



RESEARCH ARTICLE

Comparison Of Vaginal Microbiota Profiles In Patients With Endometriosis And Without Endometriosis

Sef Zani Meria¹, Haviz Yuad^{2,*}, Andani Eka Putra³

¹ Department of Obstetrics and Gynecology, Faculty of Medicine, Andalas University/ Dr. M. Djamil Hospital, Padang, West Sumatera, Indonesia

² Division of Fertility and Endocrinology, Department of Obstetrics and Gynecology, Faculty of Medicine, Andalas University/ Dr. M. Djamil, Hospital, Padang, West Sumatera, Indonesia

³ Center for Infectious Disease Diagnostic and Research Laboratory, Faculty of Medicine, Andalas University, Padang, West Sumatera, Indonesia

Corresponding Author: Haviz Yuad; yuadhaviz@gmail.com; +62 813-6313-5810

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Abstract

Introduction: Endometriosis is a complex gynecological disease affect 10% of women in reproductive age, involving inflammation and immune regulation. Diversity of vaginal microbiota is an integral part of vaginal microecology with vaginal anatomy, endocrine system and local vaginal immunity. The disruption in microecology, dysbiosis, is known to trigger inflammatory diseases. The relationship between vaginal dysbiosis and endometriosis remains controversial

Objective: To determine the comparison of vaginal microbiota profiles in patients with and without endometriosis

Method: The study was case-control design on patients with and without endometriosis at Dr. M. Djamil Hospital, conducted from December 2023 to June 2024 at Dr. M. Djamil Hospital Polyclinic and the Center for Infectious Disease Diagnostic and Research Laboratory. The diagnosis of endometriosis was confirmed via ultrasonography and histology. Microbiota were measured with miSeq Illumina Next Generation Sequencing. A comparison of microbiota profiles was carried out using the Mann-Whitney test.

Results: There were 40 respondents, with endometriosis (n=20) and without endometriosis (n=20). At the phylum level, the vaginal microbiota profile in the endometriosis group was dominated by Firmicutes, Proteobacteria, Actinobacteria, and Bacteroidetes, while in without endometriosis group dominated by Firmicutes, Actinobacteria, Bacteroidetes, and Proteobacteria. At the genus level, the endometriosis group was dominated by *Lactobacillus* sp., *Desulfovibrio* sp., *Flavobacterium* sp., and *Gardnerella* sp., while without endometriosis group were *Lactobacillus* sp., *Prevotella* sp., *Desulfovibrio* sp., and *Bifidobacterium* sp. There was no difference in the abundance of microbiota ($p>0.05$)

Conclusion: There was no difference in the abundance of microbiota between endometriosis group and without endometriosis group.

Keywords: Endometriosis, dysbiosis, vaginal microbiota, *Lactobacillus*, *Flavobacterium*, Firmicutes, Proteobacteria

INTRODUCTION

Etiology of endometriosis as a chronic inflammatory disease characterized by the presence of extrauterine endometrial-like tissue (glands and/or stroma) on the visceral surface, peritoneal tissue, and connective tissue in the extrapelvic region cannot be determined.¹⁻⁵ The formation and growth of endometriotic tissue depends on estrogen and the old theory adopted is Sampson's theory of retrograde menstruation.^{1,4}

However, since only 10% of women suffer from this illness and 90% of women experience retrograde menstruation, there are numerous alternative theories.^{6,7,17} Although many studies have not been able to identify the gene or gene sequence, epidemiological data indicates the influence of genetic predisposition. It is also believed that immune dysregulation, either alone or in conjunction with epigenetics, contributes to the development of endometriosis. Adenomyosis can result from endometrial invasion into the myometrium brought on by immunological dysregulation. It is believed that dysbiosis, a disorder of microbiota imbalance in the human body, is the cause of immune dysregulation.^{8,9,18} The incidence of endometriosis is strongly linked to an imbalance in the composition of the gut and reproductive tract microbiota, according to recent studies. This is believed to be the case because dysbiosis alters the immune response and interferes with regular immune function, leading to an inflammatory response and an increase in proinflammatory cytokines, both of which can contribute to the pathophysiology of endometriosis.^{18,19} Increased adhesions and angiogenesis are eventually made possible by chronic immune dysregulation, which develops into an endometriosis vicious circle.^{19,20,21}

