UNIVESIDAD SAN FRANCISCO DE QUITO USFQ

Colegio de Ciencias Biológicas y Ambientales

Comparative effectiveness of treatments for Bacterial Vaginosis: Network Meta-analysis

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Ingeniería en Biotecnología

Trabajo de fin de carrera presentado como requisito
para la obtención del título de
Ingeniera en Biotecnología

Quito, 14 de mayo de 2021

UNIVERSIDAD SAN FRANCISCO DE QUITO USFQ

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HOJA DE CALIFICACIÓN
DE TRABAJO DE FIN DE CARRERA

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Quito, 14 de mayo de 2021

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Lugar y fecha: Quito, 14 de mayo de 2021

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RESUMEN

La vaginosis bacteriana (VB) es una disbiosis vaginal común en mujeres en edad reproductiva. Sin embargo, la tasa de curación de la VB varía considerablemente y muchas mujeres experimentan una recaída después del tratamiento inicial. El objetivo del presente metaanálisis fue evaluar las tasas de curación clínica (CCR) en ensayos controlados aleatorios (RCTs) a través de diferentes terapias y vías de administración. Este metaanálisis incluyó un conjunto final de 25 estudios elegibles con un total de 57 RCTs, que compararon la efectividad de los tratamientos de la VB entre mujeres embarazadas y no embarazadas. El rango inicial de CCR varió mucho de 46,75% a 96,20% y el CCR combinado final fue de 75,5% (IC: 69,4 a 80,8) utilizando un modelo aleatorio. Los índices de heterogeneidad fueron Q = 418,91, I^2 = 94,27% y τ = 0,7498 (p <0,0001). No se observó sesgo de publicación según la simetría del gráfico de embudo y la prueba de regresión lineal de Egger (p = 0,1097). Para evaluar diferentes variables, también se realizaron análisis de subgrupos, meta regresiones y metaanálisis en red. Las puntuaciones P más altas en CCR se obtuvieron mediante: (1) una terapia combinada con probióticos locales y la aplicación de antibióticos por ambas vías de administración (puntuación P = 0,98); (2) una terapia combinada con administración local de antibióticos y probióticos (puntuación P = 0,86); (3) y un tratamiento probiótico local (puntuación P = 0,59). No fue posible tomar una decisión clara sobre el mejor tratamiento para la VB debido a la heterogeneidad de los resultados informados en los ensayos, lo que indica la necesidad de una mejor caracterización de los RCTs. La combinación de *L. acidophilus* con antibiótico evidenció una CCR más alta en los tratamientos de VB, mientras que la combinación de *L. gasseri* y *L. rhamnosus* con terapia antibiótica mostró CCRs significativamente menores. Finalmente, las terapias combinadas sugirieron la reducción de la concentración óptima de antibióticos y los tratamientos de antibióticos de doble fase indicaron un incremento de CCR en la VB.

Palabras Clave: Vaginosis bacteriana, antibiótico, terapía combinada, ensayos controlados aleatorios, metaanálisis.

ABSTRACT

Bacterial vaginosis (BV) is a common vaginal dysbiosis in women of reproductive age. However, the cure rate for BV varied considerably and many women experience a relapse after the initial treatment. The aim of the present meta-analysis was to evaluate the clinical cure rates (CCRs) in randomized controlled trials (RCTs) through different therapies and administration routes. This meta-analysis included a final set of 25 eligible studies with a total of 57 RCTs, comparing the effectiveness of BV treatments among non-pregnant and pregnant women. The initial range of CCRs varied greatly from 46.75% to 96.20% and the final pooled CCR was 75.5 % (CI: 69.4–80.8) using random model. The heterogeneity indices were Q = 418.91, I^2 = 94.27%, and τ = 0.7498 (p<0.0001). No publication bias was observed according to Funnel plot symmetry and Egger's linear regression test (p=0.1097). To evaluate different variables, subgroup analysis, meta-regressions, and network meta-analysis were also realized. The highest P-scores in CCR were obtained by: (1) a combined therapy with local probiotic and application of antibiotics by both administration route (P-score= 0.98); (2) a combined therapy with local administration of antibiotic and probiotic (P-score= 0.86); (3) and a local probiotic treatment (P-score= 0.59). A clear-cut decision of the best BV treatment was not possible due to the heterogeneity of outcomes reported in the trials, indicating the necessity to a better characterization of RCTs. The combination of *L. acidophilus* with antibiotic evidenced higher CCR in BV treatments, while the combination of *L. gasseri* and *L. rhamnosus* with antibiotic therapy showed significantly lower CRCs. Finally, combined therapies suggested the reduction of the optimal concentration of antibiotics and double phase treatments of antibiotics indicated an increment of CCRs in BV.

Key words: Bacterial vaginosis, Probiotic, Antibiotic, Combined therapy, Randomized controlled trials, Meta-analysis

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INTRODUCTION

The healthy vaginal microbiota of reproductive age women is composed by many bacteria species, such as *Lactobacillus* sp. *Lactobacillus* species are described as the main genus in healthy vaginal microbiota, being *L. crispatus, L. gasseri, L. jensenii* and *L. inners* reported as the numerically dominant microorganisms in most women (refs). However, little is still known about the diversity of species among women and their variability according to ethnicity and geographical ubication (Borges et al., 2014; Ma et al, 2013). Due to numerous factors among women, the vaginal microbiota is dynamic system and therefore it is possible to find different genus in the vaginal epithelia from aerobic and anaerobic bacteria.

