RESEARCH PAPER

Comparison of the Uterine Microbiome in Women with Primary Infertility and Endometriosis and Women with Infertility Due to Male Factors at Dr. Soetomo General Hospital, Surabaya, 2025

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ABSTRACT

Endometriosis is a common gynecological disorder affecting women of reproductive age and is closely associated with infertility and chronic pelvic pain. The involvement of inflammatory pathways in the development of endometriosis has led to the hypothesis that microorganisms and their metabolites contribute to disease progression. These microorganisms may reach the pelvic cavity through translocation from the gastrointestinal tract or via ascending migration from the vagina. This study aimed to analyze the characteristics and composition of the uterine microbiome in women with primary infertility associated with endometriosis and to compare them with women experiencing infertility due to male factors. The study also sought to identify differences in microbial colonization patterns that may contribute to the pathogenesis of infertility. An observational cross-sectional study was conducted at the Gynecological Endocrinology Clinic and Graha Amerta Clinic of Dr. Soetomo General Hospital between June and September 2025. A total of 70 participants were recruited using purposive sampling, comprising 35 women with primary infertility and endometriosis and 35 women with infertility due to male factors. All participants underwent uterine swab sampling for uterine microbiome analysis. Data were analyzed using logistic regression analysis. Distinct differences in uterine microbiome composition were observed between the two groups. Among women with endometriosis, *Staphylococcus aureus* was present in 28.57% of cases, whereas *Streptococcus agalactiae* was found in 2.85%. In the male factor infertility group, *Staphylococcus aureus* was detected in 5.71% of participants, while *Streptococcus agalactiae* was identified in 22.85%. Logistic regression analysis demonstrated that *Staphylococcus aureus* was strongly associated with endometriosis-related infertility (OR 6.60; 95% CI 1.33-32.84). Women with primary infertility due to endometriosis exhibit a distinct uterine microbiome profile compared with those experiencing infertility due to male factors. These findings support a potential role of the uterine microbiome in the pathophysiology of infertility and warrant further investigation.

Keywords: Uterine Microbiome; Endometriosis; Male Factor Infertility; Dysbiosis.

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INTRODUCTION

Endometriosis is a condition of concern because it affects approximately 6-10% of women of reproductive age globally in terms of fertility and, despite many efforts, the pathogenesis and progression of this chronic disease remain poorly understood.[1] The prevalence of endometriosis in infertile women with normal ovulation and normospermic partners is 47%, compared with the prevalence of non-endometriotic tubal and/or uterine pathology at 29%.[2] On the other hand, Asian women have a higher prevalence of endometriosis compared with Caucasian women among IVF patients, at 15.7% versus 5.8%.[3] Infertility is common and affects 80 million people of reproductive age worldwide. In Indonesia, infertility may affect approximately 10-15% of the fertile-age population, comprising various causes.[4]

Based on recent research data, it is stated that microbiome imbalance or dysbiosis is implicated in various human diseases; in the case of endometriosis, much evidence has emerged suggesting that there may be a urogenital-gastrointestinal crosstalk leading to the development of endometriosis. Researchers have found that the development of the microbiome and endometriosis are bidirectionally related, implying that any changes in the host microbiome may have a significant impact on the development of endometriosis.[5]

Recent studies state that the number of bacterial cells and human cells in adult humans has a ratio of 1:1, with

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findings on the potential impact of bacterial cells on human health, and that the composition of the microbiome at specific sites, namely in the gut, vagina, nose, and elsewhere, varies between healthy individuals and patients with certain diseases.[6,7] In this regard, there is growing evidence that endometriosis patients have higher levels of bacterial colonization in their endometrial tissue and menstrual blood compared with women in the general population.[8–11]

Changes in the composition of the uterine microbiome are associated with the pathogenesis of endometriosis through mechanisms of immune dysregulation, estrogen metabolism, and the bacterial contamination hypothesis.[1,5,12] Research shows that compared with non-endometriosis patients, endometriosis patients have a significant decrease in the proportion of *Lactobacillaceae* in the uterine cavity accompanied by an increase in opportunistic pathogens such as *Streptomycetaceae* and *Staphylococcaceae* as well as the pathogen Enterobacteriaceae.[13] This uterine microbiome dysbiosis causes inflammation and immune deficiency in the endometrial microenvironment, promoting infiltration of ectopic endometrial lesions, angiogenesis, and adhesions that lead to infertility.

