

# Relationship between the gut microbiome and endometriosis and its role in pathogenesis, diagnosis, and treatment: a systematic review

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## ABSTRACT

Endometriosis is a chronic inflammatory disease affecting approximately 10% of women. It is defined as endometrial tissue outside of the uterus and produces a variety of symptoms including pelvic pain, dysmenorrhea, dyspareunia, and intermenstrual bleeding. Although several theories have been postulated regarding the pathogenesis of endometriosis, no theory has provided a complete explanation, therefore limiting our progress in diagnostic tools and management of endometriosis. Recently, much attention has been paid to the importance and role of the gut microbiome in endometriosis. As defined by Joshua Lederberg — microbiome is a set of the genome of microorganisms inhabiting a human body, including commensal, symbiotic and pathogenic microorganisms. The aim of this systematic review was to conduct a search in the Embase, Medline, and PubMed databases for literature from July 2013 to July 2023 regarding the relationship between the gut microbiome and endometriosis. 147 records were screened, of which 26 met the eligibility criteria, and 16 were included in this review. Our review concludes that patients with endometriosis show an altered gut microbiome, and that this has the potential to provide insight for pathogenesis, markers for diagnosis, as well as therapeutic options for treatment of endometriosis. Future research is necessary to confirm this and further investigate the relationship between the gut microbiome and endometriosis.

**Keywords:** endometriosis; gut microbiome; pathogenesis; diagnosis; treatment

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## INTRODUCTION

Endometriosis is a chronic gynecological disease that affects approximately 10% of women [1]. Diagnosis is based on the presence of endometrial tissue outside of the uterine cavity [2]. Patients typically present with symptoms such as pelvic pain, dysmenorrhea, dyspareunia, and abnormal uterine bleeding (AUB) [3]. Additionally, 30 to 50% of women with endometriosis experience infertility [4]. Not only does the disease cause many debilitating physical symptoms, but it is also associated with significant psychological, so-

cial, and economic implications. In fact, the annual global economic burden of endometriosis is estimated to be over \$80 billion [5].

Over the past several decades, many theories have emerged regarding the development of endometriotic lesions. One of the most well-known theories, the retrograde menstruation theory, describes the regurgitation of endometrial cells into the pelvic cavity during menstruation [6]. This theory may explain superficial lesions but fails

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to explain deep infiltrating lesions or lesions outside of the pelvic cavity. Another theory, the coelomic metaplasia theory, proposes the transformation of mesothelial cells into endometrial cells [7]. Moreover, immune dysregulation and inflammation have been proposed to contribute to the development of endometriosis [8]. Theories regarding stem cell involvement, hormonal imbalance, and alterations in epigenetic regulation have also attempted to provide meaningful insights [9–11]. Despite numerous theories regarding pathogenesis and progression, none of them provide a complete explanation, which in turn, has limited progress in diagnostic tools and treatment of endometriosis.

Most recently, many studies have investigated the role of the gut microbiome in endometriosis. The gut microbiome has an impressive number of microorganisms, far beyond the number of cells that make up the human body, and plays a role in various metabolic, immunological, neuronal, and endocrine processes. Furthermore, gut dysbiosis has been implicated in the occurrence and development of many diseases [12, 13]. In particular, gut microbiota disturbances are associated with disorders such as inflammatory bowel disease, diabetes mellitus, polycystic ovary syndrome and cancer [14–16]. Emerging evidence suggests alterations in gut microbiota may also be involved in endometriosis. The aim of this systematic review is to evaluate the latest available literature and investigate the role of the gut microbiome in pathogenesis, diagnosis, as well as treatment of endometriosis.

## METHODS

A literature search was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Two authors (A.S. and I.W.) performed the search in the Embase, Medline, and PubMed databases using the following search strategy: (endometriosis\* OR adenomyosis\*) AND (microbiome\* OR dysbiosis\*) AND (gut OR faecal\* OR gastrointestinal\* OR intestinal\* OR enteric). Medical Subject Headings (MeSH) terms were additionally utilized to optimize the search process. The search was limited to articles published in English from June 2013 to June 2023. The identified articles were de-duplicated using EndNote software. Subsequently, the titles and abstracts of the articles were screened to determine their relevance. Finally, the eligible papers underwent full-text analysis. A flow diagram of the systematic review is shown in Figure 1, adhering to the PRISMA template.

