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VAGINAL MICROBIOTA COMPOSITION PROFILES IN WOMWN AT DIFFERENT LIFE STAGES

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Abstract

The vaginal microbiome is a reproductive organ-specific association that harbors a unique collection of anaerobic and aerobic microorganisms. It plays a crucial role in woman`s reproductive well-being and prevention of urogenital diseases including bacterial vaginosis (BV), infections with opportunistic microorganisms, yeast infections, urinary tract infections and sexually transmitted diseases.

The composition of the vaginal microbiota undergoes significant changes during different stages of women's life cycle and is influenced by variations in sex hormone levels, physiological factors (e.g. the menstrual cycle and pregnancy) and individual lifestyle choices. Evidence shows that these various factors can influence the vaginal microbiome, potentially leading to an imbalance in the microbial community and genital infections.

The aim of the present study was to compare the composition of vaginal microbiota in women at different life stages. A total of 2032 vaginal and cervical swab samples from women of different age, submitted for routine testing at the Institute for Microbiology and parasitology were analyzed. Vaginal microbiota was evaluated by means of quantitative PCR in real time (Femoflor Screen, DNA-Technology).

The results showed a dominance of lactobacilli in majority of the samples from the first two groups young women and women in reproductive years (72% and 69%, respectively), whilst a decrease of normocenosis was noted in women over 45 years (47%).

Moderate dysbiosis was detected in 18% of both 15-25 yrs and 25-45 yrs age groups compared to 21% of the women of the > 45 yrs group. Severe dysbiosis prevailed among women of the >45 yrs group (32%) compared to 13% and 10% in the 15-25 yrs and 25-45 yrs age groups, accordingly.

The results from this study provide a comprehensive picture of our current knowledge of the composition and abundance of the microbiota of the female reproductive tract during different life phases.

The tremendous importance of the microbiome for the reproductive health imply the necessity of future studies focused on providing more detailed information its composition and susceptibility to external influences.

Keywords: vaginal microbiota, reproductive health, Lactobacillus.

Introduction

The vaginal microbiome is a reproductive organ-specific association that harbors a unique collection of anaerobic and aerobic microorganisms. These “vaginal residents” live in harmonious and balanced relationship with its human host. The host provides a suitable habitat and nutrients for the microbes, whereas the resident microbiota produces antimicrobial and anti-inflammatory factors, providing protection against pathogens and maintenance of the vaginal homeostasis [1].

Considering previously mentioned, vaginal microbiota plays a crucial role in woman`s reproductive wellbeing and prevention of urogenital diseases including bacterial vaginosis (BV), infections with opportunistic microorganisms, yeast infections, urinary tract infections and sexually transmitted diseases [2].

The vaginal microbiome (VMB) has complex composition of variety of microbes, among which bacteria are most prevailing. In the majority of healthy reproductive age women, the vaginal microbiota is dominated by different types of *Lactobacillus* spp., including *L.crispatus, L. gasseri, L. iners* and *L. jenseni;* although these communities can vary significantly between individuals and over time. In addition of lactobacilli, many other microorganisms are part of vaginal microbiota, such as obligate anaerobes (*Gardnerella* spp., *Prevotella* spp., *Mobiluncus* spp, *Bacteroides* spp., *Veilonella* spp., *Porphyromonas* spp., *Fannyhessea vaginae* and other), facultative anaerobes (*Enterobacterales*, *Staphylococcus* spp, *Streptococcus* spp), *Ureaplasma* spp., *Mycoplasma* spp., *Candida* spp. and many others [3-5].

Lactobacillus spp. has crucial role in maintaining homeostasis in the vaginal environment and preventing the establishment or excessive growth of other microorganisms that may become potentially harmful for the host [6].

By production of lactic acid, utilization of glycogen and competition for resources with potential pathogens, lactobacilli provide acidic vaginal microenvironment and stability of the vaginal microbiota. Additionally, lactobacilli produce various antimicrobial compounds, i.e hydrogen peroxide and bacteriocins with well known microbiocidal and microbiostatic effect [7 -9].

Taking into account their multiple roles, the proportion of lactobacilli is considered an important indicator for the status of the vaginal microbiocenosis [10].

It is important to note that the vaginal microbiota is not constant ecosystem, but rather a dynamic one, influenced by various factors which generally can be classified in two groups: non-modifiable and modifiable factors.

