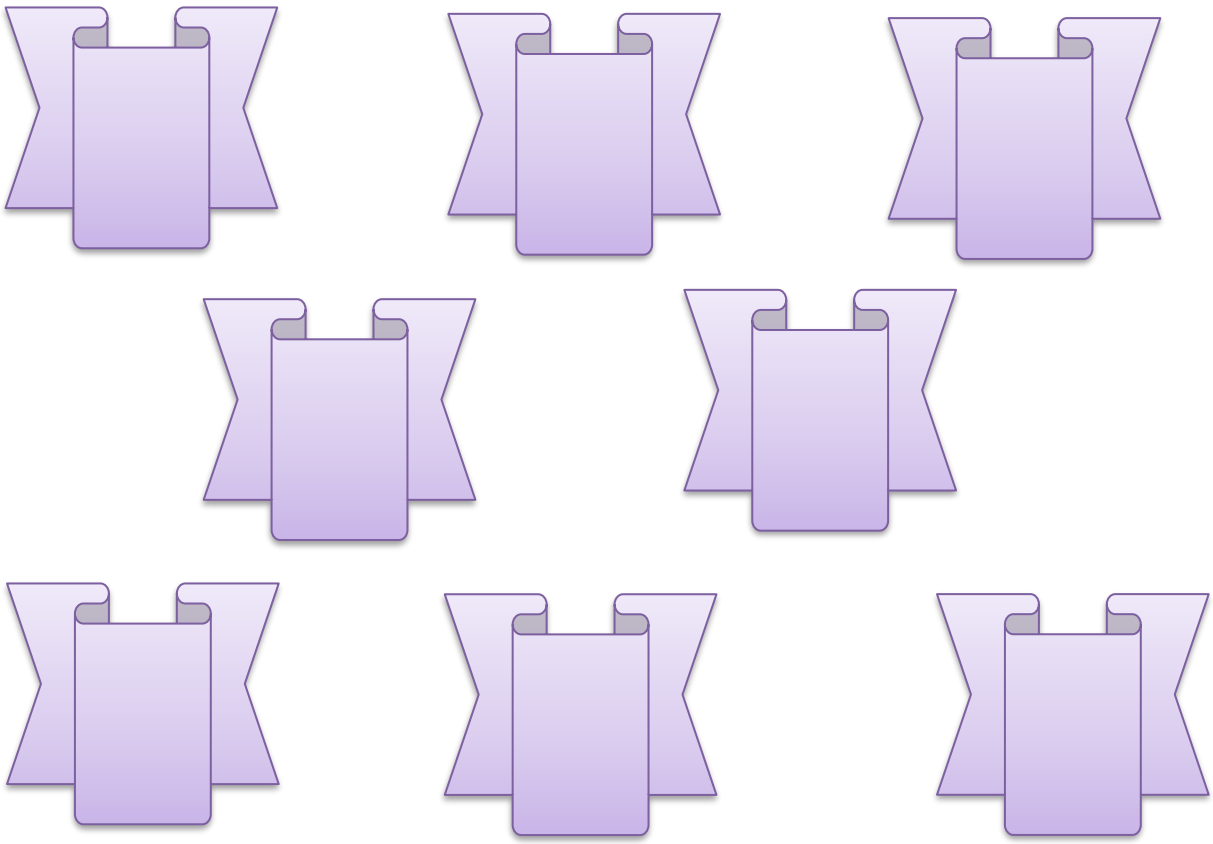
International Journal of Medical Arts

Volume 5, Issue 8, August 2023

https://ijma.journals.ekb.eg/



Print ISSN: 2636-4174

Online ISSN: 2682-3780

Available online at Journal Website
https://ijma.journals.ekb.eg/
Main Subject [Gynecology and Obstetrics]

Original Article

Comparison of The Vaginal Microflora in Relation to The Menopausal Status

Emad Mohamed Ibrahim Atallah *, Abdel-Monsef Abdel-Ghaffar Moustafa, Ahmed Abdelkader Eltabakh

Department of Obstetrics and Gynecology, Faculty of Medicine, Al-Azhar University, Cairo, Egypt

Article information

Received: 17-06-2023

Accepted: 11-09-2023

DOI: 10.21608/IJMA.2023.189805.1608.

