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Title:

Endometriosis and the microbiome: a systematic review

Short title:

Endometriosis and the microbiome

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Abstract

Background: The aetiology and pathogenesis of endometriosis is still under investigation. There is evidence that there is a complex bidirectional interaction between endometriosis and the microbiome.

Objective: To systematically review the available literature on the endometriosis-microbiome interaction, with the aim of guiding future inquiries in this emerging area of endometriosis research.

Search strategy: MEDLINE, Embase, Scopus, and Web of Science were searched through May 2019. A manual search of reference lists of relevant studies was also done.

Selection criteria: Published and unpublished literature in any language describing a comparison of the microbiome state in mammalian hosts with and without endometriosis.

Data collection and analysis: Identified studies were screened and assessed independently by two authors. Data was extracted and compiled in a qualitative synthesis of the evidence.

Main Results: Endometriosis appears to be associated with an increased presence of *Proteobacteria*, *Enterobacteriaceae*, *Streptococcus* and *Escherichia coli* across various microbiome sites. The phylum *Firmicutes* and the genera *Gardnerella* also appear to have an association, however this remains unclear.

Conclusions: The complex bidirectional relationship between the microbiome and endometriosis has begun to be characterised by the studies highlighted in this systematic review. Laboratory and clinical studies demonstrate that there are indeed differences in the microbiome composition of hosts with and without endometriosis.

Keywords: Endometriosis; Microbiota; 16S ribosomal RNA gene sequencing; Systematic review; Dysbiosis

Tweetable abstract

Review findings show endometriosis associated with increased *Proteobacteria, Enterobacteriaceae, Streptococcus and E. coli* across various microbiome sites.

INTRODUCTION

Endometriosis is an inflammatory disease process, characterized by lesions of endometrial-like tissue outside the uterus, commonly affecting women of reproductive age[1]. Primarily, it causes dysmenorrhoea and subfertility, but can also yield non-cyclical or chronic pelvic pain, deep dyspareunia and dyschezia[3–5]. The severity of patient symptomatology and disease state does not correlate, even to the extent that a person can be asymptomatic with advanced endometriosis[6,7].

Aetiology

Sampson's theory of retrograde menstruation remains the most convincing hypothesis for the origin of endometriosis[8–11]. Other theories such as the coelomic metaplasia, embryonic rest, stem cell, and immune dysfunction theories may fill gaps left by Sampson's theory. A dysregulated immune response[14,15], characterised by increased production of pro-inflammatory cytokines, auto-antibodies, growth factors, oxidative stress, decreased T cell and natural kill (NK) cell reactivity, increased activation and presence of peritoneal macrophage, B cells, antibody production and angiogenesis, may contribute to an

immunosuppressive environment that enables the growth of escaped ectopic endometrial cells outside the uterus[16], potentially explaining why some women develop endometriosis following retrograde menstruation, while others do not.

The microbiome

The microbiome encapsulates all the genetic material of the microbes, including bacteria, fungi, viruses, and archaea that live within the host and regulate several physiological functions[19]. The influence of the microbiome on immunomodulation and the development of several inflammatory diseases is well-established[20]. Much is known on how the gut microbiome maintains the integrity of the gastrointestinal epithelial lining as well as immune homeostasis, preventing bacterial translocation, which can cause low-grade systemic inflammation[21,22]. Immune homeostasis ensures that the immune system shows tolerance towards commensals and self-antigens but is still responsive to pathogens[21].

Conversely, little is known about the presence and composition of the microbiome along the female reproductive tract and its role in the development of endometriosis or other gynaecological conditions. Considering the altered inflammatory status in endometriosis, postulating the microbiome is involved is logical. Chen *et al*. have recently described the existence of unique bacterial communities along the female reproductive tract from the vagina to the ovaries[23]. Interestingly, the microbiome influences oestrogen metabolism and oestrogen influences the gut microbiota[24]. Considering that endometriosis is an oestrogen dominant condition[25], gut dysbiosis leading to abnormal levels of circulating oestrogen could potentially contribute to the development of this disease[24].

