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## INFLAMMATORY DISEASES OF PELVIC ORGANS IN WOMEN (ON THE EXAMPLE OF THE KHOREZM REGION)

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### ABSTRACT

**Introduction.** The research covers an analysis on prevalence and etiological structure of inflammatory diseases of pelvic organs in women on the example of the Khorezm region. **Methods of research.** Bacteriological, immunological and statistical research methods were applied in conducting of this study. **The object of the research** was 304 women of reproductive age with inflammatory diseases of the pelvic organs living in the Khorezm region. **Results and discussion.** A comparative characteristic of the basic biological properties of the sown strain and pathogenicity factors were determined. Besides, the degree of sensitivity to the antimicrobial medicines was found, as well as the main etiological agents (causative agents) of inflammatory disease of the pelvic organs in women and their relationship with infections of the genital tract were identified. **Conclusion.** In 273 patients positive samples (89.8%) of antibodies titer to the antigens Mycoplasma spp, Chlamydia spp, CMV, HSV 1,2, T.gondi, U.urealyticum were found. In most cases, positive antibody titer samples were for antigens Mycoplasma spp, Chlamydia spp, relatively few positive antibody titer samples for T.gondi and U.urealyticum antigens were detected.

**Key words:** inflammatory processes of female genital organs, diseases caused by pathogenic and opportunistic microorganisms, antibiotic resistance of circulating strain, monocultures and associations of microorganisms, pathogenicity factors.

### Introduction

Inflammatory diseases of pelvis organs (IDPO) in women present a complex, insufficiently studied problem associated with serious medical, social and economic losses worldwide. In the structure of gynecological diseases, inflammatory processes of female genital organs occupy the first place, making 60-65% of all gynecological pathology. The highest rate of morbidity is observed in patients aged 15-24 years, when after 30 years old its frequency decreases.

During the years of independence in Uzbekistan, the process of transforming the health-care system obtains the status of a state policy. Progress and success have been achieved in the field of protecting public health and reducing diseases, including obstetric and gynecological diseases in women. However, still there were certain issues concerning the health system. Determination of extra genital pathology, especially IDPO in women of reproductive age were most important among them. The Strategy of Actions on Five Priorities of Development of the Republic of Uzbekistan for 2017-2021 states about "further implementation of a set of measures for improvement of family health, protection of motherhood and children, expansion of qualified health care for mothers and children, providing them with specialized and high-tech medical care and reducing child mortality." In this regard improvement of public health and reduction of the factors contributing to the IDPO increase and prevention of its complications is considered to be quite relevant and important.

At the world level, the trend of decreasing of obstetric and gynecological pathology, particularly IDPO, is hardly observed; there is a gradual growth of diseases caused not only by pathogenic but also by opportunistic microorganisms (APM), thus reducing the quality of women, which is being transformed into one of the important medical and social problems. Various factors of the external environment and the organism itself are involved in the emergence and development of this pathology, and different cause-and-effect relationship appear between agents and the human organism, which are of great importance. The general reason for the IDPO growth in women is untimely diagnosis, including microbiological diagnosis. Early diagnostics, development of microbiological criteria and improvement of prognostic approaches to the treatment determine the relevance of this issue. All the above mentioned, as well as the establishment of causal link between the occurrence and development of IDPO in women of reproductive age, remains a priority of scientific research.

The present research serves in the execution of tasks, set out in the Decree of the President of the Republic of Uzbekistan PP-1652 dated November 28, 2011 "On measures for further deepening of the reform in the health-care system" and the Decree of the Cabinet of Ministers dated March 29, 2012 "On measures of further strengthening of the material and technical base and improvement of the activities organization of the medical institutions, as well as in the Decree of the President of the Republic of Uzbekistan UP-4947 dated February 7, 2017 "On the Strategy for the Further Development of the Republic of Uzbekistan," and in other normative and legal documents adopted in this field.

The term "inflammatory diseases of pelvic organs" unites the entire spectrum of the inflammatory processes in the upper reproductive tract of women. They can occur in individual nosological forms as well as in various combinations: endometrite, salpingitis, oophoritis, salpingoophoritis, tuboovarian abscess, pelvic peritonitis, pyosalpinx.[1,2]

Almost all microorganisms in a woman's vagina, with the exception of lactobacilli and bifidobacteria, can participate in inflammatory processes. In the present stage, about 400 species of bacteria and 150 viruses can be identified from human biotopes, causing diseases without symptoms. Therefore, identification of opportunistic microorganisms (OM) is not always a basis for diagnosis of a purulent-inflammatory disease (PID).

