The Eurasian Journal of Life Sciences 2026; 2 (1): 57–68

journal homepage: https://www.eajls.com
https://doi.org/10.47093/3033-5493.2026.2.1.57-68

Microbiomics and Metagenomics

Original article

Is there an association between vaginal, urethral and urinary microbiota in women with urogenital tract diseases?

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The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.
All authors read and approved the final version of the manuscript.

Informed consent statement: All patients provided written informed consent to participate in the study.

Funding: The study was not sponsored (own resources).

Competing interests: Anastasia Ivanova and Maria Petrunicheva are employees of the company, reagents of which were used to obtain the results. The authors state that this fact does not affect the quality of the data obtained or their interpretation.

Ethical approval: The study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Sechenov University Institutional Reviewer Board, protocol No. 152, dated 20 April 2022.

Data sharing: The data confirming the findings of this study are available from the

ABSTRACT

Urinary tract infections (UTI), including recurrent cystitis, are usually interpreted in relation to dominant uropathogens. However, the microbial context of adjacent urogenital sites may also be relevant.

Aim. To evaluate the association between UTI and the microbiota of different parts of the urogenital tract in order to provide insights into disease pathogenesis and treatment.

Material and methods. The study included three groups: healthy volunteer group ($n = 34$); patients at risk of developing UTI (women with micronephrolithiasis

authors upon reasonable request. Data and statistical methods used in the article were examined by a professional biostatistician on The Eurasian Journal of Life Sciences editorial staff.

Manuscript source: Unsolicited manuscript.

Country/territory of origin: Russia

Received: 06.04.2026
Accepted: 02.06.2026
Date of publication: 30.06.2026

and/or bacterial vaginosis; *n* = 16); and patients with a history of recurrent lower UTI (*n* = 100). Four types of biomaterial were used: first-pass and midstream urine samples, urethral and vaginal swabs. All samples were analyzed by multiplex real-time polymerase chain reaction reagent kits Femoflor®16 and BacScreen OM.

Results. Genomic DNA and total bacterial quantities increased while relative lactobacilli decreased in patients with a risk of UTI and in those with recurrent lower UTI. This was only the case in midstream and first-pass urine samples. Relative lactobacilli levels in the urethral and vaginal swabs were only slightly but statistically significantly reduced in patients with recurrent lower UTI. Facultative anaerobes predominated in urine samples of patients with a risk of UTI, while in patients with recurrent lower UTI an increase in both facultative and obligate anaerobes was observed.

Conclusion. Midstream and first-pass urine samples can reliably assess inflammation in the urogenital tract. No strict correlation was observed between the vaginal and urinary microbiota of patients with recurrent lower UTI, meaning that UTI do not necessarily affect the vaginal biotope.

Key Words: RT-PCR; dysbiosis; lactobacilli deficiency; recurrent lower UTI; type specimen

Citation: Boldyreva M, Ivanova A, Petrunicheva M, Morozov A, Moskvina Z, Rossolovskaya K, Spivak L. Is there an association between vaginal, urethral and urinary microbiota in women with urogenital tract diseases? *The Eurasian Journal of Life Sciences*. 2026;2(1):57–68. https://doi.org/10.47093/3033-5493.2026.2.1.57-68

MeSH terms:
URINARY TRACT INFECTIONS — MICROBIOLOGY
URINARY TRACT INFECTIONS — PHYSIOPATHOLOGY
VAGINOSIS, BACTERIAL — MICROBIOLOGY
VAGINOSIS, BACTERIAL — PHYSIOPATHOLOGY
MICROBIOTA

Introduction

It is beyond doubt that *Enterobacterales* representatives, mainly *E. coli*, are the leading cause of urinary tract infections (UTI). Vazquez-Montes et al. show that *E. coli* was detected in 65% of positive urine cultures overall and in 68% of index episodes among women with recurrent UTI in a large community-based cohort of women [1]. However, it is also undeniable that UTI and recurrent UTI have a broader etiological spectrum [2].

