Microbial dysbiosis and disease pathogenesis of endometriosis, could there be a link?

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Abstract

Endometriosis is an estrogen-dependent inflammatory condition in women that is characterised by the ectopic growth of endometrial glands and stroma outside of the uterine cavity. Although there exists many theories for the pathogenesis of endometriosis, none has been successively confirmed as a direct cause for disease development. The human body comprises a diverse microflora across all tissues that can have fundamental roles in health and disease. The microbial flora in a healthy individual can vary remarkably between anatomical sites due to the physical and chemical properties of specific tissues. This includes the female reproductive tract, notably the vagina, which harbors a microbiota dominated by *Lactobacilli* species. In addition, a core unique microbiome has been defined for the endometrium that also includes *Lactobacilli* spp. In this review we examine the possibility that endometriosis could result from microbial dysbiosis, whereby significant changes to the natural microflora within the endometrium could reduce mucosal immune regulation in this tissue with concomitant expansion of pathogenic bacteria that trigger local tissue inflammation that could perpetuate the development of endometrial disease.

Keywords: Endometriosis, Retrograde menstruation, *Lactobacilli* spp., Microbial dysbiosis,Gynaecological diseases.

Accepted on 28 December, 2016

Introduction

Endometriosis is a disorder characterised by benign, ectopic growth of estrogen-dependent endometrial tissue outside of the uterine cavity, commonly in the pelvic region. The prevalence of endometriosis is more frequent in women of reproductive age and those who exhibit pelvic pain and infertility. Endometriosis seems to be most common in women aged around 25 to 45 years [1-4]. Moreover, 45 to 49% of women who presented with pelvic pain, 33% with dysmenorrhea and 42% of women aged between 25 to 34 years with infertility had endometriosis [2,5]. The disorder is generally associated with dysmenorrhoea (painful menstruation), dyspareunia (painful sexual intercourse) dysuria (painful urination), pelvic pain and infertility [6-9]. In addition, risk factors for endometriosis include early menarche and late menopause, short menstrual cycle and heavy menstrual bleeding, along with prolonged exposure to endogenous estrogen and exposure to chemicals that disrupt normal endocrine homeostasis within the reproductive tract [7,9,10].

Endometriosis can be classified based on the anatomical location of lesions and severity and this can assist clinicians with sequential treatment and management of the condition. Diagnostic methods for the early identification of endometriosis are still lacking which means that clinicians must rely heavily on invasive surgical procedures for confirmation of the disease [11,12]. In addition, there a few treatment and management options for patients with endometriosis, and surgical intervention remains the main option for most patients. Curative treatments for the disease are absent and this is due mainly to a poor understanding of the cellular and molecular basis of disease pathogenesis.

Several studies have attempted to measure the impact of endometriosis on the quality of life of affected women and the subsequent cost across countries and ethnicities [13-20]. These studies reveal that the disease bears a significant social, physical, psychological and economic burden on those affected, given that it negatively impacts an affected woman's health related quality of life, reproductive capacity and work productivity. Severe pelvic pain is the predominant contributor to a loss of work productivity among affected women and this can greatly impede other daily activities [19]. Endometriosis thus can have a significant negative impact on a woman's life in a multi-factorial manner which urges a closer examination of the pathogenesis of the disease.

Several theories have been proposed for the pathogenesis of endometriosis, however the contribution of microbial dysbiosis to the development of the disease has been poorly examined. It is now understood that the human body has an extensive microbial flora which is established early in life [21] and each tissue displays a unique microbial flora which is determined by both the physical and chemical properties of the individual tissue [22-24]. Scientists have discovered that the normal microflora can have direct health benefits to the host and if the balance between healthy bacteria and pathogens ensues (i.e. microbial dysbiosis), this can have a direct impact on disease pathogenesis [21]. In addition, the role of the microbiota has extended from the gut and the skin which are the two major mucosal sites of microbial inhabitation. It is now apparent that the microbiota plays an important role in both health and disease in humans and can impact on various body tissues, to the extent that is has been implicated in diseases such as type 2 diabetes [28], autoimmune diseases like rheumatoid arthritis and multiple sclerosis [29,30] and for metabolic diseases such as kwashiorkor [21]. This review will provide an overview of some recent studies which have examined the makeup of the microbial flora of the female reproductive tract but also explore how microbial dysbiosis could provide a link to the development of endometriosis.

