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Vaeinal microbiota in war-affected Ukrainian women

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ABSTRACT

Introduction: This study aims to investigate the state of the vaginal microbiota and the effectiveness of its correction in women with benign uterine tumors who are under the influence of stress factors caused by the armed conflict in Ukraine.

Material and methods: Bacteriological studies were conducted to assess the species and quantitative composition of the vaginal microflora in women. The research groups consisted of women who had been in the combat zone (30 women) and women who were under the influence of the armed conflict's factors (Kyiv city and Kyiv region) (30 women).

Results: The results indicate a significant deficiency of *Lactobacillus* spp. in all groups of women, accompanied by excessive growth of conditionally pathogenic facultative anaerobic and obligate anaerobic microorganisms. The most pronounced dysbiotic changes were observed in women of Group I, who experienced the most intense and prolonged stress.

Conclusion: The findings suggest that chronic psychosocial stress is not only a trigger for dysbiosis but also a factor that modulates the effectiveness of treatment. This points to the need for developing integrated therapeutic approaches that combine antimicrobial therapy with measures to reduce psychoemotional stress.

Keywords: vaginal microbiota, stress, benign uterine neoplasms, *Lactobacillus* spp., *Candida*, conditionally pathogenic microorganisms

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Introduction

The full-scale invasion of Ukraine by the Russian Federation has had a significant impact on the lives and health of Ukrainians. Since the beginning of the full-scale invasion, the issue of chronic stress has acquired a new, more urgent context, becoming one of the most pressing concerns for obstetrician-gynecologists [1].

It is well-known that powerful stress loads during armed conflict can lead to the failure of adaptive systems and cause significant negative changes in the state of immunity and the functioning of endocrine organs [2, 3].

Psychoemotional stress contributes to the activation of persistent viral-bacterial infections and, in particular, can be accompanied by a disruption of the microecology of the genital organs and a change in the ratio of associates within the microbiocenosis of the reproductive tract [4–7].

On the modern stage, the molecular mechanism behind the development of uterine tumors remains insufficiently studied [8, 9]. Changes in the endocrine system, immune status, stress, and genital dysbiosis may play a role in the pathogenesis of benign tumors in women residing in a war conflict zone [10, 11].

According to the literature, vaginal microbiota disorders in women with uterine tumors can manifest as vaginitis, cervicitis, and urethritis. Candidiasis and bacterial vaginosis constitute a significant proportion of the pathological changes in the vaginal microbiota of patients with tumors [12, 13].

According to literature, changes in the microecology of the genital tract in patients with benign uterine tumors are caused by mixed infections, often associated with the active proliferation of facultative anaerobic microflora (*Staphylococci*, *Streptococci*, *Enterobacteria*) [14–16].

In recent years, the use of genomic research methods in microbiological practice has expanded the spectrum of obligate anaerobic microflora that contaminates the female reproductive tract [7, 17].

Specifically, the following anaerobic microorganisms are isolated from the genital and urethral tracts: *Anaerococcus prevotii* [18], *Actinomyces odontolyticus* [19], *Paraclostridium bifermentans* [20], and *Clostridium novyi* [21].

Given the negative impact of stress on the human body and the microbial imbalance that forms in patients with benign uterine neoplasms, it is appropriate to study the state of the vaginal microbiota and to improve existing methods of therapy for uterine leiomyoma in women, taking into account the factors of the state of war [22–24].

The purpose of this study was to investigate the characteristics of the vaginal microbiota and evaluate the effectiveness of its correction in women with benign uterine tumors who are under the influence of stress factors from the armed conflict in Ukraine.

Material and methods

Bacteriological studies were conducted to assess the species and quantitative composition of the facultative anaerobic and obligate anaerobic microflora of the vagina in women with benign uterine tumors (leiomyoma) under conditions of a state of war in Ukraine.