A decisive place in the formation of the purulent-inflammatory process is occupied by virulence of the causative agent, the massivity of infection and the state of the macro-organism. [3,4,5,6]

According to the recent studies, IDPO is characterized by polymicrobial etiology. [7]

However, the leading place is occupied by gram-negative bacteria (enterobacteria, primarily *E. coli*) and Gram-positive cocci, the main ones of which are the representatives of the genus *Staphylococcus*. Researches carried out in recent years show that anaerobic microorganisms also occupy a certain place, but almost all researchers agree in the opinion that they are rarely met and cannot be the main causative agents. Bacterioids, clostridia, peptostreptococci are most commonly identified. [8,9]

The cause of IDPO can also be the causative agent of sexually transmitted infections (STIs). According to data of WHO experts, in 60-70% of cases, inflammatory diseases are caused by chlamydia and gonococcus. *T. Vaginalis*, myco- and ureaplasma infection are also capable of IDPO causing. The etiology of diseases can also be associations of non-spore-forming gram-negative anaerobes, anaerobic gram-negative and in rarely gram-positive microbial flora [10,11,12]

Microbiological researches revealed positive results in 62.9% of cases, of which association was shown in 31.8% of cases, and microorganisms monoculture - in 68.2% of cases. [7,13]

Despite the intensive search for new antibacterial medicines, the problem of PIDs fight remains one of the most complex and relevant. Generally it is due to changes in the etiological structure of causative agents, the rapid development and antibiotic resistance relevance among circulating strains. [14,15]

Complicated forms of genitalia PIDs make about 5-7%, but they can lead not only to the loss of specific functions of the female body, but also to disability.

It should be noted that there are few works on a comprehensive assessment of the microbial flora of IDPO, the relationship between the causative agents of STIs and OMs of the genital tract, and the microbiological aspects of the IDPO formation. All these factors led to the need to conduct the present research. Considering the unfavorable situation of the Aral Sea region, our attention was

paid to the study of IDPO in women of this region, particularly, living in the Khorezm region. [18,19,20]

The results of the work allowed to expand the degree of theoretical knowledge on studying the current state of the microbial flora of the IDPO causative agents, a comparative assessment of the taxonomic signs of etiological agents, and the mechanism of the formation of IDPO caused by OM. Prerequisites were created for conducting in-depth studies on determination of the microbiological aspects for formation and development of this pathology, the features of the link between IDPO causative agents and genital tract infections.

The practical importance of the research is that the main etiological agents of IDPO in women and their link with genital tract infections were identified, which made it possible to establish the main criteria for antimicrobial therapy and the prognosis of the disease course. Determination of microbiological aspects and causal relationships of IDPO formation and development in women reduced the risk of complications from this pathology.

**Methods of research.** To achieve the objective, 304 women of reproductive age with IDPO, constantly living in the Khorezm region were examined. In the age aspect they were distributed as follows: 18-21 years old - 17 patients ( $5.6 \pm 1.3\%$ ), 22-29 years old - 89 patients ( $29.3 \pm 2.6\%$ ), 30-35 years old - 92 patients ( $30.3 \pm 2.6\%$ ), 36-49 years old - 99 patients ( $32.5 \pm 2.7\%$ ) and 50 years and older - 7 patients ( $2.3 \pm 0.9\%$ ). 230 ( $75.7 \pm 2.5\%$ ) of them were rural, and 74 ( $24.3 \pm 2.5\%$ ) were urban residents.

The following diagnoses were verified for women with IDPO: double-sided acute salpingoophoritis ( $60.8 \pm 2.8\%$ ,  $n = 185$ ), left-sided salpingoophoritis ( $6.9 \pm 1.5\%$ ,  $n = 21$ ), right-sided salpingoophoritis ( $11.2 \pm 3.3\%$ ,  $n = 34$ ), double-sided acute pyosalpinx ( $13.2 \pm 1.9\%$ ,  $n = 40$ ), left-sided acute pyosalpinx ( $3.0 \pm 1.0\%$ ,  $n = 9$ ), right-sided acute pyosalpinx ( $4.9 \pm 1.2\%$ ,  $n = 15$ ); chronic adnexitis ( $2.0 \pm 0.8\%$ ,  $n = 6$ ). Chronic adnexitis occurred together with acute salpingoophoritis. Diagnoses were verified by clinical, clinical-instrumental and laboratory methods according to the International Statistical Classification of Diseases and Related Health Problems 10th Revision Version (2007).

