Impact of oral administration of four *Lactobacillus* strains on Nugent score – systematic review and meta-analysis

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Abstract

We aimed at assessing the evidence for an effect on vaginal dysbiosis by oral administration of a mixture of *Lactobacillus* strains isolated from vaginal microbiota. For this purpose, we systematically reviewed the literature for randomised clinical trials (RCTs) in which the effect of oral administration of a mixture of four *Lactobacillus* strains (*Lactobacillus crispatus* LbV 88 (DSM 22566), *Lactobacillus gasseri* LbV 150N (DSM 22583), *Lactobacillus jensenii* LbV 116 (DSM 22567) and *Lactobacillus rhamnosus* LbV96 (DSM 22560)) on vaginal dysbiosis was examined based on Nugent score. Four RCTs were identified: a double-blind (DB)-RCT in 60 male-to-female transsexual women with neovagina; an open label RCT in 60 pregnant women with herpes virus infection; a DB-RCT in 36 women with bacterial vaginosis; a DB-RCT in 22 postmenopausal breast cancer patients receiving chemotherapy. Only in the three DB-RCTs Nugent score was assessed. The meta-analysis of these trials showed a significant reduction of Nugent score by probiotics compared to placebo in the fixed (standardised mean differences (SMD) -0.561; confidence interval (CI) -0.935 to -0.186; P=0.004 and random effect models (SMD -0.561; CI -0.935 to -0.186; P=0.004). The odds ratio (OR) of the cases presenting with improved Nugent score after probiotics compared to placebo treatment showed a significant effect in the fixed (OR=3.936; CI 1.702 to 9.100; P=0.001) and random effect model (OR=3.902; CI 1.681 to 9.059; P=0.001) Cochran’s Q and I² statistics showed no heterogeneity. This meta-analysis indicates that the oral intake of the pertinent *Lactobacillus* strains improves the microbial pattern in vaginal dysbiosis.

Keywords: probiotics, lactobacilli, vaginal microbiota, Nugent score

1. Introduction

The normal, acidic, vaginal milieu of adult women is maintained by microbiota predominantly consisting of lactobacilli (particularly of *Lactobacillus crispatus* and *Lactobacillus jensenii*). Under various conditions, such as bacterial vaginosis and chemotherapy, the vaginal microbiota may be impaired. Bacterial vaginosis (BV) is characterised by a depletion of lactobacilli in favour of anaerobic bacteria, by decreased H₂O₂- and lactic acid concentrations and by an increase in pH above 4.5. This is associated with increased risk for urogenital infections, obstetric complications and (in pregnant women) spontaneous abortions (Donders et al., 2000; Harmanli et al., 2000; Hauth et al., 2003; Hillebrand et al., 2002; Hillier et al., 1996; Klebanoff et al., 2004; Larsson et al., 1990; Leitich et al., 2003; Macones et al., 2004; Petricevic et al., 2014; Simhan et al., 2005; Swidsinski et al., 2013).

Oral and/or local administration of lactic acid producing bacteria and in particular of (so-called probiotic) lactobacilli such as *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 has been demonstrated to facilitate positive alterations of vaginal microbiota in healthy women, to improve or restore an impaired vaginal microbiota or to augment the efficacy of antibiotic treatment in women.
with BV (Anukam et al., 2006; Martinez et al., 2009; Reid and Bocking, 2003). In order to improve the efficacy of the treatment of BV, potential candidate lactobacilli were isolated from the vaginal microbiota of healthy women using a number of selection criteria, namely:

Catalase and oxidase activity, growth under aerobic and anaerobic conditions, acidification capacity, production of extracellular hydrogen peroxide, glycogen utilisation, bile salt tolerance and growth inhibition of pathogens (Escherichia coli, Gardnerella vaginalis, Candida krusei, Candida albicans and Candida glabrata strains), absence of antibiotic resistances, mucin degradation, β-haemolysis as well as glycosidase and arylamidase activity (Domig et al., 2014).

Four Lactobacillus strains (L. crispatus LbV 88, Lactobacillus gasseri LbV 150N, L. jensenii LbV 116 and L. rhamnosus LbV 96, fulfilling these criteria (De Seta et al., 2014; Domig et al., 2014) were isolated by Kiss et al. (2007). This mixture was tested in several RCT pilot studies (Anoshina, 2016; Kaufmann et al., 2014; Laue et al., 2018; Marschalek et al., 2017) showing positive effects on the vaginal microbial pattern and, in one study, an improvement of the cure rate in BV. It is commercially available as a food supplement (European Patent EP2509610B1). However, due to the low number of women examined, the studies mostly missed the required level of significance.

So, we aimed at assessing the overall evidence for an effect of oral administration of these Lactobacillus strains isolated from vaginal microbiota on vaginal dysbiosis. For this purpose, we performed a systematic review and meta-analysis of randomised clinical trials (RCTs) in which the effect of oral administration of a mixture of these Lactobacillus strains on vaginal dysbiosis was examined based on Nugent score.

