



New Bacteriophages for Treating Canine External Otitis Caused by *Pseudomonas aeruginosa* and *Staphylococcus pseudintermedius*

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ABSTRACT

Phage therapy presents a promising approach to combating bacterial infections; however, its effectiveness is constrained by limitations such as weak lytic activity, narrow host range, and stability issues. Overcoming these challenges requires further research aimed at isolating new specific phages, developing phage cocktails, and expanding the host range to maximize the effectiveness of phage therapy. The present study aimed to isolate and characterize bacteriophages targeting *Pseudomonas aeruginosa* and *Staphylococcus pseudintermedius*, which are the key pathogens responsible for canine external otitis. The study, conducted from 2023 to 2024 in veterinary clinics, involved 496 dogs of different ages, genders, and breeds. The current result revealed that three *P. aeruginosa* phage strains (Pa3, Pa7, Pa15) and three *S. pseudintermedius* phage strains (Sp6, Sp10, Sp17) showed high specificity and lytic activity against the corresponding pathogenic microorganisms isolated from dogs with signs of external otitis. All phages formed clear plaques on nutrient media, indicating their ability to destroy bacterial cells. The lytic activity of the phages was higher for Pa7 and Pa15, which lysed up to 92.8% of *P. aeruginosa* strains, while the Pa3 phage was active only against 71.4% of the strains. While phages Sp6 and Sp10 demonstrated superior lytic activity against *S. pseudintermedius*, Sp17 indicated the highest efficacy against canine-derived *S. aureus* isolates. The optimal phage-to-bacteria ratio for maximum effect was 0.0001 for all the studied phages. The latent period of the phages was up to 30 minutes, and virions were released in significant amounts within an hour after infection. The results demonstrated that all the phages exhibited high stability at temperatures ranging from +4°C to +40°C and within a pH range of 5 to 10, confirming their potential for use in the treatment of external otitis dogs. The results suggested that bacteriophages Pa3, Pa7, and Pa15 for *P. aeruginosa*, and Sp6, Sp10, and Sp17 for *S. pseudintermedius* are promising candidates for use in phage therapy for bacterial infections in dogs, as they exhibit high lytic activity, stability, and specificity towards their hosts.

Keywords: Dog, Otitis, Phage specificity, Phage stability, *Pseudomonas aeruginosa*, *Staphylococcus pseudintermedius*

INTRODUCTION

External otitis in dogs, a common condition in veterinary practice, involves inflammation of the external ear canal and affects approximately 10–20% of canine patients (Corb et al., 2024). Factors that increase the risk of otitis media include anatomical features such as stenotic (narrow) ear canals, excessive hair, blocked canals, and increased production of sulfur earwax. Other factors, such as excessive ear cleaning, trauma, and changes in environmental temperature and humidity, contribute to inflammation (Corb et al., 2024). Prolonged inflammation of the external ear causes excessive growth and enlargement of the glands, hyperkeratosis, and excessive cerumen secretion, leading to changes in moisture and pH that also favor the development of secondary infections (Yoon and Park, 2024). However, the primary factors that exacerbate clinical signs and facilitate disease progression are the bacteria *Staphylococcus* and *Pseudomonas* (Mills et al., 2016; Kwon et al., 2025). Currently, most epidemiological studies focus on methicillin-resistant *Staphylococcus* due to the transfer of resistance genes between humans and animals, while *Pseudomonas* bacteria are often associated with contamination of food and the environment (Morris et al., 2023; Kukhtyn et al., 2024). However, the resistance mechanisms in these pathogens, such as beta-lactamase production, efflux pumps, and biofilm formation, significantly complicate the treatment of infections caused by *Staphylococcus* and *Pseudomonas*, highlighting the need for alternative therapeutic approaches (Tseng et al., 2025). This highlighted the urgent need to develop new, more effective treatment methods, particularly alternative approaches that address the current challenges of antimicrobial resistance.

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Lytic bacteriophages (phages) are viruses that specifically target bacteria and cause their lysis (Kwon et al., 2025). Phage therapy stands out due to its high specificity for pathogens without affecting the normal microbiota, minimal side effects, bactericidal activity even against antibiotic-resistant strains, and ease of administration. These advantages position phages as a promising alternative to traditional antibacterials treatments. The effectiveness of phages against *P. aeruginosa* and *S. pseudintermedius* has been confirmed by several *in vitro* and *in vivo* studies (Morello et al., 2011; Waters et al., 2017; Horiuk et al., 2021a). A study by Kim et al. (2021) demonstrated the significant efficacy of phages pSp-J and pSp-S against biofilm formation of methicillin-resistant *S. pseudintermedius* isolated from dogs. Another study indicated that intranasal administration of phage P3-CHA markedly decreased mortality and bacterial load in the lungs of mice infected with a lethal dose of *P. aeruginosa* CHA (Vieira et al., 2012). Thus, bacteriophages demonstrate strong therapeutic potential, especially in the context of rising antibiotic resistance. Phage therapy has recently shown significant progress in veterinary medicine, particularly in the treatment of companion animals (Stroich and Horiuk, 2024). An experimental study on dogs with external otitis caused by *P. aeruginosa* demonstrated that a specially developed phage cocktail eliminated 90% of the pathogen strains (Kwon et al., 2025). Other studies have confirmed the effectiveness of phages in combating uropathogenic strains of *Escherichia coli*, which could serve as an alternative to antibiotics in the treatment of urinary tract infections (Jończyk-Matysiak et al., 2019). In the case of methicillin-resistant *S. pseudintermedius* strains, phages isolated from dog feces were found to be lytic against the resistant pathogen. However, their effectiveness against susceptible strains was limited (Stefanetti et al., 2024).