An acceptable and modern clinical diagnostic method is the approach to IDPO, proposed by the US National Center for Disease Control and Prevention - CDC, USA.

Hospital morbidity was researched considering official data from the medical centers and documents of the healthcare authorities of the Khorezm region. During the observation period from 2004 to 2015 patient case histories, reporting documentation, patient registers, and the reporting form 12-Health care were studied.

Traditional microbiological methods were applied to complete the tasks. The collection of biological material and its delivery to the bacteriological laboratory was carried out according to traditional methods (Zubkov MN, 2004). Seeding of biological material (puncture material - aspirate from the uterus, material from the posterior fornix of the vagina and cervical canal) was carried out

according to Gold, causative agents sown at a concentration of more than 104-105 CFU/ml were considered etiologically significant. Further identification was carried out according to Bergy's Manual Systematic Bacteriology (1997). [16]

Identification was performed by the taxonomic features of the Enterobacteriaceae family, Staphylococcus spp, Streptococcus spp, Enterococcus spp, Candida spp. Media produced by the company HiMedia (India) were used.

For determination of pathogenicity factors of the identified microorganism strains, plasmocoagulase and hemolytic ability, lecithin and hyaluronidase activity were studied.

A disk diffusion method was applied for study of antimicrobial resistance. The essence of the method is based on the diffusion of the antibacterial medicine from the carrier (paper disk) into a solid nutrient medium (Müller-Hinton medium) and on inhibition of the studied culture growth at a concentration of  $1.5 \times 10^8$  CFU / ml in the area where the concentration of the antibacterial medicine exceeds the minimum inhibitory concentration (MI 4.2.1890-04; Iskhakova H.I. et al. 2015). Interpretation of the results was carried out according to MI 4.2.1890 - 04. Sensitive (S), conditionally resistant (SR), and resistant (R) strains were evaluated by growth diameter on a nutrient medium.

Diagnostic test systems were used to conduct immune enzymatic analysis for determination of blood serum antibodies to Chlamydia spp, Toxoplasma gondii, Mycoplasma spp, Cytomegalovirus, Herpes simplex virus types 1 and 2 antigens (test systems by XEMA, RF), Ureaplasma urealyticum (test system by "Vector-West", RF). The studies were carried out on an immunoenzyme analyzer "Human Reader HS" by "Human Gesellschaft Biochemica und Diagnostica" (Wiesbaden, Germany).

For determination of epithelial cells and leukocytes in the secretions from the female genital tract, a smear was prepared on a slide at the patient's bed which was delivered to the bacteriological laboratory. Smears were stained according to Romanowsky-Giemsa, were microscopied with an increase of 630 times and considered the result. The presence of mucus and the intensity of its determination were studied during the examination of the patient; the evaluation criterion was the amount of identified mucus; the intensity was expressed as "+", "++" and "+++".

All studies were carried out in the bacteriological laboratories of the Central Center for Safety and Health at the Urgench station of the Uzbekiston Temir Yollari JSC and the Urgench District Central Testing Station.

The materials were statistically processed by the methods of variation statistics on a personal computer using programs for biomedical researches. In the process of research organizing and conducting, the principles of evidence-based medicine were observed.

**Results and discussion.** It is established that most often in the form of etiological agents, *S. epidermidis* ( $22.5 \pm 2.1\%$ ) and *S. aureus* ( $22.3 \pm 2.0\%$ ) were sown, then *Candida* spp ( $16.2 \pm 1, 8\%$ ) and *E. coli* ( $14.8 \pm 1.7\%$ ) sowing followed. The sowing rate of these causative agents was significantly higher comparing with other microorganisms; anaerobes (*Bacteroides* spp) were sown at a smaller degree

( $2.7 \pm 0.8\%$ ). Their low sowing rate at IDPO in women is a distinctive feature of the formation and development of IDPO in women permanently residing in our region.