The aim of this systematic review is to understand the bidirectional interaction between the microbiome and endometriosis and establishing possible concordance between the various studies.

METHODS

A comprehensive systematic review was performed to identify observational studies that compared the microbiome in humans or other species with endometriosis to those without. The review was performed according to recommended methods for systematic reviews and reported according to PRISMA guidelines[26].

Search strategies

The following databases were searched from inception until May 2019: MEDLINE and Embase via OvidSP, Web of Science Core Collection, and Scopus. OpenGrey was used to search for grey literature. The electronic search algorithm consisted of terms relating to key concepts of "endometriosis" and "microbiome" (Appendix S1).

Reference lists of relevant articles and related reviews were manually searched to identify papers not captured by the electronic searches. Authors were contacted for further information when necessary. There were no language restrictions in the search or selection of papers. Studies were uploaded to Covidence (Veritas Health Innovation, Melbourne, Australia)[27].

Selection of studies

All studies, published and unpublished in any language at any time, were considered for inclusion. Eligible studies were selected if the focus of the paper was the interaction of endometriosis and the microbiome in mammalian hosts. Only studies that included a cohort of cases (i.e. with endometriosis) and a cohort of control (i.e. without endometriosis) were considered eligible. Outcomes included any comparison of the microbiome composition in mammalian hosts' tissues with and without endometriosis. Where same cases and controls were included in more than one publication (e.g. abstract and full-text manuscript), only the publication offering the most detailed information was included. Abstracts were considered eligible if no full-text manuscript was available.

Eligibility Assessment and Data Extraction

Two authors (M. L. and G. C.) independently screened titles and abstracts. Discrepancies were resolved by consensus between M.L. and G.C. Full-text assessment was then done by M.L. and G.C. Again, discrepancies were resolved by consensus between M.L. and G.C. Data extraction was completed by C.H. for the following: study design, research objectives, setting (laboratory, field), case and control subjects/conditions, host type (and source, for animal subjects), endometriosis state (when relevant/documented), microbial community (e.g., intestinal, reproductive tract), method of characterisation of the microbiome, phylotype and/or other relevant features of the microbiome, and study findings.

Data analysis

Findings of relevant studies were organized in a qualitative synthesis according to host type, method of characterisation of the microbiome, and phylotype. The general direction of association was sought from the included publications.

Quality assessment

For human studies and the sole Rhesus monkey study[28], quality was assessed on the basis of Newcastle-Ottawa Scale (NOS) for case-control studies[29]. For mice model studies, the SYstematic Review Centre for Laboratory animal Experimentation (SYRCLE) risk of bias assessment tool was utilised[30]. Risk of bias assessment was done by M.L.

Patient and Public Involvement

There was no patient or public involvement in this study.

RESULTS

Number of retrieved papers

The systematic search, depicted in Figure S1, resulted in 251 records. These were uploaded to Covidence and 106 duplicates were immediately removed. Titles and abstracts were screened and 112 studies were deemed irrelevant and therefore excluded. A full-text review was done for the remaining 33 studies. Two studies included participants from the same study and therefore only the report including the most detailed information needed for this review was included[31], while the other was excluded[32]. Eighteen studies published between 2002 and 2019 were included (Table S1)[23,28,31,33–46]. Excluded studies are shown in Table S2.

Quality assessment

Risk of bias assessment using the NOS and SYRCLE tools revealed overall poor-moderate study quality, with many criteria not being met or not being clearly stated in the text (Table S3A/B). Unclear or absent from the full texts in all but one human study[47] was whether study participants were consecutively recruited, permitting the closest representation of patients with endometriosis. Human studies that had a NOS score of ≥ 6 (out of 8, as the non-response rate category was not applicable for these study methodologies) had reliably strong definitions of cases versus controls, appropriate selection of controls, and laparoscopic evidence of endometriosis presence or absence[34,35,40,41,46,47].

