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Association of bacterial vaginosis with the most common sexually transmitted infections

Повезаност бактеријске вагинозе и најчешћих узрочника сексуално преносивих инфекција

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SUMMARY

Introduction/Objective Bacterial vaginosis (BV) is the most common vaginal dysbiosis that enlarge a possibility of getting sexually transmitted infections (STI). The aims of this research is to examine association between BV and the nine most common causes of STIs (*Chlamydia trachomatis*, *Mycoplasma genitalium*, *Mycoplasma hominis*, *Ureaplasma spp*, *Trichomonas vaginalis*, *Neisseria gonorrhoeae*, high-risk *Human papilloma* viruses and *Herpes simplex* virus type 1 and 2) and to determine if the presence of BV increase the probability of coinfection with any of the STIs microorganisms.

Methods This study involved 235 patients of reproductive age. One sample swab was collected each for vaginal and cervical testing. The vaginal swabs were used for detection of BV by RT-PCR test. The cervical swabs were used for the detection of the most common STIs which were tested by four different multiplex RT-PCR tests. Pearson's χ^2 test and Fisher's probability test were used for statistical analysis of the results.

Results Comparison of the total number of STIs and the condition of the vaginal flora has shown that STIs are the most common in patients with BV 80 (89.9%). Women with BV have a higher frequency of infections with *Ureaplasma spp*. and *M. Hominis*, 71 (78.9%) and 50 (44.4%), respectively. The presence of detected STI pathogens and relation with the state of vaginal flora indicate that mono infections are present most often in patients with normal flora 51 (42.1%), while coinfections are mostly present in BV patients 50 (55.6%).

Conclusion This study has confirmed the association of *M. hominis* and *Ureaplasma* spp with BV as well as an association of coinfections with this dysbiosis. Better understanding of the association between various STIs and the status of vaginal flora is necessary to enable better diagnosis, prevention of diseases and women's health protection.

Keywords: bacterial vaginosis; sexually transmitted infections; coinfection; RT-PCR

САЖЕТАК

Увод/Циљ Бактеријска вагиноза (БВ) је најчешћа вагинална дисбиоза која повећава могућност настанка сексуално преносивих инфекција (СПИ). Циљ овог истраживања је утврђивање повезаности БВ и 9 најчешћих узрочника СПИ (*Chlamydia trachomatis*, *Ureaplasma* spp., *Mycoplasma genitalium*, *Mycoplasma hominis*, *Trichomonas vaginalis*, *Neisserria gonorrhoeae*, високо ризични типови хуманог папилома вируса, херпес симплекс вирус тип 1 и 2) и да ли присуство БВ повећава вероватноћу за постојањем коинфекције неким од узрочника СПИ.

Методе У студију је укључено 235 жена у репродуктивном периоду. Један вагинални и један цервикални брис коришћени су за молекуларну анализу. Вагинални брисеви коришћени су за детекцију БВ и процену вагиналне флоре уз помоћ мултиплекс квантитативног *RT-PCR* теста. Цервикални брис коришћен је за доказивање присуства сексуално преносивих патогена који су испитани са четири различита комерцијална *RT-PCR* теста. За статистичку анализу резултата коришћени су Пирсонов χ^2 и Фишеров тест вероватноће.

Резултати Поређењем присуства укупног броја узрочника СПИ у зависности од стања вагиналне флоре резултати показују да су СПИ најчешће код пацијената са БВ 80 (89,9%). Жене са БВ имају повећану учесталост инфекција са *Ureaplasma spp.* и *M. hominis*, 71 (78,9%) односно 50 (44,4%). Присуство свих детектованих узрочника СПИ у односу на стање вагиналне флоре показује да су моноинфекције најчешће присутне код пацијената са нормалном флором (42,1%), док су коинфекције (55,6%) највише присутне код жена са БВ.

