Article's History: Received: 06.01.2025; Revised: 17.04.2025; Accepted: 27.05.2025

Bulletin of Medical and Biological Research

Vol. 7, No. 2
2025

DOI: 10.63341/bmbr/2.2025.31

Journal homepage: https://bmbr.com.ua/en

UDC 615.33:615.282:578.7:579.841.1

Microbiological rationale for alternative strategies to combat infections caused by antibiotic-resistant *Pseudomonas aeruginosa*

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Abstract. This study aimed to investigate the activity of the drug Pyofag against clinical isolates of *Pseudomonas aeruginosa* and to evaluate the effectiveness of the combined action of surface-active antiseptics and bacteriophages. To achieve this aim, classical methods for the isolation and identification of bacteria were employed. Antibiotic susceptibility of *Pseudomonas* isolates was determined using the disc diffusion method, while susceptibility to surface-active antiseptics (decamethoxine, benzalkonium chloride, chlorhexidine bigluconate, octenidine dihydrochloride, and polyhexanide) was assessed using the broth dilution method. The susceptibility of clinical isolates to Pyofag was evaluated based on the optical density of bacterial suspensions after 18 hours of incubation with the preparation. The nature of the combined effect of bacteriophages and antiseptics on *P. aeruginosa* was assessed by calculating the lytic index of the phage on planktonic bacterial forms cultured in media containing sub-bacteriostatic concentrations of antiseptics. The results showed that all 54 isolated clinical strains of *P. aeruginosa* retained high susceptibility only to reserve antibiotics – colistin (94.4%) and cefiderocol (75.9%). Resistance to other antipseudomonal antibiotics (cefepime, ceftazidime, piperacillin-tazobactam, imipenem, and ciprofloxacin) was observed in 96.3%-100% of isolates. However, aminoglycosides (gentamicin, tobramycin, amikacin) and meropenem remained effective against 29.6%-44.4% of strains. Antiseptic agents containing

Suggested Citation:
Bebyk V, Vovk I, Nazarchuk H, Bahniuk N, Nazarchuk O. Microbiological rationale for alternative strategies to combat infections caused by antibiotic-resistant *Pseudomonas aeruginosa*. Bull Med Biol Res. 2025;7(2):31–9.. DOI: 10.63341/bmbr/2.2025.31

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surface-active compounds demonstrated strong antipseudomonal properties and are capable of inhibiting bacterial proliferation at concentrations ranging from 16.4-22.5 μg/mL (octenidine dihydrochloride, decamethoxine, chlorhexidine bigluconate) to 65-145.7 μg/mL (polyhexanide, benzalkonium chloride). It was confirmed that decamethoxine, octenidine, and chlorhexidine exhibit significantly greater antibacterial activity than polyhexanide and benzalkonium chloride ($p < 0.01$). The isolated *Pseudomonas* strains showed high susceptibility to the pharmaceutical preparation Pyofag: the lytic activity index (*Is*) of Pyofag exceeded 0.5 in 70.4% of strains, indicating that 50% of the bacterial population was destroyed during the dynamic interaction between bacterial growth and phage replication. In media containing sub-bacteriostatic concentrations of decamethoxine, chlorhexidine, or octenidine, both susceptible ($n = 7$, $Is = 0.69$) and resistant ($n = 8$, $Is = 0.15$) strains were lysed more intensively by the bacteriophage. This was evidenced by an increase in the susceptibility index to 0.80-0.87 in susceptible strains and to 0.54-0.70 in phage-resistant strains, respectively

Keywords: surface-active antiseptics; bacteriophages; antibiotics; Pyofag; opportunistic microorganisms

INTRODUCTION

During the period from 2015 to 2025, the problem of antibiotic resistance has assumed global importance. Existing limitations in the effectiveness of antibacterial therapy have led to an increase in the number of severe infections, higher mortality rates, and significant economic costs associated with treatment. Against the backdrop of the growing number of infections caused by superpathogens that demonstrate multidrug resistance (MDR), significant attention is being paid to the search for alternative means of antibacterial therapy, among which natural destroyers of bacteria – bacteriophages – play an increasingly prominent role. The prolonged war in Ukraine and the fullscale russian aggression launched in 2022 have led to a sharp rise in the number of severe wound infections. The widespread use of antibiotics for prophylactic and therapeutic purposes during evacuation and the provision of medical care has contributed to the selection of antibiotic-resistant strains. Therefore, the issue of identifying alternative strategies for combating antibiotic-resistant microorganisms – particularly non-fermenting Gram-negative bacilli (*Pseudomonas* spp., *Acinetobacter* spp.) – and introducing new methods of antibacterial therapy using bacteriophages, which have demonstrated their effectiveness, is particularly relevant.

