Acta Biomed 2025; Vol. 96, N. 1: 16158 DOI: 10.23750/abm.v96i1.16158

Original article

The impact of wound microbiota on chronic pain development in limb injuries: A microbiological perspective

Kateryna Ksenchyna[2], Dmytro Dmytriiev[1], Oleh Ksenchyn[2], Oleksandr Nazarchuk[2]

[1]Prometei Pain Research center, Vinnytsia, Ukraine; [2]National Pirogov Memorial Medical University, Vinnytsya, Vinnytsia, Ukraine

Abstract. *Background and aim of the work:* Undoubtedly, studying the clinical features of wound infection is relevant in the modern world, especially in Ukraine due to war. The main goal was to prevent possible remote consequences of wound healing and we studied the impact of wound microbiota in this aspect. *Research design and Methods:* We conducted a microbiological study of wounds among 51 patients with limb injuries. The age characteristics of the groups did not differ from each other. Also, 30 patients were interviewed using VAS pain to conduct an association between pain severity and the wound microbiota composition. Statistical calculations were performed using a computer program MedCalc®Software**.** *Results:* We divided patients into two groups: 1 group with one isolated an opportunistic wound pathogen and group 2 in which two or more types of opportunistic wound pathogens were isolated. The number of microbial associations formed by gram-negative species of microorganisms was determined in 1.78 times more often than the colonization of wounds by gram-positive opportunistic microorganisms. The surveyed respondents were divided into subgroups 1.1 and 2.1. The average value of pain in subgroups 1.1 and 2.1 was 38.46 (SD ± 13.44) and 39.16 (SD ± 13.78) and did not differ statistically ($p > 0.05$). *Conclusions:* The predominance of gram-negative microorganisms as *Klebsiella pneumoniae, Acinetobacter baumannii*, *Staphylococcus spp., Pseudomonas aeruginosa* was established. The sensation of pain did not depend on the number of colonizing species. The obtained data encourage further research into the role of microorganisms in the healing process. (www.actabiomedica.it)

Key words: wound microbiota, chronic pain, limb trauma, combat wound healing, microbial profiling, pain and microbiota.

Introduction

In recent years, the frequency of armed conflicts has increased in the world, which is accompanied by an increase in the number of injuries with various localization, which require special approaches to the treatment of these patients at all stages of providing medical care. Therefore, knowledge of tactical medicine's important aspects and infection control in wartime conditions are quite relevant issues. The war has been going on in Ukraine for many years, and therefore the Healthcare organization has faced new challenges and questions. Compared to the years of peacetime, there are significantly more people with injuries who need specialized help. One of the key factors is compliance with the principles of infection control during evacuation and the subsequent stages of providing medical care to reduce the number of infectious complications and their possible long-term consequences (1,2). Of course, the main source of infection is an open wound surface, since a wound received as a result of combat often comes into contact with dirt, torn clothing, debris, and other unfavorable factors. Therefore, special attention should be paid to the microbiota of wounds and the surrounding microenvironment (3,4). The biggest problems that arise before ensuring an adequate

