Isolation, Characterization and in vitro Evaluation of Specific Bacteriophages Targeting Extensive Drug Resistance Strains of Pseudomonas aeruginosa Isolated from Septic Burn Wounds

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ABSTRACT

Background: Antibiotic resistant bacteria and various infections caused by them especially extensive drug resistance (XDR) strains and worrying statistics of mortality due to these strains and also the lack of a clear vision for development and production of new effective antibiotics have made the necessity of using alternative therapies more apparent.

Materials and Methods: In this study, specific phages affecting the Pseudomonas aeruginosa XDR strain were extracted from hospital wastewater and their laboratory characteristics along with lysis effect on 40 XDR strains of P. aeruginosa were investigated.

Results: The results indicated that three isolated phages (PaB1, PaBa2 and PaBa3) belonged to the Myoviridae and Podoviridae families and were specific to Pseudomonas aeruginosa strains. More than 98% of phages absorbed their host in less than 10 minutes (Adsorption time <10 min) and completed their lytic cycle after 40 minutes (latent time = 40 min). Burst size of PaBa1, PaBa2 and PaBa3 was 240, 250 and 220 pfu/cell, respectively. PaBa1 lysed 62.5% of the XDR strains with the highest efficiency. The three Phage cocktail was effective against 67.5% of the studied strains.

Conclusion: The results of this study indicate the significant potential of these phages for therapeutic use and prophylaxis of infections caused by this bacterium.

Keywords: Bacteriophage; Pseudomonas aeruginosa; Drug resistance; Antibiotic resistance

INTRODUCTION

Pseudomonas aeruginosa as an opportunistic bacterium can cause serious infections in people with deficient immune systems. This group of patients includes people with severe burn wounds, patients with cystic fibrosis, cancer patients and patients suffering from acquired immunodeficiency syndrome [1-3]. Various studies have reported a significant prevalence of
infection and mortality caused by this bacterium in these patients [3-5]. According to data from National Nosocomial Infections Surveillance System, \textit{P. aeruginosa} is the second most common organism isolated in nosocomial pneumonia and the third most common pathogen associated with all nosocomial infections, accounting for 10.1\% of all hospital acquired infections and is associated with a high mortality rate [6, 7].

Innate and acquired resistance to most antibiotic agents, has exacerbated treatment of \textit{P. aeruginosa} infections as well as the presence of various virulence factors, including biofilm production, as Studies in burn patients with \textit{P. aeruginosa} sepsis have reported a mortality rate of over 75.0\%, while incurable infections have been predominant in patients infected with this bacterium [8].

Multiple drug resistance, first reported in 1970, has become a serious threat to the treatment of infections caused by these bacteria and an obstacle to advancing infection control goals worldwide [9].

From this point of view, and through reports from different countries that have reported resistant \textit{P. aeruginosa} isolates repeatedly as a cause of nosocomial infections and the lack of a clear vision for the development of new antibiotics to treat infections caused by these types of bacteria has motivated researchers to use alternative therapies. Accordingly, bacteriophages have been considered as one of the alternative therapies. Bacteriophages, commonly referred to as phages, were first discovered and introduced independently by two scientists, Frederick Twort and Felix d'Herelle in 1915 and 1917. Phages are one of the most abundant and common organisms on Earth, known as natural controllers of bacteria. Based on their life cycle, phages are divided into two classes; lytic and lysogenic phages. Due to the fact that lytic phages are very specific and attack and kill only certain bacterial species regardless of their antibiotic susceptibility, the possibility of their destructive effect on human microbial flora and other antibiotic-related side effects reduces. Since that phages are not able to infect eukaryotic cells, phage therapy is suitable for use in humans but, it must be characterized for some laboratory characteristics, like; morphological properties, host range, latent time and burst size before clinical use.

In this study, specific phages affecting extensive drug resistance (XDR) strain of \textit{P. aeruginosa} isolated from burn wound infection were extracted from hospital sewage sources and morphological characteristics, host range, latent time and burst size were determined.

\textbf{MATERIALS AND METHODS}

\textbf{1. Bacterial strain isolation and characterization}

XDR strain of \textit{P. aeruginosa} was isolated from an infected burn wound of a patient admitted to burn hospital, Tabriz-Iran. Bacterial isolate were identified using standard conventional bacteriological tests including Gram staining, colony morphology, pigment production, oxidase test and use of differential media. Antibiotic resistance pattern of isolated strain against gentamicin (GEN: 10\( \mu \)g), amikacin (AMK: 30\( \mu \)g), ceftazidime (CAZ: 30\( \mu \)g), cefepime (FEP: 30\( \mu \)g), imipenem (10\( \mu \)g), meropenem (MEM: 10\( \mu \)g), ciprofloxacin (CIP: 5\( \mu \)g) and piperacillin/tazobactam (TZP: 30+6\( \mu \)g) antibiotics (Rosco, Høje-Taastrup, Denmark) were evaluated by disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) standards [10]. To determine the susceptibility and resistance of bacterial isolate
against the studied antibiotics, the ranges stated in the CLSI instructions were used. This isolated strain was used as a host to extract specific lytic phage.

2. Ethics statement
This research was approved by the Research Ethics Committee of Qazvin University of Medical Sciences with the ID: IR.QUMS.REC.1398.402. Also all isolates were obtained from bacterial bank of Infectious and Tropical Diseases Research Center (Tabriz, Iran) and patient information was kept confidential.

