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Efficacy of bacteriophages against Pseudomonas aeruginosa with diverse antibiotic resistance profiles

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

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With the rising prevalence of multidrug-resistant (MDR), extensively drug-resistant (XDR), and pandrug-resistant (PDR) strains of *Pseudomonas aeruginosa*, the development of alternative therapeutic approaches has become increasingly important. Among these, the use of bacteriophages represents a particularly promising strategy for the treatment of infections caused by multidrug-resistant pathogens.

The aim of this study was to isolate and characterize bacteriophages lytically active against *P. aeruginosa*, to determine their activity spectrum against clinical isolates, and to assess the potential of combining bacteriophages with antibiotics.

Materials and methods. The lytic activity of bacteriophages was tested against 23 clinical isolates of *P. aeruginosa* obtained from pediatric bloodstream infections, including strains carrying antibiotic resistance genes $bla_{NDM\text{-}1}$, bla_{IMP}, bla_{VIM}, and bla_{TEM}. Antimicrobial susceptibility of bacterial isolates was determined using the disk diffusion method according to EUCAST recommendations. Bacteriophages were isolated from wastewater samples collected in Kyiv. Lytic activity was evaluated by the Spot test and a modified agar overlay assay. Morphological characteristics were examined using transmission electron microscopy. Bacteriophage-antibiotic interactions were assessed by a modified disk diffusion method.

Results. Between 2021 and 2024, a total of 62 bacteriophages were isolated from wastewater, belonging to the *Myoviridae-like*, *Siphoviridae-like*, and *Podoviridae-like* morphotypes. Their lytic spectra varied considerably, ranging from narrow specificity (2–4 strains) to the ability to lyse more than 20 clinical bacterial isolates. Overall, 29.0 % of bacteriophages showed activity against multiple strains, and some isolates exhibited a broad host range, with specific lysis observed in up to 91.3 % of tested strains. Combination testing revealed a predominantly synergistic interaction between bacteriophages and β-lactam antibiotics, manifested by an increase in both plaque size and number.

Conclusions. The isolated bacteriophages demonstrated substantial variability in lytic activity and have potential applications in the treatment of infections caused by multidrug-resistant *P. aeruginosa* strains. The findings support the feasibility of establishing bacteriophage collections and applying combined bacteriophage-antibiotic therapies. Considering the current epidemiological situation in Ukraine, these results are of practical importance for the development of new antimicrobial strategies.

Ключові слова:
Pseudomonas aeruginosa, бактеріофаги, спектр літичної активності, фагово-антибіотикова взаємодія.

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Ефективність бактеріофагів проти Pseudomonas aeruginosa з різними профілями стійкості до антибіотиків

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В умовах зростання частоти появи MDR, XDR та PDR штамів *Pseudomonas aeruginosa* особливого значення набуває пошук альтернативних підходів до терапії інфекцій, що ними спричинені. Одна з перспективних стратегій – використання бактеріофагів для лікування інфекцій, що спричинені мікроорганізмами з множинною лікарською стійкістю.

Мета роботи – ізолювати та схарактеризувати бактеріофаги, які літично активні щодо *P. aeruginosa*, визначити їхній спектр активності щодо клінічних ізолятів та оцінити потенціал поєднання фагів з антибіотиками.

Матеріали і методи. Для дослідження літичної активності фагів використано 23 клінічні ізоляти *P. aeruginosa*, що виділені у дітей з інфекціями кровотоку, включаючи штами, які містили гени антибіотикорезистентності $bla_{NDM\text{-}1}$, bla_{IMP}, bla_{VIM}, bla_{TEM}. Чутливість бактеріальних ізолятів до антибіотиків визначали диско-дифузійним методом, відповідно до рекомендацій EUCAST. Бактеріофаги ізолювали зі стічних вод міста Києва. Літичну активність оцінювали методом Spot test і модифікованим методом агарових шарів. Морфологію вивчали за допомогою трансмісійної електронної мікроскопії. Для визначення фагово-антибіотикової взаємодії використано модифікований диско-дифузійний метод.

Результати. Упродовж 2021–2024 рр. зі стічних вод ізольовано 62 бактеріофаги, що належали до морфотипів *Myoviridae-like*, *Siphoviridae-like* та *Podoviridae-like*. Встановлено варіабельність їхнього літичного спектра: від вузької специфічності (2–4 штами) до здатності лізувати понад 20 клінічних бактеріальних ізолятів. Загалом 29,0 % фагів були активними щодо кількох бактеріальних штамів, а окремі фагові ізоляти характеризувалися широким спектром дії (специфічний лізис до 91,3 % протестованих бактеріальних штамів). Тестування комбінованої дії дало змогу встановити переважно синергічний ефект взаємодії між фагами та β-лактамними антибіотиками, що виявили за збільшенням і діаметра, й кількості бляшок.

