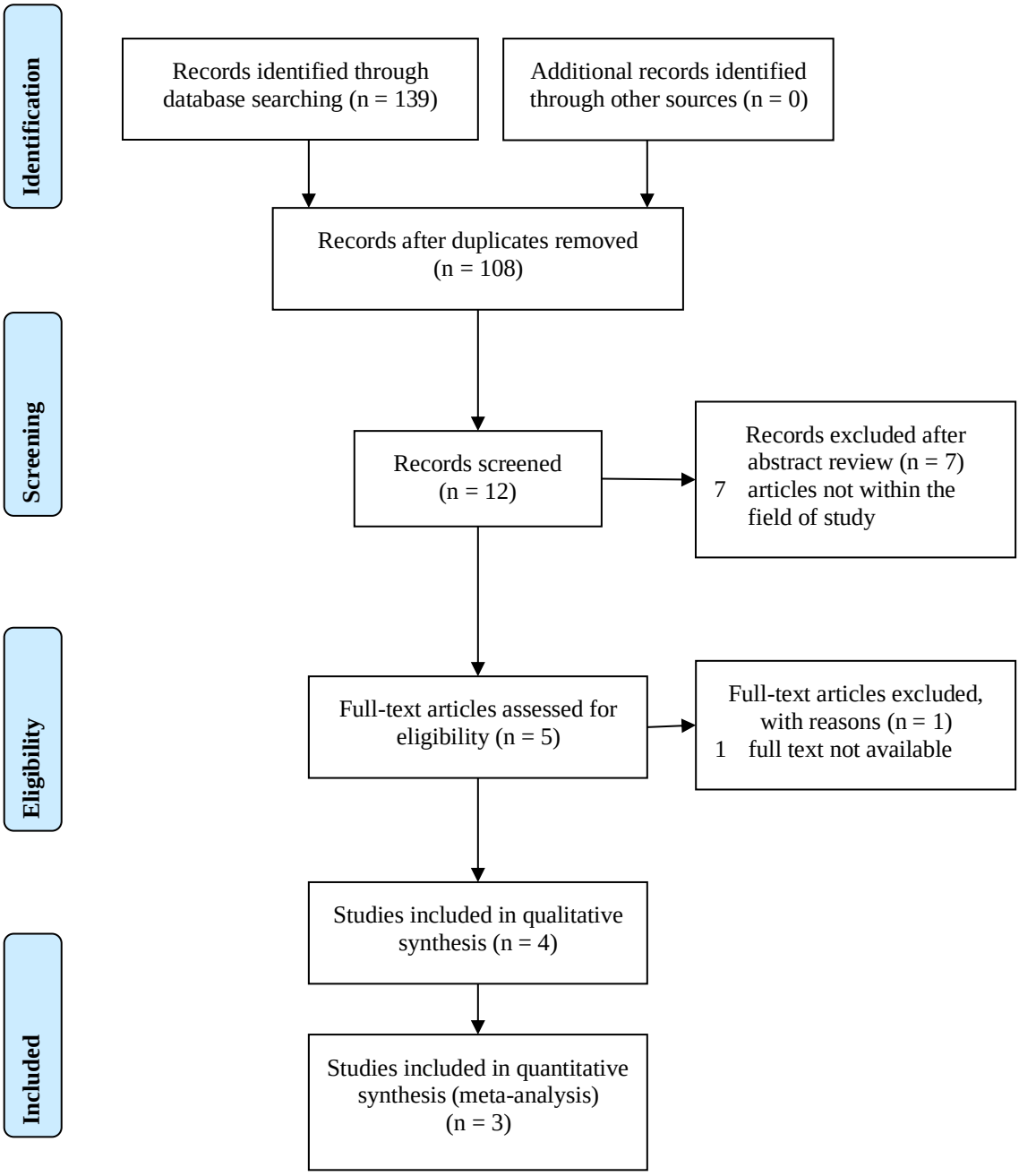

Figure 1. PRISMA Flow Diagram

Table 1. Characteristics of Included Studies

No	Authors, Year	Country	Sample Size	Study Population	Interventions		Study Outcome	Frequency	Duration of intervention
					Intervention Group	Comparison Group			
1	Hemmerling et al, 2010	USA	24	Premenopausal women 18 to 50 years old	*L. crispatus* CTV-05 (Lactin-V)	placebo	**primary**: to assess the colonization efficiency of Lactin-V on either day 10 or day 28 **secondary**: to measure the safety, tolerability, and acceptability of the product **tertier**: to assess trends for the rate of BV recurrence	Once daily for 5 days (days 1–5) followed by once weekly for 2 weeks (days 12 and 19)	19 days
2	Bohbot et al, 2018	France	98	Women over 18 years old	*L. crispatus* IP 174178 (Physioflor)	placebo	**primary***:* recurrence of bacterial vaginosis at 4th follow-up (day 112) **secondary**: the time to first recurrence, the number of patients presenting with at least a clinical recurrence, adverse event, and compliance	Once a day for 14 days over the first two menstrual cycles and another 14 days of the same treatment for the following two menstrual cycles	4 menstrual cycles
3	Cohen et al, 2020	USA	228	Premenopausal women 18 to 45 years old	*L. crispatus* CTV-05 (Lactin-V)	placebo	**primary**: the percentage of participants who had recurrent bacterial vaginosis at any follow-up visit up to and including the week 12 visit **secondary**: the percentage of participants who had recurrent bacterial vaginosis at any follow-up visit up to and including the week 24 visit, after completion of the 12-week post treatment phase of the trial	Four consecutive daily doses during week 1, followed by twice weekly doses for 10 weeks	11 weeks