*Corresponding author

Email: emadatalla1983@gmail.com

Citation: Atallah EMI, Moustafa AA, Eltabakh AA. Comparison of The Vaginal Microflora in Relation to The Menopausal Status. IJMA 2023 August; 5 [8]: 3517-3524. doi: 10.21608/IJMA.2023.189805.1608.

ABSTRACT

Background: Numerous bacteria live in the human vagina; because of this, it has been discovered that the vaginal microbiome is directly related to both vaginal and overall health.

Aim of the work: In this study, we sought to evaluate the vaginal microbiomes of premenopausal and post-menopausal women.

Patients and Methods: This was a comparative cross section study, was carried out on 120 patients who divided in to two groups as regard menopausal state in to Group A: 60 women in Premenopausal group and Group B: 60 women in Post-Menopausal group, vaginal samples were obtained from all studied women.

Results: As regard bacterial distribution according to Phylum; there was a significant difference between two groups regarding Firmicutes and Proteobacteria which were higher among premenopausal women.

Conclusion: Women in each study group had different vaginal ecosystems in terms of the relative quantity and composition of the dominating bacterial species. While species diversity greatly increased in postmenopausal women, species richness significantly declined. After menopause, the most significant difference between the two groups was caused by a decline in Lactobacillus taxa, which are represented by all taxon ranks of the vaginal microbiome.

Keywords: Microflora, Premenopausal, Postmenopausal, Menopause, Microbiome, Vaginal atrophy.

INTRODUCTION

Menopause, defined as the cessation of menstruation for a period of 12 consecutive months, typically occurs in the fourth or fifth decade of life [1]. Estrogen levels fall as a result of decreased ovarian activity during menopause, which typically causes symptoms such as hot flashes, night sweats, impaired cognitive function, and mood swings that women frequently experience in the years leading up to and during menopause [2]. A high concentration of Lactobacillus species is associated with good vaginal health. Even though the vagina is home to numerous aerobic and anaerobic bacteria, Lactobacillus is the most common species. Different Lactobacillus species produce anti-microbial compounds such as lactic acid and hydrogen peroxide [3].

The resultant environment is essential for preventing a number of anaerobic and micro-aerophilic pathogens from colonizing the vagina, which can lead to lactobacilli depletion and dysbiosis [an imbalance of the vaginal population], also known as bacterial vaginosis [BV] [4]. BV has been connected to a number of adverse obstetric and gynecologic health outcomes in both pre- and postmenopausal women [5]. Sexually transmitted diseases and gynecologic infections are more likely to affect postmenopausal women when the vaginal microbiome is disrupted. Additionally, vulvo-vaginal atrophy [VVA]-related genitourinary tract problems affect at least 50% of menopausal women [6].

Dryness, redness, itching, dyspareunia, and, rarely, discharge or bleeding are the most frequent signs of VVA [7]. Postmenopausal women have traditionally received local and systemic low-dose estrogen treatment to treat VVA and vaginal dryness. Following meno-pause, the vaginal environment changes, and the species makeup of the vaginal microbiome also varies [8]. This usually shows up as a reduction in the ratios of lactobacilli and lactic acid-producing bacteria, elevating the pH of the vagina, and perhaps enhancing the risk of infection as well as intensifying the vaginal symptoms of VVA [9]. Only a few studies have examined the vaginal microbiomes of post-menopausal women who receive hormone therapy [HT] compared to those who do not. In this study, the vaginal microbiomes of pre-menopausal and postmenopausal women were compared. The findings indicate that the vaginal microbiomes of HT-using women often resemble those of premenopausal women, as they successfully restore high levels of lactobacillus. However, it remains unclear how this restoration occurs.

PATIENTS AND METHODS

This study was conducted at Al-Hussein Hospital, Al-Azhar University, from November 2021 to May 2022. The study included 120 subjects who were all followed at the Microbiology and Gynecology and Obstetrics Department at the Faculty of Medicine, Al-Azhar University.