Висновки. Ізольовані бактеріофаги характеризуються значною варіабельністю літичної активності та потенційно можуть бути використані для лікування інфекцій, спричинених штамами *P. aeruginosa* з множиною лікарською стійкістю. Отримані результати підтверджують доцільність створення колекцій фагів і комбінованого застосування їх з антибіотиками. Враховуючи епідеміологічну ситуацію в Україні, ці дані мають практичне значення для розробки нових протимікробних стратегій.

Pseudomonas aeruginosa is a Gram-negative bacterium that ranks among the leading causative agents of opportunistic infections in humans. This microorganism is capable of inducing both acute and chronic infectious processes, particularly in immunocompromised individuals, such as those with chronic obstructive pulmonary disease, cystic fibrosis, malignancies, severe trauma, and burns. *P. aeruginosa* may serve as the etiological agent of sepsis, ventilator-associated pneumonia, and infectious complications in patients with COVID-19. Importantly, *P. aeruginosa* is one of the principal pathogens of hospital-acquired infections, frequently associated with the use of medical devices. It is often implicated in catheter-associated urinary tract infections, diabetic foot infections, and ear infections, including otitis externa and chronic suppurative otitis media [1,2].

According to ECDC data, *P. aeruginosa* is the most frequently isolated microorganism in cases of pneumonia acquired in intensive care units across the European Union (20.3 % of all isolated pathogens) [3]. The situation is further complicated by the fact that treating infections caused by *P. aeruginosa* is extremely challenging due to its high mutation rate and ability to adapt through the acquisition of antibiotic resistance [4]. In 2024, the World Health Organization classified carbapenem-resistant *P. aeruginosa* strains as belonging to the “High Priority Group,” for which there is an urgent need to develop new antibacterial agents for clinical use [5]. Globally, the incidence of *P. aeruginosa* infections has shown a general upward trend. This can be partly attributed to the increasing prevalence of risk factors for *P. aeruginosa* infections, such as population aging, a growing burden of chronic diseases, more intensive use of medical devices, and the rising number of immunosuppressed persons [6].

P. aeruginosa is also one of the leading nosocomial pathogens in Ukraine, demonstrating a high potential for the development of multidrug resistance, particularly to carbapenems. According to ECDC data for 2021, resistance rates of *P. aeruginosa* in Ukraine to antimicrobial agents were as follows: carbapenems – 78.0 %, piperacillin-tazobactam – 75.5 %, ceftazidime – 81.4 %, and fluoroquinolones – 81.4 %. At the same time, it was noted that the representativeness of isolates remained low, which limits the ability to fully reflect the epidemiological situation in the country [7].

Under the conditions of war in Ukraine, due to the substantial increase in the burden on healthcare facilities and the large number of wounded individuals as a result of active hostilities, a marked rise in the prevalence of multidrug-resistant *P. aeruginosa* strains has been observed. In military and combat settings, *P. aeruginosa* was detected in 14.8 % of postoperative wound samples and in 7.1 % of intra-abdominal samples. Resistance to key antibiotics was identified as follows: ciprofloxacin – 45.6 %, meropenem – 67.4 %, ceftazidime – 52.3 %, and piperacillin/tazobactam – 48.7 % [8].

According to the data from enhanced epidemiological surveillance of antimicrobial resistance in microorganisms causing purulent-inflammatory wound infections among combat-related casualties, conducted by the Public Health Center of the Ministry of Health of Ukraine in 2024, *P. aeruginosa* ranked among the four most common pathogens of wound infections. During 2024, this microorganism was isolated in 13.7 % of clinical specimens obtained from wounded individuals. The antibiotic resistance of the isolated *P. aeruginosa* strains was characterized by high rates, specifically: imipenem – 62.5 %, meropenem – 50.6 %, levofloxacin – 60.7 %, ciprofloxacin – 59.3 %, ceftazidime – 58.8 %, cefepime – 62.1 %, tobramycin – 55.8 %, and piperacillin-tazobactam – 51.3 % [9].

Phage therapy, which employs lytic viruses as antimicrobial agents, holds promise as an adjunctive tool in the treatment of infections caused by *P. aeruginosa*. Resistance mechanisms that limit the effectiveness of antibiotics are less likely to restrict the activity of bacteriophages, including against bacteria residing within biofilms. Over the past decade, several reports have documented successful applications of bacteriophages in infections caused by *P. aeruginosa*. Specifically, bacteriophages have been used in the treatment of antibiotic-resistant infections in cystic fibrosis [10], chronic pulmonary infections [11], prosthetic vascular graft infections [12], bone and joint infections [13], as well as skin ulcers and wounds [14].

