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 to 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 дисбиоз 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 дисбиоза 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 лактобацилл 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 лактобацилл зафиксировали 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 лактобациллы, *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 microbiological 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

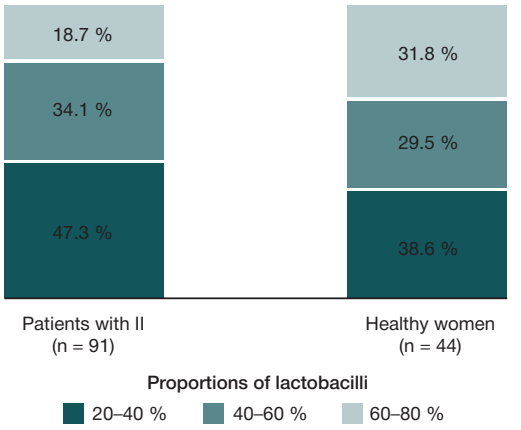

Fig. 1. Proportions of lactobacilli in the vaginal microbiota of women with moderate dysbiosis in the presence and absence of clinical signs of inflammatory infection (n = 135)

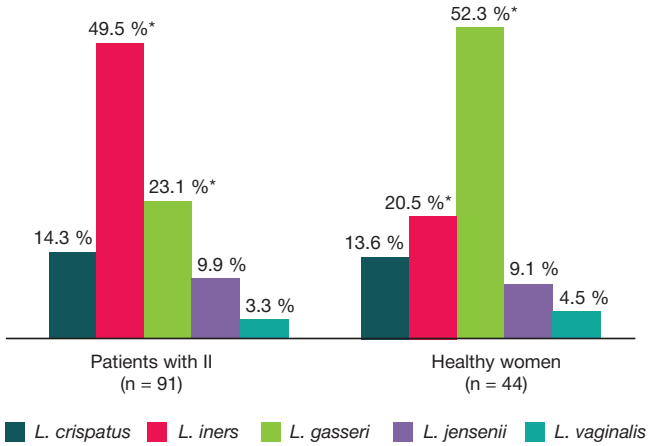

Fig. 2. Prevalence of lactobacilli species in the vaginal microbiota of women with moderate dysbiosis in the presence and absence of clinical signs of inflammatory infection (n = 135). Asterisks represent significantly different values (p < 0.01)

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)

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

(healthy women) than in group 1: 23 patients (52.3 %) vs. 21 (23.1 %), respectively (p = 0.001). Prevalence of *L. crispatus*, *L. jensenii* and *L. vaginalis* in both groups was comparable.

Opportunistic pathogens were represented by dominant *Gardnerella vaginalis/ Prevotella bivia/Porphyromonas spp*. (GPP) in every second woman with or without clinical signs of II. Other OMs were far less common. No significant difference was revealed between OM prevalence in groups 1 and 2 (see the Table).

DISCUSSION

Study results demonstrate that lactobacilli inhabiting the vagina of reproductive-age women are represented mainly by *L. crispatus*, *L. iners*, *L. gasseri*, and *L. jensenii*, which is consistent with the results of other studies [1, 2, 12, 13]. It is noteworthy that *L. iners* and *L. gasseri* dominate the *Lactobacillus* community in patients with moderate dysbiosis. A number of researchers have demonstrated that the presence of these lactobacilli is associated with an increased risk of bacterial vaginosis and poor pregnancy outcome [14–16]. Previously, we showed that *L. gasseri* can dominate the vaginal microbiota of patients with moderate dysbiosis [11]. Frequent detection of *L. gasseri* as a dominant species in patients without clinical signs of II, whose microbiota can be described as moderately dysbiotic, prompts us to assume that it can be a normal variant of the healthy vaginal flora and does not require any treatment. At the same time, moderate dysbiosis characterized by dominant *L. iners* is very often accompanied by clinical signs of II. Moreover, *L. iners* dominance is associated with an increased risk of marked vaginal dysbiosis [11]. Recent studies show that *L. iners* are higly adaptable and can survive in the presence of abundant Oms [17, 18]. Therefore, dominance of *L. iners* is a very unfavorable factor and requires medical correction.

