MODERN METHODS FOR ASSESSING VAGINAL MICROBIOCENOSIS.

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Abstract.
The literature review is devoted to the history of studying the microflora of the genital tract and modern ideas about the microbiota of the vagina. The article defines the state of the vaginal microflora in normal and in its disorders, such as bacterial vaginosis and vaginitis. The information about the main methods of laboratory diagnostics used to study the microbiocenosis of the vagina is summarized. The conclusion is made about the need for further optimization and standardization of modern methods for assessing the vaginal biotope.
Key words: vaginal microflora; microbiocenosis; microbiota; laboratory diagnostics; microbiological methods.

Introduction.

Vaginal microbiocenosis is a collection of microorganisms inhabiting this biotope, normally represented mainly by lactobacilli and some other microorganisms. The physiological microbiocenosis of the vagina is defined as a complex, dynamically changing microecosystem that forms the colonization resistance of this locus and performs barrier, enzymatic, vitamin-forming, immune and other functions. the composition of vaginal microbiocenosis varies depending on age, race, hormonal status, hygiene habits, and taking antibacterial drugs.

The state of microbiocenosis of the vaginal mucosa is of great importance for reproductive health. Changes in the qualitative and quantitative composition of microorganisms of this locus, as well as their relationship with the cells of the macroorganism, can lead to the development of vaginal dysbiosis or vulvovaginitis. The main representative of the dysbiotic state of the vagina is bacterial vaginosis, which, when microscopically examined by the vaginal mucosa, is described as a decrease or complete disappearance of lactobacilli and excessive reproduction of other, mainly anaerobic bacteria. Bacterial vaginosis is often asymptomatic, since this condition is not accompanied by a pronounced inflammatory reaction, unlike vulvovaginitis. The most common inflammatory diseases of the vagina are trichomoniasis and candidiasis [20]. Such types of vaginitis as aerobic or nonspecific vaginitis and atrophic colpitis are much less common [17].

The term "aerobic vaginitis" is currently used to refer to inflammatory diseases of the vagina caused by staphylococci, streptococci and bacteria of the Enterobacteriaceae family [17]. The role of Streptococcus agalactiae and Escherichia coli in the development of maternal and newborn infections is well known [18]. However, their significance in the etiology of vaginitis has not been sufficiently studied. Violations of the microecology of the vagina are related to septic complications of the postpartum period, neonatal infections and inflammatory diseases

of the pelvic organs, miscarriages and premature birth, increased risk of infection and further transmission of sexually transmitted infections (STIs), including HIV [12, 24].

The vaginal microflora of most women in different periods of life is quite stable. Scientists disagree on how the diversity of the vaginal microflora, regarded as a violation, affects the stability of the microecology of this locus. There are reports that bacterial vaginosis can be a fairly long and permanent condition [8].

Various types of lactobacilli play an important role in maintaining the constancy of the vaginal microflora. The results of studies have been published in which it is reported that women whose vaginal biotope is dominated by L. crispatus are less likely to develop bacterial vaginosis than women with the presence of L. iners [14]. A more detailed study of the vaginal microflora showed that L. crispatus-dominated microbiocenosis more often passes into L. iners-dominant or mixed lactobacillar than into bacterial vaginosis. In turn, vaginal microbiocenosis with a predominance of L. iners is twice as likely to turn into bacterial vaginosis than L. Crispatus dominant [12]. Data on the transformation of vaginal microbiocenoses associated with lactobacilli of other species (L. gasseri, L. jensenii, L. vaginalis) are rare and this issue requires further study [22].

To assess the state of vaginal microbiocenosis, almost the entire arsenal of laboratory methods and technologies is currently used, each of which has its own advantages and limitations. Under these conditions, the choice of the optimal method or set of methods will allow timely and qualitative diagnosis and appropriate treatment to be prescribed [3, 7].

The history of studying the microbiocenosis of the female genital tract.