## RESULTS AND DISCUSSION

The results of the studies involved in this comprehensive review are summarized in Table 1 [17–32].

Among subjects with endometriosis, differences in gut microbiome composition are evident (Tab. 2) [17, 18, 20, 21, 23, 26, 28–32]. While specific bacteria may differ

between studies, the overall evidence strongly suggests distinct microbiome composition in individuals or mice with endometriosis compared to controls. Only one study found no alterations in the microbiome of endometriotic mice compared to controls [22]. Changes in the gut microbiome can be influenced by many factors including diet, lifestyle, and the environment [33], therefore, further studies across more populations and including more human samples are needed to confirm these results.

## ROLE OF THE GUT MICROBIOME IN ENDOMETRIOSIS PATHOGENESIS

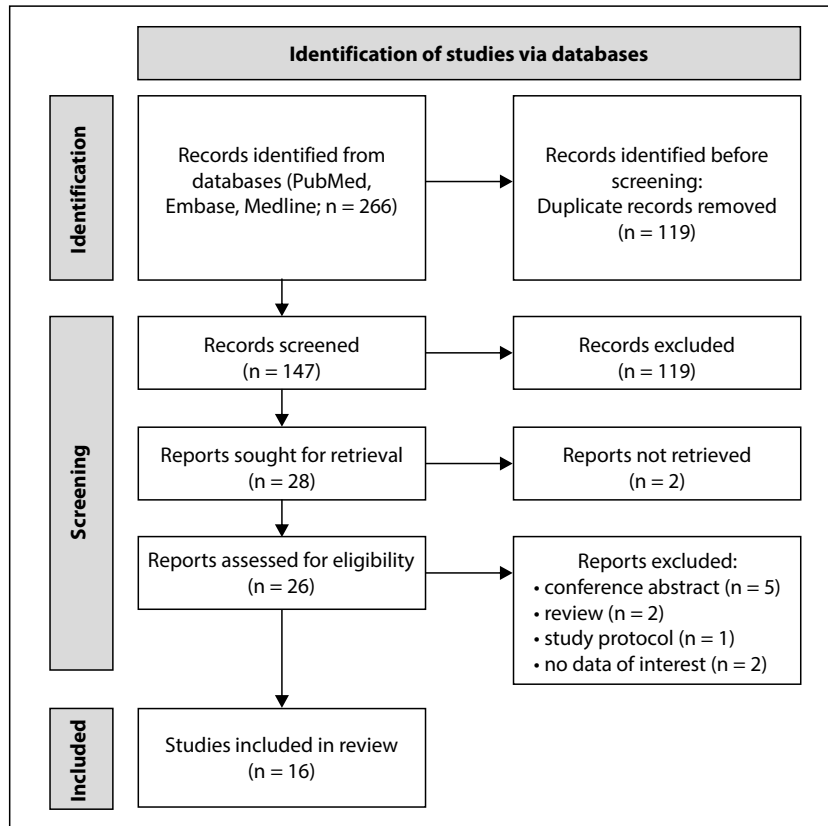
Not only have studies found that the gut microbiome is altered in endometriosis, but evidence also suggests that this alteration may contribute to endometriosis pathogenesis, particularly through the involvement of immune cells.

A study by Chadchan et al. [30] illustrated that peritoneal fluid of microbiota-depleted mice has fewer macrophages, B and T lymphocytes compared to control mice. The authors thus hypothesize that gut microbiota might influence endometriosis growth through the modulation of peritoneal immune cell populations.

Wei et al. [32] investigated the role of microbiome-produced beta-glucuronidase in endometriosis pathogenesis. Beta-glucuronidase is produced by Gram-negative bacteria and activates macrophages. Overactivation of macrophages leads to persistent inflammation and has been shown in other conditions like intestinal bowel disease and Barrett's oesophagitis [34], prompting the authors to investigate its role in endometriosis. They found that higher levels of beta-glucuronidase derived from the gut were present in both patients and mice with endometriosis compared to controls. Moreover, the study found higher levels of several classes of Gram-negative bacteria, capable of producing beta-glucuronidase, as well as increased macrophage levels in endometriosis patients. The authors also went on to show that medium conditioned by glucuronidase-treated macrophages promoted proliferation of endometrial stromal cells and that beta-glucuronidase increased the number and volume of lesions and number of macrophages in the endometriotic mouse models. Taken together, the authors hypothesize that gut dysbiosis in endometriosis patients can lead to abnormal expression of beta-glucuronidase, which in turn aggravates endometriosis development.

