Comparative analysis of vaginitis and endometrial cancer microbiomes using next-generation sequencing

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Received November 26, 2024; Accepted May 9, 2025

DOI: 10.3892/wasj.2025.357

Abstract. Bacterial vaginosis is widespread in women from developing regions, particularly where genital hygiene is inadequate. The balance between pathogenic and non-pathogenic microbes is essential for maintaining a healthy vaginal ecosystem. The present study aimed to compare the vaginal microbiota of women with vaginitis and endometrial cancer using next-generation sequencing (NGS). A total of 84 vaginal swab samples were collected between September, 2022 and July, 2023: A total of 42 from women diagnosed with various gynecological cancers (16 with endometrial cancer) and 42 from women with vaginitis. The ages of the patients ranged from 18 to 83 years, with mean ages of 54.14 (cancer group) and 38.42 (vaginitis group). In total, six samples were selected for NGS analysis (four from vaginitis cases and two from endometrial cancer). NGS of vaginitis samples yielded 51,459 reads, classified into 32 operational taxonomic units (OTUs). The dominant phyla were Proteobacteria (46.90%) and Firmicutes (43.02%), while major bacterial families included *Pseudomonas* (39.15%), *Lactobacillaceae* (51.12%) and *Enterobacteriaceae* (30.87%). Endometrial cancer samples yielded 23,817 reads, classified into 16 OTUs. Firmicutes (81.39%) was the most abundant phylum, and the dominant families were *Streptococcaceae* (80.03%) and *Lactobacillaceae* (39.70%). These findings indicate significant differences in vaginal microbiome composition between women with vaginitis and those with endometrial cancer, suggesting that microbiota profiles may reflect underlying reproductive tract conditions and influence vaginal ecosystem stability.

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Key words: vaginitis microbiomes, endometrium microbiomes, next-generation sequencing

Introduction

The female reproductive system comprises internal and external organs essential for menstruation and procreation. This organ system is responsible for producing gametes (termed eggs or ova), regulating sex hormones, and maintaining fertilized eggs as they develop into mature fetuses ready for delivery (1).

The vaginal microbiota is a complex group of bacteria that populate the vaginal environment and are vital to maintaining the reproductive health of women. The vaginal microbiota is less diverse than that in other regions of the body and is mostly composed of *Lactobacillus* species, which produce antimicrobial compounds, including lactic acid, hydrogen peroxide and bacteriocins, regulate vaginal pH levels and inhibit pathogenic colonization (2).

Bacterial vaginosis (BV) is caused by dysbiosis, or an imbalance in the vaginal microbiota. This condition is characterized by a decline in *Lactobacillus* abundance and an overgrowth of pathogens, such as *Gardnerella vaginalis*, *Atopobium vaginae*, *Megasphaera* spp., *Prevotella* spp., and *Sneathia* spp. (3,4). These microbial imbalances underscore the importance of maintaining a healthy vaginal microbiota to prevent gynecologic complications and reduce the transmission of sexually transmitted infections.

Of note, two other prevalent vaginal illnesses are candidiasis caused by *Candida albicans* and trichomoniasis, which is caused by *Trichomonas vaginalis* (5). Until recently, the endometrium was considered to be a sterile environment. A functional microbiome is present in the endometrium in physiological settings. Some research indicates that *Lactobacillus* is the major and representative genus of a healthy endometrium, while other research suggests different bacterial genera (6). After analyzing endometrial samples, Franasiak *et al* (7) found that the most prevalent genera were *Lactobacillus* and *Flavobacterium*. *Lactobacillus* (71.1%) was the most common bacteria found in endometrial fluid samples from fertile women, followed by *Gardnerella*, *Bifidobacterium*, *Streptococcus* and *Prevotella* (8).