UTIs are significantly more prevalent in women compared to men [3]. Clearly, the urinary tract microbiota of women differs from that of men: normally, the former is dominated by lactobacilli (LB), as is the adjacent genital biotope [4]. Evidence from numerous clinical studies summarized in reviews shows that a woman's vaginal microbiota affects her susceptibility to UTI [2, 5]. For example, women with bacterial vaginosis (BV) have a higher risk of developing UTI compared to women with a predominance of LB in the vaginal microbiota [2, 6–8]. In addition, clinical trials and reviews have shown that treatments affecting the microbiota (e.g., vaginal probiotics and estrogens) may protect against new relapse episodes of recurrent UTI [2, 9, 10]. Thus, these findings support an association between the female genitourinary microbiota and the development of UTI. However, the precise mechanisms underlying this relationship remain the subject of ongoing clinical research.

With the introduction of molecular techniques, there emerged more possibilities for detecting difficult or unculturable bacteria compared to classical culture methods. Non-culture-based methods also make it possible to assess

quantitative ratios of bacteria in a sample, irrespective of their viability and culturing requirements [11]. Thus, these findings support an association between the female genitourinary microbiota and the development of UTI. However, the precise mechanisms underlying this relationship remain the subject of ongoing clinical research. Moreover, the use of such methods enables a broader microbiological assessment involving different types of biological specimens. Previously, we investigated the contribution of urinary microbiota assessed in midstream urine samples in patients with chronic cystitis [12]. Building on these findings and on evidence that the urinary and vaginal microbiomes are closely linked, we performed an extended evaluation of microbiota from additional sources to assess their diagnostic value [13, 14]. This approach may provide a more comprehensive understanding of the microbial composition of the female genitourinary tract in the context of the infectious process.

The aim of this study was to evaluate the association between urinary tract infections and the microbiota of different biotopes of the urogenital tract in order to provide insights into disease pathogenesis and treatment.

Materials and methods

Study design

This multicenter, observational, cross-sectional comparative study was conducted to evaluate and compare microbiota profiles in four types of urogenital specimens obtained from women with recurrent lower UTI, women at increased risk of UTI, and healthy controls. The study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Sechenov University Institutional Reviewer Board, protocol No. 152, dated 20 April 2022.

Setting

The study was conducted at “On Clicic Lux LLC” (Moscow), State Budgetary Institution “GP 46 DZM” (Moscow), and Semeynaya Poliklinika #4 LLC (Korolev), Russia, between April 2022 and May 2023. Participant recruitment and specimen collection were performed during routine outpatient evaluation. Given the nature of the cross-sectional design, no longitudinal follow-up was planned.

Participants

An informed consent, signed and dated by a patient, was obtained prior to conducting any study-related procedures. Non-pregnant women of reproductive age from 18 to 45 years old were enrolled in the study. The exclusion criterion for all participants was acute (and relapses of chronic) vulvovaginitis due to sexually transmitted infections. Three study groups have been formed.

The first group (“Control”) included healthy volunteers, with no dysuria and/or other complaints indicative of UTI, absence of symptoms and signs of chronic inflammatory urogenital tract diseases; absence of changes in general urinalysis and non-use of antibacterial medications within the last 3 months. Participants in this group were excluded from the study if they had received glucocorticosteroids or any other immunosuppressant (due to autoimmune diseases) or intravaginal medications, including contraceptives, within the last 3 months.

The second group (“Risk of UTI”) included patients with no dysuria and/or other complaints indicative of UTI, but with micronephrolithiasis or/and BV

verified during assessment and non-use of antibacterial medications within the last 3 months. Participants in this group were excluded if they had received glucocorticosteroids or any other immunosuppressant (due to autoimmune diseases) or intravaginal medications, including contraceptives, within the last 3 months.

The third group included patients with a history of recurrent lower UTI "rLUTI" defined as two relapse episodes within six months or three episodes within one year; clinically and laboratory confirmed relapse of rLUTI; incapable of childbearing, or capable of childbearing but with negative pregnancy test at the primary visit and agreement to consistently and appropriately use one of the acceptable contraception methods. Exclusion criteria were as follows: postcoital cystitis; complicated UTI; pregnancy planning in the next 6 months; absence of laboratory verification of rLUTI; identification of sexually transmitted infections; non-adherence to the prescribed medication regimen or presence of side effects; patient withdrawal from further participation in the study.