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 *Atopobium 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

1. Ravel J, Gajer P, Abdo Z, Schneider GM, Koenig SS, McCulle SL, et al. Vaginal microbiome of reproductive-age women. Proc Natl Acad Sci U S A. 2011 Mar 15; 108 Suppl 1: 4680–7. DOI: 10.1073/pnas.1002611107.
2. Zhou X, Bent SJ, Schneider MG, Davis CC, Islam MR, Forney LJ. Characterization of vaginal microbial communities in adult healthy women using cultivation-independent methods. Microbiology. 2004 Aug; 150 (Pt 8): 2565–73. DOI: 10.1099/mic.0.26905-0.
3. Zhou X, Brown CJ, Abdo Z, Davis CC, Hansmann MA, Joyce P, et al. Differences in the composition of vaginal microbial communities found in healthy Caucasian and black women. ISME J. 2007 Jun; 1 (2): 121–3. DOI: 10.1038/ismej.2007.12.
4. Zhou X, Hansmann MA, Davis CC, Suzuki H, Brown CJ, Schütte U, et al. The vaginal bacterial communities of Japanese women resemble those of women in other racial groups. FEMS Immunol Med Microbiol. 2010 Mar; 58 (2): 169–81. DOI: 10.1111/j.1574-695X.2009.00618.x.
5. Fredricks DN, Fiedler TL, Marrazzo JM. Molecular identification of