Table 1. Current theories on the pathogensis of endometrosis.

Theory	Proposed mechanism of action	References
Retrograde menstruation	Reflux of endometrial tissue and cells through the fallopian tubes to the ovaries.	[31]
Stem cell implantation	Somatic stem cells: epithelial progenitor cells (eEPC) and endometrial mesenchymal stem cell (eMSP) populations undergo retrograde migration into the peritoneum, because of cervical obstruction by mucus plug. After migration, they lie dormant until menarche, when estrogen levels rise and stimulate the growth of endometriosis.	[94,95]
Coelomic metaplasia	Strongly accepted for the pathogenesis of ovarian endometriosis. Here metaplastic change occurs to the coelomic epithelium, covering the ovary and the serosa of the peritoneum, such that peritoneal tissue transforms into endometrial-like tissue.	[96]
Müllerian remnant abnormalities	Abnormal differentiation or migration of the embryonic Müllerian ducts (which develop into the uterus, fallopian tubes and upper vagina) cause cells to spread to atypical pelvic locations, particularly the uterosacral ligaments and pouch of Douglas.	[97-99]

Disease Pathogenesis

Endometriosis is identified as a complex disease given that it lacks a clear process of disease pathogenesis, which subsequently impedes on diagnosis and treatment. The most-widely supported theory for disease pathogenesis of endometriosis is that of retrograde menstruation (Table 1) [31]. This theory supports the notion that during the normal process of menstruation, there is a reflux of endometrial tissue and cells through the fallopian tubes to the ovaries, where it subsequently enters into the peritoneal cavity and grafts ectopically to genitourinary tissue in the peritoneal cavity. Burney and Giudice [32] explained that menstrual blood is quite commonly found in the peritoneal fluid of healthy women and this can be a common occurrence in adolescent girls with congenital outflow obstruction. Moreover, retrograde menstruation has been induced in non-human primates, the *Papio anubis* baboon, through supracervical ligation, which resulted in histologically-confirmed endometriosis [33,34]. However, in challenge to the retrograde flow theory, it is observed that approximately 90% of women are known to exhibit retrograde menstruation, whilst only 15% of women have endometriosis [9]. This implies that there are other factors that contribute to the pathogenesis of the disease.

Thus, given that the retrograde menstruation theory is not conclusive, many other theories have been hypothesised. These include the stem cell implantation theory, the coelomic metaplasia theory and the Müllerian remnant abnormalities theory summarised in Table 1. Although these theories are supported to a degree by scientific evidence, they lack an absolute association to the development of endometriosis. Despite these proposed theories, a clear definite pathogenesis of endometriosis has yet to be established.

Endometriosis is characterised as an inflammatory condition, given that the peritoneal fluid of women with the disease has a heightened number of activated macrophages, as established through immune-histochemical analysis of endometrial tissue, plasma and peritoneal fluid among women with and without endometriosis [35]. Further associated to the inflammatory-state of endometriosis is an increase in a range of soluble mediators including:

Chemokines: Macrophage inhibitory factor (MIF), MCP-1, RANTES [32,36]

Proinflammatory cytokines: TNF-α, IL-6, IL-1β, INF-γ IL-8, IL-9, IL-17 [37,38].

Growth factors: Platelet-derived growth factor (PDGF), nerve growth factor (NGF) and fibroblast growth factor (FGF), also angiogenic and neurogenic factors, G-CSF [38].

Increased nuclear factor kappa beta (NF-kB) activation has been observed in peritoneal macrophages and peritoneal endometriotic lesions of patients with endometriosis resulting in up-regulation of inflammation and cell proliferation and down-regulation of endometrial cell apoptosis [39-41].

Genome-wide association studies have established certain single nucleotide polymorphisms (SNPs) associated with the disease, namely those found on chromosomes near *Wnt4*, *Greb1*, *Vezt* and *Kdr* genes as summarised in Table 2 [42-50]. How these putative susceptibility genes impact on the establishment of ectopic tissue growth or on the immune inflammatory responses within the affected individual is currently not understood. With significant correlations found between SNPs and endometriosis, genetic factors are regarded as important contributors to the development of the disease.

