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Microbiota and probiotics in endometriosis: a review study.

Microbiota y probióticos en pacientes con endometriosis

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Abstract

OBJECTIVE: To describe the diversity of the gut-vaginal microbiota in women with endometriosis compared with those without the disease, and to assess whether probiotic supplementation reduces pain scales.

METHODOLOGY: Study in two parts, the first is a narrative review focusing on the composition of the gut-vaginal microbiota in women with and without endometriosis. The second is a qualitative systematic review of the scientific evidence regarding probiotic supplementation in patients with endometriosis. For the narrative review, the STROBE statement criteria for observational studies were considered. For the systematic reviews, the PRISMA statement was used to know whether the points of these statements were integrated or not, without the intention to undermine the studies for the review, but to determine how closely they adhered to international guidelines.

RESULTS: Differences in strain diversity were found between women with and without endometriosis. Qualitatively, a greater predominance of *Firmicutes*, *Pseudomonas* spp, *Clostridiales*, *Prevotella* spp, Atopobium spp, and *Actinobacteria* was observed. About probiotics, the one that coincided in both studies with a positive effect on the reduction of pain perception was Lactobacillus gasseri OLL2809, a lactic acid producing probiotic bacterium that stimulates the production of interleukin-12 (IL-12).

CONCLUSIONS: The overall composition of the intestinal and vaginal microbiome differs between women with and without endometriosis. Probiotic supplementation, particularly with *Lactobacillus gasseri*, may reduce symptoms, including pain, which are characteristic of endometriosis.

KEYWORDS: Vaginal microbiota; Endometriosis; Probiotic supplementation; Pain; Microbiota; Lactobacillus gasseri; Dietary Supplements; Prevotella.

Resumen

OBJETIVO: Describir la diversidad de la microbiota intestinal-vaginal en pacientes con endometriosis en comparación con quienes no tienen la enfermedad y evaluar si la suplementación con probióticos disminuye las escalas del dolor.

METODOLOGÍA: Estudio en dos secciones: la primera es una revisión narrativa enfocada en la composición de la microbiota intestinal-vaginal en pacientes con endometriosis y sin ésta. En la segunda se hace una revisión sistemática cualitativa de la evidencia científica respecto de la suplementación con probióticos a pacientes con endometriosis. Para la revisión narrativa se consideraron como criterios de análisis lo que se señala en la declaración STROBE para los estudios observacionales. Para las revisiones sistemáticas se recurrió a la declaración PRISMA con la finalidad de saber si se integraron o no los puntos de estas declaraciones, sin afán de menoscabar estudios para la revisión, pero sí para establecer cuán apegados están a las directrices internacionales.

RESULTADOS: Se encontraron diferencias en la diversidad de cepas en mujeres con endometriosis y sin ésta. Cualitativamente se observó mayor predominio de *Firmicutes, Pseudomonas* spp*, Clostridiales, Prevotella* spp*, Atopobium* spp y *Actinobacteria*. En relación con los probióticos, el que coincidió en ambos estudios con efecto positivo

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en la reducción de la percepción del dolor fue *Lactobacillus gasseri OLL2809*, una bacteria probiótica productora de ácido láctico que estimula la producción de interleucina-12 (IL-12).

CONCLUSIONES: La composición general del microbioma intestinal y vaginal difiere entre mujeres con endometriosis y las que no tienen la enfermedad. La indicación de probióticos, específicamente *Lactobacillus gasseri,* puede disminuir los síntomas, entre ellos el dolor característico de la endometriosis.

PALABRAS CLAVES: Microbiota vaginal; endometriosis; suplementos probióticos; dolor; microbiota; *Lactobacillus gasseri*; suplementos dietéticos; *Prevotella* spp.

BACKGROUND

Endometriosis is an estrogen-dependent chronic inflammatory process, characterized by tissue that is similar to that of the endometrium, outside of the uterus. It is a condition that affects the quality of life because of pain during sexual relations, evacuations, or general activities, while patients can also experience depression and anxiety.[1-2]

Symptoms often initiate at an early age, going undiagnosed for up to 10 years or more.[3] Endometriosis has been associated with dysmenorrhea, dyspareunia, non-menstrual pelvic pain, nerve pain, and infertility, which vary among patients, and it is possible that the aforementioned are not correlated with the severity of the disease.[1] Also, endometriosis, in itself, implies a higher risk of autoimmune, cardiovascular, and psychiatric comorbidities.[4]

The strategy for treatment must be adapted to each patient according to their characteristics, and it includes surgery, medication, and assisted reproduction techniques.[5] The main objective must be to alleviate pain, correct infertility, and perhaps avoid, or delay consequences associated with endometriosis, such as fibrosis, adherence, and malignant transformation.[6]