Broad-spectrum antibiotics have the potential to help modulate the microbiome in the gut and in the reproductive tract related to bacterial contamination.[14] Several animal model studies have demonstrated the efficacy of antibiotics in reducing the growth and development of endometriosis.[15]

This study aimed to determine the characteristics and composition of the uterine microbiome in infertile women with endometriosis and infertile women due to male factors, as well as to analyze the differences in the uterine microbiome between the two groups. Specifically, this study was conducted to identify the characteristics and composition of the uterine microbiome and to prove the existence of differences between infertile women with endometriosis and infertile women due to male factors.

RESEARCH METHODS

Study Design

This study is an observational study with a cross-sectional design involving patients diagnosed with primary infertility due to endometriosis and infertile patients due to male factors, who underwent uterine swab examination at Dr. Soetomo General Hospital during the period of June to September 2025.

Time and Place of Study

This study was conducted from June 2025 to September 2025. The study was conducted at the Gynecological Endocrinology Clinic and the Microbiology Laboratory of the Diagnostic Center Building, Dr. Soetomo General Hospital, Surabaya.

Population, Sample, and Sampling Technique

The study population consisted of all cases of primary infertility and infertile patients due to male factors at Dr. Soetomo General Hospital, Surabaya, from June 2025 to September 2025.

The study sample consisted of all cases of primary infertility with endometriosis and cases of infertility due to male factors at Dr. Soetomo General Hospital, Surabaya, from June 2025 to September 2025, that met the inclusion criteria. Sample selection was performed using purposive sampling. The sample size was determined using purposive sampling technique. Sample size calculation was performed using a sample size formula with a Za value of 1.96 at a significance level of 5%, an infertility prevalence in Indonesia of 10% based on the study by Harzif et al. (2019)[4], and an absolute deviation rate of 10%. Based on these calculations, a sample size of 34.57 respondents was obtained and rounded up to 35 respondents.

Study Subjects

The inclusion criteria in this study included primary infertile patients with endometriosis and infertile patients due to male factors who underwent examination at the Gynecological Endocrinology Clinic of Dr. Soetomo General Hospital during the period of June 2025 to September 2025, patients of reproductive age 20-45 years, and patients in the secretory phase of the menstrual cycle. The exclusion criteria included patients with secondary infertility with or without endometriosis, as well as patients with infertility due to ovulation disorders, tubal factors, and idiopathic infertility. The study variables consisted of the independent variable, namely primary infertile patients with endometriosis and infertile patients due to male factors, while the dependent variable was the uterine microbiome composition.

Operational Definitions

The operational definition of primary infertile patients with endometriosis is women who have been married for more than one year with regular sexual intercourse without contraception and have been diagnosed with endometriosis based on ultrasound and/or laparoscopy or laparotomy results. Meanwhile, infertile patients due to male factors are women who have been married for more than one year with regular sexual intercourse without contraception, with the cause of infertility originating from abnormalities in the husband’s sperm analysis. Uterine microbiome composition is defined as the collection of microorganisms living inside the uterus, while uterine dysbiosis is an imbalance of the microbial community inside the uterus characterized by *Lactobacillus sp.* levels below 90%. Data collection was carried out through medical record documentation, transvaginal ultrasound and/or laparoscopy examination, sperm analysis, and culture on agar media to detect the types of microorganisms and the composition of Lactobacillus sp. in the uterus.

Research Instruments

This study used patient data from the Gynecological Endocrinology Clinic of Dr. Soetomo General Hospital, microbiological culture, and SPSS software for data processing.