One of the most common types of gynecological cancer is endometrial cancer (EC), which is highly related to the endocrine system (9). Numerous variables, including environmental circumstances, genetic susceptibility, hormonal imbalances (particularly involving estrogen and progesterone), heavy periods,

being overweight, or being at an advanced age, may contribute to the development of EC (10). For instance, previous studies have demonstrated that the vaginal microbiota may play a role in the development of EC by directly interacting with endometrial tissue that is susceptible to the disease or by generating metabolites and inflammatory molecules that affect the course of cancer (11,12). *Atopobium vaginae* and *Porphyromonas*, among other bacteria that raise the vaginal pH levels, are more prevalent in the vaginal flora of women with endometrial hyperplasia or EC (13). This is considered to promote chronic endometrial inflammation that stimulates the carcinogenesis process (14). Therefore, the aim of the present study was to perform a comparison of the semi-quantitative and qualitative dynamics between the vaginal microbiota in women with vaginitis and EC in Sulaymaniyah, Iraq using next-generation sequencing (NGS). The present study focused on EC, excluding other cancer types.

Patients and methods

Sample collection. A total of 100 high vaginal swab samples were collected from Al-Sulaymaniyah Governorate, Iraq at Sulaimani Teaching Hospital, Sulaimaniyah, Iraq and Uper Arbat Health Center, Sulaimaniyah, Iraq , between September, 2022 and July, 2023. A total of 48 various types of gynecological cancer samples were collected and diagnosed through clinical findings and a histological examination; 16/58 samples were excluded due to negative pathohistological results. The ages of the women with gynecological cancers ranged from 18 to 83 years; there was a total number of 42 cancer cases. Of these, 16 cases were EC. Simultaneously, 42 other samples were collected from females with vaginitis, aged 21 to 66 years.

The College of Science Ethics Committee, College of Science, University of Baghdad, approved the research proposal in the present study. The College of Science Ethics committee expects to be informed about the study's progress, any serious adverse events occurring during the study, any revision in the protocol, and patient information/written informed consent and ask to be provided with a copy of the final report.

Molecular detection of vaginal swabs: Genomic DNA extraction. Genomic DNA was extracted from 6 vaginal specimens, including 2 cases of EC (D79-S27 and D80-S28) and 4 specimens from patients with vaginitis (D11-S18, D13-S19, D14-S20 and D65-S25) using the BIORON GmbH kit [BIORON Genomic DNA Mini kit (for Cells and Tissues) cat. no. 112005]. The selected samples depended on the similarity in some parameters (data are not published now) to reduce the difference as much as possible among the tested samples. These DNA samples were used to detect the diversity of the vaginal microbiome from patients with EC and vaginitis by sequencing the 16S rRNA gene using the iSeq method.

Measuring the DNA concentration and purity. DNA concentrations of the extracted samples were measured using the QuantiFluor dsDNA System kit from (cat. no. E2670, Promega Corporation) and analyzed using a Quantus fluorometer device (cat. no. E6150, Promega Corporation). The Quantus fluorometer yielded the following concentrations for the 4 infected subjects: (90, 103, 100 and 124 ng/μl). The concentrations for the 2 samples from patients with EC were 125 and 123 ng/μl.

Primer preparation. The present study used a primer set to identify the V3-V4 region of 16SrRNA (forwardD, 5'-TCG TCGGCAGCGTCAGATGTGTATAAGAGACAG-3' and reverse, 5'-GTCTCGTGGGCTCGGAGATGTGTATAA GAGACAG-3'). Primers were synthesized and prepared by Macrogen, Inc., delivered in lyophilized form and reconstituted to a working concentration with nuclease-free water (Table I).

Library purification. The 16S ribosomal RNA gene amplicons for the Illumina MiSeq System kit were used to PCR amplify the 16S libraries using primers targeting the 16S rRNA gene hypervariable V3 and V4 regions with overhang adapter sequences (15). The thermal amplification profile included an initial denaturation at 95˚C for 3 min, followed by 8 cycles of 95˚C for 30 sec, 55˚C for 30 sec and 72˚C for 30 sec, with a final extension at 72˚C for 5 min and a hold at 4˚C. Following PCR amplification preparation, agarose gel electrophoresis was used for PCR amplicon purification, sample preparation, tiny fragment removal and dual-size DNA fragment selection.