A link between microbiota and endometriosis has been made in recent years, particularly gut and vaginal microbiota. Endometriotic microbiota has been consistently associated with a decrease in the dominance of *Lactobacillus*, the high abundance of bacteria related to bacterial vaginosis, and other opportunistic pathogens. Possible explanations for the implications of the dysbiosis in endometriosis include the theory of bacterial contamination, self-immune activation, intestinal function altered by cytokines, metabolism and altered signaling of estrogen, and aberrant stem and progenitor cell homeostasis.[7]

Because of these irregularities, some researchers have suggested that the intestinal microbiota modulation, with probiotics for example, offer a potential path to control symptoms and delay progress of the disease. Considering this, the present study emerged with the goal of describing the diversity of gut and vaginal microbiota in women with endometriosis, comparing to women without the disease, and to evaluate if probiotic supplementation lowers the scales of pain.

MATERIAL AND METHODS

Study in two parts, first a narrative revision focused on the composition of the gut-vaginal microbiota in women with and without endometriosis. In the

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second part, a qualitative systematic review was made of the scientific evidence regarding probiotic supplementation to patients with endometriosis.

The STROBE (Strengthening The Reporting of Observational Studies)[8] declaration was used as a guide for the analysis criteria of the observational studies, and PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses)[9] was used to analyze the systematic reviews, to know whether they were integrated or not, with the points mentioned in both declarations, without any desire to undermine studies for the review, but to establish how they adhered to international guidelines. The Web of Science, PubMed, Cochrane, LILACS, BIREME, NICE, SIGN, and CENETEC databases were used.

The main result to be assessed was the composition of the gut-vaginal microbiota in women with and without endometriosis.

Inclusion criteria: analytical cross-sectional studies, case-control studies, or systematic revisions which compared the diversity in the gut microbiota of patients with endometriosis and without it. Studies with a maximum date of publication between the years 2013 and 2023, with patients of any race, in reproductive age, and published in English or Spanish.

Exclusion criteria: Studies with incomplete text, or in languages other than English or Spanish.

Elimination criteria: Randomized controlled studies made with patients with endometriosis where there was no intervention with probiotics compared with placebo, studies which mixed different types of supplement interventions, clinical trials without double blind or triple blind, clinical studies without the determination of the effect of the supplement on pain parameters.

Eligibility criteria: controlled, randomized studies, carried out with patients with endometriosis who received probiotics compared to placebo, clinical studies that included patients of any age with endometriosis, clinical studies that used any dosage of probiotics, double blind, triple blind, with no distinction in the temporality of the supplementation, and which determined the effect of the supplementation on pain parameters.

Search strategy: articles published between 2013 and 2023 on the PubMed, Cochrane, LILACS, BIREME, NICE, and CENETEC databases, with the MeSH: endometriosis, gut microbiome, and vaginal microbiome. **Table 1**

Study selection process: the literature search results were obtained in two phases, based on the eligibility criteria. In the first phase, the titles and abstracts of the articles were reviewed to identify those eligible for the second phase. In the second phase, the full, potentially eligible texts, were evaluated by each member of the research team individually, considering how many of the STROBE points were followed in the observational studies, and PRISMA points in the systematic reviews. Quality was not evaluated with the guidelines because this was not the purpose, they were only used as guides to document how many points they covered. After the study selection, they were assessed for inclusion potential.

As a second phase of the study, we undertook a qualitative systematic review of the scientific evidence about probiotic supplementation to patients with endometriosis. This review adhered to PRISMA, and Cochranes's PICO strategy. The EVIPNET, PubMed, BIREME, NICE, Cochrane, Scielo, PAHO, LILACS, and Portal of evidence databases were used. The main outcome to evaluate was the pain scale.

Study selection: After thoroughly searching, the data was collected in a manner that allowed the identification of every study by author, in alphabetical order. This prevented from working with

Table 1. PICO search strategy

PICO term	Search terms	Search strategy
Population	Women of reproductive age	Women of childbearing age OR Women of reproductive age
Intervention	Probiotic supplementation	Probiotic supplementation and endometriosis
Result	Pain	Pain
Study design	Randomized clinical trial	Randomized clinical trial

duplicates, and it allowed for a review by title, and filter them based on their characteristics.

Data extraction and analysis: all the relevant data from the chosen studies was collected in a database generated for that purpose, and then descriptive data was extracted from it, such as objective, population, study design, intervention, length of the intervention, and results which were evaluated afterwards.