A study from the Department of Obstetrics and Gynecology at Ramatibodi Hospital, Mahidol University from August 2020 to January 2021 compared the composition of the vaginal microbiota and operational taxonomic units (OTU) between adenomyosis and non-adenomyosis patients. It was concluded that there were differences in the vaginal microbiota profiles of adenomyosis and non-adenomyosis patients. In adenomyosis patients community state types are predominant (CST) III and IV, while in non-adenomyosis patients it is dominated by CST IV. *Lactobacillus* was found in abundance in both groups. However, this study did not describe endometriosis patients in general but was limited to adenomyosis.^{20,22,23}

Lactobacillus species demonstrate a microbial role in competitively eliminating opportunistic pathogens that inhabit functional niches in the intestine and vaginal mucosa, blocking the attachment of pathogens to the epithelium and killing pathogens directly by producing lactic acid, acetic acid, propionic acid, bacteriocins and reactive oxygen species (ROS). (Dempsey and Corr, 2022). In addition to its microbial role, commensal *Lactobacillus* species regulate adaptive and innate immune responses by inducing T cells, Natural killer (NK) cells, macrophage differentiation, cytokine production and stimulating Toll Like Receptors (TLRs). They also show immunomodulatory effects by increasing the expression of immunoglobulin-A (IgA)-producing B cells in Peyer's patches in the lamina propria, where they block the adhesion of pathogens to the intestinal epithelium (Cristofori et al, 2021). Increasing evidence supports the role of *Lactobacillus* in suppressing inflammatory responses by decreasing T-helper 17 (Th17) and cytokines, especially interleukin-17F

(IL-17F) and tumor necrosis factor - alpha (TNF- α).^{21,24,25}

Furthermore, β -glucuronidase and β -glucosidase, which are secreted by the gut microbiota, degrade estrogen, increasing the amount of free estrogen that is reabsorbed and in circulation. *Bacteroid*, *Bifidobacterium*, *Escherichia coli*, and *Lactobacillus* are among the bacteria in the gut microbiota that produce β -glucuronidase, according to microbial genome analysis. *E. Coli* was detected in high concentrations in the feces of patients with endometriosis. One study found that the gut microbiota causes circulating estrogen levels to rise, creating a hyperestrogenic environment in the event of endometriosis (Qi et al., 2021).^{22,26,27}

METHODS

This research was conducted at the Polyclinic of Dr. M. Djamil Hospital and the Center for Infectious Disease Diagnostic and Research Laboratory from December 2023 to June 2024. This descriptive-analytical research uses a case-control method approach to the comparison of vaginal microbiota profiles in patients with endometriosis and without endometriosis. Populations in this study were all RSUP Dr. M. Djamil Padang patients with and without endometriosis diagnoses who satisfied the inclusion criteria; there were no exclusion criteria.^{1,2}

Reproductive-aged women who had a histologic diagnosis and ultrasound of endometriosis comprised the study group, whereas asymptomatic reproductive-aged women who presented for a routine well-woman visit or preconception counseling were included in the control group. Exclusion criteria were: <18 or >45 years of age on the day of sample collection, Being ever pregnant including the day of sample collection, Postmenopausal status, Having clinical signs and symptoms suggestive of endometriosis, i.e. dysmenorrhea, dyspareunia, dyschesia and/or infertility precluded recruitment to the control group. These symptoms were assessed with the Biberoglu-Behrmann (B&B) scale and women with a score > 0 were not included in the control group, having taken antibiotics or probiotics within the last 8 weeks, inflammatory bowel disease, functional bowel disease, history of gastrointestinal cancer or surgery, having vaginal douching last 5 days, a smoker, many sexual partners, body mass index > 30 kg/m².

The sampling technique in this study was a difference between two proportions using paired numerical scales. The total sample was 40, 20 samples of cases and 20 samples of control from the vaginal swab. The diagnosis of endometriosis was confirmed via ultrasonography and histology. Microbiota were measured with miSeq Illumina Next Generation Sequencing.

The data analysis used was the chi-square test. A comparison of vaginal microbiota profiles was carried out using the independent T-test. However, previously a normality test was carried out using Shapiro Wilk. If the data is normally distributed, then the independent T-test can be used. However, if the data is not normally distributed, an alternative test is used, namely the Mann-Whitney test. The degree of significance used is 0.05 with a confidence level of 95%. Decisions from statistical test results use p-value. If the p-value $\leq$ 0.05 then H_0 is rejected or there is a significant relationship between the two variables.

