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FEATURES OF SENSITIVITY TO ANTIBACTERIAL DRUGS IN PATIENTS WITH NONSPECIFIC SALPINGOOPHORITIS

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The features of microflora of the vagina in women patients with chronic nonspecific salpingoophoritis were studied, on the basis of which the main etiological factors of the disease, the features of the formation of associations of microorganisms which cause inflammation and the priorities for the adjustment of antimicrobial therapy were set. The investigations made indicate that in patients with chronic salpingoophoritis the changes of microflora of the vagina occur, accompanied by incoordination of its functioning as a single ecosystem, what is manifested by the disorders of microbiological status and widespread antibiotic resistance of the identified pathogens.

KEY WORDS: chronic salpingoophoritis, microflora, microbial associations, antibiotic resistance

ОСОБЛИВОСТІ ЧУТЛИВОСТІ ДО АНТИБАКТЕРІАЛЬНИХ ПРЕПАРАТІВ ПАЦІЄНТОК ХВОРИХ НА НЕСПЕЦИФІЧНИЙ САЛЬПІНГООФОРИТ

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Вивчені особливості мікрофлори піхви у жінок хворих на неспецифічний хронічний сальпінгофорит, на підставі чого виявлені основні етіологічні чинники розвитку захворювання, особливості формування асоціацій мікроорганізмів, які викликають запальний процес та визначені основні напрямки коригування протимікробної терапії. Проведені дослідження свідчать про те, що у пацієнток з хронічним сальпінгоофоритом відбуваються зміни мікрофлори піхви, які супроводжуються дискоординацією її функціонування як єдиної екосистеми, що проявляється порушеннями мікробіологічного статусу та поширеною антибіотикорезистентністю виявлених збудників.

КЛЮЧОВІ СЛОВА: хронічний сальпінгоофорит, мікрофлора, мікробні асоціації, антибіотикорезистентність

ОСОБЕННОСТИ ЧУВСТВИТЕЛЬНОСТИ К АНТИБАКТЕРИАЛЬНЫМ ПРЕПАРАТАМ ПАЦИЕНТОК БОЛЬНЫХ НА НЕСПЕЦИФИЧЕСКИЙ САЛЬПИНГООФОРИТ

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Изучены особенности микрофлоры влагалища у женщин с неспецифическим хроническим сальпингофоритом, на основании чего выявлены основные этиологические факторы развития заболевания, особенности формирования ассоциаций микроорганизмов, которые вызывают воспалительный процесс и определены основные направления коррекции противомикробной терапии. Проведенные исследования свидетельствуют о том, что у пациенток с хроническим сальпингоофоритом происходят изменения микрофлоры влагалища, сопровождающиеся дискоординацией ее функционирования как единой экосистемы, проявляющейся нарушениями микробиологического статуса и обширной антибиотикорезистентностью выявленных возбудителей.

КЛЮЧЕВЫЕ СЛОВА: хронический сальпингоофорит, микрофлора, микробные ассоциации, антибиотикорезистентность

INTRODUCTION

The actuality of preservation and rehabilitation of women's reproductive health

has recently acquired special significance due to the increased number of chronic inflammatory diseases of the pelvic organs in women, lack of information about the pathogenic mechanisms of inflammatory reactions in presence or absence of the microbial factor, with some features of clinical course of chronic salpingoophoritis, what creates considerable difficulties in early diagnostics, with low efficiency of routine methods of treatment. Chronic inflammatory diseases of female pelvic organs are prevalent in the structure of gynecological pathology at the reproductive age with an incidence of 60-65%, and they are the cause of female infertility and menstrual dysfunctions [1, 2]. In modern conditions mixed infections of internal reproductive organs become increasingly important in the etiology of the diseases of the female reproductive system. Perhaps the change of the etiologic factors of infectious inflammatory diseases of the pelvic organs is caused by the adaptability of opportunistic microorganisms to the influence of environmentally unfavorable factors, chemical drugs, etc., by the creation of the conditions for the emergence of the strains resistant to drug influence, which acquired the complex of qualitatively new properties that make them high virulent and resistant to the influence of the immune system [3, 4].