According to the findings of **Gliniewicz *et al.*** [10], two groups of 60 healthy adult women were prescreened for participation in the study based on their vaginal pH and vaginal atrophy score. Subject selection and sample collection were conducted at a different location.

Patients were divided into two groups: **Group A** included 60 women in the pre-menopausal group, and **Group B** included 60 women in the post-menopausal group.

Healthy adult females who completed informed permission forms, and their ages ranged from 23 to 67, were included. However, participants who had undergone a partial or total hysterectomy, had an irregular menstrual cycle, had vulvar skin abnormalities, had diabetes, kidney, heart, or circulatory disease, were pregnant or nursing, were participating in another clinical trial, or were taking specific immunosuppressive or anti-inflammatory drugs that might alter test outcomes were not permitted to take part.

Vaginal samples were taken from the vaginal wall, approximately two inches into the vagina, using the diagnostic equipment. The swab was then sealed in a cryogenic tube, kept in an 80 °C freezer, and transported to the lab for further analysis using dry ice.

Microbial Community Analysis

After transferring each sample to dot beating, we added 100 L of lytic protein cocktail [50 L lysozyme 500 kU/mL, 6 L mutanolysin 25 kU/mL, 4 L lysostaphin 3000 kU/mL, and 41 L combination of 10 mM Tris-HCl and 50 mM EDTA pH 8.0] before incubating the mixtures at 37 °C for 60 minutes. Subsequently, the tubes

were subjected to a Small Bead Beater-96 at room temperature briefly after adding 750 mg of 0.1 mm diameter zirconia-silica beads to all samples.

A short centrifugation step followed the completion of bead beating. Following the manufacturer's instructions, a DNA Mini Kit was used to isolate the genomic DNA of the bacterium. DNA precipitations were carried out using a dsDNA kit and a Turner TBS-380 micro fluorometer. DNA size and integrity were assessed using an Agilent DNA 1000 kit and an Agilent Bioanalyzer 2100, according to the manufacturer's instructions. To enhance the V1-V3 region of the bacterial 16S rRNA gene [Escherichia coli regions 27F534R], a series of overlapping primers flanking the variable regions were employed. Amplicons were generated through PCR in two successive rounds. The first round of PCR amplified the V1-V3 region of the 16S rRNA gene, while the second round of PCR attached the sample barcode and sequencing adapters. Amplicon concentrations were determined using a PicoGreen assay and fluorometer, and then equal amounts [approximately 100 ng] were pooled into a single tube. Short unwanted fragments were removed from the amplicon pool using the following procedure: after the pool was appropriately sized, the product was run on a 1% gel, extracted from the gel, and purified using a purification kit. The pool was PCR amplified using Illumina adapter-specific primers, and the size of the amplicons was assessed using a DNA1000 chip and the appropriate Bioanalyzer. The purified amplicon pool was quantified using an Applied Biosystems StepOne Plus real-time PCR instrument and a quantification kit. After assigning the reads to samples based on both the expected barcode and primer sequences using the dbcAmplicons1 custom Python software, demultiplexing and sorting of raw DNA sequencing reads were performed. Barcode mismatches and primer mismatches [Leven-shtein distance] are allowed as long as the final 4 bases of the primer exactly match the target sequence.

After removing their primer sequences, sequencing reads were then assembled into a single amplicon sequence using Streak [11]. Subsequently, the RDP Bayesian classifier was employed to assign phylotypes to the sequences [12]. The first RDP classification level was used to group reads with a bootstrap score of 50. Further analysis of reads assigned to this group identified the Lactobacillus species present in the samples. The number of reads assigned to each taxon was tallied, the best match for each read was determined, and an estimation of the overall abundance of each bacterial species in each sample was calculated.