Although commercial bacteriophage preparations effective against *P. aeruginosa* currently exist and their successful clinical applications have been documented, the phage collections from which these preparations are derived require regular updating. Since virulent bacteriophages are characterized by narrow host specificity and can lyse only a limited number of strains, updating collections necessitates the inclusion of bacteriophages capable of effectively targeting currently circulating bacterial isolates.

Aim

The aim of the present study was the isolation and characterization of *P. aeruginosa* bacteriophages that may be utilized both for the development of commercial phage preparations and for personalized therapy.

Materials and methods

A total of 23 clinical isolates of *P. aeruginosa*, obtained from pediatric patients with bloodstream infections, were used in this study. In selected experiments, the reference strain *P. aeruginosa* DSM 50071 from the Leibniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures GmbH was also employed.

The susceptibility or resistance of clinical isolates to antimicrobial agents was assessed following the recommendations of EUCAST [15]. Susceptibility testing was performed using the disk diffusion method on Mueller–Hinton agar, with antibiotic disks supplied by HiMedia® [16]. After incubation, the diameters of inhibition zones were measured to determine the susceptibility of the strains. Further classification of isolates as susceptible, multidrug-resistant (MDR), or extensively drug-resistant (XDR) was carried out according to the criteria proposed by A. P. Magiorakos et al. [17]. Analysis of antimicrobial resistance profiles was conducted using the WHONET 2024 software.

All bacteriophages were isolated from wastewater samples collected in Kyiv. Phage isolation was carried out as follows: to remove coarse particulate matter, the samples were first filtered through a paper filter, followed by decontamination of bacterial flora through the addition of 5–10 drops of chloroform per 10 mL of sample, with subsequent

Table 1. Characteristics of primers used in this study for the detection of antibiotic resistance genes

Gene	Primer	Sequence	Tm, °C	Amplicon size	Reference
bla_{TEM}	F	ATGAGTATTCAACATTTCCG	58	867	[19]
	R	CTGACAGTTACCAATGCTTA			
bla_{NDM-1}	F	GGTTTGGCGATCTGGTTTTC	52	621	[20]
	R	CGGAATGGCTCATCACGATC			
bla_{IMP}	F	CTACCGCAGCAGAGTCTTTG	57	587	[21]
	R	AACCAGTTTTGCCTTACCAT			
bla_{VIM-1}	F	AGTGGTGAGTATCCGACAG	58	261	[22]
	R	ATGAAAGTGCGTGGAGAC			

exposure for 2 hours at room temperature. After chloroform treatment, to eliminate lysed bacterial cells, the samples were centrifuged at 3,000 rpm for 10 minutes.

To increase the likelihood of phage recovery, a pre-enrichment step was performed: the wastewater sample was incubated together with a broth culture of a susceptible bacterial strain in liquid medium for 18–24 hours at 36 ± 1 °C. Upon completion of incubation, the sample was centrifuged at 12,000 × g for 10 minutes to remove lysed bacterial cells. The supernatant was then sequentially passed through membrane filters with pore sizes of 0.45 μm and 0.22 μm (MF-Millipore™, Millex® GS MCE Membrane).

The presence of lytic bacteriophages in the sample was determined using the Spot test method [18].

A pure phage lineage was obtained by triple sequential re-plating of individual phage plaques (clear zones) on a lawn of the corresponding susceptible bacterial culture, ensuring clonality and purity of the phage isolate.

For quantitative determination of phage titers, a modified agar overlay method with certain simplifications was used. Instead of the conventional double-layer agar, a single layer was prepared, consisting of a commercial nutrient medium (Nutrient Agar, HiMedia®, India), a 24-hour culture of the target microorganisms (up to 1 mL), and an aliquot of the corresponding phage dilution. For qualitative assessment of the specific activity of isolated bacteriophages, the Spot test method was employed [18].

Formvar-coated support grids were used to prepare films for electron microscopy. Samples were contrasted using 2 % phosphotungstic acid at pH 6.8, with an exposure time of 1–2 minutes. Phage-containing samples were applied to the film by the drop method, with a phage concentration of at least 10^9 PFU/mL. Prepared specimens were examined using a JEOL JEM-1230 transmission electron microscope.

In this study, DNA obtained from bacterial cell thermolysates was used as the template for PCR. To prepare bacterial strains for nucleic acid extraction, overnight cultures (1.5 mL) in Eppendorf tubes were centrifuged for 5 minutes at 3,000 rpm. The supernatant was discarded, and the resulting cell pellet was resuspended in 100 μL of phosphate buffer. Bacterial DNA was subsequently extracted using the commercial ExToPCR™ kit (A & A Biotechnology, Poland).