Закључак Истраживање је показало асоцијацију *M. hominis* и *Ureaplasma spp.* са БВ као и повезаност коинфекција са овом дисбиозом. Разумевање повезаности између различитих СПИ и стања вагиналне флоре је неопходно како би се омогућила боља дијагностика и превенција болести, као и заштита здравља жена.

Кључне речи: бактеријска вагиноза, сексуално преносиве инфекције, коинфекције, *RT-PCR*

INTRODUCTION

The vaginal microbiome consists of various microorganisms which coexist in dynamic balance, establishing complex interconnections not only among themselves, but also with a host. In healthy women of reproductive age, the vaginal microbiome predominantly contains bacteria of the genus *Lactobacillus*. These bacteria support vaginal homeostasis and prevent colonization and growth of unwanted microorganisms including Sexually transmitted infections (STI) [1, 2].

The most common imbalance of vaginal flora is bacterial vaginosis (BV). It is a microbial dysbiosis in which normal microflora, consisting of predominantly *Lactobacillus* microflora, is replaced with numerous anaerobic bacteria, here in referred to as bacterial vaginosis associated bacteria (BVAB). Symptoms of BV are increased gray or white vaginal discharge, itching or local discomfort, although symptoms are absent in 50% of patients [3, 4].

According to World Health Organization (WHO) data, 376 million people get infected with STIs globally every year, which indicates the importance of this public health problem [5]. STIs include more than 30 bacterial, viral and parasitic pathogens which can be transmitted via vaginal, anal or oral sex. Some of the most common STIs are *Chlamydia trachomatis*, *Mycoplasma genitalium*, *Mycoplasma hominis*, *Ureaplasma spp*, *Trichomonas vaginalis*, *Neisseria gonorrhoeae* and *Human papilloma viruses* [6]. There is a great number of clinical manifestations caused by STIs in female upper and lower reproductive tract, but some of the most common ones are: pelvic inflammatory disease, cervicitis, ectopic pregnancy, miscarriage, chronic pelvic inflammatory disease, neonatal infections, genital cancer, etc [7]. Asymptomatic STIs are quite challenging as those infections are difficult to identify, while they are easily transmissible in a sexually active population [5].

Various studies have indicated that BV increases the chances of STIs [8, 9]. Reduction of protective *Lactobacillus* types and changes in the vaginal environment such as increased pH, or reduction of lactic acid concentration enables the survival of vaginal pathogens. BVAB produce mucin-degrading enzymes (such as sialidase) which degrade the mucosal membrane of the vaginal epithelium and cervix which are considered one of the most important components of the barrier against infection. The degradation of mucin and glycogen may cause microabrasions and changes in epithelial cells which can make pathogen attachment to the

receptors on epithelial cells easier. In addition, during BV the immune balance is affected in a way that causes increased levels of proinflammatory cytokines which make women more susceptible to STIs [6].

The aims of this research paper are to establish an association between BV and the nine most common causes of STIs (*Chlamydia trachomatis, Ureaplasma* spp, *Mycoplasma genitalium*, *M.hominis*, *Trichomonas vaginalis*, *Neisserria gonorrhoeae*, high-risk Human Papiloma Virus types (HR-HPV-16,18,31,33,35,39,45,51,52,56,58,59), Herpes simplex virus type 1 and 2 (HSV-1, HSV-2) and to examine if the presence of BV increases the probability of coinfection with any of the STIs microorganisms.

METHODS

This cross-sectional study was conducted on women of reproductive age who attended regular gynecological examination in the Centre for Gynecology and Human Reproduction Military Medical Academy (MMA), Belgrade, Serbia, during the period of November 2018 to December 2019. Patients included asymptomatic cases and those with various vaginal complaints. Exclusion criteria includes recent antibiotic use (> 2 weeks) prior to sample collection. All patients provided full informed consent for participation. The research has been approved by the Ethical Board of the MMA.