Maintaining the stable qualitative and quantitative composition of the vaginal microbiocenosis is important in providing normal physiological status of the female organism. Normal vaginal bacterial flora is represented by different types of microorganisms, many of which are not yet identified. Quantitative bacteriological analysis of healthy women showed that 1 g of vaginal fluid contains 10^8 cells of aerobic and 10^9 cells of anaerobic bacteria. Leading microorganisms are *Lactobacillus, Peptococcus, Bacteroides, Staphilococcus epidermidis, Corinebacterium spp., Peptostreptococcus spp., Eubacterium*. This list represents the rank location of the dominant microbiota based on concentrations of more than 10^5 CFU per gram [5, 6].

It is reasonable to consider microbial consortium of vaginal mucous membranes as an organized biofilm which specifically changes into pathogenic condition that includes a set of permanent agents such as *Pseudonocardia, Fusobacterium, Haemophylus, Klebsiella, Streptococcus, Staphylococcus epidermidis* and *Clostridium perfringens* and other periodically active community members [7].

Thus, the above-said suggests actuality of further deep study of the pathogenetic features of nonspecific salpingoophoritis on the background of identification of its microbiological features, all the more so since scientific sources suggest the lack of effectiveness of existing methods.

OBJECTIVE

The study aims to improve the treatment of the patients with nonspecific salpingoophoritis on the basis of determination of susceptibility of vaginal microflora to antibiotic drugs.

MATERIALS AND METHODS

The study evaluated the features of vaginal microflora in 70 patients aged 25 to 39 suffering from nonspecific chronic salpingoophoritis compared with the control group (35 healthy women).

The test groups were distributed as follows: group 1 – female patients with a history of chronic salpingoophoritis lasting up to 10 years (n = 35), group 2 – patients with chronic salpingoophoritis lasting more than 10 years (n = 35); group 3 – almost healthy women (comparison group).

For the assessment of the microorganism content in genital secretions of women the tested material was taken from the posterior vaginal vault and subjected to bacteriological examination. The microflora was evaluated by the method of H. Haenel (1979) in the modification of S. K. Kanareykina (1981), under which the following was taken into account: 1) the frequency of occurrence of the microorganisms in this biotope; 2) general dissemination; 3) the quantity and type composition of: a) lactobacilli; b) streptococci; c) staphylococci; d) enterobacteria; e) fungi of the genus Candida; 4) microbial associations [6]. Removal of isolates from vaginal secretions and cervical scrape was made by the conventional methods in microbiology. Enzymatic identification was made with the help of identification sets MICRO-LA-TEST®, designed for providing standard identification using micro methods. They allow identification of most clinically important microorganisms in short terms.

The sensitivity of the isolates to antimicrobial agents with different mechanisms of action on a microbial cell was studied by

means of microtest system «TNK test» with semiquantitative registration of results.

The data were processed after building the databases in Microsoft Excel, Statistica 7.0. For statistical evaluation of the results parametric criteria were used (mean – M, standard deviation sd) and non-parametric criteria (absolute (n, number) and relative (percentage (p, %) and the average error of rate (sP), criterion $\chi 2$) units). Statistical reliability of differences between groups was evaluated by nonparametric U-Mann-Whitney test. The results were considered adequate at the significance level $p < 0,05$ and $p < 0,01$.

RESULTS AND DISCUSSION

As a result of the study of the female patients of groups 1 and 2 violations of the vaginal microbiocenosis were found. Analyzing the obtained data, we can prove that the most frequent microorganisms removed from the vaginal discharge were: *Peptostreptococcus spp* – 78 % and 75 %, *Enterococcus* – 69 % and 57 %, *S.aureus* – 62,9 % and 60,1 %, *E.coli* – 64,2 % and 69,3 %, *Fusobacterium spp* – 61,0 % and 58.0 %; *S. pyogenes* – 58,0 % and 60 %, *Candida spp* – 47,0 % and 44 %.

