# ANALYSIS OF BODY MASS INDEX AND VAGINAL MICROBIOTA IN REPRODUCTIVE AGE WOMEN

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**KEYWORDS**

Body Mass Index; vaginal microbiota; Lactobacillus; 16S rRNA gene sequencing; reproductive-age women.

**ABSTRACT:**

**Introduction**: The balance of vaginal microbiota plays a crucial role in maintaining women's reproductive health. Changes in Body Mass Index (BMI) can affect the composition of the vaginal microbiota.

**Objectives**: This study aims to analyze the relationship between BMI and vaginal microbiota diversity in healthy reproductive-age women.

**Methods**: This is an analytical observational study with a cross-sectional design conducted at the Obstetrics and Gynaecology Polyclinic of Dr. Wahidin Sudirohusodo General Hospital and its network hospitals in Makassar. The sample consisted of 50 women aged 18-44 with normal and overweight BMI, selected using consecutive sampling. Microbiota profiles were analyzed through 16S rRNA gene sequencing.

**Results**: The results showed that Lactobacillus dominated the vaginal microbiota in both groups, with a higher abundance observed in the overweight-obese group. Additionally, pathogenic genera such as Megasphaera, Dialister, Sneathia, and Veillonella were found more frequently in the overweight-obese group compared to the normal BMI group. Diversity analysis showed higher Shannon and Simpson indices in the overweight-obese group, reflecting greater microbiota diversity.

**Conclusions**: This study demonstrates that although there is an increased abundance of pathogenic bacteria in the overweight-obese group, the presence of abundant Lactobacillus plays a crucial role in maintaining vaginal microbiota balance and inhibiting pathogen growth, thus ensuring that the subjects remain healthy without symptoms of infection.

## 1. Introduction

The functional balance in the composition of vaginal microbiota is crucial for maintaining gynaecological and reproductive health. The vaginal microbiota is predominantly dominated by Lactobacillus species. Disruption of this balance, known as vaginal microbiota dysbiosis, can potentially lead to various conditions, such as infections (bacterial vaginosis, atrophic vaginitis, etc.), miscarriage, preterm birth, infertility, polycystic ovary syndrome (PCOS), and menstrual disorders. [1] The investigation of vaginal ecosystem composition involves collecting samples from smears of the posterior vaginal fornix, ectocervix, and endocervix,[2] using next-generation sequencing (NGS) analysis. This method employs partial primer sequencing targeting highly conserved regions of the bacterial 16S rRNA gene.[3]

To date, more than 120 Lactobacillus species have been identified.[4] Among these, particularly *Lactobacillus crispatus, Lactobacillus iners, Lactobacillus gasseri*, and *Lactobacillus jensenii* are commonly found in the vaginas of women of reproductive age. Under the influence of oestrogen, glycogen accumulates in the vagina and is subsequently degraded by the enzyme α-amylase into

maltose, malt triose, and α-dextrin. Lactobacillus utilizes these glycogen breakdown products to produce lactic acid, acidifying the vaginal environment to a pH range of 3.0–4.5.[5]
A high Body Mass Index (BMI) is associated with an imbalance in vaginal microbiota, it creates an environment favourable to pathogenic bacteria due to disruptions in hormonal, metabolic, and immune systems.[6] High BMI and metabolic syndrome influence insulin resistance, carbohydrate metabolism, and formation, reducing glycogen levels while increasing plasma glucose levels. A decrease in glycogen levels makes *Lactobacillus spp.* Less competitive compared to other bacterial community members. Several pathogenic bacteria, such as *Finegoldia, Corynebacterium, Coriobacteriaceae, Megasphaera, Saccharibacteria, Prevotellaceae,* and *Mobiluncus mulieris,* have been reported to dominantly colonize the vaginal microbiota in women with high BMI and vaginal pH. [7,8,9,10]
The mechanism described above differs from the findings by Lokken et al. (2019), that obesity has a protective effect against vaginal microbiota dysbiosis. Excessive adipose tissue can produce oestrogen, increase oestrogen levels, and enhance glycogen availability in vaginal epithelial cells, which in turn supports the colonization of *Lactobacillus spp.* These findings create a controversy regarding obesity as a risk factor for vaginal microbiota dysbiosis, highlighting the need for further investigation.[11]
Data from the 2018 Basic Health Research (Riskesdas) indicates that the prevalence of overweight adult women aged 18 and above in Indonesia reached 15.1% of the total population.[12]

## 2. Objectives

Considering the significant proportion of overweight adult women globally and in Indonesia, along with the conflicting research findings, this study aims to analyzed further the differences in vaginal microbiota composition between reproductive-age women with normal BMI and those who are overweight in Makassar City, Indonesia.