bacteria associated with bacterial vaginosis. N Engl J Med. 2005 Nov 3; 353 (18): 1899–911. DOI: 10.1056/NEJMoa043802.
6. Handelsman J. Metagenomics: application of genomics to uncultured microorganisms. Microbiol Mol Biol Rev. 2004 Dec; 68 (4): 669–85. DOI: 10.1128/MMBR.68.4.669-685.2004.
7. Oakley BB, Fiedler TL, Marrazzo JM, Fredricks DN. Diversity of human vaginal bacterial communities and associations with clinically defined bacterial vaginosis. Appl Environ Microbiol. 2008 Aug; 74 (15): 4898–909. DOI: 10.1128/AEM.02884-07.
8. Zozaya-Hinchliffe M, Martin DH, Ferris MJ. Prevalence and abundance of uncultivated Megasphaera-like bacteria in the human vaginal environment. Appl Environ Microbiol. 2008 Mar; 74 (5): 1656–9. DOI: 10.1128/AEM.02127-07.
9. Falsen E, Pascual C, Sjoden B, Ohlen M, Collins MD. Phenotypic and phylogenetic characterization of a novel Lactobacillus species from human sources: description of Lactobacillus iners sp. nov. Int J Syst Bacteriol. 1999 Jan; 49 Pt 1: 217–21. DOI: 10.1099/00207713-49-1-217.
10. Voroshilina ES, Tumbinskaya LV, Donnikov AE, Plotko EA, Khayutin LV. [Vaginal biocenosis with a view to quantitative polymerase chain reaction: what is its norm?] Obstetrics and Gynecology. 2011; (1): 57–65. Russian.
11. Zornikov DL, Tumbinskaya LV, Voroshilina ES. [Relationship vaginal lactobacilli species with common proportion of Lactobacillus spp. in vaginal microbiocenosis and amounts of microorganisms, associated with dysbiosis]. Journal of Ural Medical Academic Science. 2015; 4 (55): 99–105. Russian.
12. Biagi E, Vitali B, Pugliese C, Candela M, Donders GG, Brigidi P. Quantitative variations in the vaginal bacterial population associated with asymptomatic infections: a real-time polymerase chain reaction study. Eur J Clin Microbiol Infect Dis. 2009 Mar; 28 (3): 281–5. DOI: 10.1007/s10096-008-0617-0.
13. Shi Y, Chen L, Tong J, Xu C. Preliminary characterization of vaginal microbiota in healthy Chinese women using cultivation-independent methods. J Obstet Gynaecol Res. 2009 Jun; 35 (3): 525–32. DOI: 10.1111/j.1447-0756.2008.00971.x.
14. Antonio MA, Hawes SE, Hillier SL. The identification of vaginal Lactobacillus species and the demographic and microbiologic characteristics of women colonized by these species. J Infect Dis. 1999 Dec; 180 (6): 1950–6. DOI: 10.1086/315109.
15. Petricevic L, Domig KJ, Nierscher FJ, Sandhofer MJ, Fidesser M, Krondorfer I, et al. Characterisation of the vaginal Lactobacillus microbiota associated with preterm delivery. Sci Rep. 2014 May 30; 4: 5136. DOI: 10.1038/srep05136.
16. Verstraelen H, Verhelst R, Claeys G, De Backer E, Temmerman M, Vaneechoutte M. Longitudinal analysis of the vaginal microflora in pregnancy suggests that L. crispatus promotes the stability of the normal vaginal microflora and that L. gasseri and/or L. iners are more conducive to the occurrence of abnormal vaginal microflora. BMC Microbiol. 2009 Jun 2; 9: 116. DOI: 10.186/1471-2180-9-116.
17. Macklaim JM, Fernandes AD, Di Bella JM, Hammond JA, Reid G, Gloor GB. Comparative meta-RNA-seq of the vaginal microbiota and differential expression by Lactobacillus iners in health and dysbiosis. Microbiome. 2013 Apr 12; 1 (1): 12. DOI: 10.1186/2049-2618-1-12.
18. Macklaim JM, Gloor GB, Anukam KC, Cribby S, Reid G. At the crossroads of vaginal health and disease, the genome sequence of Lactobacillus iners AB-1. Proc Natl Acad Sci U S A. 2011 Mar 15; 108 Suppl 1: 4688–95. DOI: 10.1073/pnas.1000086107.
19. Machado A, Jefferson KK, Cerca N. Interactions between Lactobacillus crispatus and Bacterial Vaginosis (BV)-Associated Bacterial Species in Initial Attachment and Biofilm Formation. Int J Mol Sci. 2013 Jun 5; 14 (6): 12004–12. DOI: 10.3390/ijms1406120004.
20. Patterson JL, Stull-Lane A, Girerd PH, Jefferson KK. Analysis of adherence, biofilm formation and cytotoxicity suggests a greater virulence potential of Gardnerella vaginalis relative to other bacterial-vaginosis-associated anaerobes. Microbiology. 2010 Feb; 156 (Pt 2): 392–9. DOI: 10.1099/mic.0.034280-0.
21. Swidsinski A, Dörffel Y, Loening-Baucke V, Schilling J, Mendling W. Response of Gardnerella vaginalis biofilm to 5 days of moxifloxacin treatment. FEMS Immunol Med Microbiol. 2011 Feb; 61 (1): 41–6. DOI: 10.1111/j.1574-695X.2010.00743.x.
22. Swidsinski A, Mendling W, Loening-Baucke V, Ladhoff A, Swidsinski S, Hale LP, et al. Adherent biofilms in bacterial vaginosis. Obstet Gynecol. 2005 Nov; 106 (5 Pt 1): 1013–23. DOI: 10.1097/01.AOG.0000183594.45524.d2.
23. Bradshaw CS, Tabrizi SN, Fairley CK, Morton AN, Rudland E, Garland SM. The association of Atopobium vaginae and Gardnerella vaginalis with bacterial vaginosis and recurrence after oral metronidazole therapy. J Infect Dis. 2006 Sep 15; 194 (6): 828–36. DOI: 10.1086/506621.
24. Ferris MJ, Masztal A, Aldridge KE, Fortenberry JD, Fidel PL Jr, Martin DH. Association of Atopobium vaginae, a recently described metronidazole resistant anaerobe, with bacterial vaginosis. BMC Infect Dis. 2004 Feb 13; 4: 5. DOI: 10.1186/1471-2334-4-5.
25. Menard JP, Fenollar F, Henry M, Bretelle F, Raoult D. Molecular quantification of Gardnerella vaginalis and Atopobium vaginae loads to predict bacterial vaginosis. Clin Infect Dis. 2008 Jul 1; 47 (1): 33–43. DOI: 10.1086/588661.
26. Trama JP, Pascal KE, Zimmerman J, Self MJ, Mordechai E, Adelson ME. Rapid detection of Atopobium vaginae and association with organisms implicated in bacterial vaginosis. Mol Cell Probes. 2008 Apr; 22 (2): 96–102. DOI: 10.1016/j.mcp.2007.08.002.