One sample swab was collected each for vaginal and cervical testing (FLOQ Swabs, COPAN) for molecular analysis using multiplex RT-PCR. After collection, the swabs were placed into transport medium, vortexed and stored at -20^{o}C until DNA extraction (DNA-sorb-AM, AmpliSens), which was performed according to manufacturer's instructions.

The vaginal swabs were examined for the presence and quantification, as well as interrelationship between *Lactobacillus spp*, *Gardnerella vaginalis*, *Atopobium vaginae*, and total concentration of bacterial DNA using a quantitative real time PCR (RTQ-PCR) (AmpliSensFlorocenosis/Bacterial vaginosis-FRT). Microsoft Excel software was used to calculate three coefficients. The coefficients RC1=log (Lac DNA)-log (Gv+Av DNA), RC2=log (Bac DNA)-log (Lac DNA), RC3=log(Bac DNA- log(Gv+Av DNA) were

determined by the mutual relations between those bacteria. Based on coefficients, the patients were grouped in the following categories: normal vaginal flora (RC1>1, *Lactobacillus spp.* is the dominant flora); intermediate flora (0,5≤RC1≤1 the same number of *Lactobacillus spp.*and aerobic bacteria); BV (RC1<0.5, dominant *G.vaginalis* and *A.vagine*); vaginal flora of non-specific etiology (RC2>1, RC3>2, any RC1 value, small concentration of *Lactobacillus spp*, but also *G.vaginalis, A.vagine*).

The cervical swabs were used for the detection of STIs and were tested by four different commercial RT-PCR tests. The first one detected *C. trachomatis, Ureaplasma, M.genitalium, M.hominis*, the second one detected *T.vaginalis* and *N.gonorrhoeae*, the third test identified HSV-1/HSV-2, and the fourth RT PCR was used for detection of HR-HPV (16,18,31,33,35,39,45,51,52,56,58,59). All PCR reactions were undertaken on PCR thermocycler (Sa-Cycler 96, Sacace, Biotechnologies).

Statistical analysis

To assist statistical analysis, the PCR tests results for BV were categorised in 3 groups: normal, BV and abnormal non-BV flora. The flora of nonspecific etiology and intermediary flora were considered to be abnormal non-BV flora. A result of 'bacterial load decreased' was considered normal findings.

Association between dependent and independent variables was tested using Pearson's chi-squared test or Fisher's probability test. Statistical hypotheses were tested at the level of statistical significance (Alpha level) of 0.05. All data were processed in software package IBM SPSS Statistics 22 (SPSS Inc., Chicago, IL, USA).

RESULTS

In total, 235 patients of reproductive age were recruited for this study with the average age being 29.39 years (±6.685). The results (RT PCR) for BV showed that 121 (51.4%) women

had normal (healthy) flora, 90 (38.2%) had BV, 9 (3.8%) were denoted as intermediate and 15 (6.4%) had vaginal flora of non-specific etiology.

Using 4 different RT PCR tests, the frequency of the most common STIs were examined. The tests detected *C.trachomatis* 16 (6.8%), *Ureaplasma spp.* 143 (60.9%), *M.hominis* 66 (28.1%), *M.gentialium* 2 (0.9%), HR-HPV 12 types 70 (29.8%), *T.vaginalis* 3 (1.3%), HSV-1 2 (0.9%), HSV-2 *2* (0.9%) while *N.gonorrhoeae* was not detected in any sample.

Out of 235 patients, 179 (76%) had one of the STIs microorganisms detected, while 56 (23.8%) were negative for all microorganisms. Simultaneous presence of more than one microorganism were detected in 93 patients (39.6%). The distribution of mono and coinfections is presented in Figure 1.

Results have shown there is a statistically significant difference ($\chi 2 = 15.380$, $p = 0.001$). between the total number of STIs and the condition of the vaginal flora obtained by RT PCR BV. STIs are most common in patients with BV 80 (89.9%) and then in women with intermediate flora 7 (77.8%) (Table 1).