During bacteriological study from biomaterial the isolates were typically removed in associations (Fig.).

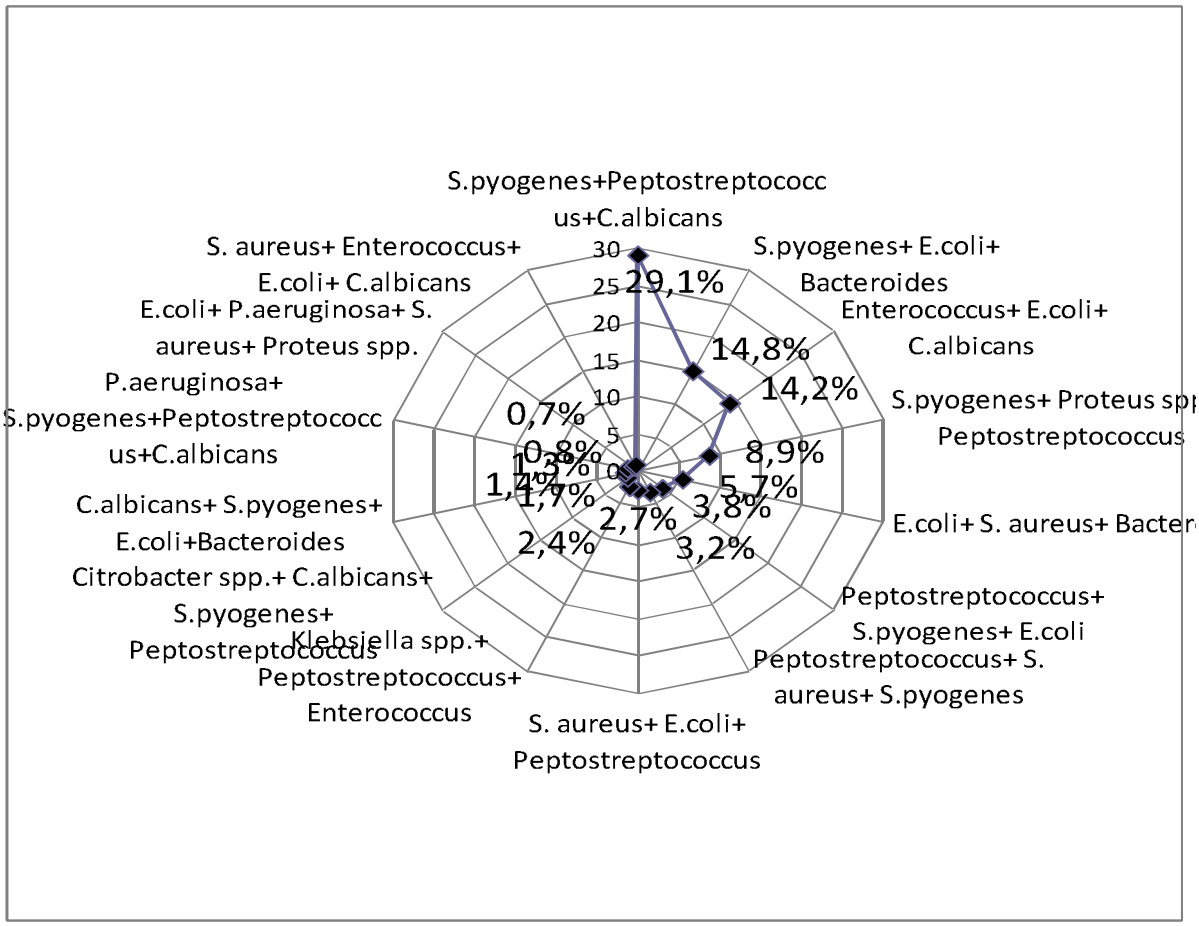

Fig. Associations of isolates separated in salpingoophoritis

Among the coccal flora there were staphylococci, which were found in the patients of two test groups. Dissemination with staphylococci was averaged $1,7 \cdot 10^5 \pm 1,2 \cdot 10^4$ and $4,1 \cdot 10^4 \pm 1,5 \cdot 10^3$ CFU per unit substrate respectively, the most frequent was *S.aureus* with the density of microbial colonization $6,3 \cdot 10^4 \pm 1,2 \cdot 10^3$ and $7,5 \cdot 10^4 \pm 1,3 \cdot 10^3$ CFU per unit substrate respectively. Microbiocenosis of anaerobic flora in patients was diverse. Thus, in the vaginal microflora of the test groups *Veillonella spp* and *Prevotella spp.* were found with the dissemination equal to $1,8 \cdot 10^4 \pm 1,2 \cdot 10^4$ and $2,9 \cdot 10^4 \pm 1,6 \cdot 10^3$, and

$1,5 \cdot 10^4 \pm 1,6 \cdot 10^4$ and $3,7 \cdot 10^3 \pm 1,4 \cdot 10^2$ CFU per unit substrate. *Bacteroides spp* was detected with dissemination of $4,4 \cdot 10^2 \pm 1,8 \cdot 10^1$ and $1,3 \cdot 10^3 \pm 1,6 \cdot 10^2$ CFU per unit substrate. At the same time in the microbiocenosis of vagina *Propionibacterium spp.* was registered. Its colonization density was equal to $1,9 \cdot 10^5 \pm 1,6 \cdot 10^4$ and $5,7 \cdot 10^4 \pm 1,2 \cdot 10^3$ CFU per unit substrate in accordance.