Nugent score (>3) was used for defining vaginal dysbiosis, since the global burden of vaginal dysbiosis was assessed in studies based on Nugent score and since the extent of dysbiosis (no or low abundance of lactobacilli; increased bacterial diversity) in molecular studies correlated well with the Nugent score and with vaginal pH (Van de Wijgert and Jespers, 2017).

2. Materials and methods

Systematic literature searches and data extraction were performed by two investigators (JS and MV) independently from each other.

Search strategy and data extraction

We searched PUBMED/MEDLINE, EMBASE, and the COCHRANE library for eligible articles without publication date or language restrictions. Using the search term (in Boolean notation) ‘(lactobacillus OR lactobacilli) AND (vaginal OR vagina) AND (microbiota OR microflora), we found 685 potentially relevant articles. The number decreased to 215 papers when the search was restricted to the above-mentioned lactobacilli species (full search term ‘(lactobacillus OR lactobacilli) AND (vaginal OR vagina) AND (microbiota OR microflora) AND (crispatus OR gasseri OR jensenii OR rhamnosus)’). If the search was limited to articles that examined the combination of all four species, or if the four lactobacilli strains were added to the search term either individually or in combination (additional term ‘LbV OR LbV88 OR LbV150N OR LbV116 OR LbV96’), only six or four articles remained. Of these, two studies (Kiss et al., 2007; Martinez-Pena et al., 2013) were rejected on the basis of the abstract because they had not investigated health effects of all four species.

A full-text search for one or all of the four strains mentioned using Google Scholar yielded the same result. A manual search of the lists of references of retrieved articles as well as inquiries to individual authors provided no further articles. A request to the patent holder of the mixture of the four lactobacilli strains mentioned above confirmed the completeness of our list of four RCTs carried out according to the criteria for good research and publication practice (Figure 1).

Eligibility criteria

We included randomised clinical trials (RCTs) in which the effect of oral administration of the complete mixture of the four Lactobacillus strains (L. crispatus LbV 88 (DSM 22566), L. gasseri LbV 150N (DSM 22583), L. jensenii LbV 116 (DSM 22567) and L. rhamnosus LbV96 (DSM 22560)) on vaginal dysbiosis was assessed based on Nugent score.

Quality assessment

The methodological quality of each study was estimated independently by both investigators (MV and JS) with respect to the method of randomisation, random allocation concealment, blinding of treatment allocation, and study withdrawals.

Publication bias

To visually identify publication bias or other small study effects, we used funnel plots of the standard error versus the standardised mean difference (SMD) or the odds ratio (OR), respectively (Light and Pillemer, 1984).
Calculation of effect measures and data synthesis

Statistical evaluation and meta-analysis were done using the program MedCalc Statistical Software version 18.2.1 (MedCalc Software, Ostend, Belgium; http://www.medcalc.org). The authors of all three lastly identified studies provided their raw data. For effect measures, we considered the mean difference ± standard deviation in absolute change of the Nugent score between baseline and first medical examination after probiotics administration between the intervention and the control group.

We calculated SMD, including 95% confidence intervals and P-values, by two approaches: the fixed effect method (using the Hedges g statistic, whereby Hedges g is the difference between the two means divided by the pooled standard deviation and, with an adjustment for small sample bias) and the random effect method (DerSimonian and Laird, 1986). The results are visualised by a forest plot.

The weakness of this approach is, that the Nugent score (Nugent et al., 1991) has been developed for the diagnosis of bacterial vaginosis, its verification is based on the practical suitability, also in comparison with the Amsel Score (Amsel et al., 1983). Thus, a linear relationship between the Nugent Score scale and the concentration of bacterial groups relevant to vaginal dysbiosis may at best exist in a semi-quantitative sense and in the central section of the scale.

In order to address this weakness, we calculated as an additional effect measure the OR from the numbers of women (in the intervention and control group) in whom probiotics treatment had improved or not improved Nugent scores. We calculated weighted summary OR, including 95% CI and P-values, by both, the fixed effects model (Mantel and Haenszel, 1959) and the more conservative random effects approach (DerSimonian and Laird, 1986), visualising the results by a forest plot.

Heterogeneity

Between-study heterogeneity was assessed using the Cochran’s Q test with a significance level at $P<0.10$ (Hedges and Olkin, 1985). $I^2$ and its 95% CI was also evaluated to quantify the proportion of inconsistency across studies (Higgins et al., 2003).

Subgroup analysis

To test the robustness of the results against the effect of an individual study, and to investigate the sources of between-study heterogeneity, we performed subgroup analyses.

3. Results

Studies identified

Overall, the literature search initially identified four RCTs, representing data from 178 subjects (Figure 1 and Table 1):

1. Kaufmann et al. (2014), a DB-RCT in 60 male-to-female transsexual women with penile linked neovagina;
2. Anoshina (2016), an open label RCT in 60 pregnant women with herpes virus infection (HVI) plus 50 healthy controls;
3. Laue et al. (2018), a DB-RCT in 36 women with bacterial vaginosis;
4. Marschalek et al. (2017), a DB-RCT in 22 postmenopausal breast cancer patients receiving chemotherapy.