Characteristics of included studies

Animal model studies

Five of the eighteen studies identified were conducted using animal models. One study involved the use of rhesus monkeys as a non-human primate model[28], while the remaining four studies used murine or rodent models[39,42,44,45]. This included Sprague-Dawley[39,44] and C57BL[45] models, however one study did not declare which murine model was used[42]. Endometriosis was surgically induced in the murine/rodent models via intraperitoneal transplantation however there was slight variation amongst the methods used in each study. Each of the models used were homologous, and tissue was transferred from the same animal.

Clinical studies

Thirteen of the eighteen studies identified were clinical studies that examined various tissue types, including the gut, vagina, cervix, endometrium, fallopian tubes, ovarian, peritoneum, peritoneal fluid, follicular fluid, menstrual blood, and ectopic endometriosis lesions. In all studies except one[31], the presence or absence endometriosis was confirmed in both patient and control cohorts via laparoscopy.

Microbiome quantification

Various methods were used to quantify the microbiome. Five of the eighteen studies utilised the conventional culturing and colony count method to determine the presence and abundance of microbial communities[23,28,35,36,39]. 16S rRNA amplicon sequencing was utilised in seven studies[23,31,34,40,43,45,46] in order to sequence the genomic material, while one study utilised macrogenomic sequencing[42]. The various kits and sequencing methods used are outlined in Table 1. Out of the seven studies that used 16S rRNA amplicon sequencing, only five discussed the targeted sequencing region[23,31,40,45,46]. Each study targeted different regions, including region V3/V4[31], region V4[45], region V3-V5[46], region V4/V5[23], and region V5/V6[40]. The remaining studies used an unknown sequencing region. Additionally, four studies used qPCR to detect the presence of viruses, in particular, human papilloma virus (HPV)[33,37,38,41], while one study used qPCR to detect the presence of mollicutes[47].

Diversity assessments

Microbial communities can be characterised through the use of diversity indices[48]. Alpha (α) diversity is used to describe the diversity of a microbial community within a single sample or site whereas beta (β) diversity is an index used to compare the diversity of microbial communities across different samples or sites[49]. Five of eighteen studies assessed α-diversity[23,31,40,44,45]. Three studies utilised Shannon's Diversity Index to assess α-diversity[31,40,45]. The study by Yuan *et al*. additionally used Simpson's index, Chao 1, ACE, Observed Species and Good's coverage to assess diversity of the communities[45]. One study utilised UniFrac analysis in QIIME to assess α-diversity[23] and one study did not include what method was used for analysis[39], though this was simply an abstract.

β-diversity was assessed in three studies[31,40,45]. Two studies utilised UniFrac analysis to assess β-diversity[23,40] and two studies used Principle Coordinate Analysis[31,40]. Additionally, one study used Bray-Curtis dissimilarity index matrices to assess the diversity[31].

Outcomes of the included studies

Microbiome

Thirty-six bacterial taxa were identified as being significantly different between endometriosis and control groups (Tables 2A and 2B). Twelve distinct areas of the body were sampled in the studies included in this review (Table S4). The most common sites were the gastrointestinal tract/stool and endometrium. When differences were identified, the site of those differences has been highlighted in Tables 2A and 2B. The only human studies that demonstrated statistically significant differences in the bacterial taxa originated from Asian countries (China, Japan, Turkey).

At the **phylum** level, *Actinobacteria*[45], *Firmicutes*[45], *Proteobacteria*[42], and *Verrucomicrobia*[42] were identified as being significantly higher in the endometriosis cohort, compared to controls. However, *Firmicutes* was also reported to be significantly decreased in the endometriosis cohort in one study, however this was not confirmed as statistically significant as no *p* value was provided in the abstract[42].

At the **class** level, *Betaproteobacteria* was reported as being significantly higher in the endometriosis population of mice[45]. At the order level, *Bifidobacteriales* and *Burkholderiales* were also reported to be significantly higher in the endometriosis group of mice, while *Bacteroidales* predominated in the mock group[45].

At the **family** level, *Bifidobacteriaceae* and *Alcaligenceae*[45] were found to be significantly increased in animal models of endometriosis, compared to controls. *Staphylococaeae* and *Streptococcaceae* were found to be significantly increased in women with endometriosis who had been treated with a GnRH agonist compared to controls[34]. In contrast, *Lactobacillacae* was found to be significantly decreased in the same cohort[34]. *Enterobacteriaceae* was also reported to be significantly increased in the endometriosis population of two studies, including one involving treatment of endometriosis with a GnRH agonist[34,40].