NGS. In NGS, and according to the manufacturer's recommendations (Illumina, Inc.), the amplicons were pooled in equimolar concentrations of 100 pM for iSeq sequencing. Pooled libraries of dsDNA were diluted with 10 mM Tris (pH 8.5) solution to a concentration of 1 nM. Subsequently, 15 μl pooled dsDNA libraries were diluted with 85 μl (resuspension buffer) RSB volume to yield a 150 pM loading concentration. A total of 20 μl of the library containing 14 pooled indexed samples was loaded into the iSeq 100 system. Sequencing was performed using iSeq 100 i1 Reagent v2 (300-cycle) manufactured by Illumina, Inc. The datasets used and analyzed during the current study are available from the corresponding author at NCBI and the accession no. PRJNA1222970 (https://www.ncbi.nlm.nih.gov/sra/PRJNA1222970).

Statistical analysis. All were analyzed using IBM SPSS Statistics for Windows, version 26.0. (IBM Corp.). The results were analyzed using the Chi-squared test and Fisher's exact test to compare the means of the parameters where necessary. A P-value <0.05 was considered to indicate a statistically significant difference.

Results and Discussion

Types and prevalence of gynecological cancer among the patients. Of the 58 patients with gynecological cancers histologically examined, 16 suspected cases were excluded following a histological examination; thus, 42 different gynecological cancer cases remained to be included in the present study, along with an additional 42 cases of vaginitis. The results of the histological diagnosis of the 42 gynecological cancer specimens revealed that only 16 (38.09%) patients with an age range between (30-67 years) and a mean age of 49.68 years had EC, 14 (33.33%) cases had ovarian cancer and 8 (19.05%) cases had polyps; in addition, there were 1 cases each of cervical cancer, fallopian tube cancer, endometrial-ovarian cancer and endometrial-ovarian-fallopian tube cancer.

The findings of the present study are in contrast to those of a recent study by Priyadarshini *et al* (16), which found that

Table I. Primer sequence, annealing temperature and size.

Primer name	Sequence[a]	Annealing temperature (˚C)	Product size (bp)
16S amplicon PCR forward primer[b]	5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-CCTACGGGNGGCWGCAG-3'	55	550
16S amplicon PCR reverse primer[b]	5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-GACTACHVGGGTATCTAA TCC-3'		

[a]International Union of Pure and Applied Chemistry (IUPAC) nucleotide nomenclature: N=any base; W=A or T; H=A or C or T; V=A or C or G. [b]The primer sequence before the hyphen is the Illumina overhang adapter sequence. The primer sequence after the hyphen corresponds to the locus-specific sequence.

uterine cancer was the third most common type of cancer. As a result, various poorly known, diverse causes may likely play a role in these events (17).

Within the same framework, the study by Knudsen *et al* (18) from 2014 elucidated that the elderly had a potentially reduced survival rate and an age-dependent mortality rate for gynecological cancers. Additionally, Piechocki *et al* (19) noted an increase in the incidence of breast cancer, ovarian and uterine cancer, and a decrease in the incidence of cervical and vaginal cancer in Poland. The study by Somasegar *et al* (20) conducted in the United States analyzed trends in the mortality rates of patients with uterine cancer over a period of 50 years, with an emphasis on age and race and ethnicity. They suggested that the incidence of uterine cancer was 6-fold higher in women aged ≥70 years than in those aged 50 to 59 years. According to Duska *et al* (21), elderly individuals typically experience poorer outcomes and are diagnosed at a later stage. Thus, one of the primary risk factors for cancer is an older age.

Molecular identification of the vaginal microbiome: Detection of 16S rRNA gene in the vaginal microbiome using a PCR template. A total of 6 vaginal specimens were selected to amplify the conserved region of the thermo-stable nuclease that encodes for the 16S rRNA gene using universal primers. To detect the genotype of bacterial communities in vaginal swabs, the good quality and concentration DNA samples that were extracted from the vaginal specimens of patients with gynecological cancers, including EC and vaginitis, were used to confirm the type of bacteria using the PCR technique. The primer set (Table I), was used to cover the V3-V4 region in the 16S rRNA gene. This region is known to have higher resolution for low-rank taxa (bacteria and archaea) (22). The results presented in Fig. 1 revealed specific 550 bp bands, indicating the correct amplicon.