For amplification, the commercial PCR Mix Plus Green kit (A & A Biotechnology, Poland) was employed. The total reaction volume was 25 μL, comprising 12.5 μL of PCR Mix Plus Green, 1 μM of each forward and reverse primer, 1 μL of bacterial DNA, and deionized water up to the final volume. PCR was performed on a Perkin Elmer GeneAMP PCR System 2400 thermocycler (USA) for 30–35 cycles (depending on the primers), using the following program: DNA denaturation at 95 °C for 30 s, primer annealing at 52–78 °C for 30–60 s *(Table 1)*, elongation at 72 °C for 15–60 s, followed by a final elongation at 72 °C for 7 minutes.

To study the effect of antibiotics on bacteriophage replication, a modified Kirby–Bauer method was used, performed as follows: up to 10 mL of molten nutrient agar, cooled to 40–45 °C, was supplemented with up to 1 mL of an overnight bacterial culture and 100 μL of the corresponding bacteriophage at a concentration of 1–5 × 10^3 PFU/mL. The mixture was thoroughly mixed and poured into sterile Petri dishes. After the agar layer solidified, the dishes were placed in an incubator with the lid slightly ajar for 15 minutes to allow surface drying. Commercial antibiotic discs (no more than five discs per dish) were then evenly applied to the surface of the prepared medium. The dishes were subsequently incubated at 35 °C for 18–20 hours. Nutrient Agar (HiMedia®, India) was used as the growth medium.

After incubation, the effect of antibiotics on bacteriophage replication was evaluated according to the following criteria:

1. Synergy – the combined use of phage and antibiotic produces a stronger effect than either agent alone. This was manifested as an increase in the diameter and/or number of phage plaques within the sublethal concentration zone of the antibiotic (i. e., in the area of antibiotic diffusion immediately beyond the bacterial growth inhibition zone);
2. Antagonism – the combined application results in a reduced effect compared to the action of each agent individually. This was observed as a decrease in the diameter and/or number of phage plaques within the sublethal antibiotic zone;
3. Additive (indifferent) effect – one component does not alter the effect of the other. This was manifested by the absence of changes in the diameter and/or number of phage plaques in the presence of the antibiotic.

Results

Between 2021 and 2024, a total of 62 bacteriophages were isolated from wastewater samples, exhibiting varying degrees of lytic activity. Clinical *P. aeruginosa* strains, collected from healthcare facilities in Kyiv during the same three-year period, were used for phage isolation.

To evaluate the activity of the isolated bacteriophages against antibiotic-resistant strains, an additional 23 *P. aeruginosa* isolates obtained from bloodstream infections were selected, representing diverse levels of antimicrobial resistance. The susceptibility of these strains to antimicrobial

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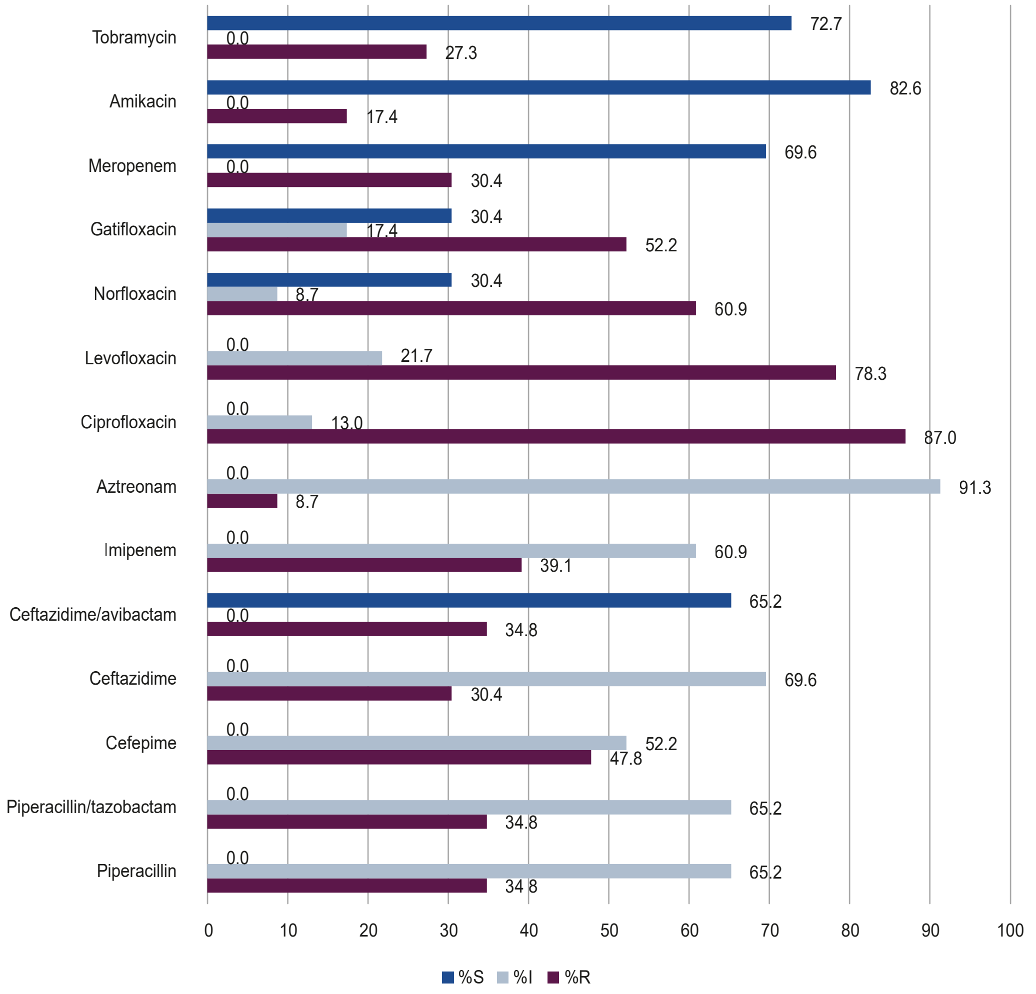