Relatively less in relation to other strains *Proteus* spp ( $1.7 \pm 0.6\%$ ), *P. aeruginosa* ( $2.4 \pm 0.8\%$ ), *Klebsiella* spp ( $2.7 \pm 0.8\%$ ) and *S. saprophyticus* ( $3.1 \pm 0.9\%$ ) were sown. In 18 cases ( $4.4 \pm 0.2\%$ ), *Neisseria* spp (non-pathogenic) was sown. Among the identified strains of IDPO agents in women, gram-positive cocci predominated ( $59.6 \pm 2.4\%$ ), which were sown 2.8 times more often than gram-negative bacteria ( $21.5 \pm 2.0\%$ ,  $P < 0.001$ ).

At the next stage, the etiological structure of causative agents was analyzed depending on nosological units (Table 1).

Reliable differences were observed only by the *S. epidermidis* ( $P < 0.05$ ) and *Bacteroides* spp ( $P < 0.05$ ) sowing. In other cases, there were no significant differences in the sowing rate of cultures depending on nosology. An analysis of the results showed that all sowed strains were detected both in the form of a monoculture and as an association of microorganisms (Table 2).

The following distinctive features for this indicator were determined: only *S. epidermidis*, *Streptococcus* spp and *Klebsiella* spp were significantly more sown as monocultures, than in associations; *S. aureus*, *E. coli* and *Candida* spp, on the contrary, were sown more often as associations than as monocultures; *Neisseria* spp was sown only in associations, and were qualified as “transient microflora” not assigning to etiologically significant IDPO agents; associations are sown 1.2 times more than monocultures of IDPO agents in women. Microorganism associations consisted of 2 ( $n = 96$ ) and 3 ( $n = 13$ ) cultures.

**Table 1.**

**Comparative parameters of causative agents sowing at salpingo-ophoritis ( $n = 240$ ) and pyosalpinx ( $n = 64$ ) in women**

Causative agents	Salpingo-ophoritis		Piosalpinx	
	Abs	%	Abs	%
<i>S. epidermidis</i>	80	24,4±2,4	13	15,3±3,9* ↓
<i>S. aureus</i>	72	22,0±2,3	20	23,5±4,6↑
<i>Streptococcus</i> spp	13	4,0±1,1	2	2,4±1,7↓
<i>Neisseria</i> spp (non-pathogenic)	16	4,9±1,2	2	2,4±1,7↓
<i>Enterococcus</i> spp	12	3,7±1,0	3	3,5±2,0↔
<i>S. saprophyticus</i>	9	2,7±0,9	4	4,7±2,3↑
<i>E. coli</i>	46	14,0±1,9	15	17,6±4,1↑
<i>Klebsiella</i> spp	10	3,1±1,0	1	1,2±1,1↓
<i>P. aeruginosa</i>	7	2,1±0,8	3	3,5±2,0↑
<i>Proteus</i> spp	6	1,8±0,7	1	1,2±1,1↔
<i>Bacteroides</i> spp	6	1,8±0,7	5	5,9±2,6* ↑
<i>Candida</i> spp	51	15,5±2,0	16	18,8±4,2↑
Total of sown strains	328	100,0	85	100,0

No growth	6	85,7±13,2	1	14,3±13,2
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Note: \*, ↑, ↓ and ↔ - signs of reliability and direction of changes in indicators;

**Abs** - are absolute numbers.

Distinctive features of causative agents sowing from different biomaterials at IDPO in women were revealed. In general differences mainly concerned *S.aureus* and *E.coli*, which were sown 2.9 and 6.0 times respectively in the puncture material rather than in the vaginal material.

The results confirm that gram-positive cocci, as pathogenic agents of PIDs, including IDPO, begin to regain their leading positions as the main etiological agents of IDPO in women in comparison with gram-negative bacteria and other OM.

The obtained results contradict the data of some authors (Krasnopolsky V.I. et al., 2001; Utkin E.V., Kulavsky V.A., 2008), which indicate the leading position of the association of gram-negative bacteria and anaerobes in the development of the inflammatory process in women with IDPO. In their opinion, gram-positive cocci as etiological agents are sown less frequently. [1,17] This revealed fact is adopted as a regional difference and the feature of the region, which should be considered when prescribing therapeutic measures for IDPO in women.