Quantifying 16S rRNA Gene Copy Number

We utilized a wide-coverage 16S qPCR test, developed by **Liu *et al.*** [13], to quantify the bacterial count in the samples. Each 10 µL reaction consisted of 0.2 ng of template DNA, 1.8 M forward and reverse primers, 225 nM TaqMan R probe, and 1 Platinum Quantitative Assay, along with molecular-grade water for fluorometric assessment of DNA yields. For each test, a negative control and a set of standards [no DNA template] were included. Three separate assays were performed. The cycling conditions included 40 cycles of uracil-N glycosylase [UNG] treatment at 50 °C for three minutes, Taq activation at 95 °C for ten minutes, denaturation at 95 °C for fifteen seconds, and extension at 60 °C for one minute.

Ethical Approval: After obtaining approval from the university's ethical committee, written informed consent was obtained from each participant in the study and collected. The study strictly adhered to the ethical guidelines outlined in the World Medical Association's code of ethics, known as the Declaration of Helsinki.

Statistical Analysis: Data analysis was carried out using IBM-SPSS version 24. [May 2016]. The statistical significance was assessed using the independent t test and Chi square test. Each variable was evaluated in accordance with the sort of data it held [parametric or not]. If the P-values were less than 0.05, we regarded the results as statistically significant.

RESULTS

The two groups were comparable regarding BMI, however there is a significant difference regarding age [table 1].

Regarding Phylum bacterial distribution, there was a significant difference between both groups regarding Firmicutes [more frequent in premenopausal women] and Proteobacteria [more frequent in postmenopausal women] as shown in table [2].

There is a significant difference between the groups regarding phylum, class, and order of Firmicutes "Bacilli [Lactobacillales] and Tissierellia" [table 3].

There is a significant difference between the groups regarding class of Actinobacteria [Actinobacteria and Coriobacteriia] [table 4]. There is a significant difference between the groups regarding phylum, class, order, and family Proteobacteria [table 5].

There is no significant difference between the groups regarding phylum, class, order, and family of Bacteroidetes [table 6].

There is no significant difference between the groups regarding phylum, class, order, and family of Fusobacteria [table 7].

Table [1]: Demographic data of studied cases

	Pre	Post	P
Age [years]	37.52 ± 4.33	54.18 ± 6.29	**<0.001**
BMI [kg/m^2]	26.53 ± 2.37	27.32 ± 2.84	0.101

Table [2]: Bacterial distribution according to Phylum

	Pre	Post	P
Firmicutes	46 [76.7%]	27 [45%]	**0.0004**
Actinobacteria	10 [16.7%]	16 [26.7%]	0.184
Proteobacteria	0	11 [18.3%]	**0.001**
Bacteroidetes	2 [3.3%]	4 [6.7%]	0.402
Fusobacteria	2 [3.3%]	2 [3.3%]	1

Table [3]: Distribution of Firmicutes [phylum, class, order, and family]

	Pre	Post	P
Firmicutes	46 [76.7%]	27 [45%]	**0.0004**
Bacilli	45 [75%]	20 [33.3%]	**0.002**
Lactobacillales	45 [75%]	18 [30%]	**0.031**
Lactobacillaceae	39 [65%]	13 [21.6%]	0.063
Enterococcaceae	1 [1.7%]	2 [3.3%]	
Streptococcaceae	5 [8.3%]	3 [5%]	
Bacillales	0	2 [3.3%]	--
Bacillaceae	0	2 [3.3%]	--
Tissierellia	1 [1.7%]	4 [6.7%]	**0.039**
Tissierellales	1 [1.7%]	4 [6.7%]	--
Peptoniphilaceae	1 [1.7%]	4 [6.7%]	--
Clostridia	0	2 [3.3%]	0.061
Clostridiales	0	2 [3.3%]	--
Lachnospiraceae	0	2 [3.3%]	--
Negativicutes	0	1 [1.7%]	0.189
Veillonellales	0	1 [1.7%]	--
Veillonellaceae	0	1 [1.7%]	--

Table [4]: Distribution of Actinobacteria [phylum, class, order, and family]