Sampling and Data Collection Procedures

First, examination of primary infertile patients with endometriosis and infertile patients due to male factors was performed. Data collection was carried out in accordance with the inclusion criteria for age, parity, menstrual phase (secretory phase), gynecological ultrasound and/or laparoscopy results, and family history of endometriosis, followed by uterine swab examination at the Gynecological Endocrinology Clinic of Dr. Soetomo General Hospital during the period of June to September 2025.

Subsequently, in collaboration with the Microbiology Laboratory of Dr. Soetomo General Hospital, microbiological culture examination was performed to observe the microbiome composition, followed by documentation of the uterine microbiome composition and microbiome percentages.

After the results of the uterine microbiome composition and uterine microbiome percentage examination were available, tabulation was performed and data were entered into the SPSS application, divided into two groups, namely primary infertility with endometriosis cases and infertile patients due to male factors. Data analysis was then performed to determine whether there were differences in the uterine microbiome composition between the two groups. After the analysis results were obtained, the data and results were recorded and reported in the research report.

Data Analysis Plan

Data analysis began with descriptive analysis to describe the basic characteristics of the study subjects and the distribution of the studied variables. Categorical variables are presented as frequencies and percentages. Subsequently, the relationship between uterine microbiome characteristics and type of infertility was analyzed using logistic regression. The results of the analysis are presented as odds ratios (OR) with 95% Confidence Intervals. A p-value of less than 0.05 was considered statistically significant. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software.

Ethical Clearance

To fulfill ethical requirements, approval from the ethics committee for basic/clinical research at Dr. Soetomo General Hospital/Faculty of Medicine, Universitas Airlangga, Surabaya, was required prior to conducting this study. To maintain the confidentiality of study subject data, the identity of study subjects was written using initials. The study subject data and research results are used solely for the purpose of scientific knowledge.

RESEARCH RESULTS

Research Data Characteristics

Table 1. Patient Characteristics

Variable	n	%
Age (years)		
<35 years	35	50
35-39 years	23	32.85
>40 years	12	17.14
Total	70	100
BMI		
Underweight	5	7.1
Normal	19	27.14
Overweight	10	14.28
Obese	36	51.42
Total	70	100
Uterine Microbiome Composition		
Staphylococcus aureus detected	12	17.14
Streptococcus agalactiae detected	9	12.85
Escherichia coli detected	7	10
Lactobacillus sp. detected	6	8.57
Enterococcus faecalis detected	3	4.28
Candida albicans detected	1	1.42
Eikenella corrodens detected	1	1.42
Bacillus flexus detected	1	1.42
Macrococcus caseolyticus detected	1	1.42

Variable	n	%
Staphylococcus haemolyticus detected	1	1.42
Staphylococcus hominis detected	1	1.42
No aerobic/anaerobic bacteria detected	27	38.57
Infertile Patients		
Endometriosis	35	50
Male Factors	35	50

The majority of the study sample was in the age group of less than 35 years (50%), followed by the 35-39 years age group (32.85%), and the more than 40 years age group (17.14%). Body mass index (BMI) showed that more than half of the respondents were in the obese category (51.42%). The proportion of respondents with normal BMI (27.14%) was followed by overweight (14.28%). The underweight proportion was recorded as the lowest (7.10%).

The variety of microorganisms that appeared was quite diverse, consisting of Gram-positive bacteria, Gram-negative bacteria, and one fungal species. The most frequently identified microorganism was *Staphylococcus aureus*, found in 12 study subjects (17.14%). This was followed by *Streptococcus agalactiae* in 9 study subjects (12.85%) and *Escherichia coli* in 7 study subjects (10%). Meanwhile, *Lactobacillus sp.*, which physiologically constitutes the dominant and protective flora in the female reproductive environment, was only found in 6 study subjects (8.57%). This finding indicates a uterine microbiome composition that is relatively not dominated by normal flora, but rather occupied more by potentially pathogenic bacteria. Other microorganisms such as *Enterococcus faecalis*, *Candida albicans*, *Eikenella corrodens*, *Bacillus flexus*, *Macrococcus caseolyticus*, *Staphylococcus haemolyticus*, and *Staphylococcus hominis* were each found in 1-3 study subjects (1.42% to 4.28%). The largest proportion was in the category of “no aerobic/anaerobic bacteria detected,” as approximately two-fifths of respondents showed no microorganism growth on uterine culture.