No randomised controlled trials directly comparing bacteriophage therapy with standard antibiotic treatment for antibiotic-resistant *Pseudomonas aeruginosa* infections in intensive care units (ICUs), burn, or polytrauma patients have been published since 2020. However, several wellcharacterised case reports and small cohort studies indicate that phage-antibiotic combinations are feasible, generally well tolerated, and may be clinically beneficial in selected challenging cases, with molecular and phenotypic resistance dynamics documented in a subset. A retrospective, observational study on device-related or systemic infections conducted by S.I. Green *et al*. [1] evaluated 12 cases of customised phage therapy, showing a 66% favourable response rate, with 42% bacterial eradication. Phage therapy was safe, though immunological neutralisation occurred in some instances.

J.-P. Pirnay *et al*. [2] conducted a multicentre, multinational, retrospective observational study focusing on individualised phage therapy. The researchers analysed 100 cases of individualised phage therapy across 12 countries and observed clinical improvement in 77.2% of cases and bacterial eradication in 61.3%. The use of antibiotics alongside phage therapy increased the likelihood of success. N. Cesta *et al*. [3] published a case report describing a 62-year-old patient with chronic *P. aeruginosa* infection who was successfully treated with customised phage therapy and meropenem, showing no recurrence of infection over a period of two years.

A case series on anti-*S. aureus* therapy for diabetic foot infection conducted by M.J. Young *et al*. [4] tested anti-*S. aureus* phage therapy on 10 patients at high risk of amputation. Nine out of ten patients benefited, although one patient showed no response to treatment. L. Rahimzadeh Torabi *et al*. [5] demonstrated success using personalised intravenous/nebulised phage and antibiotics in an extremely drug-resistant (XDR) burn patient, with clinical cure, detailed adverse event monitoring, and resistance documentation. The findings of K. Racenis *et al*. [6] indicated that the combination of phages, antibiotics, and surgical intervention holds considerable potential for treating left ventricular assist device (LVAD)-associated *Pseudomonas aeruginosa* infections, with biofilm formation and resistance phenotype evolution tracked *in vitro*.

Across all reports, phage therapy (often in combination with antibiotics) was well tolerated, with no severe infusion reactions or cytokine storms clearly attributable to phage administration. Current evidence remains limited to salvage case series and a single non-randomised cohort; phageantibiotic combinations are promising yet unproven alternatives for resistant *Pseudomonas aeruginosa* infections in critical care settings. This underscores the urgent need for well-powered, rigorously monitored clinical trials with standardised endpoints for resistance and safety. This research aimed to investigate the activity of the drug Pyofag and to assess the effectiveness of its combined action with surfactant-based antiseptics against clinical isolates of *Pseudomonas aeruginosa* obtained from wounded servicemen with complicated wound infections.

MATERIALS AND METHODS

Characteristics of the antiseptic compounds and biological preparations used in the study:

- Decamethoxine is an antiseptic from the group of surfactants and a derivative of bisquaternary nitrogen. In the study, the substance decamethoxine – a fine-grained white powder readily soluble in water and alcohol – was used. A working solution at a concentration of 0.1% was prepared by dissolving 100 mg of decamethoxine in 100 mL of sterile, purified water.
- Benzalkonium chloride (alkyldimethylbenzylammonium chloride) is a cationic surfactant and an antiseptic

from the group of quaternary ammonium compounds. In this research, a 50% solution of benzalkonium chloride (produced in China) was used. To prepare a 0.5% working solution, a 1:10 dilution was made using sterile purified water.

- Octenidine dihydrochloride is a surface-active antiseptic with a broad spectrum of microbicidal activity. To evaluate the susceptibility of clinical bacterial strains, the commercial preparation Octenisept was used. It contains 0.1 g of octenidine dihydrochloride and 2.0 g of phenoxyethanol per 100 mL of water (produced in Germany).
- Chlorhexidine bigluconate, a biguanide derivative, acts via a surface-active mechanism that disrupts microorganisms. It is widely employed as an antiseptic (0.1% solution) and as a disinfectant (0.25% and 0.5% solutions), both in complex formulations and as aqueous or aqueous-alcoholic solutions. In this study, a 20% solution of chlorhexidine (produced in Ukraine) was used to prepare a 0.2% working solution by diluting 10 mL of the drug in 90 mL of sterile purified water (1:10 dilution).
- Polyhexanide (polyamidopropylbiguanide) is a polymeric cationic surfactant with antiseptic and disinfectant properties. Susceptibility was assessed using the drug Prontosan, a wound irrigation solution produced in Germany, which contains 0.1% polyhexanide, 0.1% betaine, and purified water.
- Pyofag is a polyvalent bacteriophage preparation produced by Infuzia PJSC, Ukraine, for NEO PROBIO CARE INC., Canada. One millilitre of the preparation contains specific bacteriophages at a concentration of at least 1×10^5 phage particles, targeting the following microorganisms: *Streptococcus pyogenes*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Proteus mirabilis* [7].