After screening (n = 172), a total of 150 participants were enrolled in the study: 34 participants in the "Control" group, 16 patients in the "Risk of UTI" group, and 100 patients in the "rLUTI" group.

Variables

The primary study variables were the human genomic DNA signal, total bacterial load, the relative abundance of *Lactobacillus* spp., and the relative abundance of facultative and obligate anaerobic microorganisms in each specimen type.

Data sources / measurement

The clinical specimen for the study were the first-pass and midstream samples of morning freely discharged urine obtained after genital toilet, urethral swabs obtained prior to urination and swabs from the posterolateral vaginal wall. All specimens were collected on the same day.

DNA extraction was performed from freshly obtained urine samples and urethral and vaginal swabs using a PREP-MB MAX reagent kit (DNA Technology, Moscow). The extracted DNA was stored in –40 °C prior to polymerase chain reaction (PCR). PCR was performed using a DT Prime amplifier (DNA Technology, Moscow) in accordance with the manufacturer's user manual. The number of target DNA copies (lg10) in the sample was calculated using the threshold cycle comparison method, also known as the $\Delta\Delta C_T$ method [15].

The DNA samples were analyzed by multiplex real-time PCR using Femoflor®16 and BacScreen OM reagent kits (DNA Technology, Moscow). Both tests are based on multiplex real-time PCR, which allows to determine the amount of DNA of the sought microorganism in a sample, expressed in genome equivalents. The quantity of genome equivalents is proportional to the number of microorganism cells. Femoflor®16 test is used to determine the concentration of bacterial DNA — total bacterial mass (TBM). The detailed description of each test is available within our previous publication [12].

Bias

Several measures were taken to reduce potential bias. Participants received standardized instructions for urine collection to minimize contamination. Urethral and vaginal specimens were collected according to a standard procedure. The same laboratory workflow was used for all groups. Nevertheless, contamination of voided urine samples, personnel variation in swab sampling, and residual confounding could not be fully excluded

Study size

No formal a priori sample size calculation was performed. The study to the exploratory nature of the study, the final sample size was determined by the number of eligible women recruited during the predefined study period. Overall, 150 participants were enrolled.

Quantitative variables

Quantitative PCR-derived variables were analyzed as logarithmic values where applicable. For microbiota composition analyses, the abundance of individual taxa or bacterial groups was normalized to TBM and expressed as a percentage of the total bacterial signal. For the reason that the distributions were anomalous, continuous variables were summarized using medians and interquartile ranges.

Statistical methods

Statistical analysis was performed using IBM SPSS Statistics version 29 for Windows (IBM, USA). The distribution of continuous variables was assessed using the Shapiro–Wilk test, which showed no normal distribution of the analyzed data. The Mann–Whitney *U*-test was used to analyze the differences between the groups.

Results

Quantitative characteristics of the specimens across the study groups

A total of 150 participants met the eligibility criteria for this study. Out of them, 34 healthy volunteers were in the "Control" group, 16 participants were in the "Risk of UTI" group, and 100 patients were in the "rLUTI" group.

The quantitative assessment of the specimens showed differences in the overall microbial characteristics across the study groups. The data presented

Table 1. Genomic DNA levels in participants

Type specimen	Control *n* = 34	Risk of UTI *n* = 16	rLUTI *n* = 100
Midstream urine	0.0 (0.0;3.4)	3.3 (0.8;3.8)*	4.0 (3.3;4.9)#
First-pass urine	3.5 (0.0;4.0)	4.0 (3.6;4.1)*	4.4 (3.6;5.1)#
Urethral swab	4.1 (3.7;4.4)	4.2 (3.7;4.3)	4.1 (3.7;4.5) (*n* = 99)
Vaginal swab	4.9 (4.4;5.2)	4.6 (4.5;5.2)	4.9 (4.6;5.2) (*n* = 98)

Note: values are presented as median (*Q*1; *Q*3) of logarithmic values; * – $p < 0.05$ compared with "Control"; # – $p < 0.001$ compared with "Control". Pairwise comparisons were performed using the Mann–Whitney *U* test.