4	Reznichenko et al, 2020	Ukraine	166	Women were 18 to 45 years old	*L. crispatus* LMG S-29995 60%, *L. brevis* 20%, *dan L. acidophilus* 20% (verum)	placebo	**primary**: the percent of recurrences of BV in the verum and placebo groups during 16 weeks of intervention **secondary**: Nugent scores at weeks 0 (baseline visit), 8 (interimvisit), 16 (final visit), time to recurrence of BV, and rates of survival without BV	2 times daily for the first 7 days and 1 time daily for the next 8 to 120 days	120 days (16 weeks)

Tabel 2. Recurrence rates on groups with *Lactobacillus crispatus* probiotics and placebo groups

No	Studies	Control Type	Probiotics		Placebo		*Risk Ratio*
			Recurrence rates	n (subjects)	Recurrence rates	n (subjects)	
1	Hemmerling et al, 2010	Placebo	4	18	0	6	3,32
2	Bohbot et al, 2018	Placebo	8	39	16	39	0,50
3	Cohen et al, 2020	Placebo	59	152	41	76	0,72

Sixty-one percent of the 18 women randomized to the Lactin-V group were colonized with *L. crispatus* CTV-05 at Day 10 or Day 28. Among Lactin-V users with complete adherence to the study regimen, 78% were colonized at day 10 or day 28. Of the 120 adverse events (AEs) that occurred, 108 (90%) and 12 (10%) were of mild and moderate severity, respectively. AEs were evenly distributed between the Lactin-V and placebo group. Lactin-V colonized well, and was safe and acceptable in women treated for BV. Using the same diagnostic criteria for BV as at enrollment in order to diagnose a recurrent BV episode (Nugent scores ≥ 7 and 3 or 4 Amsel criteria), 4 women in the Lactin-V group had a BV recurrence at the day 28 visit, 2 of them with symptoms. Only 1 of the 4 women with recurrent BV had used all 7 doses of Lactin-V. This study was not powered to evaluate recurrence of BV because the sample size is too small.

