

## Microbiological Study of Vaginal Microbiota and Endometrium in Women with Chronic Endometritis

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### Abstract

**The research objective** was to study the spectrum of the vagina and endometrium microorganisms in women with chronic endometritis (CE) in order to take adequate therapeutic measures.

**Methods and Results:** We did a cross-sectional study in 47 women (average age of 35.38±5.19 years) with histologically confirmed CE. The vaginal microbiota and endometrial biopsies were assessed using microbiological research methods in accordance with the medical technology “Integral assessment of the state of the vaginal microbiota.” To identify the share of different types of microorganisms in the structure of the biocenosis, the coefficient of constancy of the species (C) was used.

Only 19% of patients had a titer of *Lactobacillus* spp. within the age norm, while the deficit was observed in 80% of women. Among the representatives of Enterobacteriaceae, *Escherichia coli* and *Klebsiella aerogenes* were sown, which are considered to be random species (C=11% and C=2.1%, respectively). The average titer for *E. coli* was 3.6±1.3 lg CFU/swab and for *K. aerogenes* - 2.14 lg CFU/swab. An atypical variant of *E. coli* with hemolytic properties was found in only one sample. All isolates of the genus *Staphylococcus* were also random species (C did not exceed 25%). Coagulase-negative staphylococci (CoNS) were detected in 7 patients (C=15%), while the average titer was 2.1±0.4 lg CFU/swab. *S. aureus* was isolated from only one patient at a titer of 5 lg CFU/swab. *Corynebacterium* spp. were isolated in 11% of cases (C=11% - random species), in a titer of 3.2±0.8 lg CFU/swab. *Enterococcus* spp. also belonged to random species (C=23.4%). At the same time, *E. faecalis* was inoculated in 19% of cases and *E. faecium* was sown in 4.3%, the average titer of which was 3.1±0.9 and 5 lg CFU/swab. *Streptococcus* spp. were recorded in only one case at a concentration of 5 lg CFU/swab. Fungi of the *Candida* were isolated as a random species in 8.5% of cases. The growth of microorganisms in endometrial samples was obtained only in 3 examined women with CE (6.4% of cases). The endometrial microbiota were represented only by random species, for which the C index ranged from 2.1% to 4.3%.

**Conclusion:** The microbiological study of the microbiota of vaginal discharge showed the presence of dysbiotic disorders with a significant deficiency of lactobacilli (80%) without the dominance of representatives of the *Lactobacillus* spp. In the structure of opportunistic microflora, *Escherichia coli*, coagulase-negative staphylococcus, *Enterococcus* spp., and *E. faecalis* prevailed as random species. Representatives of the microbiota in endometrial biopsies were identified only in 6.4% of cases, and are represented by random species. (**International Journal of Biomedicine. 2021;11(4):511-514.**)

**Key Words:** chronic endometritis • microbiota • vagina • endometrium

**For citation:** Voropaeva NM, Lazareva LM, Danusevich IN, Belkova NL, Nemchenko UM, Grigorova EV. Microbiological Study of Vaginal Microbiota and Endometrium in Women with Chronic Endometritis. International Journal of Biomedicine. 2021;11(4):511-514. doi:10.21103/Article11(4)\_OA17

### Abbreviations

CE, chronic endometritis; CPB, conditionally pathogenic bacteria; CFU, colony-forming units; CoNS, coagulase-negative staphylococci; STIs, sexually transmitted infections.

### Introduction

Chronic endometritis (CE) is a clinical and morphological syndrome, in which, as a result of persistent

damage to the endometrium by an infectious agent, multiple secondary morphofunctional changes occur, disrupting cyclic biotransformation and receptivity of the mucous membrane of the uterine body.<sup>(1)</sup> CE is detected in about 10% to 21%

of women of reproductive age, and its presence is associated with infertility and miscarriage.<sup>(2-10)</sup>

For a long time, the uterine cavity was considered a sterile biotope.<sup>(2)</sup> However, at present, the use of molecular genetic research methods makes it possible to identify associations of difficult-to-cultivate and uncultured microorganisms on the surface of the endometrium in women of reproductive age.<sup>(11-16)</sup> There are no concurrent views on the influence of certain groups of conditionally pathogenic bacteria (CPB) on the development of the endometrium inflammatory pathology, and this raises doubts about the advisability of prescribing antimicrobial therapy for CE.<sup>(4,17-19)</sup> For a more complete assessment of the state of the vaginal microbiota, it is necessary not only to assess the total quantitative characteristics of *Lactobacillus* spp., but also to determine the species composition and functional parameters of lactobacilli in various gynecological diseases.<sup>(20)</sup>

**The research objective** was to study the spectrum of the vagina and endometrium microorganisms in women with CE in order to take adequate therapeutic measures.