In 2021, Huang et al. [26] found a significantly decreased abundance of *Ruminococcus* in faecal samples of patients with endometriosis and hypothesize that this reduction results in decreased protective metabolites in the gut, thus contributing to the pathogenesis of endometriosis.

Furthermore, a study by Xu et al. [17] illustrated the potential effects of stress on endometriosis pathogenesis. The authors looked at two populations of women with



**Figure 1.** Flow diagram showing the search strategy, screening, eligibility, and exclusion criteria

endometriosis: with and without chronic stress. The study did not only find significant differences in the gut microbiome of the two populations, but also higher expression levels of inflammatory cytokines, nuclear factor- $\kappa$ B p65, and cyclooxygenase-2 in the patients with chronic stress. The authors suggest that these results indicate that the dysbiosis of the gut microbiome activates inflammatory pathways through the gut-brain axis. However, the relationship may be bidirectional, with inflammation potentially inducing gut dysbiosis.

The effect of stress on endometriosis was also investigated by Chompre et al. [19]. The authors found that mice subjected to stress developed more endometrial lesions and of a larger size compared to stress-free mice.

#### Potential for the gut microbiome in diagnosis of endometriosis

As the pathogenesis of endometriosis is poorly understood, diagnostic methods are limited. However, alterations of the gut microbiome in endometriosis, if treated as results of molecular changes caused by the disease, provide a potential target for diagnostic investigations, particularly metabolites produced by the microbiome.

In a recent study, Chadchan et al. [30] examined gut microbiota metabolites in the faeces of endometriotic mice and found that six metabolites were differentially present in the faeces of mice with endometriosis compared to control mice. The authors found an elevated level of quinic acid (QA) in the faeces of the endometriotic mice and showed that this metabolite was able to significantly increase the proliferation of immortalized human endometriotic epithelial cells in *in vitro* testing. The authors further investigated the effects of QA by orally administering it to mice induced with endometriosis and found that it resulted in significantly larger endometriotic lesions compared to placebo mice. The study's findings suggest that gut microbiota-derived metabolites, particularly QA, could be important predictive markers for endometriosis in future research.

In an earlier study, Chadchan et al. [25] also found that mice with endometriosis had significantly lower levels of short-chain fatty acid and n-butyrate in their faeces compared to control mice. The authors suggest that the development of endometriosis is associated with altered short-chain fatty acid composition in the gut and that the microbial metabolite n-butyrate is particularly important in protecting against endometriosis disease progression. These findings