Evaluation of bias risk: every study was assessed with 2 tools. The JADAD scale, or Oxford score system, and the scale to evaluate Randomized Clinical Essays (RCE). This questionnaire offers a score in a scale of 0 to 5, where the greater the score, the better the methodological quality. A randomized clinical essay with five points was considered rigorous. A clinical essay with lower score than 3 was considered of poor quality. The Physiotherapy Evidence Database (PEDro), which is comprised of 10 items to evaluate randomized clinical essays, was also used with the following score: under 4 meant poor quality, between 4 and 5 meant fair quality, 6 to 8 meant good quality, and 9 to 10 meant excellent quality.

Ethical considerations: only studies which met the current regulation about the ethical treatment of patients, and which used an informed consent form were selected.

RESULTS

The results were expressed based on the revision under consideration:

Narrative revision: The initial search yielded 59,244 studies, 15,995 were duplicates, so there were 43,249 articles left, out of which 43,181 did not match our criteria. In the end only 14 articles that matched the criteria for our revision remained.

General characteristics of the disease

Endometriosis is a gynecologic disorder which is dependent on hormones, it is defined by histological lesions generated by the growth of endometrium type tissue (glands and endometrial stroma), outside of the uterine cavity, frequently inserted in the peritoneum cavity; this is to say, in the peritoneum wall, ovaries, sigmoid rectum, and bladder, although it can also be found in distal organs such as lung, liver, pleura, diaphragm, eye, and brain.[10]

The disease is characterized by the presence of glandular, stromal, or endometrial epithelium outside of the uterus, and it is classified in three distinct types, superficial, ovarian, and deep endometriosis. It affects 10 to 15% of women in reproductive age. Up to 70% of the patients present pelvic pain, and 48% fertility issues. One of the more widely accepted hypotheses is the one made by Sampson, which suggests that retrograde menstruation, through the Fallopian tubes, goes back to the peritoneum cavity, followed by the colonization of endometrial tissue. Other theories suggest that fragmented endometrial tissue can migrate and be implanted through blood or lymph circulation, and hormone-induced metaplasia.[11] However, it is likely that other factors

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(anatomical, genetic, environmental, lifestyle, menstrual cycle dynamics, aberrant autoimmune responses, etc.) are implied, as 90% of women experience retrograde menstruation, but only 10% end up with endometriosis. Other studies suggest the alteration of serum metabolites, genetic-epigenetic changes caused by retrograde menstruation which contribute to the apparition of the lesion, and a genetic predisposition that can differ in relation to ethnic origin.[12]

The diagnosis of endometriosis is complex, it often requires invasive techniques, such as laparoscopic surgery to observe macroscopic conditions, or histological confirmation by biopsy.[10] Hormone suppression is the pillar of the treatment, and it is associated with pregnancy prevention and unwanted side effects, although it is not always successful. However, pain relief is the most burdensome symptom, and the one which drives patients to consultation.[13]

Dysbiosis and endometriosis

The study by Zizolfi et al.[14] suggests an association between dysbiosis, i.e. the alteration of the gut-microbiota, and endometriosis. An abnormal inflammatory response and the activation of immune cells in the peritoneum cavity are thought to play a role in pathogenesis. There are studies that show that dysbiosis leads to an increase of estrogen concentrations in blood, and that there is a relation of higher exposure to estrogens and higher growth of ectopic endometriotic focal points, and the inflammatory activity between them.[15] Gut microbiome dysbiosis, caused by an imbalance between commensal and pathogenic microbiomes, or a reduced microbial diversity, de-regulates the two-way communication between intestine and uterus, which results not only in an immune dysfunction, but also in an alteration of estrogen signaling. Microbial diversity and the decrease of dysbiosis reduce the activity of β-glucuronidase. On the contrary, the strobolome, through a greater abundance of β-glucuronidase producing bacteria, increases circulating estrogens.[16] Bailey et al.[17] showed that the altered composition of the gut microbiota and intestinal inflammation in Rhesus monkeys, are associated with endometriosis.

Lee et al.[18] compared the vaginal, cervical, and gut microbiota of women with advanced stage endometriosis and without it, and they found that some potentially pathogenic species increased in the cervical and fecal microbiome in women at said stage of endometriosis. These outcomes point to a dysbiosis in the intestine and the reproductive system of patients with confirmed pathologic endometriosis. Endometriotic lesions not only alter the amount of each microbial species, but also the composition of existing microbial species.[19] Patients with endometriosis have a higher proportion of *Firmicutes-Bacteroidetes* in comparison with control groups.[19]

