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MICROFLORA OF COMBAT WOUNDS OF THE MALE PELVIC ORGANS AND DYSBACTERIOSIS OF THE URINARY SYSTEM

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Summary

Introduction. Identification of microorganisms that colonize combat wounds and cause wound infection is of primary importance for the subsequent successful treatment of the patient. The resistance of microorganisms to antimicrobial drugs makes the efforts of modern medicine in the fight against infectious agents ineffective. The problem of infertility is closely related to combat injuries, their infection, stress, and neurotic disorders.
Aim. Obtaining and summarizing data on microbial colonization of mine-blast wounds of pelvic organs and the microbiome of the genitourinary system of combatants.
Materials and methods. 84 smears were taken from 56 wounds of 36 patients with injuries of the pelvic organs who were being treated. 73 patients with injuries were examined for the presence of mycoflora in the urogenital tract. Isolation of pure bacterial cultures was carried out by inoculating the studied material using meat-peptone agar, blood agar, chromogenic agars. For the diagnosis of urogenital or other infections by the PCR method, a scraping from the back wall of the urethra was taken from the patients.
Results. Predominant microorganisms in positive cultures of smears were non-fermenting gram-negative rods, which in 28 % of cultures belonged to the genus *Acinetobacter*, in 26 % to the genus *Pseudomonas.* As for associated infections, 20 % of them consisted of the genus *Acinetobacter, 32 % – Enterobacter, 4 % – Klebsiella and 29 % – Pseudomonas.* Gram-positive cocci were isolated in 37 % of positive smear cultures. The frequency of isolation of the genus *Streptococcus* in monoinfection was 2.5 %, followed by the genus *Clostridium* – 2 %, *Bacillus* – *3 %, Enterococcus* – *4 % and Actynomycceas* – *4 %*. In associated infections, the frequency of isolation of the genus *Streptococcus was 4 %, followed by the genus Clostridium – 2 %, Bacillus – 4 %, Enterococcus – 3 % and Actynomycceas – 5 %*. When analyzing the microflora of the genitourinary system, it was found that the priority role belongs to the combined infection, when there are associations of specific pathogens such as *Ureaplasma spp., Mycoplasma spp., Chlamidia spp., Neisseria gonorrhoeae, Trichomonas vaginalis, Streptococcus spp., Enterococcus faecalis, which is 80 % of the entire microbiome.*
Conclusions. *Acinetobacter baumanii* and *Klebsiella pneumoniae* are the dominant microflora complicating the course of combat wounds during almost two years of Russia's full-scale war against Ukraine. Probably, the duration of hostilities, the large number of wounded, and the forced mass unsystematic use of various antibiotics lead to rapid changes in the spectrum of pathogens of combat wounds. Combat wounds and their infection, stress, and nervous disorders lead to an imbalance of microflora, in particular microflora of the genitourinary system, which can be one of the causes of infertility. *Chlamydia* and *Ureaplasma* are the most common microorganisms that colonize the urogenital tract of men injured as a result of hostilities.

Keywords: **sombat wound, microbial flora, genitourinary system, infertility**

INTRODUCTION

Advances in the study of the human microbiome have shown its important role in various physiological and pathophysiological processes, including those associated with the onset, progression, and treatment of many diseases, including wound healing [5, 6, 8, 9, 10, 20, 21, 26]. Any wound has a significant risk of infection. In case of

infection, the wound does not heal and the general practice of wound treatment requires more resources. In this case, the identification of microorganisms that colonize combat wounds and cause wound infection is of primary importance. Understanding the complex microbiome of the wound is an important element of effective treatment and improvement of patient care [5, 6, 9, 12, 15].

In this aspect, the issue of prevention and treatment of purulent complications is acute, because wounds received during hostilities are a priori infected. Thus, according to various authors, 44-75 % of wounded people develop infectious complications of gunshot wounds [5, 6, 12].