**Table 2**

**Indicators of causative agents sowing of IDPO in women, depending on the number of strains**

Causative agents	Monoculture		Association of microorganisms	
	Abs	%	Abs	%
<i>S.epidermidis</i>	52	13,6±1,7	41	9,9±1,5* ↓
<i>S.aureus</i>	39	9,4±1,4	53	12,8±1,6* ↑
<i>Streptococcus spp</i>	11	2,7±0,8	4	1,0±0,5* ↓
<i>Neisseria spp (non-pathogenic)</i>	0	0	18	4,4±1,0↑
<i>Enterococcus spp</i>	8	1,9±0,7	7	1,7±0,6↔
<i>S.saprophyticus</i>	7	1,7±0,6	6	1,5±0,6↔
<i>E.coli</i>	21	5,1±1,1	40	9,7±1,5* ↑
<i>Klebsiella spp</i>	9	2,2±0,7	2	0,5±0,3* ↓
<i>P.aeruginosa</i>	6	1,5±±0,6	4	1,0±0,5↑
<i>Proteus spp</i>	5	1,2±0,5	2	0,5±0,3↓
<i>Bacteroides spp</i>	7	1,7±0,6	4	1,0±0,5↓
<i>Candida spp</i>	22	5,3±1,1	45	10,9±1,5* ↑
Total strains	187	45,3±2,4	226	54,7±2,4* ↑

Note: \*, ↑, ↓ and ↔ - signs of reliability and direction of changes in indicators;

**Abs** - absolute numbers.

It was determined that the number of epithelial cells was high in a smear taken from the posterior vaginal fornix (57.2%), and in smears taken from the uterine cavity and cervical canal, they were detected, respectively, 2.6 and 2.4 times less than from the posterior fornix. The presence of intensive determination of epithelial cells in the smear is an indirect indicator and additional criterion for the formation and development of the inflammatory focus in the pelvic organs of sick women. Leukocyte identification was significantly more in smears taken from the posterior vaginal fornix than in smears taken from the cervical canal. Here, the same tendency was observed as in the determination of epithelial cells. The identification of mucus in smears taken from an aspirate, posterior vaginal fornix, and cervical canal practically has the same tendency as in the determination of epithelial cells and leukocytes.

It is established that the prevalence of salpingo-oophoritis among women of reproductive age is kept at a consistently high level compared with data from foreign researchers. Women in rural areas get sick 2.3-3.5 times more often than urban women. Distinctive features of our region are: the absence of IDPO "rejuvenation" in women; reliable less number of unmarried and nonparous women with IDPO, which is observed in European countries; close, direct connection of the IDPO increase with active sexual life, the number of pregnancies, childbirth and abortion, and the level of women education. Considering these features, groups of factors have been formulated for increasing of the IDPO prevalence in women: medical, social and environmental.

A comparative characteristics of the main biological properties of sown strains causative agents at inflammatory diseases of the pelvic organs in women was carried out.

**S.aureus.** 92 strains were identified, the genus *Staphylococcus* was identified by 8 identification indicators, and according to these characteristics, the strains sown both from the puncture material and vagina were identical. From the interspecific identification of *Staphylococcus* spp, the results were obtained by the following tests: coagulase activity ( $96.7 \pm 1.9\%$ ); flocculation on nutrient broth ( $93.5 \pm 2.6\%$ ); fermentation of mannite in anaerobic conditions ( $98.9 \pm 1.1\%$ ); sensitivity to novobiocin ( $85.9 \pm 3.6\%$ ); positive reaction of Foges-Proskauer ( $95.6 \pm 2.1\%$ ); lecithovitellase activity ( $78.3 \pm 4.3\%$ ); decomposition of glucose (100%), lactose ( $97.8 \pm 1.5\%$ ), maltose ( $98.9 \pm 1.1\%$ ), sucrose ( $91.3 \pm 2.9\%$ ), hemolytic activity ( $84.8 \pm 3.7\%$ ). Atypical reactions were obtained according to some identifying indicators, i.e. those strains differed from the typical *S.aureus* strains (indicators not reaching 100%). But, the percentage of those cultures was low, each of the cultures had no more than 1-2 deviations from the typical characteristics of *S.aureus*, so their belonging to that species was not in doubt.

The study of pathogenicity factors showed that if the number of strains producing hemolysin increased in women with IDPO in 2.1 times compared to healthy individuals, then the lecithinase activity was 5.8 times higher, hyaluronidase activity 13.5 times, plasmacoagulase activity 14.4 times ( $P < 0.001$ ).