It is well-known that *Lactobacillus* species are able to protect the vaginal epithelia against pathogens through the production of lactic acid, bacteriocins and H_2O_2 (Kumar et al., 2011). Also, biofilm formation of healthy microbiota contributes to the mucous protection by the specific recognition of adhesines to the epithelium (Martín et al., 2008; Romero & Andreu, 2016). However, when healthy microbiota is disrupted, pathogens may then proliferate and eventually substitute healthy microorganisms, thus causing a dysbiosis by the overgrowth of pathogens (Chee et al., 2020). A well-known vaginal dysbiosis is bacterial vaginosis (BV), where some fastidious bacteria are able to overgrowth lactobacilli and establish a pathogenic biofilm in vaginal epithelia, such as *Gardnerella vaginalis* (Gupta et al., 2017). BV is a common cause of dysbiosis, showing with a prevalence of 29% among women in USA and a prevalence of 4 to 14% among women in Europe (Romero & Andreu, 2016). This dysbiosis affects mostly women in reproductive age. Although the predominant microorganism is *G. vaginalis,* other anaerobic bacteria are also associated with BV establishment, such as *Mobiluncus mulieris, Atopobium vaginae, Prevotella bivia, Fusobacterium nucleatum, Mycoplasma hominis and Ureaplasma urrealyticum* could be present (Romero & Andreu, 2016). Several risk factors are usually associated with BV development, such as various sexual

partners, pregnancy, douching, use of contraceptives, cigarette smoking, and many others (Kumar et al., 2011). In addition, BV women are more prone to suffer of sexual transmission infections, such as HIV and pelvic inflammatory disease. Meanwhile, pregnant women could also be more susceptible to have a pre-term labor or postpartum infections (Jones, 2019; Kamga et al., 2019).

Nowadays, there are different methods to diagnose bacterial vaginosis in women, such as Nugent Score and Amsel criteria. Amsel criteria is the main diagnostic methods among clinical physicians due to its simplicity, being able to diagnose BV in women when 3 of 4 symptoms are positive. More exactly, Amsel criteria evaluated (1) the presence of grayish color associated with vaginal discharge, (2) vaginal pH superior than 4.5, (3) positive whiff test (presence of amine odor with the application of 10% potassium hydroxide to the wet sample), and (4) the presence of clue cells (vaginal epithelial cells coated with anaerobic bacteria). Other well-known diagnostic method for BV is Nugent score, being mainly used by research and technician diagnostic laboratories, Nugent score requires a Gram stain evaluation of the vaginal swab. According to the presence or absence of *Lactobacillus* species and Gram-variable microorganisms the vaginal sample, the microbiologic evaluation is scored between 0-10, being classified as normal healthy microbiota (0-3), intermediate microbiota (4-6) and BV (7-10). The BV score is characterized by the absence of *Lactobacillus* and the overgrowth of *G. vaginalis* and *Mobiluncus* sp. (Money, 2005).

The golden standard treatment for BV includes the administration of 500 mg of oral metronidazole and other nitroimidazoles (such as tinidazole and clindamycin) by oral or local administration. Local administration routes may vary through gel, cream or ovules applications. However, probiotics have also been evaluated as plausible BV treatment and several studies reported positive outcomes by itself and also combined with antibiotics (Jones, 2019; Kovachev, S., & Vatcheva-Dobrevski, 2013; Larsson et al., 2011).

The aim of the present study was to evaluate the effectiveness of treatments for BV after an initial therapy on women, analyzing the efficiency and significant differences between therapies and administration routes. Therefore, clinical cure rates (CCRs) of different clinical treatments were collected and the evaluated. These randomized controlled trials (RCTs) were based on BV treatments with antibiotics, probiotics, and combined therapies from published studies around the world. This study attempted to obtain a general picture of the effectiveness and trends among BV treatments through meta-analysis.

METHODS

Data selection, search strategy and study guidelines

This study was conducted following Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) strategies (Liberati et al., 2009). Scopus, PubMed, and Cochrane Library databases were searched for English papers using the following medical subject heading terms (MESH): *"*bacterial vaginosis"; "treatment"; "probiotic"; "antibiotic"; and, "cure rate". No restrictions on year of study or participants' ages were imposed.

In each electronic database, a combination of MESH terms was used to conduct the search applying the following strategy (for example, in the MEDLINE): ''("Bacterial Vaginosis") AND (Treatment) AND ("Cure rate")''. All studies published until, 30th December 2020, were retrieved. The articles reporting the clinical cure rate, type of treatment, administration route and place of study were included. The references of all included studies were also checked for finding additional records. The search was limited to human clinical control trials. All references were compiled into a database Mendeley Library, then managed using Excel.

Screening process

Duplicates were initially identified and eliminated in Mendeley after entering all the recognized studies into an Excel self-created database. All articles were assessed by Alison Melissa Munoz Barreno (AMM-B) by screening titles, abstracts, topics, and finally full texts. An additional examination of the selected articles was realized by a second Antonio Machado (AM) focused on the homogeneity of the eligibility criteria of both reviewers in the initial data set. Discrepancies were resolved by discussion between all authors before finalizing the records for the evaluation of eligibility criteria.

Eligibility criteria

Reviews, editorials, congress or meeting abstracts, literatures in languages other than English, case reports, clinical trials and letters to editors were excluded of the final data set. Duplicate reports on different databases, and studies with unclear and missing data were also omitted.