Association Analysis

Table 2. Dominant Microbiota in Infertile Patients with Endometriosis

Variable	Infertile Patients	
Uterine Microbiome Composition	**Endometriosis**	
	N	**%**
Staphylococcus aureus	10	47.62
Streptococcus agalactiae	1	4.76
Escherichia coli	5	23.80
Lactobacillus sp.	5	23.80
Total	21	100

Table 3. Distribution of Microbiota in Infertile Patients with Endometriosis

Variable	Infertile Patients	
Uterine Microbiome Composition	**Endometriosis**	
	N	**%**
Aerobic/anaerobic bacterial growth detected	21	60
No aerobic/anaerobic bacteria	14	40
Total	35	100

The proportion of microbiota in infertile patients with endometriosis can be seen in Tables 2 and 3. A total of 21 patients (60.0%) showed the presence of aerobic or anaerobic bacterial growth on uterine microbiome examination, while 14 patients (40.0%) showed no bacterial growth. Among patients with identified bacteria, the most frequently found microorganism was *Staphylococcus aureus*, detected in 10 patients (28.57%). *Escherichia coli* and *Lactobacillus sp.* were each found in 5 patients (14.28%), showing equal proportions and relatively high compared with other microorganisms. *Streptococcus agalactiae* was only identified in 1 patient (2.85%), indicating a very low frequency in this group. The distribution of the uterine microbiome in infertile patients with endometriosis shows a pattern dominated by opportunistic bacteria, particularly *Staphylococcus aureus*, accompanied by the presence of Gram-negative bacteria and *Lactobacillus* in comparable proportions.

Table 4. Dominant Microbiota in Infertile Patients due to Male Factors

Variable	**Infertile Patients**	
Uterine Microbiome Composition	**Male Factors**	
	N	**%**
Staphylococcus aureus	2	5.71
Streptococcus agalactiae	8	22.85
Escherichia coli	2	5.71
Lactobacillus sp.	1	2.85
Enterococcus faecalis	3	8.57
Candida albicans	1	2.85
Eikenella corrodens	1	2.85
Bacillus flexus	1	2.85
Macrococcus caseolyticus	1	2.85
Staphylococcus haemolyticus	1	2.85
Staphylococcus hominis	1	2.85
No aerobic/anaerobic bacteria	13	37.14
Total	35	100

Table 5. Distribution of Microbiota in Infertile Patients due to Male Factors

Variable	**Infertile Patients**	
Uterine Microbiome Composition	**Male Factors**	
	N	%
Aerobic/anaerobic bacterial growth detected	22	62.86
No aerobic/anaerobic bacteria	13	37.14
Total	35	100

Tables 4 and 5 describe that out of 35 infertile patients due to male factors, more than one-third of infertile patients due to male factors had negative uterine swab culture results; 22 patients (62.86%) showed the presence of aerobic or anaerobic bacterial growth on uterine microbiome examination, while 13 patients (37.14%) showed no bacterial growth.

In the group with bacterial growth, the most frequently found microorganism was *Streptococcus agalactiae*, detected in 8 patients (22.85%). *Enterococcus faecalis* was found in 3 patients (8.57%), while *Staphylococcus aureus* and *Escherichia coli* were each identified in 2 patients (5.71%). Several other microorganisms were found with lower frequency, each in 1 patient (2.85%), namely *Lactobacillus sp., Candida albicans, Eikenella corrodens, Bacillus flexus, Macrococcus caseolyticus, Staphylococcus haemolyticus*, and *Staphylococcus hominis*. Although there is variation in the types of microorganisms in the uterine microbiome of infertile patients due to male factors, most bacteria were found in relatively small proportions. The distribution of the uterine microbiome in infertile patients due to male factors is dominated by opportunistic Gram-positive bacteria, particularly Streptococcus agalactiae. The proportion of study subjects showing no bacterial growth was considerable.

