

# NORMAL VAGINAL MICROBIOTA: PATIENT'S SUBJECTIVE EVALUATION, PHYSICAL EXAMINATION AND LABORATORY TESTS

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Criteria of normality for the vaginal microbiota of healthy women are still a subject of discussion. A decision to assign a study participant to a group of healthy individuals is quite subjective if based on the absence of complaints and physical examination only, which renders study results ambiguous. Below we compare occurrence of the normal vaginal flora and vaginal dysbiosis in women divided into 3 groups according to the examination type (patient's subjective evaluation of her condition, physical examination, and laboratory tests). We examined 234 women of reproductive age from Yekaterinburg (mean age was  $30.3 \pm 6.6$  years). Microbiota composition and lactobacillus diversity (*L. crispatus*, *L. iners*, *L. jensenii*, *L. gasseri*, *L. johnsonii*, *L. vaginalis*) were evaluated by real-time polymerase chain reaction using the Femoflor assay and reagent kits by DNA-Technology, Russia. One in 5 women of reproductive age who had no health complaints was found to have dysbiosis. The normal microbiota of those women was dominated mostly by *L. iners*, while dominant *L. crispatus* were observed in every third participant. Prevailing *L. crispatus* were also found in the normal microbiota of 46.2 % of women who were considered healthy based on the doctor's examination and laboratory tests. Thus, clinical evaluation of the female lower reproductive tract can be compromised by doctor's subjectivity if not supported by laboratory tests and may overlook vaginal dysbiosis in the patient.

**Keywords:** vaginal microbiota, vaginal lactobacilli, *Lactobacillus iners*, *Lactobacillus gasseri*, *Lactobacillus crispatus*, normal vaginal flora, dysbiosis

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## НОРМАЛЬНОЕ СОСТОЯНИЕ МИКРОБИОЦЕНОЗА ВЛАГАЛИЩА: ОЦЕНКА С СУБЪЕКТИВНОЙ, ЭКСПЕРТНОЙ И ЛАБОРАТОРНОЙ ТОЧЕК ЗРЕНИЯ

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Критерии нормы для микробиоценоза влагалища здоровой женщины остаются предметом для дальнейшего изучения. Субъективный характер формирования группы «здоровых женщин» при проведении исследований не позволяет считать получаемые результаты однозначными. В данной работе мы сравнивали частоту встречаемости нормоценозов различных типов и дисбиоза влагалища у женщин, поделенных на группы в зависимости от характера их обследования (мнение женщины, осмотр врача, микроскопическое исследование). Были обследованы 234 женщины репродуктивного возраста из г. Екатеринбурга (средний возраст —  $30,3 \pm 6,6$  лет). Оценку микробиоценоза и определение видового состава лактобацилл (*L. crispatus*, *L. iners*, *L. jensenii*, *L. gasseri*, *L. johnsonii*, *L. vaginalis*) провели методом полимеразной цепной реакции с детекцией результатов в режиме «реального времени» с использованием тест-системы «Фемофлор» («НПО ДНК-Технология», Россия) и наборов реагентов для научного применения той же компании-производителя. У каждой пятой женщины репродуктивного возраста, считающей себя здоровой, был выявлен дисбиоз. Нормоценоз в этой группе чаще всего характеризовался преобладанием *L. iners*, а вариант нормоценоза с преобладанием *L. crispatus* определяли только у каждой третьей женщины. В то же время вариант нормоценоза с преобладанием *L. crispatus* был обнаружен у 46,2 % женщин, которые были отнесены к группе клинически здоровых на основании опроса, осмотра врача и микроскопического исследования. Это говорит о том, что клиническая оценка состояния нижних отделов гениталий без учета данных микроскопии носит субъективный характер и в ряде случаев не позволяет выявить у пациентки дисбиоз влагалища.

**Ключевые слова:** микробиоценоз влагалища, вагинальные лактобациллы, *Lactobacillus iners*, *Lactobacillus gasseri*, *Lactobacillus crispatus*, нормоценоз, дисбиоз

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Criteria have not been ultimately defined yet for a normal vaginal microbiota of a healthy woman. A microbiota of an individual woman beneficial for her reproductive health may not be a close match to the population norm which is largely determined by a study design, since it describes the frequency of vaginal flora variations in women recruited according to certain inclusion criteria, such as ethnicity, area of residence, age, job, etc.