Fire or shrapnel wounds are microbially contaminated, and identification of microbial agents and primary surgical treatment of wounds is important here. Delayed treatment leads to rapid colonization of wounds by microorganisms and their gradual suppuration. The further evacuation of the wounded to medical facilities leads to tax contamination of wounds with hospital antibiotic-resistant strains of bacteria and the spread of antibiotic-resistant microorganisms. The resistance of microorganisms to antimicrobial drugs renders ineffective the efforts of modern medicine in the fight against infectious agents. According to the WHO, antibiotic-resistant strains of microorganisms such as *Acinetobacter baumannii, Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli* are the most priority for study [5, 6, 12, 21].

Research conducted with the aim of monitoring microorganisms contaminating combat wounds confirmed the global trend of spread and dominance of these microorganisms [5, 6, 12]. These data were obtained by means of permanent bacteriological examination of combat wounds in the wounded being treated in military hospitals and they coincide with those obtained in intensive care units where *Acinetobacter baumannii* prevails [5]. Overall, the understanding of microbial contamination of wound surfaces is ambiguous due to the complex and dynamic nature of the microbiota and the lack of clear microbial indicators.

According to the experience of wars and local conflicts of the last decades, the number of injuries to the pelvic area is on average 5-9 % of all injuries [21]. Based on the analysis of data on the structure of victims during military conflicts, it was established that about 12 % have injuries to the genitourinary system [1, 21].

According to the American-Ukrainian Medical Foundation (2017), infertility is closely related to combat injuries, stress, and neurotic disorders [39, 40]. Now there are a number of studies on the development of erectile disfunction (ED) in combatants (Vietnam, Afghanistan, Iraq), which is associated with constant stress, injuries, etc. [18, 19].

The modern scientific understanding of erectile dysfunction and infertility in general indicates the predominant secondary nature of sexual disorders in relation to the diseases that cause them [10]. This especially applies to military personnel who are participants in combat operations. Among the many pathological conditions that precede or complicate erectile disfunction, neurotic disorders of the psyche/central psychogenic disorders, blood vessel diseases, metabolic disorders, and partial androgen deficiency take precedence [20].

The aim was to obtain and generalize data on the microbial colonization of mine-blast wounds of the pelvic organs and the microbiome of the genitourinary system of combatants.

MATERIALS AND METHODS

The studies were conducted at Danylo Halytsky Lviv National Medical University and the Military Medical Clinical Center of the Western Region. Smears were taken from 84 wounds out of 56 wounds of 36 patients with injuries of the pelvic organs who were being treated. 73 patients with injuries were examined for the presence of mycoflora in the urogenital tract. The study was conducted in September 2023–January 2024.

Isolation of pure bacterial cultures was carried out by inoculating the studied material using meat-peptone agars, blood agars, chromogenic agars. Identification of clinical strains of microorganisms was carried out in accordance with generally accepted microbiological methods by morphological, tinctorial, cultural, and biochemical properties. Bacteriological examination of ejaculate. The ejaculate was collected in a sterile rabbit container and cultured on blood and chromogenic agars. For the diagnosis of urogenital or other infections by the PCR method, scrapings from the back wall of the urethra, semen samples or swabs from the nasopharynx were taken from patients. Indication and identification of *Chlamydia trachomatis, Mycoplasma hominis, Mycoplasma genitalium, Ureaplasma urealyticum, Herpes simplex virus 1,2 types, Cytomegalovirus,* etc. was carried out by the PCR method with hybridization-fluorescence detection of the fluorescence signal level. All studies on genodiagnosis of pathogens were performed on the automatic luminescence analyzer «ALA-1/4» (VioSan, Latvia) using appropriate diagnostic kits.

RESULTS AND DISCUSSION

The average time from a combat injury to the first sowing was 3-4 days. 84 positive cultures were obtained from 56 wounds of 36 patients. Among the isolated microorganisms, 63 % (53 strains) were gram-negative rods, 37 % (31 strains) were gram-positive cocci and gram-positive rods.

Non-fermenting Gram-negative bacilli were the predominant microorganisms in positive cultures of smears, which in 28 % of cultures belonged to the genus *Acinetobacter*, in 26 % to the genus Pseudomonas (fig. 1).