	Pre	Post	P
Actinobacteria	10 [16.7%]	16 [26.7%]	.184
Actinobacteria	10 [16.7%]	11 [18.3%]	**0.049**
Bifidobacteriales	10 [16.7%]	10 [16.7%]	.329
Bifidobacteriaceae	10 [16.7%]	10 [16.7%]	--
Actinomycetales	0	1 [1.7%]	.329
Actinomycetaceae	0	1 [1.7%]	--
Coriobacteriia	0	5 [8.3%]	**0.049**
Coriobacteriales	0	5 [8.3%]	--
Coriobacteriaceae	0	5 [8.3%]	--

Table [5]: Distribution of Proteobacteria [phylum, class, order, and family]

	Pre	Post	P
Proteobacteria	0	11 [18.3%]	**0.001**
Gammaproteobacteria	0	11 [18.3%]	
Pseudomonadales	0	4 [6.7%]	
Pseudomonadaceae	0	4 [6.7%]	
Enterobacterales	0	6 [10%]	
Morganellaceae	0	4 [6.7%]	
Enterobacteriaceae	0	2 [3.3%]	
Pasteurellales	0	1 [1.7%]	
Pasteurellaceae	0	1 [1.7%]	

Table [6]: Distribution of Bacteroidetes [phylum, class, order, and family]

	Pre	Post	P
Bacteroidetes	2 [3.3%]	4 [6.7%]	.402
Bacteroidia			
Bacteroidales			
Prevotellaceae			

Table [7]: Distribution of Fusobacteria [phylum, class, order, and family]

	Pre	Post	P
Fusobacteria	2 [3.3%]	2 [3.3%]	1
Fusobacteria	2 [3.3%]	2 [3.3%]	**1**
Fusobacteriales	2 [3.3%]	2 [3.3%]	**1**
Leptotrichiaceae	2 [3.3%]	2 [3.3%]	1

DISCUSSION

The ovulatory cycle significantly modifies the microbial composition due to the effects of estrogen and progesterone on the stratified squamous epithelium. This modification is characterized by an increase in epithelial thickness, glycogen storage, and influence on local immunology. In healthy women, there are various community state types [CSTs], including type "L" with a majority of Lactobacillus crispatus, type II with a predominance of Lactobacillus gasseri, type III with a preponderance of Lactobacillus iners, and type V with a predominance of Lactobacillus jensenii. Type IV, on the other hand, represents a diverse community of bacteria instead of lactobacilli [14].

There are more microorganisms in the vagina and fewer lactobacilli, which might be related to the side effects of dryness. With menopause, there appears to be a decrease in lactobacilli, which is associated with higher serum levels of follicle-stimulating hormone [FSH] and lower levels of estrogen. The evaluation of Gram-stained vaginal smears in postmenopausal women should take into account the clinical-laboratory association [15]. Numerous studies have been conducted on the vaginal microbiome and microbiota, whose composition is dynamic and influenced by factors such as age, nationality, medication use, and sexual behavior. Vaginal dysbiosis refers to an imbalance in this microenvironment, which can have negative pregnancy outcomes as well as increase the risk of pelvic inflammatory disease, HIV infection, and human papilloma-virus [HPV] infection. Lactobacilli species may dominate the vaginal microbiota during the reproductive stage, but eventually, this dominance gives way to a polymicrobial flora without lactobacilli [16].

The ovulatory cycle considerably modifies the microbial composition due to an increase in epithelial thickness, glycogen storage, and influence on local immunology, resulting from the effects of estrogen and progesterone on the stratified squamous epithelium [17].

In this study, our aim was to evaluate the vaginal microbiomes of premenopausal and postmenopausal women. The mean age was 37.52 ± 4.33 years in the premenopausal group and 54.18 ± 6.29 years in the postmenopausal

group. Additionally, the mean BMI was 26.53 ± 2.37 kg/m² in the premenopausal group and 27.32 ± 2.84 kg/m² in the postmenopausal group.

The average age of the women who participated in **Gliniewicz *et al.*** [10]'s study was 60.5 years for postmenopausal women, and 33 [6.4] years for premenopausal women [PRE], who comprised the only group. The averages for the groups' height, weight, and BMI were similar.