## Materials and Methods

From 2020 to the present, we have been conducting a cross-sectional study under the auspices of the Scientific Centre for Family Health and Human Reproduction Problems. Our study included 47 women with histologically confirmed CE. The average age of the patients was 35.38±5.19 years. Inclusion criteria were the presence of histologically verified CE, reproductive age (18-45 years). Exclusion criteria were the use of antibacterial, hormonal, or immunomodulatory drugs, and the presence of STIs.

The patients were included in the study after signing a written informed consent. All women who agreed to participate answered a questionnaire survey and underwent general clinical, gynecological, and laboratory-instrumental examination. The sampling of material from the uterine cavity was made on the fourth to ninth day of the menstrual cycle (middle proliferative phase) using a pipelle biopsy of the endometrium with a disposable intrauterine probe (Taizhou Kechuang Medical Apparatus Co., Ltd, China), followed by pathological examination of the endometrial tissue for verification of CE signs.

The vaginal microbiota and endometrial biopsies were assessed using microbiological research methods in accordance with the medical technology "Integral assessment of the state of the vaginal microbiota."<sup>(21)</sup> Vaginal discharge and endometrial biopsy homogenate were inoculated on standard culture media; CFU were counted. Microbiota were identified through the use of standardized bacteriological algorithms, taking into account morphological, cultural and biochemical properties.

To identify the share of different types of microorganisms in the structure of the biocenosis, the coefficient of constancy of the species (C) was used according to the formula:  $C = p \times 100 / P$ , where p is the number of observations containing the studied species, P is the total number of observations. At values of  $C \geq 50\%$ , microorganisms were considered constant;

at values of  $25\% \leq C \leq 50\%$  they were considered additional; at values of  $C < 25\%$  they were considered random.<sup>(22)</sup>

All data have been entered into the REDCap system. Statistical processing was carried out using the STATISTICA Version 6.1 (StatSoft USA). Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean±SD for continuous variables.

The study was carried out in compliance with Ethical Principles for Medical Research Involving Human Subjects, Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the 64th WMA General Assembly, Fortaleza, Brazil, October 2013. The study was approved by the Ethics Committee of the Scientific Center for Family Health and Human Reproduction Problems. Written informed consent was obtained from each participant.

## Results and Discussion

According to the results of the microbiological study of vaginal discharge in women with CE, *Lactobacillus* spp. were identified as permanent representatives of the vaginal microbiota (C=79%). Moreover, only 19% of patients had a titer of *Lactobacillus* spp. within the age norm, while the deficit was observed in 80% of women. Bacterial vaginal communities dominated by *Lactobacillus* spp. have been considered for a long time a sign of vaginal health.<sup>(2,23,24)</sup> Among the representatives of the genus *Lactobacillus*, *L. crispatus*, *L. gasseri*, *L. iners*, and *L. jensenii* dominate in the vaginal microbiota of healthy women. The prevalence of *L. crispatus* indicates the stability of the vaginal microbiota, as this species produces lactic acid, hydrogen peroxide and bacteriocins. *L. iners* is a marker of vaginal dysbiotic disorders. This species does not produce lactic acid and hydrogen peroxide, which are necessary for maintaining eubiosis and cannot provide a transition to the prevalence of *L. crispatus*.<sup>(25)</sup> Due to the structure of the genome, *L. iners* is able to quickly adapt to changing environmental conditions, switching its metabolism and using other substances, not glycogen, as food resources. Thus, under conditions of dysbiosis, *L. iners* produces cholesterol-dependent cytolysin, which destroys cell walls, and uses glycerol of destroyed cell membranes as a new food substrate. This leads to the death of other species of *Lactobacillus* spp., a decrease in the concentration of lactic acid and an increase in the pH of the vaginal environment.<sup>(26)</sup> The significance and role of *L. gasseri* and *L. jensenii* species in maintaining the normal state of the vaginal microbiota still remain controversial.<sup>(20)</sup>

