

Aerobic skin microbiota study in patients with paratraumatic eczema developed as a result of combat injuries

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ABSTRACT

Aim: To establish taxonomic composition, population level and microecological indicators of the “macroorganism-microbiota” ecosystem by determining the expression levels of individual pathogenicity factors of microorganisms isolated from the skin areas of patients with paratraumatic eczema developed as a result of combat injuries.

Materials and Methods: Studied microbiologically eczematous lesions content in 54 military men to determine the qualitative and quantitative composition of microbiocenosis to analyze the taxonomic composition, population-species level, microecological indicators and expression levels of individual pathogenicity factors.

Results: The results indicate that most eczematized areas are contaminated with *S.aureus* (48.1%) with the highest level of quantitative dominance. The rate of quantitative dominance of other strains is significantly lower: *S.epidermidis* – 2.56 times, *S.haemolyticus* – 4.56 times, *S.pyogenes* – 5.86 times, *E.faecalis* and *K.pneumoniae* – 20.56 times. When determining the sensitivity of *S. aureus* clinical strains to antibacterial drugs, we established high levels of sensitivity to mupirocin, fusidic acid, and oxazolidinones – 95.1%, 90.2%, and 87.8%, respectively. Moreover, *S. aureus* dominated among the strains with a high ability to form biofilms – 68.2%, with an average ability – *S.epidermidis*, 28.8%.

Conclusions: *S.aureus* is the leading pathogen in the infectious-allergic process with paratraumatic eczema according to the constancy index of each taxon, frequency of manifestation, Margalef’s species richness index, Whittaker’s species diversity, the value of Simpson’s and Berger-Parter’s species dominance indices, as well as the population level of each taxon, the coefficient of quantitative dominance and the coefficient of significance. We should consider this fact when prescribing therapy.

KEY WORDS: combat injuries, paratraumatic eczema, aerobic microbiota

Wiad Lek. 2025;78(1):45-52. doi: 10.36740/WLek/197131 DOI

INTRODUCTION

Combat injuries are more complicated and severe compared to peacetime injuries. The microbiology of military wounds changes with the development of both medicine, and warfare. The resulting wounds are mostly characterized by the presence of a large defect area, impaired blood supply, infection, and slow healing. Such injuries require effective medical care for quick recovery [1, 2]. Paratraumatic eczema, which occurs around wounds that do not heal for a long time after injuries, cuts, skin burns or combat wounds, is most often registered among the clinical varieties of microbial eczema [3]. In most patients, dermatosis is asymmetrical, localized on open areas of the skin (hands, forearms, lower legs, face, neck). Lesions have clear boundaries with peeling of the epidermis along the edge of the foci in the form of a border. In the center of the foci against the background of erythema and edema, there is moderate wetting, point erosions, multiple serous-purulent

crusts, while on the periphery – pustular elements. On the lower extremities, erythema in foci has a bluish tint. One cannot help but be alarmed by the fact that the course of dermatosis in recent years has become more severe with frequent relapses, significant generalization of the process on the skin, and resistance to treatment [4, 5]. The most frequent complication of the eczematous process is the addition of a secondary pyococcal and fungal infection, associated with a decrease in the antimicrobial resistance of the skin. The therapy of infectious lesions is complicated due to the growing resistance of the main causative agents of pyoderma - *Staphylococcus aureus* and *Staphylococcus epidermidis* - to widely used antibiotics. The main principle of eczematous manifestations therapy, considering the polyetiological nature of this disease, is a complex effect on the body, considering the severity, nature, localization of the pathological process, the duration of the disease, previous treatment and its effectiveness,

the age of the patient and the available concomitant pathology [6].

The ability of staphylococci to migrate, survive in adverse conditions, and exchange genetic loci of acquired antibiotic resistance leads to the spread of "aggressive" strains, which causes the development of severe complications. Such strains, as a rule, are resistant to several groups of antibacterial drugs, which significantly complicates therapy [7]. In addition, staphylococci are characterized by the presence of a large number of pathogenicity factors that allow them to survive under various adverse environmental influences, one of which is the ability to form biofilms. We commonly understand biofilms as a living community of several types of bacteria, fixed on a substrate and surrounded by a protective matrix. The presence of a well-designed consortium of bacteria in the biofilm matrix ensures their survival. Treatment with antibiotics can destroy only the planktonic forms of this bacterium, while pathogens immobilized in a biofilm can survive and multiply after therapy discontinuation [8, 9].