RESULT

Table 1. Characteristics of Research Respondents

Characteristics		Normal		Endometriosis	
		f	%	f	%
Age	20-29 years old	3	15	6	30
	30-35 years old	8	40	4	20
	36-45 years old	9	45	10	50
Body Mass Index	Normoweight	20	100	20	100
	Overweight	0	0	0	0
Work	House Wife	1	5	14	70
	Civil Servant	5	25	4	20
	Employees	2	10	0	0
	Health Workers	2	10	0	0
	Teacher	10	50	1	5
	Businesswoman	0	0	1	5
Parity	Nulipara	1	5	20	100
	Primipara	5	25	0	0
	Multipara	14	70	0	0

Based on Table 1, most women without endometriosis were aged 36-45 years (9; 45%), the remainder had ages in the range of 30-35 years (8; 40%) and 20-29 years (3; 15%). In the endometriosis group, most respondents were also aged 36-45 years and the remainder were aged 20-29 years (6; 30%) and 30-35 years (4; 20%). All patients in this study had a BMI in the normoweight category (100%). Regarding work, the majority of research respondents in the group without endometriosis worked as teachers (10; 50%), the remainder worked as civil servants (5; 25%), employees (2; 10%), health workers (2; 10%) and IRT (1; 5%). Meanwhile, in the group of endometriosis sufferers, most of the women were domestic workers (14; 70%), the rest worked as civil servants/first aid workers (4; 20%), teachers (1; 5%) and traders (1; 5%). Most women without endometriosis were multiparous (14; 70%), the remainder were primipara (5; 25%) and nullipara (1; 5%). In the endometriosis group, all respondents were nulliparous (20; 100%).

Table 2. Comparison of Vaginal Microbiota Profiles Endometriosis and Non Endometriosis Patients based on phylum

Vaginal Microbiota Profiles	EMS	Non EMS	<i>*P value</i>
	Mean $\pm$ SD	Mean $\pm$ SD	
<i>Firmicutes.</i>	77,71 $\pm$ 30,27	66,98 $\pm$ 28,41	0,110
<i>Proteobacteria</i>	8,44 $\pm$ 11,39	10,11 $\pm$ 7,94	0,223
<i>Actinobacteria</i>	7,90 $\pm$ 15,74	10,85 $\pm$ 12,64	0,144
<i>Bacteroidetes</i>	5,77 $\pm$ 14,65	10,32 $\pm$ 17,26	0,363
<i>Fusobacteria</i>	0,04 $\pm$ 0,15	1,15 $\pm$ 3,20	0,122
<i>Tenericutes.</i>	0,07 $\pm$ 0,15	0,33 $\pm$ 1,02	0,803
<i>Nitrospira</i>	0,00 $\pm$ 0,00	0,23 $\pm$ 0,65	^a 0,038
<i>Spirochaetes</i>	0,04 $\pm$ 0,15	0,01 $\pm$ 0,02	0,696

**Mann Whitney*

^a*p*<0,05

Table 2. shows that there is no difference between the percentage of Firmicutes and Bacteroidetes in endometriosis and non-endometriosis patients ($p=0.110$). The mean percentage of Firmicutes in endometriosis patients was higher (77.71 ± 30.27) compared to non-endometriosis (66.98 ± 28.41). Nitrospira was the only phylum-level microbiota that showed significant differences between groups. Other microbiota also did not show significant differences in the Mann-Whitney test with $p>0.05$.