Animal Models of Endometriosis

One of the major barriers to understanding the cellular, molecular and genetic basis of disease pathogenesis for

endometriosis is the lack of a suitable animal model. Non-human primates have been used extensively as a model of the disease and as preclinical models, due to their spontaneous development of endometriosis. Moreover, endometriosis can also be established in non-human primates through the induction of retrograde menstruation [51]. Non-human primates are considered the most suitable model for the study of endometriosis, yet there are ethical and high-cost limitations that limit their use. Murine models offer an alternative for the study of endometriosis as they are more cost-effective and easily maintained. However, mice are unable to develop endometriosis spontaneously as they lack the ability to menstruate.

Table 2. Single nucleotide polymorphisms (SNPS) in genes associated with endometrosis.

Gene	Role	SNPs in endometriosis	Role in endometriosis
WNT4	A ligand of the Wnt signalling pathway. Associated with the normal development of the female reproductive tract, follicular development, steroidogenesis and with endometrium proliferation, decidualisation and implantation.	rs16826658 (noncoding) rs3820282 (noncoding)	WNT4 protein expression downregulated
GREB1-Growth Regulation by Estrogen in Breast Cancer 1	Encodes a protein that is a co-activator of estrogen receptor-α (ER-α) transcription factor. Promotes estrogen induced growth.	rs13394619 (noncoding) rs1898003 (noncoding) rs11674184 (noncoding) rs1865574 (noncoding) rs2884374 (noncoding)	Increased gene expression in ectopic endometrial tissue
VEZT	Encodes the adherens junction transmembrane protein vezatin that plays a role in cell-cell adhesion during embryogenesis. The protein can also enter the nucleus and regulate the expression of target genes for cell adhesion and invasion. It has also been suggested as a tumour suppressive gene.	rs10859871 (noncoding)	Increased VEZT protein expression in blood and endometrium
KDR-Kinase Insert Domain Receptor	Encodes vascular endothelial growth factor (VEGF) receptor 2. This protein is the main signal transducer in the VEGF/ VEGF receptor signaling pathway, responsible for inducing angiogenesis.	rs17773813 (noncoding)	Increased expression of VEGF receptor 2 in blood vessels in endometrium

In order to replicate the disease in mice, recipient endometrial tissue must be introduced into mice, either from syngeneic animals or through xenogeneic donor tissue [51,52]. However, recent discoveries have identified the spiny mouse (*Acomys cahirinus*) as the first rodent species known to menstruate spontaneously, with subsequent cyclic endometrial shedding and repair [53]. This provides a more suitable, yet still accessible and cost-effective murine model for the future study of endometriosis. A significant limitation with the spiny mouse strain is that it is an outbred strain which would limit the capacity to transfer cells or tissues between the spiny mouse and other inbred strains. The development of gene editing technology through the CRISPR-Cas9 system would offer one potential method by which specific gene mutations could be introduced into the spiny mouse strain and the mutations could be evaluated for their ability to induce endometriosis and its impact on innate and adaptive immune responses *in vivo*.

Host Microbiota

The healthy human body comprises of a unique, diverse and relatively stable habitation of microorganisms (bacteria, eukaryotes, archaea and viruses), whose symbiotic relationship with the host contributes to general health and wellbeing. These microorganisms that reside in and on the human body are collectively termed 'microbiota,' with their assembled genomic sequences termed 'microbiome'. The importance of microbiota to human health and physiology is essential, so much so that an individual's collective microbial community has previously been regarded as a 'neglected' and 'forgotten' organ [22-24]. The significance of an individual's microbiota stems from its physiological, immunological and metabolic functional capacity [21].

To further define the importance of an individual's microbiota, an imbalance or disruption to an otherwise commensal or mutualistic relationship with the human host, results in a state of microbial dysbiosis which can affect host biology and contribute to disease. Following the direction of Robert Koch in 1890, with his postulations that microorganisms were causative agents for disease, many contemporary diseases have been associated with microbial level-changes [25]. For instance, microbial dysbiosis has been associated with inflammatory bowel disease including Crohn's disease and ulcerative colitis [26], metabolic disease such as obesity [27] and type 2 diabetes [28], asthma [54], breast cancer [55], autoimmune disease [29,30], allergies [56] and autism spectrum disorder[57]. These growing correlations between microbial dysbiosis and disease are a product of an early 21st century scientific momentum to re-evaluate the role of microbiota in human health [58]. This topical approach to understanding disease has been made possible through advancements in high-throughput metagenomic sequencing technology alongside global efforts to characterise the healthy human microbiome [58].