The object of the study was microbial cultures obtained from the museum of clinical isolates at the bacteriological laboratory of the Department of Microbiology, National Pirogov Memorial Medical University, Vinnytsya. These isolates were collected between 2022 and 2024 from patients with purulent-inflammatory wound complications who were undergoing treatment at the Communal nonprofit enterprise Vinnytsya Regional Clinical Hospital named after N.I. Pirogov Vinnytsia Regional Council, specifically at the Centre for Thermal Trauma and Plastic Surgery and other medical institutions in Vinnytsia. The study protocol was approved by the Committee on Bioethics, National Pirogov Memorial Medical University, Vinnytsya, Ukraine (Protocol No. 3; 07 April 2022).

In total, 152 isolates of Gram-negative bacteria were obtained during the 2022-2024 period. Differentiation of Gram-negative bacteria was conducted according to generally accepted criteria, based on the following indicators: oxidase test results, the ability to produce a water-soluble pigment, morphological characteristics of colonies formed on tryptic soy agar (TSA), and biochemical activity determined using diagnostic test systems Neferm-test 24 and Entero-test 24 (PLIVA-Lachema a.s., Brno, Czech Republic). Antibiotic susceptibility testing of the isolated strains was performed following the Order of the Cabinet of Ministers of Ukraine No. 116-p [8] and the recommendations of EUCAST (version 13) [9].

Determination of the susceptibility of isolated *P. aeruginosa* strains to the drug Pyofag polyvalent bacteriophage was conducted using a simplified, original method involving the calculation of a susceptibility index for each isolated culture. From cultures of clinical *P. aeruginosa* strains grown on TSA for 18-20 hours at 36°C ± 1°C, a bacterial suspension was prepared at a concentration of 1.5×10^8 CFU/mL, corresponding to 0.5 McFarland units, in isotonic sterile sodium chloride solution. The turbidity of the suspension was adjusted to the required concentration using a DensiLa-Meter densitometer. For the experiment, 200 µL of the prepared bacterial suspension was added to sterile plastic tubes (16 mm diameter) containing 2 mL of sterile meat-peptone broth (MPB). The tubes were incubated in a thermostat for 2 hours to reach the logarithmic growth phase, after which 200 µL of the phage cocktail Pyofag was added to the test tube. Simultaneously, 200 µL of sterile isotonic sodium chloride solution was added to the control tube. The experiment included a medium control (2 mL of MPB), which was incubated alongside both the growth control and the test tube for 18 hours at 36°C ± 1°C. Following incubation, the optical density of the suspension in the test culture – cultivated in the presence of 10^4 phage particles – was measured, taking into account the dilution factor (*Df*), the control culture (*Dk*), and the nutrient medium (*Dc*), all expressed in McFarland units. The assessment of the susceptibility of clinical isolates to the biological preparation Pyofag was performed by calculating the susceptibility index to the bacteriophage (*Is*) using formula (1):

$$Is = (Dk - Df)/(Dk - Dc), \quad (1)$$

where *Dk* – the density of the bacterial suspension in the control tube, in McFarland units; *Df* – the density of the bacterial suspension in the medium containing bacteriophage, in McFarland units; *Dc* – the density of the sterile culture medium, in McFarland units.

This index, as illustrated by the formula, enables an estimation of the proportion of the bacterial suspension destroyed by the bacteriophage after 18 hours of incubation, relative to the control. The susceptibility of the bacterial cultures to antiseptic agents was determined by the method of serial dilutions, establishing the minimum inhibitory concentration (MIC, µg/mL) and the minimum bactericidal concentration (MBC, µg/mL) [8, 9].

To identify the synergistic bactericidal effect of surfactant-based antiseptics and lytic bacteriophages on clinical isolates, nutrient media were prepared containing sub-bacteriostatic concentrations (25% of the MIC) of the antiseptic for the test strain, and the lytic activity of the phage was re-evaluated using the method described above. The experiment included several controls: a medium control, a culture growth control in the presence of the sub-bacteriostatic concentration of antiseptic (sub-MIC, µg/mL), and a microbial population growth control in the presence of phage in an untreated medium. Data were considered valid if the density of the culture control in the presence of the subMIC antiseptic remained within ±15% of the control density without antiseptic. The resulting *Is* values were compared with the baseline data to assess the effect of inactive concentrations of antiseptic on the lytic activity of the phage.

Statistical analysis was conducted using Microsoft Excel 2010, applying methods of variational statistics [10]. The mean values of the parametric data, along with the

standard deviation (M ± s), were calculated, and the frequency of occurrence of non-parametric features in the study group was determined. Individual samples were tested for normality of distribution (F(x)), and the reliability of results across different groups was assessed using FTEST, TTEST, and Student's t-test. Comparisons between multiple samples were made using the Bonferroni correction, depending on the number of samples being analysed. Results were considered statistically significant at a p-value of ⩽0.05, with high statistical reliability confirmed for p-values of p ⩽ 0.01, following the correction.

RESULTS

Based on morphological, cultural, and biochemical characteristics, 54 clinical strains (35.5% of all isolated microorganisms) were identified as *Pseudomonas aeruginosa*. The results of antibiotic susceptibility testing for these isolates are presented in Figure 1.