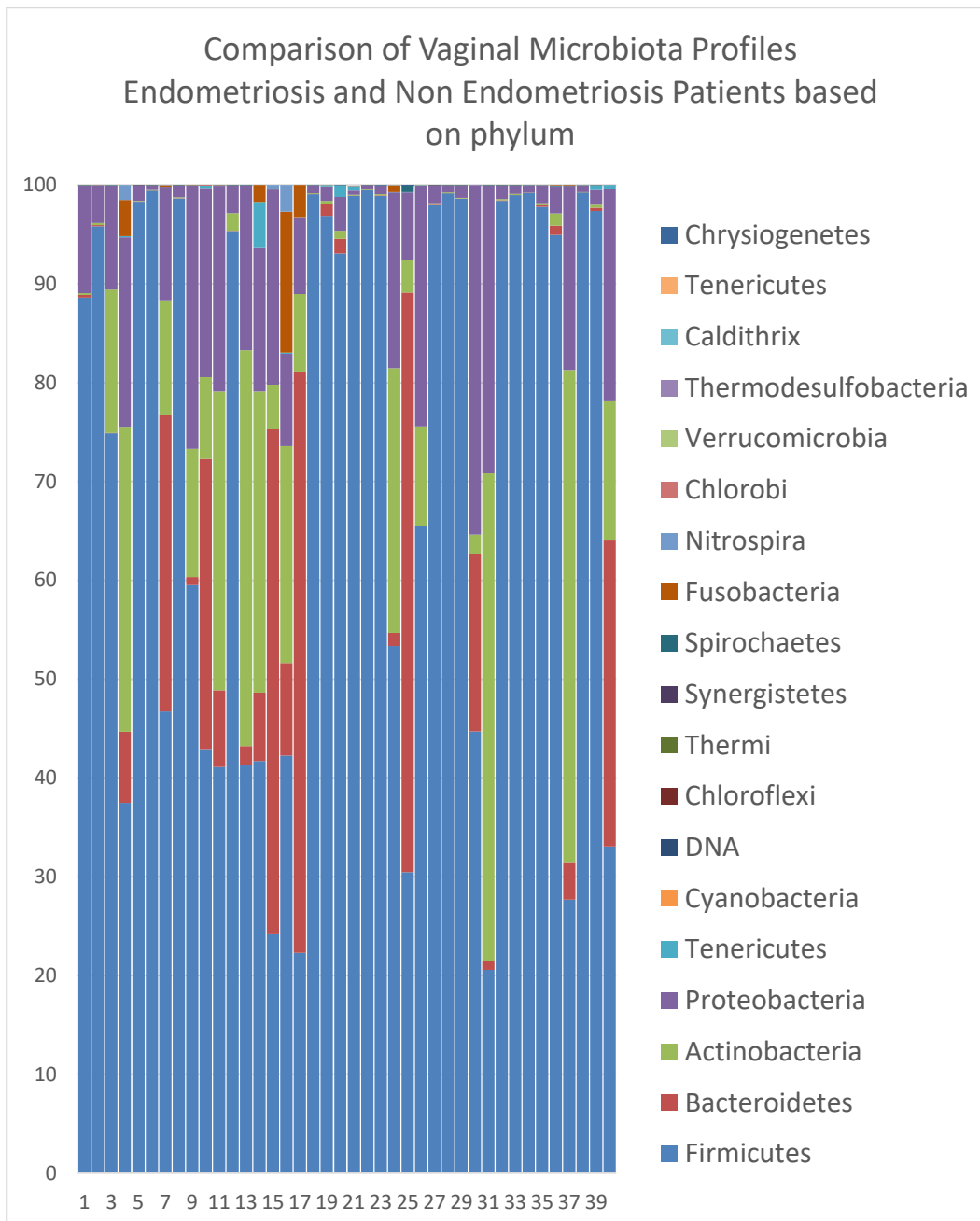


Figure 1. shows overall vaginal microbiota profile of respondents based on phylum including endometriosis and non endometriosis patients. Numbers 1-20 show the vaginal microbiota profile of non endometriosis patients, while numbers 21-40 show the vaginal microbiota profile endometriosis patients. There is an abundance of Firmicutes in endometriosis patients.

Table 3. Comparison of Vaginal Microbiota Profiles Endometriosis and Non Endometriosis Patients based on genus

Vaginal Microbiota Profiles	EMS	Tidak EMS	<i>*P value</i>
	Mean $\pm$ SD	Mean $\pm$ SD	
<i>Lactobacillus sp.</i>	64,98 $\pm$ 39,03	47,93 $\pm$ 41,30	0,212
<i>Desulfovibrio sp.</i>	6,60 $\pm$ 11,46	6,49 $\pm$ 8,24	0,521
<i>Bifidobacterium sp</i>	3,58 $\pm$ 8,28	5,60 $\pm$ 9,18	0,258
<i>Prevotella sp.</i>	1,52 $\pm$ 4,17	9,63 $\pm$ 19,50	0,416
<i>Gardnerella sp.</i>	3,69 $\pm$ 8,77	4,78 $\pm$ 6,20	0,222
<i>Streptococcus sp.</i>	3,12 $\pm$ 11,82	3,59 $\pm$ 10,96	0,713
<i>Flavobacterium sp.</i>	4,86 $\pm$ 12,14	2,33 $\pm$ 6,38	0,929
<i>Pediococcus sp.</i>	3,21 $\pm$ 5,74	0,91 $\pm$ 1,29	0,115