A total of 90 patients were examined: Group I consisted of civilian women who had been in combat zones (30 women); Group II consisted of civilian women who were under the influence of armed conflict factors (Kyiv city and Kyiv region) (30 women). All study participants were civilians and had regular access to basic personal hygiene products.

Data obtained from the examination of 30 healthy women before the beginning of the armed conflict in Ukraine were used as a control. It should be noted that no women from regions unaffected by hostilities during the war were available for recruitment. This constitutes an important limitation of the study.

The diagnosis of uterine leiomyoma was verified using clinical and instrumental methods of examination. The ICD-10, created in accordance with World Health Organization expert recommendations and adopted in Ukraine, was used for establishing gynecological diagnoses in women.

The age of the study participants ranged from 26 to 40 years, with a body mass index of 18.5–24.9. At the time of the examination, the women were not pregnant

and were not taking hormonal contraceptives or any other medications.

Based on the gynecological history, 46.6% of women had previously been treated for colpitis of various etiologies, 33.3% of patients suffered from menstrual cycle disorders, and 16.6% had a history of cervical erosion.

Somatic history was burdened in 30% of women: 16.6% were diagnosed with cardiovascular pathology, while 13.4% of the examined women had diseases of the gastrointestinal tract (gastritis, cholecystitis).

Microbiological studies and the recording of their results were carried out in accordance with Order No. 1614 of the Ministry of Health of Ukraine dated 03.08.2021, "On the procedure for epidemiological surveillance and the recording of healthcare-associated infections," as well as other relevant regulatory documents.

For culturing vaginal mucus, the following differential diagnostic media were used: blood agar, yolk-salt agar, Endo agar, Lactoagar for lactobacilli, a chromogenic medium for Group B streptococci (bioMérieux, France), Sabouraud agar, and the chromogenic medium "Chromagar Candida" (SANIMED-M LLC, Ukraine), which allows for species-level differentiation of *Candida* fungi.

Cultures were performed using the quadrant streak method on solid nutrient media, which allows for the determination of the degree of microbial contamination and the identification of the widest possible spectrum of aerobic and facultative anaerobic microflora.

The anaerobic microflora of the genital tract was studied with strict adherence to anaerobic cultivation techniques.

Material obtained without oxygen exposure was cultured on solid nutrient media with subsequent subculturing. The material was then submerged at the bottom of a test tube containing an enrichment medium. For the study, solid and liquid nutrient media were used (thioglycolate medium, blood agar with glucose, liver broth, Kitt-Tarozzi medium, and a selective agar for detecting *Gardnerella vaginalis* with a 7% sheep blood content).

All cultures were placed in an anaerobic incubator at 37°C for 7 days. In parallel, cultures were grown under aerobic conditions. The "Anaerocult" system (Merck, Germany) and "Anaerogas" gas packs were used to create anaerobic conditions. When signs of bacterial growth appeared, smears were prepared, Gram-stained, and examined under a microscope.

The identification of the isolated microorganisms was carried out based on their cultural, morphological, tinctorial, and biochemical properties using an automated microbiological analyzer, the Vitek 2 Compact (France).

In addition to the culture method, gardnerellosis was diagnosed via bacterioscopy by Romanowsky staining of smears, followed by the counting of "clue" cells, an amine test, and pH determination.

For the treatment of vulvovaginal candidiasis, a regimen was developed that included: Clotrimazole (topical, suppositories), the use of a multi-strain probiotic for women with a dual mechanism of action, "Probisfemina," and the phytocomplex with caprylic acid, "ReproCandid".

For the treatment of bacterial vaginosis, a therapeutic regimen was used that included: Secnidazole, Serratiopeptidase, topical therapy with the combined drug "Gynex Forte" (containing metronidazole and miconazole nitrate), and the use of the multi-strain probiotic "Probisfemina".

Repeat studies of the vaginal microbiota after treatment were performed 14 days after the end of the treatment.