For instance, age, ethnicity, genetics are non-modifiable, whereas the use of antibiotics, stress, smoking, diet, hygiene habits, sexual activity, contraceptives and many other external factors are defined as modifiable. Nevertheless, evidence shows that these various factors can influence the vaginal microbiome, potentially leading to an imbalance in the microbial community and genital infections [11].

Variations of vaginal microbiome in different age periods

The composition of the vaginal microbiota undergoes significant changes during different age periods and is influenced by change in sex hormone levels, physiological factors (e.g. the menstrual cycle and pregnancy), and individual lifestyle choices.

From the earliest developmental stages, reproductive organs of the newborn are influenced by the maternal transplacental estrogen, leading to rich glycogen supply in the vaginal epithelium and lower vaginal pH. Another important event at this life stage is the type of birthing method. Infants born by vaginal delivery are exposed to microorganisms from mother's birth canal, resulting in microbiome similar to the one of the mother`s vagina, while according to data from several studies, cesarean section newborns obtain microbial population comparable to their mother`s skin (*Staphylococcus* spp., *Corynebacterium* spp., *Proprionibacterium* spp.) [12].

With estrogens biotransformation, vaginal glycogen levels decrease promoting neutralization and/or alkalization of the vaginal microenvironment. These changes modulate the vaginal microbiome composition and at prepubertal stage VMB comprises mainly of diphteroids, *Staphylococcus epidermidis*, *Enterococcus* spp., *Escherichia coli*, as well as of anaerobic microbes such as *Peptococcus* spp., *Prevotella* spp., *Porphyromonas* spp. and others [12,13].

By reaching puberty, maturation of adrenal glands and gonads occurs, with accordant rise in estrogen levels, resulting with hyperplasia of the vaginal epithelium and enrichment in the vaginal glycogen content. The outcome is establishment of *Lactobacillus* spp. dominated microbiome and this state is sustained during the reproductive period [14].

Of course, inter-individual differences in the composition, as well as changes during the menstrual cycle exist [15].

Even though it is considered that the greater part of healthy women in reproductive period have lactobacilli dominant flora, some have high diversity flora with low abundance of lactobacilli, which is predicting factor for development of dysbiosis [16].

The decline in circulating estrogen during menopause, leads to diminishment of glycogen supplies, hence, loss of the Lactobacilli dominant vaginal microbiome and rise in vaginal pH. At this point, the microbiome shows high diversity and is dominated by anaerobes, whereas the lactobacillus portion of the flora is very low. During and after menopause these changes can be reversed by using hormone replacement therapy [17,18].

Aim

The aim of the present study was to compare the composition of vaginal microbiota in women at different life stages.

Materials and methods

The study included 2,032 vaginal and cervical swab (dacron swabs from different manufacturers) samples from women at different ages, collected from April to October 2023, submitted for routine laboratory testing at the Institute of Microbiology and Parasitology, Faculty of Medicine, University Ss Cyril and Methodius – Skopje, Republic of North Macedonia.

The vaginal specimen was used for assessment of vaginal microbiota (*Lactobacillus* spp., obligate anaerobes typical for women`s urogenital tract - *Gardnerella vaginalis, Prevotella bivia, Porphyromonas* spp., mycoplasmas - *Mycoplasma hominis*, *Ureaplasma* spp., fungi of the genus *Candida* spp.) and detection of *Trichomonas vaginalis, Cytomegalovirus*, *Herpes simplex virus*-1 and *Herpes simplex virus*-2, while the cervical swab specimen was used for the detection of *Ureaplasma* spp., *Mycoplasma hominis*, *Mycoplasma genitalium*, *Chlamydia trachomatis* and *Neisseria gonorrhoeae.*

Swab samples were collected by experienced gynecologist and transported to the molecular microbiology laboratory within two hours of collection. In the sample 2000 μL of physiological saline solution was added, the sample was vortexed and then 1500 μL transferred to the 1.5 mL plastic tubes for further manipulation.

Automated DNA extraction from vaginal and cervical samples was performed on abGenix automated DNA & RNA extraction system, using abGenix Bacterial DNA Extraction Kit III**.** The presence of the target genes of the above-mentioned microorganisms was detected by multiplex real-time polymerase chain reaction (PCR) with fluorescent-labeled probes.

