

Association of microorganisms of reproductive tract of women with vaginal microbiome disorders

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Summary

Disorders of the microbiome of the reproductive tract of women is quite common and can have significant consequences for the woman and for her sexual partners or to the fetus during pregnancy. The study of vaginal microbiome and biological properties of same microorganisms is a necessary step for choice the treatment strategy. The aim of research was to analyze qualitative and quantitative composition of microbiome of the vagina of women using the test-system *Femoflor-16* and to study the ability of staphylococci strains to biofilm-formation.

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Contributions: all authors participated in planning of experiments, discussion of results and make conclusions. AIV is a chef of department of Microbiology, Virology and Biotechnology and the head of experimental group; MHP is the head of diagnostic department of Independent laboratory INVITRO, makes study of female reproductive tract microbiome with real-time PCR; TMS is a chef of department of Clinical Laboratory Diagnostic, where the study of biofilm formation was done; TVS, OSV, OVK make isolation and identification of staphylococci strains and studied their ability to form biofilm.

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Using molecular genetic methods for detecting of microorganisms disorders of vaginal microbiome was confirmed in 92.7% of the surveyed women. In our study, 21 (41.2%) cases of dysbiosis were found to accompany infectious diseases. *Candida spp.*, *Mycoplasma hominis*, *M. genitalium* and *Ureaplasma (urealyticum + parvum)* showed overwhelming. Most of the pathogens detected were a monoinfection (71%), with two-agent associations accounting for only 29% of the cases. The predominant associations of opportunistic bacteria, detected during disorders of vaginal microbiome, were *Gardnerella vaginalis* + *Prevotella bivia* + *Porphyromonas spp.* that found in 50.9% of patients and *Megasphaera spp.* + *Veillonella spp.* + *Dialister spp.* – found in 34.5% of patients. Among cases of monoinfection the most often found opportunistic pathogens were *Eubacterium spp.* – 50.9% of cases and *Staphylococcus spp.* – 49.1% of cases: 92.6% of these were identified as *Staphylococcus epidermidis* and 7.4% – as *Staphylococcus saprophyticus*. 44% of *S. epidermidis* strains were able to form biofilm. None of *S. saprophyticus* strains was film-forming.

Introduction

Reproductive health of women depends on many factors, including the composition of the vaginal microbiome.

Disorders of microbial composition can cause some adverse effects, including pregnancy loss, infertility and other problem. Disorders of the microbiome can be expressed as non-specific inflammation, caused by opportunistic microorganisms, and pathological processes, caused by pathogens (6).

The study of organism's microbiome includes both qualitative analysis of bacteria and determine the biological properties of them. Quick indication of presence of certain microorganisms or their associations with use of molecular genetic techniques do not provide information about the properties of the identified bacteria. Therefore there is a need for further research to determine the pathogenicity factors of isolated strains, antibiotic resistance, the ability to form biofilm and other.

Biofilm-formation is important process, because this state is typical for microorganisms, colonized different biotops of human body. In biofilm bacteria becoming enhance the manifestation of different characteristics, including resistance to antibiotics. In the biofilm bacteria are less susceptible to antimicrobial agents, maintaining their action in concentration exceeding the 50 MIC and more (5,10,14). Lesions, caused by these strains, require special attention in choosing of therapeutic strategy, and so the study of the biological properties of pathogens able to form biofilm is obligate step in study of clinical strains.

The aim of research was to analyze qualitative and quantitative composition of microbiome of the vagina of women using the test-system *Femoflor-16* and to study the ability of staphylococci strains to biofilm-formation.

Materials and Methods

The study was conducted at the Department of Microbiology of Independent Laboratory INVITRO LLC (Dnipropetrovsk, Ukraine). The composition of the microbiome was determined in samples of vaginal secretions, obtained from 55 women of 20 to 54 years old. Studied samples were got from women that need to make an analysis of vaginal microbiome composition by clinical prescription. The study of biological material was made by quantitative polymerase chain reaction (PCR) with use of Thermocyclers DT-96 / DT-322 (DNA-Technology LLC, Russian Federation) mode Real time with use of the kit *Femoflor-16* (DNA-Technology LLC, Russian Federation), which provides to receive a complete quantitative characterization of vaginal microbiome and differentiate the state of physiological balance and dysbiosis (9). The simultaneous presence of pathogenic and opportunistic microorganisms in the vaginal microbiome was determine. With use of kit can determine next pathogenic microorganisms: *Candida spp.*, *Mycoplasma hominis*, *Ureaplasma (urealyticum + parvum)*. Detection in samples of biological material these microorganisms in a quantity of more than 4 lg CFU/mL indicating the candidiasis, mycoplasmosis or ureaplasmosis. The presence of *M. genitalium* in any quantity indicates the mycoplasmosis. Next bacteria also determined: *Enterobacteriaceae*, genus *Staphylococcus spp.*, *Streptococcus spp.*, *Eubacterium spp.*, *Peptostreptococcus spp.*, species *Atopobium vaginae* and bacterial associations: *Gardnerella vaginalis + Prevotella bivia + Porphyromonas spp.*; *Sneathia spp. + Leptotrichia spp. + Fusobacterium spp.*; *Megasphaera spp. + Veillonella spp. + Dialister spp.*; *Lachnobacterium spp. + Clostridium spp.*; *Mobiluncus spp. + Corynebacterium spp.* The state of microbiome determined in accordance with the criteria set out in the instructions for use of kit (15) and the general clinical criteria (12), according to which the decrease in quantity of the lactobacilli

below minimum standards (4 lg CFU/mL) was determine as dysbiosis.