Besides, the sensitivity of *S.aureus* cultures to antimicrobial agents ( $n = 16$ ) was studied. The cultures were most sensitive to cefazolin ( $S = 81.5 \pm 4.0\%$ ), cefobid ( $S = 69.6 \pm 4.8\%$ ), ciprox ( $S = 66.3 \pm 4.9\%$ ), rifampicin ( $S = 66.3 \pm 4.9\%$ ). The sensitivity of the other antibiotics was below 50.0%. Low sensitivity was found in streptomycin ( $S = 3.3 \pm 1.9\%$ ), penicillin ( $S = 6.5 \pm 2.6\%$ ), chloramphenicol ( $S = 6.5 \pm 2.6\%$ ), carbenicillin ( $S = 10.9 \pm 3.2\%$ ), erythromycin ( $S = 13.0 \pm 3.5\%$ ), kanamycin ( $S = 14.1 \pm 3.6\%$ ) and ampicillin ( $S = 14.1 \pm 3.6\%$  )

**S.epidermidis.** 93 strains were identified in total. According to the positive reaction of Voges-Proskauer, the fermentation of Hiss media and other parameters there was less quantity of typical colonies than atypical colonies compared with *Staphylococcus aureus*. The main differentiating features of *S.epidermidis* from *S.aureus* were: the absence of a “golden pigment”, coagulase activity, mannite fermentation and hemolytic activity. The sensitivity of *S.epidermidis* to antibacterial medicines was low, and in terms of resistance had the same tendency as *S.aureus*.

**Streptococcus spp.** 15 strains were identified. The main differentiating indicators of streptococci: location in the field of view in the form of chains (100%); staining with primary dyestuff at Gram staining (100%); the formation of small, gray, flat, S-shaped colonies (100%); absence of growth on cant (meat-peptone agar) MPA - for differentiation with *Enterococcus spp* (100%); catalase-negative (100%); the presence of the same, complete hemolysis zone around the colony on 5% blood agar (100%). Antibiotic resistance of *Streptococcus spp* is low.

**Enterococcus spp.** 15 strains were identified in total. The main identification features: gram-positive cocci (100); location in the field of view in the form of chains (100%); the formation of accreted, S-shaped, black colonies in bile-esculin agar (100%); growth on cant PA - for differentiation with *Streptococcus spp* (100%); growth on a nutrient medium containing 6.5% of NaCl (100%); fermentation of lactose, glucose and mannite to the acid (100%). The main differentiation is carried out from staphylococci and streptococci.

**Enterobacteriaceae family.** In the form of etiological agents, *E. coli* ( $n = 61$ ), *Klebsiella spp* ( $n = 11$ ) and *Proteus spp* ( $n = 7$ ) were sown. For reliable identification of enterobacteria, 9 differentiating indicators were used: the presence of lysine decarboxylase, urease; the formation of hydrogen sulfide, indole; fermentation of glucose, lactose; utilization of sodium citrate, sodium malonate; the presence of bacterial motility. All sown strains fully corresponded to the taxonomic characteristics of the specified genus and species.

**P.aeruginosa.** 10 strains were sown in total. During differentiation, attention was paid to the following basic biological properties: growth on MPA (100%); motility (100%); formation of pyocyanin (100%); gelatin liquefaction at 22°C (100%); Hugh-Leifson positive test ( $90.0 \pm 9.5\%$ ). As an additional taxonomic characteristic, the formation of a “rainbow aureole” (a mucous film on the surface of the colony, flashing different colors) was detected in 9 from 10 cases ( $90.0 \pm 9.5\%$ ). By the sensitivity to the antibiotics *P.aeruginosa* did not differ

significantly from the data presented in the literature (Yakovlev S.V., 2004; Sidorenko S.V., 2004; Kondratova Sh.Yu., 2007).

**Bacteroides spp.** 11 strains were sown in total. Identification was carried out according to the following differentiating indicators: all strains were Gram-negative (100%); motionless in sight (100%); formed small, cloudy, S-shaped colonies in blood agar, with the absence of hemolysis zone around the colonies (100%); secreted hydrogen sulfide, fermented glucose, lactose and sucrose (100%); gave growth in severe anaerobic conditions (100%).

**Candida spp.** 67 strains were identified in total. Identification was mainly carried out by cultural and enzymatic properties. During differentiation, attention was also paid to morphological (100%) and tinctorial features (100%). It was established that the sown *Candida* sp strains did not cleave lactose, when glucose and maltose were cleaved in  $97.0 \pm 2.1\%$  of cases, sucrose in  $22.4 \pm 5.1\%$ , and galactose in  $83.6 \pm 4.5\%$ , respectively.