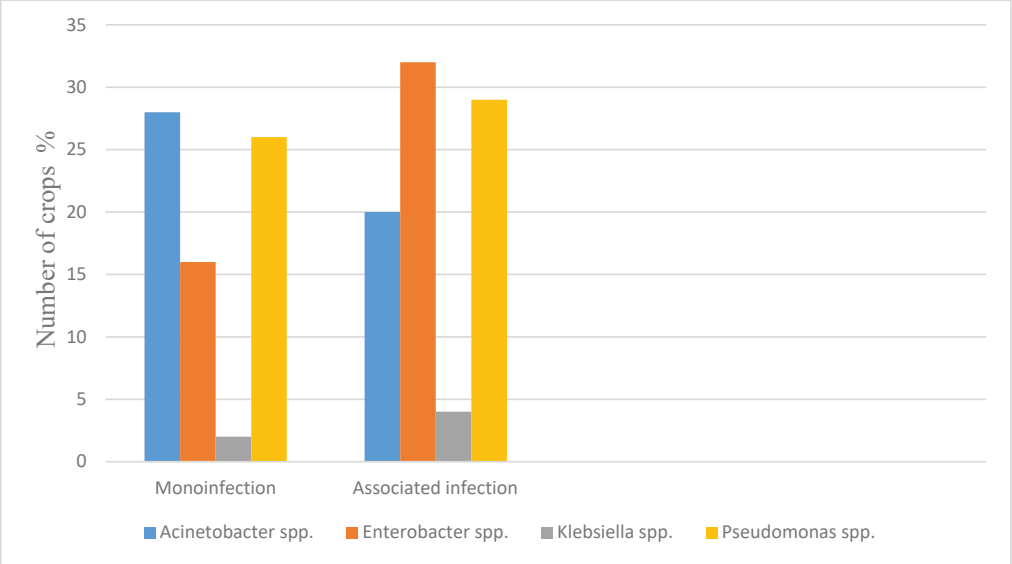

Figure 1. Predominance of gram-negative bacteria in the spectrum of wound pathogens.

Microorganisms of the genus *Enterobacter* were isolated from 16 % of positive smear cultures. In 2 % of crops, strains belonging to the *Klebsiella* genus were found, in 3 % – to Sasmonela, and 1 % – to Vibrio. As for associated infections, 20 % of them consisted of the genus *Acinetobacter*, 32 % – *Enterobacter,* 4 % – *Klebsiella* and 29 % – *Pseudomonas.*

Gram-positive cocci were isolated in 37 % of positive smear cultures. The frequency of isolation of the genus *Streptococcus* in monoinfection was 2.5 %, followed by the genus *Clostridium* – 2 %, *Bacillus* – 3 %, *Enterococcus* – 4 % and *Actynomycceas* – 4 % (fig. 2).

In associated infections, the frequency of isolation of the genus *Streptococcus* was 4 %, followed by the genus *Clostridium* – 2 %, *Bacillus* – 4 %, *Enterococcus* – 3 % and *Actynomycceas* – 5 %.

According to the American-Ukrainian Medical Foundation (2017), combat injuries, stress, nervous disorders can cause infertility [12, 18]. On the other hand, one of the causes of infertility are infections of the genitourinary tract [4, 7, 9, 13, 14, 17, 23, 24, 25].

Among the diseases of the urogenital tract in men, there is an infectious-inflammatory process, the etiological factor of which is extracellular and intracellular microorganisms. The intensity and duration, as well as the localization of the inflammatory process, affects the degree of impaired reproductive function, and in some cases leads to autoimmune reactions.