Identification of staphylococci to the species carried out with use of kit ApiStaph (bioMérieux, France).

The ability of staphylococcus strains to form biofilm was determined with use of method of cultivation on plastic plates (2). The following quantitative ratio of microorganisms and culture medium were used: suspension of the daily culture of the studied strains (50 µL with 1×10^5 CFU/mL cells) injected into the hole with meat-peptonic broth (100 µL). Culture incubated in a moist chamber for 72 h with addition of culture medium if it necessary. The ability to form biofilm determine after removing of medium remnants: the film hung on the walls of plate cell considered as positive response.

Statistical analysis was performed by the program Origin Lab Pro 7.0.

Results and Discussion

The composition of the vaginal microbiome of 55 surveyed women were determined with use of the kit *Femoflor-16* (Table 1). It is established that a disorders of the reproductive tract microbiome occurred in 92.7% of cases, that characterized as the prevalence rate over the normal amount of opportunistic microorganisms with keeping the amount of lactobacilli within 4 lg CFU/mL or more. In other 4 samples (7.3%) lactobacilli was not found that in all cases accompanied by a complete absence of pathogens on the background, but with presence of opportunistic microorganisms and their associations. As it shown in our previous materials (13) during dysbiosis bacteria of genus *Eubacterium*, *Peptostreptococcus*, *Atopobium*, associations of *Sneathia*, *Leptotrichia* and *Fusobacterium*, *Megasphaera*, *Veillonella* and *Dialister*, *Lachnobacterium* and *Clostridium*, *Mobiluncus* and *Corynebacterium*, *Gardnerella*, *Prevotella* and *Porphyromonas* were determined. Quantity of opportunistic bacteria of different genus exceed the value of 5.5 lg CFU/mL for each of the listed bacteria and their associations.

On the basis of the percentage of lactobacilli according to the criteria of dysbiosis (15) as normobiosis defined the state of microbiome from 71% of the samples, as relative normobiosis defined 25% of the samples. In 4% of samples part of lactobacilli in total amount of bacteria

Table 1. Microorganisms and their association in composition of women's reproductive tract microbiome, identified with use of the kit *Femoflor-16*.

Microorganisms	The detection rate, %	Quantitative index, lg CFU/mL
Normal flora		
<i>Lactobacillus spp.</i>	92.7	8.18±7.72
Pathogens		
<i>Candida spp.</i>	20.0	5.32±4.72
<i>Mycoplasma hominis</i>	3.6	5.33*
<i>M. genitalium</i>	0	-
<i>Ureaplasma (urealyticum + parvum)</i>	29.0	5.73±4.95
Opportunistic microorganisms and their association		
<i>Enterobacteriaceae</i>	14.5	5.16#
<i>Staphylococcus spp.</i>	49.1	5.05±4.65
<i>Streptococcus spp.</i>	34.5	6.11±5.16
<i>Atopobium vaginae</i>	16.4	7.36±6.86
<i>Peptostreptococcus spp.</i>	16.4	6.74±5.51
<i>Eubacterium spp.</i>	50.9	6.57±5.96
<i>Gardnerella vaginalis + Prevotella bivia + Porphyromonas spp.</i>	50.9	6.93±6.18
<i>Sneathia spp. + Leptotrichia spp. + Fusobacterium spp.</i>	10.9	7.86±6.60
<i>Megasphaera spp. + Veillonella spp. + Dialister spp.</i>	34.5	6.86±6.16
<i>Lachnobacterium spp. + Clostridium spp.</i>	23.6	6.53±6.02
<i>Mobiluncus spp. + Corynebacterium spp.</i>	18.2	7.00±6.25

*Data obtained for 1-2samples, #statistical error not performed, because the large difference in quantitative results of obtained samples took place.

was not more 20%, although their quantity was higher the lower limit of normal (lg 4 CFU/mL). This data indicate the state of vaginal microbiome as dysbiosis. Besides the reducing percentage of lactobacilli in one of these two samples was noted the predominance of facultative anaerobic and obligate anaerobic opportunistic bacteria. The following percentages of association of obligate anaerobic organisms were determined: *Gardnerella vaginalis* + *Prevotella bivia* + *Porphyromonas spp.* – 30-41% of the total microbial mass, *Megasphaera spp.* + *Veillonella spp.* + *Dialister spp.* – 3.4%; and bacteria: *Atopobium vaginae* – 21-28%, *Eubacterium spp.* – 10-14%, *Peptostreptococcus spp.* – 7-9%. Besides them yeast of genus *Candida* also found, but in an amount not higher than 4 lg CFU/mL. According to the criteria of dysbiosis, in this cases defined typical anaerobic-aerobic dysbiosis, with the ratio of aerobes: anaerobes – 1: 4000.