## 3. Methods

*Study Design and Population*

This study was observational analytical research with a cross-sectional design, involving healthy reproductive-age women aged 18–44 years who were married, with either a normal BMI or overweight-obesity. The inclusion criteria required participants to be aged 18–44 years, married, have a normal BMI (18.5–25.0 kg/m²) or overweight BMI (≥25.1 kg/m²), present with complaints of physiological vaginal discharge, not being menstruating, and being at least 14 days post their menstrual cycle. Participants were also required to be willing to participate in the study and to sign informed consent.
Had a history of bariatric surgery, pathological vaginal discharge indicative of reproductive organ infections, sexually transmitted infections, or systemic diseases such as diabetes mellitus, hypertension, and heart disease, pregnant, breastfeeding, had a history of immunosuppressive or anti-inflammatory treatments, hormonal therapy or hormonal contraceptive use, recent antibiotic use, or those using intrauterine devices (IUDs) were excluded in this study. Additional exclusions were for participants engaged in other studies, those who had engaged in sexual activity within 72 hours prior, and those with a history of vaginal douching.

*Study Procedure*

Samples would be collected from December 2023 to October 2024 from patients visiting the Obstetrics and Gynecology Clinic at the Centre Hospital and its network hospitals in Makassar. Sampling would be conducted using consecutive sampling, and all subjects who arrived consecutively and met the inclusion criteria would be included in this study until the required number of subjects was reached.

*Sample Collection and Deoxyribonucleic Acid (DNA) Extraction*

Sample collection was performed by using a cotton swab from the posterior vaginal fornix, ectocervix, and endocervix under direct observation during a speculum examination by an obstetrics and gynecology resident. The specimens were then placed in transport medium tubes, stored in a larger

bag, and kept at a temperature of 2-8°C, using dry ice within the box for a maximum of two hours, before being sent to the Clinical Microbiology Laboratory at Universitas Hasanuddin Hospital. Afterward, the specimen could be stored at -20°C for up to 14 days after collection. Alternatively, specimens that had been collected could be stored and sent to the laboratory frozen at -20°C, within 180 days after collection.[13]

*DNA Amplification Using Polymerase Chain Reaction (PCR)*

DNA was extracted using an automated DNA extraction machine. A total of 200 µl of the sample was incubated with 200 µl of Chemagen lysis buffer and 10 µl of proteinase K at 56°C while centrifuged at 500 rpm. DNA was then extracted from the solid phase by adding 100 µl of Chemagen elution buffer (DNA elution).[13]

DNA concentration of the samples was measured using a dsDNA assay. PCR amplification was performed on the hypervariable regions of V1/V2, V3/V4, V1-V3, and V3-V5 of the 16S ribosomal Ribonucleic Acid (16S rRNA) gene to achieve better identification of vaginal microbiota. Amplification for each sample utilized a dual-index primer set specifically developed to distinguish low-diversity microbiota in each sample.[13] The universal primer sets used were as follows: for the V1/V2 region, 27F/338R; for the V3/V4 region, 319F/806R; for the V1-V3 region, 27F/515R; and for the V3-V5 region, 341F/926R. [14,15]

The PCR conditions were as follows: initial denaturation for 30 seconds at 98°C, followed by 30 cycles of 10 seconds at 98°C, 15 seconds at 58°C, and 15 seconds at 72°C, with a final extension step of 3 minutes at 72°C. [16]

The DNA amplification products were purified using a gel extraction kit and quantified in nanomolar (nM) concentrations using the formula:

**[nM DNA] = DNA concentration (ng/µl) × $10^6$ (µl/L) / (Sample fragment size in bp × 656.4 (g/mol))**

The concentration was then standardized to 12 nM. To ensure quality, pooled DNA samples with concentrations below 8 nM were excluded from the 16S rRNA gene sequencing analysis. Sequencing of the 16S rRNA gene from the pooled samples was performed using a sequencing machine. The resulting data were processed using QIIME software to remove primers and index sequences. [17,18]

The quality threshold for the Phred score was set at a minimum of five. Paired-end reads without errors in barcode matching, a minimum overlap of six nucleotides, and a combined length of at least 400 nucleotides were merged to generate identifiable sequencing data.[18]

Operational Taxonomic Units (OTUs) were selected using the Search method. Sequences were sorted based on length and the abundance of identical reads, then screened for chimeric sequences and clustered at 97% identity to filter the data. OTUs were aligned to a reference database using the PyNAST method for sequence alignment and further classified from the genus level to the species level. The remaining sequences were analyzed using BLAST (Basic Local Alignment Search Tool) and included only if they could be identified at the genus or species level.[19]

*Data Collection*

The characteristics data of this study, such as age, education level, parity, socioeconomic status, and vaginal pH. Vaginal pH, representing the acidity level of the vagina was measured using samples of vaginal fluid. For the study, the criteria used to define reproductive-age women was those who had entered their fertile period after menarche, with fully functioning reproductive organs, aged between

*Statistical Analysis*

Data analysis was conducted using the Statistical Package for Social Sciences (SPSS) version 19 (IBM, Armonk, NY, USA). Numerical data were presented as means and standard deviations, while categorical variables were displayed as frequencies and percentages.

The chi-square test was used to compare the proportions of vaginal microbiota between healthy reproductive-age women with normal BMI and overweight. A p-value of $< 0.05$ was considered

statistically significant. Multivariate logistic regression analysis was used to assess the role of other sample characteristics that also influenced vaginal microbiota diversity, such as race/ethnicity; socioeconomic status; education level; smoking; alcohol consumption; and others, in analyzing the relationship between the diversity of vaginal microbiota composition influenced by BMI in healthy reproductive-age women. The results of the vaginal microbiota composition analysis were presented in tables and graphs.

*Ethical Approval*

Each procedure was carried out with the consent of the participants or their families through the signing of an informed consent form and declared to meet the ethical requirements for conducting research by the Biomedical Research Ethics Committee on Humans, Faculty of Medicine, Hasanuddin University, with an ethical approval recommendation letter, and the Health Research Ethics Committee (KEPK) of RSPTN UH-RSWS, Number: 745A/UN 4.6.4.5.31 / PP36 / 2A23.

## 4. Results

A total of 50 reproductive-age women completed the study, divided into 25 samples for each group: normal BMI and high BMI. The average age of the normal BMI group was 26.52 ± 2.92 years, while in the high BMI group were 35.65 ± 4.17 years. (Table 1) summarized the characteristics of the study population, divided into two categories: the normal BMI group and the high BMI group.

**Table 1.** Characteristics Data of The Study Subjects.

| Variables | Body Mass Index (BMI Kg/M$^2$) | |
|---|---|---|
| | **Normal (Mean ± SD)/n (%)** | **High (Mean ± SD)/n (%)** |
| **Age** | 26.52 ± 2.92 | 35.65 ± 4.17 |
| **Education** | | |
| Elemantary School | 2 (8%) | 2 (8%) |
| Secondary school | 13 (52%) | 10 (40%) |
| Bachelor's degree | 10 (40%) | 13 (52%) |
| **Parity** | | |
| Nulipara | 9 (36%) | 0 (0%) |
| Primipara | 12 (48%) | 3 (12%) |
| Multipara | 4 (16%) | 22 (88%) |
| **Vagina pH** | 4.10 ± 0.3 | 4.70 ± 0.5 |
| **Economic Status** | | |
| Low | 14 (56%) | 9 (36%) |
| Middle – High | 11 (44%) | 16 (64%) |

(Table 2) classifies the vaginal microbiota profile in women categorized by normal and high BMI. In both groups, *Lactobacillus* was found in all subjects (100%). However, the other bacterial genera exhibited different variations.