Only in the 3 DB-RCTs (1, 3 and 4) Nugent score was assessed. In the study of Anoshina (2016), 2×30 pregnant women with HVI have been examined. In these women, HVI and changes in vaginal acidity under the influence of gestation hormones as well as physiological immuno-suppression had led to vaginal microbial dysbiosis.
Table 1. Characteristics of the four RCTs reporting effects on vaginal dysbiosis of a mixture of four strains of lactobacilli selected for positive effects on vaginal microbiota.

<table>
<thead>
<tr>
<th>Source</th>
<th>Type of study</th>
<th>Study characteristics</th>
<th>Baseline values</th>
<th>Administration of the four <em>Lactobacillus</em> (L) strains</th>
<th>Results</th>
<th>Main outcome verum-control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kaufmann et al., 2014</td>
<td>DB-RCT</td>
<td>Male to female transsexual women with penile linked neovagina</td>
<td>41.2±1.3 Age (years) (mean ± SD) or median (IQR)</td>
<td>Oral capsules² (verum)</td>
<td>Δ Nugent score (before/after intervention)</td>
<td>BV incidence ↓; Nugent score ↓ (P&lt;0.05)</td>
</tr>
<tr>
<td>Anoshina, 2016</td>
<td>OL-RCT</td>
<td>Pregnant herpes-virus infected women (plus a control group of 50 healthy pregnant women)</td>
<td>No information presented</td>
<td>Oral capsules² (verum)</td>
<td>Δ <em>Gardnerella</em> (before/after intervention)</td>
<td>Lactobacilli ↑; pathogens ↓; BV symptoms ↓</td>
</tr>
<tr>
<td>Laue et al., 2018</td>
<td>DB-RCT</td>
<td>Women with 7d antibiotic-treated bacterial vaginosis</td>
<td>35.8±12.1 Age (years) (mean ± SD) or median (IQR)</td>
<td>In yogurt³ (2×125 g/d)</td>
<td>Δ Nugent score (before/after intervention)</td>
<td>BV ↓ (P&lt;0.05); Nugent score ↓ (n.s.)</td>
</tr>
<tr>
<td>Marschalek et al., 2017</td>
<td>DB-RCT</td>
<td>Postmenopausal breast cancer patients receiving chemotherapy</td>
<td>59(53-69) (verum) 62(50-77) (control)</td>
<td>Oral capsules² (verum)</td>
<td>Δ Nugent (verum) ↓ Δ Nugent (control) ↑ (P not shown)</td>
<td></td>
</tr>
</tbody>
</table>

1 DB/OL-RCT = double-blinded/open label randomised controlled trial; SD = standard deviation; IQR = interquartile range; (v) = verum group; (c) = control group; n/v/n = number of participants at baseline: all/verum group/control group; n.s. = not significant; BV = bacterial vaginosis.

2 *Lactobacillus crispatus* LbV88 (DSM 22566) 1×10⁹ + *Lactobacillus rhamnosus* LbV96 (DSM 22560) 1×10⁹ + *Lactobacillus jensenii* LbV116 (DSM 22567) 0.2×10⁹ + *Lactobacillus gasseri* LbV150N (DSM 22583) 0.3×10⁹ cfu/dose.

3 *L. crispatus* LbV88 (DSM 22566) 1×10⁹ + *L. rhamnosus* LbV96 (DSM 22560) 1×10⁹ + *L. jensenii* LbV116 (DSM 22567) 1×10⁹ + *L. gasseri* LbV150N (DSM 22583) 1×10⁹ cfu/ml yogurt.

4 Women had been selected for intermediate vaginal microbiota.
Unlike in the other three studies, the effects of one week administration of the probiotics capsules were not quantified on the basis of the Nugent score, but instead by measuring the change in concentration of individual bacterial species/groups in the intestine and vagina and on the basis of clinical manifestations of vaginal microbiota disorders. Therefore, the only link to the Nugent score was the assessment of cfu/ml of lactobacilli and Gardnerella. No microscopic counting of lactobacilli, Gardnerella and Bacteroides morphotypes in Gram-stained vaginal smears as in Nugent's scoring system was reported.

Since the relationship between the vaginal lactobacilli and Gardnerella cfu/ml and the Nugent score has not been investigated in this paper or elsewhere in the literature, and because we also were unable to reach the author and obtain the raw data of the study, we found no parameter common with the other studies to measure the effect size. Therefore, and also because it was not a double-blind RCT, we excluded this paper from the meta-analysis.