The results of the comparative evaluation of the sensitivity to antimicrobial drugs of therapeutic purpose of the isolates which were more frequently disseminated in patients with salpingoophoritis (staphylococci, streptococci, enterobacteria and obligate anaerobes) showed that the incidence of identification of the isolates of Staphylococcus resistant to antimicrobial drugs was much different and depended on the duration of an inflammatory process (tab. 1).

Table 1

Antibiotic resistance of Staphylococcus isolates

Antibiotics	Rate of resistant strains (M ± м)		
	Group 1	Group 2	Group 3
Cefepime	5,6 ± 5,4	4,4 ± 16,6*#	2,0 ± 3,3
Ceftazidime	11,1 ± 7,4	7,0 ± 6,2* ❋	3,0 ± 3,3
Ciprofloxacin	12,0 ± 3,6	11,1 ± 2,5**	7,0 ± 3,3
Rifampicin	76,0 ± 3,6	44,4 ± 16,6* ❋	31,0 ± 3,3
Erythromycin	88,9 ± 7,5	91,2 ± 6,2*	59,0 ± 1,2
Vancomycin	15,0 ± 3,6	13,0 ± 6,2**	9,0 ± 3,3
Lincomycin	77,7 ± 2,8	94,4 ± 6,6*	50,0 ± 5,2
Gentamicin	91,1 ± 5,5	91,3 ± 6,2*	69,0 ± 3,3*
Amikacin	10,0 ± 3,6	11,1 ± 2,5*	2,0 ± 3,3
Kanamycin	93,3 ± 4,1	93,0 ± 6,2*	57,0 ± 4,9
Ampicillin	61,1 ± 4,5	92,8 ± 6,2* ❋	50,0 ± 5,2
Oxacillin	95,6 ± 5,4	96,3 ± 2,2*	70,0 ± 3,3
Penicillin	72,2 ± 10,6	97,2 ± 1,2* ❋	50,0 ± 1,2
Amoxicillin	90,0 ± 3,6	93,1 ± 2,2*	40,0 ± 3,3
Chlorhexidine	6,0 ± 1,6	4,9 ± 0,6* ◙	1,0 ± 0,3

Note: *p<0,001; **p<0,01; # p<0,05 compared with the control group (group 3);
❋ p<0,001; ◙ p<0,05 compared with the patient group 1.