The decrease in concentration of *Bacteroides* and *Prevotella* spp (*Bacteroidetes* phylum), and high concentrations of *Clostridium* spp *(Firmicutes* phylum) observed in patients with endometriosis, can play a role in the inflammation associated with the disease through the induction of $Foxp3^+$ regulatory T cells (main transcription factor which determines the destiny and identity of colon Treg CD4+ CD25+ cells, and the activation of the production of T-cell dependent immunoglobulin A). These results could point to a dysbiosis in the gastrointestinal and urogenital microbiome of patients with endometriosis, concomitant with alterations in estrogen production and its metabolites.[19]

Dysbiosis favors inflammation through the recruitment of specific inflammatory immune cells, the production of proinflammatory cytokines, and a compromise of immunovigilance.[16]

Gut microbiota alterations in endometriosis

It is possible that gut microbiota, with its estrogen cycle regulation role, is associated with endome-

triosis.[15] Changes in the composition of the gut microbiota, and therefore in the beta-glucuronidase activity, could alter or stop regulating the circulating concentrations of estrogens, and promote estrogen-mediated conditions. This could contribute to hyper or hypo-estrogenic states as it promotes inflammation and hormonal deregulation through strobolome. This would alter the cellular proliferation-apoptosis, the metabolism, and the oxidative stress, and it would increase vascularization and angiogenesis.[13] The hypothesis of the bacterial contamination suggests that microbial pathogens activate the immune response by binding to Toll-like receptors.[20]

Vaginal microbiota alterations

The growth and maintenance of endometriosis are influenced by the metabolism of estrogens and are modulated by strobolome. Gut microbiota dysbiosis, caused by an imbalance between commensal and pathogenic microbiomes, or a reduced microbial diversity, alters the two-way communication between the intestine and the uterus. This not only results in an immune disfunction, but also in an alteration of estrogen signaling. The strobolome, through a greater abundance of β-glucuronidase producing bacteria, increases circulating estrogens, and it can thus lead to endometriosis.[16] The pathogenesis of endometriosis could start from the intrauterine chronic inflammation reaction induced by a specific bacterial contamination, and the immune response would accelerate endometrial cell generation.[21]

Retrograde menstrual blood could be a great substrate for pelvic bacteria, and the bacterial colonization could greatly increase the risk for endometriosis.[21]

The study by Wei et al.[22] shows that the proportion of *Lactobacillus* in the inferior and posterior vaginal reproductive system of patients with endometriosis, was lower than in the controls, and the number of cases of microbiota not dominated by *Lactobacillus* significantly increased in cervical mucus. Also, distinctive operative taxonomic units gradually increased from the lower part of the reproductive system to the upper part, and they reached a peak at the pelvic cavity. These findings suggest that there is a distinct microbiota in the genital tract of patients with endometriosis, and this represents a predicting factor for women prone to endometriosis. *Sphingobium sp* and *Pseudomonas viridiflava* were more abundant in the endometrium and in peritoneal fluid, which shows that the abundance of these two species could play a decisive role in the pathogenesis of endometriosis.[22]

In the study by Ata et al.,[15] the sensibility analyses of vaginal microbiota after the exclusion of species of *Lactobacillus,* showed that *Gardnerella* amounted to a significantly larger proportion of the remaining microbiota in the endometriosis group than in the controls (72.9, compared to 36.8%, $p<0.05$). *Escherichia-Shigella* were also more abundant in the endometriosis group, and *Prevotella* and *Dialister* were lower in patients with endometriosis than in the control group.

Diversity in the microbiota of women with endometriosis

Reproductive health tends to be associated with a *Lactobacillus* dominated vaginal microbiota, while intestinal health tends to be associated with a diverse microbiota. It has been suggested that alterations in the gut microbiota contribute to the pathogenesis of endometriosis through the regulation of inflammatory processes and estrogen metabolism, while intestinal permeability also increases in patients with endometriosis, which can contribute to the systemic nature of this condition.[12]

Endometriosis has been associated with a higher coexistence of *Proteobacteria*, *Enterobacteriaceae*, *Streptococcus*, and *Escherichia coli* at various points of the microbiome. The *Firmicutes* phylum

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and the *Gardnerella* genera also seem to be associated, although this is not yet fully clear.[20]

The uterine endometrial microbiota of patients with endometriosis seems to be more diverse than in that of the symptomatic controls (with pelvic pain, but without endometriosis surgery). The relative abundance of enriched bacterial taxa in the endometrial microbiota in patients with endometriosis was associated with the phylum *Actinobacteria*, the families *Oxalobacteraceae* and *Streptococcaceae*, and the genus *Tepidimonas,* while the enriched symptomatic controls belonged to the family *Burkholderiaceae* and the genus *Ralstonia*.[12]

Hernandes et al.[23] didn't find significant differences in the analysis of diversity of the microbiome profile of controls and endometriosis patients. However, deep endometriotic lesions seemed to have a different bacterial composition with smaller dominance of *Lactobacillus*, and a greater abundance of *Alishewanella, Enterococcus* and *Pseudomonas*.