At the **genus** level, *Atopobium, Barnesella, Prevotella, Gemella, Lactobacillus, Dialister, Megasphaera,* and *Sneathia* were found to be significantly decreased in endometriosis cohorts, compared to control cohorts[31]. In contrast, *Alloprevotella, Enterococci, Parasuterella, Shigella, Ureaplasma,* and *Ruminococcaeae* were found to be significantly increased in the endometriosis cohort, compared to controls[31,45].

The **genera** *Streptococcus* and *Escherichia* were found to be significantly increased in the endometriosis population compared to controls, in more than one study[31,35,40]. *Gardnerella* was found to be significantly increased in the endometrial, vaginal and cervical microbiota across two studies[31,35], yet significantly decreased in the stool of another[31].

At the species level, *Escherichia coli* was found to be significantly increased in two studies[35,36]. *Blautia, Coprococcus, Lachnospira, Peptococcaceae,* and *Tyzzerella* increased in endometriosis mice following treatment with Nei Yi Fang (NYF), a Chinese medicine compound, however this data is not confirmed to be statistically significant as it was derived from an abstract[42].

The nine detected taxa belonging to the phylum *Proteobacteria,* were all reported to be significantly increased in endometriosis cohorts, compared to controls, across seven different studies[31,34–36,40,42,45].

Six of the eighteen studies did not specify a significant difference in microbial taxa between endometriosis and control cohorts[23,39,43,44,46,47]. Campos *et al.* looked exclusively at the Mollicutes class and specific species (*M. hominis*, *M. genitalium*, *Ureaplasma urealyticum*, and *U. parvum*)[47]. Chen *et al.* describe a microbiota-based model that can distinguish infertile patients with and without endometriosis, but they do not highlight the differences in taxa[23]. Wang *et al*. exclusively assessed the peritoneal fluid using a backward sequencing technique (V5→V4)[43]. Cregger *et al.* applied 16S rRNA gene amplification and sequencing of the hypervariable V3-V5 region to cervical and uterine samples in a small sample size (n = 18)[46]. Appleyard *et al.* used culture media to determine total bacteria, total lactobacilli, and total gram-negative bacteria numbers in the jejunum or the distal colon of mice[39]. Lastly, the Chompre *et al.* abstract states that faecal bacterial composition of mice was analysed before and after induction of endometriosis, but the results do not highlight the differences[44].

Virome

Four of the eighteen studies analysed the virome to determine whether there is an association between HPV and endometriosis[33,37,38,41] (Table 3). Three studies found that the HPV detection was higher and therefore associated with endometriosis[33,37,41]. However, one study found that there was no association at all[38].

Diversity analyses

Out of the eighteen studies, four assessed α-diversity between endometriosis and control cohorts[31,40,44,45]. There was no significant difference between endometriosis and mock mice in one study[45], and this was also reported in a clinical study[31]. Another study found that α-diversity was lower in stressed animals[44]. Finally, one study found that α-diversity was significantly higher in the endometriosis population[40].

Only three studies assessed ß-diversity[31,40,45]. It was reported in one study that the ß-diversity index was significantly higher in the endometriosis mice group, compared to controls[45]. It was also found that the diversity between vaginal, cervical and gut sites was similar between endometriosis and control groups in Ata *et al*[31].

Bacteroidetes/Firmicutes ratio

Two studies measured the Bacteroidetes/Firmicutes ratio[44,45]. One study found that in a mice model of endometriosis, the ratio was two-fold higher than in control mice[45]. Another study found that the ratio was altered, but it is unclear in which direction this is in[44].

Endometriosis stage

Table S1 outlines how the endometriosis in patients in the human studies was classified. Only three studies specifically stated there was no difference in their specific microbiome findings between groups[35,46,47]. Cregger *et al*. specify that American Society of Reproductive Medicine (ASRM) stage III exhibited "differences" from the other stages, yet there was only one patient classified as ASRM stage III in their study[46]. None of the studies included in this review planned a comparison between ASRM stages as their primary study design.