healing process are not only the resistance of microorganisms to antibiotics, but also the nature of the action of microbial factors of pathogenicity on local structures and tissues that produce bacteria and, as a result, long-term inflammation (5). The effectiveness and success of wound infection treatment also depends on the qualitative composition of the microbiota on the wound surface (gram-positive, gram-negative). Most types of gram-negative bacteria are highly pathogenic, as their cell wall contains their own endotoxins. In published works on the study of chronic wound infection, it is said that in comparison with the normal microbiota of the skin, gram-negative rods, anaerobes and gram-positive cocci are found more often. In vivo studies have also been conducted in animal models. Based on these results, it was established that the changed composition of the wound microbiome can directly initiate healing disorders (6). According to many studies, the most frequent causes of a prolonged inflammatory process and wound infection are gram-negative bacteria *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. These bacteria are also included in the group of ESKAPE multiresistant pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter species*). The ability to produce extended-spectrum β-lactamases makes them universal persistent damaging agents, as this feature forms resistance to the most frequent first-line therapy: the group of penicillins and cephalosporins (7,8,9). For a long time, the potential occurrence of postoperative pain was directly associated only with damage to the nerve endings, but in many patients, subsequent studies confirmed the absence of nerve involvement. In today's conditions, it is considered that postoperative pain is not associated with isolated changes, but with a combination of nerve damage and inflammatory reaction (10). Early prevention of wound infection is a significant factor that affects the healing process and its duration. The longer the wound heals, the longer the soft tissues, blood vessels, and peripheral nerve fibers suffer. Such a long-term condition can significantly affect the sensitivity, the mechanisms of perception of pain stimuli by receptors and the transmission of nerve impulses (11,12). In addition, complex regional pain syndrome is described in the literature as a chronic condition occurring in 7% of patients with limb injuries requiring surgical treatment (13). Although the basic mechanisms of wound healing are known, its impairment at the cellular and molecular levels continues to be studied. In recent years, there have been studies describing the mechanism of the tissue damage influence on immediate inflammatory reactions. However, it is believed that the role of microorganisms in the development and course of chronic wound infection is not sufficiently studied, and it remains unknown whether the processes of the response to tissue damage and the innate immune response to infection are similar. It is also repeatedly mentioned the importance of the early use of local antimicrobial agents, and only later, if there are indications, the use of antibiotic therapy (6,14,15).

The difficulty of treating chronic wounds is, of course, the frequent combined colonization of the wound by several microorganisms and their ability to form biofilms (16,17). The microbiota of the wound can probably be considered as a direct predictor of the chronic pain syndrome formation. Chronic pain, like infected wounds, belongs to the problematic issues of providing medical care. On the one hand, a group of patient-centered consequences: decrease in the quality of life, an increased percentage of unfavorable treatment prognosis, the impact on the course of concomitant pathology, and on the other hand, the difficulties of solving the tasks of diagnosis and treatment before the medical staff. Changes in psychological state and behavioral reactions are also often observed (18).

Therefore, such clinical cases significantly increase the need for financing medical care, and this also creates an additional burden on the economic system, especially in less developed countries. The aim of our study was to study the composition of the wound microbiota and evaluate the potential relationship between the nature of microbial colonization of wounds and the formation of prerequisites for secondary chronic pain.

Patients and methods

The study included 51 patients. All individuals were male. The criteria for inclusion in the study were: age from 22 to 59 years, presence of limited injuries of

the extremities, and assessment of pain after surgical treatment no earlier than 1 month and no later than 3 months. It was also mandatory for the patient to sign a personal informed consent of the research participant. Exclusion criteria were age younger than 18 and older than 60 years, concomitant diseases in the decompensation stage, combined severe trauma, and cognitive disorders. In our work, we used questionnaire methods, microbiological examination of wound contents, and statistical methods.

Microbiological examination

The collection of material for further research from the wound surface was carried out according to the following steps: 1) hygienic treatment of hands and availability of personal protective equipment (gown, mask, gloves); 2) confirmation of the patient's identity and clarification of the methodology and purpose of the procedure; 3) after removing the bandage from the wound, changing gloves; 4) washing the wound with a sterile physiological solution; 5) removal of surface contamination; 6) system opening and sample transportation; 7) sampling with a swab from viable tissues with an area of 1 cm2; 8) further transportation to the laboratory in a sterile test tube Test tubes with Amies transport medium and accompanying referral for bacteriological examination. In the accompanying document, the patient's data, the time, date and place of material collection, data on antibiotic therapy and the time of sending to the laboratory were necessarily indicated. Transportation was carried out in accordance with all requirements. The actual microbiological research was carried out at the University Microbiological Laboratory of National Pirogov Memorial Medical University, Vinnytsya.