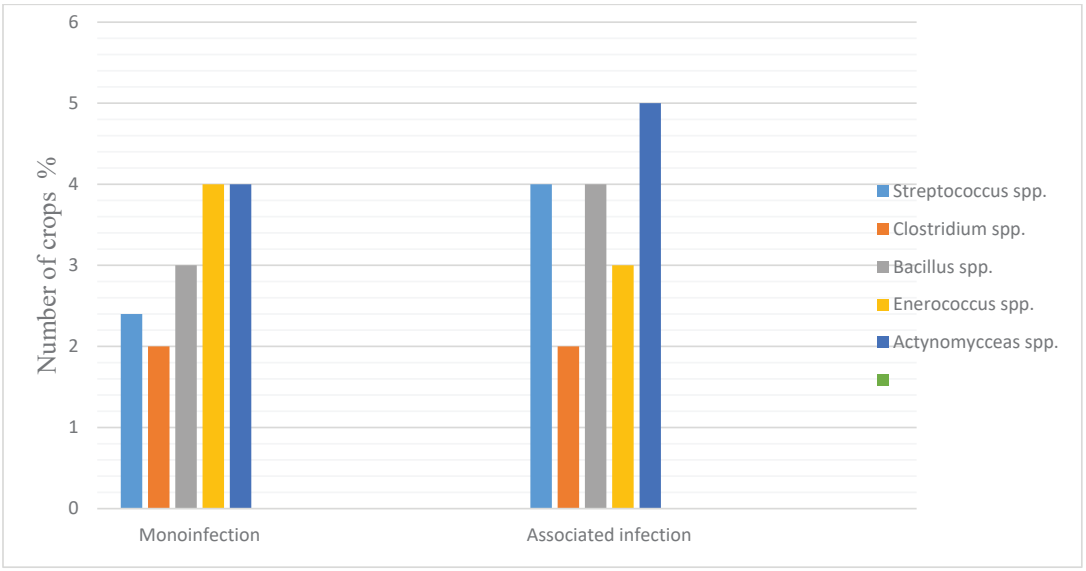

Figure 2. Predominance of gram-positive bacteria in the spectrum of wound pathogens.

The most common method of identifying a bacterial infection of the male urogenital tract is microbiological culture. Bacteriological diagnosis in each specific case allows to determine the degree of insemination of the urogenital tract by quantitative and qualitative indicators, and to determine the degree of biofilm formation. It is believed that an increase in the number of pathogenic microorganisms, especially when there are grouped associations of different genera and families, leads to a thickening of the biofilm in the genitourinary system of men. Using the method of bacteriological seeding, it was found that the species spectrum of conditionally pathogenic microflora in the urogenital tract in diagnostic titers was diverse.

All ill patients with infertility were analyzed for the localization of the inflammatory process and the extent of its spread in the genitals and urinary tract (table 1).

Table 1

Localization of chronic inflammatory processes in infertile men

Localization of inflammatory lesions	Number of patients	
	Absolute number	%
Urethritis + prostatitis	33	46.0
Prostatitis	23	32.0
Urethritis + prostatitis + vesiculitis	6	8.2
Urethritis + prostatitis + vesiculitis + epididymitis	10	13.8
In total	72	100

The analysis of inflammatory processes of the urogenital tract and their ranking by localization and spread showed that chronic inflammatory processes of the male genital organs have the multifocal lesions character. Colonization of the anterior urethra and its glandular apparatus by conditionally pathogenic and pathogenic microflora and successive involvement in the inflammatory process of the posterior urethra, seminal tubercle, excretory ducts, and later the parenchyma of the prostate gland, seminal vesicles and testicular appendages is directly dependent on the intensity the inflammatory process, the general reactivity of the organism and the state of local immunity, which correlates with the topography of biofilms of microorganisms in the case of mixed infections. In order to determine the bacterial spectrum, which is the leading etiological factor of infections of the genitourinary system of men, the frequency of detection of monoinfections and microbial associations was monitored (table 2).

Table 2

Etiological factor of urogenital diseases causing infertility

Etiological factor	The number of patients	% of patients
Chlamydiosis	16	22.2
Chlamydiosis+ureaplasmosis	13	18.1
trichomoniasis + chlamydiosis	10	13.89
Ureaplasmosis	8	11.1
Gonorrhea	4	5.6
Trichomoniasis	6	8.3
Trichomoniasis+gonorrhea+other etiologic factors	15	20.8
In total	72	100