Table 1 . Studies investigating association between gut microbiome and endometriosis included in the current study [17–32]				
Authors and date	Type of study and country	Sample size and characteristics	Methods	Results
Xu et al., 2017 [17]	Case control study, China	10 women: 5 women with endometriosis and chronic stress and 5 women with endometriosis and no chronic stress (controls) (Sorted into respective groups using GAD-7 and PHQ-9 questionnaires)	<ul style="list-style-type: none"> <li>— faecal and tissue specimens collected</li> <li>— gut microbiota analysed using 16S rRNA gene sequencing</li> <li>— IHC performed to identify activation of inflammatory pathways in endometriotic tissues</li> </ul>	<ul style="list-style-type: none"> <li>— levels of <i>Paraprevotella</i>, <i>Odoribacter</i>, <i>Veillonella</i>, and <i>Ruminococcus</i> were significantly lower in women with endometriosis and chronic stress</li> <li>— prevalence of <i>Prevotella</i> was higher in women with endometriosis and chronic stress vs controls</li> </ul>
Yuan et al., 2017 [18]	Experimental study, China	42 mice: 22 mice with endometriosis and 20 mice without endometriosis (controls)	<ul style="list-style-type: none"> <li>— mice sacrificed at 4 different time points for model confirmation and faecal specimen collection</li> <li>— 16S rRNA gene sequencing used to analyse gut microbiota</li> <li>— alpha diversity: assess complexity and species diversity</li> <li>— beta diversity: assess differences in species complexity</li> </ul>	<ul style="list-style-type: none"> <li>— no differences in gut microbiota between mice with endometriosis and control mice before 42 days</li> <li>— increase of <i>Bifidobacterium</i> in mice with endometriosis</li> <li>— <i>Firmicutes/Bacteroidetes</i> ratio elevated in mice with endometriosis</li> </ul>
Chompre et al., 2018 [19]	Experimental study, Puerto Rico	Rats (11–12/group) with induced endometriosis and control rats	<ul style="list-style-type: none"> <li>— endometriosis surgically implanted, PI or Pro administered in drinking water until sacrifice</li> <li>— S rats subjected to lack of water for 60 mins/day (days 14–20)</li> <li>— NS rats kept in a clean cage</li> <li>— anxiety measured before and after stress using open field</li> <li>— faecal bacterial composition analysed at 4 times: before surgery, before stress, after stress, and at sacrifice</li> </ul>	<ul style="list-style-type: none"> <li>— ESPI rats had more anxiety and developed more endometriotic lesions (91.27% of larger size (38.31 ± 6.90 mm) vs ENSPI (62.50%; 17.94 ± 3.90 mm)</li> <li>— administration of probiotic reduced number and size of endometriotic lesions, as well as damage of colon (p &lt; 0.05)</li> </ul>
Ata et al., 2019 [20]	Prospective cohort study, Turkey	28 women: 14 women with histologic diagnosis of stage 3/4 endometriosis and 14 healthy controls	<ul style="list-style-type: none"> <li>— DNA extracted from faecal samples using QIAamp DNA Stool Mini Kit</li> <li>— 16S rRNA gene sequencing and diversity analysis performed</li> </ul>	<ul style="list-style-type: none"> <li>— sneathia, <i>Barnesella</i> and <i>Gardnerella</i> significantly decreased in the endometriosis patients (p &lt; 0.01 for all)</li> <li>— increased <i>Escherichia/Shigella</i> observed in 2 endometriosis patients who later required segmental colon resection due to colonic involvement</li> </ul>
Chadchan et al., 2019 [21]	Experimental study, USA	14 mice: 5 mice without endometriosis, 5 mice with endometriosis + vehicle (water with aspartame), and 4 mice with endometriosis + VNMA (water with aspartame and antibiotics)	<ul style="list-style-type: none"> <li>— mice treated with broad-spectrum antibiotics (VNMA), subjected to surgically induced endometriosis, and assayed 21 days later</li> <li>— differences in faecal bacterial composition analysed in mice with and without endometriosis, and faecal microbiota transfer studies performed</li> <li>— DNA extraction from faecal samples after 21 days</li> <li>— 16S rRNA gene sequencing and diversity analysis performed</li> </ul>	<ul style="list-style-type: none"> <li>— mice treated with VNMA had smaller endometriotic lesions (5-fold, p &lt; 0.01) with reduction of proliferating cells (p &lt; 0.001) vs mice treated with vehicle</li> </ul>
Hantschel et al., 2019 [22]	Experimental study, Germany	16 mice: 8 mice transplanted with endometriotic lesions and 8 healthy control mice	<ul style="list-style-type: none"> <li>— endometriotic lesions injected from donor mice</li> <li>— bacterial analysis using 16S rRNA gene sequencing performed from fecal samples from transplanted and donor mice</li> </ul>	<ul style="list-style-type: none"> <li>— no significant effect of endometriosis induction on bacterial microbiota in mice</li> </ul>

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**Table 1 (cont.). Studies investigating association between gut microbiome and endometriosis included in the current study [17–32]**