Genitourinary Microbiota and Dysbiosis of the Microflora

The colonisation of microorganisms in and on body surfaces occurs at birth, with the in-utero environment considered axenic (germ free) [59-61]. It has been reported that infants are initially exposed to microorganisms upon birth, with maternal cervical mucus and immunoglobulins, as well as the placenta, providing a degree of barrier defence and antimicrobial activity from ascending vaginal infective agents [62].

Recent scientific efforts to characterise the human microbiome have determined key microorganisms that constitute the vaginal flora. *Lactobacilli* species were the most dominant species and their ability to produce lactic acid subsequently contributes to the low pH of the vagina (Figure 1) [63]. Furthermore, hormonal changes associated with the menstrual cycle have been shown to alter the composition of the vaginal microbiome. Varying levels of estrogen and progesterone have been reported to impede on microbial flora stability, particularly during menses when the flora presents with a lack of stability. However, hormonally fluctuated microbial community changes occur without effecting the functional and metabolic capacity of the vaginal flora [64].

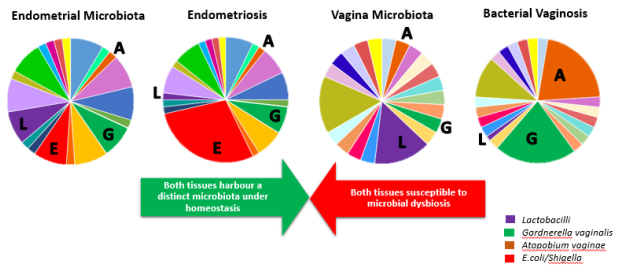

Figure 1. Summary of the microbial dysbiosis that occurs in response to endometriosis and bacterial vaginosis. The pie charts show representations of various microbial species within the endometrium and vagina of females in health disease – For simplicity we have focused on 4 main species *Lactobacilli* (purple), *Gardnerella vaginalis* (green) *Atopobium vaginae* (brown) and *E. coli/Shigella* which are members of the *Enterobactericiae* family (red) that are discussed in the text. The microbiota of women with endometriosis show a large expansion of *E. coli/Shigella* which are pathogenic and a reduction in the proportion of *Lactobacilli* spp. compared to healthy endometrium. In women with bacterial vaginosis there is an expansion of *Gardnerella vaginalis* and *Atopobium vaginae* species at the expense of *Lactobacilli* which could facilitate local tissue inflammation. The charts highlight that both the endometrium and vagina have a distinct microflora during normal tissue homeostasis, but this can change during the onset of disease such as endometriosis or bacterial vaginosis respectively.

Microbial dysbiosis is not an alien term when discussing diseases of the female reproductive tract. In fact, particular gynaecological diseases have been shown to develop in response to bacterial imbalances that can lead to bacterial vaginosis. Bacterial vaginosis is a bacterial infection of the vagina that commonly presents as abnormal grey vaginal discharge with a strong unusual 'fishy' odour. This disease can cause discomfort and unease in affected women and can reduce the frequency of successful pregnancy in patients undergoing *in-vitro* fertilisation (IVF) [65] and is also associated with premature births [66,67]. *Lactobacilli* spp. are the most abundant commensal bacteria in the vagina and the clinical onset of bacterial vaginosis is characterised by a decrease in the population of *Lactobacilli* species [65]. In contrast there is a substantial increase in growth of residing anaerobic or facultative anaerobic bacteria, such as *Gardnerella vaginalis* and *Atopobium vaginae* (Figure 1). This microbial population shift introduces heterogeneity into the resident microbial community, resulting in microbial imbalance and subsequent disease [65,67].

Table 3. Bacterial species identified in the endometrium.