The statistical processing of the obtained research results was carried out using the standard computer package "Data Analysis" in Microsoft Excel for Windows 2007. The values calculated were the arithmetic mean (M), the standard error of the mean (m), and the probability value (p). The significance of the obtained data was assessed using the generally accepted Student's t-test. The results were considered statistically significant if their probability was no less than 95% ($p < 0.05$).

Results

An examination of vaginal material from women in Group I, who were in the combat zone, revealed negative changes in the state of the vaginal microbiota.

The results of the bacterioscopic study of the vaginal contents indicate enhanced desquamation, vacuolization of epithelial cells, an increased number of leukocytes (30–100 per field of view), a significant density of Gram-negative and Gram-positive microflora, a deficiency of morphologically normal microflora, and an increased frequency of yeast cells and pseudomycelia. An increase in the number of "clue" cells and the presence of microflora morphologically similar to vaginitis-associated species were also observed.

Culture-based bacteriological examination of vaginal contents from Group I patients revealed microflora from the phylum *Firmicutes*, family *Enterobacteriaceae*, *Proteobacterium*, and fungi of the genus *Candida* (Tab. 1).

The detected microflora was found in three- and four-component associations. The frequency of three-

-component associations was 66.6%, while four-component associations were found in 33.3% of cases.

The examined patients showed an increased frequency and quantitative level of *E. coli* (hem+), *Klebsiella pneumoniae*, and *Enterobacter cloacae*. The quantitative levels of these microflora species were statistically significantly higher than the norm (Fig. 1).

In the spectrum of the vaginal microflora of women in Group I, an increased frequency of pathogenic *Firmicutes* was found: *S. epidermidis* hem+, *S. aureus*, *Str. pyogenes*, and *Str. agalactiae*. The quantitative levels of Gram-positive coccal microflora exceeded the diagnostic level ($> 10^4$ CFU/mL).

An increased frequency and quantitative level of *Candida* fungi were registered in the examined women of Group I (20% and 10^5 CFU/mL, respectively).

The results of the vaginal material study from women in Group I revealed a decrease in the quantitative level of *Lactobacillus spp.* The frequency of normal microflora registration in patients of Group I did not reach the level found in healthy women.

One of the objectives of the study was to evaluate the degree of vaginal contamination with obligate anaerobic microflora. According to the results, the most frequently isolated species from women's vaginas were *Peptostreptococcus spp.*, *Fusobacterium spp.*, *Veillonella spp.*, *Paraclostridium bifermentans*, *Clostridium novyi*, and *Anaerococcus prevotii*. The quantitative level of anaerobic microorganisms in the vagina also exceeded the level found in healthy women.

The increased frequency of *Gardnerella vaginalis* and *Atopobium vaginae* (26.7% and 43.3%, respectively) within the vaginal microbiota is a negative finding, which may indicate the development of bacterial vaginosis.

Given the negative changes in the vaginal microbiocenosis of women in Group I, a complex therapy was applied aimed at correcting the detected microflora disorders.

The results of a bacterioscopic examination of vaginal contents from women in Group I after treatment indicated positive changes in the microbiota. These included a decrease in the number of leukocytes (10–25 per field of view), reduced epithelial cell desquamation, a lower frequency of "clue" cells, decreased microbial density, and fewer yeast cells. An increase in the presence of microflora morphologically resembling *Lactobacillus spp.* and a decrease in the presence of vaginitis-associated species were also established.

The results of the culture-based bacteriological examination of vaginal discharge in Group I patients after treatment allowed for a decrease in the frequency of associative forms of bacterial contamination of the

Table 1. Microorganisms predominant in the vaginal microbiota of women with benign uterine tumors who were in occupied territories, before and after treatment (group I) [%]