The human microbiome is anticipated to function as a novel and beneficial tool for classifying human epithelial materials, as Yao *et al* (23) found in 2021. Vaginal secretions are the most common biological specimen in several sexual assault cases; the characterization of these fluids is crucial to accurately determining the nature of the case. Knowledge of vaginal microbiota across different regions, physiological conditions and ethnic populations is constantly being improved. The present study performed high-throughput sequencing of the V3-V4 hypervariable regions of the 16S rRNA gene of vaginal

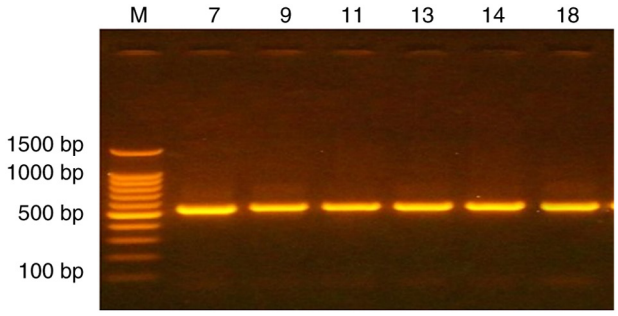

Figure 1. The amplification bacterial products of the *16s RNA* gene with a specific primer gene (550 bp) separated using 2% agarose gel electrophoresis (100 v/mAmp, TAE buffer 1X) for 60 min after staining with ethidium bromide, conceived under UV light. M, molecular size marker (100-1,500 bp).

samples collected from patients in the Al-Sulaymaniyah region, Iraq, who had EC and vaginitis to obtain comparable information about the vaginal microflora and its association with cancer sequelae in women.

Illumina iSeq sequencing for the *identification of bacteria in the vaginal microbiome*. A total of 6 DNA samples were extracted from the patients with gynecological cancers, including 2 cases of EC (NCBI Accession nos. SRX27671438 and SRX27671439) another 4 cases of vaginitis (NCBI Accession nos. SRX27671443, SRX27671442, SRX27671444 and SRX27671448) used as an indicator for vaginal microbiome, these data have been deposited in (NCBI). The DNA samples were sent to Macrogen Inc. to identify the bacterial community among these groups by NGS (iSeq sequencing). All DNA samples used in this experiment were passed through the quantification analysis.

One of the primary techniques in studying the taxonomy of the microbiome community is the (16S rRNA). It plays a crucial role in the survival and functions of the cell, and it is considered a golden standard in microbial genotyping. The 16S rRNA gene is comprise of two hypervariable regions bordered by more conserved sequences. Microbial diversification studies typically employ the dual-indexed custom primer 16S rRNA gene sequencing technology for the V4 hypervariable region. This region was used by the Human Microbiome Project to provide adequate information for the taxonomic classification

of microbial communities obtained from specimens related to human microbiome research. However, the approach outlined could be extended to any location (24,25), yet it was designed for iSeq sequencing. Griffin *et al* (26) demonstrated the utility of NGS for resolving polyploid complexes. The advantage of this technique is discovering the low-abundant microbe. It allows covering large numbers of reads with high depth in a single experiment. The mean percentages of bacterial phyla for the 4 patients in the present study suffering from different types of vaginosis are presented in Fig. 2.

A total of iSeq 51,459 reads were created in the present study and were used for downstream analyses (Fig. 2). The reads were classified into 32 operational taxonomic units (OTUs) representing the individual bacterial species. The most abundant phyla detected in the present study were Proteobacteria (46.90%), Firmicutes (43.02%), Actinobacteria (8.84%), Bacteroidetes (0.05%), Tenericutes (0.03%) and unclassified at the phylum level (1.14%). The values in parentheses represent the average percentage of bacteria at a specific taxonomic level in the vaginitis cases. As demonstrated in Fig. 2, the most dominant phyla are Proteobacteria and Firmicutes; these findings are in accordance with those in the study by Sroka-Oleksiak *et al* (27) who also revealed 4 phyla: Firmicutes, Actinobacteria, Proteobacteria and Bacteroidetes. It can be suggested that the differences between the percentages of the two studies may be related to the disruptions among vaginal microbiomes as a result of infections, as previously mentioned by Srinivasan *et al* (28).