Литература

1. Ravel J, Gajer P, Abdo Z, Schneider GM, Koenig SS, McCulle SL, et al. Vaginal microbiome of reproductive-age women. Proc Natl Acad Sci U S A. 2011 Mar 15; 108 Suppl 1: 4680–7. DOI: 10.1073/pnas.1002611107.
2. Zhou X, Bent SJ, Schneider MG, Davis CC, Islam MR, Forney LJ. Characterization of vaginal microbial communities in adult healthy women using cultivation-independent methods. Microbiology. 2004 Aug; 150 (Pt 8): 2565–73. DOI: 10.1099/mic.0.26905-0.
3. Zhou X, Brown CJ, Abdo Z, Davis CC, Hansmann MA, Joyce P, et al. Differences in the composition of vaginal microbial communities found in healthy Caucasian and black women. ISME J. 2007 Jun; 1 (2): 121–3. DOI: 10.1038/ismej.2007.12.
4. Zhou X, Hansmann MA, Davis CC, Suzuki H, Brown CJ, Schütte U, et al. The vaginal bacterial communities of Japanese women resemble those of women in other racial groups. FEMS Immunol Med Microbiol. 2010 Mar; 58 (2): 169–81. DOI: 10.1111/j.1574-695X.2009.00618.x.
5. Fredricks DN, Fiedler TL, Marrazzo JM. Molecular identification of bacteria associated with bacterial vaginosis. N Engl J Med. 2005 Nov 3; 353 (18): 1899–911. DOI: 10.1056/NEJMoa043802.
6. Handelsman J. Metagenomics: application of genomics to uncultured microorganisms. Microbiol Mol Biol Rev. 2004 Dec; 68 (4): 669–85. DOI: 10.1128/MMBR.68.4.669-685.2004.
7. Oakley BB, Fiedler TL, Marrazzo JM, Fredricks DN. Diversity of human vaginal bacterial communities and associations with clinically defined bacterial vaginosis. Appl Environ Microbiol. 2008 Aug; 74 (15): 4898–909. DOI: 10.1128/AEM.02884-07.
8. Zozaya-Hinchliffe M, Martin DH, Ferris MJ. Prevalence and abundance of uncultivated Megasphaera-like bacteria in the human vaginal environment. Appl Environ Microbiol. 2008 Mar; 74 (5): 1656–9. DOI: 10.1128/AEM.02127-07.
9. Falsen E, Pascual C, Sjoden B, Ohlen M, Collins MD. Phenotypic and phylogenetic characterization of a novel Lactobacillus species from human sources: description of Lactobacillus iners sp. nov. Int J Syst Bacteriol. 1999 Jan; 49 Pt 1: 217–21. DOI: 10.1099/00207713-49-1-217.
10. Ворошилина Е. С., Донников А. Е., Плотко Е. Э., Тумбинская Л. В., Хаютин Л. В. Биоценоз влагалища с точки