2. Bohbot et al, 2018[5]

A prospective, multi-centre, double blind, randomised phase III trial in women over 18 years and women with at least two documented episodes of BV in the previous year (diagnosis confirmed by presence of three Amsel criteria and a Nugent score ≥ 7), and who had been clinically cured (i.e., no Amsel criteria) after oral metronidazole treatment (1 g/day x 7 days). The patients were randomised to receive vaginal capsules of either *L. crispatus* IP 174178 (10^9 CFU per gram) or placebo, once a day, for 14 days over the first two menstrual cycles and another 14 days of the same treatment for the following two menstrual cycles. The primary efficacy endpoint was the number of patients with at least one bacteriologically confirmed recurrence of BV.

Out of 98 assessable patients (mean age 35,7 years), 78 women were evaluated (20 patients had missing data). During the treatment period, 16/39 patients (41%) had at least one recurrence in the placebo group versus 8/39 patients (20,5%) in the Lc group (p = 0,0497). The time to recurrence was longer by 28% in the Lc group (3,75 ± 0,16 months) vs. the placebo group (2,93 ± 0,18 months) (p = 0,0298). Tolerability and safety were good in both groups. In women with recurrent BV after antibiotics, treatment with Lc IP 174178 administered over four menstrual cycles, could significantly reduce the rate of recurrence and increase the time to recurrence.

3. Cohen et al, 2020[2]

The study was a randomized, double-blind, placebo-controlled, phase 2b trial to evaluate the ability of *Lactobacillus crispatus* CTV-05 (Lactin-V) to prevent the recurrence of bacterial vaginosis. Women 18 to 45 years of age who had received a diagnosis of bacterial vaginosis and who had completed a course of vaginal 0,75% metronidazole gel as part of the eligibility requirements were randomly assigned, in a 2:1 ratio, to receive vaginally administered Lactin-V at 2 x 10^9 CFU per dose or placebo for 11 weeks; follow-up occurred through week 24. The primary outcome was the percentage of women who had a recurrence of bacterial vaginosis by week 12.

A total of 228 women underwent randomization: 152 to the Lactin-V group and 76 to the placebo group; of these participants, 88% in the Lactin-V group and 84% in the placebo group could be evaluated for the primary outcome. In the intention-to-treat population, recurrence of bacterial vaginosis by week 12 occurred in 46 participants (30%) in the Lactin-V group and in 34 participants (45%) in the placebo group (risk ratio after multiple imputation for missing responses, 0,66; 95% confidence interval [CI], 0,44 to 0,87; p = 0,01). The risk ratio for recurrence by week 24 (also calculated with multiple imputation for missing responses) was 0,73 (95% CI, 0,54 to 0,92). At the 12-week visit, *L. crispatus* CTV-05 was detected in 79% of participants in the Lactin-V group. The percentage of participants who had at least one adverse event related to Lactin-V or placebo by week 24 did not differ significantly between the groups. The percentage of participants with local or systemic adverse events was similar in the two groups. The use of Lactin-V after treatment with vaginal metronidazole resulted in a

significantly lower incidence of recurrence of bacterial vaginosis than placebo at 12 weeks.

4. Reznichenko et al, 2020[14]

The authors conducted a phase-2 randomized parallel group prospective placebo-controlled study at 7 clinical centers enrolled 18 to 45-years-old women with recent symptomatic BV cured with metronidazole. Within 48 hours after completion ofmetronidazole therapy, eligible women received 1 capsule of the verum (5.4 billion *Lactobacillus crispatus* LMG S-29995, *Lactobacillus brevis*, and *Lactobacillus acidophilus* in proportion of 60%, 20%, and 20%, respectively), or the placebo supplement 2 times daily for the first 7 days and 1 time daily for the next 8 to 120 days. The primary outcome measurewas the percentage of recurrence of BV, which was defined as 3 of 4 Amsel criteria plus abnormal vaginal discharge/vulvar odor during 4 months of intake of the test dietary supplement. Differences between the groups were assessed with Z test for proportions.