Fig. 1. Antimicrobial susceptibility of *P. aeruginosa* strains used to assess the activity of the isolated bacteriophages. **S:** susceptible; **I:** susceptible, increased exposure; **R:** resistant.

agents was assessed using the disk diffusion method, and molecular identification of selected genes associated with antibiotic resistance was performed using PCR.

To determine susceptibility patterns, antimicrobial agents from six main groups recommended for the treatment of *P. aeruginosa* infections were used [15]: penicillins (piperacillin, piperacillin/tazobactam), cephalosporins (cefepime, ceftazidime, ceftazidime / avibactam), carbapenems (imipenem, meropenem), monobactams (aztreonam), fluoroquinolones (ciprofloxacin, levofloxacin, norfloxacin, gatifloxacin), and aminoglycosides (amikacin, tobramycin).

Analysis of antimicrobial resistance profiles using WHONET 2024 software revealed that four isolates (strains No. 1, 6, 7, 22) were classified as “putative PDR,” two isolates (strains No. 14, 21) as “confirmed XDR,” and 17 isolates as “possible XDR,” requiring additional validation. Overall, the tested strains exhibited moderate levels of resistance to β-lactam antibiotics and fluoroquinolones *(Fig. 1)*.

PCR analysis revealed that 52.2 % of *P. aeruginosa* isolates carried the $bla_{\text{NDM-1}}$ gene (encoding the NDM-type metallo-β-lactamase), 8.7 % harbored the bla_{IMP} gene (IMP-type metallo-β-lactamase), 47.8 % contained the bla_{VIM} gene (VIM-type metallo-β-lactamase), and 8.7 % possessed the bla_{TEM} gene (TEM-type β-lactamase).

Thus, the obtained bacterial isolates were consistent with the study objectives and exhibited diverse antibiotic resistance profiles.

In the subsequent stage of the experimental study, the specific activity of the isolated bacteriophages, lytically active against *P. aeruginosa* , was evaluated according to two main criteria: the number of bacterial strains lysed by each individual bacteriophage (i. e., the spectrum of its lytic activity) and the number of bacteriophages capable of lysing a single bacterial strain (i. e., the sensitivity of the bacterial isolate to the bacteriophages).

The results of the assessment of the specific activity of the bacteriophages demonstrated considerable variability in their lytic spectrum, with lysis observed in 4.2 % to 87.5 % of the total tested clinical multidrug-resistant *P. aeruginosa* isolates.

A total of 18 phage isolates, representing 29 % of the entire phage collection, exhibited activity against individual bacterial cultures. Specifically, 9 isolates (14.4 %) lysed only two bacterial strains, corresponding to a lytic spectrum of 8.3 %. Four isolates (6.5 %) lysed three strains, and one isolate (1.6 %) lysed four strains, accounting for 13 % and