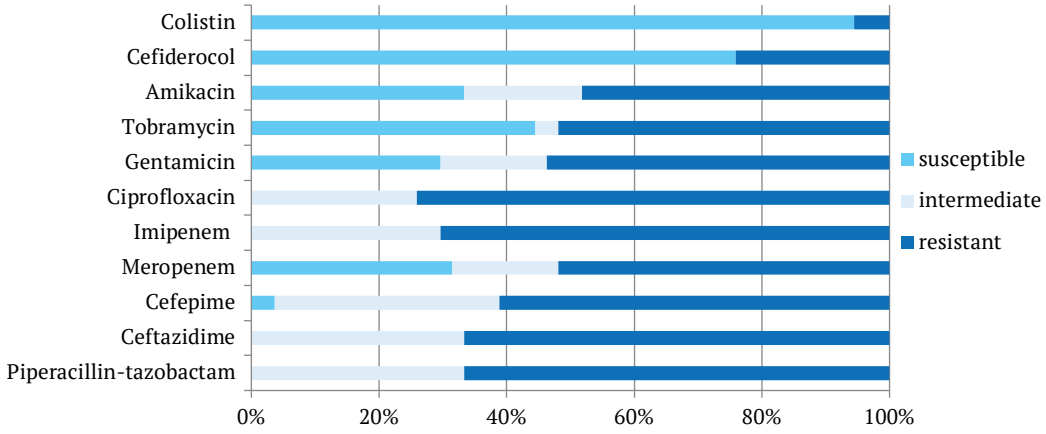

Figure 1. Antibiotic susceptibility profile of isolated *P. aeruginosa* strains (n = 54)
Source: compiled by the authors

According to the obtained results, clinical isolates of *Pseudomonas aeruginosa* demonstrated high susceptibility to polymyxin (94.4%) and cefiderocol (75.9%), and moderate susceptibility to the aminoglycosides amikacin, tobramycin, and gentamicin, for which the combined proportion of susceptible and intermediate strains was 51.85%, 48.1%, and 46.3%, respectively. Among these, tobramycin exhibited the highest proportion of susceptible isolates – 44.4%, compared to 33.3% for amikacin and 29.6% for gentamicin. A total of 31.5% of isolates remained susceptible to meropenem, whereas no isolates were fully susceptible to imipenem; only 29.6% of clinical strains were classified as moderately resistant. The susceptibility of *Pseudomonas aeruginosa* to other antipseudomonal beta-lactams revealed low susceptibility to piperacillin-tazobactam and ceftazidime: 33.3% of isolates were moderately resistant, while 66.7% were resistant. Only 2 out of 54 strains remained susceptible to cefepime, with 35.2% moderately resistant and 42.6% resistant. Thus, resistance to antipseudomonal cephalosporins and penicillins reached 96.3%-100%. Cationic surfactants demonstrated considerable activity against Gram-negative non-fermenting bacteria. The susceptibility of the clinical isolates to quaternary ammonium compounds (decamethoxine, benzalkonium chloride), as well as octenidine dihydrochloride, chlorhexidine bigluconate, and polyhexanide, was assessed. The results are summarised in Table 1.

Table 1. Susceptibility of clinical isolates of *P. aeruginosa* to antiseptics (n = 54)

Antiseptic	Average concentrations of antiseptic (M ± s, μg/mL)	
	Minimum inhibitory concentration (MIC)	Minimum bactericidal concentration (MBC)
Decamethoxin	22.52 ± 2.83[4,5]	73.74 ± 15.06[4,5]
Benzalkonium chloride	145.7 ± 32.1[1,2,3]	418.75 ± 78.89[1,3]
Chlorhexidine bigluconate	22.5 ± 2.36[4,5]	72.09 ± 2.06[5]
Octenidine dihydrochloride	16.4 ± 2.43[4,5]	102.5 ± 10.75[4,5]
Polyhexanide	65.0 ± 5.08[1,2,3]	192.5 ± 27.70[1,2,3]

Notes: [1] – statistically significant difference compared to decamethoxine (p ⩽ 0.01); [2] – statistically significant difference compared to chlorhexidine (p ⩽ 0.01); [3] – statistically significant differences compared to octenidine (p ⩽ 0.01); [4] – statistically significant differences compared to benzalkonium (p ⩽ 0.01); [5] – statistically significant differences compared to polyhexanide (p ⩽ 0.01)
Source: compiled by the authors

It was found that the greatest antipseudomonal activity was exhibited by decamethoxine, chlorhexidine bigluconate, and octenidine dihydrochloride, which inhibited bacterial growth at MICs of 22.52 ± 2.83 μg/mL, 22.5 ± 2.36 μg/mL, and 16.4 ± 2.43 μg/mL, respectively. The MBCs of these antiseptics ranged from 72.09 ± 2.06 μg/mL

(chlorhexidine) to 102.5 ± 10.75 μg/mL (octenidine). No statistically significant differences in antimicrobial activity were found among decamethoxine, chlorhexidine, and octenidine; however, all three exhibited significantly greater antipseudomonal activity than polyhexanide and benzalkonium chloride ($p \leqslant 0.01$). The antiseptics decamethoxine, chlorhexidine, and octenidine exhibited a bacteriostatic effect at concentrations 2.9-4 times and 6.5-8.9 times lower than those required for polyhexanide and benzalkonium chloride, respectively. It was found that the death of *P. aeruginosa* occurred at minimum concentrations of 192.5 ± 27.70 μg/mL for polyhexanide and 418.75 ± 78.89 μg/mL for benzalkonium chloride, which significantly exceeded the corresponding values for decamethoxine, chlorhexidine, and octenidine by 1.9-2.7 times and 4.1-5.8 times, respectively ($p \leqslant 0.01$).