**Table 2.** Vaginal Microbiota Profile in Women Based on BMI.

| Bacterial Genus | Body Mass Index (BMI Kg/M$^2$) | |
|---|---|---|
| | **Normal n (%)** | **High n (%)** |
| ***Lactobacillus*** | 25 (100%) | 25 (100%) |
| ***Megasphaera*** | 12 (48%) | 15 (60%) |
| ***Dialister*** | 16 (64%) | 18 (72%) |
| ***Sneathia*** | 7 (28%) | 10 (40%) |
| ***Veillonella*** | 13 (52%) | 19 (76%) |

*Megasphaera* was found in 48% of the normal BMI group, which increased to 60% of the high BMI group. *Dialister* was also more prevalent in the high BMI group (72%) compared to those with normal BMI (64%). *Sneathia* was less prevalent in the normal BMI group (28%) but higher in the high BMI group (40%). Similarly, *Veillonella* was more prevalent in the high BMI group (76%) compared to normal BMI (52%).

(Figure 1) showed the relative abundance of five bacterial genera among the 50 female samples. In this analysis, the five bacterial genera such as *Lactobacillus*, *Megasphaera*, *Dialister*, *Sneathia*, and *Veillonella* were found to have a higher relative abundance in overweight-obese *group* compared to those normal.

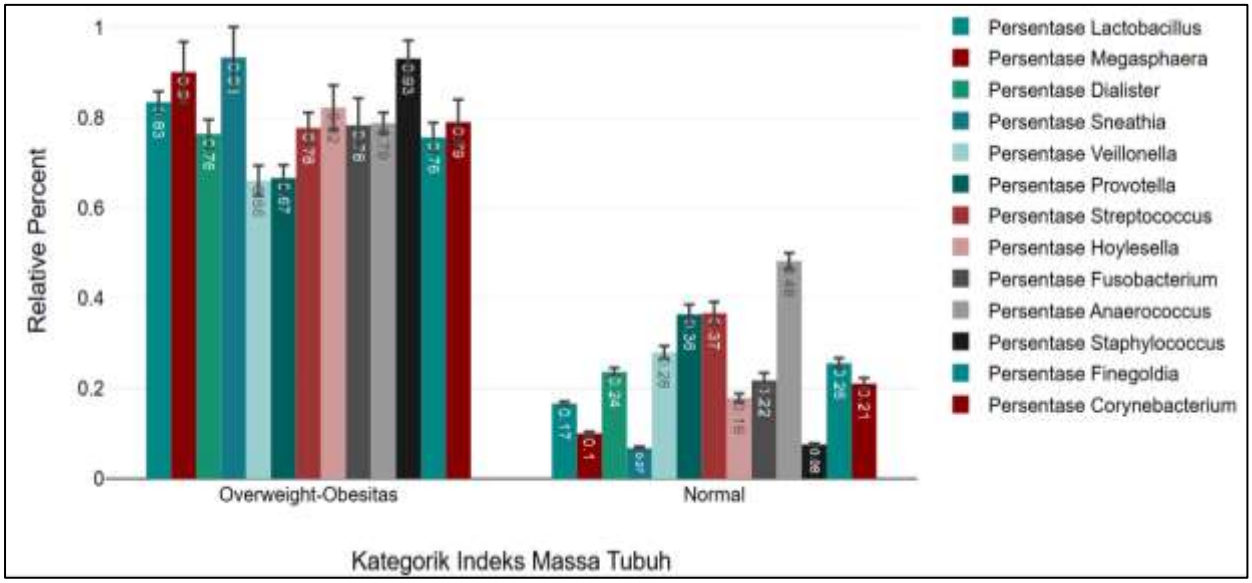


**Figure 1.** Comparison of Microbiota Profiles.

(Table 3) compared the vaginal microbiota profile based on BMI categories. In both groups, *Lactobacillus* had a high relative abundance (0.0067 ± 0.0012 in the normal BMI group and 0.0333 ± 0.0060 in the high BMI group), with no statistically significant difference (p=0.083). This suggested that *Lactobacillus* remained the dominant bacterium in the vaginal microbiota regardless of BMI status, consistent with its role in maintaining vaginal health.