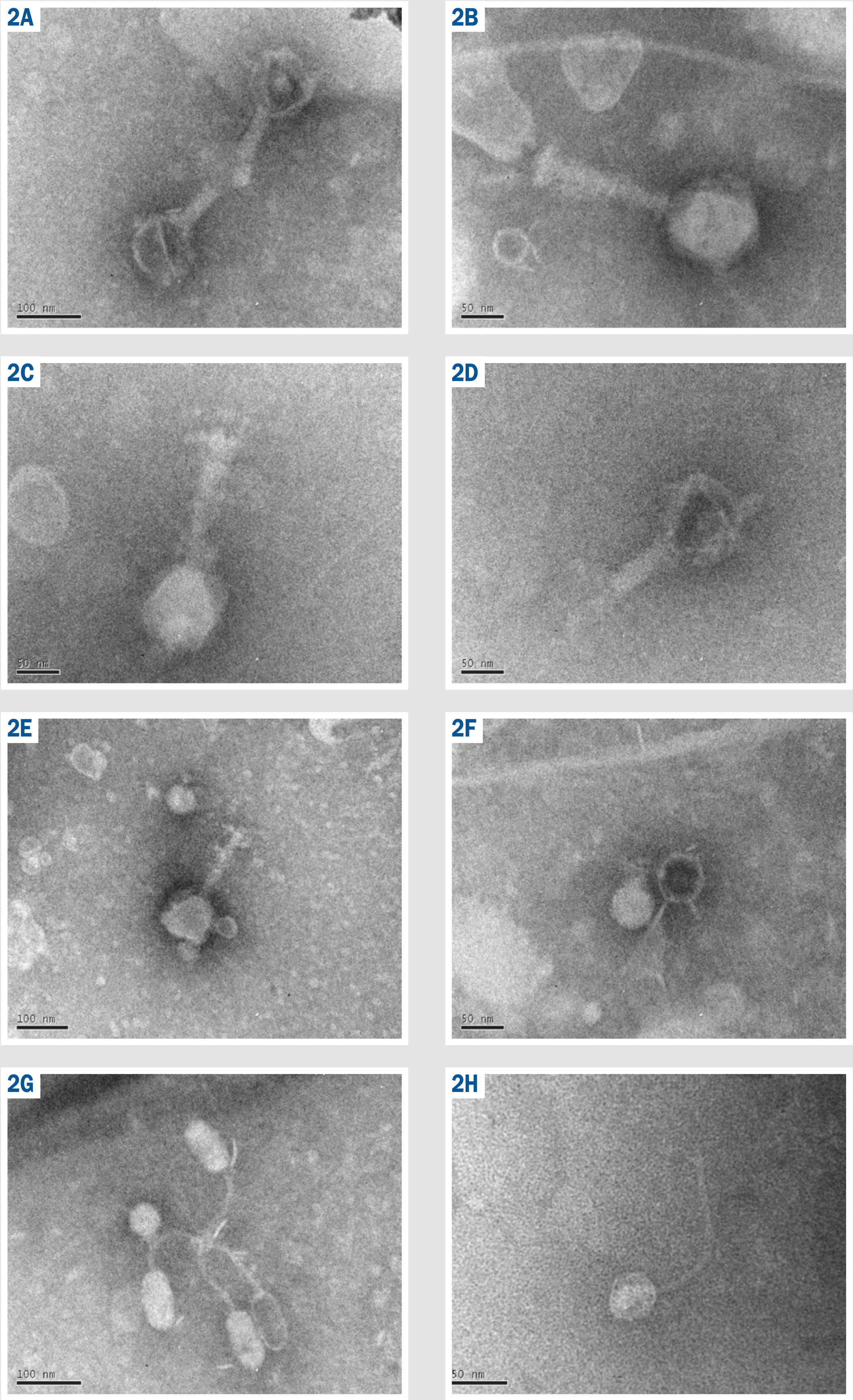

Fig. 2. Transmission electron microscopy images of bacteriophages lytically active against clinical isolates of *P. aeruginosa*. Staining with 2 % phosphotungstic acid: **A–E** – *Myoviridae-like* type; **F** – *Podoviridae-like* type; **G, H** – *Siphoviridae-like* type.

