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Sensitivity of biofilms formed by Staphylococcus *aureus* bacteria isolated from patients with chronic osteomyelitis to antiseptics and disinfectants

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Abstract

Introduction In the management of osteomyelitis, one of the first and important stages is the treatment of wounds with antiseptics and disinfectants. However, there are conflicting opinions in the literature about their effectiveness against biofilms formed by bacteria. It indicates the need to study the structure and functioning of biofilms formed by leading pathogens in chronic osteomyelitis, monitoring their sensitivity to antiseptics drugs.

Purpose To evaluate the bactericidal activity of hydrogen peroxide and alcohol-based skin antiseptic against biofilms formed by S. *aureus* strains isolated from patients with chronic osteomyelitis.

Materials and methods Biofilms of S. *aureus* bacteria were grown in 96-well plates and on coverslips for 24 hours. Twenty-four hour old biofilms were treated with disinfectants according to the developed protocol. The effect of antiseptics and disinfectants on monospecies biofilms of S. aureus was assessed visually and quantitatively on glass coverslips using an Axio Lab. A1 microscope (Carl Zeiss, Germany) and an ELx808 photometer (BioTek, USA) at a wavelength of 630 nm. All experiments were repeated four times.

Results Disks impregnated with 3 % H_2O_2 or 6 % H_2O_2 skin antiseptics had a bactericidal effect against all clinical isolates studied. S. *aureus* strains formed biofilms with varying intensity on the surface of coverslips and in 96-well plates. Impregnation with 3 % or 6 % hydrogen peroxide decreased the intensity of *MSSA* biofilm formation by 1.2 times compared to control values and of *MRSA* by 1.5 times. The application of a skin antiseptic to 24-hour old biofilms did not lead to a significant decrease in the level of biofilm formation. Biofilms in the groups differed in structure and morphology.

Discussion The varying activity of disinfectants against monospecies biofilms depends on their type and concentration. Low concentrations of H_2O_2 disrupt cell membranes, oxidize DNA, and destabilize enzymes and proteins. Sublethal concentrations of H_2O_2 can lead to the emergence of cells with the small colony variant (SCV) phenotype due to increased mutation rates and subsequent replication, which will facilitate the survival of the pathogen in body tissues.

Conclusion Hydrogen peroxide at a concentration of 3 % and 6 % suppresses the activity of biofilm formation by clinical strains of S. *aureus* and *MRSA*, but does not completely remove them from the abiotic surface, which indicates the bacteriostatic nature of the action of the disinfectant. The skin antiseptic is not effective against the biofilm form of bacteria, but can have a bactericidal effect on single adhered cells around which the exopolysaccharide matrix has not formed yet.

Keywords: biofilm, chronic osteomyelitis, alcohol-based antiseptic, hydrogen peroxide, Staphylococcus *aureus*, *MRSA*

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INTRODUCTION

Osteomyelitis accounts for 10% of all purulent inflammatory diseases, with a recurrence rate of 10 to 40 % [1, 2]. Staphylococcus *aureus* bacteria are among the pathogens most frequently isolated from wound discharge in chronic osteomyelitis. They are usually found as part of a biofilm characterized by high heterogeneity of cells with an altered phenotype resulting from their reduced proliferative and metabolic activity [3, 4]. It is known that the virulence and pathogenicity of all pathogens increases in the biofilm [5, 6]. The biofilm matrix is capable of retaining and inactivating antibiotics [6, 7]. One of the ways to increase the effectiveness of antibiotic therapy is their simultaneous use with drugs that act on the structure of the biofilm (enzymes, phages, various disinfectants), the activity of which can be aimed at suppressing the adhesion of bacteria to the surface, blocking the synthesis or destruction of the polymer matrix, disrupting intercellular information exchange, etc. [8–10].

Various methods have been developed to prevent the formation and control of biofilms, including their physical removal and wound irrigation with disinfectant solutions [11]. In clinical practice, hydrogen peroxide- and alcohol-based disinfectants are traditionally used for wound treatment in most cases. Antimicrobial disinfectants are also used to treat surfaces and medical equipment to prevent the transmission of pathogens to patients [12–14].