In another study by **Kim *et al.*** [18], they recruited 30 women aged 18-70 years for the trial, consisting of 11 premenopausal and 19 postmenopausal women. The mean age was 39.4 ± 2.4 years in the premenopausal group and 58.5 ± 3.6 years in the postmenopausal group. The mean BMI was 23.2 ± 3.3 kg/m² in the premenopausal group and 25.9 ± 3.6 kg/m² in the postmenopausal group.

In the current study, a significant difference was observed between two groups in terms of bacterial distribution. The phyla Firmicutes and Proteobacteria were found to be higher among premenopausal women. In a study conducted by **Kim *et al.*** [18], the taxonomic classification at the phylum level showed that Firmicutes comprised the largest portion, followed by Actinobacteria, Proteobacteria, Bacteroides, and Fusobacteria. Among pre-menopausal women, the proportions of each phylum were 77.8%, 15.2%, 0.0%, 2.4%, and 3.7%, respectively, while among post-menopausal women, they were 46.1%, 24.9%, 18.6%, 8.0%, and 2.5%, respectively. There was a significant decrease in Firmicutes [p = 0.012] and a significant increase in Proteobacteria and Bacteroidetes [p = 0.0004 and p = 0.019, respectively] among post-menopausal women compared to premeno-pausal women.

In the ongoing study, there was a tremendous contrast between the groups regarding the phylum, class, and order of Firmicutes "Bacilli [Lactobacillales] and Tissierellia," which were higher among premenopausal women. This finding is in accordance with a past report by **Brotman *et al.*** [19], which examined American ladies and found that the Lactobacillus type diminished from 83% to 54% after menopause. However, there was a quantitative difference between the two studies, and it appears that additional research in different ethnic groups is necessary.

Moreover, **Kim *et al.*** [18] found that post-menopausal ladies had a higher species assortment and a lower species abundance compared to pre-menopausal women. Less Lactobacillus, an antibacterial, is likely the cause of this imbalance in the vagina.

Microorganisms that have recently colonized the vagina have steadily increased or acquired new irresistible qualities. In **Wang *et al.*** [20]'s study on a female patient with pelvicitis in China, a reduction in lactobacilli was accompanied by polymicrobial disease, which is indistinguishable from the current findings. According to **Gliniewicz *et al.*** [10]'s study from 2019, only three out of fifteen ladies' networks were dominated by lactobacilli, which is consistent with **Shen *et al.*** [21]'s findings from 2016, where only seven out of thirty ladies were affected [dominance was defined as >25% of a community]. It should be noted that despite being "drained" of lactobacilli, other studies indicate that postmenopausal ladies' vaginal networks are quite stable in terms of species composition [22].

These broad assumptions appear to be inaccurate. On the contrary, and in agreement with other studies, our findings demonstrate that strictly anaerobic bacteria co-dominate in significant numbers within the vaginal ecosystems of postmenopausal women [10/15, group D] [21]. Furthermore, we observed significant differences in the phylum, class, order, and family of Proteobacteria between the groups. Conversely, there were no notable distinctions between the groups regarding the phylum, class, order, and group of Bacteroidetes. Additionally, the phylum, class, order, and group of Fusobacteria did not exhibit significant variations among the groups.

Gliniewicz *et al.* [10]'s study reveals that in Group A [n = 12], the average proportion of L. Crispatus was 0.92 [95% CI: 0.92, 0.92], while in Group B [n = 12], Gardnerella and L. had an average proportion of 0.66 [95% CI: 0.65, 0.66]. In Group C [n = 6], it was 0.73. Groups E and F were predominantly populated by Bifido-bacterium and L., each with n = 2. In Group D, which showed greater diversity, co-existing taxa included Gasseri, Atopobium, Finegoldia, Gardnerella, Prevotella, and Streptococcus. Additionally, **Gliniewicz *et al.*** [10] used principal coordinate analysis to visualize the patterns of different community compositions, highlighting significant differences between

communities dominated by L. Iners or L. Crispatus G. In most postmenopausal women receiving hormone therapy [HT], lactobacilli, specifically L. L., dominated the vaginal communities. L. Crispatus [8/15], L. Iners [1/15], and L. Gasseri [1/15] belonged to Groups A, C, and F, respectively. In the remaining five communities of women receiving HT, either Gardnerella [3/15; Group B] or Bifidobacterium [2/15; Group E] had high concentrations. Only a small percentage of postmenopausal women who did not receive HT had vaginal communities completely dominated by Lactobacillus species [2/15].