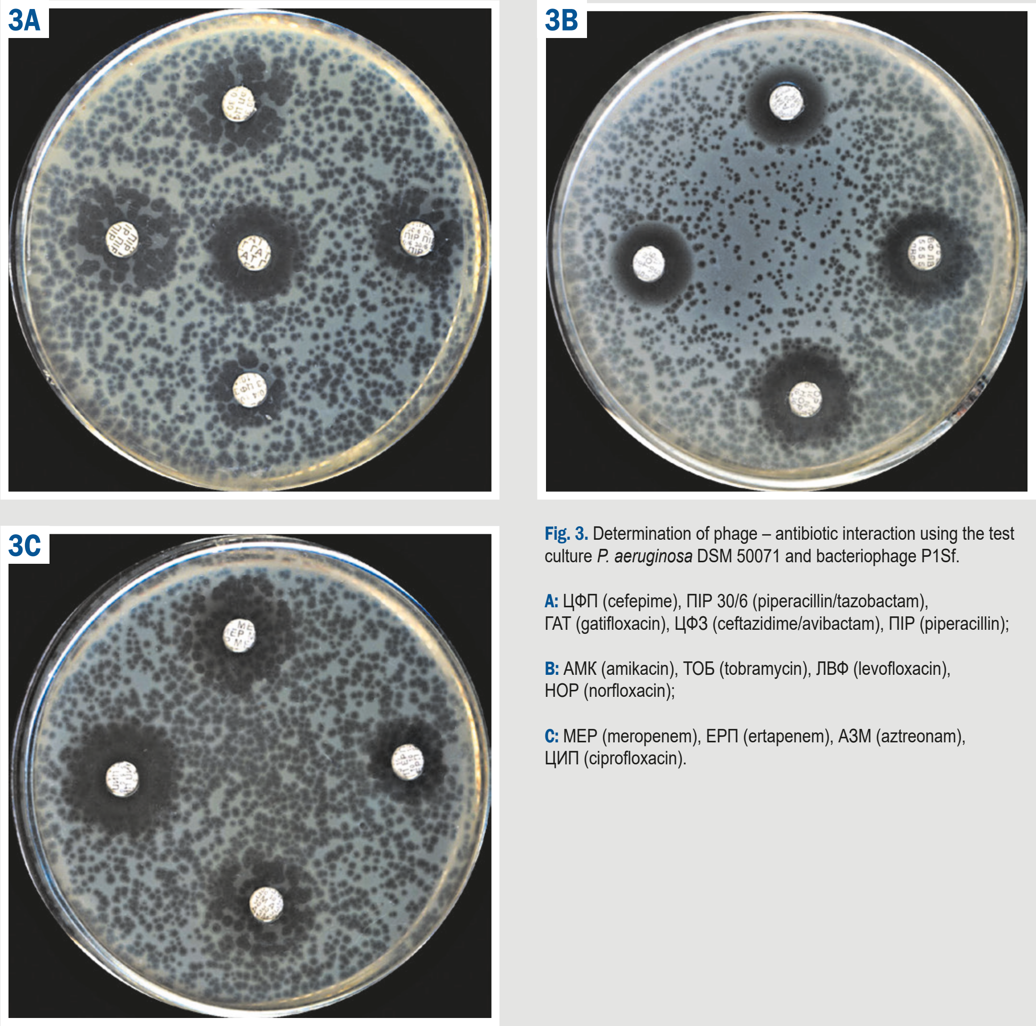

Fig. 3. Determination of phage – antibiotic interaction using the test culture *P. aeruginosa* DSM 50071 and bacteriophage P1Sf.

A: ЦФП (cefepime), ПІР 30/6 (piperacillin/tazobactam), ГАТ (gatifloxacin), ЦФЗ (ceftazidime/avibactam), ПІР (piperacillin);

B: АМК (amikacin), ТОБ (tobramycin), ЛВФ (levofloxacin), НОР (norfloxacin);

C: МЕР (meropenem), ЕРП (ertapenem), АЗМ (aztreonam), ЦИП (ciprofloxacin).

17.4 % of the spectrum, respectively. Two isolates (3.2 %) demonstrated activity against five strains (21.7 % of the spectrum), one isolate (1.6 %) against six strains (26 %), and two isolates (3.2 %) lysed seven bacterial strains (30.4 %). Three isolates (4.8 %) exhibited activity against eight strains (34.8 %), another three (4.8 %) against nine strains (39.1 %), and five isolates (8.1 %) lysed ten strains (43.5 %). Two isolates (3.2 %) lysed eleven strains (47.8 %), and three more (4.8 %) lysed twelve strains (52.2 %). Four isolates (6.5 %) lysed thirteen strains (56.5 %), two isolates (3.2 %) lysed fifteen strains (65.2 %), and single isolates (1.6 % each) lysed sixteen (69.6 %), twenty (86.9 %), and twenty-one (91.3 %) strains, respectively. This variability in the lytic spectrum reflects the substantial diversity of the phage collection, ranging from narrow-host-specific isolates to those capable of lysing a broad range of bacterial strains.

Concurrently, the sensitivity of the clinical *P. aeruginosa* strains to the phage isolates was analyzed. This parameter also exhibited variability, with bacterial culture susceptibility ranging from 6.3 % (lysis of a single bacterial strain by four bacteriophages) to 47.6 % (lysis of a single bacterial strain by 30 different bacteriophages).

During the electron microscopy study, it was shown that the isolated bacteriophages belonged to several morphological types, including *Myoviridae-like, Siphoviridae-like*, and *Podoviridae-like* types. The majority of the bacteriophages were classified as *Myoviridae-like* types *(Fig. 2)*.

In addition to determining the lytic spectrum of the isolated bacteriophages, a study was conducted to evaluate the potential for combined use of *P. aeruginosa* bacteriophages and antibiotics. The modified disk diffusion method allowed the assessment of how different antibiotics, applied on paper discs, affected the replication of the bacteriophage *P1Sf* on a culture of *P. aeruginosa* DSM 50071 as they diffused through the agar.