Study characteristics

The remaining three studies are DB-RCTs in which vaginal dysbiosis was measured (exclusively or inter alia) on the basis of the Nugent score. Their characteristics are outlined in Table 1. The studies are heterogenous (1) in the patients studied, (2) in the causes and extent of their vaginal dysbiosis, (3) in the mean Nugent score before treatment, (4) in the modalities and duration of treatment, and one may discuss, how well the Nugent score, which was ultimately developed to facilitate the diagnosis of bacterial vaginosis (BV), generally reflects the nature, strength, and changes in vaginal dysbiosis.

In particular the male-to-female transsexual women studied by Kaufmann et al. (2014) have a neovaginal microbiota which, on the one hand, has similarities with the vaginal microbiome in BV, but on the other hand differed significantly from the microbiota of a normal vagina, especially with respect to the very limited number of lactobacilli (Weyers et al., 2009, 2010). Therefore, the aim of the study was not the treatment of BV, but the shift of the (neo)vaginal microbiota towards a normal vaginal microbiota, as assessed by the Nugent score, by oral administration of a lactobacilli preparation (Petricevic et al., 2008; Reid et al., 2003). Exclusion criteria for this study were inter alia clinical signs of vaginal or urinary tract infection, abnormal neovaginal discharge, and antibiotic therapy in the four weeks before enrolment, so that the mean Nugent score of the women at the beginning of treatment had a relatively low value of 5.

Participants in the study by Laue et al. (2018) were middle-aged women with bacterial vaginosis (based on the Amsel criteria and Nugent scores) and consequently with an increased mean Nugent score of 8 before treatment. In contrast to the other two studies, the treatment period (28 days) was relatively long. Between days one and seven, the patients also received metronidazole for the treatment of BV, such that, the Nugent score also improved in the control group. A weakness of this study is that in the verum group the women were given the probiotics test product in yoghurt, while in the control group chemically acidified and curdled milk was given, hence, a possible effect of yoghurt bacteria cannot be controlled.

Marschalek et al. (2017) examined 22 postmenopausal breast cancer patients with vaginal atrophy (due to chemotherapy and oestrogen deprivation therapy), an intermediate vaginal microbiota and a Nugent score between 4 and 6 (which was one of the inclusion criteria). The author calculated the effect of the 14-day administration of the probiotics preparation on the Nugent score separately for the verum and the placebo group, without statistical comparison of both groups (we made this comparison, using the raw data, in the context of this meta-analysis).

Study quality

Regardless of quality losses due to the differences between the studies with regard to the tested subjects, study design and effect measure, the individual studies have some weaknesses (Table 2). In fact, all of them were pilot studies with a small number of subjects.

Publication bias

We included all published RCTs on the topic of the meta-analysis and were ascertained by the patent owner that no more studies were finalised with these strains. Because of this and the small number and manageability of the articles, we assume that a publication bias does not exist. This assumption is substantiated by the fact, that the funnel plots of mean study effects, i.e. standard error versus SMD or OR, are relatively symmetric (Figure 2). However, this finding is not very meaningful with only three papers included in the test (Sedgwick, 2013).

Meta-analyses of the effect of the lactobacilli preparation on Nugent score

The meta-analysis of the three DB-RCTs which were included in final statistical evaluation show a significant reduction of Nugent score by probiotics compared to placebo in the fixed (SMD -0.561; confidence interval (CI) -0.935 to -0.186; P=0.004) and random effect model (SMD -0.561; CI -0.935 to -0.186; P=0.004) (Table 3A and Figure 3A). The OR of the cases presenting with improved Nugent score after probiotics compared to placebo in the fixed (SMD -0.561; confidence interval (CI) -0.935 to -0.186; P=0.004) and random effect model (OR=3.936; CI 1.702 to 9.100; P=0.001) and random effect model (OR=3.902; CI
1.681 to 9.059; \( P = 0.002 \) (Table 3C and Figure 3B). There are no signs of heterogeneity between studies according to visual inspection of the forest plots (Figure 3A,B), as well as according to Cochran’s \( Q \) and to the \( I^2 \) statistic (inconsistency), neither when testing changes in Nugent score (\( Q=0.574, P=0.751, I^2=0.0\% \); Table 3B) nor OR (\( Q=0.245, P=0.885, I^2=0.0\% \); table 3d). Here heterogeneity is confirmed when \( P<0.1, Q>2 \) (df) and/or \( I^2 >>0\% \) (Higgins et al., 2003).

**Subgroup analyses**

To test firstly to what extent the specific conditions of an artificial (neo)vagina compared with a natural vagina had affected the results of the meta-analyses and secondly whether the weakness of the placebo group in the study of Laue et al. (2018) has had a significant effect on the results, we performed two subgroup analyses.

In the first subgroup analysis, we removed the study by Kaufmann et al. (2014) from the meta-analysis, in the second one, only the studies by Kaufmann et al. (2014) and Marschalek et al. (2017) were included. Table 4 and table 5 show the results of both analyses.