The analysis was carried out and an evaluation of the results of the immune-enzymatic diagnostic for inflammatory diseases of the pelvic organs in women was performed, as well as determination of the positive samples parameters, which revealed antibody titers in blood serum against antigens of different microorganisms. Antibodies to antigens were not found in the blood of 304 women and 31 patients ( $10.2 \pm 1.7\%$ ). In total 373 positive samples were identified in 273 patients.

In most cases, antibodies to *Mycoplasma* spp were revealed in the observed patients. A positive antibodies titer to *Mycoplasma* spp was determined in  $32.4 \pm 2.4\%$  of cases ( $25.1 \pm 2.2\%$  for one microorganism and  $7.3 \pm 1.2\%$  in the association). *Chlamydia* spp ( $24.2 \pm 2.2\%$ ) occupied the second place by its identification, but unlike *Mycoplasmas* in *Chlamydia* between the determination of antibodies to antigens of one microorganism ( $13.7 \pm 1.8\%$ ) and associations ( $10.5 \pm 1.6\%$ ) no significant differences were found ( $P > 0.05$ ). Quite often, antibodies to Cytomegalovirus (CMV) and Herpesviruses of types 1 and 2 (HSV 1,2) were identified, which were determined in  $20.0 \pm 2.1\%$  and  $15.1 \pm 1.9\%$  of cases, respectively. In both cases, no differences were found between the number of positive samples of antibodies to antigens of one microorganism and associations. Relatively less number of positive antibody titers to *T.gondi* were identified ( $5.6 \pm 1.2\%$ ). Positive antibody titers to *U. urealyticum* were found in  $2.7 \pm 0.8\%$  of cases and only as associations. By the factor of identification of positive samples of the antibodies titer to antigens of the above mentioned six microorganisms, no significant differences were found between the compared nosological units ( $P > 0.05$ ).

On the next stage a comparative analysis was performed to establish causal relationships between the sowing rate of the main causative agents of IDPO in women with identification of positive antibody titer samples to *Mycoplasma* spp, *T.gondi*, *Chlamydia* spp, CMV, HSV (1,2), and *U.urealyticum* antigens (Table . 3).

Percentage of positive samples on identification of antibodies to antigens of all microorganisms was significantly high in women with IDPO, where *S.aureus* ( $P < 0.001$ ) was the causative agent of the disease in relation to *S.epidermidis*. It was found that the higher the virulence of the causative agent of the disease, the greater the identification of positive samples of antibodies to antigens of genital infections agents.

Close indicators to *S.epidermidis* parameters, but with some statistically insignificant differences, were also noted for *Streptococcus* spp. Almost identical results with *S.aureus* parameters were also obtained with *E. coli* sowing ( $P > 0.05$ ).

But, when the causative agents of IDPO were *Bacteroides* spp, a significantly high percentage of positive samples of antibodies titer to antigens CMV, HSV 1,2, *Mycoplasma* spp and *Chlamydia* spp ( $P < 0,05$ ) was noted.

**Table 3**

**Comparative indicators of antibodies identification depending on the main etiological agents of IDPO in women, %**

Causative agents	Mono-culture, n = 187	identification of antibody titer			
		<i>Mycoplasma</i> spp, n=120	<i>Chlamydia</i> spp, n=82	CMV, n=62	HSV1,2, n=38
<i>S.epidermidis</i>	27,8±3,3	7,5±2,4	6,1±2,6	3,2±2,2	5,3±3,6
<i>S.aureus</i>	20,9±3,0	25,8±4,0	20,7±4,5	29,0±5,8	23,7±6,9
<i>Streptococcus</i> spp	5,9±1,7	10,0±2,7	7,3±2,9	4,8±2,7	5,3±3,6
<i>E.coli</i>	11,2±2,3	30,8±4,2	22,0±4,6	22,6±5,3	18,4±6,3
<i>Bacteroides</i> spp	3,7±1,4	5,8±2,1	6,1±2,6	14,5±4,5	5,3±3,6
<i>Candida</i> spp	11,8±2,4	10,0±2,7	6,1±2,6	6,5±3,1	21,1±6,6

It is explained by the fact that IDPO, the etiological agent of which are anaerobes, is more severe and the addition of CMV against its background increases the risk of an unfavorable outcome of this pathological process. Similar data were obtained on *Candida* spp, with the difference that the percentage of positive samples of antibody titer was high for HSV 1.2, which significantly differed from the parameters of *Chlamydia* spp, CMV and *Mycoplasma* spp ( $P < 0.05$ ).