*Megasphaera*, *Dialister*, *Sneathia*, and *Veillonella* showed higher relative abundance in the high BMI group compared to the normal group. Megasphaera had a higher relative abundance (0.0360 ± 0.0172) in the overweight-obese group compared to the normal group (0.0040 ± 0.0010, p= 0.038), showing a significant difference. Similarly, Dialister was higher in the overweight-obese group (0.0305 ± 0.0079) compared to those normal (0.0095 ± 0.0023, p=0.040), which might reflect changes in the vaginal microbiota ecosystem associated with BMI.

The relative abundance of *Sneathia* and *Veillonella* also significantly increased in the high BMI group. *Sneathia*, with a relative abundance of (0.0373 ± 0.0173) compared to (0.0027 ± 0.0098), showed a significant difference (p=0.046). Similarly, *Veillonella* was more abundant in the overweight-obese group (0.0264 ± 0.0852) compared to the normal group (0.0112 ± 0.0036, p=0.035). Both of these genera had a stronger association with increased BMI, which could potentially affect the microbiota balance in women with overweight obesity.

**Table 3.** Analysis of Comparative Vaginal Microbiota Profiles in Women Based on BMI.

| Bacterial Genus | Body Mass Index (BMI Kg/M$^2$) | | p-value |
|---|---|---|---|
| | Normal (Mean ± SD) | High (Mean ± SD) | |
| *Lactobacillus* | 0.0067 ± 0.0012 | 0.0333 ± 0.0060 | 0.083 |
| *Megasphaera* | 0.0040 ± 0.0010 | 0.0360 ± 0.0172 | 0.038 |
| *Dialister* | 0.0095 ± 0.0023 | 0.0305 ± 0.0079 | 0.040 |
| *Sneathia* | 0.0027 ± 0.0098 | 0.0373 ± 0.0173 | 0.046 |
| *Veillonella* | 0.0112 ± 0.0036 | 0.0264 ± 0.0852 | 0.035 |

Description: *Values are n (%) or mean ± SD, unless stated otherwise. Continuous variables were compared using independent-samples t-test, categorical variables with Pearson Chi-square test. Non-normally distributed data were analyzed with Mann-Whitney U test. *p < 0.05*

(Table 4) showed the comparison of alpha diversity in the vaginal microbiota between normal and high group, measured by the Shannon and Simpson diversity indices. The Shannon and Simpson indices measured species diversity and dominance, respectively, were both higher in women with overweight-obesity (Shannon: 1.01 ± 0.62, Simpson: 0.47 ± 0.23) compared to women with normal BMI (Shannon: 0.80 ± 0.60, Simpson: 0.37 ± 0.25) (p= 0.009 and 0.008). These findings suggested the vaginal microbiota diversity was higher in the overweight-obese group, which attributed to changes in the microbiota ecosystem related to BMI status.

**Table 4.** Vaginal Microbiota Alpha Diversity Based on BMI.

| *Alpha Diversity* | Body Mass Index (BMI Kg/M$^2$) | | p-value |
|---|---|---|---|
| | Normal (Mean ± SD) | High (Mean ± SD) | |
| **Shannon diversity index** | 0.80 ± 0.60 | 1.01 ± 0.62 | 0.009 |
| **Simpson's index** | 0.37 ± 0.25 | 0.47 ± 0.23 | 0.008 |

Description: *Values are n (%) or mean ± SD, unless stated otherwise. Continuous variables were compared using independent-samples t-test, categorical variables with Pearson Chi-square test. Non-normally distributed data were analyzed with Mann-Whitney U test. *p < 0.05*

(Table 5) showed the alpha diversity of the vaginal microbiota measured using the Shannon and Simpson diversity indices to compare groups based on socioeconomic status. The average Shannon diversity index value in the low socioeconomic status group was 1.04 ± 0.56, slightly lower than the middle-high socioeconomic status group (1.13 ± 0.47). This difference approached statistical significance with a p-value of 0.078.

For the Simpson's index, the low socioeconomic status group had an average value of 0.58 ± 0.35, while the middle-high socioeconomic status group had a higher average value (0.63 ± 0.28). However, this difference was not statistically significant (p= 0.104). These results suggested a tendency for higher microbiota diversity in the middle-high socioeconomic status group, although the difference was not statistically significant.