Table 2. Levels of total bacterial mass in participants

Type specimen	Control *n* = 34	Risk of UTI *n* = 16	rLUTI *n* = 100
Midstream urine	5.1 (3.7;6.0)	6.1 (5.2;6.7)*	6.8 (5.1;8.1)#
First-pass urine	5.7 (4.4;6.6)	6.2 (5.7;7.4)*	6.8 (5.9;8.1)#
Urethral swab	5.3 (4.6;6.0)	5.1 (4.6;5.8)	5.1 (4.5;5.9) (*n* = 99)
Vaginal swab	6.9 (6.6;7.3)	6.8 (6.5;7.4)	6.8 (6.2;7.2) (*n* = 98)

Note: values are presented as median (*Q*1; *Q*3) of logarithmic values; * – $p < 0.05$ compared with "Control"; # – $p < 0.001$ compared with "Control". Pairwise comparisons were performed using the Mann–Whitney *U* test.

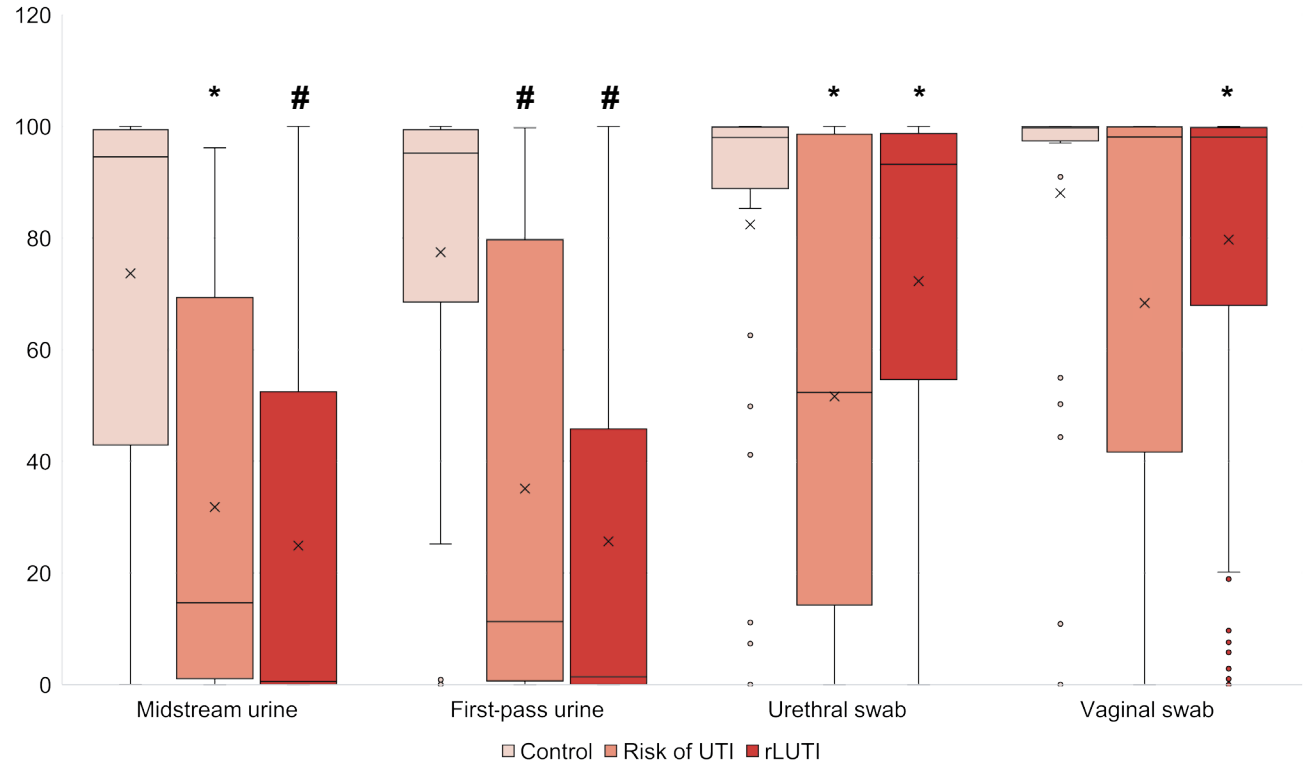
show an increase in genomic DNA and bacterial quantities in patients with rLUTI as well as in patients with microlithiasis and/or BV compared to healthy controls, but only in midstream and first-pass urine samples. No differences were observed in urethral and vaginal swabs between the study groups. The quantitative values of genomic DNA and TBM for the midstream urine, urethral swabs, and vaginal swabs are presented in Tables 1 and 2.