AIM

The purpose of the study was to establish taxonomic composition, population level and microecological indicators of the «macroorganism-microbiota» ecosystem by determining the expression levels of individual pathogenicity factors of microorganisms isolated from the skin areas of patients with paratraumatic eczema developed as a result of combat injuries.

MATERIALS AND METHODS

The authors conducted a laboratory microbiological study of eczematous lesions content of 54 military men treated in the dermatological department of the State Institution «Institute of Dermatology and Venereology, National Academy of Medical Sciences of Ukraine». 77 strains of microorganisms, representatives of 6 taxonomic groups were isolated. All patients were male. The patients were from 19 to 57 years old, the average age was (34.3 ± 1.1) years. The comparison group consisted of 19 people of representative age and gender.

The authors collected and primarily inoculated biological material by generally accepted methods. Moreover, the material was microbiologically studied immediately after collection, no later than 2 hours. Morphological, tinctorial, cultural, and biochemical properties helped identify the selected taxa [10]. In addition, to characterize the population-species and microecological indicators, we determined index of

constancy, frequency of occurrence according to the value of Margalef's species richness index, the Whittaker's species diversity index, as well as Simpson's and Berger-Parter's indices of species dominance [11].

The study also determined separate factors of pathogenicity (resistance to antibiotics and the ability to form biofilms). Using the method of adhesion to polystyrene in flat-bottomed plastic tablets, we confirmed the ability to form a biofilm. The optical density (OD) was measured on a microplate reader at 540 nm, comparing the OD 540 of the samples with that of the negative control. Absence of biofilm was recorded when the OD of the sample \leq OD of the control, a weak degree of biofilm production - when the OD of the control $<$ OD of the sample \leq 2 OD of the control, the average degree of biofilm production - at 2 OD of the control $<$ OD of the sample \leq 4 OD of the control, a high level of biofilm production - at 4 OD of the control $<$ OD of the sample, in accordance with the recommendations of Rodrigues et al., 2010. All experiments were performed in six replicates [12, 13]. Statistical data analysis was carried out using MS Excel packages.

RESULTS

54 patients took part in bacteriological examination with the aim to isolate and identify aerobic bacteria in eczematous contents obtained from patients with paratraumatic eczema. In total, we isolated 77 strains of opportunistic bacteria belonging to 6 different taxonomic groups. During the examination of the comparison group (19 people), we isolated a total of 21 strains of microorganisms belonging to the genus *Staphylococcus*. Table 1 shows the taxonomic composition of the «macroorganism-microbiota» ecosystem of the aerobic lesions microflora in patients with paratraumatic eczema and practically healthy individuals.

54 patients with paratraumatic eczema resulting from combat injuries (wounds) had 77 strains of conditionally pathogenic aerobic bacteria isolated and identified. This confirms that associations of these bacteria are involved in the eczematous process in some patients. In comparison, in the control group, only 2 people showed the presence of two-component associations including types of staphylococci. Fig. 1 shows quantitative characteristics of opportunistic aerobic microbiota associations in the eczematous process.

Qualitative characterization of associations is important for the eczematous process. Table 2 shows qualitative characteristics of opportunistic bacteria association contaminating eczematization foci, as compared to the skin of practically healthy individuals.

To identify the leading causative agent of the inflammatory process caused by associations, it is