While human studies haven't yet shown that the microbiome may play a role in the cause of endometriosis, it may be speculated that signaling pathways associated with bacteria, including lipopolysaccharide (LPS) and toll-like receptor 4 (TLR4), the metabolome, strobolome, and immune mediators, play a role in the induction of a host environment that can promote endometriosis, and the possible two-way interaction between endometriosis, infertility and pelvic pain, and the microbiome.[13] While the general composition of the microbiome in the cervix, vagina, and gut seem to be similar in women with endometriosis in stages 3-4, and healthy controls, there seem to be some differences in genera. **Tables 2 and 3**

Systematic revision

The initial search yielded 179 studies, after the elimination of 81 duplicates, 15 were analyzed to review inclusion and exclusion criteria. Eleven were excluded because they didn't meet the inclusion criteria. Four articles were subsequently evaluated according to the main result and the PICO search strategy characteristics, to verify that everything established was met. Two of the four articles met the pre-established criteria for this revision. **Figure 1**

Regarding the evaluation of methodology quality, the two selected studies were assessed again. For the first one, the PEDro scale was referenced, and both studies had 10 points out of 10.[24-25] The JADAD scale was used to analyze and evaluate the studies, and both studies had 5 points out of 5. **Table 4** shows the included randomized clinical studies and the main characteristics of the studies selected.

In general, the participants of the studies were patients with a clinical diagnosis of endometriosis, between 18 and 45 years of age. The study by Khodaverdi et al.[24] included LactoFem, which contains 10^9 colony forming units (CFU) of 4 different strains of *Lactobacillus* (*Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus fermentum*, and *Lactobacillus gasseri*). In the study by Itoh et al.,[25] a 250 mg tablet of lyophilized L. gasser OLL2809, killed by heat (75°C for 60 minutes) was used for a period between 8 and 12 weeks. Both studies evaluated pain reports as a main outcome. Table 4 shows the effects on the pain intensity scores in patients with endometriosis. Statistically significant positive results are shown with (+), and when there were no significant results, the (-) is shown.

Pain scale results

The supplementation of *Lactobacillus* showed to have a positive effect in pain scale results in patients with endometriosis, in both studies included here. Itoh et al.[25] carried out a randomized, double blind, and placebo controlled clinical trial, at a single institution (Gynecol-

Table 2. Gut microbiota dominance results for patients with endometriosis compared with women without it

Study	Country (ethnicity)	Strain dominance in women with endometriosis	Strain dominance in women without endometriosis	STROBE	PRISMA
Le et al.[22]	U.S.A. (Caucasian, Hispanic and others)	*Firmicute/Bacteroidetes*	NS	19/22	
Huang et al.[32]	China (NS)	*Pseudomonas spp., Prevotella spp. y Xanthomonadaceae Luteimonas spp.*	NS	19/22	
Shan et al.[33]	China (NS)	*Firmicute/Bacteroidetes. Corynebacterium spp.: 1.27% Prevotellaceae: 1.39% Allisonella spp.: 1.81% Ruminococcaceae: 2.35% Eubacterium spp.: 2.77%*	*Clostridiales: 1.49% Synergistes: 2.79% Peptococcus spp: 3.00% Mollicutes: 8.53% Rumminiclostridium spp:17.59%*	19/22	
Svensson et al[3]4	Sweden (NS)	*Lachnobacterium sp. Lactococcus spp. Prevotella spp.*	*Firmicute/Bacteroidetes*	18/22	
Leonardi et al.[20]	Japan (NS)	*Actinobacteria, Firmicutes, Proteobacteria y Verrucomicrobia. Bifidobacteriaceae y Alcaligenaceae.*			20/27
Ata et al.[15]	Turkey (NS)		*Sneathia spp. Barnesiella spp.*	18/22	

NS: Not specified.

ogy Department of the Longhua Hospital of the Chinese Traditional Medicine University of Shanghai, in Shanghai, China), between September 2008 and February 2009. Sixty-six patients were clinically diagnosed with endometriosis and were sequentially recruited for the study. The patients were randomly divided in two groups, 1 (placebo group), and 2 (active group), by a controller who did not participate directly in the study. The patients received two active pills with L. gasseri OLL2809, or placebo pills, daily for 12 weeks (three menstrual periods). One tablet (250 mg) contained 50 mg of lyophilized *L. gasseri* OLL2809, killed by heat (75° for 60 minutes). The placebo tablet contained dextrin instead of lyophilized L. *gasseri* OLL2809, and it was identical in color and taste to the active tablet.