The study of vaginal microbiocenosis began at the end of the XIX century, shortly after the improvement of the light microscope and the introduction of the microscopy method into medical practice. Scientists from different countries published their first works on this topic almost simultaneously. In 1892 Albert Döderlein wrote a dissertation on the topic "Das Scheidensekret und seine Bedeutung für das Puerperalfieber" ("Vaginal secret and its significance for puerperal fever"). Using a microscopic method to study native drugs, he compared the vaginal microflora of healthy women and maternity patients with postpartum endometritis. For the first time, drawings of bacteria were published in the work, which were later called Doderlein sticks, and currently belong to the genus Lactobacillus. As a result, an idea was obtained about the dominance of lactobacilli in the vagina of healthy women [15].

Maunu Ossian af Heurlin in 1910 published the work "Bakteriologische Untersuchungen des Keimgehaltes im Genitalkanale der fiebernden Wöchnerinnen mit Berücksichtigung der Gesamt morbidität im laufe eines Jahres" ("Bacteriological studies of the content of microbes in the genital tract of parturient women with inflammation, taking into account the general incidence during the year"), in which he described four degrees of purity of the vagina. When assessing the vaginal microbiocenosis, he took into account not only the morphotypes of vaginal secretion bacteria, but also the leukocyte response and the number of epithelial cells. According to this classification, the I degree of vaginal cleanliness is the predominance of doderlein rods, the absence of leukocytes and the presence of single epithelial cells, the II degree is a decrease in the number of lactobacilli and the appearance of single leukocytes, the III degree is single lactobacilli and the predominance of other microflora, the presence of gram—negative diplococci and trichomonads, moderate leukocytosis and single epithelial cells, IV — complete disappearance of lactobacilli, their replacement with other

microorganisms, the presence of trichomonads, pronounced leukocytosis and an increase in the number of epithelial cells [10].

Karl Schröder published his classification of vaginal microbiocenosis in 1921. According to this classification, three degrees of vaginal microflora were distinguished. Degree I, defined as a "healthy" microflora, is the predominance of the morphotype of lactobacilli of different sizes in the vaginal discharge. Grade II is an intermediate picture of microbiocenosis with partial replacement of lactobacilli by other bacteria. Grade III — complete replacement of lactobacilli with other bacteria [16]. The modified Schroeder classification with the changes made by G. Donders in 1999 is used in clinics to this day. The author divided the intermediate II degree into IIa and IIb sub-stages depending on the severity of the predominance of lactobacilli in the mixed vaginal microflora [19].

British scientists Robert Cruickshank and Albert Sharman in 1934 in the work "The Biology of the Vagina in the Human Subject" described three degrees of purity of the vagina depending on the types of microorganisms in the microbiocenosis, consistency and pH values of the vaginal contents. Grade I was defined as "homogeneous bacterial flora represented by Doderlein bacilli, possible presence of yeast–like fungi, low pH values (4.0-4.4), creamy-white, curd-like consistency of the vaginal discharge." Degree III — "the disappearance of doderlein sticks and the presence of several types of bacteria, such as diphteroids, enterococci, staphylococci, vibrions and coliform bacteria, the pH of the secretions is increased (pH 5.6–7.6), they can be abundant and purulent or rather sparse and watery." Grade II is "intermediate between the first and third: doderlein sticks may be present in combination with one or more types of bacteria, pH varies from 4.6 to 5.6" [14].

In 1948, Otto Irovec et al. (O. Jírovec at al.) proposed their classification of vaginal microbiocenosis, in which six paintings were distinguished. The first picture is "norm", with the predominance of Doderlein sticks, the second picture is "non—purulent bacterial colpitis": the detection of numerous non—foreign bacteria without leukocytes. Microscopic signs of bacterial vaginosis fit this description. The third picture is "purulent bacterial colpitis" with the detection of a large number of leukocytes and pyogenic microbes in the preparation. The IV picture, according to Irovets et al., corresponds to the microscopic picture of gonococcal infection, the V picture corresponds to trichomonas vaginitis, the VI picture corresponds to candidiasis vaginitis [23]. further study of the microecology of the vagina is associated with the discovery by Gardner H. L. and Duke C. D. in 1955 of a new species of bacteria, which they named Haemophilus vaginalis. until the 50s of the XX century, the microbiological etiology of the condition corresponding to the III degree of vaginal microbiocenosis, described as "nonspecific vaginitis" or "non-purulent bacterial colpitis", was still unknown. Gardner and Duke showed that the microorganism they described is predominantly present in the vaginal discharge of women with bacterial vaginosis. Further studies of the new species of bacteria led to the fact that it was isolated into a new genus Gardnerella by the name of one of the discoverers [23].