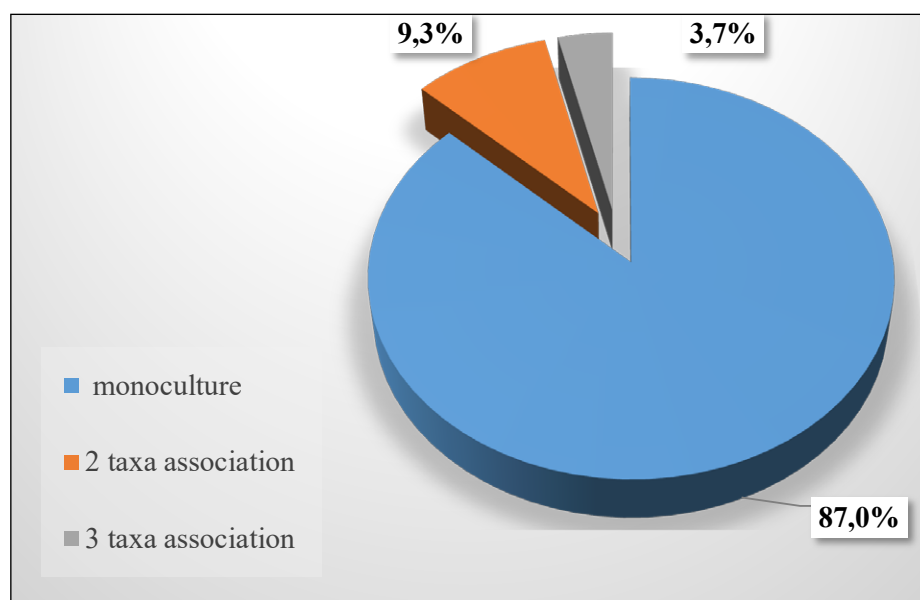


Fig. 1. Quantitative characteristics of opportunistic aerobic microbiota associations in lesions of patients with paratraumatic eczema.

Table 1. Taxonomic composition of the aerobic lesions microflora in patients with paratraumatic eczema and practically healthy individuals

Microorganisms	Typology of dominant microorganisms						Indices of species dominance						P*
	Affected skin (n=54)						Healthy skin (n=19)						
	Determined and identified strains	Constancy index (%)	Index of species dominance	Whittaker's species diversity index	Simpson's species dominance indices	Berger-Parter's species dominance indices	Determined and identified strains	Constancy index (%)	Index of species dominance	Whittaker's species diversity index	Simpson's species dominance indices	Berger-Parter's species dominance indices	
<i>S.aureus</i>	41	53,2	0,761	0,878	0,282	0,53	2	9,5	2,88	9,5	0,009	0,991	≤ 0,05
<i>S.epidermidis</i>	16	20,8	2,267	3,812	0,044	0,21	17	80,9	0,71	0,23	0,65	0,35	≤ 0,05
<i>S.haemolyticus</i>	9	11,7	3,09	7,555	0,014	0,12	2	9,5	2,88	9,5	0,009	0,991	
<i>S.pyogenes</i>	7	9,1	3,46	10,0	<0,001	0,09	-	-	-	-	-	-	
<i>E.faecalis</i>	2	2,6	5,267	37,5	<0,001	0,03	-	-	-	-	-	-	
<i>K.pneumoniae</i>	2	2,6	5,267	37,5	<0,001	0,03	-	-	-	-	-	-	

* p given in comparison with a control group of practically healthy individuals.

necessary to determine the population level of each component of the microbial community. Table 3 shows the population level and microecological indicators of opportunistic bacteria contaminating foci of eczematization.

The next stage of the study relates to sensitivity determination of clinical strains of *S.aureus* isolated from affected areas of the skin of military personnel with paratraumatic eczema to antibacterial drugs, as the most represented and pathogenic representative of the genus *Staphylococcus*. Fig. 2 shows the obtained data.

The study of various formation (or destruction) aspects of bacterial biofilms is a relevant and promising

direction. It will enable scientists to optimize approaches to the diagnosis and treatment of a number of infections, including purulent-inflammatory complications of paratraumatic eczema [8]. Table 4 presents the summarized adhesion degree data to polystyrene of the extracted clinical strains of staphylococci as dominant representatives in the structure of eczematous lesions microbiocenosis.

DISCUSSION

Using the species constancy index and Whittaker's species diversity index, the authors analyzed micro-