As we can see from the analysis, the priority role belongs to the combined infection. There are associations of specific pathogens such as *Ureaplasma spp., Mycoplasma spp., Chlamidia spp., Neisseria gonorrhoeae, Trichomonas vaginalis, Streptococcus spp., Enterococcus faecalis*, which is 80 % of all microbiome in inflammatory diseases of the genitourinary system in men. Approximately 10 % are microorganisms such as *Enterobacter sp., Streptococcus sp., Morganella morganii, Bacteroides ureolyticus, Mycobacterium smegmatis, Pseudomonas aeruginosa, Corynebacterium glucuronolyticum, Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus,* and *Candida* sp. Although the question of the importance of these microorganisms as monoinfections in the development of inflammatory diseases of the genitourinary system of men has not been strictly proven due to their biological characteristics. Changing the structure of the biofilm by adding more than 25 % of allochthonous microflora of various etiologies: bacterial, fungal and viral, which as a result of their vital activity change the pH, local immunity and hormonal background affect the topography of the inflammatory process in the organs of the reproductive system.

For a better understanding of the inflammatory process of the urogenital tract of men caused by TORCH-complex infection, it is best to use the polymerase chain reaction method. It is the slow pathogens that can cause orchitis, testicular atrophy, obstruction of the vas deferens and are triggers for the formation of antisperm antibodies. In addition, they affect the mobility of spermatozoa, the production of pathological forms and the number of spermatozoa. All these changes can be seen on the spermogram, but in most cases it is impossible to

establish the cause by the classic bacteriological method. It was determined by the PCR method that *Chlamydia trachomatis* is the highest indicator in all three groups (table 3). Most often, associations of *Chlamydia spp.* are found in the ecosystem of the urogenital tract with *Trichomonas vaginalis*, as well as with herpes viruses.

Table 3

Microbiome of the urogenital tract of infertile men

Bacteriological factor	% of the species spectrum of microflora
Ureaplasma parvum	8.2
Mycoplasma hominis	7.9
Mycoplasma genitalium	9.8
Mycoplasma fermentans	2.8
Ureaplasma urealyticum	27.6
Chlamidia hominis	15.3
Chlamidia trachomatis	31.2

Chlamydia trachomatis, which occurred in 31.2 % of cases, is most often localized in the testicle and its appendage, seminal vesicle, urethra, prostate gland and affects spermatogenesis [27]. In addition, slowly persistent infection of the urogenital tract increases the activity of production of oxygen free radicals during inflammation and thus increases oxidative stress. While moderate oxidative stress helps to eliminate the pathogen, excessive oxidative stress can cause damage to the testes or even lead to infertility [2, 11, 14, 17, 19]. As a monoinfection, according to literature reviews, *Chlamydia trachomatis* accounts for 12 % of cases. Our practice shows that there are mainly two or three component associations.

Timely provision of medical assistance and carrying out the full range of necessary medical measures is directly related to the subsequent medical rehabilitation of the soldier. In this aspect, the issue of prevention and treatment of purulent complications is acute, because wounds received during hostilities are a priori infected. Thus, according to various authors, 44-75 % of wounded people develop infectious complications of gunshot wounds [1, 5, 6, 12, 21].

During the last decades of armed conflicts, gram-negative microorganisms (*Acinetobacter baumannii, Enterobacter aerogenes, Klebsiella pneumoniae, Pseudomonas species, Proteus species* and *Escherichia coli*) became the main contaminants of combat wounds [5, 6].

The dominance of gram-negative bacteria in the spectrum of wound pathogens confirms the trend of recent years and correlates with the data obtained during the ATO/OS in the period 2014-2021 [5, 6, 12]. The isolates of bacteria belonged to two groups: enterobacteria and gram-negative non-fermenting bacteria. The dominance of *Acinetobacter baumannii* among the isolated pathogens draws attention. These microorganisms contaminated the gunshot wound in 63 % of the wounded. For the first time, the role of these microorganisms in the development of wound infection was recorded during military operations in Iraq and Afghanistan (2001-2014). Then this type of pathogens was isolated from the wounds of 50 % of the wounded [12]. The proportion of *Acinetobacter baumannii* isolated during ATO/OS was about 45 % [5, 6]. Thus, there is an obvious increase in the specific gravity of *Acinetobacter baumanii* in the microbial spectrum of microorganisms – contaminants of a modern gunshot wound during the period from the beginning of ATO/OOS to the full-scale war against Ukraine. This is explained by the formation and spread of hospital strains of this type of bacteria in the process of long-term movement of the wounded through the stages of medical evacuation. The peculiarity of these bacteria is the speed of development of antibiotic resistance, a high degree of ability to form a film in the wound. Due to these properties, *Acinetobacter baumanii* is included by the WHO in the list of priority microorganisms that pose the greatest threat to human health [12].