The results presented in Fig. 3 revealed that the most predominant bacterial families, in descending order, were as follows: *Lactobacillaceae* (51.12%), *Pseudomonas* (39.15%), *Enterobacteriaceae* (30.87%), *Streptococcaceae* (7.44%), *Staphylococcaceae* (0.33%), *Clostridiaceae* (0.15%), *Enterococcaceae* (0.13%) and others under unclassified at family level (4.88%).

The results of the present study were completely incompatible with those documented in the study by Srinivasan *et al* (29), which revealed that ~20% of pregnant women may suffer from BV, the most prevalent vaginal condition in patients. In BV, the overgrowth of characteristically non-*Lactobacillus* anaerobic bacteria, such as *Mobiluncus* spp., *Gardnerella vaginalis*, and *Atopobium vaginae*, leads to a disruption of the ecosystem of vaginal balance and the modification of the vaginal milieu, which may result in developing clinical signs, such as vaginal discharge and itchiness. Nevertheless, almost 50% of the patients who have BV are asymptomatic or have fewer clinical signs. However, Yao *et al* (23) demonstrated that there was minimal variation in the makeup of the main bacteria in the vagina, which were primarily *Lactobacillus* and *Gardnerella* species.

In the same context, Fig. 4 illustrates the mean percentages of bacterial phyla for 2 patients with EC. A total of iSeq 23,817 reads were created in the present study and were used for downstream analyses (Fig. 4). The reads were classified into 16 OTUs representing the individual bacterial species. The most abundant phyla detected in the present study were Firmicutes (81.39%), Actinobacteria (7.84%), Proteobacteria (6.00%), unclassified at the phylum level (4.54%) and Tenericutes (0.11%); these percentages represent the average percentage of bacteria at a specific taxonomic level in the endometrial cancer cases. From these phyla, as illustrated in Fig. 4, the phylum, Firmicutes, was predominant in EC, as also demonstrated by Lee *et al* (30), who documented that vaginal microbiota are related to the health of the reproductive system of women. When *Lactobacillus* levels are reduced, the majority of the vaginal microbiota tend to become imbalanced, which can lead to the development of bacterial vaginosis (31). Srinivasan *et al* (28) reported that there are several clinical signs related to vaginal infection, such as a vaginal pH >4.5, the presence of clue cells, amine odor under the whiff test, and an increased thin homogenous vaginal discharge linked with specific microorganisms such as *Atopobium vaginae*, *Gardnerella vaginalis*, and *Leptotrichia amnionii*. Tamrakar *et al* (32) indicated that the *Lactobacillus*

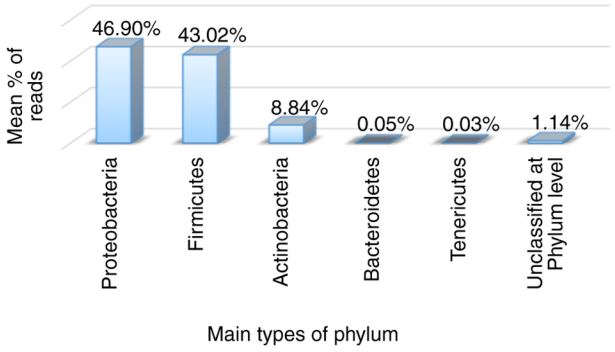

Figure 2. The main types of bacterial phyla dependent on the mean percentages of reads in 4 patients with vaginitis.