Site-specific microbiota composition in urine, urethral, and vaginal samples

A detailed comparison of the participants' microbiota was then performed. The microbiota was compared between the studied groups based on the number of bacteria/bacterial groups normalized to the TBM for each biotope.

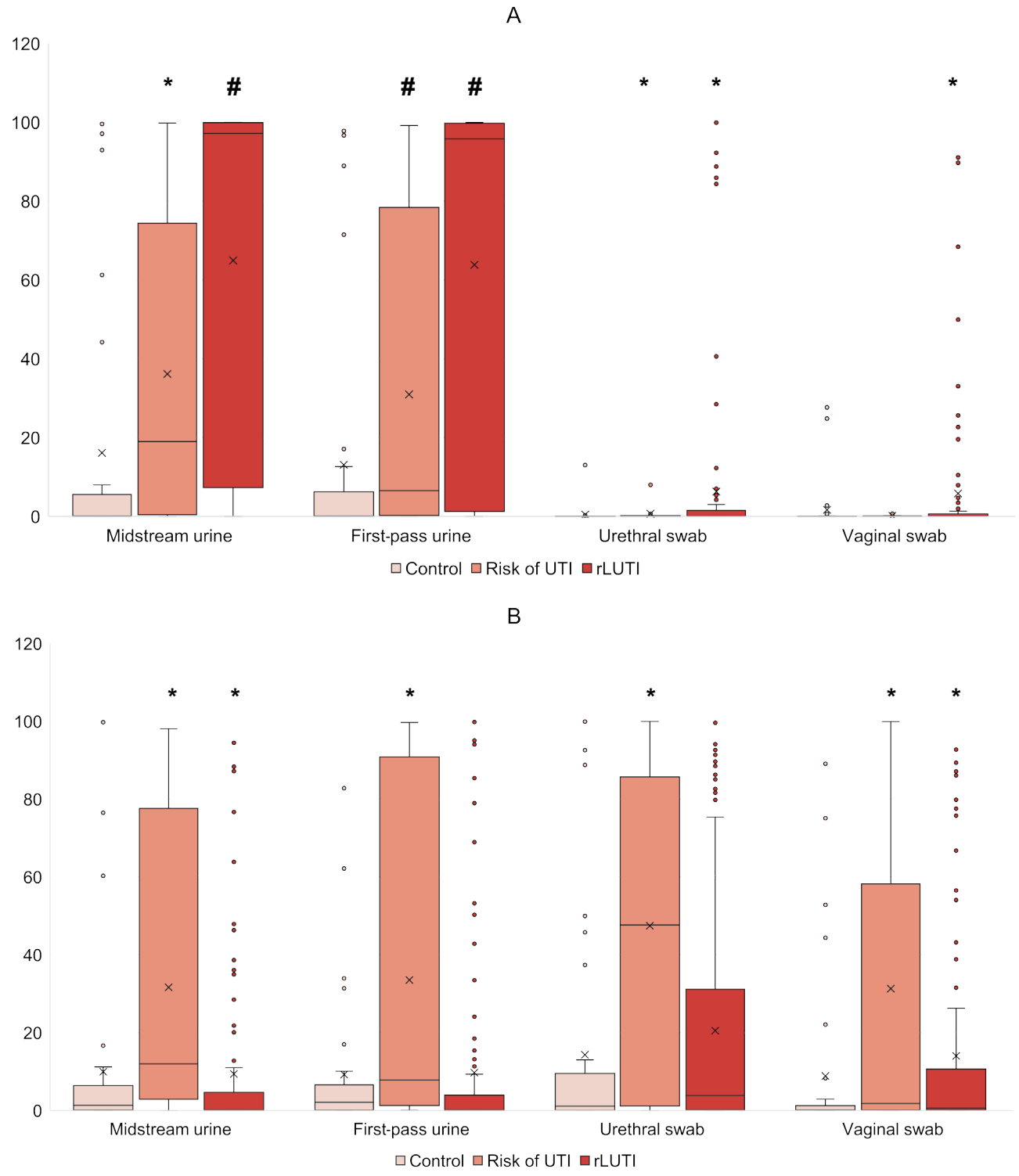
The relative presence of LB, a major contributor to urogenital normobiota in women, was critically reduced in both urine samples in patients with rLUTI compared with healthy volunteers. In patients with rLUTI, LB levels were slightly but statistically significantly reduced in urethral and vaginal swabs compared with in the "Control" group ($p = 0.022$ and $p = 0.008$, respectively). A decrease in relative LB levels was observed in the urethral swabs of patients with risk of UTI, compared with in the "Control" group ($p = 0.024$). Their vaginal swabs also showed a decrease in LB compared with the "Control" group, but the difference was not statistically significant ($p = 0.086$), but only in a minority of patients. Relative levels of *Lactobacillus* spp. in midstream urine, first-pass urine samples and urethral, vaginal swabs of participants are presented in Figure 1.