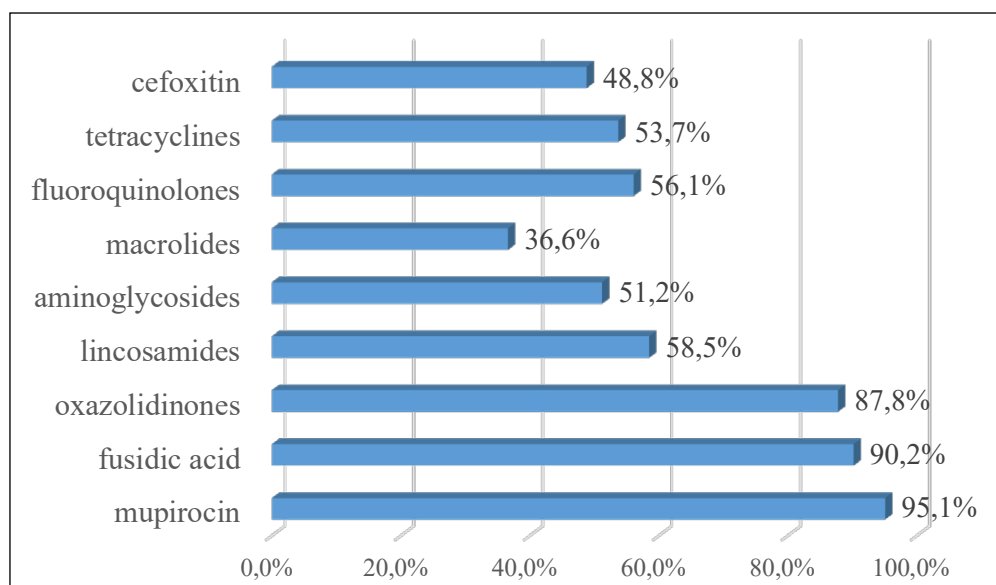


Fig. 2. Sensitivity of *S. aureus* clinical strains isolated from the affected skin areas in military personnel with paratraumatic eczema, (n = 41).

Table 2. Qualitative characteristics of opportunistic bacteria association, contaminating foci of eczematization compared to the skin of practically healthy individuals

Affected skin (n=54)				Healthy skin (n=19)			
Association composition	Association forming bacteria	abs.		Association composition	Association forming bacteria	abs.	
monoculture	<i>S.aureus</i>	37	48,1	monoculture	<i>S.aureus</i>	1	5
	<i>S.epidermidis</i>	13	16,9		<i>S.epidermidis</i>	15	71,4
	<i>S.haemolyticus</i>	5	6,5		<i>S.haemolyticus</i>	1	5
	<i>S.pyogenes</i>	6	7,8				
2- taxa associations	<i>S.aureus</i> + <i>S.epidermidis</i>	2	2,6	2- taxa associations	<i>S.aureus</i> + <i>S.epidermidis</i>	1	5
	<i>S.aureus</i> + <i>S.haemolyticus</i>	2	2,6		<i>S.haemolyticus</i> + <i>S.epidermidis</i>	1	5
	<i>S.pyogenes</i> + <i>E.faecalis</i>	1	1,3				
3- taxa associations	<i>K.pneumoniae</i> + <i>E.faecalis</i> + <i>S.haemolyticus</i>	1	1,3	3- taxa associations			
	<i>K.pneumoniae</i> + <i>S.haemolyticus</i> + <i>S.epidermidis</i>	1	1,3				

ecologically the selected associations of microorganisms. Depending on the value of the constancy index, microorganisms were divided into permanent (with a constancy index of 50% or more), additional (with a constancy index of 25% - 50%) and random (with a constancy index of 25% and below). A low index means the homogeneity of the system, that is, the predominance of one group of microorganisms.

The combination of a low diversity index with a high constancy index of one species indicates a decompensated dysbiosis or a disease most likely caused by this species. The study of the taxonomic composition of the «macroorganism-microbiota» ecosystem of the aerobic lesions microflora in

patients with paratraumatic eczema (Table 1) shows that the absolute majority of eczematized areas are contaminated with *S.aureus* (53.2% of patients). However, *S.epidermidis*, *S.haemolyticus*, *S.pyogenes* are less often isolated and identified – 20.8%, 11.7% and 9.1%, respectively.

Gram-negative bacillus (*K.pneumoniae*) and gram-positive cocci (*E.faecalis*) occurred in 2.6% of the examined. According to the constancy index, the frequency of occurrence, the value of Margalef's species richness index, Whittaker's species diversity index, and Simpson's and Berger-Parter's species dominance indices, the main part of the microbiocenosis of eczematization centers contained

Table 3. Population level and microecological indicators of opportunistic bacteria contaminating foci of eczematization (n=54)

Microorganisms	Population level in lg CFU/ml	Coefficient of quantitative dominance	Significance coefficient
<i>S.aureus</i>	5,4 ± 0,3	53,25	0,024
<i>S.epidermidis</i>	4,87 ± 0,35	20,78	0,063
<i>S.haemolyticus</i>	3,67 ± 0,36	11,69	0,11
<i>S.pyogenes</i>	4,27 ± 0,21	9,09	0,14
<i>E.faecalis</i>	4,52 ± 0,05	2,59	0,5
<i>K.pneumoniae</i>	4,39 ± 0,21	2,59	0,5

Table 4. Intensity assessment of biofilm formation by clinical strains of staphylococci