Data extraction and quality assessment

Methodological quality assessment of the studies was performed using a checklist for necessary items as outlined in the Critical Appraisal Skills Programme (CASP) checklists (Zeng et al., 2015). For each article, a series of critical questions was asked. If the pertinent data was given, the question was scored as ‘‘yes.’’ If there was any doubt or no information in the study, that question was marked as ‘‘no’’. A data extraction form was designed to extract the relevant characteristics of each study. The extracted information included the authors’ names, time of the study, year of publication, location, sample size, clinical curation rate, and type of treatment (such as, antibiotic, probiotic, and conjugate). The first author (AMM-B) extracted all data, further confirmation and final evaluation were realized by AM, Eduardo Terán (ET) and Fausto Sebastián cabezas Mera (FSC-M)

Data analysis and statistical methods

Meta-analysis was performed using the RStudio software (Version 1.4.1103; https://rstudio.com/), using several R packages (meta, metafor, dmetar, poibin, stringr and netmeta). The clinical cure rate was computed, and values reported with confidence intervals (CI) of 95%. The heterogeneity was assessed by the Cochrane Q and I^2 tests. Considering the heterogeneity indices, the random-effects model was used, the logit transformation was applied to calculate the pooled frequencies. Subgroup analyses and meta-regressions were performed according to type of treatment, pregnancy status, and geographic distribution. Outliers’ analysis was done with Baujat diagram. Egger test, funnel plot and *p*-curve analysis were used to

explore publication bias. As recommended by Sterne and colleagues (Sterne et al., 2011), funnel plot asymmetry tests were only performed when the number of studies were at least ten ($k \geq 10$). All *p*-values <0.05 were considered as statistical significance threshold, with the exception of Egger's test (<0.10) (Song et al., 2002). A network meta-analysis was used to compare the efficacy of all pairs of interventions that included placebo, antibiotic, probiotic, and conjugate or combined treatments. Random effects model was used in subgroup analyses. Odds Ratios (OR) were used to report the effect size for assessing efficacy. Also, inconsistency between direct and indirect evidence was evaluated based on the Z test and provide a *p*-value to indicate inconsistency ($p < 0.05$). Treatment efficacy rank was determined by P-scores in a manner that the larger P score suggested a better treatment based on efficacy.

RESULTS

Study inclusion criteria and characteristics of the eligible studies

A total of 658 studies were retrieved and 72 full texts were reviewed. Twenty-nine studies met our inclusion criteria. The final data set include studies covering different global regions (most of them in Europe). All available and relevant data was extracted of each study, more exactly, type of treatment, route of administration, clinical cure rate, reinfection rate, and pregnant or non-pregnant state. This data was then used to create another file base, selecting only information reported in five or more papers, and consequently each paper was cited more than once (Appendix A). A total data set of 27 studies was obtained for the present meta-analysis following the eligibility criteria, screening process, and quality assessment, being further processed to evaluate CCR reports.

Overall efficiency of bacterial vaginosis treatments

The data set reported CCR of bacterial vaginosis treatments between 2000 and 2018 in several countries worldwide. As shown in Table 1, the values of CCR varied greatly from 46.75% to 96.20% among eligible studies. Different types of treatment also described, evaluating the exclusive therapy by antibiotics (AB:23/27) or probiotics (PB: 6/27), and even combined therapies (AB+PB: 11/27) (Table 1).

Most of the data set belonged to studies realized in Europe (14/27), followed by Asia (5/27), America (4/27), Africa (3/27), and finally Oceania (1/27). However, three fourths of the studies in America belonged to United States of America (USA) and just one study was from Brazil. Likewise, three fifths of the studies in Asia belonged to India and two thirds of the studies in Africa were from Nigeria. Finally, four studies in our data set reported CCR of bacterial vaginosis treatments among pregnant women.

After removing the two outliers of the initial data set of 27 studies (Bradshaw et al., 2012; Schwebke & Desmond, 2015), the final pooled clinical cure rate was 75.5 % (CI: 69.4–80.8) and the heterogeneity indices computed using random model were: Q = 418.91, I2 = 94.27%, and $\tau = 0.7498$ ($p<0.0001$), as shown in Figure 1.

Funnel plot was then realized to evaluate the existence of publication bias in the final data set (Figure 2). Egger's linear regression test was also used to reveal any publication bias and possible asymmetric data distribution in the selected studies. No publication bias was observed according to Funnel plot symmetry and Egger's linear regression test ($p=0.1097$).

Also, to evaluate different variables in the effectiveness of BV treatment, subgroup analysis, meta-regressions, and network meta-analysis were realized among our data set. However, the presence of publication bias could lead to data mining and so an evaluation of p-curve was realized (Figure 3). The p-curve analysis supports the absence of publication bias in our overall and subgroup results. The detection of p-hacking allowed to observe the distribution of statistically significant p values in our data set.

Effectiveness of BV treatment types, administration routes and pregnancy state

The effectiveness of BV treatment between antibiotic, probiotic and conjugate or combined therapies was evaluated through subgroup analysis. As shown in Table 2, 57 randomized controlled trials (RCTs) were considered from our final data set for the evaluation of the CCR among different treatment types. Although the CCRs of probiotic therapy overpassed the effectiveness of both antibiotic and conjugate or combined therapies, no statistically significant difference was obtained among the pooled CCR between treatment types ($p=0.845$).

No publication bias was found in the evaluated subgroups according to Egger's linear regression test among conjugate or combined therapy. However, it was not possible to apply Egger's linear regression test in probiotic therapy due to the low number of trials ($k\leq 10$). Also,

antibiotic therapy showed a low *p*-value (*p*=0.091) compiling CCRs of 35 trials, where it was possible to detect some heterogeneity among the results. Also, the regression model for this moderator did not explain any of the variability among the result tests. Further evaluation of the administration routes and pregnancy state among the pooled CCRs were realized by meta-regression. Meta-regression models revealed no significant association between pregnancy and CCR (beta (β) = 0.2250, *SE* = 0.3384, *p* = 0.5060), neither between administration routes of different types of treatment and CCR (*p*=0.5248). However, in the pregnancy subgroup (k = 8), the CCR was higher with the oral administration when compared to local application (88.2% *versus* 64.4%, respectively), but it was not statistically significant (*p* = 0.0797).