Authors and date	Type of study and country	Sample size and characteristics	Methods	Results
Cao et al., 2020 [23]	Experimental study, China	38 rats: 30 rats with induced endometriosis and 8 healthy control mice	<ul style="list-style-type: none"> <li>— rats were subjected to surgically induced endometriosis, treated with letrozole and SFZYD, and assayed after 28 days</li> <li>— divided into 4 groups: blank (no endometriosis), model (endo), letrozole (endo), and SFZY (endo); the first 2 received normal saline and the later 2 letrozole and SFZYD, respectively</li> <li>— DNA extraction from faecal samples after 4 weeks, 16S rRNA gene sequencing and diversity analysis performed</li> </ul>	<ul style="list-style-type: none"> <li>— letrozole and SFZYD reduced size of endometriotic lesions and lowered COX-2 expression</li> <li>— endometriotic rats vs control rats: alpha diversity decreased, <i>Firmicutes/Bacteroidetes</i> ratio increased, and <i>Ruminococcaceae</i> reduced</li> </ul>
Ni et al., 2020 [24]	Experimental study, China	Mice with induced endometriosis and control mice	<ul style="list-style-type: none"> <li>— 16S rRNA gene sequencing for microbiome analysis</li> </ul>	<ul style="list-style-type: none"> <li>— decreased variety and abundance of gut microbiota in mice with endometriosis</li> <li>— increased abundance of chenodeoxycholic acid and ursodeoxycholic acid and decreased ALA and 12, 13-EOTRE detected in faeces of mice with endometriosis</li> </ul>
Chadchan et al., 2021 [25]	Experimental study, USA	Mice with induced endometriosis and control mice	<ul style="list-style-type: none"> <li>— mice treated with broad-spectrum antibiotics (VNMA) every 12 hours for 7 days, subjected to surgically-induced endometriosis, followed by oral gavage with faeces</li> </ul>	<ul style="list-style-type: none"> <li>— faeces of mice with endometriosis had less short-chain fatty acid and n-butyrate vs faeces of mice without endometriosis</li> <li>— treatment with n-butyrate reduced growth of endometriotic lesions in mice</li> </ul>
Huang et al., 2021 [26]	Case control study, China	41 women: 21 women with endometriosis and 20 healthy controls	<ul style="list-style-type: none"> <li>— samples of faecal, cervical mucus, and peritoneal fluid microbiota collected and analysed using 16S rRNA gene sequencing</li> </ul>	<ul style="list-style-type: none"> <li>— endometriosis patients have distinct composition of microbiota, especially in feces and peritoneal fluid</li> <li>— increased level of pathogens in peritoneal fluid and reduced number of protective microbes in faeces in women with endometriosis</li> <li>— <i>Ruminococcus</i> and <i>Pseudomonas</i> identified as potential biomarkers in gut and peritoneal fluid, respectively</li> </ul>
Le et al., 2021 [27]	Case control study, USA	29 women: 20 women with endometriosis and 9 healthy controls	<ul style="list-style-type: none"> <li>— urine, faecal and vaginal samples collected</li> <li>— liquid chromatography/tandem mass spectrometry used to evaluate oestrogen levels from urine samples</li> <li>— 16S rRNA gene sequencing of faecal and vaginal samples performed</li> </ul>	<ul style="list-style-type: none"> <li>— differences in gut microbiome between patients with endometriosis and controls; GI tract bacterial communities were influenced by estrogen and its metabolites</li> <li>— endometriosis patients receiving OCP/hormonal therapy had significantly different bacterial communities vs those not receiving OCP/hormonal therapy</li> <li>— after surgery, GI bacterial communities of endometriosis patients who used hormonal therapy were more similar to those of healthy controls</li> </ul>