**Mann Whitney*

In Table 3, the percentage of *Lactobacillus sp.* in endometriosis patients compared to non-endometriosis patients is the same ($p=0.212$). However, the percentage of *Lactobacillus sp.* in endometriosis patients was higher (64.98 ± 39.03) compared to non-endometriosis patients (47.93 ± 41.30). Other microbiota also did not show significant differences in the Mann-Whitney test with $p>0.05$.

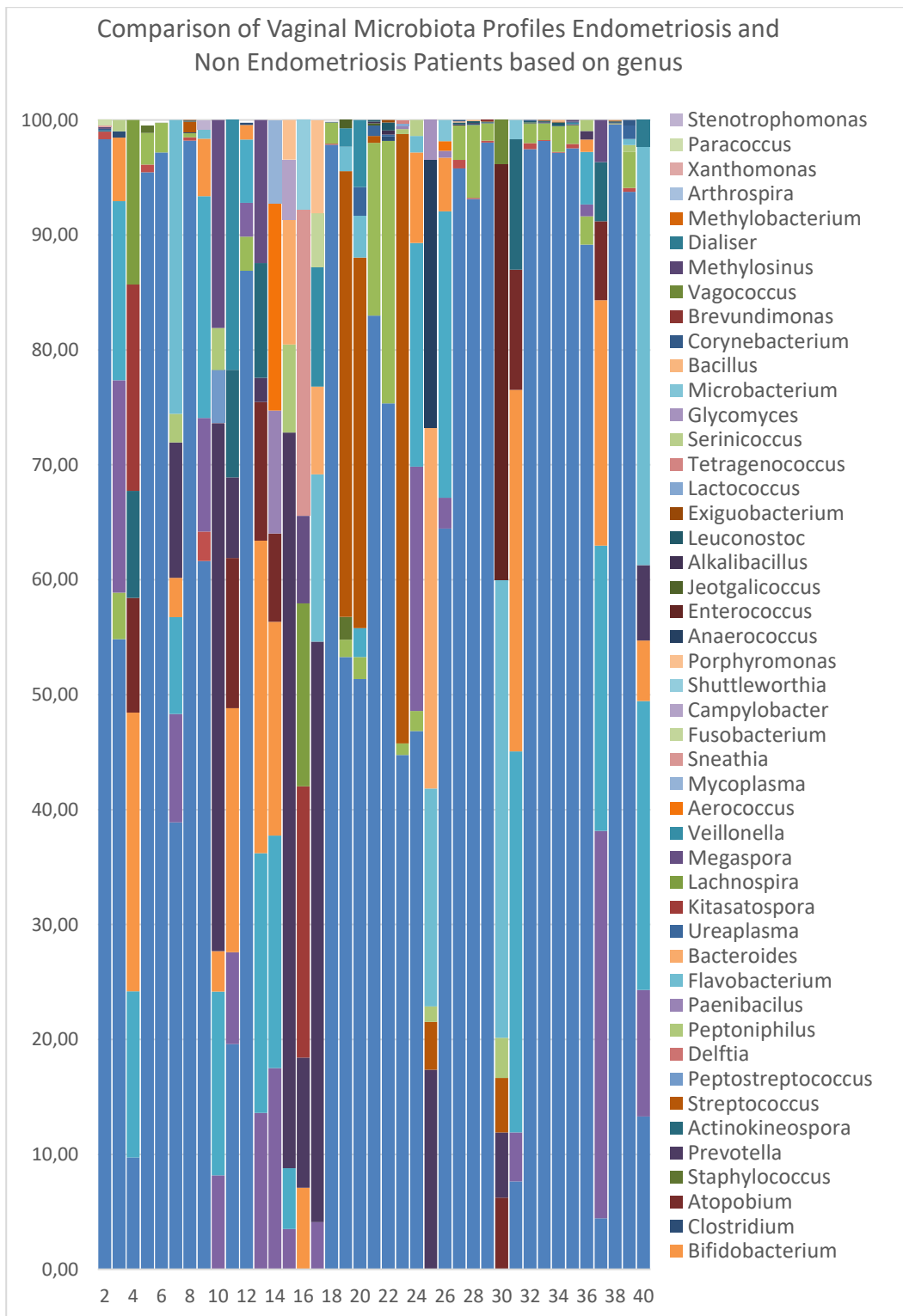


Figure 2. shows the vaginal microbiota profile of endometriosis and non endometriosis patients. Numbers 1-20 show the vaginal microbiota profiles of non endometriosis patients, while numbers 21-40 show the vaginal microbiota profile of endometriosis patients.