Distribution of the presence of various STI pathogens in correlation with the state of vaginal flora is shown in Table 2. Statistical significance of correlation of STI pathogens related to the state of vaginal flora exists only with *Ureaplasma spp.* and *M.hominis,* while no such significance was identified with other pathogens. Although there is no significant difference in distribution of correlation with the state of vaginal flora, it has been confirmed that the largest percentage of positive STIs pathogens are with patients with BV.

The association between BV and the presence of mono and coinfection is presented in Table 3. There is statistical significance between the presence of mono infections and coinfections compared to the state of vaginal flora (Chi-square = 23.677, $p<0.001$). In most cases, women with normal flora had mono infection 51 (42.1%), while women with BV had coinfection 50 (55.6%).

DISCUSSION

This paper employs molecular methods to investigate the prevalence of BV in women of reproductive age but also the association between vaginal dysbiosis and some of the most frequent STI pathogens. Investigations into the association of BV and STIs traditionally use Nugent or Amsel which are the gold standard in BV diagnostic method [10–13]. We have used RT-PCR diagnostic testing for BV in the study because there are reaserch that demonstrate shortcomings in the application of the Amsel and Nugent criterion methods [14, 15]. AmpliSensFlorocenosis/Bacterial vaginosis-FRT in relation to Nugent, Amsel, vaginal culture and BD MAX Vaginal panel shows the highest degree of association related to 16S rRNA genome sequencing with microbiome analysis which was presented in research paper by *Ellen et al.* [16]. The RT PCR test that was used is based on detection and quantification of *G.vaginalis* and *A.vaginae* which are important marker for BV diagnostic. On the grounds of the relationship of these two anaerobes, lactobacilli and total number of bacteria, the test enables assessment of the status of vaginal flora. Our results indicate that BV prevalence is 38.2% in our study population.

This research has shown that women with BV have a higher frequency of infections with *Ureaplasma spp* and *M.hominis.* Since *M. hominis* and *Ureaplasma* spp can be found in both healthy individuals and women with BV there is an ongoing debate and disagreement on the detection of these pathogens. Due to this fact, the presence of *M. hominis* and *Ureaplasma* spp in the urogenital tract is not definitive proof of infection and as such,can be a significant clinical problem. It is considered that the identification of these two pathogens is not adequate without the assessment of the status of vaginal flora. On account of these pathogens association with various reproductive problems (chorioamnionitis, endometritis, postpartum fever, low birth weight, and preterm delivery) we consider that their identification is of great importance, particularly if BV is present as well in a patient [17, 18].

Some STIs pathogens (*C.trachomatis* and HR-HPV) have been detected in women with all three states of vaginal flora. However, we have confirmed that the highest percentage of BV patients is positive with *C.trachomatis* (11.1%). At the same time the highest percentage of HPV positive patients are in the intermediate vaginal flora (75%) and BV (50%) groups.

In this study, some of the pathogens such as *M.genitalium, T.vaginalis,* HSV-1/HSV-2 have been detected in a very small percentage of swabs or have not been identified at all (*N. gonorrhoeae*). Considering these results, the opportunity to compare these STI pathogens with various states of vaginal flora has been limited.

The presence of detected STI pathogens and relation with the state of vaginal flora indicate that mono infections are present most often in patients with normal flora (42,1%), while coinfections with 2, 3 or 4 pathogens are mostly present in BV patients (55,6%). Other research studies have also confirmed association of coinfection and certain STI pathogens with BV [19, 20, 21]. A large number of studies examine the association between STI and BV, but they rarely includeas many pathogens as the current study.