Certain limitations hinder the application of phage therapy, including low lytic activity, burst size, stability, and host range (Fitzpatrick et al., 2025). The narrow host range of phages, while beneficial for precision medicine, necessitates prior pathogen identification and complicates immediate application (Tsonos et al., 2014; Horiuk et al., 2021b). The reduction in the titer of lytic phages at the site of inflammation due to environmental factors, such as enzymes and acidity, reduces their ability to interact with target bacteria, which can lead to treatment ineffectiveness (Dąbrowska, 2019). To overcome these limitations, further research is necessary to isolate new, specific phages and investigate their properties, develop phage cocktails to expand the host range, and optimize therapeutic efficacy.

The present study aimed to identify and examine new bacteriophage strains that exhibit lytic activity against the primary pathogens responsible for otitis externa in dogs, namely *P. aeruginosa* and *S. pseudintermedius*, to develop effective new therapeutic strategies.

MATERIALS AND METHODS

Ethical approvals

Ethical approval for conducting experimental studies, as well as consent from animal owners and veterinary staff, was granted by the Ethics and Bioethics Committee of the Faculty of Veterinary Medicine and Animal Husbandry Technologies at Podillia State University, Protocol No. 2, dated March 15, 2023.

Bacterial strains

The bacterial strains used in the present study were previously isolated from dogs with clinical manifestations of external otitis or pyoderma. The study also included strains of microorganisms isolated from the environment of veterinary clinics and their staff. The study was conducted from 2023 to 2024 in veterinary medicine clinics. A total of 496 dogs, representing different ages, genders, and breeds, were studied. The bacterial strains were stored in the working collections at the Faculty of Veterinary Medicine and Animal Husbandry Technologies of the Podillia State University (Kamianets-Podilskyi, Ukraine) and the laboratory of the Ternopil Research Station of the Institute of Veterinary Medicine (Kyiv, Ukraine) at -80°C in 30% glycerol. Bacterial strains isolated from dogs were revived on nutrient medium (MPB) immediately before the experiments. Subsequently, they were subcultured onto solid medium (MPA), a single colony was selected, and the culture was re-incubated in liquid medium. The cultures were incubated at 30°C for 24 hours. Fourteen strains of *P. aeruginosa*, eight strains of *S. pseudintermedius*, and two strains of *S. aureus* were studied, which were isolated from dogs with otitis, as well as two strains of *S. aureus* isolated from humans. These bacterial strains were typical representatives of the microflora isolated from dog and human habitats. Museum strains of *S. aureus* ATCC 25923 and *P. aeruginosa* 27/99 were also utilized as controls.

Samples for bacteriophage isolation

The 132 samples were collected from the ears and feces of dogs with clinical signs of external otitis and used for bacteriophage isolation. Immediately after collection, samples were placed in thermoboxes and sent to the laboratory. The samples were sent for analysis within four hours after collection at a temperature of 4 to 6°C.

Isolation and purification of bacteriophages

The bacterial strains used as hosts for bacteriophage isolation were initially cultured in liquid medium (meat-peptone broth; Pharmaktiv, Ukraine). Samples taken from the ears and feces were eluted with sterile physiological saline, followed by centrifugation at 4°C and 8000 g for 15 minutes to remove impurities. The resulting supernatant was filtered through filters with pore sizes of 0.45 and 0.22 µm. A few drops of the filtrate were applied to solid nutrient agar plates covered with host bacteria. When plaques appeared on the plate, they were carefully detached using a sterile spatula and transferred into Standard Medium (SM) buffer (Pharmaktiv, Ukraine) at 4°C for 12 to 14 hours. The mixture was then combined with a 24-hour culture of host bacteria and incubated for 18 hours at 30°C with shaking at 150 rpm using a thermoshaker. The resulting supernatant was centrifuged and filtered following the same procedure. To determine the morphology of bacteriophage-negative colonies, the double-layer agar method was used. For this, 0.1 mL of phage lysate was mixed with 0.1 mL of a 24-hour bacterial culture and incubated for 10 minutes. This mixture was then combined with 5 ml of semi-solid medium (0.6% agar) and poured onto LB nutrient agar plates (HiMedia, India). After the agar solidified, the plates were incubated for 12-14 hours at 30°C. Carefully selected, well-formed plaques were transferred to SM buffer and incubated at 4°C for 12 hours. This procedure was repeated 3 to 5 times until the plaques achieved uniformity and density, indicating the purification of the phage solution (Qin et al., 2017).