Network analysis

The studies selected for the network meta-analysis showed comparisons between placebo and treatments or between treatments (see Figure 4). The antibiotic treatments (AB) included 5-nitroimidazoles derivatives or clindamycin while the probiotic treatments (PB) included different lactobacilli, such as *Lactobacillus reuteri, L. gasseri, L. acidophilus, L. rhamnosus, L. brevis, L. salivarius, L. plantarum, L. fermentum,* and combinations between them. We classified the different therapies according to treatment type (AB or PB) and administration route (oral and local) by itself or combined therapies to avoid the generation of sub-networks. Different treatments have been compared to placebo in many trials, appointing two therapies ("*Oral AB & Local AB & Local PB*" and "*Oral AB & Oral PB*") as more far from control ("*placebo*") (Figure 5). It is important to note that there are no multi-arm trials (trials with more than two arms) in our network avoiding inference and incorrect correlations. Further evaluation was realized through P-scores, allowing to generate a ranking of treatments from most to least beneficial among patients accordingly to CCRs. These P-scores measures the certainty that one treatment is better than another treatment and averaged over all competing treatments. The highest P-score (Table 3) was also achieved by the combined therapy of antibiotic by both

administration routes plus local probiotic (*Oral AB & Local AB & Local PB*, P-score= 0.9758), followed by oral administration of antibiotic and probiotic (*Oral AB & Oral PB*, P-score= 0.8645), and local probiotic (*Local PB*, P-score= 0.5890). These results appointed to a better effectiveness from orally combined therapies and local administration of probiotics. However, when comparing the effectiveness outcomes between different treatments and placebo in trials, it was possible to observe treatments with considerable overlapping confidence intervals (Figure 6).

Evaluation of probiotic therapy in BV treatment

In the data set, the probiotic treatments contained a greater variability of different lactobacilli, when compared to antibiotic treatments (nitroimidazoles derivatives or clindamycin). These probiotic lactobacilli were evaluated by itself or combined with other probiotic species or antibiotics. The number of lactobacilli species or strains showed statistically significant differences in the CCRs of BV treatment (p<0.0001). Probiotic or combined therapies containing with one or two lactobacilli demonstrated similar high CCRs and no statistically significant difference between them (p=0.4455). However, CCR in BV treatment dropped in studies using three probiotic lactobacilli. Several combinations of two and three lactobacilli species were evaluated among trials, being *L. rhamnosus, L. reuteri, L. acidophilus,* and *L. gasseri* the most frequently used species. However, no statistically significant differences were found among a specific combination of two and three lactobacilli. Furthermore, when analyzing lactobacillus species individually, the absence of *L. gasseri* in the probiotic administration and the co-use of antibiotics with *L. acidophilus* showed higher CCRs in BV treatment demonstrating statistically significant differences, more exactly, p=0.0051 and p<0.0001, respectively. Finally, no correlation was found among CCRs of the remaining lactobacilli species (Table 4).

DISCUSSION

This meta-analysis included a final set of 25 eligible studies with a total of 57 randomized controlled trials (RCTs), comparing effectiveness of different types of BV treatments including non-pregnant and pregnant women. All treatments evaluated the CCRs after an initial treatment. The CCRs differences were analyzed between treatments (antibiotics, probiotics, and conjugates) and routes of administration (oral and local), assessing therapies with higher effectiveness in BV treatment.

Effectiveness of BV treatments among women

Initially, the highest CCRs in our data set were achieved by Hantoushzadeh et al. (96.20%) and Raja et al. (93.86%) among pregnant and non-pregnant women, respectively. In Iran, Hantoushzadeh et al. applied two different treatments in each group set of 250 pregnant women involving one probiotic treatment with the consumption of a mixed-lactobacilli yogurt and another antibiotic treatment with an oral ingestion of clindamycin (Hantoushzadeh et al., 2012). No statistically differences were found among these treatments and both showed CCRs above 90%. While, in India, Raja et al. applied an oral antibiotic treatment with metronidazole and tinidazole (Raja et al., 2016). However, a further evaluation of the 57 RCTs in our data set was realized through network meta-analysis allowing to identify certain therapies with better effectiveness in BV treatment. The best CCRs based on P-scores were an *Oral AB & Local AB & Local PB* (P= 0.9758) and *Oral AB & Oral PB* (P=0.8645). The first type of treatment combined an antibiotic orally administrated with a local administration of an antibiotic (1000 mg metronidazole) and a vaginal cream with probiotic lactobacilli, more exactly, *L. acidophilus* and *L. rhamnosus* (1.00E+09 CFU) (Kovachev, S., Dobrevski-Vacheva, 2013). The second type of treatment administrated an oral antibiotic (tinidazole, metronidazole, ofloxacin and ornidazole) with an oral probiotic, more exactly, *L. rhamnosus* GR-1 and *L. reuteri* RC-14 (1.00E+09 CFU) (Anukam et al., 2006; Martinez et al., 2009) or *B. coagulans* (Ratna Sudha et

al., 2012). Anukam and colleagues applied in patients a combined therapy with oral metronidazole and oral *L. rhamnosus* GR-1 plus *L. reuteri* RC-14, while Martinez and colleagues administrated a single dose of tinidazole supplemented with two capsules containing *L. rhamnosus* GR-1 and *L. reuteri* RC-14 every morning for 4 weeks. Finally, Ratna Sudha and colleagues assigned a dose of antibiotic therapy (Ofloxacin–Ornidazole with vaginal co-kimaxazol peccaries) simultaneously with two probiotic capsules (1.00E+09 CFU of *Bacillus coagulans*

On the other hand, the lowest average of CCR among pregnant woman in our data set was reported by Darwish et al. (58.33%), which included four different treatments for pregnant woman (Darwish et al., 2007), more exactly: two oral treatments with metronidazole and clindamycin and two local treatments with local metronidazole an clindamycin. Meanwhile in non-pregnant group the lowest CCR was reported by Larsson et al. (46.85%), which included a combined treatment with oral clindamycin and local metronidazole plus the interaction of different strains of lactobacilli such as *L. rhamnosus, L. jensenii, L. gasseri and L. crispatus.* (Larsson et al., 2011).However, low CCRs in BV treatment was also detected in non-combined therapies through network meta-analysis. Based on P-scores, some low CCRs were found in certain treatments of oral administration of antibiotics (*Oral AB*, P=0.3780) and local administration of antibiotics (*Local AB*, P=0.2536) when compared to placebo. Although general results in network meta-analysis indicated local administration route as preferential therapy for probiotic treatment, the average CCR was higher with oral administration when compared to local application among pregnant women despite no statistically significant differences were found.