**Table 5.** Vaginal Microbiota Alpha Diversity Based on Economic Status.

| *Alpha Diversity* | Economic Status | | p-value |
|---|---|---|---|
| | Low (Mean ± SD) | Middle - High (Mean ± SD) | |
| **Shannon diversity index** | 1.04 ± 0.56 | 1.13 ± 0.47 | 0.078 |
| **Simpson's index** | 0.58 ± 0.35 | 0.63 ± 0.28 | 0.104 |

Description: *Values are n (%) or mean ± SD, unless stated otherwise. Continuous variables were compared using independent-samples t-test, categorical variables with Pearson Chi-square test. Non-normally distributed data were analyzed with Mann-Whitney U test. *p < 0.05*

## 5. Discussion

In this study, *Lactobacillus* was found to dominate the vaginal microbiota, with a prevalence of 100% among all subjects. This is consistent with the findings of Ravel et al. (2011), who analysed the vaginal microbiota in a cohort of 396 healthy non-pregnant women from four ethnic groups in North America. The bacterial communities were grouped into five major types (Community State Types or CSTs), of these five CST types, four types, which accounted for 73% of all subjects, were dominated by different species of *Lactobacillus* (*L. crispatus* in CST I, *L. gasseri* in CST II, *L. iners* in CST III, and *L. jensenii* in CST V). Meanwhile, CST IV was found in 27% of subjects, and exhibited a more diverse community, predominantly consisting of a large proportion of obligate anaerobic bacteria such as *Atopobium*, *Gardnerella*, *Prevotella spp*., and other bacterial species.[20]

The vaginal microbiota profile based on BMI categories showed the relative abundance of five bacterial genera. *Lactobacillus* had a higher relative abundance in the overweight-obese group compared to the normal group, but this difference was not statistically significant (p=0.083). This suggests that although there is a tendency for increased *Lactobacillus* abundance in the overweight-obese group, *Lactobacillus* remains the dominant genus in the vaginal microbiota of women, regardless of their BMI status. This finding contrasts with the results of Vongsa et al. (2019), who reported a significant difference in *Lactobacillus* abundance between the normal BMI and overweight-obese groups (0.40 ± 0.32 vs 0.13 ± 0.24, p=0.00). This indicates that in the overweight-obese group, *Lactobacillus* abundance tends to be significantly lower compared to the normal BMI group. Vongsa et al. also emphasized that although there is a correlation between the vulvar microbiome and BMI, other factors may influence the state of the microbiome, such as ethnicity and diet, which were not controlled in their study. Further research is needed to understand how these factors may alter the vulvar microbiome.[21]

One explanation for the shift in the microbiome is the potential change in the carbon balance provided by the body. *Lactobacillus* is known for its ability to alter the pH of its environment. Under the right conditions and adequate carbohydrate availability for fermentation, *Lactobacillus* produces lactic acid, providing a competitive advantage over other bacteria, such as *Escherichia coli*, which cannot survive in such acidic environments. However, in subjects with high BMI, this condition seems to have changed, which may affect the abundance of Lactobacillus. [22, 23]

Increasing the number of *Lactobacillus* in the high BMI women in this study is consistent with the findings by Lokken et al. (2019), which outline several biological effects that could explain this relationship. Women with obesity have higher oestrogen levels due to adipose tissue, which subsequently increases glycogen content in the vaginal epithelial cells. Increasing glycogen supports the colonization of *Lactobacillus* and lactic acid production, which can create a more optimal vaginal environment.[11]

*Megasphaera* showed significant variation in abundance between the normal and high BMI groups (48% vs. 60%), indicating that BMI may influence the presence of *Megasphaera* in the vaginal microbiota. Based on relative abundance analysis, *Megasphaera* ranked second after *Lactobacillus*, with a significantly higher relative abundance in the overweight-obese group and a relatively even distribution, although not as large as *Lactobacillus*. These results suggest that an increase in BMI affects the composition of the vaginal microbiota, particularly in increasing the abundance of *Megasphaera*, associated with changes in the vaginal environment in the overweight-obese group.