Questionnares: Visual Analogue Scale

To assess the pain syndrome, we chose a visual analog scale. Although this is a method based on the patient's subjective feelings, this scale is one of the most reliable methods for assessing the intensity of both acute and chronic pain (19,20). The scale has the form of a 10 cm ruler, where "0" means no pain, and "10" reflects as much pain as possible (21). The cut-off point for the distribution of patients into different groups was taken as the value "5", which characterizes the average intensity of pain. All patients were interviewed within a specified period: 1-3 months after surgical treatment due to the received injury.

Statistical analysis

The statistical calculation of the obtained data was carried out using the standardized program MedCalc®Software. We determined the following statistical indicators under the condition of normal distribution: arithmetic mean (M), arithmetic mean error (m), mean error (t), and the reliability of the difference (p). Differences were considered statistically significant at $p \leq 0.05$.

Bioethical commission

The study was performed in accordance with the World Medical Association Declaration of Helsinki on the ethical principles for medical research involving human subjects, the Council of Europe Convention on the Human Rights and Biomedicine, relevant laws, orders of the Ministry of Health of Ukraine. The Study protocols were approved by the Committee on Bioethics, National Pirogov Memorial Medical University, Vinnytsya, Ukraine (Protocol № 7, 27.05.2024).

Results

Our study included 51 men with limited limb injuries. The participants were divided into two groups depending on the results of the microbiological examination of the material taken from the wound contents. The principle underlying the division into different observation groups was the number of isolated pathogens in the studied material (Figure 1).

Group 1 included 26 patients, the average age of men in this group was 35.69 (SD ± 9.84) years. Group 2 included 25 patients, the average age of patients in this group was 32.88 (SD ± 9.73) years. There was no statistically significant age difference between the groups. As a result of the study, the predominance of

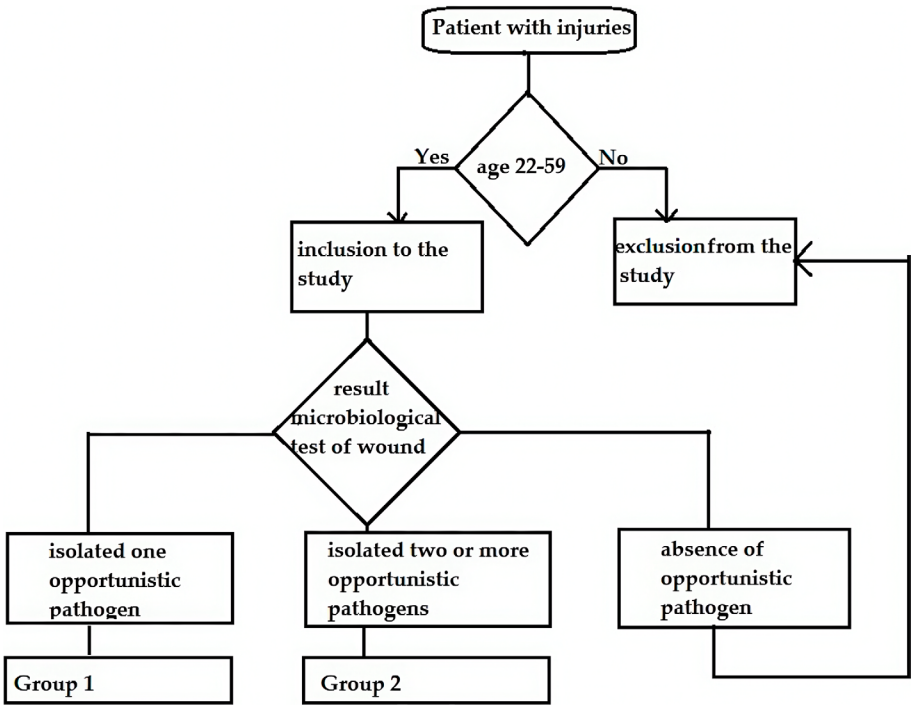

Figure 1. An algorithm for dividing participants into groups.