Table 6. Logistic Regression of the Uterine Microbiome in Infertile Patients

Infertile Patients				
	Odds Ratio (Endometriosis vs Male Factors)	**95% CI Lower**	**95% CI Upper**	**p-value**
Staphylococcus aureus	6.6	1.326	32.843	0.021
Streptococcus agalactiae	0.099	0.012	0.843	0.034
Escherichia coli	2.750	0.496	15.246	0.247
Lactobacillus sp.	5.667	0.626	51.267	0.123
No aerobic/anaerobic bacteria	1.128	0.431	2.955	0.806

Reference: infertile patients due to male factors.

Table 6 shows the results of the logistic regression analysis examining the relationship between the presence of various microorganisms and the group of infertile patients due to male factors and the group of primary infertile patients with endometriosis. By treating the presence of each type of bacteria as the dependent variable and infertility status (endometriosis compared with male factors) as the independent variable, a picture emerges that the uterine microbiome composition in primary infertile patients with endometriosis differs significantly compared with infertile patients due to male factors.

Staphylococcus aureus showed a significant association with endometriosis status, with an odds ratio of 6.6 (95% CI: 1.33-32.84; p=0.021). Infertile patients with endometriosis had a higher likelihood of *S. aureus* being found in the uterus compared with infertile patients due to male factors. *Streptococcus agalactiae* showed a significantly lower odds ratio in the endometriosis group (OR 0.099; 95% CI: 0.01-0.84; p=0.034), indicating that this bacterium is far less commonly found in endometriosis patients compared with infertile patients due to male factors.

Escherichia coli and *Lactobacillus sp.*, although the odds ratios showed a tendency toward a higher likelihood in the endometriosis group (OR 2.75 and OR 5.67, respectively), these associations did not reach statistical significance. The wide confidence intervals for both bacteria reflect limitations in sample size and data variability. The tendency for increased *E. coli* and *Lactobacillus* in endometriosis may indicate changes in uterine microbiome balance that are heterogeneous in nature and do not always lead to dominance of protective flora. The absence of aerobic or anaerobic bacteria did not show a significant association with infertility status (OR 1.13; p=0.806), indicating that the absence of bacterial growth on uterine culture can be found in both endometriosis patients and infertile patients due to male factors.

DISCUSSION

The data from this study illustrate that the types of bacteria detected on culture were quite varied, ranging from Gram-positive bacteria, Gram-negative bacteria, to fungi. Several bacteria commonly found in pelvic infections and endometrial disorders, such as *Staphylococcus aureus, Streptococcus agalactiae*, and *Escherichia coli*, appeared in varying numbers. Recent literature indicates that the uterine microbial community may play a role in the implantation process, pelvic inflammation, and infertility, particularly when there is an imbalance of normal flora or dysbiosis.[16]

Understanding of the uterine microbiome has undergone a significant paradigm shift. The uterus, which for many years was considered a sterile organ, is now recognized as having its own microbial community that can play a role in reproductive physiology, implantation, and the pathogenesis of infertility. The analysis results show an interesting relationship between the uterine microbiome composition and two causes of infertility, namely male factor infertility and infertility due to endometriosis. Several publications confirm that *Lactobacillus* dominance is a marker of a healthy uterine environment, while an increase in opportunistic pathogenic bacteria is associated with implantation failure, chronic endometritis, and infertility.[17]

The findings of this study provide an empirical picture consistent with developments in the literature, while also adding evidence that the relationship between the uterine microbiome and infertility is complex, multifactorial, and likely differs between infertility etiologies.