In the management of osteomyelitis, one of the first and most important steps is wound treatment with antiseptics and disinfectants. However, the literature contains conflicting opinions regarding their effectiveness against bacterial biofilms [12–15]. This indicates the need to study the structure and function of the biofilms formed by the leading pathogens causing chronic osteomyelitis and monitor their sensitivity to antiseptics.

Purpose To evaluate the bactericidal activity of hydrogen peroxide and alcohol-based skin antiseptic against biofilms formed by S. *aureus* strains isolated from patients with chronic osteomyelitis.

MATERIAL AND METHODS

The object of the study was clinical isolates of Staphylococcus *aureus* ($n = 38$), including methicillin-resistant staphylococci (*MRSA*, $n = 12$), isolated from wounds and fistulas during surgery in patients with chronic osteomyelitis.

Bacteria were isolated using standard microbiological methods. Species identification was performed using a BactoScreen bacteriological analyzer, which includes a MALDI mass spectrometer and BactoScreen-ID software for microorganism management, analysis, and identification. The reference strain S. *aureus* 25923 from the American Type Culture Collection (ATCC) was used as a control.

To evaluate the bactericidal activity of disinfectants and of a skin antiseptic against the isolated clinical strains, a disk diffusion method was used. Working suspensions of 24-hour S. *aureus* bacterial cultures grown on a dense indicator medium (DIM) at 37 °C were prepared by directly suspending pure culture colonies in a sterile isotonic solution. The suspension density corresponded to a turbidity standard of 0.5 according to McFarland. Sterile disks made of technical filter cardboard (GOST 6722-75), soaked in 1–3 % or 2–6 % H_2O_2 solutions and skin antiseptic (SA), were placed on the surface of a lawn obtained from a bacterial culture suspension on Muhler-Hinton agar. The dishes were incubated at a temperature of (35 ± 1) °C for 24 hours. To assess the effectiveness of each disinfectant, the diameter of the microbial inhibition zone around the discs was measured. Each test was repeated three times, and the results were averaged.

Biofilms formed by S. *aureus* bacteria were grown in 96-well plates and on coverslips for 24 hours. The 24-hour old biofilms were treated with disinfectants according to a previously developed protocol [9] and placed in an incubator for 24 hours (48 hours of experiment).

The experiment had four series:

— Series 1: determination of biofilm forming ability (BA) of clinical isolates S. *aureus* without applicayopn of antiseptics (control group);

— Series 2: BA determination under the effect of 3 % H_2O_2;

— Series 3: BA determination under the effect of 6 % H_2O_2;

— Series 4: BA determination under the effect of a skin alcohol-based antiseptic (60 % 2-propanol, 10 % 1-propanol).

Sterile meat-peptone broth (MPB) was used to control the quality of the study.

The effect of antiseptics and disinfectants on monospecies S. *aureus* biofilms was assessed visually on coverslips using an Axio Lab.A1 microscope (Carl Zeiss, Germany) and quantitatively using an ELx808 photometer (BioTek, USA) at a wavelength of 630 nm. All experiments were performed in quadruplicate. Biofilm formation activity was characterized as high (OD630 > 0.360), medium (0.180 < OD630 ⩽ 0.360), weak (0.090 < OD630 ⩽ 0.180), and no activity (OD630 < 0.090).

Using the modular software ZEN (Carl Zeiss, Germany) and the ImageJ software (USA), we digitalized the fields of view with the biofilm formed on the surface of the cover glass, measured the area of the field of view, the number and area occupied by single adherent cells (SAC) and microcolonies (MC).

Gnumeric 1.12.17 was used to process and statistically analyze the obtained results. Digital data are presented as median (Me) and quartiles (Q25–Q75). The nonparametric Wilcoxon test was used to determine differences between the groups. Differences were considered significant at $p < 0.05$.

