PREVALENCE OF *LACTOBACILLUS INERS* IN THE VAGINAL MICROBIOTA OF WOMEN WITH MODERATE DYSBIOSIS IS ASSOCIATED WITH CLINICAL SYMPTOMS OF INFECTIOUS INFLAMMATORY CONDITION OF THE VAGINA

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Moderate vaginal dysbiosis is a shift in normal vaginal microbiota composition characterized by increased levels of opportunistic microbes and an ordinary high proportion of lactobacilli that make up 20–80 % of the total microbial population of the vagina. Some women with vaginal dysbiosis do not show any symptoms of the infectious inflammatory condition (IIC), which raises the question of whether their dysbiosis should be corrected. We studied the association between some parameters of the microbiota and clinical symptoms of IIC in female patients with moderate vaginal dysbiosis. Participants were distributed into two groups: group 1 included patients with clinical symptoms of IIC (n = 91), group 2 was comprised of asymptomatic patients (n = 44). Mean age was 26.9 ± 6.9 years. Vaginal microbial communities were studied using real-time polymerase chain reaction assays. Levels of six Lactobacillus species were measured in the vaginal discharge: *Lactobacillus crispatus*, *L. iners*, *L. jensenii*, *L. gasseri*, *L. johnsonii*, and *L. vaginalis*. We found that *L. iners* dominated the microbiota of 45 (49.5 %) symptomatic patients and only 9 (20.5 %) asymptomatic individuals (p = 0.002), unlike *L. gasseri* that significantly prevailed in the samples of asymptomatic patients: 23 (52.3 %) women vs 21 (23.1 %) in the group of patients with clinical signs of IIC (p = 0.001).

**Keywords:** vaginal microbiota, vaginal lactobacilli, *Lactobacillus iners*, *Lactobacillus gasseri*, moderate vaginal dysbiosis

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ПРЕОБЛАДАНИЕ *LACTOBACILLUS INERS* В МИКРОБИОЦЕНОЗЕ ВЛАГАЛИЩА ЖЕНЩИН С УМЕРЕННЫМ ДИСБИОЗОМ АССОЦИИРОВАНО С НАЛИЧИЕМ КЛИНИЧЕСКИХ ПРИЗНАКОВ ИНФЕКЦИОННО-ВОСПАЛИТЕЛЬНОЙ ПАТОЛОГИИ ВЛАГАЛИЩА

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Умеренный дисбисоз влагалища является переходным типом вагинального микробиоценоза, для которого характерно увеличение количества и доли условно-патогенных микроорганизмов при сохранении высокой доли лактофлоры — на уровне 20–80 % от общей микробной биомассы. У части женщин данное состояние микробиоценоза не сопровождается клиническими признаками инфекционно-воспалительной патологии влагалища (ИВП), и возникает вопрос о целесообразности коррекции умеренного дисбисоза в этом случае. В исследовании оценивали взаимосвязь между отдельными микробиологическими показателями и наличием клинических проявлений ИВП у пациенток с умеренным дисбиозом вагинальной микробиоты. Были сформированы две группы участниц: группа 1 — пациентки с клиническими признаками ИВП (n = 91), группа 2 — клинически здоровые женщины (n = 44). Средний возраст женщин составил 26,9 ± 6,9 лет. Микробиоценоз исследовали методом полимеразной цепной реакции в режиме “реального времени”. Провели количественную оценку 6 видов лактообацилл в вагинальном отделяемом: *Lactobacillus crispatus*, *L. iners*, *L. jensenii*, *L. gasseri*, *L. johnsonii*, *L. vaginalis*. Было установлено, что *L. iners* преобладает в микробиоценозе 45 (49.5 %) пациенток с признаками ИВП, тогда как у клинически здоровых женщин преобладание данного вида лактообацилл зафиксировано в 9 (20.5 %) случаях (p = 0.002), *L. gasseri*, наоборот, достоверно чаще преобладал в образцах, полученных от клинически здоровых пациенток: 23 (52.3 %) случая против 21 (23.1 %) в группе пациенток с клиническими признаками ИВП (p = 0.001).