Strains of microorganisms/ quantity	Index of film formation (OD ₅₄₀)					
	low		average		high	
	Quantity of strains	OD ₅₄₀	Quantity of strains	OD ₅₄₀	Quantity of strains	OD ₅₄₀
<i>S. haemolyticus</i> / 9			4	0,31±0,01	5	0,41±0,04
<i>S.epidermidis</i> / 16	2	0,179±0,02	10	0,29±0,01	4	0,44±0,03
<i>S. aureus</i> / 41			5	0,33±0,01	36	0,369±0,01
Total	2		19		45	

S.aureus. *S. epidermidis* occupied the second position, considering the tendency to its frequent extraction within the limits of the constancy index values, as an additional microorganism. Other bacteria (*S.haemolyticus*, *S.pyogenes*, *E.faecalis*, *K.pneumoniae*) were rare and accidental, according to the data of the study. The data we obtained were compared with similar studies conducted with the participation of civilian patients with the study of the microbiological status of venous ulcers of the lower extremities. There were, that alert pathogens are defined as drug-resistant, life-threatening microorganisms that constitute a significant epidemiologic problem. Among other microorganisms, the group of alert pathogens includes *S.aureus*, *Pseudomonas aeruginosa*, *Enterococcus* spp., *Streptococcus pneumoniae*, *Acinetobacter* spp., and *Enterobacteriaceae*, which were isolated from 28.6% of culture-positive ulcers. Alert pathogens, especially *P.aeruginosa* and *S.aureus*, are commonly isolated from venous leg ulcers [14]. It should be noted that the microflora of eczematous ulcers that developed as a result of combat injuries had higher degrees of colonization and population level. Due to the lack of data on the determination of statistical microbiological parameters in the study of the severity of combat injuries, a comparative analysis was not conducted

77 strains of conditionally pathogenic aerobic bacteria were isolated and identified in 54 patients with paratraumatic eczema, resulting from combat injuries (wounds). This is evidence that associations of these bacteria take part in the eczematous process in some

patients. The quantitative characteristics of aerobic bacteria associations isolated from lesions (Fig. 1) show that a monoculture contaminated 87.0% of patients with paratraumatic eczema; 9.3% had associations of aerobic bacteria consisting of two different taxa; in 3.7% - contamination consisted of three different taxa. The study also determines qualitative characteristics of aerobic bacteria associations extracted from areas of eczematization in patients with paratraumatic eczema. (Table 2). The research has established that the inflammatory process in most patients is due to monoculture of *S.aureus* (48.1%). Other microorganisms in monoculture were rare: *S.epidermidis* in 16.9% of the examined, *S.haemolyticus* in 6.5% and *S.pyogenes* in 7.8%. In 9.3%, an association consisting of 2 different taxa: *S.aureus*+ *S.epidermidis*, *S.aureus*+ *S.haemolyticus* and *S.pyogenes*+ *E.faecalis* (2.6%, 2.5% and 1.3%, respectively) supported the inflammatory process. Associations consisting of three different taxa contained *K.pneumoniae* + *E.faecalis* + *S.haemolyticus* and *K.pneumoniae* + *S.haemolyticus* + *S.epidermidis*. Thus, *S.aureus* is the main cause of the inflammatory bacterial process that develops in paratraumatic eczema.

The study of the population level and microecological indicators of aerobic bacteria in the microbiota of eczematous lesions (Table 3) shows that the highest population level among them is in *S.aureus*. The values of the rating indicators (Margalef's and Whittaker's indices) were the highest among aerobic bacteria and characterized the spatial and nutritional resources of the biotope as well as the habitat conditions of

S.aureus. All other anaerobic bacteria have a lower population level - *S.epidermidis* by 23.9%, *S.haemolyticus* by 42.7%, *S.pyogenes* by 33.3%, *E.faecalis* and *K.pneumoniae* by 42.3%. The revealed value of the population level determines different dominance levels of these bacteria. Thus, the highest level of quantitative dominance was in *S.aureus*. The quantitative dominance of other species was much smaller: *S.epidermidis* - 2.56 times, *S.haemolyticus* - 4.56 times, *S.pyogenes* - 5.86 times, *E.faecalis* and *K.pneumoniae* - 20.56 times.