FIG. 1. *Lactobacilli* proportion in different samples



Note: relative levels of *Lactobacillus* spp. are presented as percentages of the total bacterial mass. "Control" group, $n = 34$ for all types of specimens; "Risk of UTI" group, $n = 16$ for all types of specimens; "rLUTI" group, $n = 98$ for first-pass urine, vaginal swab and urethral swab, $n = 100$ for midstream urine.
* – $p < 0.05$ compared with "Control"; # – $p < 0.001$ compared with "Control". Pairwise comparisons were performed using the Mann–Whitney *U* test.

FIG. 2. Type of microorganism proportion in different samples



Note: data are presented as median (*Q*1; *Q*3). Relative levels of facultative and obligate anaerobic microorganisms are expressed as percentages of the total bacterial mass. "Control" group, *n* = 34 for all types of specimens; "Risk of UTI" group, *n* = 16 for all types of specimens; "rLUTI" group, *n* = 97 for urethral swab, *n* = 98 for first-pass urine and vaginal swab, *n* = 100 for midstream urine. Pairwise comparisons were performed using the Mann–Whitney *U* test; * — $p < 0.05$ compared with "Control"; # — $p < 0.001$ compared with "Control"; A — Facultative anaerobic microorganisms in different samples (%); B — Obligate anaerobic microorganisms (%).

The relative LB quantities were also reduced in patients with risk of UTI in both urine portions, but to a lesser extent compared to patients with rLUTI. The normalized values for the midstream urine, first-pass urine samples, urethral and vaginal swabs are presented in Supplementary Tables 1–4, respectively (supplementary materials on the journal website https://doi.org/10.47093/3033-5493.2026.2.1.57-68-annex).

The decrease in the relative quantities of LB was accompanied by an increase in other bacteria/bacterial groups. Figures 2A and 2B present comparative plots of the relative quantities of total facultative and obligate anaerobic microorganisms in all studied groups.

In patients with rLUTI, both urine portions were dominated by facultative anaerobes, primarily bacteria of the order The predominance of *Enterobacterales* was accompanied by a significant decrease in *Lactobacillus* spp. in both urine portions ($p < 0.001$). Among the other bacterial groups, significant differences from healthy controls were limited to *Lachnobacterium* spp./*Clostridium* spp. and *Mobiluncus* spp./*Corynebacterium* spp. in both urine samples, with an additional significant difference in *Staphylococcus* spp. in first-pass urine. Thus, the main microbiological pattern in patients with rLUTI was the marked replacement of lactobacilli by *Enterobacterales*-dominated facultative anaerobic microbiota.