The main criteria to evaluate efficacy were the patient's response to the treatment for menstrual pain, and the dysmenorrhea score associated with endometriosis, as evaluated by the Visual Analog Scale (VAS) for pain intensity, and the Verbal Rating Scale (VRS) for dysmenorrhea, respectively.

After three months of ingestion, the decline in the VAS for pain intensity in the active group (-3.2 ± 0.36) was significantly greater than in the placebo group (-2.00 ± 0.29; $p \leq 0.01$). The active group showed a tendency to lower the score in the VRS for dysmenorrhea after three months of intake, in a more effective way than in the group with placebo pills (-1.44 ± 0.17 in comparison with -1.03 ± 0.16) ($p \leq 0.05$).

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Table 3. Vaginal microbiota dominance results for patients with endometriosis compared with women without it

Study	Country (ethnicity)	Strain dominance in women with endometriosis	Strain dominance in women without endometriosis	STROBE
Akiyama et al.[35]	Japan (NS)	Corynebacterium spp.: 2% Flavobacterium spp.: 3% Enterobacteriaceae: 8% Pseudomonas spp.: 4%	Corynebacterium spp.: 0% Flavobacterium spp.: 1% Enterobacteriaceae: 5% Pseudomonas spp.: 2%	17/22
Perrotta et al.[36]	U.S.A. (NS)	Lactobacillus crispatus.	NE	18/22
Wei et al.[22]	China (NS)	Veillonellaceae.	*Lactobacillus (II).*	15/22
Lee et al.[18]	South Korea (NS)	*Pseudomonadales (0.105).*	*Actinobacteria (0.11).* *Actinomycetales (0.080).*	12/22
Chen et al.[37]	China (NS)	*Coriobacteriales, Coriobacteriaceae, Atopobium spp.*	NE	16/22
Chao et al.[6]	China (NS)	*Clostridium butyricum, Clostridium disporicum, Alloscardovia omnicolens y Veillonella montpellierensis,*	*Lactobacillus jensenii, Lactobacillus reuteri y Lactobacillus iners*	16/22
Ata et al.[15]	Turkey (NS)	*Alloprevotella spp.*	NE	18/22
Wessels et al.[12]	Canada (NS)	*Actinobacteria, Oxalobacteraceae, Streptococcaceae, Tepidimonas.*	*Burkholderiaceae, Ralstonia spp.*	18/22
Hernandes et al.[23]	Brazil (NS)	*Lactobacillus spp. (100%), Enterococcus spp. (35%), Gardnerella sp. (70%), Pseudomonas spp. (55%), Alishewanella spp. (90%), Ureaplasma spp. (45%) y Aerococcus spp. (65%) en*	NE	16/22

NS: Not specified.

Khodaverdi et al.[24] carried out a placebo-controlled pilot project with randomized assignation, and triple blind, at a reference center for endometriosis at a university hospital in Teheran, Iran, between October 2016 and October 2017. Thirty-seven patients with endometriosis were randomly assigned to one of the two groups: 1 capsule per day of LactoFem, (Zist Takhmir Co. Theran, Iran), or placebo (as control group). Each LactoFem capsule had 10^9 colonies of 4 different strains of *Lactobacillus (Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus fermentum* and *Lactobacillus gasseri)*. Pain intensity, dysmenorrhea, and dyspareunia were evaluated according to the VAS with 0 (no pain) to 10 (severest pain). The two groups continued to take medication for 8 weeks, and pain intensity was evaluated at 8 and 12 weeks after the intervention, with a follow-up visit or a phone call. NSAIDs were used as a rescue therapy during the follow-up time. The initial mean measurements for pain for dysmenorrhea, dyspareunia, and pelvic pain were 6.53 ± 2.88, 4.82 ± 3.76 and 4.19 ± 3.53, respectively for the LactoFem group, and 5.60 ± 2.06, 3.67 ± 2.64 y 2.88 ± 2.80, respectively for the control group. The two groups had comparable score results. There was a greater decrease in the pain scores for dysmenorrhea and pain in general, after 8 weeks of treatment for the LactoFem group, in comparison with the control group.