Huge gaps in our knowledge of STI etiology remains an issue including coinfection and its links with certain clinical manifestations. Bacterial coinfections impact significantly on the process of pathogenesis and appearance of clinical manifestations [22, 23]. It is also indicative that coinfections may be present in asymptomatic patients [24]. Compared to infections, coinfections change the process of inflammation in different ways. They also provide fertile ground for the multiplication of opportunistic mycoplasma and its pathogenic effects. The mediators freed by the process of inflammation can cause stagnation in the development cycle of *C.trachomatis* and lead the process into inactive, persistent form. Urogenital mycoplasmacan prolong the inflammation of the urethra even after the elimination of *C. trachomatis* if thereis a resistance to antibiotic therapy. A great number of microorganisms reduce bioavailability of applied medication during therapy of infection. For this reason, it isimportant that further STI research targets coinfections, their pathogenesis,eradication, and efficiency of therapy [22].

In patients with abnormal flora (not related to BV) there was a high percentage of coinfections (41.7%), but also no presence of STI pathogens (37.5%). However, since we have, while using PCR tests, detected a small percentage of patients with intermediate vaginal flora and flora of non-specified etiology, our results cannot adequately confirm association of abnormal non-BV vaginal flora and STI pathogens.

Although a lot of longitudinal and cross-sectional studies have examined the association between BV and STI, the results are different [8, 21, 25, 26, 27]. The problem arises from the

fact that BV is a polymicrobial syndrome whose diagnosis is not precise because a lot of these studies used Amsel or Nugent as the gold standard. On the other side, the identification of STIs pathogens depends on laboratory tests, geographic region and characteristics of the examined populations.

The limitation of this study is that the presence of some of STIs pathogens have been detected in a very small percentages or have not been detected at all and that impedes full examination of their association with BV. Besides BV, association of anaerobic vaginitis with various STIs pathogens also needs to be researched as there are a few studies on this topic in the current literature [17].

CONCLUSION

This study has confirmed the association of *M. hominis* and *Ureaplasma* spp with BV as well as an association of coinfections with this dysbiosis. Considering that the total frequency of STI pathogens in the examined swabs is 76%, it is important to pay attention to prevention and eliminating the spreading of STI. It is also important to continue with education, screening and raising awareness of STI and the health issues they may cause. Better understanding of the association between various STIs and the status of vaginal flora is necessary to enable better diagnosis, prevention of diseases and women's health protection.