When analyzing the RCTs among pregnant women, the difference in both CCRs could be attributed to an oral probiotic treatment used by Hantoushzadeh et al. (Hantoushzadeh et al.,

2012). This study reported the best CCR among the subgroup set of trials on pregnant women, where Hantoushzadeh and colleagues administrated a probiotic yogurt (100 g twice a day for one week) containing *Lactobacillus bulgaricus, Lactobacillus acidophilus,* other probiotic lactobacilli, *Streptococcus thermophilus* and *Bifidobacterium lactis* (Hantoushzadeh et al., 2012). This probiotic yogurt was chosen due to the persistence of its probiotic bacteria in gastrointestinal tract (resistance against bile and gastric acid), and its similarity to the common yoghurts consumed in daily life.

Characterization of the lactobacilli species in probiotic therapies

Despite of the diversity among probiotic treatments, most therapies used of *Lactobacillus* species in the treatment of bacterial vaginosis through oral and local administration routes. As previously referred, our data set showed that these probiotic lactobacilli can be applied by itself or combined with antibiotics or other probiotic species (such as, *Bacillus coagulans, Streptococcus thermophilus* and *Bifidobacterium lactis*) (Darwish et al., 2007; Hantoushzadeh et al., 2012; Larsson et al., 2011; Ratna Sudha et al., 2012). Several lactobacilli species were evaluated among the 57 RCTs of this meta-analysis, such as, *L. acidophilus, L crispatus, L. rhamnosus, L. reuteri, L. delbrueckii, L. gasseri, L. fermentum, L. brevis, L. salivarus* and *L. plantarum.* Although our subgroup analysis reported non statistical difference between treatments, the highest CCR was shown by probiotic treatment. Therefore, network metanalysis was realized to identify treatments with higher effectiveness in BV treatment when compared to placebo assays (control). According to P-scores, treatments with local probiotic application have higher P-score (P=0.5890) when compared to exclusively oral (P=0.3780) and local (P=0.2536) application of antibiotics. Based on this information it is important to characterize the lactobacilli species in probiotic and combined treatments. Although the number of lactobacilli on probiotic treatments showed statistically significant differences in the CCRs (p<0.0001), this evaluation only considered *Lactobacillus* species. Probiotic products with one

or two lactobacilli demonstrated higher CCRs in BV treatment. Also, combined therapies between antibiotics and two lactobacilli demonstrated high CCRs in BV treatment, such as *L. acidophilus* plus *L. rhamnosus* (79.7%) and *L. rhamnosus* plus *L. reuteri* (85.1%). In 2013, Kovachev and Dobrevski-Vacheva successively treated BV women with 600 mg of oral clindamycin, 1000 mg of local metronidazole, and local application of *L. acidophilus* plus *L. rhamnosus* (1.00E+09 CFU) achieving a CCR of 87.5% (Kovachev, S., Dobrevski-Vacheva, 2013). Meanwhile, the effectiveness of the probiotic treatment with *L. rhamnosus* plus *L. reuteri* was evaluated by itself (Vujic et al., 2013) and combined with antibiotic treatment (tinidazole and metronidazole) through local and oral administration routes (K. Anukam et al., 2006; K. C. Anukam et al., 2006; Martinez et al., 2009). Once again, the CCRs of the combined therapies (87.5-90.0%) surpassed the CCRs of the monotherapies with these lactobacilli combination (61.5%), showing better outcomes when the probiotic treatment was applied through local administration route. It is also important to mention that *L. gasseri* was present in 5 of 7 trials in combination with metronidazole and clindamycin as an aggressive treatment against BV (Larsson et al., 2011). However, Larsson and colleagues reported low CCRs in BV treatment (55.6%). Likewise, the probiotic combination of *L. rhamnosus* and *L. gasseri* showed the lowest CCR (63.0%) in our data set among combinations with two lactobacilli (Table 4). Finally, statistically significant differences were found (p=0.0051) between the presence and the absence of *L. gasseri* in RCTs for BV treatment, showing a greater CCR among RCTs without this species. Another significant p value was reported between the presence and the absence of *L. acidophilus* among combined therapies with antibiotics ($p< 0.0001$), evidencing higher CCRs in treatments with *L. acidophilus* (90.4%). However, further studies should evaluate the effectiveness of treatments with *L. acidophilus* plus antibiotics among BV women.

CONCLUSIONS

The present meta-analysis allowed to characterize patterns of CCRs in BV treatment and consequently to identify better therapies. However, a clear-cut decision of the best BV treatment was not possible due to the heterogeneity of outcomes reported in the trials, indicating the necessity to a better characterization of RCTs. Nonetheless, Network meta-analysis allowed to identify certain BV treatments with high CCRs among women. More exactly, certain combined therapies (such as, (1) local probiotic and application of antibiotics by both administration route, and (2) local administration of antibiotic and probiotic) outperformed monotherapies. In addition, the increase of the number of lactobacilli species does not rise the cure rate among probiotic or combined therapies. The presence of *L. acidophilus* in treatments with antibiotics showed a higher cure rate in BV treatment, while *L. gasseri* together with antibiotics evidenced low CCRs among BV women. Numerous factors, such as concentration, administration route, antibiotic, *Lactobacillus* species, and possible combinations, are key to the effectiveness in BV treatment. Future studies should analyze new combination therapies, different lactobacilli strains and probiotic potential of other microorganisms.