In order to predict the epidemic situation and develop anti-epidemic measures, in particular, empirical antibiotic therapy in each specific hospital and even department, it is important to constantly monitor not only the species composition and levels of resistance to antibiotics of the causative agents of purulent-inflammatory diseases of the wounded, but also the genes encoding this resistance. In addition, a comparative analysis data of the phenotypic signs of microorganisms resistance to antibiotics (antibiotic resistance profiles) with the presence of certain antibiotic resistance genes in their genome will help predict possible expression of genes that are in an inactive state. Such studies are especially relevant in countries with military conflicts. Therefore, the number of patients with wounds of various severity infected with microorganisms, in particular, resistant to antibiotics, is increasing. When determining the sensitivity of clinical strains of *S. aureus* isolated from patients with paratraumatic eczema to ABP, we established high levels of sensitivity to mupirocin, fusidic acid and oxazolidinones - 95.1%, 90.2% and 87.8%, respectively (Fig. 2). At the same time, the number of polyresistant strains was 54.2%, with extensive resistance - 4.7%. Analysis of the sensitivity of pathogens to individual groups of drugs, obtained in another study, showed that *K. pneumoniae* is sensitive mainly to tetracyclines, fluoroquinolones, moderately sensitive to cephalosporins. Among other pathogens (*A.lwoffii*, *K.oxytoca*, *P.mirabilis*, *S. aureus*, *S. epidermidis*, *S. haemolyticus*, *S.hominis*, *S. maltophilia*), the general pattern of sensitivity was the following: most of them were resistant to penicillins, almost half - to carbapenems and fluoroquinolones, and the distribution of sensitivity did not depend on tinctorial properties [1].

Bacteria are able to adapt to changes in nutrition, the presence of stresses caused by external conditions, presence of inhibitory compounds, as well as to immune protection. One of the particularly important examples of bacterial adaptation mediated by the systematic action of genes is the ability to reproduce in immobile polymicrobial communities known as biofilms. Significant changes in gene expression

and synthesis of additional proteins, manifested by resistance to antimicrobial agents and immune defense factors, accompany existence in the form of biofilms. The data in Table 4 show that the ability to form biofilms of varying degrees of density characterizes clinical strains of microorganisms. Such strains accounted for 68.2% (45 strains), among which *S.aureus* dominated (three quarters of the total number of these strains). 28.8% of strains had average ability to form biofilms, and dominated in *S. epidermidis* - 52.6%. Due to the lack of data on the determination biofilm parameters in another study of combat injuries microorganisms, a comparative analysis was not conducted.

CONCLUSIONS

1. *S.aureus* is the leading pathogen in the infectious-allergic process with paratraumatic eczema according to the constancy index of each taxon, frequency of manifestation, Margalef's species richness index, Whittaker's species diversity, the value of Simpson's and Berger-Parter's species dominance indices, as well as the population level of each taxon, the coefficient of quantitative dominance and the coefficient of significance. This microorganism causes the inflammatory process in 48.1% of patients in monoculture. Therefore, the therapeutic tactics of treating paratraumatic eczema should take into account sensitivity of *S.aureus* to antibacterial drugs.
2. When determining the sensitivity of *S. aureus* clinical strains isolated from patients with paratraumatic eczema, the study established high levels of sensitivity to mupirocin, fusidic acid, and oxazolidinones - 95.1%, 90.2%, and 87.8%, respectively. In case of necessity, it is possible to prescribe these drugs empirically before obtaining the results of a bacteriological study with the actual determination of the pathogens sensitivity to antibacterial drugs.
3. The study reveals that clinical strains of staphylococci have the ability to form biofilms of varying degrees of density. Strains with a high ability to form biofilms made up 68.2%, with *S.aureus* dominating (three quarters of the total number of these strains). Strains with an average ability to form biofilms accounted for 28.8% and contained *S. epidermidis* strains.

Prospects for further research the need for further research into the taxonomic composition of aerobic microorganisms with further study of the population level of autochthonous and allochthonous microbiota, their sensitivity to antibacterial agents for therapeutic and preventive purposes.

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The study was carried out within the framework of the National Research Council P 54-23 «Development of new means and methods of external treatment of combat traumatic injuries of the skin and adjacent tissues depending on the phase of the wound process», state registration No. 0123U101367, approved by the Resolution of the Presidium of the National Academy of Medical Sciences of Ukraine dated 12.30.22 No. 16/1.

CONFLICT OF INTEREST


The Authors declare no conflict of interest


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RECEIVED: 07.05.2024

ACCEPTED: 09.12.2024