DISCUSSION

At the phylum level, vaginal microbiota profiles of endometriosis patients showed an increase in Firmicutes followed by an increase in Proteobacteria. Meanwhile, in non-endometriosis, Firmicutes were followed by Actinobacteria in large quantities. This is consistent with the findings of Chang et al, who discovered an increase in Firmicutes and Proteobacteria followed by a decrease in Actinobacteria, whereas, in controls, Actinobacteria remained the dominant microbiota after Firmicutes.^{28,29,32} According to a recent systematic review by Leonardi et al, there has been an increase in the abundance of bacteria such as Enterobacteriaceae, *Escherichia coli*, Proteobacteria, and *Streptococcus* in various locations that appear to be linked to endometriosis.³³ D'Alterio et al. (2021) also reported that species from the phylum Proteobacteria, Bacteroidetes, and Negativicutes, which are distinguished by Gram-negative staining, increased significantly in the endometriosis group. Proteobacteria are gram-negative bacteria.^{30,31} Lipopolysaccharide (LPS), a component of gram-negative bacteria's outer membrane, can be the initial trigger and contamination of bacteria from the intrauterine environment, as well as the primary cause in the regulation of endometriosis growth, either alone or in combination with ovarian steroids through their inflammation.^{16,33}

At the genus level, *Lactobacillus* sp. was abundant, followed by *Desulfovibrio* sp., *Flavobacterium* sp., and *Gardnerella* sp., which was higher in endometriosis but not statistically significantly different from the vagina of non-endometriosis patients. Meanwhile, in non-endometriosis patient profiles, *Prevotella* sp, *Desulfovibrio* sp, and *Bifidobacterium* sp were found to be predominant. This is consistent with the findings of Akiyama et al. (2019), who discovered an abundance of *Flavobacterium* in endometriosis other than *Corynebacterium*, Enterobacteriaceae, *Pseudomonas*, and *Streptococcus*.³² Another study of endometrial samples by Wessel et al discovered greater diversity in endometriosis patients.³⁴ Wei et al discovered that the presence of *Lactobacillus* was homogeneous in the lower genital tracts of both control and endometriosis patients, but this number decreased in the endometrium compared to cervical mucus.³¹ This study only looked at the vaginal microbiota profile, so the microbiota profile in the endometrium was unknown.

Aside from inflammation caused by non-*Lactobacillus* microbiota, another mechanism for microbiota linked to endometriosis is estrogen regulation. Gut microbiota plays a role in estrogen metabolism and regulates systemic estrogen levels via β -glucuronidase activity. *Lactobacillus* is a microbiota that controls the β -glucuronidase enzyme. β -glucuronidase converts estrogen into its active form, allowing it to bind to the estrogen receptor. Microbial secretion of β -glucuronidase leads to increased estrogen levels. Active estrogen can then be transported to distant mucosal sites, such as the endometrium, via the bloodstream. Gut microbiota regulates estrogen homeostasis in the intestinal and distal regions in this manner. Endometriosis patients may have more β -glucuronidase-producing bacteria in their gut microbiota. On the other hand, estrogen levels have been shown to affect the microbiota. High estrogen levels eventually lead to an increase in *Lactobacillus* sp. in the vagina.^{16,19,22}

CONCLUSION

The Vaginal microbiota of endometriosis patients mostly consists of Firmicutes, Proteobacteria, Actinobacteria, Bacteroidetes, Fusobacteria, Tenericutes, Spirochaetes, and Thermi. Meanwhile, at the genus level, it is dominated by *Lactobacillus* sp., *Desulfovibrio* sp., *Flavobacterium* sp., *Gardnerella* sp., *Bifidobacterium* sp., *Pediococcus* sp., *Streptococcus* sp., and *Bacteroides* sp. The dominant species that make up the dominance of the genus *Lactobacillus* sp. endometriosis consists of *Lactobacillus taiwanensis*, *Lactobacillus ultunensis*, *Lactobacillus apis*, *Lactobacillus gegiorum*.