TABLES

Table 1: General information extracted from the data set selected to the present meta-analysis.

First author, year	Region	Country	Pregnancy	Clinical cure rate (%)	Treatment assays*
Raja, 2016	Asia	India	No	107/114 (93.86)	AB
Darwish, 2007	Africa	Egypt	Yes	91/156 (58.33)	AB
Ling, 2012	Asia	China	No	45/55 (81.81)	AB, PB
Larsson, 2008	Europe	Norway	No	24/37 (64.86)	AB+PB
Kekki, 2002	Europe	Finland	Yes	123/187 (65.77)	AB
Martínez, 2009	America	Brazil	No	44/64 (68.75)	AB, AB+PB
Voorspoels, 2002	Europe	Belgium	No	49/76 (64.47)	AB
Brandt, 2008	Europe	Germany	No	240/263(91.25)	AB
Schwebke, 2011	America	USA	No	168/287 (58.53)	AB
Thulkar, 2012	Asia	India	No	304/344 (88.37)	AB
Eriksson, 2005	Europe	Sweden, Finland & Norway	No	111/187 (59.35)	AB, AB+PB
Schwebke, 2015	America	USA	No	144/308 (46.75)	AB
Paavonen, 2000	Europe	Europe	No	172/233(73.82)	AB
Kurkinen, 2000	Europe	Finland	Yes	54/62 (87.09)	AB
Sobel, 2001	America	USA	No	270/342 (78.94)	AB
Larsson, 2011	Europe	Sweden	No	35/63 (55.55)	AB+PB
Hantoushzadeh, 2012	Asia	Iran	Yes	481/500 (96.20)	AB, PB
Kovachec, 2013a	Europe	Bulgaria	No	485/539 (89.98)	AB, AB+PB
Kovachec, 2013b	Europe	Bulgaria	No	224/381 (58.79)	AB, PB, AB+PB
Vujic, 2013	Europe	Croatia	No	243/395 (61.52)	PB
Anukam, 2006a	Africa	Nigeria	No	30/40 (75.00)	AB, PB
Bradshaw, 2012	Oceania	Australia	No	381/408 (93.38)	AB, AB+PB
Anukam, 2006b	Africa	Nigeria	No	82/106 (77.35)	AB, AB+PB
Mastromarino, 2009	Europe	Italy	No	12/18 (66.66)	PB
Marcone, 2008	Europe	Italy	No	63/84 (75.00)	AB, AB+PB
Ratna, 2011	Asia	India	No	25/40 (62.50)	AB, AB+PB
Bohbot, 2018	Europe	France	No	52/76 (68.42)	AB, AB+PB

* AB: Antibiotic, PB: probiotic, AB+PB: Conjugate or combined therapies. Clinical cure rate was calculated with 95% CI through random-model and significance level ≤0.05 (*p*-value). The sample size and prevalence were used to calculate the combined clinical cure rate. The complementary proportion of each study was considered as reinfection or non-cure.

Table 2: Pooled CCR of treatments for bacterial vaginosis.

Treatment type	k=57 (trials)	Clinical cure rate (95% CI)	Egger's test	Random effects model			
			*p**	t	Q	I^2	*p*
Only Antibiotics	35	74.6 (69.1 – 79.3)	0.091	0.7396	283.42	88.0	0.8453
Conjugate (antibiotic + probiotic)	16	74.1 (63.1 - 82.7)	0. 296	0.9101	89.10	83.2	
Only Probiotics	6	79.7 (59.3 – 91.4)	-	1.1347	105.03	95.2	

The trials considered (k = 57) from 25 studies. * Test for subgroup difference Egger's test was not realized for treatments with less than 10 trails (k<10) due to lack of statistical power in the detection of publication bias.

Table 3: P-scores ranked from different types of treatments**.**

Treatment	P-score
Oral AB & Local AB & Local PB	0.9758
Oral AB & Oral PB	0.8645
Local PB	0.5890
Local AB & Local PB	0.5607
Oral AB & Local PB	0.4533
Oral PB	0.4206
Oral AB	0.3780
Local AB	0.2536
Placebo	0.0077

Table 4: Subgroup analysis of the efficacy in BV treatment with probiotic lactobacilli.