The study was conducted at the Federal State Budgetary Institution Ilizarov National Medical Research Center of Traumatology and Orthopedics of the Russian Ministry of Health at the Microbiology Laboratory and the Department of Preclinical and Laboratory Research.

RESULTS

Discs impregnated with 3 % H_2O_2 or 6 % H_2O_2 and skin antiseptic had a bactericidal effect against all clinically isolated strains of S. *aureus*. A considerable zone of their growth inhibition was noted for hydrogen peroxide (Fig. 1). The diameter of the inhibition zone aganst bacterial growth of S. *aureus* depended on the disinfection agent and was (45 ± 3) mm for 3 % H_2O_2; (47 ± 2) mm for 6 % H_2O_2; and (35 ± 3) mm for skin antiseptic.

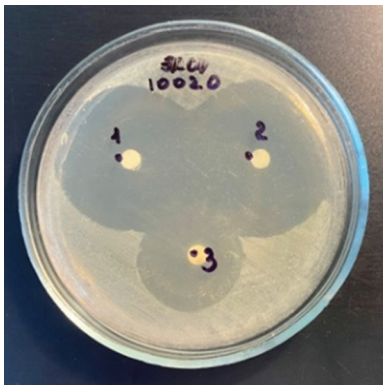

Fig. 1 Bactericidal effects of 1 — 3 % H_2O_2, 2 — 6 % H_2O_2 and 3 — skin antiseptic on clinical strains of S. *aureus*

The biofilm formation activity of methicillin-susceptible S. *aureus* isolates (*MSSA*) in the control series was 0.351 (0.311; 0.359) optical density units, while that of methicillin-resistant isolates was 0.275 (0.258; 0.290) ($p = 0.044$) optical density units, which corresponded to the average level of biofilm formation. Addition of 3 % and 6 % hydrogen peroxide decreased the intensity of biofilm formation in *MSSA* by 1.2 times compared to the control values ($p = 0.04$ and $p = 0.04$), and in *MRSA* by 1.5 times ($p = 0.033$ and $p = 0.027$). In the fourth series of the experiment, the skin antiseptic added to the 24-hour old biofilms did not lead to a significant decrease in the level of biofilm formation (Fig. 2).

All clinical isolates formed biofilms on the coverslip surface with varying intensity. Treatment of the 24-hour biofilm formed by S. *aureus* strains with 3 % and 6 % hydrogen peroxide resulted in a significant change in the MC proportion per unit area of the visual field compared to the control and the SA group. Alcohol-based skin antiseptic was less effective against microcolonies (Fig. 3).

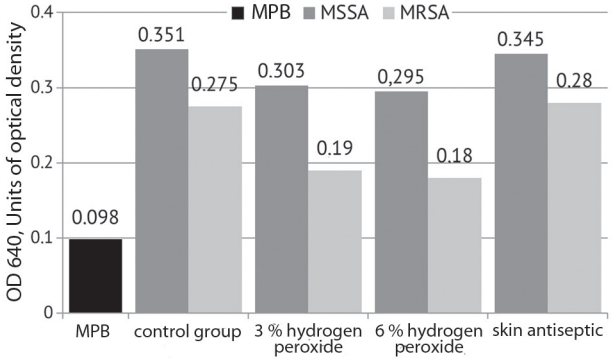

Fig. 2 Biofilm formation of *MSSA* and *MRSA* and its dependence on the disinfection agent after 48 hours

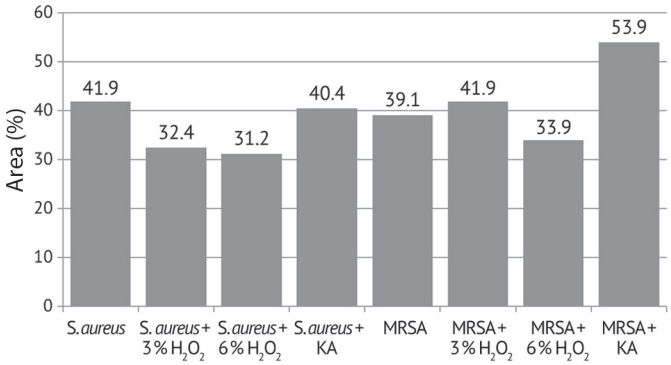

Fig. 3 Area occupied by MC S. *aureus* and *MRSA* on the surface of coverslip after 48 hours