One hundred sixty-six women were analyzed in the verum (82 patients) and the placebo group (82 patients). Recurrence of BV was documented in 15 (18,3%) of 82 women in the verum group and 27 (32,1%) of 84 in the placebo group (p = 0,014). Rates of survival without BV rates were higher in the verum group (Cox F test, p = 0,018). Both verum and placebo supplements were well tolerated. Oral intake of *L. crispatus* LMG S-29995, *L. brevis*, and *L. acidophilus* can significantly decrease percent of recurrences of BV in recently treated women and prolong time to recurrence of the disease.

Quantitative Data Result (Meta-Analysis)

Recurrence rates on groups with *Lactobacillus crispatus* probiotics and placebo groups are presented in Table 2.

Meta-analysis of effectiveness *Lactobacillus crispatus* probiotics administration compared with placebo group on bacterial vaginosis recurrence rates based on heterogeneity test results showed homogeneity of the data with Q value=2,170 df=2; p=0,338, Tau^2=0,026. Analysis was conducted with fixed effect model because the homogeneity of the data. Meta-analysis results showed the value of Q statistics is z value= -2,650; (p=0,008) and overall risk ratio is 0,694 (95% CI= 0,532 s/d 0,906) with p=0,007. This study showed that the groups with *Lactobacillus crispatus* probiotics had 0,694 times the risk of BV recurrence compared to placebo groups (figure 2).

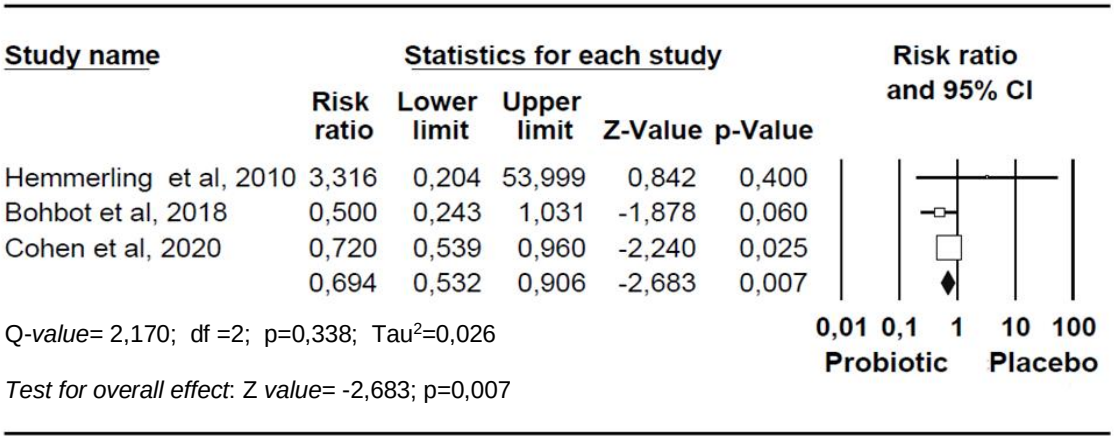

Figure 2. Effectiveness of *Lactobacillus crispatus* probiotics administration compared with placebo groups on bacterial vaginosis recurrence rates.

Time to recurrence in groups with *Lactobacillus crispatus* probiotics administration and placebo groups are presented in Table 3.

Meta-analysis time to recurrence known as Q value is significant with I^2 >50% which shows heterogeneity, although based on Hemmerling

et al, 2010 forrest-plot diagram results, it was eliminated from meta-analysis due to inadequate data. On Bohbot et al, 2018 dan Cohen et al, 2020 forrest-plot diagram studies, both were on the superior side of the probiotics thus the analysis used fixed-effect model. The overall effect test results found that placebo group time to recurrence was shorter compared to probiotics ones with time recurrence overall means between both groups were -0,960±0,20 (Z value= -4,730; p<0,001) (figure 3).