Uterine Microbiome Composition in Infertile Patients

This study found that several pathogenic bacteria such as *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Escherichia coli* appeared in different percentages in the two infertility groups. In contrast, *Lactobacillus sp.*, which physiologically constitutes the dominant flora, was found in only a small proportion of samples. Such a composition indicates a tendency toward dysbiosis. These findings are consistent with the research of Moreno and Simon (2019), which states that a decrease in *Lactobacillus* can reduce endometrial receptivity and increase the risk of infertility.[18] Foteinidou et al. (2024) also reported that the presence of pathogenic uterine bacteria is associated with subclinical inflammation, particularly in infertile patients.[16]

One important finding from this study is that some samples showed a higher presence of pathogenic bacteria in the control group compared with the case group, a phenomenon that can be explained through two aspects:

1. Natural biological variation between individuals, which does not always follow clinical patterns.
2. Limitations in the sensitivity of culture methods, which can lead to under-detection of physiological bacteria (*Lactobacillus*) and over-representation of bacteria that grow more easily, such as *Staphylococcus* or *Streptococcus*.

This is in line with the report by Balla et al. (2024), which states that conventional culture does not always reflect the true composition of the uterine microbiome.[17]

Relationship Between the Uterine Microbiome and Endometriosis

The study results show a different pattern in the endometriosis group. S. aureus had an OR of 6.6 (p=0.021), indicating a significant association. Several studies show that certain Gram-positive bacteria are capable of triggering the release of inflammatory mediators such as IL-6 and TNF-alpha, which can worsen pelvic inflammation, a condition that is already predominant in endometriosis.[19]

In contrast to male factor infertility, *S. agalactiae* had an OR of 0.099 (p=0.034) in endometriosis, showing a tendency toward a protective effect or lower prevalence in this group. This pattern reflects differences in uterine microbiome characteristics between endometriosis and other types of infertility, as reported by Ono et al. (2024), who found that endometriosis has a distinctive hallmark of a decrease in certain Gram-positive bacteria alongside an increase in specific other bacteria.[19] These differences in association patterns indicate that the relationship between the uterine microbiome and infertility is not universal, but depends on the underlying disease etiology. Therefore, clinical interpretation must take into account the characteristics of the underlying disease.

Biologically, *S. aureus* and *S. agalactiae* are bacteria that have been linked to chronic endometritis, pelvic inflammation, and disruption of the endometrial microenvironment that can affect implantation. Several studies show that colonization by pathogenic bacteria can increase the production of inflammatory cytokines, disrupt local immune balance, and alter endometrial receptivity. This provides a biologically plausible basis for why their presence may be associated with infertility.[16]

Relationship Between the Uterine Microbiome and Male Factor Infertility

The logistic regression results show that *Staphylococcus aureus* was lower and *Streptococcus agalactiae* was higher compared with endometriosis cases, and neither was significantly associated with male factor infertility. Although male factor infertility is fundamentally caused by sperm abnormalities, the presence of pathogenic bacteria in the female uterus can still play an important role indirectly. Several potentially relevant mechanisms include changes in the uterine microenvironment that disrupt the process of sperm capacitation and transit, mild endometrial inflammation that reduces the ability of the uterus to receive sperm or maintain an optimal environment, and couple-dependent interaction, where the female uterine microbiome can modulate the effectiveness of sperm from a particular partner. A study showed that an imbalanced uterine microbiome composition can affect fertilization success even when the primary contributing factor comes from the male partner.[20]

The increased risk of infertility in women with *S. aureus* or *S. agalactiae* colonization signals that uterine microbiome examination may become part of the evaluation of infertile couples, even when the primary etiology comes from the male partner. This finding reinforces the view that infertility is a couple-centered condition, not merely a characteristic of one partner.