During the study, two types of interactions between the bacteriophage and antibiotics were observed: synergistic and additive effects *(Fig. 3)*. Most of the tested antimicrobial agents exhibited a synergistic effect.

Specifically, when the following antibiotics were applied to the nutrient medium – piperacillin, piperacillin / tazobactam, gatifloxacin, ceftazidime / avibactam, cefepime, levofloxacin, norfloxacin, ciprofloxacin, ertapenem, meropenem, and aztreonam – synergism was observed. This manifested as an increase in the diameter of phage plaques in the zone of sublethal antibiotic concentrations, indicating enhanced phage replication under conditions of partial inhibition of bacterial cell growth.

Discussion

In this study, a total of 62 bacteriophages were successfully isolated from wastewater samples collected in Kyiv, demonstrating lytic activity against clinical isolates of *P. aeruginosa*, including multidrug-resistant strains. These findings are consistent with previous reports indicating that wastewater represents one of the most reliable sources for isolating bacteriophages targeting *P. aeruginosa*, due to the high density of bacterial hosts present in such environments [23]. This isolation strategy is promising for updating phage collections, which are essential both for the development or renewal of commercial phage preparations and for personalized phage therapy.

Most of the bacteriophages tested in this study lysed only a few bacterial strains (typically 2–4), and only a small proportion exhibited a broad lytic spectrum (>10 strains). This highlights the need for further selection and combination of bacteriophages into targeted preparations, continuously updating collections to account for currently circulating resistant strains. These findings also corroborate observations from other studies, indicating that bacteriophages active against *P. aeruginosa* generally possess a narrow host range, and thus effective therapy typically requires the use of mixtures (cocktails) of multiple bacteriophages [24].

The lysis of all 23 *P. aeruginosa* strains by bacteriophages from the collection demonstrates the therapeutic potential of bacteriophages against multidrug-resistant strains. Despite the presence of various resistance mechanisms in the bacterial strains tested, it was shown that the bacteriophages were capable of actively lysing these pathogenic bacteria.

It has been experimentally demonstrated that the phage – antibiotic synergy of bacteriophage *P1Sf*, which is lytically active against *P. aeruginosa* DSM 50071, most frequently occurs when using antimicrobial agents that disrupt cell wall function and inhibit DNA gyrase and topoisomerase IV. These findings are consistent with experimental studies conducted by other researchers. For instance, Dana J. Holger and colleagues showed that the combination of phage – meropenem – colistin against multidrug-resistant *P. aeruginosa* strains restored their susceptibility to meropenem [25]. Ferran A. A. et al. simulated oral ciprofloxacin treatment combined with inhaled phage administration for respiratory infections caused by *P. aeruginosa*, demonstrating that the combined use of bacteriophages and ciprofloxacin prevented the growth of resistant bacteria both during simultaneous and sequential treatment [26].

The obtained results highlight the potential of phage therapy as an adjunctive tool in the treatment of infections caused by *P. aeruginosa*, particularly in Ukraine, where the war has led to an increase in wound infections. Bacteriophages may be applied for the management of post-traumatic complications, wound infections, burns, and osteomyelitis. Local bacteriophages from our collections can also be adapted for topical therapy, for example, in wound hydrogels. The efficacy of this approach has already been demonstrated under laboratory conditions [27,28].

Limitations of the study include a focus on *in vitro* activity without *in vivo* testing, the narrow host specificity of some bacteriophages, and a limited number of bacterial strains.

Conclusions

1. Overall, the obtained data emphasize the importance of further research on bacteriophages as adjunctive therapy for combating multidrug-resistant *P. aeruginosa*.

2. Considering the current epidemiological background in Ukraine, including the increased prevalence of XDR clones due to the ongoing war, the phage isolates described here may serve as valuable tools in outpatient or inpatient management of complex nosocomial infections.

3. The findings also underscore the potential of phage – antibiotic interactions as a novel approach in the clinical treatment of severe and hard-to-treat infections, opening opportunities for the development of optimized combination therapy protocols and personalized patient care.

Perspectives of subsequent scientific research. Future studies should encompass genomic analysis of bacteriophages, evaluation of biofilm activity, and clinical trials to enable integration into treatment protocols in Ukraine. Overall, the isolated bacteriophages represent a valuable resource for the development of new therapeutics, contributing to the fight against *P. aeruginosa* antibiotic resistance.

Ethics statement

The study was reviewed and approved by the Commission on Bioethical Expertise and Ethics of Scientific Research of the Bogomolets National Medical University (Protocol No. 195, May 26, 2025). According to the submitted documentation, the experimental research was conducted during 2019–2025 in full compliance with relevant bioethical regulations and scientific standards.

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