Staphylococcus isolates were characterized by variable sensitivity to the studied drugs. The most resistant to antimicrobial drugs Staphylococcus strains were taken from the patients of groups 1 and 2. Regarding penicillin the percentage of resistant strains in the patients of group 2 was 97.2 %; the percentage of majority of cephalosporins – from 2.0 % to 11.1 %. Generally, most Staphylococcus isolates were poly-resistant.

As shown in Table 2, among Enterobacteria extracted from the patients of test groups, the rate of resistant strains was high in all groups of study. Before cephalosporins resistant strains percentage was higher for Enterobacterium isolates from the patients of group 1 – 9.3 to 16.7 %.

Table 2

Antibiotic resistance of Enterobacterium isolates

Antibiotics	Rate of resistant strains (x ± Sx)		
	Group 1	Group 2	Group 3
Cefepime	11,3 ± 0,8	16,7 ± 1,0*◙	2,0 ± 0,3
Ceftazidime	9,3 ± 0,8	6,7 ± 1,0*◙	3,0 ± 0,3
Ciprofloxacin	81,3 ± 8,8	86,7 ± 7,0*	7,0 ± 0,3
Rifampicin	56,3 ± 2,4	96,2 ± 1,8*◙	31,0 ± 3,3
Gentamicin	93,8 ± 6,1	97,8 ± 6,2*	69,0 ± 3,3
Amikacin	93,8 ± 6,1	93,0 ± 1,8*	2,0 ± 0,3
Kanamycin	81,3 ± 8,8	88,9 ± 4,7*	57,0 ± 4,9
Ampicillin	81,3 ± 8,8	96,0 ± 1,8*◙	50,0 ± 5,2
Amoxicillin	97,0 ± 2,0	98,0 ± 1,8#	83,3 ± 6,8
Carbenicillin	75,0 ± 6,8	88,9 ± 4,7	73,3 ± 6,8
Levomycetin	98,0 ± 1,0	98,2 ± 1,8*	24,0 ± 2,5
Chlorhexidine	5,0 ± 3,6	4,4 ± 0,6	1,0 ± 0,3

Note: *p < 0,001; # p < 0,05 compared with the control group;
* P < 0.001; ◙ p < 0.05 compared with group 1

The data in Table 3 indicate that in patient groups 1 and 2 of the number of resistant strains of streptococci ranged from 11.0 % to 98.6%.

Table 3

Antibiotic resistance of Streptococcal isolates

Antibiotics	Rate of resistant strains (x ± Sx)		
	Group 1	Group 2	Group 3
Clindamycin	69,0 ± 2,5	28,6 ± 2,1*◙	1,0 ± 0,15
Erythromycin	73,3 ± 7,6	88,6 ± 2,1*◙	10,0 ± 0,9
Ceftriaxone	15,0 ± 1,1	17,7 ± 1,4*	9,0 ± 0,2
Cefotaxime	16,7 ± 1,8	15,7 ± 1,4*	8,0 ± 0,9
Ampicillin	93,3 ± 2,6	98,6 ± 1,1*	50,0 ± 5,0
Azithromycin	90,0 ± 2,1	88,6 ± 2,1*	5,0, ± 2,8
Vancomycin	11,0 ± 1,5	11,0 ± 1,1*	1,0 ± 0,1
Levomycetin	86,7 ± 6,8	92,85 ± 3,2*	24,0 ± 2,5
Ofloxacin	28,9 ± 2,5	28,6 ± 2,1*	1,0 ± 0,15
Sisomicin	12,0 ± 1,5	14,3 ± 1,4	15,0 ± 1,6
Chlorhexidine	13,0 ± 3,6	14,4 ± 4,6*◙	1,0 ± 0,3

Note: * p < 0.001; ** p < 0.01; # P < 0.05 compared with the control group;
* P < 0,001; • p < 0,01; ◙ p < 0.05 compared with group 1.