Biofilms in the groups differed in structure and morphology. In the control group, after exposure to 3 % H_2O_2 and SA, most of the coverslip surface was occupied by microcolonies larger than 1000 μm^2; after treatment with 6 % H_2O_2, their proportion was two to six times smaller. In the control group, the biofilm on the coverslip surface was represented by large MCs with smaller MCs located between them, as well as a large number of OAC. After treatment with 3 % H_2O_2, the biofilm acquired the appearance of a network with lumpy, cloud-like, large MCs having an altered dye color. After treatment with 6 % H_2O_2, MCs smaller in size than 1000 μm^2 predominated on the coverslip surface. SA, like hydrogen peroxide, changed the dye color; the biofilm was represented by large, coarse, lumpy structures (Fig. 4).

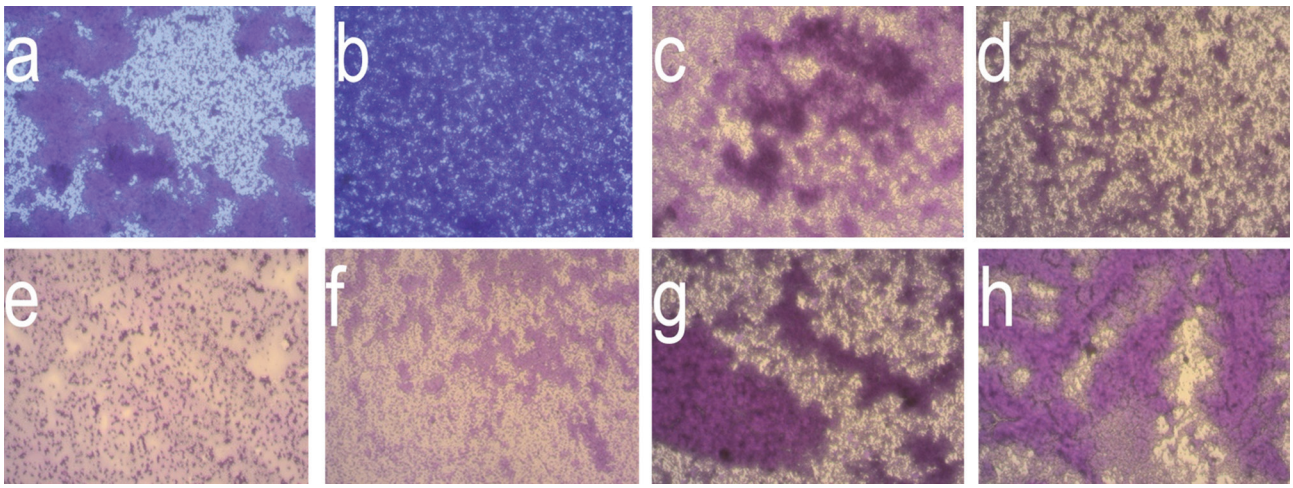

Fig. 4 Images of fields of view of the biofilm formed by clinical strains of S. *aureus* and *MRSA* on the coverslip surface: (a) S. *aureus*; (b) S. *aureus* + 3 % H_2O_2; (c) S. *aureus* + 6 % H_2O_2; (d) S. *aureus* + SA; (e) *MRSA*; (f) *MRSA* + 3 % H_2O_2; (g) *MRSA* + 6 % H_2O_2; (h) *MRSA* + SA. Light microscopy. Magnification ×400. Staining with carbolic solution of gentian violet

The amount of SAC depended on the concentration and type of antiseptic. Treatment with 6 % hydrogen peroxide and SA significantly reduced the amount of SAC on the surface of the coverslip compared to the control ($p = 0.045$; $p = 0.0014$) (Fig. 5).