DISCUSSION

Main findings

Endometriosis appears to be associated with elevated levels of *Proteobacteria, Enterobacteriaceae, Streptococcus and Escherichia coli* across various microbiome sites. The phylum *Firmicutes* and genera *Gardnerella* also appear to have an association, but the studies were sometimes conflicting. Nine different taxa were reported to be significantly increased in the endometriosis cohort, across seven separate studies (Tables 2A and 2B)[31,34–36,40,42,45].

Strengths and limitations

This study presents the first systematic review of the literature that compares the microbiome of mammalian hosts with and without endometriosis. The majority of the studies included focus on humans with laparoscopic documentation of endometriosis presence or absence, which is essential when comparing the composition between groups. This study presents a thorough summary of the included studies' findings and methodologies, which are heterogenous and can be challenging to review individually. There are limitations to the review itself. It is possible that there may be additional studies that were not identified. We found only eighteen eligible studies, many of which are of poor-moderate quality or have an unclear risk of bias. The scarcity of the literature resulted in some reliance on animal studies, which carry their own limitations.

Animal model studies

The development of endometriosis in non-human primate models is rare and slowly progresses, providing a small sample size as seen in Bailey *et al*. 2002[28]. One of the major limitations of mice models is that they do not menstruate and therefore require the surgical

induction of endometriosis. Each of the mice studies included used the homologous method (involving the transfer of tissue from the same animal), however there are variations amongst them, which may introduce some bias.

Human studies

The use of the conventional culturing and colony count method prevents the detection of microbial communities that are unculturable or low in abundance[50]. There are also some limitations of 16S rRNA amplicon sequencing, including the risk of amplification bias. Additionally, amplicon sequencing provides a broad, low-resolution view of the microbial communities present in a sample, in comparison to metagenomic sequencing[51]. Finally, it is important to consider the hypervariable region used for sequencing, as there is some evidence to suggest that the V1/V2 region is not reliable in accurately representing the microbial communities present in the female genital tract[52].

Moreover, there are several sample-specific limitations with these studies that include, but are not limited to, small sample size, representation of the cases to reflect a true patient population, definition and selection of controls (which often included other pathology, which may act as a confounder for microbiome findings), other population confounders such as timepoint in menstrual cycle, use of hormonal medications, and administration of antibiotics or probiotics. These not only create additional heterogeneity between studies, but also call into question the validity of the results.

Interpretation

The microbiome may be involved in the pathogenesis of endometriosis

A dysfunctional immune response appears to have a significant role and there is some evidence to suggest that the microbiome may modulate the immune response in endometriosis. The bacterial contamination hypothesis suggests that microbial pathogens activate the immune response by binding with Toll-Like receptors (TLRs)[53]. Lipopolysaccharide (LPS) is a bacterial endotoxin and marker of inflammation found in the cell wall of gram-negative bacteria, which has been shown to promote the onset and progression of endometriosis lesions via binding with TLR4[53–56]. Eight studies detected taxa belonging to the phylum *Proteobacteria* that were significantly increased in endometriosis cohorts[31,34–36,40,42,45,46]. Interestingly, this phylum is characterised by gram-negative staining, and hence LPS[57]. It is unclear whether bacterial contamination occurs via direct migration from the vagina into the uterine cavity. However, four studies included in this review reported the prevalence of microbial communities along the reproductive tract in women with endometriosis[23,34,35,46]. Along the lines of inflammatory markers, Campos *et al.* identified an association between *Mycoplasma genitalium* and interferon-γ and interleukin-1ß, though there was no significant difference in microbial taxa between endometriosis and control groups[47]. No other studies demonstrated associations between inflammatory markers and microbiota. Finally, a recent study found that administration of metronidazole to an endometriotic mice model resulted in a reduction in the volume of ectopic lesions as well as the magnitude of the inflammatory response[58].