Tabel 3. **Time to recurrence in groups with *Lactobacillus crispatus* probiotics administration and placebo groups**

No	Studies	Control Type	Probiotics		Placebo	
			Time to recurrence (days) mean±SB	N (subjects with recurrence)	Time to recurrence (days) mean±SB	N (subjects with recurrence)
1	Hemmerling et al, 2010	Placebo	28±0,0	18	0±0,0	6
2	Bohbot et al, 2018	Placebo	112,5±4,8	8	87,9±5,1	16
3	Cohen et al, 2020	Placebo	103,6±38,8	59	75,9±39,4	41

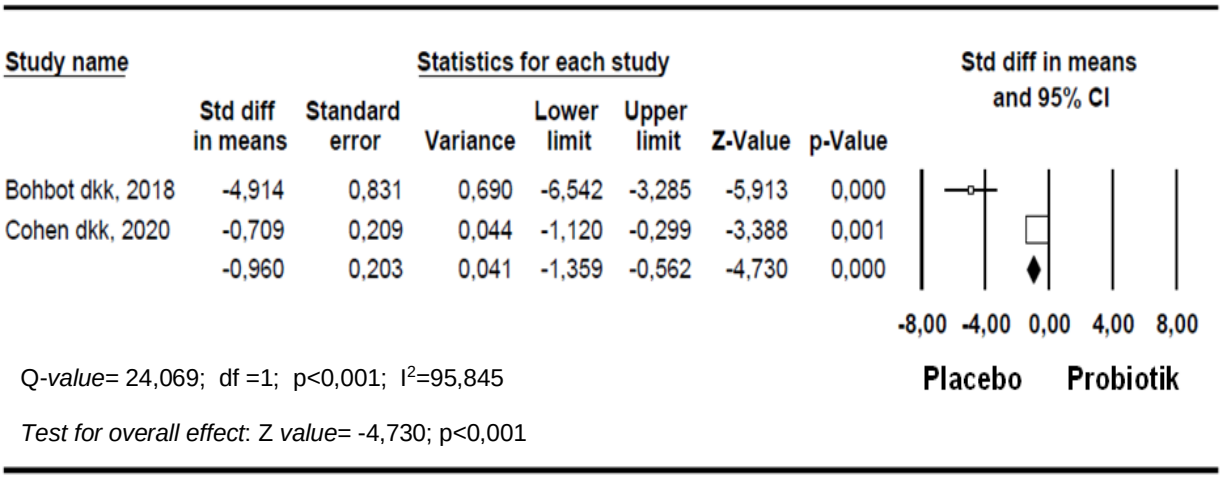

Figure 3. Meta-analysis of effectiveness of *Lactobacillus crispatus* administration compared to placebo groups on bacterial vaginosis time to recurrence.

Risk of Bias in Included Studies

Research articles included in meta-analysis were 3 studies of Hemmerling et al, 2010; Bohbot et al, 2018; dan Cohen et al, 2020; with data outcome of bacterial vaginosis recurrence rates and 2 studies of Bohbot et al, 2018; dan Cohen et al, 2020; with data outcome of bacterial vaginosis time to recurrence.[2,5,15] Study of Reznichenko et al, 2020 could not be included in quatitave analysis due to mixed probiotics administration of *Lactobacillus crispatus* with other *Lactobacillus*, which were *L. brevis* dan *L. acidophilus*.[14]

Risk of bias from the study included in the analysis (be it qualitative or quantitative) was assessed using The Cochrane Collecting data - form for RCTs only and The Cochrane Collaboration's tool for assessing risk of bias in randomized trials, including randomization technique, allocation concealment, participant blinding, blinding outcome, choice of outcomes reported, other source of bias, incomplete outcome data, and other biases. Table 3 shows the risk of bias in the included studies.