Differences in Uterine Microbiome Composition

Data analysis from this study shows significant differences in uterine microbiome composition between primary infertile patients with endometriosis and infertile patients due to male factors. The uterine microbiome literature indicates that the bacterial community in infertile patients tends to be more heterogeneous compared with fertile individuals, with a relative decrease in the *Lactobacillus* population associated with a healthy uterine environment. Several molecular studies have found that endometriosis patients have a different uterine microbiome pattern compared with groups without endometriosis, although the results have not been uniform across all studies.[21,22]

A study showed that several bacterial genera such as *Streptococcus*, *Pseudomonas*, and *Acinetobacter* are more frequently observed in endometriosis patients compared with patients with other infertility factors, although inter-study heterogeneity remains high. Data from other research also reported that in infertility not caused by tubal or ovulation factors, including endometriosis, there are changes in the uterine microbiota community.[23,24]

Changes in uterine microbiome composition in endometriosis patients compared with male factor patients can be explained through several immunological and local inflammatory mechanisms. In chronic endometrial inflammation, endometriosis is often accompanied by immune and inflammatory responses characterized by an increase in pro-inflammatory cytokines such as IL-6 and IL-1 beta, which can alter the microenvironment favorable to certain bacterial communities.[24,25] The imbalance between protective bacteria (*Lactobacillus*) and opportunistic bacteria in the uterine cavity can affect pH, local metabolites, and endometrial receptivity to implantation.[26] Some bacteria that are clearly more dominant in endometriosis patients can inhibit the colonization of protective bacteria through competition for nutrients and space, or through the secretion of antagonistic metabolites.[23,27] These mechanisms biologically explain that differences in the uterine microbiome can reflect actual changes in the microenvironment in endometriosis, which do not occur in the infertile group due to male factors.

The presence of *Staphylococcus aureus* was significantly more frequently found in the endometriosis group compared with male factor infertility, with an odds ratio of 6.6. Biologically, *S. aureus* is known as an opportunistic bacterium commonly found on the skin and male urogenital tract; colonization by this bacterium may reflect partner transmission or semen contamination that affects the female uterine microbiome without direct involvement of the more complex chronic inflammatory mechanisms of endometriosis. Meanwhile, bacteria such as *Escherichia coli* and *Lactobacillus sp.* showed higher odds ratios in endometriosis cases compared with male factor cases, reflecting a dysbiosis pattern in the uterine environment connected to this pathological condition. Previous research has shown that altered endometrial microbiota composition, particularly with reduced *Lactobacillus* dominance and increased opportunistic bacteria, is associated with dysbiosis and impaired endometrial function that can hinder implantation and contribute to infertility.[21,28,29]

E. coli is a Gram-negative bacterium whose products, particularly lipopolysaccharides, can trigger a pro-inflammatory immune response that is worsened in the context of endometriosis, a condition characterized by chronic inflammation, immune dysregulation, and widespread uterine microbiome changes.[30] Although Lactobacillus is classically considered protective flora in the reproductive tract, molecular evidence shows that the presence of lower *Lactobacillus* species or changes in certain species in the endometrium can be associated with infertility and reduced pregnancy chances.[17,31] This underscores that an OR greater than 1 for *Lactobacillus* in the context of endometriosis should not be interpreted as a protective effect, but rather as a marker of changes in the microbial community that no longer reflects a healthy eubiotic state. Such dysbiosis has been reported to cause increased microbial diversity not dominated by *Lactobacillus*, a pattern associated with impaired implantation and poor reproductive outcomes.[17,21,28]

Within the biological and clinical framework, these findings are consistent with the understanding that the uterus is no longer viewed as a sterile organ, but as a complex microbial community that can affect and be affected by pathological conditions such as endometriosis. Research has shown that endometrial dysbiosis, particularly where *Lactobacillus* dominance is disrupted, is associated with fertility problems including implantation disorders and pregnancy failure.[17,30]

This study has several methodological strengths worth noting. First, the study directly combines uterine microbiome analysis with the clinical characteristics of infertility, including male factors and endometriosis, thus enabling a more comprehensive evaluation of the relationship compared with research that assesses only one dimension. Second, the use of aerobic and anaerobic culture provides a real picture of organisms that can survive and thrive in the uterine environment, which may be clinically related to inflammatory or infectious processes. Third, this study also used logistic regression analysis, enabling assessment of the strength of associations through odds ratio values, so that interpretation of results is more informative and consistent with the study objectives. The uterine microbiome composition has the potential to become a biomarker for stratifying infertile patients who may respond better to certain therapies compared with patients with other microbiome profiles. This study opens opportunities for therapeutic approaches targeting the uterine microbiome, including the use of probiotics or local microbiota modification, particularly in endometriosis patients who may experience more severe dysbiosis.