**Ключевые слова:** микробиоценоз влагалища, вагинальные лактообациллы, *Lactobacillus iners*, *Lactobacillus gasseri*, умеренный дисбиоз влагалища

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Numerous studies show that the vaginal microbiota of healthy women is dominated by *Lactobacillus* [1–4]. Lactobacilli are thought to ensure colonization resistance of the vaginal microbial community. The vaginal epithelium can be colonized by other microbes, but they are less abundant in healthy women.

Many vaginal microorganisms are not so readily culturable or completely unculturable [5–8], including some *Lactobacillus* species that refuse to grow on standard media. It was shown that one of the most prevalent species, *Lactobacillus iners*, cannot grow on Sharpe (MRS) and Rogosa agars used to culture lactobacilli [9]. Therefore, culture-based studies provide very scarce data on the diversity of species constituting the vaginal microbial community. To date, the most comprehensive results can be achieved using methods of molecular genetics.

Health of the vaginal microbiota is determined by the abundance of lactobacilli (no less than 80 % of all species isolated from the sample) measured by real-time polymerase chain reaction assays [10]. If lactobacilli constitute 20 to 80 % of the whole microbial community, the vaginal microbiota is considered moderately dysbiotic. Moderate dysbiosis is very often asymptomatic; therefore, it presents a particular interest for researchers and health professionals and raises the question of whether it is necessary to treat this condition in the absence of signs of vaginal inflammation.

Of importance is identification of microbial markers associated with clinical signs of vaginal inflammation in patients with moderate dysbiosis. Studies of the diversity of lactobacilli in the vaginal microbiota of reproductive-age women showed that prevalence of some lactobacilli varies in patients with different types of vaginal flora [11]. Perhaps, there is an association between the diversity of lactobacilli in patients with moderate dysbiosis and the presence of subjective symptoms and objective signs of vaginal inflammation. We cannot rule out the possibility that clinical manifestations of pathology in moderate dysbiosis depend on the diversity and abundance of opportunistic bacteria inhabiting the vagina.

The aim of this study was to estimate the correlation between some microbiological characteristics and the presence of clinical signs of vaginal infection in patients with moderate vaginal dysbiosis.

METHODS

The study was carried out in 135 women with moderate vaginal dysbiosis aged 18 to 53 (mean age was 26.9 ± 6.9 years), outpatients of the Medical Center Harmony (Yekaterinburg) in 2011–2016. Exclusion criteria were HIV, parenteral hepatitis, sexually transmitted infections, namely *Treponema pallidum, Neisseria gonorrhoeae, Chlamydia trachomatis, Mycoplasma genitalium,* and *Trichomonas vaginalis,* and antimicrobial therapy started as early as 4 weeks before the study.

Samples (posterolateral vaginal wall swabs) were collected into Eppendorf tubes containing 1 ml sodium chloride solution. DNA was extracted using the Proba-GS reagent kit (R&P DNA-Technology, Russia). Abundance and diversity of species in the samples were evaluated by real-time PCR and the Femoflor reagent kit (R&P DNA-Technology). Identification and quantification of 6 *Lactobacillus* species (*Lactobacillus crispatus, L. iners, L. jensenii, L. gasseri, L. johnsonii, L. vaginalis*) was done by real-time PCR with reagent kits for scientific research (R&P DNA-Technology) and the DT-96 PCR detection system by the same vendor.

Patients were questioned about their complaints and examined to identify clinical signs of infection-induced inflammation of the lower genital tract.

Statistical analysis was performed using Microsoft Office Excel 2007. Significance of differences was estimated by the two-tailed Fisher’s test using WinPepi software.

The study was approved by the Ethics Committee of the Ural State Medical University (Protocol No. 4 dated May 05, 2015). All patients gave their informed consent.

RESULTS

All patients were divided into two groups depending on the presence of clinical signs of an inflammatory infection (II) in the lower genital tract. Group 1 consisted of 91 patients with clinical signs of II, group 2 included 44 healthy women. We attempted to establish associations between the proportion of lactobacilli in the microbiota, the dominant species of lactobacilli, the dominant species of opportunistic microorganisms (OMs), and II.

Based on the proportion of lactobacilli (20–40 %, 40–60 % and 60–80 %), all patients were divided into 3 subgroups. Then the relative share of each subgroup in groups 1 and 2 was estimated (Fig. 1). The difference between the groups was insignificant.