Endometriosis and ethnicity

It has been reported that ethnicity and geographical location have a large impact on the taxonomic composition of microbial communities. However, it is unclear whether the impact of ethnicity is due to genetic variability, or is the result of cultural practices[59]. A meta-analysis of the influence of race and ethnicity on the prevalence of endometriosis found that in comparison with white women, black women are less likely to be diagnosed with endometriosis, while Asian women are more likely to receive a diagnosis[60]. Interestingly, all human studies that demonstrated significantly different microbiota between women with endometriosis and those without originated from Asian countries (Japan, China, Turkey).

The microbiome as a non-invasive diagnostic tool

The lack of non-invasive diagnostic tools continues to be a major dilemma in diagnosis of endometriosis. Though we must exert caution to not overestimate the value of the differences in the microbiome that have been summarized in this review, there is perhaps potential for the establishment of specific microbial signature to aid in the non-invasive diagnostic process, especially for those with isolated superficial endometriosis. In particular, Khan *et al.* reported a significant increase in the levels of *E. coli* in the menstrual blood of women with ovarian endometriomas and superficial peritoneal lesions, when compared to women with ovarian endometriomas alone[36]. However, the limitations stated above highlight the importance of future studies also taking into account patient and environmental confounders on the microbiome, so that we may know how to interpret tests optimally.

The microbiome and oestrogen metabolism

Endometriosis is an oestrogen-dominant condition[25]. Within the gut microbiome exists the 'estrobolome', which encapsulates the enteric microbial genes whose products have the capacity to metabolise oestrogens in the gut[61]. The secretion of ß-glucuronidase and ß-glucosidases by enteric bacteria promotes the deconjugation of oestrogen, which may therefore increase reabsorption of free oestrogens, resulting in higher circulating levels[61,62]. An analysis of microbial genomes found that multiple genera within the gut microbiome encode for ß-glucuronidase production, including *Bacteroides, Bifidobacterium, Escherichia* and *Lactobacillus*[62]. Notably, the genus *Escherichia* was reported to be significantly higher in the stool of endometriosis patients, compared to controls in one study included in this review[31]. The role of the 'estrobolome' and ß-glucuronidase-secreting bacteria in endometriosis is currently unknown. However, it is suggested that a dysbiotic gut microbiome that promotes the deconjugation of oestrogens resulting in increased circulating levels may contribute to a hyper-oestrogen environment which promotes the progression of endometriosis[24]. Further studies investigating ß-glucuronidase activity in women with endometriosis are required in order to determine the role of the 'estrobolome' in endometriosis.

Future directions

Recent advances in multiomic technology have enabled comprehensive analysis of microbial communities. Amplicon sequencing, shotgun metagenomic sequencing and next generation RNA sequencing are powerful tools that have resulted in a greater understanding of the human microbiome[63]. The use of next generation RNA sequencing would be beneficial in order to detect changes in the expression of microbial genes and enable the discovery of the

functional profile[63]. From a clinical perspective, assessment of the microbiome of the gut and female reproductive tract in humans over a period of time, with and without intervention for endometriosis, is recommended. Considering that there is a mindset that patients with endometriosis have various phenotypes (superficial, ovarian, and deep endometriosis)[10], may have different prevalence based on ethnicity[60] and sometimes present quite different clinically (e.g. pain-related complaints versus infertility), microbiome findings could be stratified in order to decipher whether there are any differences between these groups. Another concept that warrants further exploration is the relationship between the microbiota and specific inflammatory markers that are elevated in patients with endometriosis.

CONCLUSION

The complex bidirectional relationship between the microbiome and endometriosis has begun to be characterised by the studies highlighted in this systematic review. Laboratory and clinical studies demonstrate that there are indeed differences in the microbiome composition of hosts with and without endometriosis. Additional, methodologically-sound translational studies are needed to further our understanding of the interactions of endometriosis and the host microbiome.

Disclosure of interests

None declared. Completed disclosure of interest forms are available to view online as supporting information.

Contribution to authorship

M.L., C.H., F.E-A., E.E-O., and G.C. wrote the protocol. M.L. provided the search. M.L. and G.C. independently screened and selected eligible studies. C.H. extracted data. Differences of opinion were registered and resolved by consensus between M.L. and G.C.. M.L., C.H., F.E-A., E.E-O., and G.C. took part in interpretation of the data and writing of the review.