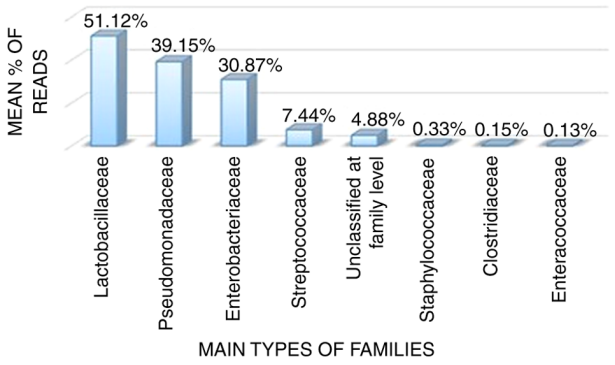

Figure 3. The main types of bacterial families according to the percentages of reads in 4 patients with vaginitis.

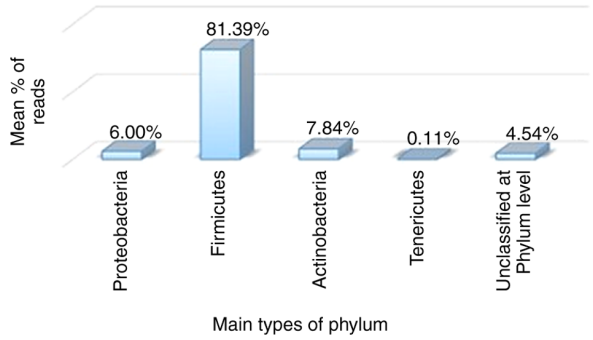

Figure 4. The main types of bacterial phyla dependent on the mean percentages of reads in 2 patients with endometrial cancer.

Table II. Chi-squared test analysis for the distribution of bacterial phyla between the vaginitis and endometrial cancer samples.

	Vaginitis		Endometrial cancer	
Phyla classification	No. of reads	% of reads	No. of reads	% of reads
Proteobacteria	5931	46.90%	820.5	5.97%
Firmicutes	5573	43.01%	10210	81.39%
Unclassified at the phylum level	156.5	1.14%	580.5	4.53%
Actinobacteria	1285	8.84%	841.5	7.84%
Tenericutes	3.5	0.03%	14	0.11%
Bacteroidetes	6.5	0.05%	38	0.23%
Chi-squared test value	5589.48	DF=5	P-value	0.001[a]

[a]Indicates a statistically significant difference (P<0.05).

iners is considered a marker of vaginal microbiome disorder as its prevalence has been linked with that of other bacterial vaginosis-related bacteria, such as *Leptotrichia*, *Eggerthella*, *Megasphaera* and bacterial vaginosis.

The results shown in Figs. 2 and 4 yielded significantly differences P<0.05). Based on the phylum classification distributions, the results are statistically significant, indicating an association between endometrial bacterial sample disorders and vaginitis. The observed differences show substantial variations in the distribution of phyla between the two cases (Table II). The observed comparable statistical analysis revealed that a P-value of 0.001, indicating a highly significant difference in the family classification distribution between the two conditions [vaginitis (Fig. 3) and EC (Fig. 5); Table III]. Based on the data presented in Fig. 5, the most common bacterial families were *Streptococcaceae* (80.03%), *Lactobacillaceae* (39.70%) and *Staphylococcaceae* (0.17%). The remaining taxa (8.41%) are not categorized at the family level. A P-value of 0.001 suggests that the family categorization distributions for EC (Fig. 5) and vaginitis (Fig. 3) cases differ significantly.

Moreno and Simon (33) demonstrated that the microbiome related to the reproductive tract comprised 9% of the human microbiota, which includes the flora in the vagina and endometrial follicular fluid in women. The composition of the vaginal microbiota depends on hormonal fluctuations, sexual behaviors, age and menstruation, as well as on the use of drugs such as antibiotics and probiotics that may lead to an imbalance. The results presented in Fig. 5 reveal the decrease in the *Lactobacillaceae* reading frequency compared to *Streptococcaceae*; these variations may be explained by Wan *et al* (34), who reported the association of hormonal factors and the development of EC. It is certain however, that excess levels of or unopposed estrogen are a major risk factor. Vajpeyee *et al* (35) documented that endometrial thickness had a significant impact on the levels of luteinizing hormone, estrogen and progesterone. In addition, microbial abundance alteration depends on the reproductive system states, regardless of the degree of pathogenicity that can affect fertilization, implantation and subsequent embryonic development. After comparing pregnant and non-pregnant patients, pregnant patients had a higher abundance of Firmicutes and Proteobacteria, and a lower abundance of Actinobacteria, Fusobacterium and