The vaginal microbiota of non-endometriosis patients mostly consists of Firmicutes, Actinobacteria, Bacteroidetes, Proteobacteria, Fusobacteria, Tenericutes, Nitrospira and Spirochaetes. Meanwhile, at the genus level, it is dominated by *Lactobacillus* sp., *Prevotella* sp., *Desulfovibrio* sp., *Bifidobacterium* sp., *Gardnerella* sp., *Streptococcus* sp., *Flavobacterium* sp., and *Atopobium* sp. The dominant species that make up the dominance of the genus *Lactobacillus* sp. Non-endometriosis patients consisting of *Lactobacillus taiwanensis*, *Lactobacillus ultunensis*, *Lactobacillus apis*, and *Lactobacillus gegiorum*.

The number of Firmicutes and *Lactobacillus* did not vary between groups. Furthermore, there was no significant difference in the number of non-Firmicute and non-*Lactobacillus* sp. microbiota between the groups. However, there were differences in the microbiota dominance after Firmicutes at the phylum level and *Lactobacillus* at the genus level between the endometriosis and non-endometriosis groups.

REFERENCES

1. F F, Utama Bi, Firdawati F. Comparison Of Women's Quality Of Life Post Abdominal And Vaginal Hysterectomy. *Andalas Obstet Gynecol J*. 2022;6(1):35-41. Doi:10.25077/Aoj.6.1.35-41.2022
2. Sa Mr, Yanti Rs, Utama Bi. Research Article Comparison Of Microbiota In Threatened Preterm. *Andalas Obstet Gynecol J*. 2024;8(Vol. 8 No. 1 (2024)):548-559. Doi:https://doi.org/10.25077/Aoj.8.1.548-559.2024
3. ESHRE Endometriosis Guideline Development Group. Endometriosis: Guideline of European Society of Human Reproduction and Embryology. 2022
4. Kim AH. Williams Gynecology 4rd edition. Gynecologic Infection. United States: McGraw Hill Education. 2020
5. Berek JS, Kraus L, Berek DL. Berek & Novak's Gynecology (Berek and Novak's Gynecology). 2020. Diakses dari <https://lccn.loc.gov/2018052861> .
6. Hestiantoro A dkk. Himpunan Endokrinologi Reproduksi dan Fertilitas Indonesia (HIFERI). 2017.
7. Taylor HS, PL, SE. Speroff's clinical gynecologic endocrinology and infertility. 2020
8. Uzuner C, Mak J, El-Assaad F, Condous G. The bidirectional relationship between endometriosis and microbiome. *Front Endocrinol*. 2023; 14.
9. Mansur SA, Trisetiyono Y, Muniroh M, Setiyorini N. Characteristics of Endometriosis Patients in RSUP Dr. Kariadi Semarang in 2018. *Diponegoro Medical Journal (Jurnal Kedokteran Diponegoro)*. 2022; 11; 100–103.
10. Bahall V, De Barry L, Rampersad A. Clear cell carcinoma arising from abdominal wall endometriosis—a report on two cases and literature review. *World J Surg Oncol*. 2022; 20.
11. Kalaitzopoulos DR, et al. Treatment of endometriosis: a review with comparison of 8 guidelines. *BMC Womens Health*. 2021; 21: 1–9
12. WHO. Endometriosis Key facts. *World Health Organisation* <https://www.who.int/news-room/fact-sheets/detail/endometriosis> (2021).
13. Moradi Y, et al. A systematic review on the prevalence of endometriosis in women. *Indian J Med Res*. 2021; 154: 446–454.
14. Lestari SPP, Annas JY, Juniastuti, J. Hubungan Usia Menarche Dengan Kejadian Endometriosis. *Indonesian Midwifery and Health Sciences Journal*. 2021; 3: 30–36.
15. Kim MR, et al. Clinical Diagnosis and Early Medical Management for Endometriosis: Consensus from Asian Expert Group. *Healthcare*. 2022; 10: 1–14.
16. Hidayat A, Hendry D. Characteristics of Endometriosis and Adenomyosis Patients In Dr . M . Djamil Hospital Padang in The Period of January 2017 - October 2018. *Andalas Obstetrics and Gynecology Journal*. 2019; 3: 1–8.
17. Barbara G, Buggio L, Facchin F, Vercellini P. Medical Treatment for Endometriosis: Tolerability, Quality of Life and Adherence. *Front Glob Womens Health*. 2021; 2: 1–6.
18. Colonetti T, et al. Gut and Vaginal Microbiota in the Endometriosis: Systematic Review and Meta-Analysis. 2023.
19. Hernandez C, et al. Microbiome profile of deep endometriosis patients: Comparison of vaginal fluid, endometrium and lesion. *Diagnostics*. 2020; 10.