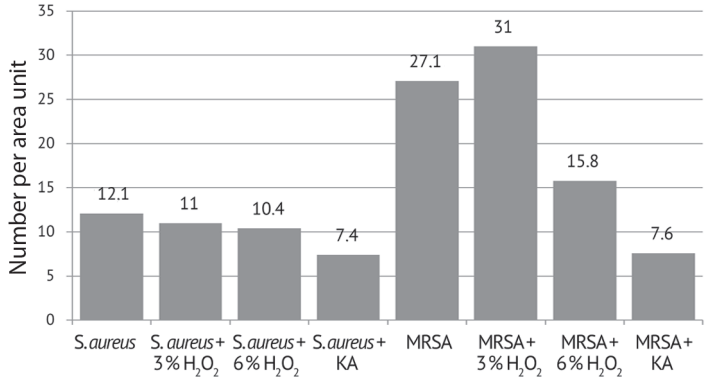

Fig. 5 Number of SACs of S. *aureus* and *MRSA* on the coverslip surface after 48 hours

Biofilm formation on the surface of the coverslip by resistant strains differed significantly. The average area and number of *MRSA* MCs in the control group did not differ significantly from the corresponding indicators of sensitive staphylococci, but differences in the biofilm structure were observed. In the biofilms formed by *MRSA*, MCs ranging in size from 10 to 10,000 μm^2 predominated, while in sensitive staphylococci the proportion of such MCs was two times smaller. Exposure of *MRSA* biofilm to 3 % H_2O_2 led to an insignificant increase in the number and proportion in the field of view of MCs and SAC compared to the control group and significant differences from *MSSA* (p = 0.0000003 and p = 0.000000004) (Fig. 3).

Treatment of *MRSA* biofilm with 6 % H_2O_2 resulted in a decrease in the area occupied by MCs on the coverslip surface compared to the control group (p = 0.042) and was significantly higher than that of *MSSA*. MCs up to 100 μm^2 in size predominated in the biofilm structure; their proportion was twice that of the *MSSA* biofilm.

As in the case of sensitive staphylococci, SA was ineffective against *MRSA* MCs (Fig. 3).

The morphology of *MRSA* biofilms differed from that of *MSSA*. In the control group and after hydrogen peroxide treatment, they were more uniform and flat, with individual fields of view completely filled with networked MCs. After SA treatment, up to 40 % of the coverslip surface was occupied by large MCs measuring over 10,000 μm^2 (Fig. 4).

In the control series, the number of *MRSA* SACs was 2.3 times higher than that of *MSSA*. After exposure to 6 % H_2O_2 and SA, a significant decrease in SACs by 1.7 and 3.4 times was observed as compared to the control. The number of *MRSA* and *MSSA* SACs after SA treatment did not differ (Fig. 5).

DISCUSSION

Biofilm is one of the ways in which microorganisms adapt to the impact of physical and chemical environment [5, 6, 16]. It is known that bacteria in the biofilm state are tolerant to disinfectants and antiseptics [8, 10]. In clinical practice, various biocides, including hydrogen peroxide, are often used for the cleaning and treatment of chronic wounds. There are few studies on the effect of H_2O_2 on chronic wound healing. It is known that hydrogen peroxide can induce natural inflammatory responses in the cells and stimulate wound healing, while its effect is dose-dependent [17]. Low concentrations of H_2O_2 destroy cell membranes, oxidize DNA, destabilize enzymes and proteins, quickly oxidizing to the hydroxyl radical (OH), which contributes to oxidative stress [14]. Exposure of S. *aureus* to sublethal concentrations of H_2O_2 can result in the emergence of cells with the small colony variant (SCV) phenotype due to an increased mutation rate and subsequent replication, which facilitate the survival of the pathogen in host tissues [18]. Several studies have shown a reduced susceptibility of biofilms to H_2O_2 compared to planktonic bacterial cells [19]. According to another

study, H_2O_2 can remove both the matrix of S. *aureus* biofilms and a viable bacterial mass [20]. Bacterial cells in the surface layer of the biofilm produce many enzymes, including catalase, peroxidase, glutathione reductase, and superoxide dismutase, which can break down H_2O_2. It has been shown that H_2O_2 is not able to effectively penetrate mature biofilms, as its destruction occurs in the surface layer, preventing effective diffusion of the disinfectant into the inner layers [21]. A common enzyme produced by bacteria is catalase. The decomposition of H_2O_2 due to catalase production may be the reason for the need for high concentrations and longer contact times of H2O2 with the surface as an antibiofilm agent [16].