Also, an increase in the specific weight of bacteria of the genus *Klebsiella spp.* in the microbial spectrum of wound pathogens was recorded. Attention should also be paid to the monomorphism of the selected *Klebsiella* in terms of species composition. All isolated strains were identified as *Klebsiella pneumoniae* [5, 6, 12].

The specific weight of another type of non-fermenting gram-negative bacteria, which in past years dominated the list of wound pathogens, namely representatives of the genus *Pseudomonas spp.*, on the contrary, decreased [5, 6, 12]. If in the period of ATO/OOS, this type of bacteria accounted for 25 % of all isolated gram-negative bacteria, then in modern conditions, the bacillus of blue-green pus was isolated from only 1 % of the wounded. Apparently, *Pseudomonas aeruginosa*, characterized by a relatively high pathogenic potential, is displaced in hospital conditions, to a greater extent, by evolutionarily flexible species of non-fermenting gram-negative bacteria, such as *Acinetobacter spp.*.

The microflora of a combat wound causes microbial dysbacteriosis of other microbial populations, their quantitative and qualitative indicators. This is characterized by changes in typical metabolic functions, induction of oxidative stress, inflammation and damage to the male reproductive system, affecting sperm quality and fertility [2, 3, 12].

The human body is largely colonized by microorganisms, the impact of which on health is becoming more and more decisive [16]. The human genital tract contains a diverse microbiota, and more and more studies indicate that bacteria play a role in male infertility, hormonal regulation, and the development of pathological conditions [10]. However, this area of research remains understudied. Studies of bacterial colonization of the male genital tract depend on many factors, in particular, the invasive nature of sampling and the low number of microbiota, which makes diagnosis difficult.

The presence of bacteria in the urogenital tract was associated with an infectious state [27]. The most common consequences of bacterial infections in the urogenital tract are orchitis, epididymitis, prostatitis, and urethritis [4, 22]. Most of these infections are caused by sexually transmitted pathogens and ascending uropathogens. *Chlamydia trachomatis,* the most common sexually transmitted microorganism. *Neisseria gonorrhoeae* predominates in epididymorrhitis and urethritis. On the other hand, acute and chronic prostatitis are mostly caused by *Escherichia coli*, as well as other members of the *Enterobacteriaceae, in particular Klebsiella spp., Proteus spp. and Pseudomonas aeruginosa* and other species of *Enterococcus spp. and Staphylococcus aureus*. The presence of bacteria in the upper parts of the genital tract is associated with active infections, viral or bacterial, with subsequent acute or chronic inflammation [22, 23]. The main bacterial agents of the urogenital tract include *Chlamydia trachomatis, Neisseria gonorrhoeae, Mycoplasma genitalium, Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus,* which, according to bacteriological studies, constitute a specific share of the etiological factor of orchitis, epididymitis, or epididymitis. Bacterial colonization of the urethra has also been studied in the context of idiopathic urethritis. The results showed that the microbiota differed significantly between the control group and men with urethritis, and that the gender of the partner also affected the composition of the microbiota.

Thus, the microbiome of combat wounds, dysbacteriosis of the male reproductive system should be considered as one of the factors of male infertility. Recognizing individual microbial imbalances is critical to medicine, allowing for personalized interventions to treat wounds and address the root causes of male infertility.