Table 1. Qualitative characteristics of microbiota in Group 1.

The microbiota type	Number	%
Gram-positive bacteria	5	19,23
Gram-negative bacteria	21	80,77

gram-negative pathogens of wound infection over gram-positive ones was established when colonizing wounds with a monoculture of microorganisms. Also, the dominance of gram-negative microorganisms was determined when colonization of wounds by several types of bacteria was present at the same time. The number of microbial associations formed by gram-negative species of microorganisms was determined 1.78 times more often than the colonization of wounds by gram-positive opportunistic microorganisms in association with gram-negative pathogens. A significant predominance of gram-negative microbiota in wound contents in the first group was established (Table 1).

Among the patients in the second group, there was also a predominance of a combination of gram-negative bacteria, there were also cases of a combination of gram-positive bacteria with gram-negative bacteria, a combination of gram-positive bacteria was not isolated (Table 2).

Table 2. Qualitative characteristics of microbiota in Group 2.

The microbiota type	Number	%
Gram-positive bacteria + Gram-negative bacteria	9	36
Gram-negative bacteria + Gram-negative bacteria	16	64
Gram-positive bacteria + Gram-positive bacteria	0	0

When conducting an analysis of the species composition of bacteria isolated from wound contents, it was established that: in the group *K. pneumoniae*, *A. baumanii*, *Staphylococcus spp.* were most often found. A smaller share was *P. aeruginosa*, *Proteus mirabilis*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Klebsiella oxytoca*, *Escherichia coli*, *Corynobacter spp.* (Figure 2).

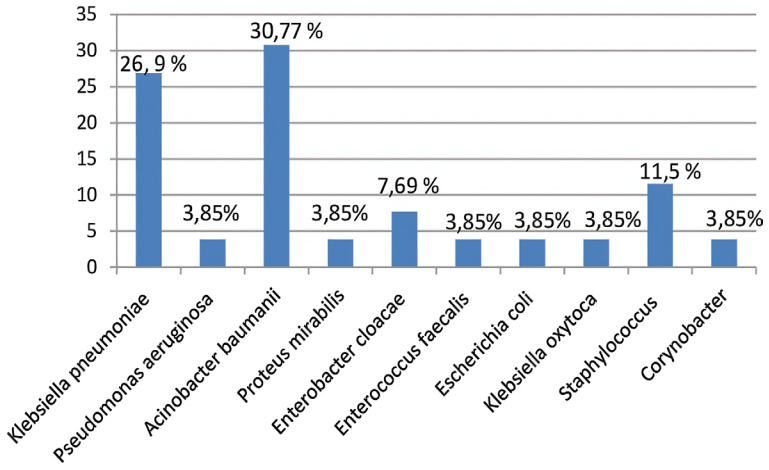

Figure 2. Species characteristics of wound microbiota composition in Group 1.