For microscopic diagnostics of the most common violation of vaginal microbiocenosis, namely bacterial vaginosis, Carol A. Spiegel and co-authors (Carol A. Spiegel at al.) in 1983 developed criteria based on the quantitative assessment of morphotypes of lactobacilli and morphotypes of Gardnerella in a smear of the vaginal mucosa, stained by Gram. The number of bacteria of each morphotype was proposed to be calculated with an increase of ×1000 using oil immersion according to the following scheme: 1+ if this morphotype is not represented in

every field of vision or less than one in the field of vision; 2+ (++) if 1-5 bacteria were detected in each field of vision; 3+ (+++), if there were 6-30 bacteria in the field of view; 4+ (++++), if >30 is in view. Large gram-positive rods were determined as the morphotype of Lactobacillus. Small gram-variable rods were represented as the morphotype of Gardnerella. other microorganisms were characterized only by morphology, namely gram-negative rods, curved rods, gram-positive cocci in chains and fusiform bacteria. if the Lactobacillus morphotype was presented in isolation or in combination only with the Gardnerella morphotype, the microbiocenosis was assessed as normal. if mixed microflora prevailed in the vaginal discharge, and the Lactobacillus morphotype was absent or the number of lactobacilli was reduced, the conditions. Later, Robert Nugent et al. (Nugent R. P. at al., 1991) returned to the Spiegel criteria and based on them developed a method for assessing vaginal microbiocenosis for the diagnosis of bacterial vaginosis, which is still used today. Nugent's method is based on a score assessment of the ratio of three morphotypes of bacteria, namely Lactobacillus (gram-positive large rods), Gardnerella/Bacteroides (gram-variable or gram-negative small rods) and Mobiluncus (curved gram-negative bacteria) in Gram-stained preparations of the vaginal mucosa. Points are assigned according to the number of each of the three morphotypes, which is evaluated similarly to the Spiegel method. the sum of points 7-10 is interpreted as the presence of bacterial vaginosis, 4-6 — an intermediate type of vaginal microbiocenosis, and 0-3 points — the absence of bacterial vaginosis [22]. Nugent's method is well suited for the diagnosis of bacterial vaginosis, but the interpretation of the intermediate variant corresponding to 4-6 points remains controversial. Some researchers associate this variant of microbiocenosis with various complications of pregnancy, no such correlations have been found in other studies. In addition, the traditional treatment of bacterial vaginosis does not have an effect in most cases, accompanied by an intermediate picture of Nugent. In 1994, E. F. Kira proposed a classification of microscopic evaluation of vaginal microbiocenosis, describing four types of its condition and the nosological forms corresponding to each type. Thus, the first type of "normocenosis" is defined as the physiological state of the vagina and is characterized by the dominance of lactobacilli, the absence of gram-negative microflora and elements of yeast-like fungi, the presence of single leukocytes and "pure" epithelial cells. The "intermediate type" is borderline and is often observed in healthy women, not accompanied by clinical manifestations and complaints. Microscopic examination reveals a moderate or reduced number of lactobacilli, the presence of gram-positive cocci and gram-negative rods, leukocytes, monocytes, macrophages and epithelial cells. "vaginal dysbiosis" is described as a complete absence of lactobacilli or a small number of them, an abundance of polymorphic gram-variable microflora, the presence of "key cells". The number of leukocytes may vary, incomplete phagocytosis is noted. This type of microbiocenosis corresponds to the microscopic picture of bacterial vaginosis. The fourth type of microbiocenosis is described as inflammatory or corresponding to the diagnosis of "Vaginitis". There is marked leukocytosis, the presence of macrophages, epithelial cells [5].