Among the representatives of Enterobacteriaceae, *Escherichia coli* and *Klebsiella aerogenes* were sown, which are considered to be random species (C=11% and C=2.1%, respectively). The average titer for *E. coli* was 3.6±1.3 lg CFU/swab and for *K. aerogenes* - 2.14 lg CFU/swab. An atypical variant of *E. coli* with hemolytic properties was found in only one sample. Vaginal discharge of such intestinal biotope residents as enterobacteria confirms the translocation of microorganisms from the intestine against the background of immunodeficiency in women with chronic endometrial inflammation.<sup>(27)</sup>

All isolates of the genus *Staphylococcus* were also random species (C did not exceed 25%). Coagulase-negative

staphylococci (CoNS) were detected in 7 patients (C=15%), while the average titer was  $2.1 \pm 0.4$  lg CFU/swab. *S. aureus* was isolated from only one patient at a titer of 5 lg CFU/swab. CoNS are part of the normal vaginal microbiota; however, under certain conditions, lactobacilli are not able to suppress the growth and production of toxins by staphylococci. The increase in pH created by the violation of the microbiota ratio promotes the production of *S. aureus* toxins.<sup>(20)</sup>

*Corynebacterium* spp. were isolated in 11% of cases (C=11% - random species), in a titer of  $3.2 \pm 0.8$  lg CFU/swab. *Enterococcus* spp. also belonged to random species (C=23.4%). At the same time, *E. faecalis* was inoculated in 19% of cases and *E. faecium* was sown in 4.3%, the average titer of which was  $3.1 \pm 0.9$  and 5 lg CFU/swab. *Streptococcus* spp. were recorded in only one case at a concentration of 5 lg CFU/swab. Streptococci are glycogen-dependent bacteria, which ensures their successful colonization of the vaginal epithelium, thereby creating competition for lactobacilli. Despite the fact that these microorganisms are part of the normal vaginal microbiota, their presence, even as an accidental one, can contribute to the development of dysbiotic disorders. Also, changes in the composition of the vaginal microbiota can lead to the spread of opportunistic microflora in the upper genital tract and contribute to the development of infertility.<sup>(28)</sup>

Fungi of the *Candida* were isolated as a random species in 8.5% of cases. The average titer was  $4.0 \pm 0.8$  lg CFU/swab.

The growth of microorganisms in endometrial samples was obtained only in 3 examined women with CE (6.4% of cases). The endometrial microbiota were represented only by random species, for which the C index ranged from 2.1% to 4.3%. Moreover, *Lactobacillus* spp. titer was 4 lgCFU/swab, for *E. coli* (one isolate) - 3 lg CFU/swab, *E. faecalis* (2 isolates) -  $3.5 \pm 0.7$  lg CFU/swab. Only one case, *E. faecalis*, was sown both from the vagina and from endometrial samples. It is possible to assume that the presence of these types of CPB probably indicates an upward spread of the infectious process.

## Conclusion

Thus, in women with CE, a microbiological study of the microbiota of vaginal discharge showed the presence of dysbiotic disorders with a significant deficiency of lactobacilli (80%) without the dominance of representatives of the *Lactobacillus* spp. In the structure of opportunistic microflora, *Escherichia coli*, coagulase-negative staphylococcus, *Enterococcus* spp., and *E. faecalis* prevailed as random species. Representatives of the microbiota in endometrial biopsies were identified only in 6.4% of cases, and are represented by random species.

Our study is a pilot. The limitation of our study is that the sample is not large enough to obtain more significant results and understand the role of changes in the microbiota of the vagina and endometrium in the development of CE.

*This work was performed with the use of equipment of the collective research center "Centre for the development of progressive personalized health technologies" SC FHHRP, Irkutsk.*

## Acknowledgments

This article contains material that has been discussed at the VIII International Research and Practical Conference "FUNDAMENTAL AND APPLIED ASPECTS OF REPRODUCTION" (December 2021, Irkutsk, Russia). The author thanks all researchers who participated in the oral discussion.

## Conflict of Interests

The authors declare that they have no conflicts of interest.

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