We compared the effects of disinfectant solutions (3 % and 6 % H_2O_2, alcohol-based antiseptic) on monospecies biofilms formed by clinical strains of *MSSA* and *MRSA*. Our results demonstrate that the different activities of disinfectants against monospecies biofilms depend on their type and concentration. Hydrogen peroxide and alcohol-based antiseptic did not exhibit bactericidal properties against biofilms but inhibited their formation. Thus, after treating the biofilms formed by S. *aureus* strains with 3 % or 6 % hydrogen peroxide, film-forming activity significantly reduced, as confirmed by the results of a study on polystyrene plates and a significant reduction in the area occupied by MC on the surface of a coverslip, compared to the control group. Similar results were obtained when treating *MRSA* biofilms with 6 % hydrogen peroxide.

After exposure of *MRSA* biofilm to 3 % hydrogen peroxide, the area occupied by MCs increases; irrigating wounds with 3 % H_2O_2, proteins, nucleic acids, and lipids of healthy cells are oxidized, which can slow down wound healing [17]. It is known that oxidative stress can induce the formation of a biofilm that is tolerant to antibiotics, and this suggests that antiseptics can reduce the effectiveness of drugs used for treatment, which in turn requires an increase in the concentration of the solution to enhance the antibacterial effect [22].

Our results are consistent with in vitro studies that show that hydrogen peroxide reduces S. *aureus* biofilm volume without exerting a bactericidal effect [22, 23].

Exposure to 6 % hydrogen peroxide significantly reduced the number of SACs of the clinical strains studied. Conversely, treatment with 3 % hydrogen peroxide resulted in an increase in the number of SACs of *MRSA*.

According to literature data, the S. *aureus* biofilm has a higher tolerance to antiseptics compared to planktonic bacteria [15, 24, 25].

An alcohol-based skin antiseptic was ineffective against biofilm MCs. The effect of the alcohol-based antiseptic on *MRSA* MCs increased their surface area on the coverslip. A more pronounced effect of the antiseptic was observed against S. *aureus* and *MRSA* strains.

Alcohols are known to have a broad spectrum of antimicrobial activity against bacteria, fungi, and viruses. The mechanism of their action is unknown, but it is believed to be related to the denaturation of membrane and enzymatic proteins. Ethanol and isopropanol (2-propanol) are commonly used as components in alcohol-based antiseptics [26, 27]. The effectiveness of alcohol-containing antiseptics depends on its concentration. The recommended alcohol concentration is usually 60–95 %. In this range, the alcohol interacts with functional proteins and inactivates them. Alcohol concentration of 60–75 % denatures proteins, while higher concentrations (> 95 %) cause coagulation of membrane proteins, which prevents alcohol from entering the cell [28]. Low concentration alcohols, which are used as disinfectants, have been shown to promote the formation of biofilms by some bacteria (S. *epidermidis*) and increase their resistance to disinfection [29].

According to the data obtained, the biofilms that are formed by clinical strains of S. *aureus* and *MRSA* have a higher tolerance to hydrogen peroxide and antiseptics compared to single adhered cells, which may be due to the absence of a complete matrix around them.

CONCLUSION

Hydrogen peroxide at concentrations of 3 % or 6 % inhibits biofilm formation by clinical strains of S. *aureus* and *MRSA*, but does not completely remove them from abiotic surfaces, indicating the disinfectant bacteriostatic action. Skin antiseptics are ineffective against biofilm-forming bacteria, but they may have a bactericidal effect on single adhered cells that have not formed an exopolysaccharide matrix around them yet.