Determination of host range

The host range was assessed using the spot test method as described by Wongyoo et al. (2023). Bacterial strains were spread onto Petri dishes containing solid nutrient agar and allowed to dry. Subsequently, 10 µl of the phage suspension was applied to the surface. The plates were then incubated at 28 to 30°C for 18-24 hours to observe and confirm plaque formation.

Determination of optimal multiplicity of infection

The optimal ratio of bacteriophages to host cells was determined by exposing bacterial suspensions to five different phage concentrations (0.1, 0.01, 0.001, 0.0001, 0.00001). The mixtures were incubated at 28 to 30°C with shaking at 150 rpm for six hours. Following incubation, the cultures were centrifuged at 8000 rpm for 10 minutes. Phage titers in the resulting supernatants were quantified using the double-layer agar method. Control experiments were conducted by culturing bacteria without adding phages in triplicate (Qin et al., 2017).

Determination of the latent period

The mixture of phages and host cells, prepared at the optimal multiplicity of infection, was incubated at room temperature for 15 minutes and then centrifuged as previously described to remove the supernatant. Fresh nutrient broth was added to the pellet, and the culture was incubated at 30°C with shaking at 90 rpm. Samples were collected at designated time intervals, and phage titers were measured using the double-layer agar method. Control experiments were carried out in triplicate using bacterial cultures without the addition of phages.

Determination of the effect of temperature and pH on phage stability

To investigate the thermal stability of the phages, 900 µg of bacterial culture broth was exposed to temperatures ranging from 4°C to 60°C. After the temperature was stabilized, 100 µg of phage was added to each sample and incubated for an hour. Afterward, the mixture was centrifuged, and the phage titer in the supernatant was evaluated using the double-layer agar method (Qin et al., 2017). The stability of phages against pH variations was evaluated by mixing 100 µl of the phage with 900 µl of host bacteria across a pH spectrum from 3 to 13 (with a gradual change of one unit). The mixtures were incubated at 30°C for an hour, then centrifuged, and the phage titer was determined using the double-layer agar method (Wongyoo et al., 2023).

Statistical analysis

The results were expressed as the mean value and standard deviation (SD) based on three measurements. Statistical analysis was performed using analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons, as implemented in SAS software (Version 9.2). A p-value of less than 0.05 was considered statistically significant.

RESULTS

Isolation of *Pseudomonas aeruginosa* phages

Three *P. aeruginosa* phage strains were isolated, including Pa3, Pa7, and Pa15. After multiple purification cycles, the isolated phage strains produced clear, transparent plaques with distinct borders edges. The diameter of the phage

colonies ranged from 0.39 to 0.42 cm, with the smallest plaques formed by phage Pa7 (0.39 cm) and the largest by Pa15 (0.42 cm), which was 1.1 times larger than those of Pa7 ($p < 0.05$). Figure 1 shows the morphology of the negative colonies formed by the isolated phages.

The host range of the bacteriophages isolated from dogs was assessed using different strains of *P. aeruginosa* (Table 1). It was found that phages Pa7 and Pa15 exhibited the best action, as they lysed 92.8% of the bacterial cultures isolated from dogs ($p < 0.05$). In contrast, phage Pa3 was only lytically active against 71.4% of the strains ($p < 0.05$). It is worth noting that none of the phages isolated in the current study were able to lyse the reference strain *P. aeruginosa* 27/99.