20. Ata B, et al. The Endobiota Study: Comparison of Vaginal, Cervical and Gut Microbiota Between Women with Stage 3/4 Endometriosis and Healthy Controls. *Sci Rep*. 2019; 9.
21. Jiang I, Yong PJ, Allaire C, Bedaiwy MA. Intricate connections between the microbiota and endometriosis. *International Journal of Molecular Sciences*. 2021; 22. Preprint at <https://doi.org/10.3390/ijms22115644>
22. Kunaseth J, et al. Vaginal microbiome of women with adenomyosis: A case-control study. *PLoS One*. 2022; 17.
23. Rastogi S, Singh A. Gut microbiome and human health: Exploring how the probiotic genus *Lactobacillus* modulate immune responses. *Frontiers in Pharmacology*. 2022; 13 Preprint at <https://doi.org/10.3389/fphar.2022.1042189>
24. Qin R, Tian G, Liu J, Cao L. The gut microbiota and endometriosis: From pathogenesis to diagnosis and treatment. *Frontiers in Cellular and Infection Microbiology*. 2022; 12 Preprint at <https://doi.org/10.3389/fcimb.2022.1069557>
25. García-Peñarrubia, P., Ruiz-Alcaraz, A. J., Martínez-Esparza, M., Marín, P. & Machado-Linde, F. Hypothetical roadmap towards endometriosis: Prenatal endocrine-disrupting chemical pollutant exposure, anogenital distance, gut-genital microbiota and subclinical infections. *Hum Reprod Update*. 2020; 26: 214–246.
26. Yen CF, Kim MR, Lee CL. Epidemiologic Factors Associated with Endometriosis in East Asia. *Gynecol Minim Invasive Ther*. 2019; 8: 4–11.
27. Ocviyanti D, et al. Gynaecologic History During Adolescence to Predict Endometriosis Earlier. *Bioscientia Medicina: Journal of Biomedicine and Translational Research*. 2022; 6: 1515–1522.
28. Tsamanioti ES, Mahdy H. Endometriosis. *StatPearls*. NCBI Bookshelf. 2023
29. Davila G. Endometriosis. *Medscape*. 2021.
30. Hendarto H. Endometriosis dari Aspek Teori sampai Penanganan Klinis. 2015
31. Gruber TM, Mechsner S. Pathogenesis of endometriosis: The origin of pain and subfertility. *Cells*. 2021; 10. Preprint at <https://doi.org/10.3390/cells10061381>
32. D'Alterio MN, et al. Possible role of microbiome in the pathogenesis of endometriosis. *Minerva Obstetrics and Gynecology*. 2021; 73: 193–214. Preprint at <https://doi.org/10.23736/S2724-606X.21.04788-2>
33. Wei W, Zhang X, Tang H, Zeng L, Wu R. Microbiota composition and distribution along the female reproductive tract of women with endometriosis. *Ann Clin Microbiol Antimicrob*. 2020; 19(15).
34. Akiyama K, Nishioka K, Khan KN, Tanaka Y, Mori T, Nakaya T, Kitawaki J. Molecular detection of microbial colonization in cervical mucus of women with and without endometriosis. *American journal of reproductive immunology*. 2019; 82(2).
35. Leonardi M, Hicks C, El-Assaad F, El-Omar E, Condous G. Endometriosis and the microbiome: a systematic review. *BJOG : an international journal of obstetrics and gynaecology*. 2020; 127(2): 239–249.
36. Wessels JM, Domínguez MA, Leyland NA, Agarwal SK, Foster WG. Endometrial microbiota is more diverse in people with endometriosis than symptomatic controls. *Scientific reports*. 2021; 11(1): 18877.