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Table 1 (cont.). Studies investigating association between gut microbiome and endometriosis included in the current study [17–32]				
Authors and date	Type of study and country	Sample size and characteristics	Methods	Results
Ni et al., 2021 [28]	Experimental study, China	Mice with induced endometriosis and control mice with intraperitoneal adipose tissue injection	<ul style="list-style-type: none"> <li>— faecal microbiota transplantation from mice with endometriosis</li> <li>— 16S rRNA gene sequencing performed using faecal samples to determine gut microbiome in mice with endometriosis, mice treated with ALA and control mice</li> </ul>	<ul style="list-style-type: none"> <li>— gut microbiome composition in mice with endometriosis differs from control groups; Firmicutes and Bacteroidota significantly increased and decreased, respectively</li> <li>— firmicutes, <i>Lactobacillus</i>, <i>Clostridium sensu stricto 1</i>, <i>Bifidobacterium</i> and <i>Candidatus Saccharimonas</i> increased, <i>Bacteroides</i>, <i>Dubosiella</i> and <i>Muribaculum</i> decreased</li> <li>— exogenous supplementation of ALA could restore the abundance of Firmicutes and Bacteroidota in EMS mice</li> <li>— ALA could increase the expression of ZO-2 protein in intestinal wall of EMS mice, reduce levels of LPS in abdominal cavity (<math>p &lt; 0.05</math>), and reduce the aggregation of peritoneal macrophages</li> </ul>
Svensson et al., 2021 [29]	Case control study, Sweden	264 women: 66 women with endometriosis ( $n = 66$ ) and each woman matched with 3 healthy controls ( $n = 198$ )	<ul style="list-style-type: none"> <li>— patients completed questionnaires regarding socioeconomic status, medical history, and GI symptoms, and passed stool samples</li> <li>— gut bacteria analysed using 16S rRNA gene sequencing; 58 bacteria observed at genus level in patients and controls</li> </ul>	<ul style="list-style-type: none"> <li>— both alpha and beta diversities were greater in controls vs patients with endometriosis</li> <li>— 2 bacteria of <i>Bacteroidia</i> class (<i>Bacteroides</i> and <i>Parabacteroides</i>) and 2 bacteria of <i>Clostridia</i> class (<i>Oscillospira</i> and <i>Coproccoccus</i>) were observed in higher abundance in patients with endometriosis</li> <li>— gut microbiota may be altered in patients with endometriosis</li> </ul>
Chadchan et al., 2023 [30]	Experimental study, USA	Mice	<ul style="list-style-type: none"> <li>— MD mice created by administering broad-spectrum antibiotics and anti-fungal agents</li> <li>— injection-based induction of endometriosis in mice; analysis performed after 21 days</li> <li>— faecal material oral administration</li> </ul>	<ul style="list-style-type: none"> <li>— MD mice demonstrated reduced growth of endometriotic lesions</li> <li>— transplantation of gut microbiota from mice with endometriosis reduced growth endometriotic lesions</li> </ul>
Chen et al., 2023 [31]	Experimental study, China	16 mice: 8 mice with AM and 8 control mice	<ul style="list-style-type: none"> <li>— pituitary transplantation performed for AM modelling</li> <li>— faeces samples obtained for microbial (16S rRNA gene sequencing) and metabolomic (LC-MS) assessment</li> </ul>	<ul style="list-style-type: none"> <li>— gut microbiota of mice with AM was altered</li> <li>— concentration of <i>Lactobacillus</i> and <i>Firmicutes/Bacteroidetes</i> ratio increased in mice with AM vs controls</li> </ul>
Wei et al., 2023 [32]	Experimental study, China	65 women: 35 women with endometriosis, 30 women without endometriosis; mouse model	<ul style="list-style-type: none"> <li>— blood and faecal samples taken the day before surgery</li> <li>— paraffin- embedded sections from 50 bowel endometriotic lesions, 50 uterosacral lesions, 50 samples without lesions, and 50 normal endometrial samples collected</li> <li>— 16S rRNA gene sequencing of stool samples and further assays performed</li> <li>— GUSB and macrophage expression analysed in mouse models where endometriosis was injected with oestrogen injections</li> </ul>	<ul style="list-style-type: none"> <li>— no difference in alpha and beta diversity between patients and controls</li> <li>— IHC revealed higher <math>\beta</math>-glucuronidase expression in bowel and uterosacral ligament lesions vs normal endometrium (<math>p &lt; 0.01</math>)</li> <li>— <math>\beta</math>-glucuronidase increased number and size of endometriotic lesions in mouse model with endometriosis</li> </ul>

GAD — generalized anxiety disorder; PHQ — patient health questionnaire; rRNA — ribosomal ribonucleic acid; IHC — immunohistochemistry; Pl — placebo; Pro — probiotic; S — stressed; NS — non-stressed; VNMA — vancomycin; neomycin; metronidazole; and ampicillin; SFZYD — Shaofu Zhuyu decoction; COX-2 — cyclooxygenase-2; EMS — endometriosis; ALA — alpha-linolenic acid; OCP — oral contraceptive pills; LPS — lipopolysaccharide; MD — microbiota-depleted; AM — adenomyosis; LC-MS — liquid chromatography mass spectrometry; GUSB — gut dysbiosis-derived  $\beta$ -glucuronidase