Despite the omission of the patient group with an artificial vagina, meta-analysis of the two remaining DB-RCTs still show a significant reduction of Nugent score by probiotics compared to placebo in the fixed (SMD -0.681; CI -1.222 to -0.139; \( P = 0.015 \)) and random effect model (SMD -0.681; CI -1.222 to -0.139; \( P = 0.015 \)) (Table 4A and Figure 4A). The OR of the cases presenting with improved Nugent score after probiotics compared to placebo show also a significant effect both in the fixed (OR=3.241; CI 1.051 to 9.992; \( P = 0.041 \)) and random effect model (OR=3.240; CI 1.051 to 9.992; \( P = 0.041 \)) (Table 4C and Figure 4B).

In the second subgroup analysis, the only study (Laue et al., 2018) that had been omitted had primarily investigated treatment options in BV patients, but suffered from the absence of yoghurt starter cultures in the placebo group. Nevertheless, meta-analysis of the two remaining RCTs once again shows the same significant reduction of Nugent score by test preparation compared to placebo in both models (SMD -0.550; CI -0.998 to -0.101; \( P = 0.017 \)) (Table 5A and Figure 5A). The OR of the cases presenting with
Table 3. Meta-analysis of the effects of a probiotic mixture of four *Lactobacillus* strains (*L. crispatus* LbV 88, *L. gasseri* LbV 150N, *L. jensenii* LbV 116 and *L. rhamnosus* LbV96) on (A) changes in Nugent score before and after treatment (continuous measure model) and (C) on the number of subjects with improvement in Nugent score (odds ratios) in women with vaginal dysbiosis. Total effects are calculated according to a fixed effect and a random effect model. Heterogeneity of underlying studies is given as well (B, D).

<table>
<thead>
<tr>
<th>A</th>
<th>Study</th>
<th>N1</th>
<th>N2</th>
<th>SMD</th>
<th>SE</th>
<th>95% CI</th>
<th>P</th>
<th>Weight (%)</th>
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<tbody>
<tr>
<td></td>
<td>Kaufmann et al., 2014</td>
<td>33</td>
<td>25</td>
<td>-0.445</td>
<td>0.265</td>
<td>-0.976 to 0.0855</td>
<td>0.265</td>
<td>50.94</td>
</tr>
<tr>
<td></td>
<td>Laue et al., 2018</td>
<td>16</td>
<td>17</td>
<td>-0.586</td>
<td>0.347</td>
<td>-1.295 to 0.122</td>
<td>0.265</td>
<td>29.60</td>
</tr>
<tr>
<td></td>
<td>Marschalek et al., 2017</td>
<td>11</td>
<td>11</td>
<td>-0.824</td>
<td>0.429</td>
<td>-1.718 to 0.0699</td>
<td>0.265</td>
<td>19.45</td>
</tr>
<tr>
<td></td>
<td>Total (fixed effects)</td>
<td>60</td>
<td>53</td>
<td>-0.561</td>
<td>0.189</td>
<td>-0.935 to -0.186</td>
<td>0.004</td>
<td>100.00</td>
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<tr>
<td></td>
<td>Total (random effects)</td>
<td>60</td>
<td>53</td>
<td>-0.561</td>
<td>0.189</td>
<td>-0.935 to -0.186</td>
<td>0.004</td>
<td>100.00</td>
</tr>
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</table>

B Q 0.5738
Significance level P = 0.7506
\(I^2\) 0.00%
95% CI for \(I^2\) 0.00 to 88.31

<table>
<thead>
<tr>
<th>C</th>
<th>Study</th>
<th>Intervention</th>
<th>Controls</th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>P</th>
<th>Weight (%)</th>
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<tbody>
<tr>
<td></td>
<td>Kaufmann et al., 2014</td>
<td>16/33</td>
<td>4/25</td>
<td>4.941</td>
<td>1.390 to 17.571</td>
<td>0.001</td>
<td>100.00</td>
</tr>
<tr>
<td></td>
<td>Laue et al., 2018</td>
<td>12/16</td>
<td>8/17</td>
<td>3.375</td>
<td>0.769 to 14.812</td>
<td>0.001</td>
<td>100.00</td>
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<td></td>
<td>Marschalek et al., 2017</td>
<td>7/11</td>
<td>4/11</td>
<td>3.063</td>
<td>0.539 to 17.402</td>
<td>0.001</td>
<td>100.00</td>
</tr>
<tr>
<td></td>
<td>Total (fixed effects)</td>
<td>35/60</td>
<td>16/53</td>
<td>3.936</td>
<td>1.702 to 9.100</td>
<td>0.001</td>
<td>100.00</td>
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<tr>
<td></td>
<td>Total (random effects)</td>
<td>35/60</td>
<td>16/53</td>
<td>3.902</td>
<td>1.681 to 9.059</td>
<td>0.002</td>
<td>100.00</td>
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</tbody>
</table>

D Q 0.2451
DF 2
Significance level P = 0.8846
\(I^2\) 0.00%
95% CI for \(I^2\) 0.00 to 72.63

Figure 3. Forest plots of (A) meta-analysis of standardised mean differences in Nugent score and (B) of the odds ratios of all included studies.
improved Nugent score after test preparation compared to placebo show a significant effect both in the fixed (OR=4.222; CI 1.524 to 11.698; \( P =0.006 \)) and random effect model (OR=4.184; CI 1.502 to 11.656; \( P =0.006 \)) (Table 5C and Figure 5B).