зрения количественной полимеразной цепной реакции: что есть норма? Акушерство и гинекология. 2011; (1): 57–65.
11. Зорников Д. Л., Тумбинская Л. В., Ворошилина Е. С. Взаимосвязь отдельных видов лактобацилл с суммарной долей лактофлоры в вагинальном микробиоценозе и группами условно-патогенных микроорганизмов, ассоциированными с дисбиозом влагалища. Вестник уральской медицинской академической науки. 2015; 4 (55): 99–105.
12. Biagi E, Vitali B, Pugliese C, Candela M, Donders GG, Brigidi P. Quantitative variations in the vaginal bacterial population associated with asymptomatic infections: a real-time polymerase chain reaction study. Eur J Clin Microbiol Infect Dis. 2009 Mar; 28 (3): 281–5. DOI: 10.1007/s10096-008-0617-0.
13. Shi Y, Chen L, Tong J, Xu C. Preliminary characterization of vaginal microbiota in healthy Chinese women using cultivation-independent methods. J Obstet Gynaecol Res. 2009 Jun; 35 (3): 525–32. DOI: 10.1111/j.1447-0756.2008.00971.x.
14. Antonio MA, Hawes SE, Hillier SL. The identification of vaginal Lactobacillus species and the demographic and microbiologic characteristics of women colonized by these species. J Infect Dis. 1999 Dec; 180 (6): 1950–6. DOI: 10.1086/315109.
15. Petricevic L, Domig KJ, Nierscher FJ, Sandhofer MJ, Fidesser M, Krondorfer I, et al. Characterisation of the vaginal Lactobacillus microbiota associated with preterm delivery. Sci Rep. 2014 May 30; 4: 5136. DOI: 10.1038/srep05136.
16. Verstraelen H, Verhelst R, Claeys G, De Backer E, Temmerman M, Vaneechoutte M. Longitudinal analysis of the vaginal microflora in pregnancy suggests that L. crispatus promotes the stability of the normal vaginal microflora and that L. gasseri and/or L. iners are more conducive to the occurrence of abnormal vaginal microflora. BMC Microbiol. 2009 Jun 2; 9: 116. DOI: 10.186/1471-2180-9-116.
17. Macklaim JM, Fernandes AD, Di Bella JM, Hammond JA, Reid G, Gloor GB. Comparative meta-RNA-seq of the vaginal microbiota and differential expression by Lactobacillus iners in health and dysbiosis. Microbiome. 2013 Apr 12; 1 (1): 12. DOI: 10.1186/2049-2618-1-12.
18. Macklaim JM, Gloor GB, Anukam KC, Cribby S, Reid G. At the crossroads of vaginal health and disease, the genome sequence of Lactobacillus iners AB-1. Proc Natl Acad Sci U S A. 2011 Mar 15; 108 Suppl 1: 4688–95. DOI: 10.1073/pnas.1000086107.
19. Machado A, Jefferson KK, Cerca N. Interactions between Lactobacillus crispatus and Bacterial Vaginosis (BV)-Associated Bacterial Species in Initial Attachment and Biofilm Formation. Int J Mol Sci. 2013 Jun 5; 14 (6): 12004–12. DOI: 10.3390/ijms1406120004.
20. Patterson JL, Stull-Lane A, Girerd PH, Jefferson KK. Analysis of adherence, biofilm formation and cytotoxicity suggests a greater virulence potential of Gardnerella vaginalis relative to other bacterial-vaginosis-associated anaerobes. Microbiology. 2010 Feb; 156 (Pt 2): 392–9. DOI: 10.1099/mic.0.034280-0.
21. Swidsinski A, Dörffel Y, Loening-Baucke V, Schilling J, Mendling W. Response of Gardnerella vaginalis biofilm to 5 days of moxifloxacin treatment. FEMS Immunol Med Microbiol. 2011 Feb; 61 (1): 41–6. DOI: 10.1111/j.1574-695X.2010.00743.x.
22. Swidsinski A, Mendling W, Loening-Baucke V, Ladhoff A, Swidsinski S, Hale LP, et al. Adherent biofilms in bacterial vaginosis. Obstet Gynecol. 2005 Nov; 106 (5 Pt 1): 1013–23. DOI: 10.1097/01.AOG.0000183594.45524.d2.
23. Bradshaw CS, Tabrizi SN, Fairley CK, Morton AN, Rudland E, Garland SM. The association of Atopobium vaginae and Gardnerella vaginalis with bacterial vaginosis and recurrence after oral metronidazole therapy. J Infect Dis. 2006 Sep 15; 194 (6): 828–36. DOI: 10.1086/506621.
24. Ferris MJ, Masztal A, Aldridge KE, Fortenberry JD, Fidel PL Jr, Martin DH. Association of Atopobium vaginae, a recently described metronidazole resistant anaerobe, with bacterial vaginosis. BMC Infect Dis. 2004 Feb 13; 4: 5. DOI: 10.1186/1471-2334-4-5.
25. Menard JP, Fenollar F, Henry M, Bretelle F, Raoult D. Molecular quantification of Gardnerella vaginalis and Atopobium vaginae loads to predict bacterial vaginosis. Clin Infect Dis. 2008 Jul 1; 47 (1): 33–43. DOI: 10.1086/588661.
26. Trama JP, Pascal KE, Zimmerman J, Self MJ, Mordechai E, Adelson ME. Rapid detection of Atopobium vaginae and association with organisms implicated in bacterial vaginosis. Mol Cell Probes. 2008 Apr; 22 (2): 96–102. DOI: 10.1016/j.mcp.2007.08.002.