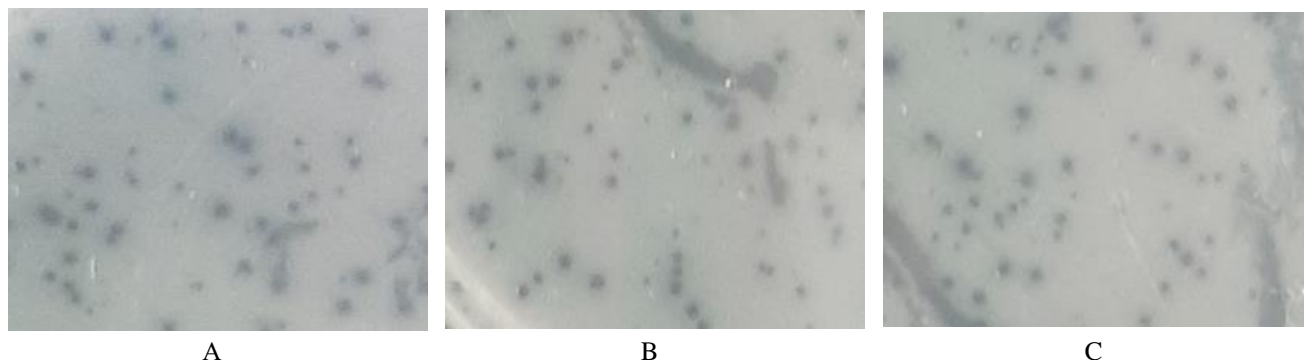


Figure 1. Morphology of negative phage colonies including Pa3 (A), Pa7 (B), Pa15 (C) isolated from dogs' otitis

Table 1. The host range for *Pseudomonas aeruginosa* bacteriophages isolated from dogs

Bacterial strains	Isolated phages		
	Pa3	Pa7	Pa15
<i>P. aeruginosa</i> 3	+	+	+
<i>P. aeruginosa</i> 4	-	+	+
<i>P. aeruginosa</i> 6	+	+	+
<i>P. aeruginosa</i> 7	+	+	+
<i>P. aeruginosa</i> 11	+	-	+
<i>P. aeruginosa</i> 12	+	+	+
<i>P. aeruginosa</i> 15	+	+	+
<i>P. aeruginosa</i> 17	+	+	-
<i>P. aeruginosa</i> 21	+	+	+
<i>P. aeruginosa</i> 26	-	+	+
<i>P. aeruginosa</i> 29	+	+	+
<i>P. aeruginosa</i> 32	+	+	+
<i>P. aeruginosa</i> 46	-	+	+
<i>P. aeruginosa</i> 48	-	+	+
<i>P. aeruginosa</i> ATCC 27853	-	-	-

+: Positive action, -: Absence of positive action

Figure 2 shows the multiplicity of phage infection. The optimal ratio of bacteriophages to bacteria in all three cases was 0.0001. Analysis of the growth curves of phages Pa3, Pa7, and Pa15 (Figure 3) showed that the latent period was up to 30 minutes, with the number of released virions after one hour ranging from 7.92 to 9.06 log PFU/ml. It should be noted that in phages Pa7 and Pa15, the peak size was 1.5 to 1.8 times larger than in Pa3.

All three isolated phages did not decrease their titer within an hour at temperatures ranging from 4 °C to 30 °C (Figure 4). At a temperature of +40°C, the number of bacteriophages was nearly 100 times lower for Pa3 and Pa7, and two times lower for Pa15. Phage Pa7 was not resistant to a temperature of 50°C. The other phages tested in the experiment were able to survive at 50°C, but their number ranged from 3.87 to 7.14 log PFU/ml. A temperature of 60°C had a detrimental effect on all isolated *P. aeruginosa* phages. The phages exhibited optimal lytic activity against their host bacteria within a pH stability range of 5 to 10. Their activity significantly decreased or ceased completely when the pH level dropped to 4 or rose above 11 ($p < 0.05$).

Thus, the current results indicated that the isolated bacteriophages Pa3, Pa7, and Pa15, due to their high lytic activity and stability under varying temperature and pH conditions, are promising candidates for therapeutic applications, particularly in the treatment of otitis in dogs caused by *P. aeruginosa*.

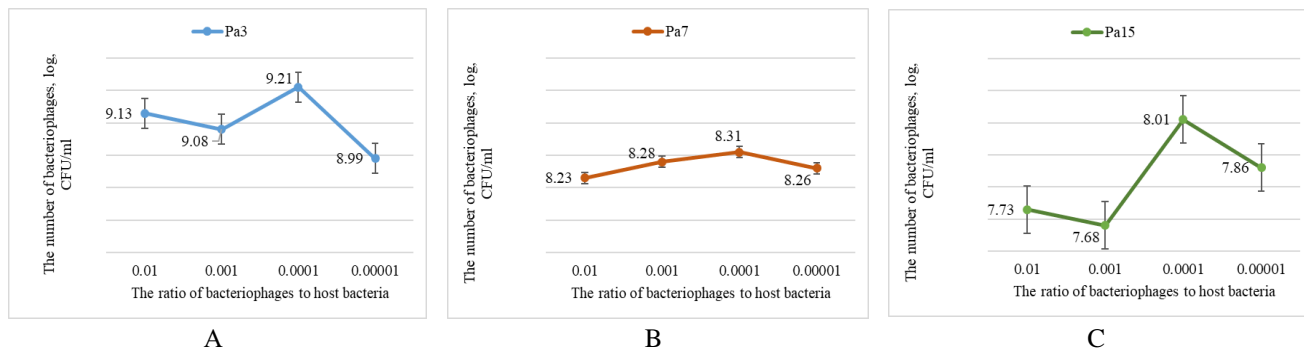


Figure 2. Multiplicity of infection of isolated phages including Pa3 (A), Pa7 (B), and Pa15 (C)