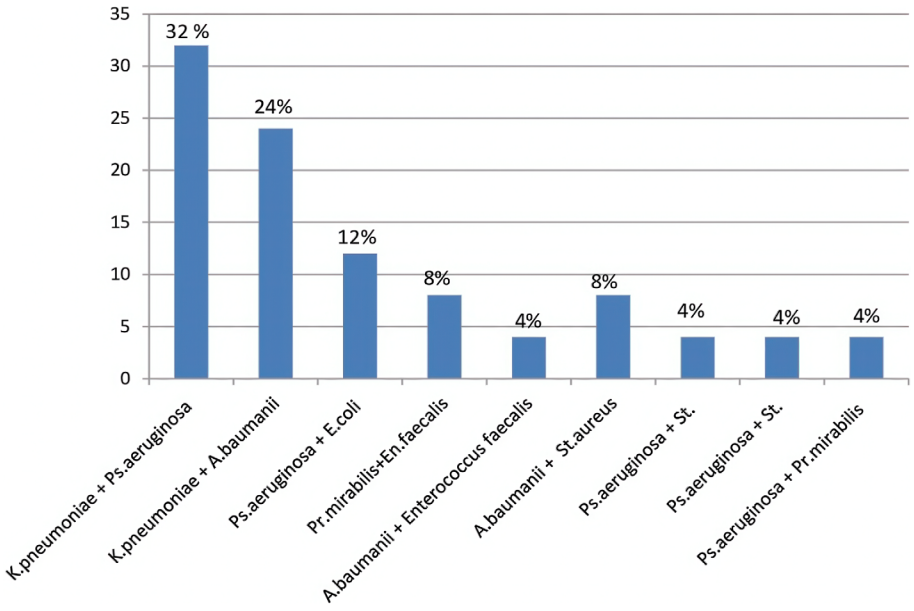

Figure 3. Species characteristics of wound microbiota composition in Group 2.

In Group 2, the combination of *K. pneumoniae* + *P. aeruginosa, K. pneumoniae* + *A. baumanii, E. faecalis* + *P. aeruginosa was most often recorded, P. aeruginosa* + *E. coli, P. mirabilis* + *E. faecalis, P. aeruginosa* + *P. Mirabilis* (Figure 3).

As a result of a survey of 15 patients 4-12 weeks after the surgical treatment in each group using the VASH pain questionnaires, the respondents were divided into subgroups 1.1 and 2.1, respectively, according to the following criterion: the distribution point was the average intensity of pain, which according to the VASH pain corresponded value "5". As a result, both subgroups of patients were divided according to the following characteristics: patients with pain < 5 on the pain scale and patients with pain ≥ 5 on the pain scale (Figure 4).

The mean value of pain in subgroups 1.1 and 2.1 was 38.46 (SD ± 13.44) and 39.16 (SD ± 13.78), respectively, and did not differ statistically ($p > 0.05$).

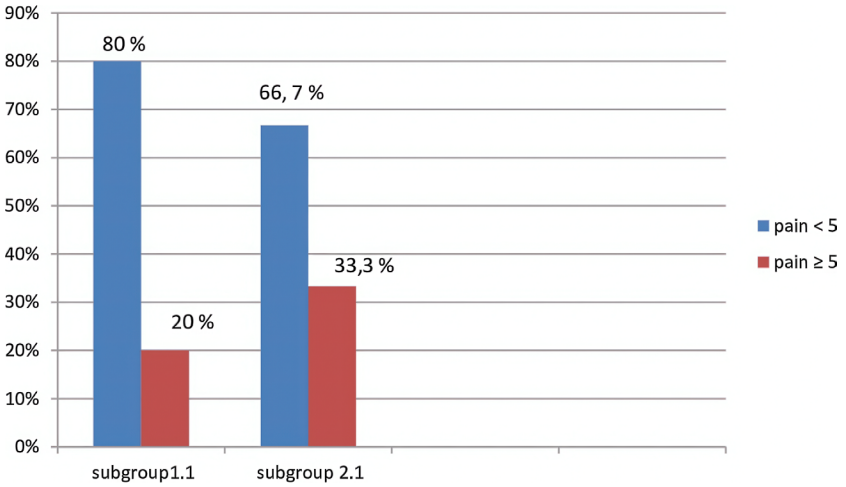

Figure 4. Characteristics of the pain level sensation in the injured depending on the wound colonization by one or more microorganism's types.