Assessment of the susceptibility of the isolated *P. aeruginosa* strains established that the biologically active preparation Pyofag displayed variable activity against the tested isolates. Calculation of the susceptibility index enabled evaluation of the lytic efficacy of the antipseudomonal phage cocktail in the preparation by determining the proportion of the bacterial population undergoing lysis after 18-20 hours of incubation. Based on the value of the susceptibility index (*Is*), the isolated strains were classified into the following categories: highly susceptible ($0.8 \leqslant Is \leqslant 0.99$), susceptible ($0.5 \leqslant Is \leqslant 0.79$), moderately resistant ($0.3 \leqslant Is \leqslant 0.49$), and resistant ($0.01 \leqslant Is \leqslant 0.29$). The distribution of isolates by susceptibility to the polyvalent bacteriophage is shown in Figure 2.

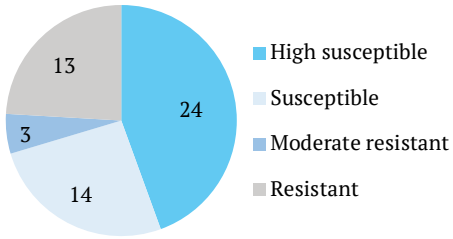

Figure 2. Susceptibility of isolated *Pseudomonas aeruginosa* strains to Pyofag (n = 54)
Source: compiled by the authors

According to the data obtained, 70.4% of *P. aeruginosa* strains (38 isolates) were classified as highly susceptible or susceptible to Pyofag, while 29.6% (16 isolates) were moderately resistant or resistant. The values of the phage lytic activity index (*Is*) for the tested *P. aeruginosa* strains are presented in Table 2.

Table 2. Susceptibility of clinical isolates of *P. aeruginosa* (n = 54) to Pyofag

Susceptibility category	Number of isolates (n)	Susceptibility index (*Is*)
Highly susceptible	24	0.95 ± 0.007*
Susceptible	14	0.65 ± 0.024*
Moderately resistant	3	0.34 ± 0.020*
Resistant	13	0.098 ± 0.026*

Notes: * – statistically significant differences between group values ($p \leqslant 0.01$)
Source: compiled by the authors

According to the results, the lytic effect of Pyofag was observed in 24 *P. aeruginosa* strains, as indicated by a susceptibility index (*Is*) of 0.95 ± 0.007. This suggests that only 5% of the population remained viable 20 hours after phage application. In the susceptible isolates (n = 14), the susceptibility index was 0.65 ± 0.024, indicating a substantial activity of Pyofag and its ability to reduce the bacterial population by approximately 65% within 20 hours of incubation. Among the moderately resistant *P. aeruginosa* strains, 66% of cells survived phage treatment, reflected by a susceptibility index of 0.34 ± 0.020. In contrast, 13 strains were classified as resistant to the polyvalent pseudomonal phage cocktail in Pyofag, with a susceptibility index of 0.098 ± 0.026. Given the results obtained, the potential synergistic detrimental effect of surfactant-active antiseptics and bacteriophages on clinical strains of *P. aeruginosa* was investigated. To this end, the lytic effect of the phage was assessed under cultivation conditions in a medium containing 25% of the sub-bacteriostatic concentration of the antiseptic specific to each *P. aeruginosa* strain. To rule out the additive effect of the antipseudomonal agents, the experiment included controls to measure the optical density (in McFarland units) of bacterial suspensions cultured in the presence of subbacteriostatic concentrations (subMIC) of antiseptics, compared with the untreated culture control (Table 3).

Table 3. Optical density of *P. aeruginosa* bacterial cultures in the presence of sub-bacteriostatic concentrations (subMIC) of antiseptics (M ± s, McFarland units)

Characteristics of the nutrient medium	Phage-resistant strains (n = 8)	Phagosensitive strains (n = 7)
Control MPB	7.44 ± 0.19	7.07 ± 0.14 (–5%)
MPB with subMIC decamethoxine	6.79 ± 0.22	6.49 ± 0.20 (–4.4%)
MPB with subMIC benzalkonium chloride	6.95 ± 0.20	6.54 ± 0.22 (–6%)
MPB with subMIC chlorhexidine bigluconate	6.81 ± 0.25	6.64 ± 0.13 (–2,5%)
MPB with subMIC octenidine dihydrochloride	6.73 ± 0.24	6.6 ± 0.17 (–2%)
MPB with subMIC polyhexanide	6.85 ± 0.17	6.46 ± 0.26 (–5.7%)

Source: compiled by the authors

As shown by the results of the control measurements, sub-bacteriostatic concentrations of antiseptic agents did not result in significant inhibition of bacterial growth, as evidenced by a 2-6% reduction in average optical density relative to the control. Therefore, these concentrations did not exert a pronounced effect on bacterial proliferation.