The findings above align with the results of Ventolini et al. (2017), who suggested that obesity, as a condition of low-grade inflammation, triggers an increase in the production of pro-inflammatory cytokines such as Tumour Necrosis Factor alfa (TNF-α), Interleukin 1 Betha (IL-1β), and IL-6, which disrupt the immune system and increase vulnerability to recurrent vulvovaginal bacterial infections (RVVBI). In Ventolini et al.'s study, women with obesity had an average BMI of 35 ± 4 kg/m², higher than the control group (26 ± 3 kg/m²), with a significant p-value of <0.001. Multivariate logistic regression analysis showed that higher BMI was significantly associated with the risk of RVVBI (OR

4.00, 95% CI 3.1 to 4.52, p=0.001).[24] Although the exact mechanisms behind the increased risk of infection are not fully understood, changes in the composition and immune function of the vaginal microbiota are suspected to be a key factor. In obese women, *Megasphaera* types I and II were found more frequently, along with elevated levels of IL-1rα, IL-6, IL-12, and IL-17, indicating immunological changes in the vaginal environment.[25]

The relative abundance of *Dialister* increased in the high BMI group, reaching statistical significance ($p = 0.040$). This reflects changes in the vaginal microbiota ecosystem associated with BMI status. These findings are consistent with the results of Raglan et al. (2021), who reported that the vaginal microbiota of obese women is characterized by a lower prevalence of *Lactobacillus*-dominant vaginal microbiome (VMB) and higher bacterial diversity, particularly a reduction in *Lactobacillus spp.* and *Gardnerella spp.*, compared to non-obese women ($p < 0.001$). In their study, obese women showed a higher relative abundance of *Dialister spp.* ($p < 0.001$). Additionally, Raglan et al. noted increased levels of pro-inflammatory cytokines, including IL-1β and IL-8, which were correlated with the diversity of vaginal microbiota species.[26]

According to Balle et al. (2018), vaginal microbiota was dominated by various anaerobic bacteria such as *G. vaginalis*, *Porphyromonas somerae*, *Corynebacterium urealyticum*, *Dialister spp.*, *Megasphaera*, *A. vaginae*, and *Prevotella disiens* are at higher risk of being infected by bacterial sexually transmitted infection (STI) agents such as *Chlamydia trachomatis* (*CT*), compared to women whose vaginal microbiota is dominated by *L. crispatus*.[27]

The relative abundance of *Sneathia* was higher in the high BMI women (p=0.046). This result is consistent with the findings of Si et al. (2017), who reported that obesity negatively impacts vaginal microbiota, leading to an increase in microbial community diversity, as well as the abundance of *Prevotella* and *Sneathia*. This study found that diet-altered vaginal microbiota triggers systemic inflammation through the release of lipopolysaccharides (LPS) from Gram-negative pathogenic bacteria.[28]

Gajer et al. (2012) stated that *Sneathia* falls under the classification of CST IV, alongside *Atopobium*, *Prevotella*, *Parvimonas*, *Gardnerella*, and *Mobiluncus*. The dominance of these anaerobic bacteria is associated with local inflammation and an increased risk of reproductive tract infections.[29] This highlights the potential role of *Sneathia* in contributing to a less favourable vaginal ecosystem, which elevates susceptibility to infections.

In this study, *Veillonella* was one of the five most abundant bacterial genera found among the 50 female samples. The abundance of *Veillonella* was higher in the overweight-obese group (p=0.035). This suggests that *Veillonella* may be more prevalent in women with higher BMI, highlighting a potential association between increasing BMI and the presence of *Veillonella* in the vaginal microbiota. This genus is typically associated with anaerobic environments, and its increased abundance in women with overweight obesity could be linked to the ecological shifts occurring in the vaginal microbiome of this population.

There has been no direct study investigating the difference in *Veillonella* abundance as vaginal microbiota based on BMI, although some studies have shown that *Veillonella* is involved in changes in microbiota composition related to certain health conditions, such as bacterial vaginosis (BV). The study by Salliss et al. (2021) provides important insight into the role of *Veillonella* in changes in the cervicovaginal microbiota. In this study, BV was identified as a shift from *Lactobacillus spp.* Dominance to a polymicrobial biofilm involving anaerobic bacteria, including several *Veillonella* species.[30]

The study assessed the immunometabolism contribution of three members of the *Veillonellaceae* family: *Veillonella atypica*; *Veillonella montpellierensis*; and *Megasphaera micronuciformis*, found that infection by *Veillonella* spp. Significantly increased polyamines and inflammatory mediators. *Veillonella* spp. were also found to consume lactate, a key metabolite associated with the occurrence of BV.[30] These findings suggest that infection by *Veillonella* spp. Impact the balance of the microbiota

and increase inflammation, which may be related to changes in vaginal microbiota in women with high BMI. However, these findings need to be expanded upon with further studies.