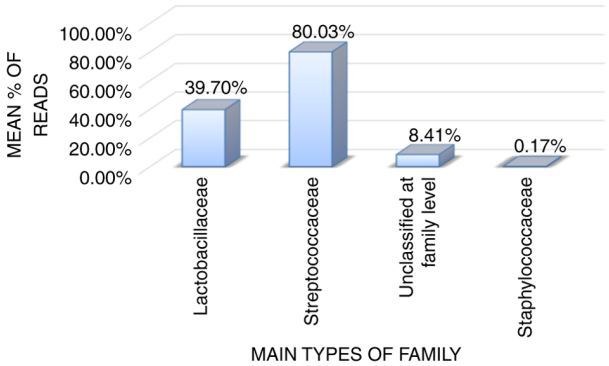

Figure 5. The main types of bacterial families dependent on the mean percentages of reads in 2 patients with endometrial cancer.

Bacteroidetes at the phylum level (36). Therefore, it can be suggested that different sexual hormonal levels may directly or indirectly affect the vaginal microbiome (37).

In short, these samples were classified into 11 and 9 phyla, 12 and 9 classes, 18 and 11 orders, 20 and 14 families, and finally, 25 and 15 genera for the vaginitis and EC specimens, respectively. The most abundant bacterial families in the present study for the vaginitis samples were *Lactobacillaceae*, *Staphylococcaceae*, *Streptococcaceae*, *Enterococcaceae* and *Clostridiaceae* (Firmicutes phylum); *Paenibacillaceae* (Bacillota phylum); *Pseudomonadaceae*, *Anaplasmataceae*, *Acetobacteraceae*, *Enterobacteriaceae*, *Desulfovibrionaceae*, *Vibrionaceae*, *Ferrimonadaceae* and *Shewanellaceae* (Proteobacteria phylum); *Bifidobacteriaceae*, *Mycobacteriaceae*, *Glycomycetaceae*, *Intrasporangiaceae* (Actinobacteria phylum); *Prevotellaceae* (Bacteroidetes phylum); and others are unclassified phylum and family levels. In addition to *Streptococcaceae*, *Staphylococcaceae*, Planococcaceae, and Leuconostocaceae (Firmicutes phylum); Paenibacillaceae (Bacillota phylum); *Brucellaceae*, *Bartonellaceae*,(Proteobacteria phylum); *Corynebacteriaceae*, *Intrasporangiaceae* and *Bifidobacteriaceae* (Actinobacteria phylum); *Desulfovibrionaceae* (Thermodesulfobacteriota phylum); *Phyllobacteriaceae* (Pseudomonadota phylum); and others are unclassified at the phylum and family levels, which represent the most abundant families in endometrial samples.

Table III. Fisher's exact test analysis for the distribution of bacterial Families between the vaginitis and endometrial cancer samples.