Conclusions

In recent years, researchers around the world have paid a lot of attention to the problems of providing medical care to patients with infected wounds, as it is required by a significant number of armed conflicts in the world. Of course, the principles of tactical medicine are somewhat different from traditional medicine, taking into account the special conditions of combat operations. However, one of the main theses of modern medicine remains unchanged: the future lies in preventive medicine. And the goal that doctors face is not only the timely provision of qualified medical care, but also the prevention of complications, which can significantly affect a patient's life. In view of this, it is important to study various probable factors influencing the course of wound healing and timely prevention of possible distant negative consequences. We considered the association of the qualitative composition of the microbiota as a possible predictor of the chronic pain syndrome formation. Wound infection directly affects and prolongs the healing time of the wound, which in turn adversely affects the soft tissues and structures located in the corresponding dermatome. According to the results, the predominance of gram-negative bacteria was established, regardless of the number of types of microorganisms present in the wound. *K. pneumoniae, A. baumanii, Staphylococcus spp., P. aeruginosa* were the most common in both groups. Since these microorganisms belong to pathogens with a high risk of developing multiresistance, this often complicates treatment and reduces a favorable prognosis. Assessing the level of pain sensation in 30 respondents 4-12 weeks after the surgical treatment, the average pain value in the subgroups did not differ statistically ($p>0.05$). Gram-negative bacteria (*K. pneumonia – 39, 2 %, A. baumannii – 31, 4 %, P. aeruginosa – 25, 5 %)* could be mainly found among the bacteria which colonize wounds of the limb after combat trauma with some predominance of their inter species associations in patient with higher pain sensation during 12 weeks after surgery. The hypothesis of microbial impact into pain prolongation is visible but insignificant ($p>0.05$). The data of pilot study encourage further research into the role of microorganisms in the formation of prerequisites for the development of chronic pain on a larger sample of participants, and to investigate in more detail the influence of not only qualitative, but also quantitative characteristics of the microbiota.

Ethic Approval: The Study protocols were approved by the Committee on Bioethics, National Pirogov Memorial Medical University, Vinnytsya, Ukraine (Protocol № 7, 27.05.2024).

Acknowledgements: We want to say thank you to all who support Ukraine in difficult time.

Funding: Funding for the research, authorship, and/or publication of this article was not received from third-party organizations.

Conflict of Interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article.

Authors' Contribution: *1 – KK:* Collecting microbial strains, their identification; Writing – original draft, conceived and designed the study; Agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy; *2 – DD:* Agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy; Drafted the article or revised it critically for important intellectual content; Approved the version to be published. 3 – *OK*: Writing – created graphical presentation and tables; Substantial contribution to the concept or design of the article; Agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy; 4 – *ON:* Substantial contribution to the concept or design of the article; Agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy; Drafted the article or revised it critically for important intellectual content; Approved the version to be published.