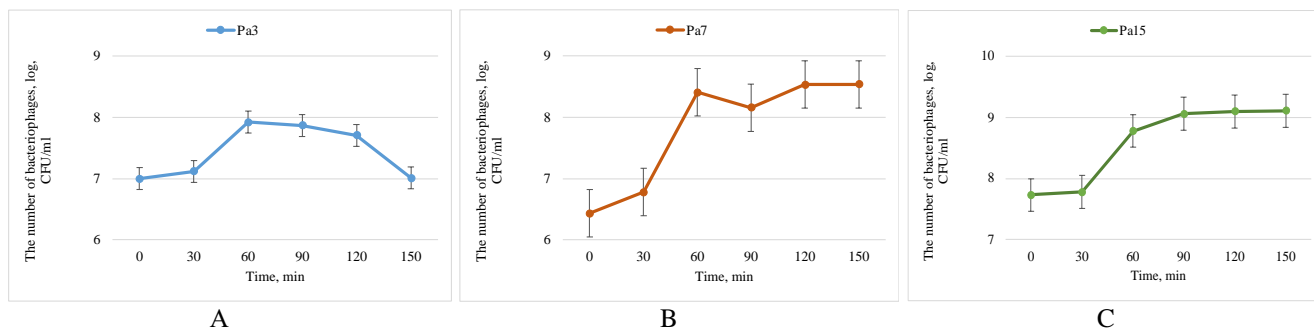


Figure 3. Latent period of phages including Pa3 (A), Pa7 (B), and Pa15 (C)

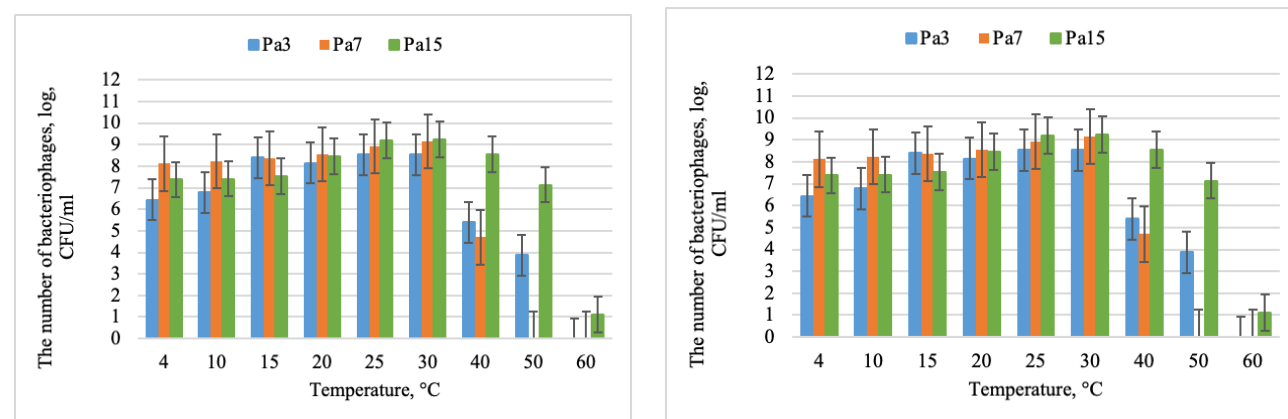


Figure 4. Stability of phages including Pa3, Pa7, Pa15 under temperature and pH fluctuations.

Three strains of *S. pseudintermedius* phages (Sp6, Sp10, Sp17) were isolated in the current study. The isolates of *S. pseudintermedius* formed clear plaques with well-defined edges on nutrient medium, indicating their high lytic activity. The plaque diameter ranged from 0.32 cm (for phage Sp6) to 0.35 cm (for Sp17), which is characteristic of this type of bacteriophage (Figure 5). Table 2 summarizes the host range of *S. pseudintermedius* bacteriophages obtained from canine samples, indicating their lytic activity against different bacterial strains.