In the "Risk of UTI" group, a decrease in urinary LB level was associated with an increase in both facultative and obligate anaerobes. In both urine samples, facultative anaerobes were represented by the order *Enterobacterales*, while obligate anaerobes comprised of *Gardnerella vaginalis*/*Prevotella bivia*/*Porphyromonas* spp., *Eubacterium* spp., *Sneathia* spp./*Leptotrichia* spp./*Fusobacterium* spp., *Megasphaera* spp./*Veillonella* spp./*Dialister* spp., *Peptostreptococcus* spp., *Atopobium vaginae.* At the same time, a decrease in LB in the urethral swabs was linked to an increase in obligate anaerobic microbiota, in contrast to healthy volunteers ($p = 0.011$). The representation of anaerobic microorganisms was almost the same as in urine samples (Supplementary Tables 1, 2). In vaginal swabs, a decrease in lactobacilli was also accompanied by an increase in anaerobic microbiota of the similar spectrum in a small proportion of patients (Supplementary Tables 3, 4).

Discussion

In the present study, the composition of microbiota of first-pass and midstream urine, urethral and vaginal mucosa in women was investigated by real-time PCR to evaluate the association between urinary tract infections and the microbiota of different biotopes of the urogenital tract. In our previous study [12] we have shown that for midstream urine sample, the biomaterial traditionally used for diagnosing cystitis, qualitative classification (AUC = 0.88 (0.81; 0.95)) of patients with rLUTI and healthy controls is based on three main indicators: the amount of genomic DNA originating from human epithelial cells and leukocytes, TBM, and the proportion of LB in the total bacterial count. Therefore, the present study employed the same indicators for other biotopes as well.

In patients of "rLUTI" group, the amount of genomic DNA in midstream and first-pass urine samples significantly increased in comparison to the "Control" group. At the same time, no differences were observed in the urethral and vaginal swabs between patients with rLUTI and healthy volunteers. It should, however, be noted that the quantity of genomic DNA in a swab depends on an external factor: the biomaterial is collected by a doctor. The amount of

genomic DNA in freely released urine is less dependent on external factors, so this indicator is more objective, and perhaps it can be used as a laboratory criterion for inflammation in the urinary tract, which is standardly assessed based on the number of epithelial cells and leukocytes in the urinalysis [16, 17]. However, within current trial we did not assess such correlation. The absence of significant differences in urethral and vaginal swabs between patients of "rLUTI" group and healthy volunteers may also be explained by the dependence of swab-based quantitative results on the technique of material collection.

TBM is an indicator of bacterial quantity in a sample [18]. In patients of "rLUTI" group, it was significantly higher than in the "Control" group in both urine samples. However, in the urethral and vaginal swabs, the bacterial quantities did not differ between patients from all groups. The same pattern is true for genomic DNA, which may reflect the dependence of the quantitative result of scraping on the technique of material collection.

Our findings support the view that the ratios between opportunistic and normobiotic bacteria may be more informative for assessing microbiota disorders than their quantitative values. Han et al. also reported on the importance of maintaining a balance between the normal microbiota and opportunistic/anaerobic bacteria in the development of gynecological diseases, including inflammatory conditions [19]. Normalizing particular species or groups of bacteria to the total bacterial quantity, i.e. calculating the proportion of a specific microorganism or a group of microorganisms in the TBM of a sample, provides such a possibility. Additionally, normalization to the TBM makes it possible to compare the microbiota from biomaterial specimens obtained using different methods, including swabs and free-flowing urine.

Thus, the relative quantity of LB, the main component of urogenital normobiota in women [20, 21], was critically reduced in both the midstream and first-pass urine samples in patients with rLUTI as compared to healthy controls. The levels of LB were also reduced in urethral and vaginal swabs, but in a very small fraction of patients. This decrease was less pronounced in swabs than in urine samples.

A decrease in LB has to be associated with increased levels of other bacteria. The main question is, "What other bacteria have so significantly 'edged out' lactobacilli in patients with rLUTI?" In both the midstream and first-pass urine samples of these patients, facultative anaerobes were dominant, with almost all of them belonging to the order *Enterobacterales*. This pattern reflected a significant decrease in *Lactobacillus* spp. and a significant increase in *Enterobacterales*-dominated facultative anaerobic microbiota compared with healthy controls. Among the other bacterial groups, significant differences from controls were limited to *Lachnobacterium* spp./*Clostridium* spp. and *Mobiluncus* spp./*Corynebacterium* spp. in both urine samples, with an additional significant difference in *Staphylococcus* spp. in first-pass urine. Conducted results support the concept that rLUTI is not necessarily limited to the predominance of one uropathogen, but may reflect a broader urogenital dysbiosis. This is also in line with previous studies in which the urinary microbiota of women with recurrent UTI/recurrent cystitis differed from that of controls, including shifts in *Lactobacillus*, *Enterobacterales*, and *Escherichia*/*Shigella* [22, 23].