All things considered, unmistakably completely anaerobic microorganisms were frequently present [group D; 10/15], while Gardnerella occasionally took the lead [group B; 3/15].

In conclusion, our study included 15 pre-menopausal women, and only eight of them had vaginal ecosystems dominated by Lactobacillus species. Out of those eight, three were L. crispatus, including L. gasseri, and L. dominates one. The dominant bacterial species varied in terms of relative abundance and composition in the vaginal ecosystems of the women in each study group. To confirm the findings, larger samples of women must be included in future research. To strengthen the case for a causal connection between VVA and atrophic vaginitis in menopause, future longitudinal research, intended to control, modify, and restore vaginal microbiota homeostasis, should build on the findings of this study.

Conflict of interest: The authors state that they have no competing interests.

Sources of funding: No specific grant was received for this research from public, private, or nonprofit funding organizations.

Author contribution: All the study's authors contributed equally.

REFERENCES

1. Santoro N, Johnson J. Diagnosing the Onset of Menopause. JAMA. 2019 Aug 27;322[8]: 775-776. doi: 10.1001/jama.2019.6250.
2. Ariadi A, Jamsari J, Yanwirasti Y, Siregar MFG, Yusrawati Y. Correlation between Estrogen Levels with Lipid Profile in Menopause Women in West Sumatera. Open Access Maced J Med Sci. 2019; 7[13]:2084-2087. doi: 10.3889/oamjms.2019.625.
3. Lewis AL, Gilbert NM. Roles of the vagina and the vaginal microbiota in urinary tract infection: evidence from clinical correlations and experimental models. GMS Infect Dis. 2020 Mar 26;8:Doc02. doi: 10.3205/id000046.
4. Joseph RJ, Ser HL, Kuai YH, Tan LT, Arasoo VJT, Letchumanan V, *et al.* Finding a Balance in the Vaginal Microbiome: How Do We Treat and Prevent the Occurrence of Bacterial Vaginosis? Antibiotics [Basel]. 2021 Jun 15;10[6]:719. doi: 10.3390/antibiotics-10060719.
5. Daniel GA, Hu Y, Tsementzi D, Jhaney CI, Hu YJ, Yeager KA, *et al.* Exploring the Vaginal Microbiome and Intravaginal Practices in Postmenopausal Women. Nurs Res. 2021;70[5]: 405-411. doi: 10.1097/NNR.0000000000000538.
6. Erel CT. Current Treatment Modalities for the Genitourinary Syndrome of Menopause. In: Pérez-López FR [ed], Postmenopausal Diseases and Disorders. Springer, Cham. 2019 [pp. 187-203]. doi: 10.1007/978-3-030-13936-0_11.
7. Labrie F, Archer DF, Koltun W, Vachon A, Young D, Frenette L, *et al.*; members of the VVA Prasterone Research Group. Efficacy of intravaginal dehydroepiandrosterone [DHEA] on moderate to severe dyspareunia and vaginal dryness, symptoms of vulvovaginal atrophy, and of the genitourinary syndrome of menopause. Menopause. 2018;25[11]:1339-1353. doi: 10.1097/GME.0000000000001238.
8. Foschi C, Alvisi S, Baldassarre M, Laghi L, Gava G, Mancini I, *et al.* Vaginal metabolites in postmenopausal women with or without vulvo-vaginal atrophy at baseline and after ospemifeme and systemic hormone treatment. Maturitas. 2022;159:7-14. doi: 10.1016/j.maturitas.2021.12.007.
9. Cancelo-Hidalgo MJ, Coello LB. Genito-urinary syndrome of the menopause: vaginal health and microbiota. In: Cano A [ed], Menopause: A Comprehensive Approach. Springer, Cham. 2017:91-107. doi: 10.1007/978-3-319-59318-0_6.
10. Gliniewicz K, Schneider GM, Ridenhour BJ, Williams CJ, Song Y, Farage MA, Miller K, Forney LJ. Comparison of the Vaginal Micro-biomes of Premenopausal and

Postmenopausal Women. Front Microbiol. 2019;10:193. doi: 10.3389/fmicb.2019.00193.