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REFERENCES

1. Ceccarani C, Foschi C, Parolin C, Antuono A, Gaspari V, Consolandi C, et al. Diversity of vaginal microbiome and metabolome during genital infections. Scientific Reports. 2019;9(1):14095. doi:10.1038/s41598-019-50410-x. PMID: 31575935.
2. Saraf VS, Sheikh SA, Ahmad A, Gillevet PM, Bokhari H, Javed S. Vaginal microbiome: normalcy vs dysbiosis. Arch Microbiol. 2021;203(7):3793-3802. doi: 10.1007/s00203-021-02414-3. PMID: 34120200.
3. Coudray MS, Madhivanan P. Bacterial vaginosis-A brief synopsis of the literature. Eur J Obstet Gynecol Reprod Biol. 2020;245:143-148. doi:10.1016/j.ejogrb.2019.12.035
4. Redelinghuys MJ, Geldenhuys J, Jung H, Kock MM. Bacterial Vaginosis: Current Diagnostic Avenues and Future Opportunities. Front Cell Infect Microbiol. 2020;10:354. doi: 10.3389/fcimb.2020.00354. PMID: 32850469
5. World Health Organization. Sexually transmitted infections (STIs); 2019. Avalible from https://www.who.int/news-room/fact-sheets/detail/sexually-transmitted-infections-(stis)
6. Petrova MI, Lievens E, Malik S, Imholz N, Lebeer S. Lactobacillus species as biomarkers and agents that can promote various aspects of vaginal health. Frontiers in Physiology. 2015;6:81. doi:10.3389/fphys.2015.00081. PMID: 25859220.
7. The Centers for Disease Control and Prevention (CDC). Sexually Transmitted Infections Treatment Guidelines, 2021
8. Sanchez G, Contreras P, Martinez A, Garcia C, Lizano M, de la Cruz E. Molecular epidemiology of bacterial vaginosis and its association with genital micro-organisms in asymptomatic women. J Med Microbiol. 2019;68(9):1373-1382. doi: 10.1099/jmm.0.001044. PMID: 31329097.
9. Ravel J, Moreno I, Simón C. Bacterial vaginosis and its association with infertility, endometritis, and pelvic inflammatory disease. Am J Obstet Gynecol. 2021; 224(3):251-257. doi: 10.1016/j.ajog.2020.10.019. PMID: 33091407.
10. Tamarelle J, Thiébaut ACM, de Barbeyrac B, Bébéar C, Ravel J, Delarocque A. The vaginal microbiota and its association with human papillomavirus, Chlamydia trachomatis, Neisseria gonorrhoeae and Mycoplasma genitalium infections: a systematic review and meta-analysis. Clin Microbiol Infect. 2019;25(1):35-47. doi:10.1016/j.cmi.2018.04.019
11. Li W, Liu LL, Luo ZZ, Han CY, Wu QH, Zhang L, et al. Associations of sexually transmitted infections and bacterial vaginosis with abnormal cervical cytology: A cross-sectional survey with 9090 community women in China. PLoS One. 2020;15(3):e0230712. doi:10.1371/journal.pone.0230712. PMID: 32214342.
12. Allsworth JE, Peipert JF. Severity of bacterial vaginosis and the risk of sexually transmitted infection. Am J Obstet Gynecol. 2011; 205(2):113.e1-6. doi: 10.1016/j.ajog.2011.02.060. PMID: 21514555; PMCID: PMC3156883.
13. Turpin R, Brotman RM, Miller RS, Klebanoff MA, He X, Slopen N. Perceived stress and incident sexually transmitted infections in a prospective cohort. Ann Epidemiol. 2019; 32:20-27. doi: 10.1016/j.annepidem.2019.01.010. PMID: 30799204
14. Lynch T, Peirano G, Lloyd T, Read R, Carter J, Chu A, et al. Molecular Diagnosis of Vaginitis: Comparing Quantitative PCR and Microbiome Profiling Approaches to Current Microscopy Scoring. J Clin Microbiol. 2019; 57(9):e00300-19. doi: 10.1128/JCM.00300-19. PMID: 31315951; PMCID: PMC6711928.
15. Scapaticci M, Da Rin G, Bartolini A. Comparison between a novel molecular tool and conventional methods for diagnostic classification of bacterial vaginosis: is integration of the two approaches necessary for a better evaluation? New Microbiol. 2020;43(3):121-126. Epub 2020 Jul 13. PMID: 32656571.
16. van den Munckhof EHA, van Sitter RL, Boers KE, Lamont RF, Te Witt R, le Cessie S, et al. Comparison of Amsel criteria, Nugent score, culture and two CE-IVD marked quantitative real-time PCRs with microbiota analysis for the diagnosis of bacterial vaginosis. Eur J Clin Microbiol Infect Dis. 2019; 38(5):959-966. doi:10.1007/s10096-019-03538-7.
17. Rumyantseva T, Khayrullina G, Guschin A, Donders G. Prevalence of Ureaplasma spp. and Mycoplasma hominis in healthy women and patients with flora alterations. Diagn Microbiol Infect Dis. 2019; 93(3):227-231. doi:10.1016/j.diagmicrobio.2018.10.001. PMID: 30344067.
18. Leli C, Mencacci A, Latino MA, Clerici P, Rassu M, Perito S, et al. Prevalence of cervical colonization by Ureaplasma parvum, Ureaplasma urealyticum, Mycoplasma hominis and Mycoplasma genitalium in childbearing age women by a commercially available multiplex real-time PCR: An Italian observational multicentre study. J Microbiol Immunol Infect. 2018; 51(2):220-225. doi: 10.1016/j.jmii.2017.05.004. PMID: 28711440.
19. Sanchez-Garcia EK, Contreras-Paredes A, Martinez-Abundis E, Garcia-Chan D, Lizano M, de la Cruz-Hernandez E. Molecular epidemiology of bacterial vaginosis and its association with genital micro-organisms in asymptomatic women. J Med Microbiol. 2019; 68(9):1373-1382. doi:10.1099/jmm.0.001044. PMID: 31329097.
20. Van Der Pol B, Daniel G, Kodsi S, Paradis S, Cooper CK. Molecular-based Testing for Sexually Transmitted Infections Using Samples Previously Collected for Vaginitis Diagnosis. Clinical Infectious Diseases: an Official Publication of the Infectious Diseases Society of America. 2019; 68(3):375-381 doi:10.1093/cid/ciy504. PMID: 30084883.