Details of ethics approval

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Table/Figure Caption List

Table 1: Summary of detection and sequencing methodology.

Table 2A: Summary of bacterial taxa part one.

Table 2B: Summary of bacterial taxa part two.

Table 3: Summary of virome detection.

Figure S1: Flow diagram for study selection.

Table S1: Characteristics of studies included in the systematic review.

Table S2: Excluded studies.

Table S3A: Risk of bias assessment (Newcastle–Ottawa Quality Assessment Scale criteria)

for human and rhesus monkey studies.

Table S3B: Risk of bias assessment (SYstematic Review Centre for Laboratory animal

Experimentation (SYRCLE)) for laboratory mice studies.

Table S4: Frequency of anatomical site/source assessment in systematic review included

studies.

Appendix S1: Search Strategy.

Table 1 Summary of detection and sequencing methodology.
Legend: PCR - Polymerase chain reaction; qPCR – quantitative PCR

Methodology	Number of studies
Detection method	
▪ Conventional culturing and colony counting	5
▪ 16S ribosomal RNA sequencing	7
▪ qPCR	5
▪ Macrogenomic sequencing	1
Data extraction kits	
▪ QIAamp DNA Stool Mini Kit (Qiagen)	1
▪ QuickGene DNA tissue kit S (Kurabo)	1
▪ Purelink Genomic DNA Mini Kit (Invitrogen)	1
▪ Power Soil® DNA Extraction Kit (Mobio)	2
▪ DNA Mini Kit (TransGen Biotech)	1
▪ CTAB/SDS method	1
▪ NucleoSpin Microbial DNA	1
Sequencing method	
▪ Illumina MiSeq	4
▪ Ion Torrent PGM	4
▪ Macrogenomic sequencing	1
▪ Illumina HiSeq2500	1
▪ 16S ribosomal RNA pyrosequencing	1
▪ qPCR	4

Table 2A Summary of bacterial taxa part one.

All bacterial taxa included reached statistical significance (p < 0.05), excluding results published by Shan et al. For Ata et al. 2019, sensitivity analyses excluding Lactobacillus were conducted on the vaginal and cervical microbiota.

Legend: ↑ = increased, ↓ = decreased, ● = completely absent, * = animal model, ★ = GnRHa-treated women.

PHYLUM	CLASS	ORDER	FAMILY	GENUS	SPECIES
Actinobacteria ↑ Yuan 2018 (stool)*	*Actinobacteria*	*Bifidobacteriales* ↑ Yuan 2018 (stool)*	*Bifidobacteriaceae* ↑ Yuan 2018 (stool)*	*Gardnerella* ↓ Ata 2019 (stool) ↑ Ata 2019 (vaginal/cervical excluding *Lactobacillus*) ↑ Khan 2014 (endometrial)	
	Coriobacteriia	*Coriobacteriales*	*Coriobacteriaceae*	*Atopobium* ↓ Ata 2019 (vaginal, cervical)●	
Bacteroidetes	*Bacteroidetes*	*Bacteroidales*	*Porphyromonadaceae*	*Barnesella* ↓ Ata 2019 (stool)	
			Prevotellaceae	*Prevotella* ↓ Ata 2019 (vaginal) ↓ Ata 2019 (cervix excluding *Lactobacillus*) *Alloprevotella* ↑ Ata 2019 (cervical)	
Firmicutes ↓ Shan 2018 (stool)* ↑ Yuan 2018 (stool)*	*Bacilli*	*Bacillales*	*n/a*	*Gemella* ↓ Ata 2019 (vaginal)●	
			Staphylococaeae ↑ Khan 2016 (endometrial, ovarian endometrioma fluid)★		
		Lactobacillales	*Enterococcaceae*	*Enterococci* ↑ Khan 2014 (endometrial)	
			Lactobacillacae ↓ Khan 2016 (endometrial)★	*Lactobacillus* ↓ Bailey 2002 (stool)*	
			Streptococcaceae ↑ Khan 2016 (endometrial, ovarian endometrioma fluid)★	*Streptococcus* ↑ Akiyama 2019 (cervical mucus) ↑ Ata 2019 (cervix excluding *Lactobacillus)* ↑ Khan 2014 (endometrial)	

Table 2B Summary of bacterial taxa part two.