Evaluation of the resistance of the isolates to antimicrobial agents using microplate «TPK G -» and «TPK G + » (tab. 4) showed that all strains were variable to antimicrobial drugs and

most strains were resistant to ampicillin and doxycycline and moderately resistant to gentamicin. The analysis of the data obtained allowed to find out that all isolates had multiple antibiotic resistance. The studies showed that the isolates were resistant to ampicillin, gentamicin and doxycycline – the growth of culture was observed in the microplate cells with higher and lower concentrations of antimicrobial preparations.

Table 4

The sensitivity of isolates to antimicrobial drugs inoculated in microplate cells «TPK G -» and «TPK G + »

Antimicrobial drugs inoculated in microplate cells «TPK G -» and «TPK G + »	Staphylococci			Streptococci			Enterobacteria		
	R %	I %	S %	R %	I %	S %	R %	I %	S %
Cefotaxime	4	4	92	4	7	89	80	9	11
Ciprofloxacin	11	1	88	30	4	66	86	2	12
Gentamicin	94	6	0	97	3	0	97	3	0
Ampicillin	69	31	0	93	6	1	98	2	0
Doxycycline	96	4	0	94	6	0	88	12	0

Thus, in determining the susceptibility of isolates to antibiotics it has been discovered that most of them were multiresistant (89.2 %). Variable sensitivity to antimicrobial agents related to glycopeptides, fucidins, rifampicin and lincozamides was observed.

Thus, the development of the diseases of microbial etiology depends on the persistent properties of microorganisms aimed for the inactivation of the factors of natural resistance of the organism. The type composition and biological properties of the vaginal microflora in women with nonspecific salpingoophoritis were studied and it was shown that inflammatory diseases of internal reproductive organs occur on the background of dysbiotic disorders characterized by the release of microorganisms with high persistent properties. The latter obviously play an important role in the pathogenesis of inflammatory diseases and dysbiotic conditions of internal reproductive organs.

Based on the above it was confirmed that the occurrence and intensity of inflammatory process depend on the individual fluctuations in qualitative and quantitative composition of microflora of the vagina.

The main practical problem of the diagnosis verification primarily concerns timely determination of the cause of inflammatory process and is associated with the demand of accurate typing of the etiological factor of salpingoophoritis. During the detection of microbial associations the difficulties which arise during the treatment of patients with salpingoophoritis have become clear. The first reason of any failure is persistent diagnostics of the etiological factors while ignoring the pathogenic potential of normal microflora. The second reason is common difficulties in transporting the drugs to the focus of inflammation. The third cause little known to clinicians is group resistance of antibiotics and other effects (quorum sensing) of locus microbiota organized in biofilm.

From the overview of the information on the distribution of microbial associations during the inflammation of the pelvic organs, their multiple antibiotic resistance, antibiotic drugs should be administered immediately after clarifying the nosological diagnosis and before getting the results of bacteriological research. After receiving the results of the bacteriological study the mode of antimicrobial therapy should be adjusted for the selected microflora and its antibiotic sensitivity. Therefore, adequate microbiological diagnosis of ascending infection which causes development of nonspecific salpingoophoritis should be given enough attention as well as to the choice of a mode of therapy.

PROSPECTS FOR FUTURE STUDIES

The current stage of the development of medicine is characterized by insufficient effectiveness of therapy of ascending infection

that leads to salpingoophoritis and then to its chronic course. To a certain extent this is explained by to the presence of mechanisms of protection of pathogens from damaging factors. One of such mechanisms is the ability to form biofilm. Therefore, the study of the ability to form biofilms by microorganisms will allow a new approach to the administration of antimicrobial therapy, creation of the conditions for further investigations on the realization of rational therapeutic measures.

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