CONCLUSIONS

Acinetobacter baumanii and Klebsiella pneumoniae are the dominant microflora complicating the course of combat wounds during almost two years of Russia's full-scale war against Ukraine. Probably, the duration of hostilities, the large number of wounded, and the forced mass unsystematic use of various antibiotics lead to rapid changes in the spectrum of pathogens of combat wounds. Combat wounds, stress, and nervous disorders lead to an imbalance of microflora, in particular the microflora of the genitourinary system, which is one of the causes of infertility. *Chlamydia* and *ureaplasma* are the most common microorganisms that colonize the urogenital tract of men injured as a result of hostilities.

The prospects for further research. These studies will be continued in the direction of: characteristics of the microflora of the wound surface and urogenital tract in the dynamics until the wound surface heals. Analysis and study of factors of pathogenicity of wound surface microorganisms and their sensitivity to antibiotics.

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COMPLIANCE WITH ETHICAL REQUIREMENTS

The research was carried out in compliance with the principles of medical ethics and the protection of patients' rights, human dignity and moral and ethical norms, in accordance with the principles of the Helsinki Declaration of Human Rights, the Council of Europe Convention on Human Rights and Biomedicine, the relevant laws of Ukraine; permission of the Bioethics Commission of Danylo Halytsky Lviv National Medical University.

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Резюме

МІКРОФЛОРА БОЙОВОЇ РАНИ ОРГАНІВ ТАЗУ ЧОЛОВІКІВ І ДИСБАКТЕРІОЗ СЕЧОСТАТЕВОЇ СИСТЕМИ

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Вступ. Стійкість мікроорганізмів до протимікробних препаратів робить неефективнми чи малоефективними зусилля сучасної медицини у боротьбі з інфекційними агентами. Із бойовими травмами, їх інфікуванням, стресами, невротичними розладами тісно пов'язана проблема неплідля.
Мета. Отримання та узагальнення даних про мікробну колонізацію мінно-вибухових ран органів тазу та мікробіом сечостатевої системи учасників бойових дій.
Матеріали та методи. Були взяті 84 мазки із 56 ран 36 пацієнтів з пораненнями органів тазу, які знаходились на лікуванні. 73 пацієнтів з пораненнями досліджували на предмет мікорофлори в урогенітальному тракті. Виділення чистих культур бактерій проводили шляхом посіву досліджуваного матеріалу з використанням м'ясо-пептидного агару, кров'яного агару, хромогенних агарів. Для діагностики урогенітальних чи інших інфекцій методом ПЛР у пацієнтів брали зішкріб із задньої стінки уретри.
Результати. Переважаючими мікроорганізмами в позитивних посівах мазків були неферментуючі грамнегативні палички, які в 28 % посівів належали до роду *Acinetobacter*, в 26 % до роду *Pseudomonas.* Щодо асоційованих інфекцій, то на 20 % вони складались із роду *Acinetobacter*, 32 % *Enterobacter,* 4 % *Klebsiella* і 29 % *Pseudomonas.* Грампозитивні коки були виділені в 37 % позитивних посівів. При асоційованих інфекціях частота виділення роду *Streptococcus* становила 4 %, потім роду *Clostridium* 2 %, *Bacillus* 4 %, Enterococcus 3 % та Actynomycceas 5 %. При аналізі мікрофлори сечостатевої системи виявлено, що пріоритетна роль належить комбінованій інфекції, коли присутні асоціації специфічних патогенів таких як *Ureaplasma spp., Mycoplasma spp., Chlamidia spp., Neisseria gonorrhoeae, Trichomonas vaginalis, Streptococcus spp., Enterococcus faecalis,* що складає 80 % усього мікробіому.
Висновки. Домінуючою мікрофлорою, що ускладнює перебіг бойових поранень, протягом практично двох років повномасштабної війни Росії проти України є *Acinetobacter baumanii* і *Klebsiella pneumoniae.* Бойові рани та їх інфікування, стреси, нервові розлади призводять до дисбалансу мікрофлори, зокрема мікрофлори сечостатевої системи, що можуть бути одними із причин розвитку неплідля. Найпоширенішими мікроорганізмами, що колонізують урогенітальний тракт чоловіків постраждалих внаслідок бойових дій є хламідії та уреаплазми.

Ключові слова: **бойова травма, мікрофлора травми, урогенітальна система, неплідність**