Most often, in positive samples for identification of antibodies to antigens of microorganisms CMV (n = 44) and *Chlamydia* spp (n = 39) were found in associations, when HCV 1,2 (n = 29), *Mycoplasma* spp (n = 27), *T.gondi* (n = 16) and *U.urealyticum* (n = 10) were found much less. Most often, in positive samples, associations were observed with the following microorganisms: CMV and HCV 1.2 in  $12.1 \pm 2.5\%$  of cases, *Chlamydia* spp and *Mycoplasma* spp in  $10.9 \pm 2.4\%$  of cases, CMV and *Mycoplasma*spp in  $9.7 \pm 2.3\%$  of cases, CMV, *Chlamydia* spp and HSV 1.2 in  $12.8 \pm 2.6\%$  of cases. The remaining associations were found from  $2.5 \pm 1.2\%$  to  $6.1 \pm 1.9\%$ . These associations were of great importance in the diagnosis of IDPO in patients. In the association there were 2

microorganisms in  $65.7 \pm 5.8\%$ , 3 microorganisms in  $28.3 \pm 5.5\%$ , 4 microorganisms in  $3.0 \pm 2.1\%$  and 5 microorganisms  $3.0 \pm 2.1\%$  cases. Monoculture was met 3.1 times more than the association of those microorganisms ( $n = 206$  versus  $n = 67$ ).

### Conclusions.

1. The main causative agents of IDPO in women were *S. epidermidis*, *S. aureus*, *Candida* spp and *E.coli*. Gram-positive cocci were sown 2.8 times more often than gram-negative bacteria; by the content of *S. epidermidis* and *Bacteroides* spp, there were significant differences between salpingo-oophoritis and pyosalpinx.

2. *S. epidermidis*, *Streptococcus* spp and *Klebsiella* spp were reliably sown more as a monoculture than in associations, as well as *S.aureus*, *E.coli*; and *Candida* spp, on the contrary. Associations were met 1.2 times more, and they consisted mainly of 2 ( $n = 96$ ) and 3 ( $n = 13$ ) cultures of microorganisms. *S. aureus* and *E.coli* were sown 2.9 and 6.0 times more in a puncture material than in biomaterials taken from the vagina.

3. Distinctive features which have importance in the formation of IDPO were: absence of "rejuvenation" of IDPO in women; reliable fewer number of unmarried and nulliparous women with IDPO; a close, direct relationship between the increase of IDPO and active sexual life, the number of pregnancies, childbirth and abortion; level of education of women. These features are of practical value in planning, organizing and financing of activities for the treatment and prevention of IDPO in women.

4. It has been established that the number of *S.aureus* strains producing pathogenicity factors is 2.1-14.4 times higher than in healthy individuals. *S.aureus* cultures were sensitive to cefazolin, cefobid, ciprox, rifampicin, and to chloramphenicol, penicillin, erythromycin, carbenicillin and were highly resistant to ampicillin. *S.epidermidis* in the degree of resistance to antibiotics had the same tendency as *S.aureus*. The degree of resistance of enterobacteria is significantly lower than that of gram-positive cocci.

5. In 273 patients positive samples (89.8%) of antibodies titer to the antigens *Mycoplasma* spp, *Chlamydia* spp, CMV, HSV 1,2, *T.gondi*, *U.urealyticum* were identified. In most cases, positive antibody titer samples were for antigens *Mycoplasma* spp, *Chlamydia* spp. Relatively few positive antibody titer samples were found for *T.gondi* and *U.urealyticum* antigens.

6. Identification of positive samples of antibody titer was reliably high in patients where *S. aureus* and *E. coli* were the causative agents of IDPO. The opposite picture was observed in women, where *S.epidermidis* and *Streptococcus* spp were the causative agents of IDPO.

7. Most often, in positive samples for determination of antibodies to antigens of microorganisms CMV, Chlamydia spp were identified in associations, when HCV (1,2), Mycoplasma spp, T.gondi, U.urealyticum were determined significantly less. In positive association samples the following was observed: CMV and HCV 1.2 in 12.1%, Chlamydia spp and Mycoplasma spp in 10.9%, CMV and Mycoplasma spp in 9.7%, CMV, Chlamydia spp and HSV 1.2 in 8% of cases. The remaining associations were found from 2.5% to 6.1%.

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