Table 3. Risk of Bias of Included Studies

	Random Sequence Generation	*Allocation Concealment*	*Blinding (participants and personnel)*	*Blinding (outcome assessment)*	*Selective reporting*	*Other sources of bias*	*Incomplete Outcome Data*	*Other bias*	*Overall*
Hemmerling et al, 2010	?	+	+	+	+	+	+	+	+
Bohbot et al, 2018	?	?	?	?	+	+	+	+	?
Cohen et al, 2020	+	+	+	+	+	+	+	+	+
Reznichenko et al, 2020	+	+	+	+	+	+	+	+	+

Discussion

This study is a meta-analytic observational study, a systematic review and meta-analysis to know the effectiveness of *Lactobacillus crispatus* probiotics administration as prophylactic therapy on bacterial vaginosis recurrence. Four studies were included in qualitative review (systematic review) and 3 of them were conducted with quantitative review (meta-analysis) in order to recognize the effectiveness of *Lactobacillus crispatus* probiotics administration in reducing bacterial vaginosis recurrence rates and prolong the time to recurrence of said infection.

Studies included in systematic review and meta-analysis had subjects with various age ranges. In Cohen et al, the age range was 18 – 45 years old, Bohbot et al, it was more than 18 years old, Hemmerling et al, was 18 – 50 years old, and Reznichenko et al, was 18 – 45 years old. According to literature, bacterial vaginosis is commonly found in reproductive age of 14 – 49 years old. Other literatures stated that age range of 15 – 44 years old are the most common age group in women with bacterial vaginosis. [6,7]

All studies in this study gave therapy for subjects with wither oral or intravaginal metronidazole before administering *L. Crispatus* probiotics. Hemmerling et al, 2010 dan Cohen et al, 2020 gave metronidazole 0,75% intravaginal gel for 5 days. Bohbot et al, 2018 dan Reznichenko, et al 2020 gave 500 mgs metronidazole orally per day in divided dose for 7 days. According to sexually transmitted infections treatment guidelines 2021 by The Centers for Disease Control and Prevention (CDC), regimen recommended for bacterial vaginosis is oral or intravaginal metronidazole and intravaginal clindamycin. [6] Both metronidazole and clindamycin in oral or intravaginal preparation are more preferred as bacterial vaginosis therapy and have similar efficacy. Oral metronidazole is more commonly used due to patient's preference rather than intravaginal preparation. Metronidazol is rarely related to *Clostridioides difficile* ninfection compared with clindamycin. Intravaginal clindamycin is also associated with greater resistence to anaerobic bacteria after treatment. [16] As a consequence, metronidazol is more preferred rather than clindamycin in all of these studies.

There were various strain of *Lactobacillus crispatus* used in the studies. Hemmerling, et al 2010 and Cohen et al, 2020 used *L. crispatus* CTV-05 (Lactin-V) strain. Bohbot et al, 2018 used *L. crispatus* IP 174178 (Physioflor) strain, while Reznichenko, et al 2020 used *L. crispatus* LMG S-29995 strain combined with *L. brevis* and *L. acidophilus*. According to literature, may of *Lactobacillus* strains in human's vagina are

being studied for possible potency in restoring vaginal normal flora which has been dominated by *Lactobacillus sp* and for protecting genital normal flora colonization against pathogens associated with BV. [15] Studies about *L. crispatus* strain against BV recurrence are still limited that it is still unknown which one is the best *L. crispatus* strain. [17]

Lactobacillus crispatus CTV-05 is the most studied *L. crispatus* strain for BV recurrence prevention and therapy and for urinary tract infections. Former study showed that *L. crispatus* CTV-05 was vaginal H_2O_2-producing strain, commonly found in vagina epithelial cells in vitro and had high success rate in colonizing vagina when given as vaginal suppository. *Lactobacillus crispatus* CTV-05 strain is considered as one of probiotics promising candidates. [13] Lactin-V is the biotechnology product name from *L. crispatus* CTV-05 in human's vagina which is currently being developed by Osel, Inc (Santa Clara, CA). Lactin-V works to restore *Lactobacillus* population in vagina on women with BV after conventional use of antibiotics with metronidazole 0,75% gel. [2,15]