The synergistic effect of surfactants (chemical lytic factors) and Pyofag (a biological lytic agent) was assessed in strains resistant (*Is* = 0.15 ± 0.05) and susceptible (*Is* = 0.69 ± 0.03) to the phage. A total of eight resistant and seven phage-susceptible *P. aeruginosa* clinical isolates were included. The results are presented in Table 4.

Table 4. Lytic effect of phage on clinical isolates of *P. aeruginosa* in the presence of subbacteriostatic concentrations of surfactant antiseptics

Antipseudomonal factor(s)	Susceptibility index (*Is*) (M ± s)	
	Phage-resistant *P. aeruginosa* (n = 8)	Phagosensitive *P. aeruginosa* (n = 7)
Pyofag	0.15 ± 0.05	0.69 ± 0.03
Pyofag with subMIC decamethoxine	0.70 ± 0.09*	0.87 ± 0.05*
Pyofag with subMIC benzalkonium chloride	0.25 ± 0.05	0.75 ± 0.04
Pyofag with subMIC chlorhexidine bigluconate	0.54 ± 0.09*	0.80 ± 0.02*
Pyofag with subMIC octenidine dihydrochloride	0.64 ± 0.12*	0.86 ± 0.03*
Pyofag with subMIC polyhexanide	0.28 ± 0.05	0.76 ± 0.04

Notes: * – statistically significant differences relative to the corresponding Pyofag-only indicator ($p \leq 0.01$)
Source: compiled by the authors

The results indicated that the presence of 25% of the minimum bacteriostatic concentration of decamethoxine, chlorhexidine bigluconate, and octenidine dihydrochloride led to a statistically significant enhancement of the lytic effect of Pyfag ($p \leq 0.01$), as reflected in increased susceptibility index values. Specifically, in the group of *P. aeruginosa* strains resistant to the phage cocktail (*Is* = 0.15 ± 0.05), the susceptibility index increased by 3.6, 4.3, and 4.7 times, respectively, in the presence of chlorhexidine, octenidine, and decamethoxine at concentrations that did not inhibit bacterial growth. These differences were statistically significant ($p \leq 0.003$). It was also demonstrated that the effectiveness of Pyofag against susceptible strains of *P. aeruginosa* increased by 1.2 times in the presence of decamethoxine and octenidine, while sub-bacteriostatic concentrations of chlorhexidine increased the susceptibility of *P. aeruginosa* by 1.16 times. According to the findings, the susceptibility of *P. aeruginosa* to Pyofag also increased in the presence of sub-bacteriostatic concentrations of benzalkonium chloride and polyhexanide; however, no statistically significant difference between the obtained values was established. It should be noted that, during the study of the synergistic interaction between surfactant antiseptics and Pyofag, alterations in the distribution of strains by phage susceptibility were observed in the experimental group (Fig. 3).

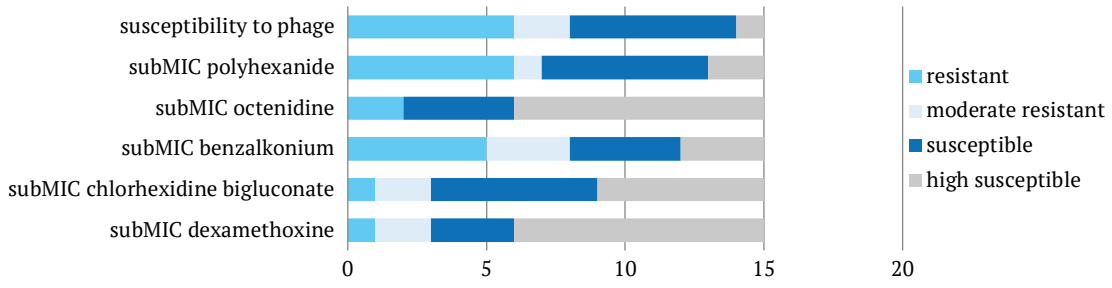

Figure 3. Susceptibility of clinical strains of *P. aeruginosa* to Pyofag in the presence of sub-bacteriostatic concentrations of surfactant antiseptics (n = 15)
Source: compiled by the authors

According to susceptibility to Pyofag, determined based on *Is* values, the tested strains were classified as resistant (6 isolates), moderately resistant (2 isolates), susceptible (6 isolates), and highly susceptible (1 isolate). When exposed to Pyofag in a medium containing subMIC concentrations of decamethoxine and octenidine, the number of highly susceptible strains increased to 9, while the number of resistant strains decreased to 1 and 2 isolates, respectively. Changes in phage susceptibility categories of *P. aeruginosa* were also observed when Pyofag was applied in the presence of other antiseptics. The number of highly susceptible strains increased to 6 in media containing subMIC concentrations of chlorhexidine bigluconate, and to 2 and 3 strains in the presence of polyhexanide and benzalkonium chloride, respectively. Notably, the number of Pyofage-resistant strains of *P. aeruginosa* decreased to one under the combined action of Pyofag and chlorhexidine. However, when clinical isolates were tested in media supplemented with polyhexanide or benzalkonium, the number of resistant strains either remained unchanged or decreased only marginally.