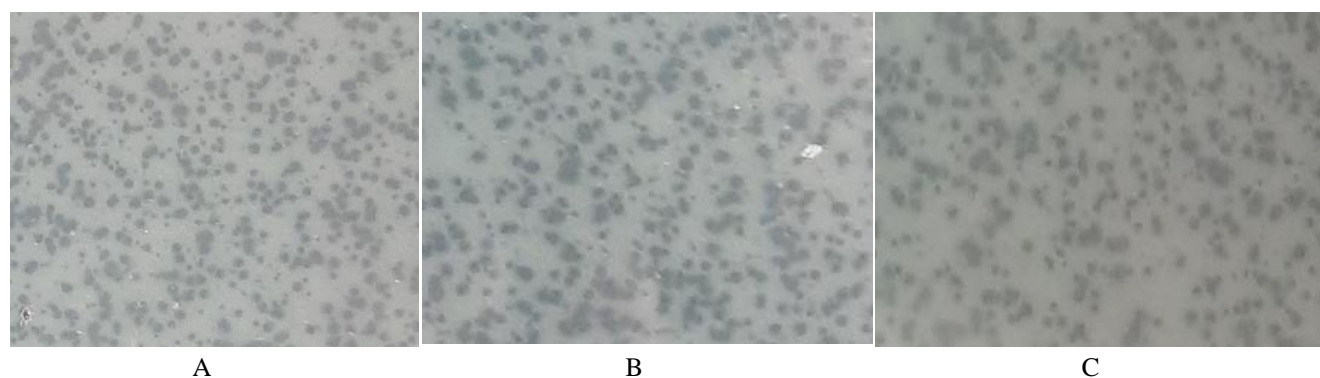


Figure 5. Morphology of negative phage colonies Sp6 (A), Sp10 (B), Sp17 (C) isolated from dogs with otitis

Table 2. The host range for *Staphylococcus pseudintermedius* bacteriophages isolated from dogs

Bacterial strains	Isolated phages		
	Sp6	Sp10	Sp17
<i>S. pseudintermedius</i> 3	+	+	+
<i>S. pseudintermedius</i> 6	+	+	+
<i>S. pseudintermedius</i> 9	-	+	+
<i>S. pseudintermedius</i> 10	+	+	-
<i>S. pseudintermedius</i> 12	+	-	+
<i>S. pseudintermedius</i> 14	+	+	+
<i>S. pseudintermedius</i> 16	+	+	+
<i>S. pseudintermedius</i> 17	+	+	+
<i>S. aureus</i> 54	+	-	-
<i>S. aureus</i> 69	+	+	+
<i>S. aureus</i> 187	-	-	-
<i>S. aureus</i> 192	+	-	-
<i>S. aureus</i> ATCC 25923	-	-	-

+: Positive action, -: Absence of positive action

As shown in Table 2, the isolated bacteriophages demonstrated a significant host range, as they lysed most *Staphylococcus* strains isolated from dogs. Specifically, phages Sp6 and Sp10 exhibited lytic activity against seven strains (87.5%) of *S. pseudintermedius* ($p < 0.05$) and one strain (50%) of *S. aureus* ($p < 0.05$) isolated from dogs. However, they did not lyse *S. aureus* strains isolated from humans. In contrast, Sp17 was more effective in lysing human-derived *S. aureus*, destroying 50% of the cultures ($p < 0.05$) and completely lysing *Staphylococcus* strains isolated from dogs (100%; $p < 0.05$). None of the phages demonstrated activity against the reference *S. aureus* strain, confirming the high specificity of the phages. To ensure maximum therapeutic efficacy, the multiplicity of phage infection caused by Sp6, Sp10, and Sp17 was determined (Figure 6).

The data presented in Figure 6 indicated that the optimal ratio of bacteriophages to *S. pseudintermedius* is 0.0001 for all studied phage strains. The latent period for all three phages ranged from 0 to 30 minutes (Figure 7). After the burst, the number of virions reached 7.56-7.72 log PFU/mL.

The phages maintained their activity over a temperature range of 4 °C to 40 °C (Figure 8). At +50°C, the activity of phages Sp10 and Sp17 decreased 3 to 5 times ($p < 0.05$), while the Sp6 phage strain completely lost its viability. A temperature of 60°C was lethal for all *Staphylococcus* phages. The highest phage activity was observed within a pH range of 5 to 10. Phage activity completely diminished when the pH fell to 4 or rose above 11, emphasizing the significance of this factor in their storage and future use.

The bacteriophages Sp6, Sp10, and Sp17 exhibited high specificity and effectiveness against most *S. pseudintermedius* and *S. aureus* strains isolated from dogs with otitis externa. The highest lytic activity was observed in phages Sp6 and Sp10, which lysed the majority of strains, whereas Sp17 was less effective against *S. aureus*. The phages have a short latent period, high replication activity, and stability under moderate temperature and pH conditions, making them promising candidates for treating bacterial infections in dogs caused by *S. pseudintermedius* and *S. aureus*.