21. Barrientos-Durán A, Fuentes-López A, de Salazar A, Plaza-Díaz J, García F. Reviewing the Composition of Vaginal Microbiota: Inclusion of Nutrition and Probiotic Factors in the Maintenance of Eubiosis. *Nutrients*. 2020; 12(2):419. doi:10.3390/nu12020419
22. Vesić S, Vukićević J , Gvozdenović E , Skiljević D , Janosević S , Medenica L. Chlamydia trachomatis and urogenital mycoplasms in nongonococcal uretritis in men. [InSerbian] Med Pregl. 2010; 63(1-2):47-50.
23. Denks K , Spaeth EL , Jõers K , Randoja R , Talpsep T , Ustav M , Kurg R . Coinfection of Chlamydia trachomatis, Ureaplasma urealyticum and human papillomavirus among patients attending STD clinics in Estonia. Scand J Infect Dis. 2007;39(8):714-8. Avalible from:https://www.ncbi.nlm.nih.gov/pubmed/17654349. doi: 10.1080/00365540701199824
24. Wang L, Yang B, Tso LS, Zhao P, Ke W, Zhang X, et al. Prevalence of co-infections with other sexually transmitted infections in patients newly diagnosed with anogenital warts in Guangzhou, China. Int J STD AIDS. 2020; 31(11):1073-1081. doi: 10 .11 77/ 09 564 624 1 9 890496. PMID: 32842906
25. Shipitsyna E, Khusnutdinova T, Budilovskaya O, Krysanova A, Shalepo K, Savicheva A, et al. Bacterial vaginosis-associated vaginal microbiota is an age-independent risk factor for Chlamydia trachomatis, Mycoplasma genitalium and Trichomonas vaginalis infections in low-risk women, St. Petersburg, Russia. Eur J Clin Microbiol Infect Dis. 2020; 39(7):1221-1230. doi: 10.1007/s10096-020-03831-w. PMID: 32036466.
26. Chen X, Lu Y, Chen T, Li R. The Female Vaginal Microbiome in Health and Bacterial Vaginosis. *Front Cell Infect Microbiol*. 2021; 11:631972. Doi:10.3389/fci mb.2 021.631972
27. Lewis FMT, Bernstein KT, Aral SO. Vaginal Microbiome and Its Relationship to Behavior, Sexual Health, and Sexually Transmitted Diseases. Obstet Gynecol. 2017; 129(4):643-654. doi:10.1097/AOG.0000000000001932. PMID: 28277350.