	Vaginitis		Endometrial cancer				
Family classification	No. of reads	% of reads	No. of reads	% of reads	Odds ratio	Fisher's exact test P-value	P-value
Lactobacillaceae	6590.66	51.12%	3479	39.70%	1.90342565	1.2857E-167	<0.001
Unclassified at the family level	636.5	4.88%	1067	8.41%	0.503141758	1.35816E-42	<0.001
Enterobacteriaceae	3556	30.87%	0	0	inf	0	0.001
Clostridiaceae	18	0.15%	0	0	inf	1.40969E-05	<0.001
Enterococcaceae	17	0.13%	0	0	inf	2.66201E-05	<0.001
Prevotellaceae	10	0.08%	0	0	inf	0.002405388	<0.002
Staphylococcaceae	45	0.33%	15	0.17%	2.604972376	0.00102524	0.001
Paenibacillaceae	10	0.08%	70	0.43%	0.123512231	1.92164E-14	<0.001
Pseudomonadaceae	5450.5	39.15%	0		inf	0	0.001
Streptococcaceae	1036.5	7.44%	13010	80.03%	0.024852306	0	0.001
Staphylococcaceae	80	0.57%	15	0.17%	4.638180141	3.55791E-10	<0.001
Anaplasmataceae	37	0.27%	0	0	inf	1.11508E-10	<0.001
Acetobacteraceae	22	0.16%	0	0	inf	1.12177E-06	<0.001
Vibrionaceae	115	1.00%	0	0	inf	1.1061E-31	<0.001
Ferrimonadaceae	8	0.07%	0	0	inf	0.00892627	0.008
Glycomycetaceae	8	0.07%	0	0	inf	0.00892627	0.008
Shewanellaceae	7	0.06%	0	0	inf	0.017288841	0.017
Bifidobacteriaceae	4950	33.92%	946	10.85%	5.516386638	0	0.001
Desulfovibrionaceae	161	1.10%	31	0.36%	4.52907633	8.54899E-19	<0.001
Intrasporangiaceae	75	0.51%	21	0.24%	3.10430152	8.93743E-07	<0.001
Mycobacteriaceae	18	0.12%	0	0	inf	1.40969E-05	<0.001
Planococcaceae	0	0	15	0.17%	0	1.00688E-05	<0.001
Leuconostocaceae	0	0	25	0.29%	0	4.67909E-09	<0.001
Corynebacteriaceae	0	0	459	2.82%	0	7.6058E-155	<0.001
Brucellaceae	0	0	328	2.02%	0	1.3312E-110	<0.001
Bartonellaceae	0	0	252	1.55%	0	4.98033E-85	<0.001
Phyllobacteriaceae	0	0	85	0.52%	0	4.45185E-29	<0.001

P-values <0.05 were considered to indicate a statistically significant difference.

Thus, nine bacterial phyla and eight families were identified as being shared between vaginitis and endometrial cancer. The shared phyla included *Proteobacteria*, *Firmicutes*, *Actinobacteria*, *Tenericutes*, *Bacteroidetes*, *Verrucomicrobia*, *Fusobacteria* and *Spirochaetes*, along with other unclassified taxa at the phylum level. Similarly, at the family level, the shared bacterial families comprised *Lactobacillaceae*, *Streptococcaceae*, *Staphylococcaceae*, *Bifidobacteriaceae*, *Intrasporangiaceae*, *Desulfovibrionaceae* and *Paenibacillaceae*, in addition to other unclassified families.

The present study identified nine shared phyla, classes, orders, and families between the two groups. Additionally, the presence of *Lactobacillus* does not necessarily suppress another vaginal microbiota. In addition, in each case, the vaginal microbiomes produce a microenvironment suitable for particular microbial growth that may affect directly or indirectly the ecosystem of the female reproductive system. Thus, it may highlight the need for further research to determine the real causes using larger sample sizes for potential therapeutic applications.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

The data generated in the present study may be found in the NCBI database under accession number PRJNA1222970 (https://www.ncbi.nlm.nih.gov/sra/PRJNA1222970).

Authors' contributions

All authors (DFA, MAA and TAH) contributed significantly to the acquisition, analysis and interpretation of the data. MAA suggested the subject of research, and both DF and TAH collected samples from the hospital for analysis and presented accurate research results. All authors have reviewed and have read and approved the final version of the manuscript. DFA and MAA confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The College of Science, University of Baghdad Ethics Committee approved the research proposal in the present study. The College of Science Ethics committee expects to be informed about the study's progress, any serious adverse events occurring during the study, any revision in the protocol, and patient information/written informed consent and ask to be provided with a copy of the final report. None of the investigators and co-investigators participating in this study took part in the decision-making and voting procedure for this study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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