**Table 2.** Changes in the gut microbiome composition in endometriosis found in papers considered in this review [17, 18, 20, 21, 23, 26, 28–32]

Increased abundance		Decreased abundance	
Organism	References	Organism	References
<b>Species level</b>			
<i>Eggerthella lenta</i>	Huang et al. 2021 [26]		
<i>Escherichia coli</i>	Ata et al. 2019 [20]		
<i>Eubacterium dolichum</i>	Huang et al. 2021 [26]; Wei et al. 2023 [32]		
<b>Genus level</b>			
<i>Bifidobacterium</i>	Ni et al. 2021 [28]; Yuan et al. 2017 [18]	<i>Barnesiella</i>	Ata et al. 2019 [20]
<i>Biopphila</i>	Wei et al. 2023 [32]	<i>Dubosiella</i>	Ni et al. 2021 [28]
<i>Candidatus</i>	Ni et al. 2021 [28]	<i>Gardnerella</i>	Ata et al. 2019 [20]
<i>Saccharimonas</i>	Ni et al. 2021 [28]	<i>Muribaculum</i>	Ni et al. 2021 [28]
<i>Clostridium sensu stricto 1</i>	Svensson et al. 2021 [29]	<i>Odoribacter</i>	Xu et al. 2017 [17]
<i>Coprococcus</i>	Wei et al. 2023 [32]	<i>Paraprevotella</i>	Svensson et al. 2021 [29]; Xu et al. 2017 [17]
<i>Desulfovibrio</i>	Ni et al. 2021 [28]; Chen et al. 2023 [30]	<i>Ruminococcus</i>	Huang et al. 2021 [26]; Xu et al. 2017 [17]
<i>Lactobacillus</i>	Svensson et al. 2021 [29]	<i>Sneathia</i>	Ata et al. 2019 [20]
<i>Oscillospira</i>	Svensson et al. 2021 [29]	<i>Turicibacter</i>	Svensson et al. 2021 [29]
<i>Parabacteroides</i>	Xu et al. 2017 [17]	<i>Veillonella</i>	Xu et al. 2017 [17]
<i>Prevotella</i>	Ata et al. 2019 [20]		
<i>Shigella</i>			
<b>Family level</b>			
		<i>Lachnospiraceae</i>	Huang et al., 2021 [26]; Svensson et al. 2021 [29]
		<i>Ruminococcaceae</i>	Cao et al. 2020 [23]
<b>Class level</b>			
		<i>Coriobacteriia</i>	Svensson et al. 2021 [29]
		<i>Clostridia</i>	Huang et al. 2021 [26]; Svensson et al. 2021 [29]
		<i>Gamma-Proteobacteria</i>	Chadchan et al. 2023 [30]; Svensson et al. 2021 [29]
<b>Phylum level</b>			
<i>Firmicutes</i>	Ni et al. 2021 [28]	<i>Firmicutes</i> [phylum]	Chadchan et al. 2019 [21]; Chadchan et al. 2023 [30]
<i>Bacteroidetes</i>			
<i>Firmicutes/Bacteroidetes ratio</i>	Svensson et al. 2021 [29]; Chadchan et al. 2019 [21]; Chen et al. 2023 [31]; Yuan et al. 2017 [18]; Cao et al. 2020 [23]	<i>Bacteroidetes</i> [phylum]	Chadchan et al. 2023 [31]; Svensson et al. 2021 [29]; Ni et al. 2021 [28]

thus suggest two further biomarkers that could be used in endometriosis diagnosis.

Altered faecal metabolite expression has also been shown in mice with adenomyosis, a subtype of endometriosis. In their study, Chen et al. [35] found sixty differential expressed metabolites in the faeces of mice with adenomyosis compared to controls. Specifically, they found altered levels of sulfur-containing amino acids.

A shortcoming of the above-mentioned studies is that they are based on findings in mice and have not been validated in humans yet.