Bacterial Phylotypes
*Acidovorax**
Aerococcus
Atopobium vaginae
*Bacteroides fragilis**
*Bacteroides thetaiotaomicron**
*Bacteroides ovatus**
*Bacteroides vulgatus**
*Bacteroides xylanisolvens**
*Betaproteobacteria**
Bifidobacterium
*Caulobacter**
*Chitinophagaceae**
Clostridium
*Escherichia/Shigella**
Flavobacterium ϕ
Gardnerella vaginalis ϕ
Lactobacillus crispatus ϕ
Lactobacillus iners ϕ
Lactobacillus jensenii
*Pelomonas**
Prevotella ϕ
*Pseudomonas**
Sphingomonas
Stenotrophomonas
Streptococcus
Veillonella

*Suggested as part of the uterine core microbiome [68].

ϕ=Abundant in at least one of the studies [68-71].

Recently Verstraelen et al. [68] examined the resident microbiota of the endometrium through 16S ribosomal RNA (16S rRNA) metagenomic sequencing of endometrial samples. They identified 183 different bacterial phylotypes, of which 15 had an abundance greater than 1% among the test subjects. The authors identified a 'uterine core microbiome' that not only enforces the presence of microorganisms in the endometrium, but further suggests a consistency of bacterial phylotypes among individuals. Belonging to this uterine core microbiome were bacteria from the *Proteobacteria*, *Firmicutes* and predominantly *Bacteroidetes* phyla (Figure 1 and Table 3). These findings have been supported by three independent

analyses that examined the composition of the uterine microbiota [69-71]. Brewster, et al. [72] examined the bacterial microbiome of fallopian tubes, fimbriae and ovaries and showed these various tissues harboured a significantly unique bacterial microbiome. Additionally, Pelzer et al. [73] revealed the characteristics of the microbiome of follicular fluid which provided insight into the microbial colonisation of the ovaries. Although contamination is common among the microorganisms of the vagina and follicular fluid, Pelzer et al. [73] identified that the microbiota of follicular fluid was distinct and unique to that of the vagina in some patients. Predominant species identified were *Lactobacillus iners*, *Actinomyces* spp., *Corynebacterium auromucosum*, *Fusobacterium* spp., *Peptinophilus asaccharolyticus*, *Peptostreptococcus* spp., *Propionibacterium* spp. *Prevotella* spp., *Staphylococcus* spp., and the yeast *Candida parapsilosi*.

The Role of *Lactobacillus* species in the Vaginal Microflora

Resident vaginal *Lactobacilli* spp. have been shown to reduce infection of pathogens and protect against microbial dysbiosis [74]. This protection is achieved through the general ability of *Lactobacilli* spp. to biosynthesise products of lactic acid, hydrogen peroxide (H_2O_2) and antimicrobial compounds, which act to inhibit the growth of pathogens (Figure 2) [75]. Lactic acid is the primary microbicide agent produced by *Lactobacilli* spp. and is responsible for maintaining a low pH environment, creating an unfavourable milieu for many bacterial species [76]. The acidification driven by *Lactobacilli* spp. has been shown to directly inhibit the growth of pathogens [74,75,77]. Accompanying lactic acid, is the production H_2O_2, which further acts to inhibit the growth of pathogens. H_2O_2 generates reactive oxygen species and induces oxidative stress which results in DNA damage and bacterial lethality [78]. It seems that *Lactobacilli* spp. are themselves protected from this H_2O_2 through the expression of anti-oxidative enzymes including catalase, superoxide dismutase 2 and glutathione peroxidase-1 [79]. Furthermore, H_2O_2 enhances host production of antimicrobial peptides secreted by epithelial cells, namely muramidase and lactoferrin (Figure 2). Muramidase is an enzyme that hydrolyses and thus cleaves the bacterial cell wall component peptidoglycan, inhibiting bacterial growth and survival, particularly that of gram positive bacteria [80]. Many, but not all, *Lactobacilli* spp. have a constitutively expressed proteinaceous surface layer (S-layer), that non-covalently binds to peptidoglycan and envelopes the entire cell wall [81,82]. This S-layer is often associated with a protective function and could thus infer protection from muramidase activity and other bacteriolytic enzymes [82]. On the other hand, lactoferrin, a host-derived glycoprotein, acts by binding to lipopolysaccharide (LPS), a component of the cell wall of gram negative that is absent in gram positive *Lactobacilli* spp., destabilising it and increasing the permeability of the bacterial outer membrane to surrounding intrinsic bactericidal agents [83]. Interestingly, decreased lactoferrin levels have been detected in the peritoneal fluid of women with endometriosis [84]. Not only does this support reduced *Lactobacilli* spp. abundance in women with endometriosis, it further reveals that women with endometriosis have a reduced anti-bactericidal capacity, which could further promote microbial dysbiosis. However, it remains unclear whether decreased lactoferrin levels indicate reduced *Lactobacilli* spp. abundance in the endometrium or the peritoneal cavity, making it difficult to ascertain where the microbial dysbiosis takes place.