Conflicts of Interest *The authors declare no obvious or potential conflicts of interest related to the publication of this article.*
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REFERENCES

1. Trushin P.V., Razin M.P. Chronic osteomyelitis of tubular bones: modern view on the problem. *Medical newsletter of Vyatka*. 77(1):114-119. (In Russ.) doi:10.24412/2220-7880-2023-1-114-119
2. Sakovich NV, Andreev AA, Mikulich EV, et al. Modern aspects of etiology, diagnostics and treatment of osteomyelitis. *Journal of Experimental and Clinical Surgery*. 2018; 11(1):7. (In Russ.) doi: 10.18499/2070-478X-2018-11-1-70-79.
3. García-Betancur JC, Lopez D. Cell Heterogeneity in Staphylococcal Communities. *J Mol Biol*. 2019;431(23):4699-4711. doi: 10.1016/j.jmb.2019.06.011.
4. Shipitsyna I, Osipova E. Monitoring of the most common gram-positive microflora and its antibiotic sensitivity in persons with chronic osteomyelitis over a three-year period. *Genij Ortopedii*. 2022; 28(2):189-193. doi: 10.18019/1028-4427-2022-28-2-189-193.
5. Savilov ED, Anganova EV, Noskova OA, Dukhanina AV. Bacteria biofilms in purulent-septic infections. *Acta biomedica scientific*. 2019;4(5):38-42. (In Russ.) doi: 10.29413/ABS.2019-4.5.6
6. Okulich VK, Kabanova AA, Plotnikov FV. *Microbial biofilms in clinical microbiology and antibacterial therapy*. Vitebsk: VSMU; 2017:300. (In Russ.)
7. Yarets Yu, Shauchenka N. A new method for the bacterial biofilms analysis in medicine. *Science and innovation*. 2016;(11):68-72. (In Russ.)
8. Aleshukina A. V., Goloshova E. V., Tverdokhlebova T. I. Study of the effect of disinfectants on biofilm-forming non-fermentative bacteria. Bulletin of higher education institutes. North caucasus region. *Natural sciences*. 2020;(1):89-94. (In Russ.)
9. Shipitsyna IV, Osipova EV. Influence of disinfectants on growth of biofilm formed by K. pneumoniae strains. *Medical alphabet*. 2022;(35):37-41. (In Russ.) doi: 10.33667/2078-5631-2022-35-37-41.
10. Bozhkova S, Oleinik Y, Artyukh V, et al. The first step of two-stage hip revision: what affects the result? *Traumatology and Orthopedics of Russia*, 2024;30(2):5-15. doi: 10.17816/2311-2905-17582.
11. Koo H, Allan RN, Howlin RP, et al. Targeting microbial biofilms: current and prospective therapeutic strategies. *Nat Rev Microbiol*. 2017;15(12):740-755. doi: 10.1038/nrmicro.2017.99.
12. Abuga K, Nyamweya N. Alcohol-Based Hand Sanitizers in COVID-19 Prevention: A Multidimensional Perspective. *Pharmacy (Basel)*. 2021;9(1):64. doi: 10.3390/pharmacy9010064.
13. Lineback CB, Nkemngong CA, Wu ST, et al. Hydrogen peroxide and sodium hypochlorite disinfectants are more effective against Staphylococcus aureus and Pseudomonas aeruginosa biofilms than quaternary ammonium compounds. *Antimicrob Resist Infect Control*. 2018;7:154. doi: 10.1186/s13756-018-0447-5.
14. Montagna MT, Triggiano F, Barbuti G, et al. Study on the In Vitro Activity of Five Disinfectants against Nosocomial Bacteria. *Int J Environ Res Public Health*. 2019;16(11):1895. doi: 10.3390/ijerph16111895.
15. Raval YS, Flurin L, Mohamed A, et al. In vitro Activity of Hydrogen Peroxide and Hypochlorous Acid Generated by Electrochemical Scaffolds Against Planktonic and Biofilm Bacteria. *Antimicrob Agents Chemother*. 2021;65(5):e01966-20. doi: 10.1128/AAC.01966-20.
16. Yin W, Wang Y, Liu L, He J. Biofilms: The Microbial "Protective Clothing" in Extreme Environments. *Int J Mol Sci*. 2019;20(14):3423. doi: 10.3390/ijms20143423.
17. Zhu G, Wang Q, Lu S, Niu Y. Hydrogen Peroxide: A Potential Wound Therapeutic Target? *Med Princ Pract*. 2017;26(4):301-308. doi: 10.1159/000475501.
18. Painter KL, Strange E, Parkhill J, et al. Staphylococcus aureus adapts to oxidative stress by producing H_2O_2-resistant small-colony variants via the SOS response. *Infect Immun*. 2015;83(5):1830-1844. doi: 10.1128/IAI.03016-14.
19. Parker DM, Koch JA, Gish CG, et al. Hydrogen Peroxide, Povidone-Iodine and Chlorhexidine Fail to Eradicate Staphylococcus aureus Biofilm from Infected Implant Materials. *Life (Basel)*. 2023;13(6):1230. doi: 10.3390/life13061230.
20. Toté K, Horemans T, Vanden Berghe D, et al. Inhibitory effect of biocides on the viable masses and matrices of Staphylococcus aureus and Pseudomonas aeruginosa biofilms. *Appl Environ Microbiol*. 2010;76(10):3135-3142. doi: 10.1128/AEM.02095-09.
21. Tibbits G, Mohamed A, Gelston S, et al. Activity of a hypochlorous acid-producing electrochemical bandage as assessed with a porcine explant biofilm model. *Biotechnol Bioeng*. 2023;120(1):250-259. doi: 10.1002/bit.28248.
22. Rowe SE, Wagner NJ, Li L, et al. Reactive oxygen species induce antibiotic tolerance during systemic Staphylococcus *aureus* infection. *Nat Microbiol*. 2020;5(2):282-290. doi: 10.1038/s41564-019-0627-y.