### Outlook for the gut microbiome in endometriosis management

The treatment of endometriosis, utilizing insights into the alterations of the gut microbiome associated with this disease, holds great promise. Recent research indicates that targeted supplementation of specific medications and substances can effectively modulate the composition of gut bacteria, subsequently leading to a reduction in inflammatory responses. This, in turn, contributes to the diminishment in both the quantity and size of developed endometriotic lesions.

In their study, Chompre et al. [19] demonstrated a significant reduction in both the quantity and size of developed lesions, along with a decrease in colonic damage, through the administration of a probiotic mixture to mice with surgically implanted endometriosis.

In 2020, Ni et al. [24] revealed a decrease in the expression of alpha-linolenic acid (ALA) in the faeces of mice with endometriosis. In a subsequent publication, Ni et al. [28] delved further into their investigations and unveiled that the supplementation of exogenous ALA led to an augmentation in the expression of the ZO-2 protein — an essential component for preserving the integrity of the intestinal barrier. This supplementation also resulted in diminished levels of lipopolysaccharide (LPS), suggesting a reduction in LPS leakage across the intestinal barrier and thereby minimizing disruption to the gut microbiome. Given that LPS is known to induce inflammatory processes [36] that could contribute to endometriosis pathogenesis, this finding holds significant potential. The researchers also observed a decrease in macrophage aggregation within the intestinal wall of mice with endometriosis as a result of exogenous ALA supplementation. Although these experiments were conducted on mice, these findings underscore the possible role of ALA in endometriosis management and the need for further in-depth investigation.

In 2020, an experimental study conducted by Cao et al. [37] in China examined the effects of letrozole, an aromatase inhibitor, and Shaofu Zhuyu decoction (SFZYD), a Chinese herbal medicine, on the gut microbiota of endometriotic rats. SFZYD, widely utilized in China as a therapeutic agent, has previously demonstrated its ability to inhibit cellular proliferation, promote apoptosis, and reduce angiogenesis in ectopic endometrial tissues [38]. Following treatment with both letrozole and SFZYD for 28 days, a reduction in the size of ectopic lesions was observed. Moreover, there was a decrease in the *Firmicutes/Bacteroidetes* ratio, which the authors propose could contribute to the mitigation of the inflammatory response in rats with endometriosis. Although these findings offer a promising avenue for potential endometriosis treatments, the study did not establish statistically significant correlations, thus necessitating further research for validation.

In 2019, Chadchan et al. [39] carried out a study where surgically induced endometriosis mice were treated with a combination of broad-spectrum antibiotics (vancomycin, neomycin, metronidazole, and ampicillin — VNMA). This treatment led not only to a reduction in the size of endometriotic lesions but also to a slower progression of the disease compared to the placebo group. The authors speculated that the therapeutic efficacy of antibiotics might be linked to the effective reduction of *Bacteroides* growth, particularly through metronidazole. Interestingly, oral ad-

ministration of faeces from mice with active endometriosis reversed the positive effect of metronidazole treatment, causing a resumption or intensification of endometriotic lesion growth and inflammation in mice previously treated with metronidazole.

In another study by Chadchan et al. [25], it was discovered that microbiota-depleted (MD) mice that received faeces from mice without endometriosis exhibited significantly smaller endometriosis lesions compared to those receiving faeces from mice with endometriosis. This suggests that faeces from mice with endometriosis might contain factor(s) that promote endometriotic lesion growth, or that faeces from mice without endometriosis might contain factor(s) that inhibit or protect against endometriotic lesion growth.

## CONCLUSIONS

Numerous studies collectively offer compelling evidence of an altered gut microbiome in relation to endometriosis, suggesting its potential significance in understanding the disease's pathogenesis, diagnosis, and management. However, it's important to acknowledge the limitations inherent in the existing research on this subject. Many of the studies available are either conducted on animal models or rely on retrospective analyses, which hinders the establishment of a definitive cause-and-effect relationship.

Despite these limitations, the exploration of the link between endometriosis and the gut microbiome holds considerable promise for future investigation. Further research in this area could yield valuable insights into the intricate interplay between these factors and offer new avenues for advancing our understanding and treatment of endometriosis.

## Article information and declarations

### Author contributions

Conceptualization, J.S. and A.U.; Literature search, A.S. and I.W.; Manuscript writing, I.W., A.U., A.S., and C.S.; Manuscript editing, J.S., P.B. and K.Cz.

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