DISCUSSION

V. Kovalchuk *et al*. [11] demonstrated that among the pathogens responsible for purulentinflammatory complications in injured servicemen during the full-scale military aggression by russia, non-fermenting Gram-negative bacilli of the genera *Acinetobacter* and *Pseudomonas*, as well as enterobacteria of the genus *Klebsiella*, are predominant. The proportion of isolated *P. aeruginosa* varies widely (from 1% to 35%), which is associated with multiple factors (nosological classification, wound type and location, duration of hospitalisation, extent of antibacterial therapy, etc.). Thus, the proportion identified in this study (35.5%) is generally consistent with contemporary data [12].

Numerous studies – including those by N. Bahniuk *et al*. [13], S. Mudenda *et al*. [14], and O. Nazarchuk *et al*. [15] – have demonstrated that *P. aeruginosa* is a multidrug-resistant (MDR) pathogen that exhibits high levels of resistance to antipseudomonal antibiotics. The strategy for addressing antibiotic-resistant bacteria, proposed by the World Health Organization as part of a global action plan, involves the rational use of antibiotics, which are classified into Access, Watch, and Reserve groups [16]. According to the data obtained, clinical strains of *Pseudomonas aeruginosa* demonstrated notable susceptibility to the reserve antibiotics colistin (94.4%) and cefiderocol (75.9%). However, no strains were identified as susceptible to the Watch group antibiotics ceftazidime, piperacillin-tazobactam, ciprofloxacin, or imipenem, although susceptibility to meropenem and tobramycin was observed in 31.5% and 44.4% of isolates, respectively. The aminoglycosides gentamicin and amikacin (Access group antibiotics) were effective against 29.6% and 33.3% of clinical isolates, respectively. T. Denysko [17] has shown that the low susceptibility of *P. aeruginosa* to Access group antibiotics results in the limited effectiveness of empirical antibiotic therapy for pseudomonal infections. J. Murugaiyan *et al*. [18], in their review, highlighted strategies currently applied or proposed as alternatives to traditional antibiotics in managing wound infections. These include the topical application of antiseptics, to which bacteria develop resistance slowly and generally remain highly susceptible. The results obtained indicate that the antiseptic activity index of agents available on the pharmaceutical market in Ukraine and recommended for topical use against clinical strains of *P. aeruginosa* ranges from 61 (Octenisept), 22.2 (0.05% chlorhexidine bigluconate solution), 15.4 (Prontosan) to 8.9 (Decasan).

In the context of rising antibiotic resistance, alternative antimicrobial strategies targeting healthcare-associated pathogens are gaining increasing importance. Interest in polyvalent phage cocktails composed of virulent bacteriophages has grown significantly, and additional phage therapy approaches are actively under development [19]. Clinical studies by A. Nawaz *et al*. [20] and C. Torres-Barceló & M.E. Hochberg [21] have yielded positive results for phage therapy in infections caused by multidrug-resistant strains of *P. aeruginosa*, supporting the development of alternative strategies to combat super-pathogens under modern conditions. S. Derkach [22] demonstrated that Pyofag is effective against several wound pathogens, including clinical strains of *S. aureus* and *P. aeruginosa*; however, numerous factors may influence phage activity both *in vitro* and *in vivo*. In the present study, 70.4% of clinical isolates of *P. aeruginosa* were identified as susceptible to Pyofag, slightly exceeding the results (46%-68%) reported by S. Derkach, possibly due to differences in susceptibility assessment methodology.

The combined effects of virulent bacteriophages and antibiotics on bacterial cells have been thoroughly examined by F. Oechslin *et al*. [23] and L. Cui *et al*. [24]. A synergistic antibacterial effect has been observed between phages and beta-lactam antibiotics; however, antibiotics that disrupt bacterial protein synthesis (such as macrolides and aminoglycosides) have been shown to inhibit phage efficacy. The concurrent use of antiseptics and bacteriophages is insufficiently covered in scientific literature, except for general recommendations to avoid simultaneous application due to the potential negative impact of antiseptics on phage activity. Nevertheless, surfactant-based antiseptics do not adversely affect non-enveloped viruses, including bacteriophages. Therefore, considering the mechanisms of action of antiseptics such as quaternary ammonium compounds, biguanides, and other surfactants, it was considered relevant to investigate the combined antimicrobial effects of phages and cationic detergents on clinical strains of *P. aeruginosa*.