23. Ernest EP, Machi AS, Karolcik BA, et al. Topical adjuvants incompletely remove adherent Staphylococcus aureus from implant materials. *J Orthop Res*. 2018;36(6):1599-1604. doi: 10.1002/jor.23804.
24. O'Donnell JA, Wu M, Cochrane NH, et al. Efficacy of common antiseptic solutions against clinically relevant microorganisms in biofilm. *Bone Joint J*. 2021;103-B(5):908-915. doi: 10.1302/0301-620X.103B5.BJJ-2020-1245.R2.
25. Christopher ZK, Tran CP, Vernon BL, Spangehl MJ. What Is the Duration of Irrigation? An *In Vitro* Study of the Minimum Exposure Time to Eradicate Bacteria With Irrigation Solutions. *J Arthroplasty*. 2022;37(2):385-389.e2. doi: 10.1016/j.arth.2021.10.013.
26. Daverey A, Dutta K. COVID-19: Eco-friendly hand hygiene for human and environmental safety. *J Environ Chem Eng*. 2021;9(2):104754. doi: 10.1016/j.jece.2020.104754.
27. Singh D, Joshi K, Samuel A, et al. Alcohol-based hand sanitisers as first line of defence against SARS-CoV-2: a review of biology, chemistry and formulations. *Epidemiol Infect*. 2020;148:e229. doi: 10.1017/S0950268820002319.
28. Berardi A, Perinelli DR, Merchant HA, et al. Hand sanitisers amid CoViD-19: A critical review of alcohol-based products on the market and formulation approaches to respond to increasing demand. *Int J Pharm*. 2020;584:119431. doi: 10.1016/j.ijpharm.2020.119431.
29. Lopez-Gigosos RM, Mariscal-Lopez E, Gutierrez-Bedmar M, Mariscal A. Effect of Long-Term Use of Alcohol-Containing Handwashing Gels on the Biofilm-Forming Capacity of Staphylococcus epidermidis. *Int J Environ Res Public Health*. 2023;20(6):5037. doi: 10.3390/ijerph20065037.

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