Extensive data have been collected under the Human Microbiome Project about the vaginal flora of women representing different ethnic groups [1–6]. The project employed whole-genome sequencing enabling identification of all microorganisms that constitute the vaginal microbiome. Some authors use a classification of vaginal microbiota types based on the proportion of dominant bacteria; if lactic bacteria are the most abundant in the community, which is an indicator of vaginal health, then microbiotas are classified according to the dominant lactobacilli species [6]. Consequently, researchers distinguish between 5 major types of microbial communities inhabiting the female reproductive tract [6]:

1. type I — normal flora dominated by *Lactobacillus crispatus*,
2. type II — normal flora dominated by *L. gasseri*,
3. type III — normal flora dominated by *L. iners*,
4. type IV — dysbiotic flora dominated by obligate anaerobes,
5. type V — normal flora dominated by *L. jensenii*.

Although all types of microbial communities listed above were identified in women of all ethnic groups, their prevalence varied depending on the ethnicity. For example, type IV (dysbiosis) was observed in 40.6 % of black and 38.1 % of Hispanic women who were shown to be at an increased risk of miscarriage associated with bacterial vaginosis (BV). At the same time, type I was more common for Caucasian participants (45.4 %). The dysbiotic microbiota was observed only in 10.3 % of white women. Correlations were established between the normal microbiota dominated by *L. crispatus* and a lower vaginal pH [6]. Strong protective effects of *L. crispatus* were associated with high colonization capacity and increased production of hydrogen peroxide and lactic acid [6–8]. To our knowledge, no similar studies have been carried out in the Russian population so far.

In most cases, descriptions of the vaginal microbiome are based on the data obtained from asymptomatic women, i.e. those who consider themselves healthy. However, complaints or the lack of thereof are always subjective, because a patient does not have a reference value to compare her condition to; besides, the idea of normality varies culturally and socially. Visual assessment of the vaginal mucosa and vaginal secretions by the doctor is subjective. Therefore, criteria for normality are incomplete without lab tests. It is unclear how much the “normal” vaginal microbiota varies across studies depending on the criteria applied.

*Femoflor*, a real-time polymerase chain reaction-based (PCR) assay, was introduced into clinical practice in 2008. About the same time, criteria were proposed for the assessment of the status of the vaginal flora [9]. Genotyping of vaginal lactobacilli entailed the need to revise previously used criteria for normality.

This study aimed to describe some compositional aspects of the vaginal microbiota, including the diversity of lactobacilli, in reproductive-age women from Yekaterinburg who considered themselves healthy, based on the criteria applied to the norm group.

## METHODS

The study recruited 234 women aged 18 to 45 (mean age was  $30.3 \pm 6.6$  years) who presented at the Medical Center *Harmony* (Yekaterinburg) for a gynecology check-up over the period from 2011 to 2015. All women considered themselves healthy and had no complaints indicative of vaginal inflammation. Exclusion criteria were: sexually transmitted obligate pathogens, HIV, HBV or HCV, and systemic or local antibiotic therapy in the preceding 4 weeks.

All women were examined once. Vaginal microbiota samples were analyzed using *Femoflor-16* real-time PCR-based assay (R&D DNA-Technology, Russia). Six species of *Lactobacilli* were quantified: *L. crispatus*, *L. iners*, *L. jensenii*, *L. gasseri*, *L. johnsonii*, and *L. vaginalis*, by real-time PCR, but for this purpose we used a reagent kit for scientific research by the same vendor.

Microscopy of the vaginal swab was performed using Romanovsky-Giemsa staining. The status of the vaginal microbiota was assessed based on the criteria proposed by Kira E. F. in 2001 [10].

The participants were questioned about their vaginal health. Vaginal examination with mirrors was conducted in all women to assess the status of the vaginal mucosa and quality of vaginal discharge.