This study also identified the alpha diversity of vaginal microbiota, assessed using the Shannon diversity index and Simpson's index, and showed a significant difference between the normal BMI group and the high BMI group (p=0.009 and 0.008). These findings are consistent with those of Vongsa et al. (2019), who observed differences in the alpha diversity of vulvar bacterial communities based on BMI categories. Vongsa et al. reported that the group with higher BMI had higher Shannon and Simpson index values compared to the average BMI group. Their study showed the following: Shannon index (mean: 3.57 ± 0.95 for average BMI and 4.57 ± 0.91 for high BMI, p=0.00010) and Simpson's index (mean: 0.78 ± 0.15 for average BMI and 0.89 ± 0.90 for high BMI, p=0.00003).[21]

Allen et al. (2022) reported that women with overweight obesity had higher alpha diversity of vaginal microbiota compared to women with normal weight. This alpha diversity was measured using the Shannon index (p=0.025) and the Inverse Simpson index (p=0.026), as well as the Chao1 index, which showed a similar pattern, highlighting the differences in species richness that contributed to the variation in alpha diversity between the groups.[7]

Pramanick et al. (2022) reported that alpha diversity, measured using Pielou's evenness, showed significantly higher microbiota diversity in bacterial vaginosis (BV) compared to normal microbiota (p=0.0165).[31]

This study suggested that individuals with a middle-high socioeconomic status tend to have higher vaginal microbiota diversity compared to those with a low socioeconomic status, as measured by both Shannon and Simpson diversity indices. Although the differences were not statistically significant (p-values= 0.078 and 0.104).

This trend is consistent with several other studies. Jansaker et al. (2022) showed that women with low family income had a higher risk of infections, which affected the vaginal microbiota.[32] Socioeconomic status influences vaginal microbiota diversity through various mechanisms. Individuals with higher socioeconomic status generally have better access to nutritious foods, such as Fiber and prebiotics, which support the growth of a healthy and diverse microbiota. Furthermore, they also have easier access to quality hygiene products and healthcare services. In contrast, individuals with low socioeconomic status often face chronic stress, which affects the immune system and creates an inflammatory environment in the vagina, thus reducing microbiota diversity. Environmental factors such as sanitation; water quality; and air quality; which are typically better in higher socioeconomic status groups also contribute to a more diverse microbiota condition. [32, 33]

It was found that women with overweight obese BMI had a higher abundance of *Lactobacillus*. *Lactobacillus* is known to play a protective role for vaginal health, acting as the primary defense against pathogens in the vagina at optimal levels. Although there was an increase in four other bacterial genera: *Megasphaera*; *Dialister*; *Sneathia*; and *Veillonella*, known as opportunistic pathogens in women, the abundance of *Lactobacillus* appears to balance the presence of these pathogenic bacteria. This suggests that *Lactobacillus* plays an important role in suppressing pathogen growth through competitive mechanisms and the production of protective metabolites. Therefore, even though women have a higher abundance of pathogenic bacteria, this condition remains under control and remain healthy without showing signs of infection.

## 6. Conclusion

This study demonstrates that although there is an increased abundance of pathogenic bacteria in the overweight-obese group, the presence of abundant *Lactobacillus* plays a crucial role in maintaining vaginal microbiota balance and inhibiting pathogen growth, thus ensuring that the subjects remain healthy without symptoms of infection.

A more in-depth study of other factors, such as diet patterns; lifestyle; and hormones, are also necessary to understand how these factors interact with BMI in influencing the vaginal microbiota. Furthermore,

it is important to expand the study population by including women from diverse ethnic backgrounds and other health conditions that may affect the composition of the vaginal microbiota, to ensure the generalizability of the findings.

## 7. Refrences

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