Prevalence of dominant *Lactobacillus* species in groups 1 and 2 was different (Fig. 2). *L. iners* was significantly more common in group 1 (patients with II) than in group 2: 45 women (49.5 %) vs. 9 (20.5 %), respectively (p = 0.002). *L. gasseri,* on the contrary, was significantly more common in group 2

![Fig. 1. Proportions of lactobacilli in the vaginal microbiota of women with moderate dysbiosis in the presence and absence of clinical signs of inflammatory infection (n = 135)](image)

![Fig. 2. Prevalence of lactobacilli species in the vaginal microbiota of women with moderate dysbiosis in the presence and absence of clinical signs of inflammatory infection (n = 135). Asterisks represent significantly different values (p < 0.01)](image)
Prevalence of opportunistic pathogens in the vaginal microbiota of women with moderate vaginal dysbiosis in the presence or absence of clinical signs of inflammatory infection (n = 135)

<table>
<thead>
<tr>
<th>Dominant opportunistic pathogen</th>
<th>Patients with II (n = 91)</th>
<th>Healthy women (n = 44)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterobacteriaceae family</td>
<td>3 (3.3 %)</td>
<td>0</td>
</tr>
<tr>
<td>Streptococcus spp.</td>
<td>6 (6.6 %)</td>
<td>6 (13.6 %)</td>
</tr>
<tr>
<td>Enterococcus spp.</td>
<td>0</td>
<td>2 (4.5 %)</td>
</tr>
<tr>
<td>Gardnerella vaginalis/Prevotella bivia/Porphyromonas spp.</td>
<td>50 (54.9 %)</td>
<td>24 (54.5 %)</td>
</tr>
<tr>
<td>Eubacterium spp.</td>
<td>8 (8.8 %)</td>
<td>5 (11.4 %)</td>
</tr>
<tr>
<td>Sneathia spp./Leptobrixia spp./ Fusobacterium spp.</td>
<td>2 (2.2 %)</td>
<td>0</td>
</tr>
<tr>
<td>Megasphaera spp./Veillonella spp. /Dialister spp.</td>
<td>4 (4.4 %)</td>
<td>3 (6.8 %)</td>
</tr>
<tr>
<td>Clostridium spp./Lachnobacterium spp.</td>
<td>3 (3.3 %)</td>
<td>0</td>
</tr>
<tr>
<td>Peptostreptococcus spp.</td>
<td>0</td>
<td>1 (2.3 %)</td>
</tr>
<tr>
<td>Atopobium vaginae</td>
<td>15 (16.5 %)</td>
<td>3 (6.8 %)</td>
</tr>
</tbody>
</table>

We were unable to identify an association between the proportion of lactobacilli in the microbiota and the presence of II in patients with moderate vaginal dysbiosis. However, the obtained results may have been influenced by a small patient sample size, which means that such an association remains a possibility.

In more than half of patients with moderate vaginal dysbiosis, opportunistic bacteria were represented by GPP. In the studies in vitro Gardnerella vaginalis, a member of the GPP group, was shown to have a high adhesion capacity [19, 20] and stimulate growth of other OMs, including Prevotella bivia, also a GPP representative [19]. It was hypothesized that G vaginalis could be the first microorganism that colonizes the vagina and prepares the environment for other pathogens [21, 22]. This can explain high prevalence of GPP as dominant opportunistic pathogens in patients with moderate dysbiosis. It is possible that as dysbiosis progresses, the contribution of other OMs to pathology increases; this may be true for Ato pobium vaginae, a microorganism associated with vaginal dysbiosis [23–26]. In our study A. vaginae was twice more common in patients with II than in healthy women. However, the difference was not statistically significant. We assume that the lack of significance was due to the small number of healthy women in group 2.

CONCLUSIONS

Dominance of Lactobacillus iners in the Lactobacillus community of the vaginal microbiota of women with moderate dysbiosis is associated with clinical signs of the infection of the lower genital tract, while dominance of L. gasseri is typical for clinically healthy women with moderate dysbiosis. Thus, these microorganisms can be used as microbiological markers when it is unclear whether dysbiosis requires treatment.

References

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