Lewis et al. analyzing the relationship between vaginal microbiota and UTI suggested that "vaginal bacteria may cause UTI, either themselves (i.e. a traditional uropathogen using the vagina as a reservoir) or by acting as a 'covert pathogens' to facilitate pathogenesis of another organism" [2]. In the present study, analysis of the microbiota of urine, urethral and vaginal swabs of patients with rLUTI showed that a variant of UTI without evident involvement

of the vaginal biotope is possible. Perhaps the described variant of UTI with a significantly altered urinary microbiota and a completely normal vaginal microbiota in patients of "rLUTI" group is a variant of the course of UTI, or it can be a stage of disease progression.

Patients in "Risk of UTI" group had no evident symptoms of urinary infections, but they were not included in the healthy "Control" group due to having microlithiasis and/or BV as a diagnosis. Since both BV and stone formation have been associated with abnormalities of the urinary tract microbiota [24–26], we deemed it reasonable to include these women in the study as a separate group of patients, whom we considered 'neither obviously ill nor healthy'.

The microbiota profile of patients in the "Risk of UTI" group can be considered as an intermediate variant between healthy volunteers and patients of "rLUTI" group. Although these women had no evident symptoms of urinary tract infection, their urine samples showed higher genomic DNA and TBM values, together with a decrease in urinary LB, compared with healthy controls. The decrease urinary lactobacilli was linked to an increase in both facultative and obligate anaerobes, in approximately equal proportions. Facultative anaerobes were represented by *Enterobacterales*, while obligate anaerobes mainly constituted of microorganisms associated with BV [27, 28]. In contrast to patients with recurrent lower urinary tract infection, in whom LB were dominant in urethral and vaginal swabs, in patients with urolithiasis and/or BV the proportion of LB in urethral swabs was reduced in some patients [29, 30]. Thus, urinary and urethral microbiota alterations may be detected even when vaginal dysbiosis is not clearly expressed, although in cases where lactobacilli were reduced in the urethra and vagina, this decrease was accompanied by an increase in a similar spectrum of anaerobic microbiota associated with BV.

A limitation of this study is clearly the small group size and heterogeneity of patients with BV and/or urolithiasis, which makes it difficult to reach definitive conclusions. Nevertheless, the obtained results suggesting that decreased lactobacilli and increased BV-associated anaerobic microbiota in urine and urinary tract mucosa in the absence of clinical UTI signs may partially support the hypothesis that BV-associated organisms may play an important role in the etiology of uropathology and uropathogenesis. However, more studies are needed to determine the causal role of these organisms in the development of symptomatic UTI.

Undoubtedly, larger sample sizes are needed for a study with more specific grouping. The use of the presented research tools is promising. Future studies may be able to identify combinations of pathotype variants of the urinary and genital microbiota in different variants/stages of disease, which, in turn, would allow the development of more specialized approaches to treatment and prevention of urinary infections.

Another potential limitation is possible contamination of the specimens. Obtaining urine with a catheter might reduce it, but we decided to avoid this invasive test. In order to standardize the procedure of specimen collection, patients were instructed in detail how to perform it properly.

Conclusion

Analyzing biomaterial samples from different segments of the urogenital tract provides a comprehensive approach to studying and diagnosing UTI. Analysis of midstream and first-pass urine samples yielded similar results, suggesting that both samples can be reliably used to determine the presence

of inflammation in the urogenital tract. At the same time, the findings from the urethral and vaginal swabs reveal a different pattern. For instance, comparing the vaginal and urinary microbiota of patients of "rLUTI" group to showed that UTI do not necessarily affect the vaginal biotope. The swabs, however, appear to be less objective as a diagnostic tool due to being dependent on the quality of the swab itself. Thus, the presented data may serve as supporting evidence when selecting the type of specimen for microbiological assessment in the diagnosis of UTI.

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