Microorganisms	Patients of group I before treatment n = 30	Patients of group I after treatment n = 30	Healthy women n = 30
	[%]	[%]	[%]
<i>S. aureus</i>	13.3	3.3	3.3
<i>S. epidermidis</i> hem+	20.0	6.7	6.7
<i>S. epidermidis</i>	10.0	6.7	10.0
<i>S. agalactiae</i>	16.7	10.0	6.7
<i>S. piogenes</i>	10.0	3.3	–
<i>E. faecalis</i>	13.3	6.7	13.3
<i>E. coli</i> hem+	16.7	3.3	6.7
<i>E. coli</i>	10.0	6.7	13.3
<i>Enterobacter</i> spp.	16.7	10.0	6.7
<i>Klebsiella</i> spp.	13.3	3.3	3.3
<i>Candida albicans</i>	20.0	13.3	10.0
<i>Gardnerella vaginalis</i>	26.7	16.7	10.0
<i>Atopobium</i> spp.	43.3	30.0	13.3
<i>Veillonella</i> spp.	23.3	16.7	6.7
<i>Prevotella</i> spp.	26.7	13.3	23.3
<i>Bacteroides</i> spp.	16.7	20.0	30.0
<i>Peptostreptococcus</i> spp.	20.0	13.3	10.0
<i>Fusobacterium</i> spp.	13.3	10.0	6.7
<i>Paraclostridium bifermentas</i>	36.7	26.7	33.3
<i>Clostridium novyi</i>	33.3	30.0	26.7
<i>Anacrococcus prevotii</i>	30.3	33.3	36.7
<i>Staphylococcus saccharalyticus</i>	26.7	30.0	30.0
<i>Lactobacillus</i> spp.	76.7	83.3	100.0

vagina. Compared to the data obtained before treatment, the frequency of four-component associations decreased to 13.3%, and three-component associations to 43.3%. The frequency of two-component associations increased (43.3%).

After treatment, a decrease was observed in both the frequency and the quantitative level of vaginal contamination by *E. coli* with hemolytic properties, *S. haemolyticus*, and *S. aureus*. The quantitative levels of these microflora species after treatment did not exceed the diagnostic level (< lg 4.0 CFU/mL).

A less favorable dynamic was observed in women of Group I after treatment regarding the indicators of vaginal contamination with obligate anaerobic microflora. The frequency and quantitative levels of these microorganisms showed only a tendency to decrease.

For instance, the isolation frequency of *Peptostreptococcus* spp. after treatment was 13.3%, and for *Fusobacterium* spp. it was 10.0%. The quantitative levels for these species were lg 5.6 CFU/mL and lg 4.8 CFU/mL, respectively.

The level of vaginal contamination with certain types of obligate anaerobic microflora (*Bacteroides* spp., *Prevotella* spp.) approached normal levels after treatment (lg 5.0 CFU/mL and lg 4.8 CFU/mL).

After the conducted treatment, complete normalization of vaginal contamination with vaginitis-associated microflora was not observed in the examined patients. Specifically, *Atopobium* spp. was isolated from women in Group I with a frequency of 30.0%, and *Gardnerella vaginalis* with a frequency of 16.7%. The quantitative levels of these microflora representatives showed a tendency to decrease.