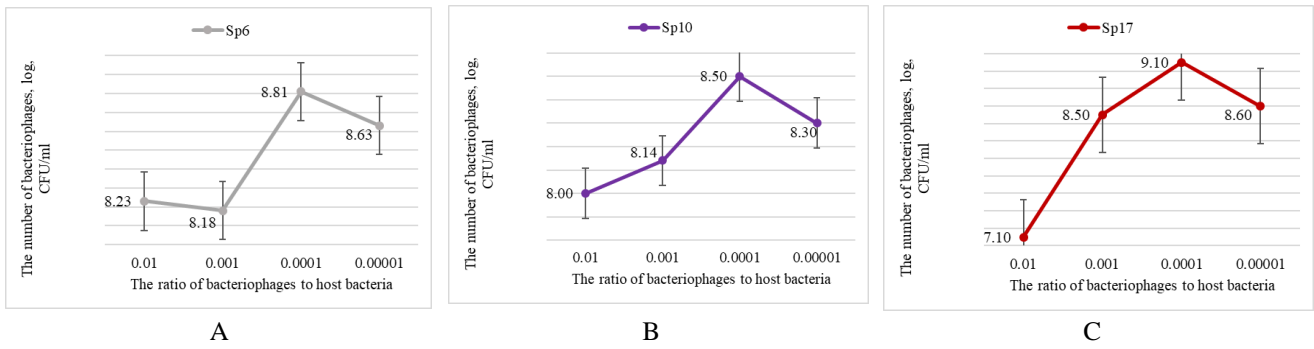


Figure 6. Multiplicity of infection of isolated phages including Sp6 (A), Sp10 (B), Sp17 (C)

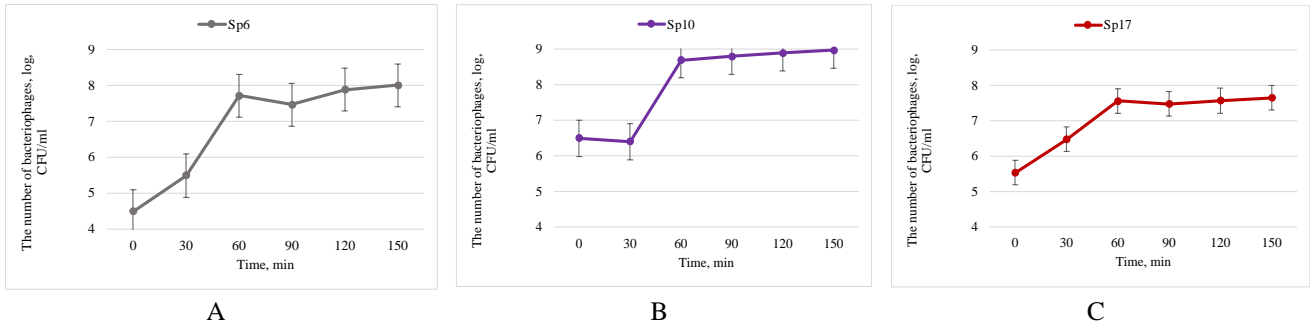


Figure 7. Latent period of phages including Sp6 (A), Sp10 (B), Sp17 (C)

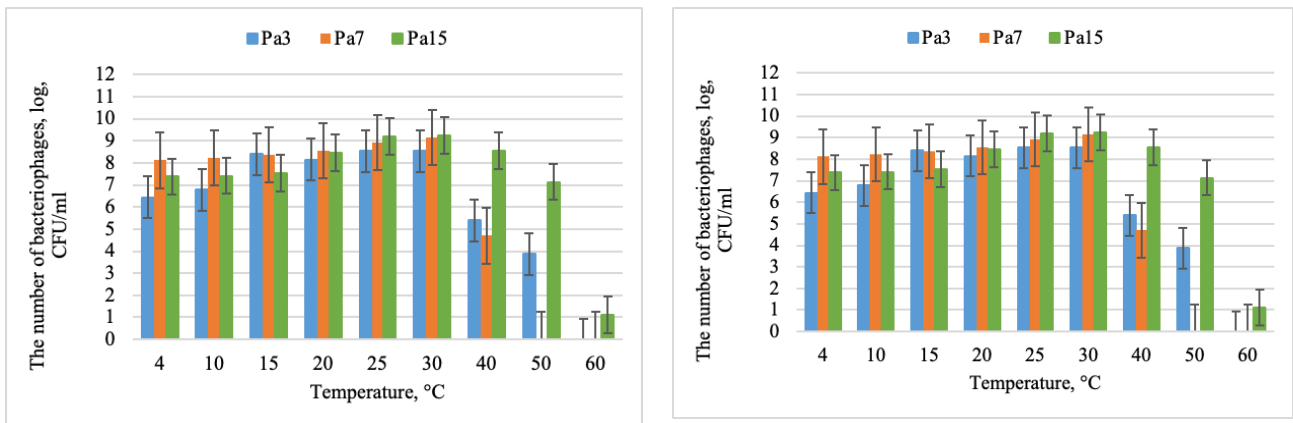


Figure 8. Stability of phages including Sp6, Sp10, Sp17 under temperature and pH fluctuations

DISCUSSION

Antibiotic-resistant *Pseudomonas* spp. and *Staphylococcus* spp., which belong to the so-called ESKAPE pathogens (Rice, 2008; Choi et al., 2024; Pipitò et al., 2025), can cause severe infectious diseases. *Pseudomonas aeruginosa* and *S. pseudintermedius* are frequently associated with the development of dermatological conditions in dogs, including otitis externa. One of the most promising approaches for eradicating strains of these multidrug-resistant bacteria is phage therapy (Eiferman et al., 2025; Luo et al., 2025). However, a major challenge of this therapeutic approach is the high specificity of bacteriophages, requiring their isolation from the site of infection and thorough investigation for safe application.