Statistical analysis was performed using WinPepi. Two-tailed Fisher test was performed to estimate differences in the prevalence of different microbial communities in the participants.

## RESULTS

All study participants (Main group) were divided into two subgroups. Subgroup 1 included 125 women who had no complaints and no signs of vaginal inflammation detected during visual examination by the gynecologist. Subgroup 1 consisted of 52 women from Subgroup 1 who had no complaints, no visual signs of vaginal pathology and no pathogenic shifts in the microbiota revealed by microscopy [10].

Depending on the proportion of lactobacilli that normally dominate the healthy microbiota and the proportion of opportunistic microorganisms (OMs), we identified 5 types of microbial communities:

1. normal flora, type I — proportion of *Lactobacillus spp.* > 80 %, dominated by *L. Crispatus*,
2. normal flora, type II — proportion of *Lactobacillus spp.* > 80 %, dominated by *L.gasseri* prevailing,
3. normal flora, type III — proportion of *Lactobacillus spp.* > 80 %, dominated by *L. Iners*,
4. normal flora, type IV — proportion of *Lactobacillus spp.* > 80 %, dominated by *L. jensenii/L. vaginalis*,
5. dysbiosis — proportion of *Lactobacillus spp.* < 80 %, proportion of OMs > 20 %.

Prevalence of different types of normal flora and dysbiosis is shown in Figure.

Dysbiosis was detected in 19.7 % of all examined women (Main group); the vaginal microbiota of 80 % of the participants was normal. Type III of the vaginal flora was the most common type observed in 38.9 % of the participants (Figure). The second prevailing type was type I (dominated by *L. crispatus*); it was found in 30.8 % of women who considered themselves healthy.

No significant differences were revealed in the microbial composition of the vaginal microbiota between subgroup 1

(women found healthy by the visual examination) and all other participants. Dysbiosis was observed in 19.2 % of the participants in subgroup 1. Type I of the vaginal flora was detected in 31.2 % of the examined women, and type III was observed in 37.6 % of the patients. Types II and IV were rare in subgroup 1 and in all the participants in general.

Compositionally, the vaginal microbiota of subgroup 1 differed significantly from that of 2 other groups: the dysbiotic type was 5 times less common (3.8 % vs. 19.7 % in main group and 19.2 % in subgroup 1,  $p < 0.01$ ). The normal flora dominated by *L. crispatus* was significantly more common in subgroup 1: this microbiota type was observed in almost half of the examined women. Prevalence of other microbiota types varied: type II (dominated by *L. gasseri*) and type IV (dominated by *L. jensenii/L. vaginalis*) were slightly more common than other types, while type III (dominated by *L. iners*) was less common. The difference, however, was insignificant, which may be due to the small number of women who carried these microbiota types.

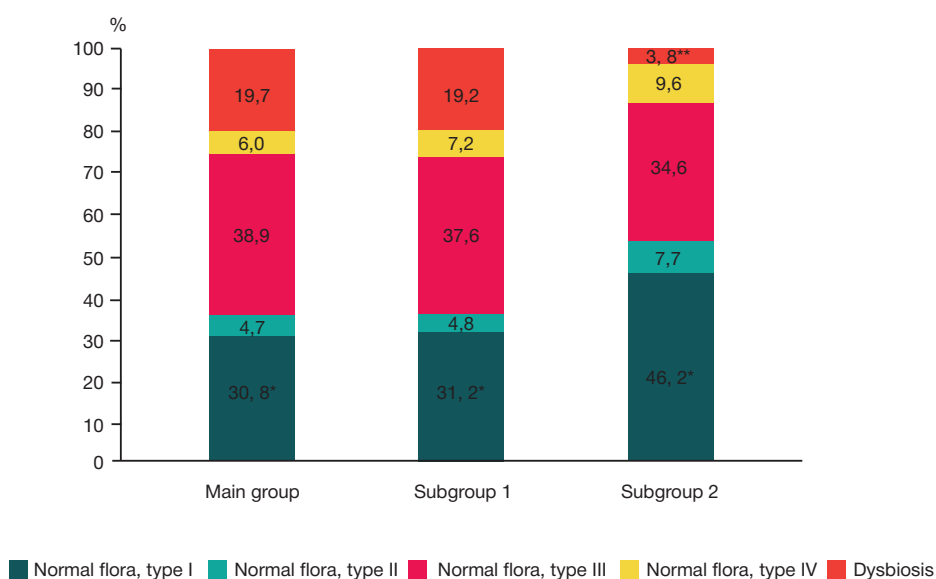
Thus, one out of 5 women who considered herself healthy (main group) was diagnosed with vaginal dysbiosis. A similar result was obtained for those women who were found healthy after the visual examination by the gynecologist (subgroup 1). In contrast, almost half of the women from subgroup 1, whose vaginal health was evaluated using microscopy, had type 1 vaginal flora — the most beneficial type of microbial community dominated by *L. Crispatus*. In this group, dysbiosis was observed in a few patients only.