Lactobacillus crispatus IP 174178 strain is believed as a biomarker for vaginal health. *Lactobacillus crispatus* IP 174178 produces lactic acid, microbicides, and virucides which facilitates the exfoliation of glycogen-rich cells in the vaginal epithelium. [5] On the other hand, *L. crispatus* LMG S-29995 strain combined with *L. brevis* and *L. acidophilus* is considered very effective and easy to use. [14]

Frequency, dosage, and duration of *L. crispatus* probiotics administration in the studies are also diverse. Hemmerling, et al 2010 and Cohen, et al 2020 used the same strain and dosage, but with different frequency and duration. Hemmerling et al, 2010 used *L. crispatus* CTV-05 (Lactin-V) at a dose of $2x10^9$ CFU/dosage for 5 consecutive days, continued on day 12 and 19, while Cohen et al, 2020 used *L. crispatus* CTV-05 (Lactin-V) at a dose of $2x10^9$ CFU/dosage for 4 consecutive days in first week, continued with 2 times per week for 10 weeks. According to literatures, safety tests on *L. crispatus* CTV-05 (Lactin-V) has been conducted with three different doses, namely $5x10^8$, $1x10^9$ dan $2x10^9$ CFU. All three doses had already proven safe and accepted by research subjects. [13] Which dosage is the most effective to prevent BV recurrence is still under research.

Bohbot, et al 2018 and Reznichenko et al, 2020 used different strain of *L. crispatus,* so they also conducted with different frequency, dosage, and duration. Bohbot et al, 2018 used *L. crispatus* IP 174178 at a dose of 10^9 CFU/gram 1 time per day for 14 days in first 2 menstrual cycles continued with the same regimen for 14 days in the next 2 menstrual cycles. Reznichenko et al, 2020 used *L. crispatus* LMG S-29995 60%, *L. brevis* 20%, and *L. acidophilus* 20% at a dose of 2 times 5,4 billions per day for first 7 days and 1 time per day for the next 8 – 120 days consecutively, after meals. It is still unknown which of both *L. crispatus* strains to have the most effective frequency, dosage, and duration in preventing BV recurrence.

Therapy for intervention groups in three studies were given *L. crispatus* probiotics intravaginally. Intravaginal route of administration for *L. crispatus* probiotics is chosen due to beneficial in dosage efficiency, usage frequency, and unaffected by gastrointestinal absorption. Intravaginal administration transferred directly into vagina, whether it is in the form of capsule or powder with vaginal applicator (similar with full-filled tampon), expected to increase probiotic species colonization in vagina in order to generate maximum benefits. [13]

Reznichenko, et al 2020 gave *L. crispatus* probiotics combined with *L. brevis* dan *L. achidophilus* orally. Randomized trials with small samples showed that *Lactobacillus* given orally could increase bacteria ecology in vagina and reduce BV recurrence in heterogenous groups of women. The reason of *Lactobacillus* oral administration indirectly is to proof that bacteria in colon can translocate into vagina. [14]

Hemmerling et al, 2010 showed that the groups with *Lactobacillus crispatus* probiotics was 3,32 times as high as the risk of BV recurrence compared to placebo groups. Bohbot et al, 2018 showed the groups with *Lactobacillus crispatus* probiotics had 0,5 times the risk compared to placebo groups. Cohen et al, 2020 showed the groups with *Lactobacillus crispatus* probiotics had 0,72 times the risk compared to placebo groups.

Meta-analysis showed bacterial vaginosis recurrence rates on groups with *Lactobacillus crispatus* probiotics were lower compared to placebo groups. The value of Q statistics is z *value*= -2,650; (p=0,008) and overall *Risk Ratio* is 0,694 (95% CI= 0,532 s/d 0,906) with p=0,007. This means the groups with *Lactobacillus crispatus* probiotics had 0,694 times the risk of bacterial vaginosis recurrence compared with placebo groups.