Number of lactobacilli species [a]	k	CCR (95% CI)	Random effects model			
			t	Q	I^2	*p**
1[b]	4	82.6 (74.5-88.5)	0	2.89	0	**<0.0001**
2	10	77.3 (62.9-87.3)	1.0376	145.15	93.8	
3	7	56.5 (48.5-64.2)	0	3.72	0.0	
Combinations (2 strains)						
L. rhamnosus + L. acidophilus	3	79.7 (37.4-96.3)	1.6510	115.94	98.3	0.2413
L. rhamnosus + L. gasseri	2	63.0 (48.3-75.6)	0	0.27	0.0	
L. rhamnosus + L. reuteri	5	80.8 (62.0-91.6)	0.9469	23.62	83.1	
Combinations (3 strains)						
L. crispatus + L. gasseri + L. jensenii	2	45.9 (22.5-71.4)	0.4557	1.52	34.4	0.6728
L. rhamnosus + L. gasseri (2 strains)	2	61.3 (33.9-83.1)	0	0.32	0.0	
Other [c]	3	57.9 (48.9-66.5)	0	0.71	0.0	
Includes *L. rhamnosus*?						
No	7	70.0 (53.9-82.4)	0.7112	15.14	60.4	0.6010
Yes	14	74.8 (63.1-83.7)	0.9333	155.50	91.6	
***L. rhamnosus* with antibiotics?**						
Yes	11	77.4 (64.4-86.7)	0.9548	74.43	86.6	0.1323
No	3	61.3 (42.0-77.5)	0.6031	21.55	90.7	
Includes *L. reuteri*?						
No	15	71.8 (57.1-83.0)	1.1589	136.89	89.8	0.6953
Yes	6	75.3 (62.2-84.9)	0.6225	26.31	81.0	
***L. reuteri* with antibiotics?**						
Yes	4	77.6(52.9-91.4)	1.0339	19.86	84.9	0.9496
No	2	76.4 (38.0-94.5)	1.1000	5.27	81.0	
Includes *L. acidophilus*?						
No	18	71.4 (63.8-78.0)	0.5355	49.68	65.8	0.6427
Yes	3	79.7 (37.4-96.3)	1.6510	115.94	98.3	
***L. acidophilus* with antibiotics?**						
Yes	2	90.4 (84.9-94.1)	0.2775	2.20	54.5	**< 0.0001**
No	1	42.7 (34.8-50.9)	-	0.00	-	
Includes *L. gasseri*?						
No	14	79.3 (68.7-87.0)	0.9565	173.70	92.1	**0.0051**
Yes[d]	7	58.4 (47.8-68.2)	0	3.89	0.0	

[a] One study was discarded because not provide information about probiotic species (Hantoushzadeh et al., 2012)

[b] *L crispatus, L. rhamnosus, L. delbrueckii and B. coagulans* (k=1).

[c] Other combinations includes*: L. crispatus (2 strains) and L. gasseri, L. rhamnosus, L. gasseri and L. fermentum, L. brevis, L. salivarus and L. plantarum* (k=1*).*

[d] Every treatment with *L. gasseri* was conducted with antibiotics.

*Test for subgroup difference.

FIGURES

Figure 1: Forest plot of the meta-analysis of CCR of treatments for bacterial vaginosis.

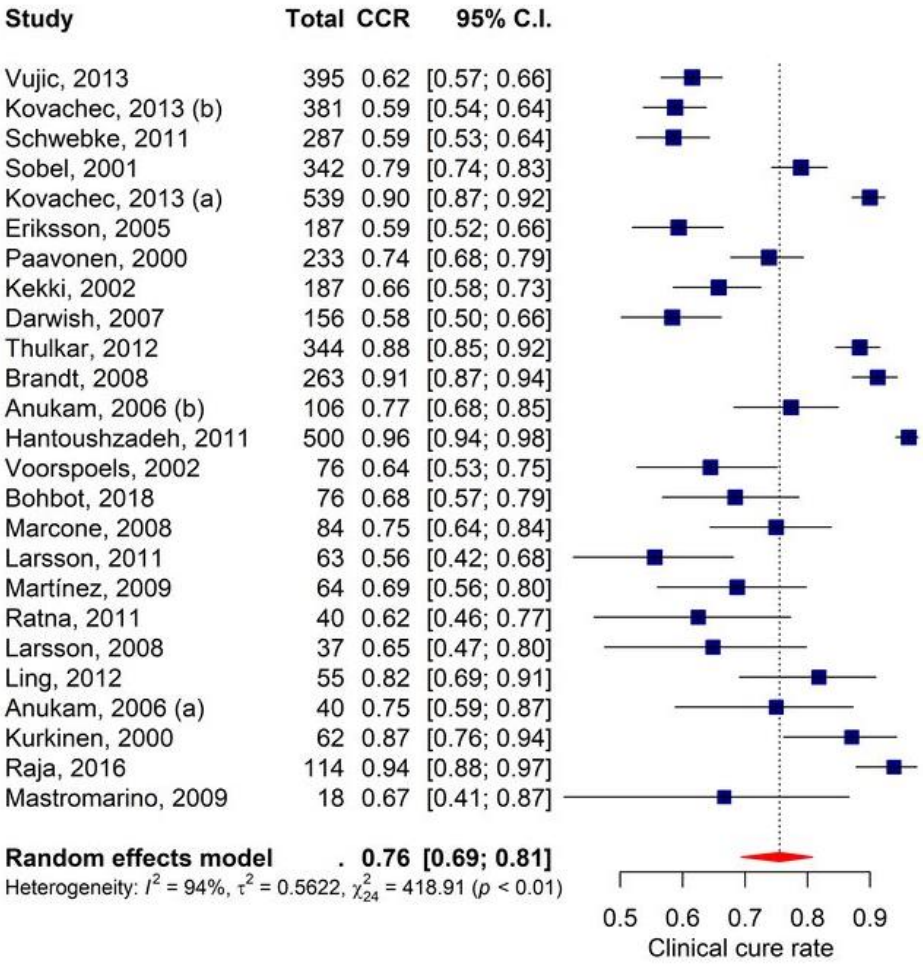


Figure 2: Funnel plot of the meta-analysis on the clinical cure rate of treatments for bacterial vaginosis.