The plaques formed by the phages typically exhibited well-defined edges and a clear center, with sizes characteristic of these phages types. Negative colonies of varying sizes can result from the incomplete purification of phage lysates and the infection of host cells at different stages of bacterial growth cycles (Storms and Sauvageau, 2015; Chu et al., 2025).

The host range and lytic potential of phage strains are key criteria for their use as antibacterial agents. The current results demonstrated a relatively narrow host range for the isolated *P. aeruginosa* phages, indicating their specificity for particular microbial strains isolated from dogs. This phenomenon is attributed to the presence of highly specific tail spike proteins (Yuan et al., 2019; Jiang et al., 2025). However, it was found that phages Sp6, Sp10, and Sp17 exhibited lytic

activity not only against *S. pseudintermedius* but also against *S. aureus*. The host range of staphylococcal phages may be broader, encompassing relevant strains, species, and even genera, as some phages can transduce DNA across species boundaries. Additionally, phages that infect *Staphylococcus* species can bind to glycopolymers of the wall teichoic acid, which exhibit diverse structures and glycosylation patterns (Braunstein et al., 2024). For example, the teichoic acid glycosylation in the opportunistic pathogen *Staphylococcus epidermidis* can also influence other coagulase-negative *Staphylococcus* species (Chu et al., 2025). The exact mechanism by which phages recognize related host cells remains unclear. However, in environments with diverse microbial populations, these characteristics offer a considerable advantage in treating animals by targeting a wider range of pathogens (Wongyoo et al., 2023; Braunstein et al., 2024).

All isolated phages of *P. aeruginosa* (Pa3, Pa7, Pa15) and *S. pseudintermedius* (Sp6, Sp10, Sp17) exhibited a short latent period (up to 30 minutes) and a significant burst size, indicating their ability for rapid replication and the release of a large number of virions. These characteristics suggest their potential as effective agents for host cell lysis (Jończyk-Matysiak et al., 2019; Stroich and Horiuk, 2024; Matheus et al., 2025).

Pseudomonas aeruginosa and *S. pseudintermedius* pathogens can be isolated from different parts of an animal's body or its surrounding environment (Mocherniuk et al., 2022; Stefanetti et al., 2024). However, for therapeutic applications, phages should remain stable in the target environment. The bacteriophages demonstrated resistance to different temperatures and pH conditions during the experiment. The number of virions remained stable within the temperature range from 4 to 40°C and pH levels between 5 and 10. These traits suggested that these phage strains could be effective for application on the skin or in the ears of dogs. Additionally, these aspects must be considered when creating application methods and storage conditions for bacteriophage-based therapies. Although phage therapy appears promising for veterinary use, extensive clinical trials are necessary to validate its effectiveness and safety. Additionally, obtaining regulatory approval from veterinary authorities will be critical prior to its broader application in animal care.

CONCLUSION

The study indicated that bacteriophages isolated for the treatment of infections caused by *P. aeruginosa* and *S. pseudintermedius* have great potential as an alternative method for combating multidrug-resistant bacteria. This is particularly relevant in veterinary medicine, especially for treating dermatological diseases in dogs, such as otitis externa. The isolated bacteriophages demonstrated high specificity for the causative agents of otitis in dogs, a crucial characteristic for their practical application. They have a short latent period, release a large number of virions, and exhibit resistance to temperatures ranging from 4 to 40°C and a pH range of 5 to 10, making them suitable for treating ear infections. Thus, phage therapy indicated significant potential as an alternative to antibiotics in the fight against resistant bacteria. However, additional studies are needed to evaluate its safety and efficacy before its clinical implementation.

DECLARATIONS

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Authors' contributions

Yuliia Horiuk, Volodymyr Tsymbalisty, and Mykola Kukhtyn initiated and implemented the research concept, designed the experimental part, analyzed the obtained data, and carried out the scientific and stylistic editing of the manuscript. Viktor Horiuk, Vladyslav Kozhyn, and Vitaly Chuhno collected the biological material, conducted a series of experiments, and made a significant contribution to the practical implementation of the research component. All authors actively participated in discussing the research findings, reviewed the final version of the manuscript, and approved it for publication.

Competing interests

The authors declared no conflict of interest.

Funding

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Ethical considerations

Plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy have been checked by the authors.

Availability of data and materials

The original data presented in the study are included in the article. For inquiries, please contact the corresponding author.

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