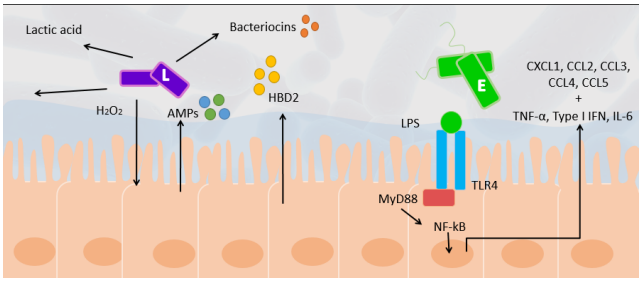

Figure 2. Mucosal immune regulation induced by the microbial flora within the female reproductive system. *Lactobacilli* spp. dominate the normal microflora in the vagina and endometrium of healthy women. These organisms can secrete lactic acid to reduce the local pH as well as H_2O_2 and bacteriocins that reduce the growth of microbial species.In addition, the presence of *Lactobacilli* can induce the secretion of antimicrobial peptides (AMPs) that include human beta defensins from epithelial cells that can directly impact on the composition and diversity of the local microflora. Emergence of pathogenic E. coli/Shigella spp. can lead to the release of lipopolysaccharide (LPS) which can bind to pattern recognition receptors (e.g. Toll-like receptor 4) on the surface of epithelial cellsto induce signalling and the release of pro-inflammatory cytokines (TNF-a, Type 1 IFN and IL-6) and chemokines that promote recruitment of inflammatory cells to the site of infection. If the microbial dysbiosis is not corrected then this could lead to chronic inflammation in the reproductive tract.

In addition to lactic acid and H_2O_2, *Lactobacilli* spp. have been found to produce a range of bacteriocins (bacteria-derived antimicrobial agents) that further inhibit the growth of surrounding bacterial and fungal pathogens [85,86]. For instance, *L. acidophilus*, produces the bacteriocin Acidophillin 801 which has a narrow inhibitory spectrum of activity against gram negative bacteria as well as some other *Lactobacilli* spp. [81] (Figure 2). *L. acidophilus* contains a S-layer that is believed to infer protection from biosynthesised bacteriocins [81]. Whilst different species vary in their capacity to produce these antimicrobial agents, the core microbiota *Lactobacilli* spp. population provides a collective effort to protect against pathogens and prevent subsequent microbial dysbiosis. These findings assert the importance of *Lactobacilli* spp. in the endometrium and their corresponding biosynthesised products in protecting against pathogens.

Bacterial Vaginosis and Microbial dysbiosis

Given that endometrial ectopic tissue has been found in the surrounding genitourinary region, as a result of retrograde menstruation, it raises a hypothesis that maybe the development of endometriosis could arise from a microbial dysbiosis. This concept has been identified more frequently and characterised in more detail with gastrointestinal diseases such as Crohn's Disease and Ulcerative Colitis [26]. Given the

proximity of the vagina to the uterus, and the recent characterisation of the microbiota along with different areas of the reproductive tract, it warrants consideration to the influence that a microbial dysbiosis might have on disease pathogenesis of endometriosis. Gynaecological diseases such as bacterial vaginosis have been shown to develop in response to an imbalance of bacterial species. As seen in Figure 1, bacterial vaginosis specifically involves a decrease in *Lactobacilli* spp. and an increase in growth of residing anaerobic or facultative anaerobic bacteria, including *Gardnerella vaginalis* and *Atopobium vaginae*. The ascent, introduction and domination of certain sexually transmitted microorganisms including *Chlamydia trachomatis* and *Neisseria gonorrhoeae*, pathogenic *Escherichia coli*, or bacterial vaginosis-related species act to alter the reproductive tract microbiota, enforcing microbial dysbiosis and subsequent disease [87-89].