In other cases of a numerically minor proportion of lactobacilli there was an increase in the number of opportunistic bacteria accompanied by a marked anaerobic dysbiosis as it classified in instructive materials to used kit (15). The following bacterial associations were found to dominate: *Sneathia spp.* + *Leptotrichia spp.* + *Fusobacterium spp.* – 58-79%, *Gardnerella vaginalis* + *Prevotella bivia* + *Porphyromonas spp.* + *Mobiluncus spp.* + *Corynebacterium spp.* – 16-22%, *Megasphaera spp.* + *Veillonella spp.* + *Dialister spp.* – 8-10% with almost complete absence of facultative anaerobes. The presence of *M. hominis* and *Ureaplasma spp.* was detected as well.

For 71% of the samples with normal contain of lactobacilli and 25% samples with relative dysbiosis state the quantity of opportunistic facultative and obligate anaerobic bacteria and presence of pathogenic microorganisms were analyzed. It was found that in all cases there is an excess of the threshold level of 4 lg CFU/mL at least for one of the studied groups of microorganisms, that corresponding with other data (3,6,8,11) about the characteristics of dysbiosis, that shows the discrepancy of vaginal microbiome state as normal.

In our study, 21 (41.2%) cases of dysbiosis were found to accompany infectious diseases. Most of the pathogens detected were a monoinfection (71%), with two-agent associations accounting for only 29% of the cases. An association of *Mobiluncus spp.* and *Corynebacterium spp.* found in 18.2% of the samples showed the maximum quantity of 10^8 CFU/mL.

It is known that often opportunistic bacteria are part of the normal vaginal microbiome. However, their excessive reproduction can cause non-specific inflammation, characterized by the lack of specific clinical picture and asymptomatic inflammation that complicate the diagnosis, leading to a chronic process and the development of complications (4).

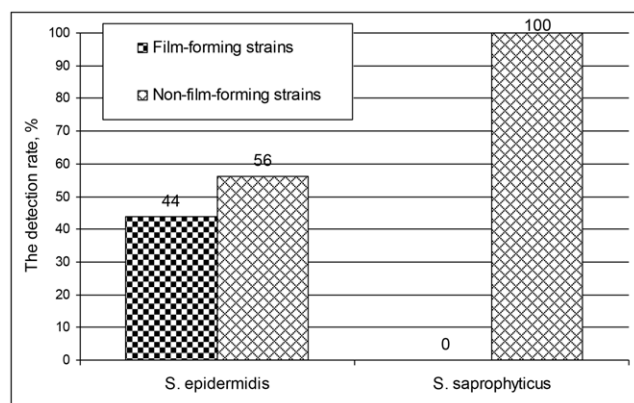


Figure 1. The detection rate of film-form ability among staphylococci strains.

At such inflammation facultative anaerobic bacteria (including staphylococci) detected in about 23% of cases (7). The results of our study showed that the incidence of staphylococci was significantly higher – 49.1% (27 strains): 92.6% of these were identified as *Staphylococcus epidermidis* and 7.4% – as *Staphylococcus saprophyticus*.

Research into the biofilm-forming ability of staphylococci strains showed that some of these are biofilm-forming (Figure 1). It was found that 44% of the *S. epidermidis* strains had the ability to form a biofilm, whereas 56% of these did not form a biofilm in laboratory conditions. Neither of the *S. saprophyticus* strains was found to be biofilm-forming.

It is widely accepted (13) that a large proportion of the detected staphylococci lesions, especially prolonged severe cases, is associated with the ability of microorganisms to form a biofilm. This mostly applies to wound surface infections, where the percentage of biofilm-forming staphylococci strains can be as high as 50-65%. The film-forming ability, however, is less typical of strains detected in rhinitis, dental plaque and on medical equipment.

The presence of biofilm-forming strains can significantly complicate treatment due to increased microbial resistance to environmental factors, including antibiotics. Research into the biological properties of the strains following their detection is, therefore, of crucial importance in diagnostics.

Conclusions

The following conclusions have been drawn.

The disorders of the vaginal flora confirmed in 92.7% of cases. Infectious pathology identified on the background of vaginal dysbiosis in 41.2% of cases, characterized by a significant prevalence of opportunistic microorganisms and decrease of the ratio aerobes: anaerobes – 1: 4000.

The predominant association of microorganisms, detected in vaginal dysbiosis, were *Gardnerella vaginalis* + *Prevotella bivia* + *Porphyromonas spp.* – 50.9% of cases and *Megasphaera spp.* + *Veillonella spp.* + *Dialister spp.* – 34.5%.

Staphylococci were isolated in 27 cases, of which 92.6% were identified as *S. epidermidis* and 7.4% – as *S. saprophyticus*.

Forty-four percent of *S. epidermidis* strains were able to form biofilm. None of *S. saprophyticus* strains was film-forming.

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