According to the data obtained, decamethoxine, chlorhexidine bigluconate, and octenidine dihydrochloride enhanced the lytic effect of Pyofag and increased the susceptibility of resistant isolates to the phage cocktail. It should also be noted that one strain remained classified as resistant even in the presence of antiseptics that exerted a synergistic effect on the lytic properties of the bacteriophage. This finding may indicate the presence of distinct resistance mechanisms in *Pseudomonas aeruginosa* against the phage cocktail in the drug Pyofag.

CONCLUSIONS

Clinical strains of *P. aeruginosa* exhibit a high level of resistance to antipseudomonal antibiotics from the Access and Watch groups, with the predicted effectiveness of empirical therapy ranging from 30% to 50%. The antiseptics decamethoxine, octenidine dihydrochloride, chlorhexidine bigluconate, and polyhexanide demonstrate strong antipseudomonal activity. Their dosage forms are likely to be effective for the local treatment of wound infections, as their activity indices exceed the MIC of the antiseptics by a factor of 8.9 to 61. The majority (70.4%) of clinical *P. aeruginosa* isolates were susceptible to the antipseudomonal phage cocktail in the composition of Pyofag, which supports recommending this drug for the treatment of antibiotic-resistant strains. The surface-active antiseptics decamethoxine, chlorhexidine, and octenidine exhibit a synergistic antipseudomonal effect when used with Pyofag, enhancing both the susceptibility of *Pseudomonas aeruginosa* to the bacteriophage and its lytic activity. Future research will focus on evaluating the ability of bacteriophages to affect the biofilm-forming properties of clinical *P. aeruginosa* strains, as well as exploring the combined effects of surfactants and bacteriophages on biofilm-associated bacterial forms.

ACKNOWLEDGEMENTS

None.

FUNDING
None.

CONFLICT OF INTEREST
None.

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Мікробіологічне обґрунтування альтернативних шляхів боротьби з інфекціями, спричиненими антибіотикорезистентними *Pseudomonas aeruginosa*

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Анотація. Метою роботи було дослідити активність препарату піофаг щодо клінічних ізолятів *Pseudomonas aeruginosa*; визначити ефективність комбінованої дії поверхнево-активних антисептиків і бактеріофагів. Для досягнення мети використовувались класичні методи виділення та ідентифікації бактерій. Визначення чутливості псевдомонад до антибіотиків проводилось диско-дифузійним методом, до поверхнево-активних антисептиків (декаметоксину, бензалконію хлориду, хлоргексидину біглюконату, октенідину дигідрохлориду і полігексаніду) – методом розведень у рідкому поживному середовищі. Чутливість клінічних ізолятів до препарату піофаг визначали за оптичною густиною бактеріальної суспензії після 18 год інкубації з піофагом. Визначення характеру комбінованого впливу бактеріофагу і антисептиків на *P. aeruginosa* проводилось шляхом визначення індексу літичної дії фагу на планктонні форми бактерій, які культивували в середовищі з суббактеріостатичними концентраціями антисептиків. За результатами встановлено, що 54 виділених клінічних штами *P. aeruginosa* зберігали високу чутливість тільки до антибіотиків резерву колістину (94,4 %) і цефідероколу (75,9 %), до інших антипсевдомонадних антибіотиків (цефепіму, цефтазидиму, піперациліну-тазобактаму, іміпенему, ципрофлоксацину) зафіксований рівень стійкості у 96,3-100 % ізолятів, однак аміноглікозиди (гентаміцин, тобраміцин, амікацин) і меропенем залишаються ефективними щодо 29,6-44,4 % штамів. Антисептичні засоби, які містять поверхнево-активні антисептики, демонструють високі антипсевдомонадні властивості і здатні пригнічувати розмноження бактерій в концентраціях від 16,4-22,5 мкг/мл (октенідину дигідрохлорид, декаметоксин, хлоргексидину біглюконат) до 65-145,7 мкг/мл (полігексанід, бензалконію хлорид). Доведено, що декаметоксин, октенідин і хлоргексидин достовірно перевищують антибактеріальну дію антисептиків полігексанід і бензалконій хлорид ($p < 0,01$). Виділені штами псевдомонад продемонстрували високу чутливість до лікарського засобу піофаг: індекс літичної дії (*Is*) піофагу був вище 0,5 у 70,4 % штамів (50 % бактерій гинули в процесі динамічної взаємодії росту бактеріальної популяції і розмноження бактеріофагів). В середовищах з суббактеріостатичними концентраціями декаметоксину, хлоргексидину, октенідину, і чутливі ($n = 7$, $Is = 0,69$), і стійкі ($n = 8$, $Is = 0,15$) штами руйнувались бактеріофагом більш інтенсивно, про що свідчило зростання індексу чутливості до 0,8-0,87 у чутливих штамів, і до 0,54-0,7 у фагорезистентних штамів, відповідно

Ключові слова: поверхнево-активні антисептики; бактеріофаги; антибіотики; піофаг; умовно-патогенні мікроорганізми