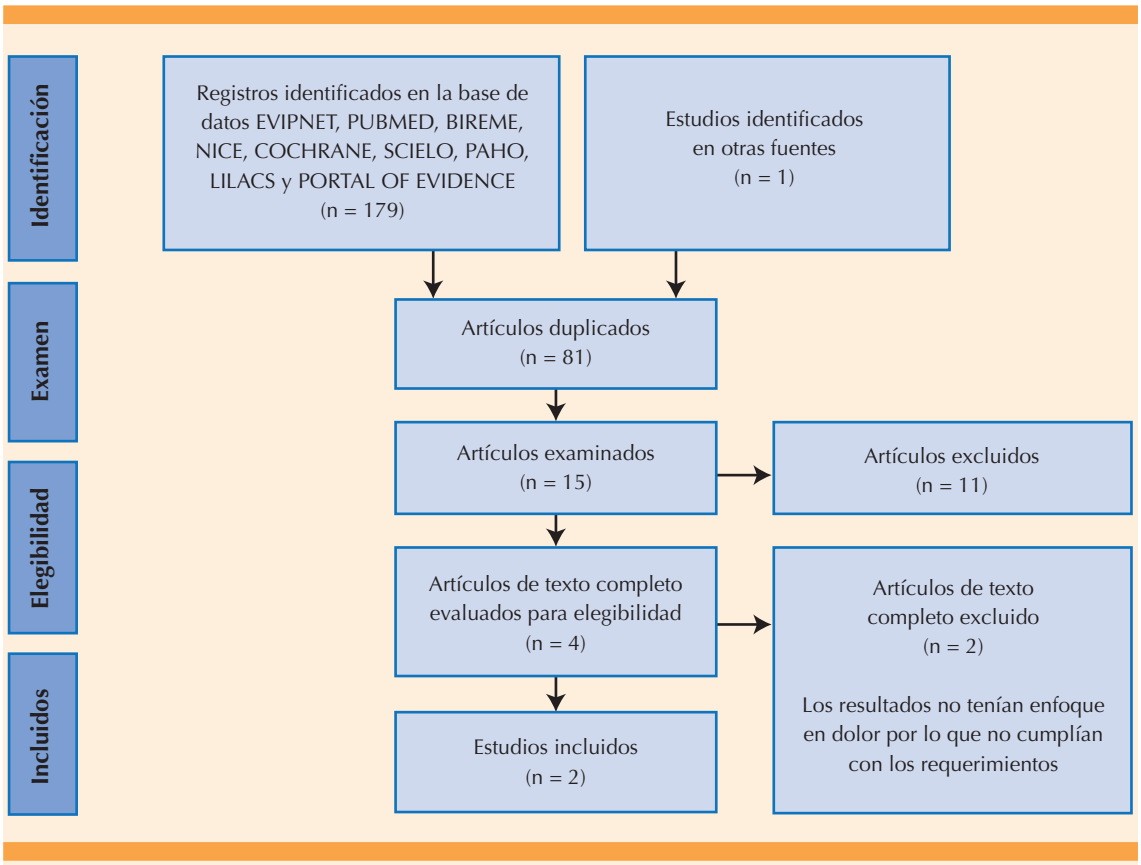


Figure 1. Identification of studies in databases. Included studies selection.

DISCUSSION

Fourteen studies were included in the narrative revision, and 2 in the systematic review. The women in the latter were patients with endometriosis diagnosis between 18 and 45 years of age. The intervention was the administration of *Lactobacillus spp* probiotics, and the timeframe of the studies was between 8 to 12 weeks. Unfortunately, no randomized clinical studies were found that referenced the indication of prebiotics or symbiotics to patients with endometriosis.

The goal of the reviews was to examine exhaustively the found literature to compare the vaginal-gut microbiota in women with endometriosis, with patients without it, and systematically evaluate the literature that aims to determine if the indication of probiotics to patients with endometriosis has any effect in pain perception.

We observed in the narrative review that the dominant strains in patients with endometriosis were *Prevotella* spp, *Pseudomonas* spp, *Gardnerella* spp, *and Atopobium* spp. These types of microorganisms have been associated with proinflammatory processes that induce an increase in concentrations of cytokines, that alter the epithelial barrier of the mucosa, through T-cells, the production of estrogen and its metabolites, and they are capable of adapting to pH, by creating an inadequate environment for pathogens. With this, they prevent them from colonizing and causing infections by producing lactic acid as a subproduct of fermentation.[26]

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Table 4. Evaluation of systematic review results

Author	Objective	n (age)	Study design	Intervention	Dose	Main outcome	PEDro scale	JADAD scale	Type of result
Khodaverdi et al.[24]	Evaluate the therapeutic effects of oral *Lactobacillus* in cases associated with endometriosis-pain	37 (18-45)	Randomized pilot study, controlled.	Oral *Lactobacillus* or placebo once a day for 8 weeks.	LactoFem 10^9 colonies.	Changes in general pain score in the LactoFem® group (7.33 ± 7.00) vs control 4.11 ± 1.68 (p<0.017).	10/10	5/5	Positive (+)
Itoh et al.[25]	Evaluate the efficacy of *Lactobacillus gasseri OLL2809* for pain.	66 (18-45)	Double blind, controlled, clinical trial.	Active *L. gasseri* OLL2809 tablets or placebo, once a day for 12 weeks	Tablet (250 mg) *L. gasseri OLL2809*	Pain intensity decrease in VAS: Intervention group (-3.28 ± 0.36) vs placebo group (-2.00 ± 0.29) (p≤0.01).	10/10	5/5	Positive (+)

VAS: Visual Analog Scale.