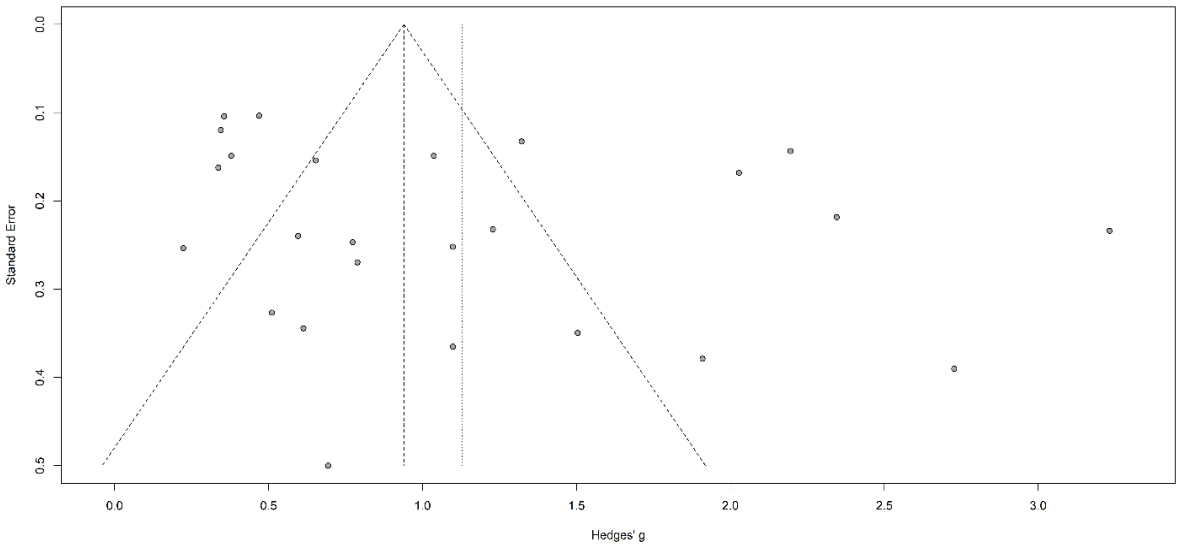


Figure 3: p-curve to assess publication bias and detect p-hacking.

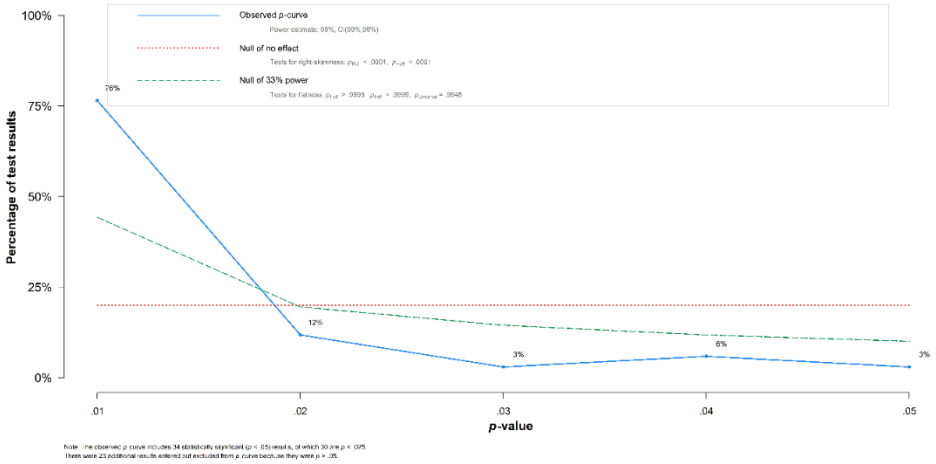


Figure 4: Network of comparisons for treatments and administration in clinical cure rate of bacterial vaginosis.

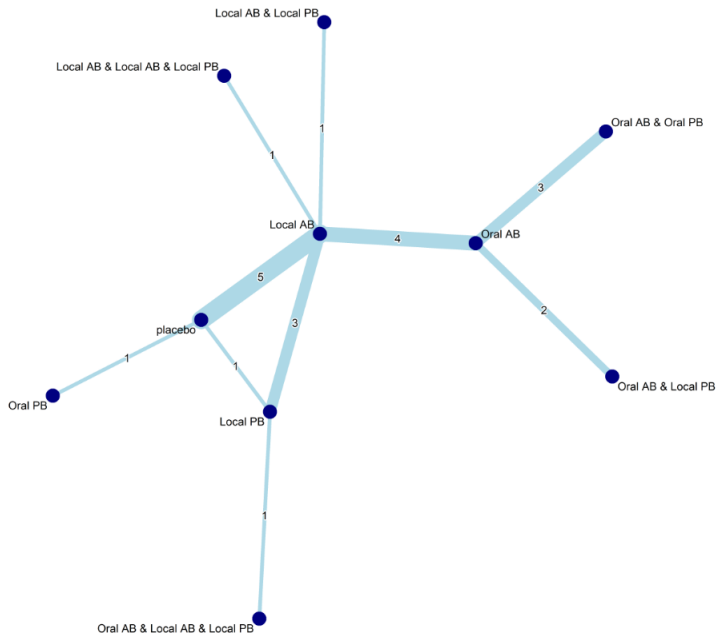
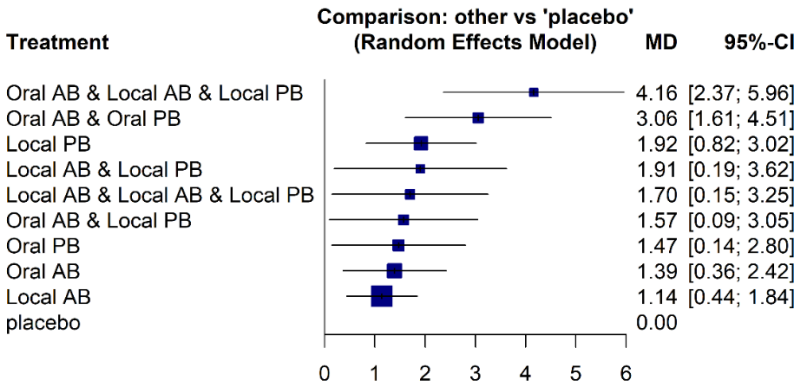


Figure 5: Forest plot of network meta-analysis results for treatment efficacy outcomes compared placebo.



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APPENDIX

Appendix 1 Prisma flow chart of the eligible studies obtained during screening process.