11. Magoč T, Salzberg SL. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics. 2011 Nov;27[21]: 2957-63. doi: 10.1093/bioinformatics/btr507.

12. Wang Q, Garrity GM, Tiedje JM, Cole JR. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol. 2007; 73[16]:5261-7. doi: 10.1128/AEM.00062-07.

13. Liu CM, Aziz M, Kachur S, Hsueh PR, Huang YT, Keim P, Price LB. BactQuant: an enhanced broad-coverage bacterial quantitative real-time PCR assay. BMC Microbiol. 2012 Apr 17;12:56. doi: 10.1186/ 1471-2180-12-56.

14. de Oliveira NS, de Lima ABF, de Brito JCR, Sarmento ACA, Gonçalves AKS, Eleutério J Jr. Postmenopausal Vaginal Microbiome and Microbiota. Front Reprod Health. 2022 Jan 14;3:780931. doi: 10.3389/frph.2021.780931.

15. Brotman RM, Shardell MD, Gajer P, Fadrosh D, Chang K, Silver MI, Viscidi RP, Burke AE, Ravel J, Gravitt PE. Association between the vaginal microbiota, menopause status, and signs of vulvovaginal atrophy. Menopause. 2018;25[11]: 1321-30. doi: 10.1097/GME.0000000000001236.

16. Lewis FMT, Bernstein KT, Aral SO. Vaginal Microbiome and Its Relationship to Behavior, Sexual Health, and Sexually Transmitted Diseases. Obstet Gynecol. 2017 Apr;129[4]:643-654. doi: 10.1097/AOG. 0000000000001932.

17. Younes JA, Lievens E, Hummelen R, van der Westen R, Reid G, Petrova MI. Women and Their Microbes: The Unexpected Friendship. Trends Microbiol. 2018 Jan; 26[1]:16-32. doi: 10.1016/j. tim.2017.07.008.

18. Kim S, Seo H, Rahim MA, Lee S, Kim YS, Song HY. Changes in the Microbiome of Vaginal Fluid after Menopause in Korean Women. J Microbiol Biotechnol. 2021 Nov 28;31[11]:1490-1500. doi: 10.4014/jmb.2106. 06022.

19. Brotman RM, Shardell MD, Gajer P, Fadrosh D, Chang K, Silver MI, *et al.* Association between the vaginal microbiota, menopause status, and signs of vulvovaginal atrophy. Menopause. 2014 May;21 [5]:450-8. doi: 10.1097/GME.0b013e3182a4690b.

20. Wang Y, Zhang Y, Zhang Q, Chen H, Feng Y. Characterization of pelvic and cervical microbiotas from patients with pelvic inflammatory disease. J Med Microbiol. 2018; 67[10]:1519-26. doi: 10.1099/jmm.0.000821.

21. Shen J, Song N, Williams CJ, Brown CJ, Yan Z, Xu C, Forney LJ. Effects of low dose estrogen therapy on the vaginal microbiomes of women with atrophic vaginitis. Sci Rep. 2016 Apr;6: 24380. doi: 10.1038/srep24380.

22. Zhang R, Daroczy K, Xiao B, Yu L, Chen R, Liao Q. Qualitative and semiquantitative analysis of Lactobacillus species in the vaginas of healthy fertile and postmenopausal Chinese women. J Med Microbiol. 2012 May;61[Pt 5]:729-739. doi: 10.1099/jmm.0. 038687-0.

International Journal of Medical Arts

https://ijma.journals.ekb.eg/
Print ISSN: 2636-4174
Online ISSN: 2682-3780