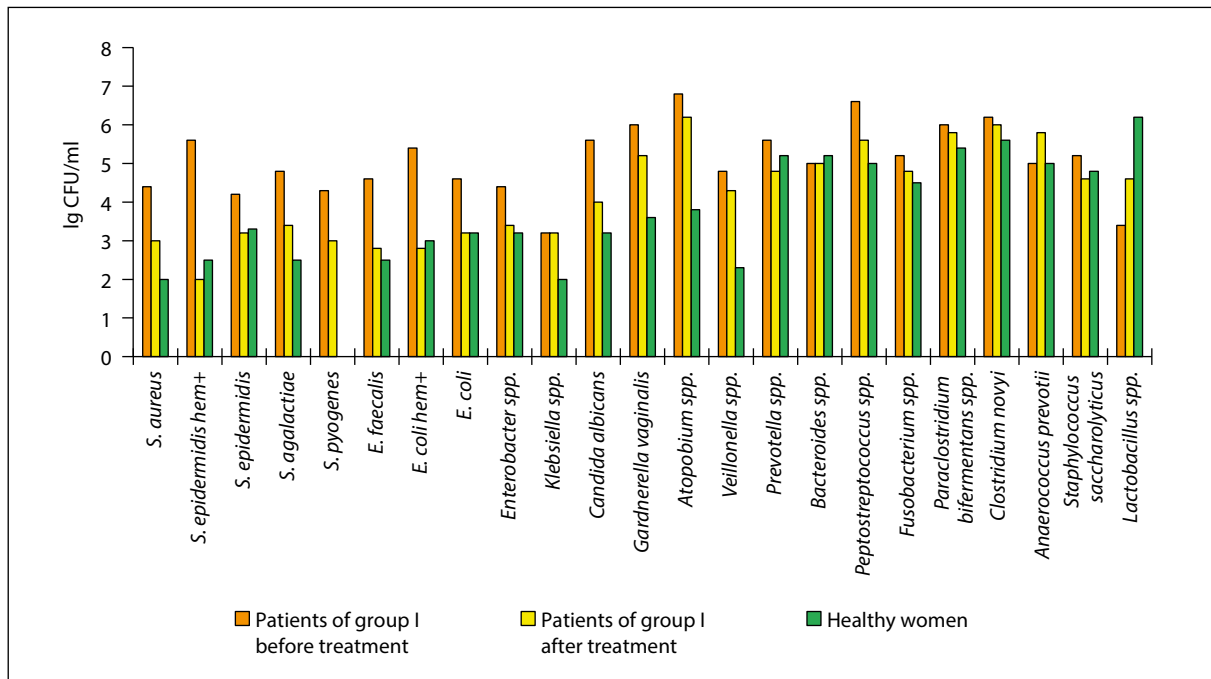


Figure 1. Vaginal microbiota of women with benign uterine neoplasms who were in the occupied territories before and after treatment (group I)

The examination of Group I patients after treatment showed a trend toward normalization of the *Candida* group indicators (a frequency of 13.3% and a concentration of lg 4.0 CFU/mL).

The conducted therapy course contributed to an increase in vaginal *Lactobacillus spp.*, but a complete restoration of their levels to normal indicators was not observed.

Thus, the results of the examination of Group I patients before treatment allowed us to establish the formation of negative changes in the composition of the vaginal microbiota, which, after the therapy course, showed a tendency toward normalization.

The study also aimed to investigate the indicators of vaginal microbiocenosis in women with benign uterine tumors who were under the influence of armed conflict factors (Kyiv and Kyiv region) (Group II).

Microscopy of vaginal contents in Group II patients before treatment revealed negative changes in microbiota indicators. An increased number of neutrophils and leukocytes, enhanced desquamation of epithelial cells, an increased number of "clue" cells, a deficiency of morphologically normal microflora, a significant density of coccal and rod-shaped microflora, and an increased presence of vaginitis-associated microflora and *Candida* fungi were observed.

Culture-based bacteriological examination of vaginal discharge before treatment revealed a significant

frequency of bacterial-fungal associations: three-component associations were found in 63.3% of cases, while two-component associations were found in 36.7%.

In the spectrum of the vaginal microbiota of women in Group II, an increased proportion of *Firmicutes* was observed: *S. epidermidis hem+*, *S. epidermidis*, and *Str. agalactiae*. The quantitative levels of these *Firmicutes* species slightly exceeded the diagnostic level (Tab. 2).

According to the study data, negative changes in the vaginal microbiota before treatment were manifested by an increased frequency and quantitative level of *E. coli hem+*, *E. coli*, and *Enterobacter cloacae* (Fig. 2).

In the composition of the vaginal microbiota of women in Group II before treatment, an increased presence of *Candida* fungi was registered against a background of a deficiency of normal microflora — *Lactobacillus spp.*

Additionally, an increase in the proliferation of obligate anaerobic microflora was observed in the vaginas of Group II patients before treatment. The quantitative levels and isolation frequency of *Peptostreptococcus spp.* and *Fusobacterium spp.* exceeded those of healthy women. The quantitative levels of the vaginitis-associated microflora, *Atopobium vaginae* and *Gardnerella vaginalis*, were statistically significantly higher than the normal range.