In none of the two subgroup analyses calculation of Cochran's Q or \( I^2 \) statistics showed evidence of heterogeneity between the remaining studies (Table 4B,D and 5B,D).

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**Table 4. Subgroup analysis. (A) Continuous measure, (C) odds ratio. (B,D) Test for heterogeneity.**\(^1\)

<table>
<thead>
<tr>
<th>A Study</th>
<th>N1</th>
<th>N2</th>
<th>SMD</th>
<th>95% CI</th>
<th>( P )</th>
<th>Weight (%)</th>
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</thead>
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<td>60.35</td>
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<td>Marschalek et al., 2017</td>
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<td>11</td>
<td>-0.824</td>
<td>-1.718 to 0.0699</td>
<td>39.65</td>
<td>39.65</td>
</tr>
<tr>
<td>Total (fixed effects)</td>
<td>27</td>
<td>28</td>
<td>-0.681</td>
<td>-1.222 to -0.139</td>
<td>0.015</td>
<td>100.00</td>
</tr>
<tr>
<td>Total (random effects)</td>
<td>27</td>
<td>28</td>
<td>-0.681</td>
<td>-1.222 to -0.139</td>
<td>0.015</td>
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</tr>
</tbody>
</table>

| B Q          | 0.1854 | 0.00% | 0.00 to 0.00 |
| Significance level | \( P =0.6667 \) | |
| \( I^2 \) (inconsistency) | |
| 95% CI for \( I^2 \) | |

<table>
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<tr>
<th>C Study</th>
<th>Intervention</th>
<th>Controls</th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>( P )</th>
<th>Weight (%)</th>
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<td></td>
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<td></td>
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<tr>
<td>Laue et al., 2018</td>
<td>12/16</td>
<td>8/17</td>
<td>3.375</td>
<td>0.769 to 14.812</td>
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<td>4/11</td>
<td>3.063</td>
<td>0.539 to 17.402</td>
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<tr>
<td>Total (fixed effects)</td>
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<td>3.241</td>
<td>1.051 to 9.992</td>
<td>0.041</td>
<td>100.00</td>
</tr>
<tr>
<td>Total (random effects)</td>
<td>19/27</td>
<td>12/28</td>
<td>3.240</td>
<td>1.051 to 9.992</td>
<td>0.041</td>
<td>100.00</td>
</tr>
</tbody>
</table>

| D Q          | 0.006967 | 0.00% | 0.00 to 0.00 |
| Significance level | \( P =0.9335 \) | |
| \( I^2 \) (inconsistency) | 0.00% | |
| 95% CI for \( I^2 \) | 0.00 to 0.00 | |

\(^1\) The study by Kaufmann et al. (2014) was omitted from this analysis.

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**Figure 4. Forest plots – subgroup analysis of (A) standardised mean differences in Nugent score and (B) odds ratios of the studies of Laue et al. (2018) and Marschalek et al. (2017).**

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Improvement of vaginal dysbiosis by probiotic lactobacilli

General outcome

Our meta-analysis of only three RCTs with 118 women included divided over verum and control group showed clear evidence that the oral intake of a probiotic mixture containing 4 Lactobacillus strains (L. crispatus LbV 88 (DSM 22566), L. gasseri LbV 150N (DSM 22583), L. jensenii LbV 116 (DSM 22567) and L. rhamnosus LbV96

Table 5. Subgroup analysis. (A) Continuous measure, (C) Odds Ratio. (B, D) Test for heterogeneity.¹

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<th>A</th>
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<td>11</td>
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<tr>
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<td>Total (fixed effects)</td>
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<td>36</td>
<td>-0.550</td>
<td>-0.998 to -0.101</td>
<td>0.017</td>
<td>100.00</td>
<td>100.00</td>
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<tr>
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<td>44</td>
<td>36</td>
<td>-0.550</td>
<td>-0.998 to -0.101</td>
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<td>100.00</td>
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<th>P</th>
<th>$I^2$</th>
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<th>C</th>
<th>Study</th>
<th>Intervention</th>
<th>Controls</th>
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<th>95% CI</th>
<th>P</th>
<th>Weight (%)</th>
<th>Fixed</th>
<th>Random</th>
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<td>1.390 to 17.571</td>
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<td>3.063</td>
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<td></td>
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<td>8/36</td>
<td>4.222</td>
<td>1.524 to 11.656</td>
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<table>
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<th>D</th>
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<td>0.1903</td>
<td>P=0.6627</td>
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</table>

¹ The study by Laue et al. (2017) was omitted from this analysis.