All bacterial taxa included reached statistical significance (p < 0.05), excluding results published by Shan et al. For Ata et al. 2019, sensitivity analyses excluding Lactobacillus were conducted on the vaginal and cervical microbiota.

Legend: ↑ = increased, ↓ = decreased, ● = completely absent, * = animal model, ★ = GnRHa-treated women, Δ = increase in endometriotic mice after Nei Yi Fang treatment.

PHYLUM	CLASS	ORDER	FAMILY	GENUS	SPECIES
Firmicutes	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia* ↑ Shan 2018 (stool)Δ *	
				Coprococcus ↑ Shan 2018 (stool)Δ *	
				Lachnospira ↑ Shan 2018 (stool)Δ *	
				Tyzzerella ↑ Shan 2018 (stool)Δ *	
			Peptococcaceae ↑ Shan 2018 (stool)Δ *	*Dehalobacterium*	
				Ruminococcaeae ↑ Yuan 2018 (stool)*	
	Negativicutes	*Selenomonadales*	*Veillonellaceae*	*Dialister* ↓ Ata 2019 (cervix excluding *Lactobacillus*)	
				Megasphaera ↓ Ata 2019 (cervix excluding *Lactobacillus*)	
Fusobacteria	*Fusobacteriia*	*Fusobacteriales*	*Leptotrichiaceae*	*Sneathia* ↓ Ata 2019 (cervical, stool)	
Proteobacteria ↑ Shan 2018 (stool)*	*Betaproteobacteria* ↑ Yuan 2018 (stool)*	*Burkholderiales* ↑ Yuan 2018 (stool)*	*Alcaligenceae* ↑ Yuan 2018 (stool)*		
			Sutterellaceae	*Parasuterella* ↑ Yuan 2018 (stool)*	
	Gammaproteobacteria	*Enterobacteriales*	*Enterobacteriaceae* ↑ Akiyama 2019 (cervical mucus) ↑ Khan 2016 (endometrial)★	*Escherichia* ↑ Ata 2019 (vaginal/cervical excluding *Lactobacillus*)	*Escherichia coli* ↑ Khan 2010 (menstrual blood) ↑ Khan 2014 (endometrial)
				Shigella ↑ Ata 2019 (vaginal/cervical excluding *Lactobacillus*)	
Tenericutes	*Mollicutes*	*Mycoplasmatales*	*Mycoplasmataceae*	*Ureaplasma* ↑ Ata 2019 (cervix excluding *Lactobacillus)*	
Verrucomicrobia ↑ Shan 2018 (stool)*					

Table 3 Summary of virome detection.

Legend: ID – identification; STI – sexually transmitted infection; PCR – polymerase chain reaction; * - control tissue samples originate from healthy-appearing tissue in patients with endometriosis

Study ID	Method	Tissue type	Virus detection rate		
			Human papilloma viruses	**Herpes virus**	**Other STIs**
Oppelt *et al.* 2010	PCR	Endometriosis lesions Tissue-matched controls	Endometriosis tissue samples - 11.3% Control tissue samples* – 27.5%	No association	No association
Heidarpour *et al.* 2017	PCR	Formalin-fixed, paraffin-embedded ovarian tissue	Endometriosis tissue samples – 26.0% Control tissue samples – 10.2%	No data	No data
Rocha *et al.* 2019	PCR	Vaginal, cervical, endometrial, ovarian, uterine tube lavage and peritoneal fluid	Endometriosis patients – 82.8% Control patients – 38.7%	No association	No association
Vestergaard *et al.* 2010	PCR	Endometrial tissue Endometriosis lesions	Endometriosis patients – 3.2% Control patients – 10.0%	Endometriosis patients – 6.3% Controls – 0%	No association