To measure pain perception, the authors of the studies used the Visual Analog Scale, which is a pain rating scale used initially by Hayes and Patterson in 1921. The scores are based in self-reported measurements of symptoms that are registered with a single mark made by hand, placed at a point along a 10 cm line which represents a continuum between the two extremes of the scale: "without pain" at the far left of the scale (0 cm), and "the worst pain" at the fat right (10 cm). The measurements are recorded in centimeters, starting from the left side of the scale to where the patients made the mark, and this is interpreted as a measurement of their pain. The values can be used for a follow-up of the evolution of a patient's pain, or to compare the pain between two patients with similar conditions.[27]

It has been shown that the VAS is valid, reliable, with high confidence interval, and can be repeated for test-retest. It is used in epidemiologic and clinical research to measure the intensity or frequency of a variety of clinical symptoms. In controlled, randomized trials, the VAS is frequently used to determine the efficacy of a treatment as a measure of the result. This scale is also very popular in the gynecology field.[28]

Regarding the administered probiotics, the one which was used in both studies was *Lactobacillus gasseri* OLL2809, a lactic acid producing probiotic bacteria which stimulates interleukin (IL)-12 to a very important degree, it is also known as a metabiotic, biogenic, or simply CFS/metabolite (cell-free supernatant). It is about soluble factors, (metabolic products or byproducts) secreted by living bacteria, or liberated after bacterial lysis.[29] They have several attractive features, such as clear chemical structures, safety dosage parameters, and a longer useful life (up to 5 years, when they are consumed as food and drink ingredients, or as nutritional supplements). They have favorable characteristics of absorption, metabolism, distribution, and excretion, which could mean that they have a high ability to send signals to

different organs and tissue of the host, provoking thus various biologic responses.[29]

Il-12 is also a cytokine known for activating natural killer cells (NKC) and inducing the differentiation of virgin T cells, into auxiliary T cells, playing thus a vital role in the immune reaction. *L. gasseri* OLL2809 has several effects on the immune system in allergies, endometriosis, NKC activity after high-intensity exercise, and stress. NKC activity decreases in peripheral blood and in peritoneal fluid of endometriosis patients. This decrease was significantly associated with an increase in the stage of the disease. Il-12 induces NKC cytotoxicity, so it is expected that *L. gasseri* OLL2809 can decrease endometriosis through the cytotoxicity of NKC with enhanced production of Il-12.[22] Nishida et al.[30] analyzed the relation between *L. gasseri* and stress and the quality of sleep. They observed a decrease in the growth of strains of *Bacteroides vulgatus*, which is responsible for intestinal inflammation, so they concluded that the strain *L. gasseri* is a good ally against stress and its side effects, as well as to promote sleep quality.[31]

This systematic review, which studies the treatment of endometriosis with probiotics, shows several strengths that contribute to its credibility and utility. Firstly, the review shows a careful and well-defined methodology. The systematic approach in the literature search, the study selection, and the synthesis of the data improves the reliability of the findings. The inclusion of multiple databases and rigorous criteria for the eligibility of the studies, guarantees an integral reach of the available evidence, and it minimized the risk of selection bias. The review's emphasis on quality assessment is another key strength; by critically evaluating the methodological rigor of the studies included, it offers an acute insight into the reliability of the evidence base. Another strength is the focus on strains, doses and mechanisms of action of specific probiotics. Lastly, the review acknowledges its limitations, which shows a balanced and careful interpretation of the findings, it improves the credibility of the review, and it provides a valuable context to understand the limits of the current evidence.

The current panorama of information, research, and articles about the indication of probiotics to treat pain in endometriosis is marked by a notable shortage. While there is growing interest in the potential of probiotics to exert an influence on the symptoms of this complex condition, the available literature is still limited, and often, preliminary. Therefore, the interpretation of the results must be made with caution for the clinical practice, given that positive effects have been seen on the pain scale in both studies analyzed, but it is clear that further longitudinal studies are needed to have a more solid evaluation.

CONCLUSIONS

The general composition of vaginal and gut microbiota differs between women with endometriosis and those without the disease. The indication of probiotics, specifically *Lactobacillus gasseri*, can reduce the symptoms, among them the characteristic pain of endometriosis.

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