Subsequently, scientists continued to modify and generalize existing classifications, an example of which is the work published in 2002 by Catherine A. Ison and Phillip E. Hay. the authors propose to evaluate Gram-stained preparations of the vaginal discharge by five degrees. At the same time, I, II, III degrees correspond to the classification of the Shredder. (1921). additionally, the zero (0) degree describes the state of vaginal microbiocenosis,

represented only by epithelial cells with a complete absence of microorganisms, and the IV degree — epithelial cells covered only with gram-positive cocci [20].

dNA sequencing technologies appeared thanks to the work of scientists Allan Maxam, Walter Gilbert and Frederick Sanger in the 70s of the last century [12,15]. In the process of genome sequencing (or whole genome sequencing, whole genome sequencing), the researcher receives information about the entire dNA contained in the genetic apparatus of a macro- or microorganism cell. Continuous improvement of these technologies led to the fact that the Human Genome sequencing project (Human Genome Project, HGP), after ten years of work, was completed in one year [4,5,6]. automated sanger sequencing is considered a "first-generation method", whereas modern methods are called "Next-Generation or second-Generation Sequencing" (NGS).

Microscopy of stained preparations. This method is used in the routine practice of most diagnostic laboratories.

The sensitivity and specificity of microscopic evaluation of stained preparations for the diagnosis of disorders of vaginal microbiocenosis are comparable to those for microscopy of native preparations, that is, quite high. As for the sensitivity of the microscopic method for the diagnosis of trichomoniasis and gonorrhea, it is lower than that of the microscopy of the native drug and depends more on the qualifications and experience of the specialist conducting the study. In case of sufficient professionalism of the researcher, the sensitivity of the microscopic method for the diagnosis of trichomonas vaginitis reaches 85.7% with 100% specificity [5]. There is a high level of false-positive diagnoses in postmenopausal patients, since cells of the parabasal and basal layers of the vaginal mucosa are often present in clinical materials, which are often mistaken for trichomonas due to similar sizes [16].

Molecular methods. In recent decades, phylogenetic analysis, primarily sequencing of the 16S rRNA gene of a microbial cell, shows that bacterial communities in the vaginal biotope are more complex than previously thought. The appearance of molecular methods in laboratory practice and their improvement have made it possible to expand the range of determined microorganisms by identifying difficult-to-cultivate and whimsical, mainly anaerobic bacteria that were not previously isolated by traditional culture analysis [22,23]. For example, this applies to the detection of certain species of lactobacilli and bacteria associated with bacterial vaginosis (Atopobium vaginae, BVAB 1, 2, 3, etc.) [13].

In the practical work of diagnostic laboratories, molecular methods are primarily represented by various modifications of the polymerase chain reaction. The sensitivity of PCr reaches single copies of the DNA of bacterial cells in the bioassay, which makes it possible to significantly increase the effectiveness of detecting infectious agents directly in the clinical sample, bypassing the stage of bacteriological seeding. if traditional PCr allows detecting dNA fragments in the test sample, then multi-dimensional PCr with real-time detection of results determines the quantitative content of several DNA targets in the test sample. It is on the basis of multi-dimensional quantitative Real-Time PCR that domestic commercial kits for assessing the vaginal microbiocenosis of women of reproductive age FeMOFLOr® and amplisens Florocenosis were created/Bacterial vaginosis-FL". The FeMOFLOr test is designed to determine the total number of bacteria in the clinical material and DNA of Lactobacillus, Gardnerella vag.

Conclusion: Evaluation of vaginal microbiocenosis is of great importance for the diagnosis of infections of the reproductive tract, bacterial vaginosis, as well as for determining the need for

treatment and adequate therapy. Laboratory diagnostics or methods used at the patient's bedside should be fast, accurate and present the picture of microbiocenosis as fully as possible. Microscopic methods, despite the fact that they have been used for a long time, have not lost their relevance, are being improved and are widely used in diagnostic and research laboratories, as well as at the reception of a practicing doctor. an urgent task and a discussed issue in modern scientific works is the need to standardize microscopic methods for assessing vaginal microbiocenosis, especially in terms of determining the number of leukocytes and bacterial morphotypes. In addition, further optimization of microscopic methods makes it possible to reduce the degree of subjectivity of the results through the use of automated analyses for research and scientific purposes.

Molecular methods for assessing the microbiocenosis of the vagina confirmed the traditional ideas about the predominance of lactobacilli in the vaginal biotope and expanded the understanding of their diversity, as well as determined the presence and ratio of microorganisms not previously isolated by traditional bacteriological methods. The variety and complexity of modern laboratory methods determines the need to choose the optimal combination of them and allows the most complete assessment of the microbiocenosis of the vagina and the urogenital tract as a whole.

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