After treatment, women in Group II showed a more favorable dynamic in their vaginal microbiota indicators

Table 2. Microflora predominant in the vaginal microbiota of women with benign uterine tumors who were in Kyiv and Kyiv region, before and after treatment (group II) [%]

Microorganisms	Patients of group II before treatment n = 30	Patients of group II after treatment n = 30	Healthy women n = 30
	[%]	[%]	[%]
<i>S. aureus</i>	10.0	3.3	3.3
<i>S. epidermidis</i> hem+	13.3	6.7	6.7
<i>S. epidermidis</i>	13.3	10.3	10.0
<i>S. agalactiae</i>	10.0	6.7	6.7
<i>S. piogenes</i>	6.7	3.3	–
<i>E. faecalis</i>	6.7	6.7	13.3
<i>E. coli</i> hem+	10.0	–	–
<i>E. coli</i>	16.7	6.7	13.3
<i>Enterobacter</i> spp.	6.7	3.3	6.7
<i>Klebsiella</i> spp.	10.0	3.3	3.3
<i>Candida albicans</i>	13.3	6.7	10.0
<i>Gardnerella vaginalis</i>	10.7	6.7	10.0
<i>Atopobium</i> spp.	10.0	3.3	3.3
<i>Veillonella</i> spp.	10.0	3.3	6.7
<i>Prevotella</i> spp.	16.7	20.3	23.3
<i>Bacteroides</i> spp.	26.7	30.0	30.0
<i>Peptostreptococcus</i> spp.	13.3	10.0	10.0
<i>Fusobacterium</i> spp.	13.3	6.7	6.7
<i>Paraclostridium bifermentas</i>	30.3	30.3	33.3
<i>Clostridium novyi</i>	33.3	20.3	26.7
<i>Anaerococcus prevorii</i>	26.7	30.0	36.7
<i>Staphylococcus saccharalyticus</i>	30.3	26.7	30.0
<i>Lactobacillus</i> spp.	93.3	96.7	100.0

compared to patients in Group I. According to the bacterioscopic results, destructive changes in epithelial cells and the number of "clue" cells decreased, the number of leukocytes normalized, and the density of Gram-positive, Gram-negative, and vaginitis-associated microflora, along with the frequency of *Candida* fungi, was reduced. The presence of microflora morphologically resembling *Lactobacillus* spp. increased.

After the therapy course, the frequency of bacterial associations decreased: three-component associations were found in 30.0% of cases, and two-component associations in 70%.

The frequency and quantitative levels of *E. coli*, *Klebsiella pneumoniae*, and *Enterobacter cloacae* in the vaginal microbial spectrum decreased. Their levels were within the normal range (<lg 4.0 CFU/mL).

After completing the therapy course, the frequency and quantitative levels of *S. aureus*, *S. epidermidis* hem+, and *Str. agalactiae* in the vaginal discharge of Group II patients also decreased to normal levels (< lg 4.0 CFU/mL).

After treatment, the quantitative levels of vaginal contamination with obligate anaerobic microflora — *Peptostreptococcus* spp., *Fusobacterium* spp., *Bacteroides* spp., and *Prevotella* spp.—also decreased to normal levels.

