Paper Accepted*  ISSN Online 2406-0895

Original Article / Оригинални рад

Sonja Atanasievska-Kujovic¹, Elizabeta Ristanovic¹, Vesna Protic-Dokić¹, Dane Nenadic²

Association of bacterial vaginosis
with the most common sexually transmitted infections

Повезаност бактеријске вагинозе
и најчешћих узроцника сексуално преносивих инфекција

¹Military Medical Academy, Institute of Microbiology, Belgrade, Serbia;
²Military Medical Academy, Centre for Gynecology and Human Reproduction, Belgrade, Serbia

Received: November 16, 2021
Revised: February 3, 2022
Accepted: February 5, 2022
Online First: February 9, 2022
DOI: https://doi.org/10.2298/SARH211116018A

*Accepted papers are articles in press that have gone through due peer review process and have been accepted for publication by the Editorial Board of the Serbian Archives of Medicine. They have not yet been copy-edited and/or formatted in the publication house style, and the text may be changed before the final publication.

Although accepted papers do not yet have all the accompanying bibliographic details available, they can already be cited using the year of online publication and the DOI, as follows: the author’s last name and initial of the first name, article title, journal title, online first publication month and year, and the DOI; e.g.: Petrovic P, Jovanovic J. The title of the article. Srp Arh Celok Lek. Online First, February 2017.

When the final article is assigned to volumes/issues of the journal, the Article in Press version will be removed and the final version will appear in the associated published volumes/issues of the journal. The date the article was made available online first will be carried over.

*Correspondence to:
Sonja ATANASIEVSKA-KUJOVIĆ
Military Medical Academy, Crnotravska 17, 11000 Belgrade, Serbia
E mail: sonja.atanasievska@gmail.com
Association of bacterial vaginosis
with the most common sexually transmitted infections

Повезаност бактеријске вагинозе
и најчешћих узроцика сексуално преносивих инфекција

SUMMARY
Introduction/Objective Bacterial vaginosis (BV) is the most common vaginal dysbiosis that enlarge a possibility of getting sexually transmitted infections (STIs). 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 χ² 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
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, Neisseria 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°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. genitalium* 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 research 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 include as 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 mycoplasmas can prolong the inflammation of the urethra even after the elimination of *C. trachomatis* if there is a resistance to antibiotic therapy. A great number of microorganisms reduce bioavailability of applied medication during therapy of infection. For this reason, it is important 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.

ACKNOWLEDGMENT

This paper is a part of a PhD thesis by Sonja Atanasievska-Kujović, titled “The importance of multiplex quantitative real-time PCR in diagnosis of vaginal dysbiosis,” Medical Faculty of the Military Medical Academy, University of Defense, Belgrade, Serbia.

Conflict of interest: None declared.
REFERENCES


7. The Centers for Disease Control and Prevention (CDC). Sexually Transmitted Infections Treatment Guidelines, 2021


DOI: https://doi.org/10.2298/SARH211116018A Copyright © Serbian Medical Society
Figure 1. Distribution of mono and coinfection
Table 1. Association between BV and presence of total STIs

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

BV – bacterial vaginosis; STI – sexually transmitted infection
**Table 2.** Distribution of sexually transmitted infection pathogens in correlation with the state of vaginal flora

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>Normal flora</th>
<th>BV</th>
<th>Abnormal nonBV</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>BV</td>
<td>Intermediate</td>
<td>Flora of unspecified etiology</td>
</tr>
<tr>
<td>C. trchomatis</td>
<td>5 (4.1%)</td>
<td>10 (11.1%)</td>
<td>0</td>
<td>1 (6.7%)</td>
</tr>
<tr>
<td>Ureplasma spp.</td>
<td>63 (52.1%)</td>
<td>71 (78.9%)</td>
<td>5 (55.6%)</td>
<td>4 (26.7%)</td>
</tr>
<tr>
<td>M. genitalium</td>
<td>0 (0.0%)</td>
<td>2 (2.2%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>M. hominis</td>
<td>20 (16.5%)</td>
<td>50 (44.4%)</td>
<td>3 (33.3%)</td>
<td>3 (20.0%)</td>
</tr>
<tr>
<td>T. vaginalis</td>
<td>1 (0.8%)</td>
<td>1 (1.1%)</td>
<td>0</td>
<td>1 (6.7%)</td>
</tr>
<tr>
<td>N. gonorrhoeae</td>
<td>0 (0.0%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HR-HPV (6, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59)</td>
<td>34 (37%)</td>
<td>25 (50%)</td>
<td>6 (75%)</td>
<td>5 (45%)</td>
</tr>
<tr>
<td>HSV-1</td>
<td>1 (0.8%)</td>
<td>1 (1.1%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HSV-2</td>
<td>0</td>
<td>1 (1.1%)</td>
<td>1 (11.1%)</td>
<td>0</td>
</tr>
</tbody>
</table>

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

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

BV – bacterial vaginosis