Figure 5. Forest plots – subgroup analysis of (A) standardised mean differences in Nugent score and (B) odds ratios of the studies of Kaufmann et al. (2014) and Marschalek et al. (2017).

4. Discussion

General outcome

Our meta-analysis of only three RCTs with 118 women

Beneficial Microbes

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A reduction of the Nugent score by 0.5 points is equivalent to 8.3% of the mean Nugent score (5.9 points) of all women in the three studies. The effect of the probiotics is therefore relevant taking the good correlation between Nugent score and the burden of vaginal dysbiosis and between Nugent score and the findings in molecular studies into account (Van de Wijgert and Jespers, 2017). In contrast, all the individual studies showed the same tendency, but were not or at most marginally significant: with one exception, their respective CIs included the zero effect values of Nugent score differences (0) or OR (1), respectively (Figure 3A,B).

The result of this meta-analysis is consistent with older studies, in which different Lactobacillus strains enabled alterations of the vaginal microbiota of healthy women (Reid and Bocking, 2003) or augmented the efficacy of metronidazole in Nigerian women with BV (Anukam et al., 2006).

The Nugent score does not imply symptoms and two of the studies (Kaufmann et al., 2014; Marschalek et al., 2017) were not targeting symptoms but only assessing the microbial pattern of vaginal smears by microscopy. The Nugent score, however, was shown to correlate with sequelae of vaginal dysbiosis and with findings in molecular studies (Van de Wijgert and Jespers, 2017) and is the gold standard in gynaecology for diagnosis of bacterial vaginosis (CDC, 2006; Sherrard et al., 2011), the most common form of vaginal dysbiosis, whereas Amsel criteria comprising symptoms, such as discharge and odour, were less predictive for bacterial vaginosis (Sha et al., 2005).

Heterogeneity

The unambiguosness of the result is surprising, taking into account that the three individual studies of this meta-analysis differ considerably in terms of study population, treatment and baseline values of the outcome measure: natural vagina versus neovagina, antibiotic treatment or not, proven bacterial vaginosis or dysbiosis with only an intermediate Nugent score. The heterogeneity tests (Figure 2A,B), however, reveal that there is very little heterogeneity between the three studies. From this the following conclusions may be drawn:

1. By choosing the two effect measures, namely (1) SMD of the Nugent score between baseline and first medical examination, and (2) OR of the cases presenting with improved Nugent score after probiotics compared to placebo treatment, as done in this meta-analysis, a certain normalisation is obtained. Thus, the meta-analysis is not based on absolute values, but rather on the alterations, i.e. differences between ‘before’ and ‘after’ treatment on a standardised scale.

2. The Nugent score measures a pattern of the vaginal microbiota even without a direct relation to bacterial vaginosis which confirms that Nugent score is a valuable measure for assessing vaginal dysbiosis independent from fulfilling the criteria for the diagnosis of bacterial vaginosis. This is in line with the good correlation between Nugent score and the burden of vaginal dysbiosis and between Nugent score and the findings in molecular studies (Van de Wijgert and Jespers, 2017). One, however, has to be aware of the fact that certain states of dysbiosis cannot be comprehended by Nugent score, e.g. candida vaginitis, which may be associated with unaltered abundance of lactobacilli (Van de Wijgert and Jespers, 2017; Van de Wijgert et al., 2014).

3. The probiotic shows similar effectiveness in a wide range of different vaginal microorganisms and vaginal environments. This can be understood if one keeps in mind, that the vaginal microbiota originates to a great extent from the gut (Antonio et al., 2005). Pathogenic bacteria infecting the female urogenital tract, as well as lactobacilli and other beneficial microorganisms, which contribute to the maintenance of a healthy vagina, emerge from the women’s intestinal microbiota (Hilton et al., 1992; Reid et al., 2001). It has been demonstrated that strains of L. rhamnosus and L. fermentum can be delivered to the vagina by oral consumption, leading to a significant increase in vaginal lactobacilli (Morelli et al., 2004; Reid et al., 2003).

Effect on neovaginal microbiota

A major objection against the present meta-analysis might be that the artificial (neo) vagina of the 60 transsexual women studied by Kaufmann et al. (2014) (that is 51% of the cases included in the meta-analysis) might not be a suitable model for the question under consideration, due to the considerable and possibly fundamental differences to the natural vagina. One of the most significant differences to the natural vagina is the (almost) complete absence of lactobacilli in the neovagina, because – beside other reasons – their neovaginal environment has been reported not to support the growth of lactobacilli (Weyers et al., 2009, 2010).
Another reason for this is that the microbial colonisation of the (natural) vagina and the neovagina occurs under completely different conditions. Acquisition of the vaginal microbiota occurs during and shortly after birth (Dominguez-Bello et al., 2010). The composition of early microbial communities of the vagina, intestines and other habitats of children shows similarities with the maternal microbiota of intestine (Ardissone et al., 2014) and vagina or skin, respectively, depending on whether the infant was delivered vaginally or by Caesarean section (Makino et al., 2013).