Following the completion of therapy in women who were under the influence of armed conflict factors in Kyiv and the Kyiv region, the quantitative levels of vaginal contamination with vaginitis-associated microflora (*Gardnerella vaginalis*, *Atopobium vaginae*) and *Candida* fungi statistically significantly decreased. The levels of

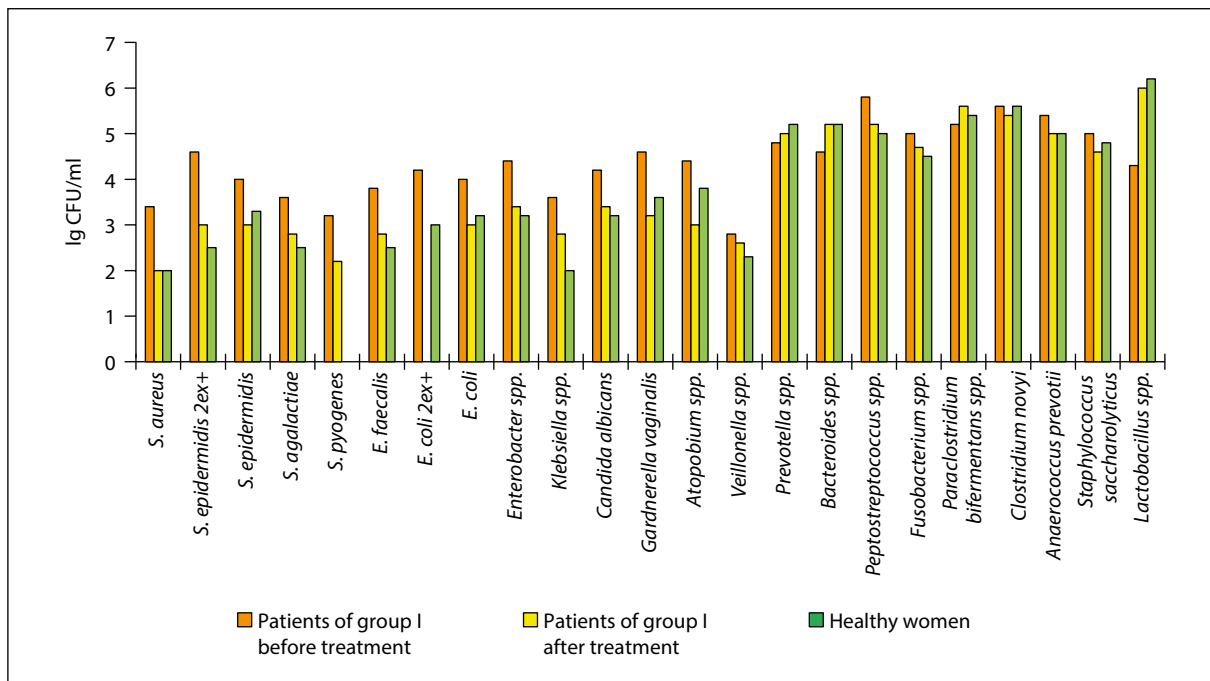


Figure 2. Vaginal microbiota of women with benign uterine neoplasms who were exposed to factors of armed conflict (Kyiv and Kyiv region) before and after treatment (group II)

Lactobacillus spp. in the vaginal cultures of Group II patients normalized after treatment (lg 6.0 CFU/mL).

Thus, the results of the bacteriological examination of vaginal material from Group II patients demonstrated the normalizing effect of therapy on the vaginal microbiota. Positive changes were noted in the composition of both facultative anaerobic and obligate anaerobic microflora. In these patients, the frequency of bacterial vaginosis and candidiasis also significantly decreased.

Discussion

The results of the present study on the vaginal microbiota in women with benign genital neoplasms who were under the influence of armed conflict-related stress factors align with the opinion of many researchers regarding the impact of psychoemotional stress on the development of dysbiotic changes [1, 4, 6]. The present analysis showed that the stress experienced by women during the armed conflict, regardless of whether they were in a direct combat zone, a displaced person, or living in relative safety, acts as a trigger for vaginal microbiota disruption. This manifests as a significant deficiency of *Lactobacillus* spp. and excessive growth of conditionally pathogenic microorganisms, which is consistent with data presented in other scientific works [5, 22, 25, 26].