References

1. Thompson KB, Krispinsky LT, Stark RJ. Late immune consequences of combat trauma: a review of trauma-related immune dysfunction and potential therapies. Mil Med Res. 2019;6(1):11. doi: 10.1186/s40779-019-0202-0.
2. Kao R. Introduction: combat damage-control resuscitation/surgery and beyond. Can J Surg. 2018;61(6):178–179. doi: 10.1503/cjs.014418.
3. Maurya S, Bhandari PS. Negative Pressure Wound Therapy in the Management of Combat Wounds: A Critical Review. Adv Wound Care. 2016;5(9):379–89. doi: 10.1089/wound.2014.0624.
4. Yao Y, Zhang H. Better therapy for combat injury. Mil Med Res. 2019;6(1):23. doi: 10.1186/s40779-019-0214-9.
5. Sen CK, Roy S, Mathew-Steiner SS, Gordillo GM. Biofilm Management in Wound Care. Plast Reconstr Surg. 2021;148(2):275e-288e. doi: 10.1097/PRS.0000000000008142.
6. Mihai MM, Bălăceanu-Gurău B, Ion A, et al Host-Microbiome Crosstalk in Chronic Wound Healing. Int. J. Mol. Sci. 2024;25(9):4629.doi: 10.3390/ijms25094629.
7. Euler CW, Raz A, Hernandez A, et al. PlyKp104, a Novel Phage Lysin for the Treatment of Klebsiella pneumoniae, Pseudomonas aeruginosa, and Other Gram-Negative ESKAPE Pathogens. Antimicrob Agents Chemother. 2023; 67(5):e0151922. doi: 10.1128/aac.01519-22.
8. Effah CY, Sun T, Liu S, Wu Y. Klebsiella pneumoniae: an increasing threat to public health. Ann Clin Microbiol Antimicrob. 2020;19(1):1. doi: 10.1186/s12941-019-0343-8.
9. Nazarchuk O, Denysko T, Dmytriiev D, et al. In vitro evaluation of antimicrobial and anti-biofilm properties of antiseptics against multidrug resistant clinical Escherichia coli strains, isolated from combat wounds. Paediatric surgery. 2023;3(80):8–20. doi.10.15574/PS.2023.80.8.
10. Lopes A, Seligman Menezes M, Antonio Moreira de Barros G. Chronic postoperative pain: ubiquitous and scarcely appraised: narrative review. Brazilian Journal of Anesthesiology (English Edition). 2021;71(6):649–55. doi: 10.1016/j.bjane.2020.10.014.
11. Vivcharenko V, Trzaskowska M, Przekora A. Wound Dressing Modifications for Accelerated Healing of Infected Wounds. Int J Mol Sci. 2023;24(8):7193. doi: 10.3390/ijms24087193.
12. Wylde V, Dennis J, Beswick AD, et al. Systematic review of management of chronic pain after surgery. Br J Surg. 2017;104(10):1293–1306. doi: 10.1002/bjs.10601.
13. Bruehl S. Complex regional pain syndrome. BMJ. 2015; 351:h2730. doi: 10.1136/bmj.h2730.
14. Tomic-Canic M, Burgess JL, O'Neill KE, Strbo N, Pastar I. Skin Microbiota and its Interplay with Wound Healing. Am J Clin Dermatol. 2020;21(S1):36–43. doi: 10.1007/s40257-020-00536-w.
15. Liu S, Hur YH, Cai X, et al. A tissue injury sensing and repair pathway distinct from host pathogen defense. Cell. 2023;186(10):2127-2143.e22. doi: 10.1016/j.cell.2023.03.031.
16. Tipton CD, Sanford NE, Everett JA, et al. Chronic wound microbiome colonization on mouse model following cryogenic preservation. PLOS ONE. 2019;14(8):e0221565. doi: 10.1371/journal.pone.0221565.
17. Zhao A, Sun J, Liu Y. Understanding bacterial biofilms: From definition to treatment strategies. Front Cell Infect Microbiol. 2023;13:1137947. doi: 10.3389/fcimb.2023.1137947. 13.
18. Crofford L. Chronic Pain: Where the Body Meets the Brain. Trans Am Clin Climatol Assoc. 2015;126:167-83. PMID: 26330672.
19. Sung YT, Wu JS. The Visual Analogue Scale for Rating, Ranking and Paired-Comparison (VAS-RRP): A new technique for psychological measurement. Behav Res

Methods. 2018;50(4): 1694-1715. doi: 10.3758/s13428-018-1041-8.
20. Thong ISK, Jensen MP, Miró J, Tan G. The validity of pain intensity measures: what do the NRS, VAS, VRS, and FPS-R measure? Scand J Pain. 2018;18(1):99–107. doi: 10.1515/sjpain-2018-0012.
21. Fillingim RB, Loeser JD, Baron R, Edwards RR. Assessment of Chronic Pain: Domains, Methods, and Mechanisms. J Pain. 2016;17(9):T10–20. doi: 10.1016/j.jpain.2015.08.010.

Correspondence:
Received: 28 June 2024
Accepted: 2 September 2024
Kateryna Ksenchyna, PhD student
Department of Microbiology,
Pirogova 56 street, Vinnytsia, 21018 Ukraine
E-mail: zaluzhna.katia@gmail.com
ORCID: 0009-0008-1506-2673