The following differentiation of the vaginal microbiota in childhood is not yet well understood Huang et al., (2014), but it has been shown that lactobacilli become a predominant genus in older girls, whereas in younger girls most frequently enteric organisms were isolated (Hammerschlag et al., 1978a). During adolescence substantial changes in the composition of the vaginal microbiota occur, which are driven by dramatic hormonal shifts. Increasing oestrogen levels during puberty increase glycogen deposition in the vaginal epithelium, increase glycogen-fermenting and lactic-acid producing bacteria, particularly lactobacilli, and decrease the vaginal pH (Paavonen, 1983), which is neutral or slightly alkaline during early childhood (Hammerschlag et al., 1978b). Other potentially relevant factors influencing vaginal microbiota are infections, birth control or sexual behaviours (Gajer et al., 2012; Mitchell et al., 2012; Witkin and Ledger, 2012).

These factors differ from those influencing the colonisation of the neovagina. Here, the bacteria for microbial colonisation of the neovagina derive mainly from the intestinal microbiota of a man (Reuter, 2001). Other factors related to surgical gender reassignment, such as antibiotic treatment before, during and after surgery, the tissue from which the neovagina was formed and its immunological and bacterial adhesion-related properties, or the time between surgery, healing of the neovagina and onset of microbial colonisation, affect vaginal colonisation as well.

Both the different composition of the intestinal microbiota as a source of vaginal bacteria and the considerably different environments of the neovagina and the natural vagina are reasons why the development of an adequate microbiota in the neovagina of transsexual women is delayed and disturbed. Therefore, oral administration of certain (probiotic) strains of lactobacilli surviving the gastrointestinal passage in sufficient quantity, might increase the reservoir of lactobacilli in the gut, which can colonise the neovagina or at least help to stabilise a healthy vaginal microbiota. The homogeneity of the results of the meta-analysis and the similarity of results after exclusion of single studies support the assumption that the suitability of the probiotic mixture considered here for improving the microbial pattern in vaginal dysbiosis also applies to the artificial (neo)vagina.

**Effect of yogurt culture**

Another fundamental objection against the present meta-analysis might be that the lack of a true placebo group in the study of Laue et al. (2018) could have led to a false-positive result not only of the study itself but also of the meta-analysis. Indeed, studies have shown that also yogurt cultures survive gastrointestinal passage (Elli et al., 2006), although at a low percentage. In the study of Laue et al. (2018), yogurt with live cultures was administered only in the verum group, while the control preparation contained chemically acidified milk without bacteria. These verum yoghurt cultures might have enhanced the favourable effect of the probiotic on bacterial vaginosis and microbial pattern, respectively.

Two findings argue against this objection. (1) In the meta-analysis of all three studies the mean effect size measured by Laue et al. (2018) lies between those found in the studies of Kaufmann (2014) and Marschalek et al. (2017) as shown by the forest plots of the SMD and OR (Figure 3A,B). (2) In the second subgroup analysis exclusion of the trial of Laue et al. (2018) did not change the results significantly. This means that the effect of the examined lactobacilli mixture on vaginal dysbiosis is significant irrespective of inclusion or exclusion of the study of Laue et al. (2018). The most likely explanation for this is that even if yoghurt bacteria should have reached the vagina and acidified the vaginal environment to a small extent, this effect was irrelevant or minor compared to that of the probiotic mixture. This is in agreement with the fact that L. crispatus, L. jensenii and L. gasseri dominate the vaginal microbiota whereas the yoghurt starter bacteria S. thermophilus and L. delbrückii ssp. bulgaricus are not found (Antonio et al., 1999; Aroutcheva et al., 2001; Fredricks, 2011; Fredricks and Giedler, 2005; Fredricks et al., 2007; Hyman et al., 2005; Ling et al., 2010; Oakley et al., 2008; Ravel et al., 2011; Srinivasan et al., 2012; Verhelst et al., 2004) and that the pertinent strains were selected from the vaginal environment and for their particular properties in maintaining the conditions of a healthy vaginal milieu (Domig et al., 2014).

**5. Conclusions**

This meta-analysis indicates that the oral intake of a probiotic containing L. crispatus LbV 88 (DSM 22566), L. gasseri LbV 150N (DSM 22583), L. jensenii LbV 116 (DSM 22567) and L. rhamnosus LbV96 (DSM 22560), either as yoghurt or in capsule form, may improve the microbial pattern (measured as Nugent score) in different forms of vaginal dysbiosis.
Conflicts of interest

M. de Vrese: no conflict of interest, C. Laue and E. Papazova: honoraria for consulting HSO GmbH, J. Schrezenmeir: Received honoraria for consulting HSO GmbH.

References


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Reid, G., Bruce, A.W., Fraser, N., Heinemann, C., Owen, J. and Henning, B., 2001. Oral probiotics can resolve urogenital infections. FEMS Immunology and Medical Microbiology 30: 49-52.