The amplification of the targets was made using the Femoflor® Screen REAL- TIME PCR Detection Kit. For the detection of previously listed microorganisms PCR reactions were conducted in DTPrime thermocycler (DNA-Technology, Russia).

The cycling protocol (provided by the manufacturer) contains the steps of the procedure, starting with initial denaturation at 94 °C for 30 seconds, followed by two-step PCR program: denaturation at 94°C for 10 seconds and annilation/extension at 64°C for 15 seconds for 45 reps. [19].

Obtained results were further analyzed following manufacturer instructions [19, 20].

Results

Study participants were classified in three groups according to their age: first group: 15 to 25 years old (young age); second group: 25 to 45 years old (reproductive age); third group: older than 45 years (women approaching menopause) [21]. 1965 samples were derived from private medical offices and 67 were from hospitalized patients from the University Clinic for gynecology and obstetrics in Skopje. Using the Femoflor Screen Real-time PCR method the following results were obtained (Table 1).

In the first group, that included women from 15 to 25 years, from 290 samples normocenosis was detected in 209 samples (72%), moderate dysbiosis was detected in 53 samples (18%) and severe dysbiosis was detected in 28 samples (10%).

In the second age group, women from 26-45 years old, from 1462 samples, normocenosis was detected in 1009 samples (69%), moderate dysbiosis was detected in 262 samples (18%) and severe dysbiosis was detected in 191 samples (13%).

In the third group, women older than 45 years, from 280 samples, in 131 samples (47%) was detected normocenosis, in 90 samples (32%) moderate dysbiosis and in 59 samples (21%) severe dysbiosis (Fig.1).

Table 1. Summary of collected data, presenting the results of vaginal microbiota state in the examined samples.

	State of vaginal microbiome			Total number of samples
Age group	**Normocenosis**	**Moderate dysbiosis**	**Severe dysbiosis**	
15-25 years	209 (72%)	53 (18%)	28 (10%)	290 (100%)
26-45 years	1009 (69%)	262 (18%)	191 (13%)	1462 (100%)
> 45 years	131 (47%)	90 (32%)	59 (21%)	280 (100%)
				2032

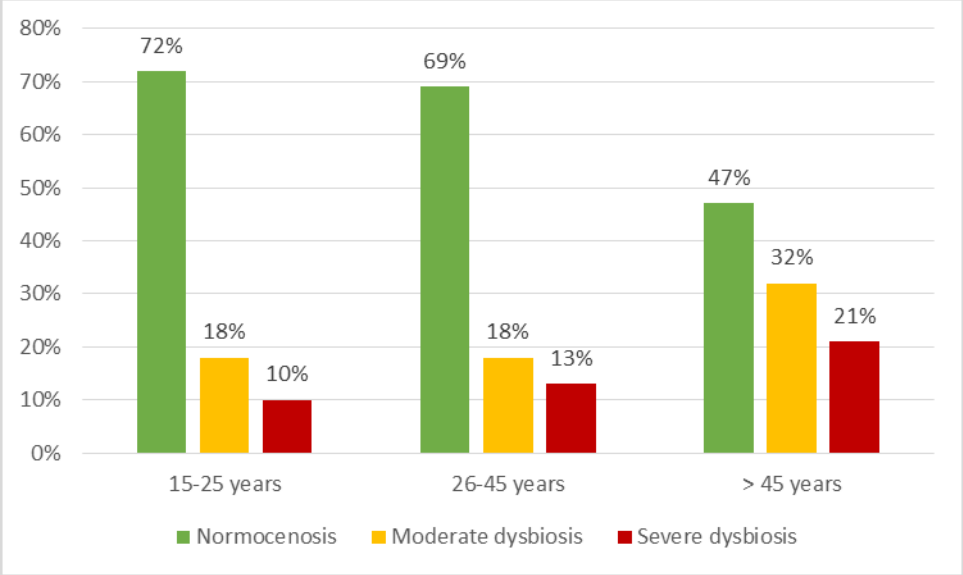

Fig.1. State of vaginal microbiota at different age periods

Discussion

From total of 2032 samples, 290 were from women at the age 15 to 25 years, 1462 samples from women at the age 25 to 45 years and 280 from women older than 45 years, in other words the biggest portion of examined samples belonged to women in reproductive period.