Although microbial dysbiosis has been associated with other gynaecological diseases, it has been poorly investigated in relation to endometriosis. Nonetheless, several studies have attempted to characterise the endometrial microbiome of women with endometriosis and investigate the role of bacteria in relation to the disease. Khan et al. [90], utilising 16s rRNA genomic sequencing, identified a significant increase in Streptococcaceae in samples from women with endometriosis, compared to control samples. The same study also recognized a slight increase in Moraxellaceae and a modest decrease in Lactobacillaceae in women with endometriosis, though these differences were statistically insignificant.

Khan et al. [91] found that women with endometriosis had a significant increase in *E. coli* in menstrual blood and endotoxin levels in menstrual and peritoneal fluid. Following this, Khan et al. [92] further revealed that endometriosis was accompanied by an increase in Gardnerella, Group A-Streptococcus, *Enterococci* and *E. coli* upon the culturing of endometrial samples on culture medium. Although quantity of bacterial growth was measured, rather than specificity of bacterial species, culture-dependent techniques still warrant caution when accepting these results. Nonetheless, excess growth of certain bacterial species or an imbalance of commensal bacteria could contribute to the development of endometriosis.

Moreover, Khan et al. [92] also found that women with endometriosis were more predisposed to a higher vaginal pH ($\geq$ 4.5) than in control subjects, inferring that this greater diversity of bacterial species in women with endometriosis was permitted due to an altered vaginal environment. This suggests that women with endometriosis could have a reduced lactic-acid producing bacterial population, namely *Lactobacilli* spp., increasing the susceptibility to microbial imbalance and dysbiosis. As aforementioned, although the results were not statistically significant, Khan et al. [90] did note a decrease in Lactobacillaceae in women with endometriosis. This decrease was also commonly seen in women with bacterial vaginosis, supporting the notion that perhaps altered *Lactobacilli* spp. levels contribute to the development of endometriosis.

Studies investigating endometriosis in non-human primates have shed similar insights into a dysbiosis of endometrium. Bailey and Coe [93] examined the concentration of popular bacterial species in the shed endometrium of female rhesus monkeys with endometriosis by means of culture-dependent techniques. It was found that rhesus monkeys with endometriosis had reduced *Lactobacilli* spp. concentrations, whilst presenting an increased concentration of gram negative anaerobic and facultative anaerobic bacteria. As samples were not taken directly from the endometrium, but instead from shed endometrium, this might not be an accurate characterisation of endometriosis-associated endometrium microbiota. However, despite the method of sample obtainment and the results being obtained from non-human primates, they resemble the findings presented in human studies [92,90].

Conclusion

Whilst microbial dysbiosis in relation to the development of endometriosis is quite a novel concept, the collective and comprehensive analysis of several direct and multiple indirect studies have allowed for an evaluation of the topic. It has been demonstrated that the endometrium harbours a microbiota and is thus susceptible to dysbiosis, although further microbiome characterisation of the peritoneal cavity and deeper genitourinary regions is poor. Additionally, microbial dysbiosis has been shown to be responsible for other gynaecological diseases, making it reasonable to deduce that microbial dysbiosis could contribute to the disease in question. Moreover, key microbes were identified in women with endometriosis, implying that endometriosis or at least the inflammatory property of endometriosis is a result of microbial imbalance. Of particular interest was reduced *Lactobacilli* spp. abundance accompanied by a high pH environment as well as an increase in gram negative bacteria abundance, commonly *E. coli*. This shift in the microbial population was shown to have the capacity to reduce host immunological capability and induce host susceptibility to pathogens. This inference supports the retrograde menstruation theory crediting the probability of retrograde migration of pathogenic bacteria into the endometrium and ectopic endometrial tissue to atypical sites and further appreciates that genetic susceptibility may also play a role in the development of the disease, in conjunction with microbial dysbiosis. Ultimately, it seems plausible that the pathogenesis of endometriosis could be related to microbial dysbiosis, predominately centred around a reduced commensal-associated immune capability as well as an ascending microbial infection. This could have important implications for the treatment of endometriosis in the future.

Acknowledgements

We would like to acknowledge grant support of the Juvenile Diabetes Research Foundation (4-2006-1025) and Diabetes Research Foundation of Western Australia awarded to GFH.

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