This study also has several important limitations that need to be considered when interpreting the results. One interesting yet methodologically challenging finding is that in the control group without endometriosis or male factors, more pathogenic and opportunistic bacteria were detected. This phenomenon may be influenced by several factors, including natural biological variation between individuals or possible technical bias in sampling and culture procedures. This finding emphasizes the need for caution in making causal inferences, given that the presence of pathogenic bacteria does not always reflect a direct pathological condition, but may be influenced by environmental factors, timing of sample collection, or specimen handling techniques.

Uterine sample collection in this study was performed using strict aseptic and antiseptic techniques to minimize the risk of contamination from the external environment or lower genital flora. All procedures were performed by trained medical personnel using sterile single-use instruments, including sterile specula and sampling instruments that did not come into contact with the vaginal wall or external cervix. Prior to sample collection, the cervical area was thoroughly cleaned using an antiseptic solution in accordance with standard clinical procedures, so that exposure to vaginal commensal flora could be minimized. Samples were then immediately placed in sterile transport media and sent to the microbiology laboratory under controlled conditions to maintain the viability of any microorganisms present. With consistent application of sterile protocols, this procedure was designed to ensure that microorganisms detected on culture reflect the actual uterine microbiota condition, not the result of contamination during collection.

Although the sampling procedure was performed under sterile conditions, the possibility of laboratory contamination still needs to be considered as an inherent methodological limitation in culture-based microbiological research. Contamination can occur at various stages, including during inoculation of samples onto culture media, incubation, or specimen manipulation in the laboratory, particularly for microorganisms that are commensal flora of the skin or environment, such as *Staphylococcus sp.*[32] All laboratory analysis processes in this study were carried out following biosafety and quality control standards, including the use of biosafety cabinets, sterile culture media, and cross-contamination prevention procedures between samples.

One of the most critical weaknesses in this study is the limitation of conventional culture methods. The process from sample collection to culture can cause bacterial death, particularly for fastidious species or those sensitive to changes in temperature, pH, and oxygen levels. This condition is very likely to contribute to the high rate of "no growth" results and the low detection of Lactobacillus sp., which is actually the dominant flora in the healthy female reproductive tract. Modern scientific literature shows that culture is only able to detect a small fraction of the total microbial community, with far lower sensitivity compared with molecular methods.

Other limitations include the relatively small sample size for some microorganism categories, which produced very low frequencies and reduced statistical power. This caused some tests to be unable to detect associations that actually exist (false negatives). In addition, this study did not include strict control of confounding variables such as history of antibiotic use, hormonal status, or recent sexual intercourse history, which can affect uterine microbiome composition.

CONCLUSION

The conclusions of this study indicate that there are specific characteristics and composition of the uterine microbiome in primary infertile patients with endometriosis as well as in infertile patients due to male factors at Dr. Soetomo General Hospital. In addition, differences were found in the specific characteristics and composition of the uterine microbiome between primary infertile patients with endometriosis compared with infertile patients due to male factors.

Based on the research results, it is recommended that further research be conducted using MALDI-TOF and 16S rRNA NGS methods to describe the uterine microbiome more accurately and comprehensively. Efforts to increase normal Lactobacilli flora in infertile women with endometriosis can also be made through the consumption of prebiotic and probiotic supplements. In addition, research on changes in pro-inflammatory cytokines and endometrial receptivity in infertile patients with endometriosis is needed to assess the causal relationship between the uterine microbiome and infertility. Fertility clinics are also advised to consider uterine microbiome examination as part of the diagnostic evaluation of infertility, particularly in idiopathic cases.

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