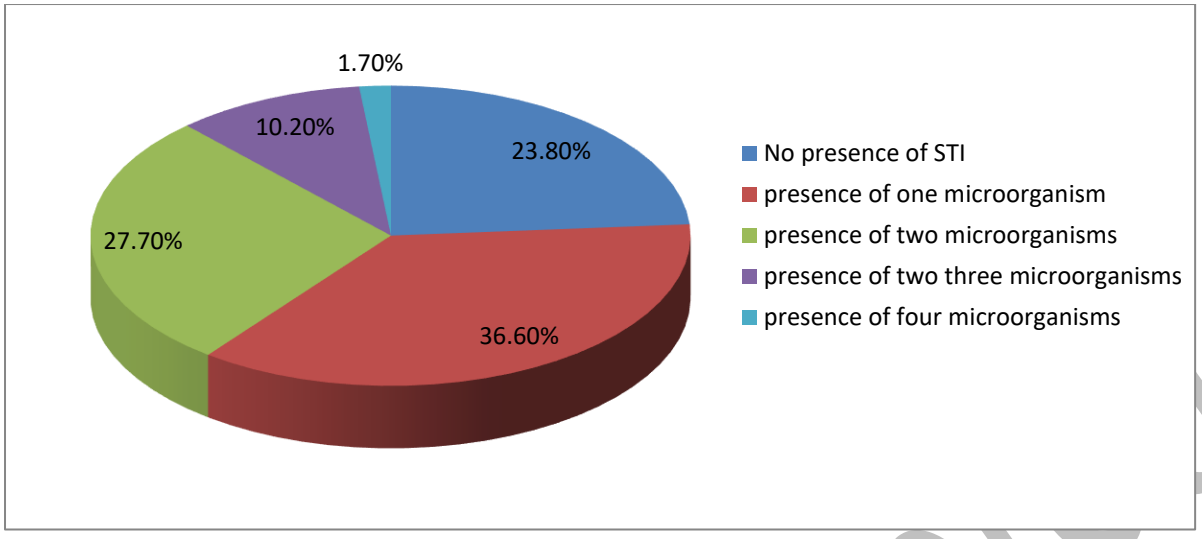

Figure 1. Distribution of mono and coinfection

Table 1. Association between BV and presence of total STIs

Total number of STIs	Presence	Normal flora	BV	Intermediate	Flora of unspecified etiology	Total
	no presence	37 (30.6%)	10 (11.1%)	2 (22.2%)	7 (46.7%)	56 (23.8%)
	presence	84 (69.4%)	80 (88.9%)	7 (77%)	8 (53.3%)	179 (76.2%)
	Total	121 (51.4%)	90(38.2%)	9 (3.8%)	15 (6.4%)	235 (100%)

BV – bacterial vaginosis; STI – sexually transmitted infection

Table 2. Distribution of sexually transmitted infection pathogens in correlation with the state of vaginal flora

Pathogens	Normal flora	BV	Abnormal nonBV		p
			Intermediate	Flora of unspecified etiology	
C.trchomatis	5 (4.1%)	10 (11.1%)	0	1 (6.7%)	0.2
Ureplasma spp.	63 (52.1%)	71 (78.9%)	5 (55.6%)	4 (26.7%)	< 0.01
M. genitalium	0	2 (2.2%)	0	0	0.34
M. hominis	20 (16.5)	50(44.4%)	3 (33.3%)	3 (20.0%)	< 0.01
T. vaginalis	1 (0.8%)	1 (1.11%)	0	1 (6.7%)	0.33
N. gonorrhoeae	0	0	0	0	-
HR-HPV (6 ,18, 31,33, 35, 39, 45, 51, 52, 56, 58, 59)	34 (37%)	25 (50%)	6 (75%)	5 (45%)	0.125
HSV-1	1 (0.8%)	1 (1.1%)	0	0	1
HSV-2	0	1 (1.1%)	1 (11.1%)	0	0.3

HSV – herpes simplex virus; HR-HPV – high-risk human papilloma virus; BV – bacterial vaginosis

Table 3. Association between BV and presence of mono and coinfection

Infection	Normal vaginal flora	BV	Abnormal non-BV microbiota	Total
No presence of STI	37 (30.6%)	10 (11.1%)	9 (37.5%)	56 (23.8%)
Mono infection	51 (42.1%)	30 (33.3%)	5 (20.8%)	86 (36.6%)
Coinfection	33 (27.3%)	50 (55.6%)	10 (41.7%)	93 (39.6%)
Total	121 (51.4%)	90 (38.2%)	24 (10.4%)	235 (100%)

BV – bacterial vaginosis