## DISCUSSION

The obtained data demonstrate that criteria used to form norm groups significantly affect the outcome of the study and should be carefully elaborated when working on a study design. How woman sees her health is subjective; the same is true for a visual examination performed by the doctor. Patient's and doctor's opinions alone unsupported by lab tests may result in the underdiagnosis of the dysbiotic state and untimely treatment of vaginal dysbiosis, which will affect woman's reproductive health.

In this light, it is interesting to compare our results with the data published by Ravel et al. [6]. They analyzed vaginal microbiotas in 4 ethnic groups (Caucasian, Asian, Hispanic and Afro-American). Study participants considered themselves healthy at the time of the examination, in spite of the fact that some of them had not seen a gynecologist for a check- for several years, similar to main group in our study. Results of the comparative analysis are presented in Table.

Vaginal microbiotas of women in our study differed from those of other ethnicities (the study was conducted in the USA, in Baltimore and Atlanta). In our opinion, it would be incorrect to estimate significance of differences between the two studies because methods used for the assessment of the vaginal microbiota status were different. However, in our study dysbiosis was detected 2 times more often than in the Caucasian group; its prevalence was comparable to the prevalence in the Asian group. The most beneficial microbiota type was 1.5 less



Prevalence of different types of the vaginal microbiota in women with no visual signs of vaginal inflammation (\* —  $p < 0.05$  and \*\* —  $p < 0.01$  when comparing main group and subgroup 1 and subgroups 1 and 2)

Prevalence of various microbiota types in women of different ethnic groups who considered themselves healthy

Vaginal flora type	Study by Ravel et al., 2011 (whole-genome sequencing) [6]				Our data (real-time PCR)
	Caucasian (n = 98)	Asian (n = 97)	Hispanic (n = 97)	Afro-American (n = 104)	Russian, Yekaterinburg (n = 234)
Normal flora, type I	45,4 %	25,0 %	14,4 %	22,1 %	30,8 %
Normal flora, type II	8,2 %	5,2 %	7,2 %	4,8 %	4,7 %
Normal flora, type III	26,8 %	42,7 %	36,1 %	31,5 %	38,9 %
Normal flora, type IV	9,3 %	7,3 %	4,2 %	1,0 %	6,0 %
Dysbiosis	10,3 %	19,8 %	38,1 %	40,6 %	19,7 %

common in the Russian women than in the Caucasian group, but more common than in other ethnic groups. On the whole, in our study vaginal microbiota composition was similar to that of Asian women. Further research is necessary to compare the results of our study with the results of similar studies conducted in other Russian regions.

## CONCLUSIONS

Vaginal dysbiosis detected by real-time PCR is common in reproductive-age women who consider themselves healthy. It was observed in one out of five patients. Normal vaginal flora

was usually dominated by *L. Iners*; another type of normal flora dominated by *L. crispatus* was observed in every third woman. Normal flora dominated by *L. crispatus* was present in 46.2 % of women who were assigned to the norm group based on their own opinion, visual examination and microscopy data. Without microscopy, clinical assessment of the vaginal status is subjective and may not detect dysbiosis in a number of patients.

Variations in the vaginal microbiota composition in women from Yekaterinburg and differences from the vaginal flora of Caucasian women may be associated with ethnic diversity of female population of the Ural region.

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