In the first age group, women from 15-25 years, normocenosis was detected in 72% of the samples, moderate dysbiosis in 18% and severe dysbiosis in 10%. In the second age group, women from 25-45 years, normocenosis was detected in 69%, moderate dysbiosis in 18% and severe dysbiosis in 13% of the samples. In the third age group, women older than 45 years, normocenosis was detected in 47 %, moderate dysbiosis in 21% and severe dysbiosis in 32% of the samples.

The results showed a dominance of lactobacilli in majority of the samples from the first two groups young women and women in reproductive years (72% and 69%, respectively), whilst a decrease of normocenosis was noted in women over 45 years (47%). Moderate dysbiosis was detected in 18% of both 15-25 yrs and 25-45 yrs age groups compared to 21% of the women of the > 45 yrs group. Severe dysbiosis prevailed among women of the >45 yrs group (32%) compared to 13% and 10% in the 15-25 yrs and 25-45 yrs age groups, accordingly (Fig.2).

The results from this study provide comprehensive picture of our current knowledge of the composition and abundance of the microbiota of the female reproductive tract during different life phases. In young women and women in reproductive period, higher circulating levels of estrogen lead to lactobacillus dominant flora.

In their study, Wessels et al., 2018 have described the changes of sex hormones during puberty, that lead to thicker vaginal epithelium with more glycogen storage, as an adequate source of nutrients for the growth and proliferation of vaginal microorganisms [22].

In 2011, Ravel et al. conducted a cross-sectional study that characterized the vaginal microbiome of reproductive-age women in the United States using molecular sequencing method.

The study has shown that most vaginal communities (73%) were dominated by one or more species of Lactobacillus spp. It was then when the CSTs (community state types) classification was introduced, categorizing the vaginal microbial communities in five groups: CST-I, CST-II, CST-III, CST-IV, CST-V [16]. In women approaching menopause, the decreasing of circulating estrogens and the lower glycogen storage in the vaginal epithelium leads to shift in the vaginal microbiome towards composition with high diversity and low lactobacilli portion, stated in several studies on that subject (Gliniewicz K. et al, 2019; Heinemann and Reid, 2005; Mirmonsef et al., 2015) [17, 18, 23].

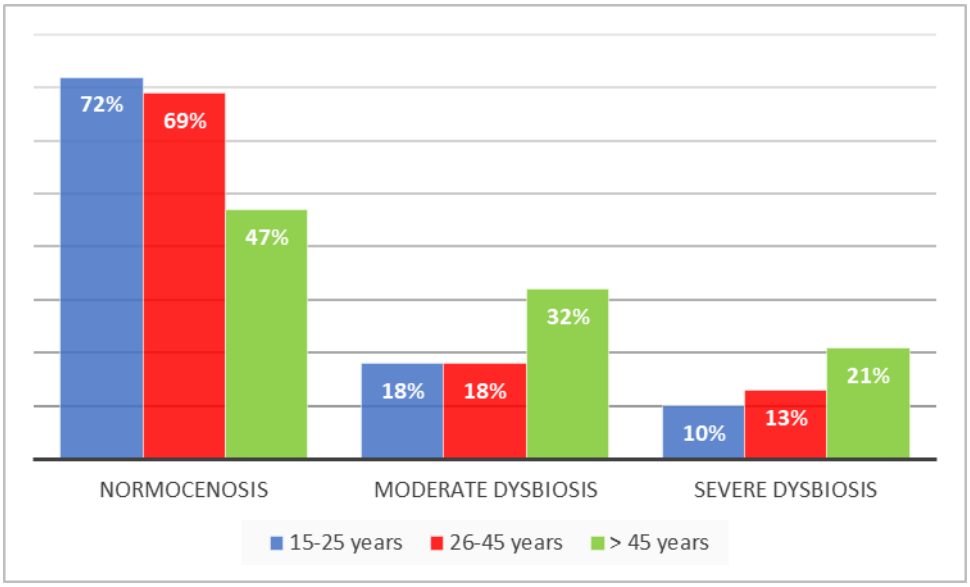

Fig. 2. Comparison of the states of vaginal microbiome at different life stages

Conclusion

Emerging molecular methodologies offer a more profound assessment of the dynamics of the vaginal microbiota at different stages of a woman's life. The tremendous importance of the microbiome for the reproductive health implies a necessity of future studies focused on providing more detailed information its composition and susceptibility to external influences.

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