Follow-up studies after antibiotics therapy administration revealed an increase in bacterial vaginosis recurrence rates. The high rates of bacterial vaginosis recurrence might be caused by 2 reasons, namely the possibilty of antibiotic resistence and the biofilm product developed by pathogenic microorganisms. [20] Some other literatures stated that recurrence can happen due to reinfection from asymptomatic male partner or female sexual partner and reinfection frequency depends on involved patient population. More than one sexual partners is one of risk factors for BV, although the transmission from single or the same sexual partner is more considered in monogamous women with BV recurrence. [19]

Prophylactic therapy to prevent bacterial vaginosis recurrence is not established yet. A multicenter prospective study with long term administration of metronidazole gel has proven effective in reducing recurrence rates, but lead to complaints of vulvovaginal candidiasis and no beneficial report after therapy is completed. Long term regimen of oral or intravaginal clindamycin is not recommended due to reports of toxicity and low efficacy. [18]

Lactobacillus crispatus is a *Lactobacillus* species which is most commoly found in vaginal ecosystem. *Lactobacillus crispatus* is considered as protective agent and biomarker of a healthy vagina. [13] Single or mixed *Lactobacillus* strains probiotics usage, especially *L. crispatus* in vagina, has many beneficial effects. *Lactobacillus crispatus* can prevent the risk of other bacteria invasion associated with bacterial vaginosis or vaginal infection, such as *G. vaginalis* and *Prevotella.* Once *Lactobacillus* strain colonized, lactic acid and bacteriocin produced can reduce vaginal pH, inhibits BV-associated pathogens, and can prevent recurrence in a long term period. [20]

The study of Bohbot et al, 2018 showed mean of time to recurrence in groups with probiotics was 112,5±4,8 days, while placebo groups experienced recurrence faster, namely in 87,9±5,1 days. Cohen et al, 2020 stated the mean of time to recurrence in probiotics groups was 103,6±38,8 days, while the reccurence in placebo groups happened faster, namely in 75,9±39,4 days. Based on those data, according to Hemmerling et al, 2010 the probiotics group had recurrence in follow-up day 28, while in placebo groups there were no recurrence. According to Bohbot et al, 2018 and Cohen et al, 2020 placebo group experienced recurrence faster compared to probiotics groups.

Meta-analysis result in Bohbot et al, 2018 dan Cohen et al, 2020 showed time to recurrence in *L. crispatus* probiotics groups were longer than in placebo groups. The results of study of Hemmerling et al, 2010 was eliminated from meta-analysis due to inadequate data. Result of test for overall effect showed that time to recurrence in placebo groups was shorter compared to probiotics groups with mean overall difference of time to recurrence between both groups are -0,960±0,20 (Z *value*= -4,730; p<0,001).

Antibiotics therapy often can not eradicate negative-Gram bacteria associated with BV and only reduce its amount, which cause BV recurrence. Former studies showed

approximately 57 – 90% patients treated with antibiotics had recurrence in 3 month-period and 34 – 51% had recurrence in 12 month-period. [8] Probiotics usage to prevent recurrence and to prolong time to recurrence in BV is still controversial. Hence, probiotics as therapy is not included yet in the treatment guidelines for sexual transmitted infections by CDC in 2021 and *L. crispatus* is not yet to be sold over the counter. Nevertheless, *L. crispatus* probiotics usage is a promising therapy to prevent bacterial vaginosis recurrence and to prolong the time to recurrence of BV. [19]

Conclusion

The result of qualitative review and meta-analysis showed that *Lactobacillus crispatus* probiotics administration after antibiotics therapy can lower the incidence of bacterial vaginosis recurrence and prolong the time of recurrence of BV. More studies with bigger sample sizes and longer observational periods are needed to confirm effectiveness of *L. crispatus* probiotics in preventing recurrence of bacterial vaginosis.

Abbreviations:

BV: Bacterial Vaginosis

RCT : Randomized Controlled Trial

Mesh: Medical Subject Headings

PRISMA: Preferred Reporting Items for Systematic Review and Meta Analysis

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