Of particular importance is the detection of a high frequency and quantitative level of vaginitis-associated microflora (*Gardnerella vaginalis* and *Atopobium vaginiae*), as well as *Candida* fungi, in all groups [27]. As research shows, the detected associations of microflora in most of the examined women are an indicator of a pronounced microbiota disruption, which can have a direct impact on the pathogenesis of benign uterine neoplasms [10, 11], and this finding is consistent with the present study's results.

The complex therapy course that was conducted proved its effectiveness, but with certain specific characteristics [28–30]. While only a tendency toward microbiota normalization was observed in Groups I and II, the results were significantly better in Group III, where the stress factor was less intense and prolonged. This manifested as a complete normalization of *Lactobacillus* spp. levels (lg 6.0 CFU/mL) and a significant decrease in the level of vaginitis-associated microflora below the diagnostic threshold. This opens a new aspect in understanding the relationship between the level of stress, the effectiveness of therapy, and the body's ability to restore the microbiome.

The data obtained allow us to conclude that stress is not only a trigger for dysbiosis but also a factor that can modulate the effectiveness of treatment [5].

One limitation of the present study is the absence of a control group from peaceful regions of Ukraine during

the war period. Instead, pre-war data from healthy women were used. While informative, this design does not fully exclude the possibility that other factors unrelated to war could have influenced the vaginal microbiota. Future studies should aim to include participants from regions minimally affected by hostilities to strengthen the comparative analysis.

Another limitation is that the study relied on conventional culture-based and bacterioscopic methods. Although these approaches are informative and widely used, molecular techniques could provide a more accurate and detailed characterization of the vaginal microbiota.

Due to the pilot nature of the study and the difficulty of patient recruitment under a state of war, the sample size was limited. Nevertheless, the data obtained are sufficiently informative for identifying key trends and forming hypotheses for future, more extensive research.

The authors' next study will focus on examining changes in the vaginal microbiota of women affected by factors of military aggression in Ukraine, depending on their level of stress.

Conclusions

1. The analysis of the vaginal microbiocenosis in women with benign uterine tumors who were under the influence of armed conflict-related stress factors confirmed significant dysbiotic changes. All women showed a deficiency of normal microflora, which was manifested by a decrease in *Lactobacillus spp.* levels against a background of a significant increase in the concentration of conditionally pathogenic microorganisms.
2. Significant vaginal contamination with pathogenic and conditionally pathogenic microorganisms was found. Specifically, Group I showed a high frequency of *Enterobacteria* as well as *Candida* fungi in concentrations exceeding diagnostic levels. Similar trends were observed in the other group, indicating the universal effect of stress on the microbiome.
3. The applied complex therapy demonstrated a positive, yet ambiguous, effect. In women of Group I, despite a decrease in the quantitative levels of conditionally pathogenic microflora, a complete restoration of the microbiocenosis did not occur. For instance, after treatment, the level of *Gardnerella vaginalis* in these women remained high, and the level of *Lactobacillus spp.* did not reach the norm.
4. The most pronounced positive dynamic was observed in Group II. After therapy, there was an

almost complete normalization of the microbiota: the *Lactobacillus spp.* count reached the normal range, and the concentration of vaginitis-associated microflora significantly decreased. This indicates a potential dependence of treatment effectiveness on the intensity and duration of stress exposure.

Article information and declarations

Data availability statement: All data are available upon request from the authors.

Ethics statement: The study was approved by the Bioethics Committee of the State Institution "All-Ukrainian Center for Maternity and Childhood of the National Academy of Medical Sciences of Ukraine", protocol No. 6, dated October 27, 2022. The study was conducted in accordance with the Declaration of Helsinki, and all patients provided their written consent to participate in this study.

Author contributions: IP — conceptualization, validation, data curation, writing — review and editing, supervision; TL — conceptualization, methodology, formal analysis, resources, writing — original draft preparation; ML — software, investigation, visualization, project administration.

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Conflict of interest: The authors declare no conflict of interest.

Supplementary material: There are no additional materials.

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