3. Wastewater sample collection and Bacteriophages isolation
Cerveny et al. enrichment method [6] with slight modifications was used to isolate specific phage against the host strain. For this purpose, several untreated wastewaters of different hospitals were sampled as phage presence sites. After collection, the samples were immediately transferred to the laboratory and examined for the presence of specific phages. Briefly, the collected samples were centrifuged (4,000 g, 10 min; Hettic- Föhrenstraße, Tuttlingen, Germany) to separate bacteria and wastes and supernatant was filtered through a 0.45 µ syringe filter (Jet-Biofil, Alicante, Spain). To isolate the phage, 2 mL of the exponential phase culture of the host bacterium (XDR strain) was mixed with 20 mL of Luria-Bertani (LB; Himedia, Mumbai, India) broth (2X) and 20 mL of the filtered waste sample and incubated overnight at 37 °C by shaking at 100 RPM (Hettic).

The culture was then centrifuged (4,000 g, 4 min) to precipitate the bacteria and supernatant was filtered through a 0.22 µ syringe filter (Jet-Biofil) and investigated for the presence of specific phage.

4. Spot test and plaque assay
The presence of lytic phage was investigated by plaque-formation after spotting 10 µl of the filtered suspension onto the top layer (soft LB agar, 0.7% agar; Himedia) of double layer agar (DLA) containing the host strain poured on the bottom layer (LB 1.5% agar; Himedia) [11].

Plaque assay was used to determination of phage titer by employing double agar overlay technique. Briefly, phage suspension was serially diluted. 100 µl diluted phage and 100 µl host bacterium (10^8 CFU mL^-1) were mixed and incubated at 37°C for 10 min for phage adsorption, then mixture was added to 5.0 mL molten soft top agar (1mM cacl2, 0.7% w/v agar at 45°C) and poured quickly on top of the solidified LB agar plate (1.5% w/v agar) [12]. The plates were incubated overnight at 37°C and phage titer was calculated.

5. Phage Purification
Purification of isolated phages was performed by the method described by Sambrook et al. [13]. Briefly: A good isolated plaque was removed from each plate by a sterile Pasteur pipette and poured into a tube containing 5 mL LB broth, 50 µl exponential phase culture of the host bacterium were added to the tube containing the plaque and incubated at 37°C for 24 h with shaking (150 RPM). Next day lysate centrifuged (10,000g, 10 min, 4°C) and filter sterilized through 0.22 µ filter and treated with 1.0% (v/v) chloroform (Himedia) to remove bacterial contamination, the filtrate lysate was evaluated for forming homogeneous plaques by double-layer method (DLA).
6. Bacteriophages concentration

Polyethylene glycol (PEG) sedimentation method was used for concentration of purified phage [13]. For this purpose, 0.58 g of NaCl (Himedia) was added to 10 mL of phage suspension (final concentration: 1M) and stirred until completely dissolved and placed in ice for an hour, suspension was then centrifuged (10,000 g, 4°C, 10 min), and 10.0% (w/v) of PEG 8,000 (Himedia) added to supernatant and dissolved, the suspension incubated at 4 °C for an hour. Bacteriophage precipitation was performed by centrifugation (10,000 g, 4°C, 10 min), supernatant removed and precipitate was dissolved in 1 mL SM buffer (100 Mm NaCl, 10 mM MgSO4, 10 Mm Tris-HCL; pH 7.5) and incubated at room temperature for one hour. To remove PEG and cell debris, an equal volume of chloroform (Himedia) was added to the suspension and vortexed 30 second and centrifuged (5,000g, 4°C, 15 min), the aqueous phase containing bacteriophages was removed.

7. Host range determination

Forty XDR P. aeruginosa clinical isolates (all resistant to GEN, AMK, CAZ, FEP, IPM, MEM, CIP and TZP) obtained from burn patients (kindly provided from Infectious and Tropical Diseases Research Center, Tabriz, Iran) were used to determine host range of isolated phages. Sensitivity of bacterial strains was assessed by spot test. In summary 2 - 3 colony of isolated strains dissolved in LB broth (5 mL) and incubated overnight at 37 °C with shaking (120 RPM), then 100 µl of bacterial culture was added to 5 mL of soft LB agar (45°C, 0.7% agar) and overlaid onto plates containing 1.5% LB agar after mixing. After solidification (10 minutes), 10 µl of isolated phages were spotted on the top layer. The plates were incubated overnight at 37°C until the formation of lysis zones. Lytic activity of extracted phages was also tested on Acinetobacter baumannii (ATCC 19606) (ATCC, Manassas, VA, USA), Escherichia coli (ATCC25922), Staphylococcus aureus (ATCC2392), Enterococcus faecalis (ATCC29212), Shigella flexneri (ATCC12022).

8. TEM analysis

To observe the morphology of the isolated phages, a drop of concentrated phage (10¹⁰ - 10¹² pfu mL⁻¹) was placed on a carbon-coated copper acid grid for 4 minutes, then additional amount was blotted with filter paper and stained negatively with 2.0% (w/v) uranyl acetate (pH 7.0). Prepared grid was examined by transmission electron microscope at accelerating voltage of 90 kv (Zeiss LEO 906 TEM, Tabriz, Iran).

9. Phage adsorption assay

Phage adsorption test was performed by the method described by Adam [12]. Briefly, prepared phage suspension [Multiplicity of Infection (MOI): 0.1] was added to fresh host bacterial culture and incubated at 37°C for 15 minutes. At one minute intervals (up to 15 minutes) 100 µl were taken from the solution and the titration of infectious free phages was calculated by DLA method.

10. One step growth curve

The method described by Pajunen et al. [14] were used to investigate One step growth curve with slight modifications. Isolated phage with MOI: 0.1 was added to exponential phase culture of the host bacterium and incubated for 10 minutes at room temperature, then the suspension was centrifuged at 4°C for 10 minutes at 10,000 g. The precipitate containing infected cells was dissolved in 50 mL LB broth and incubated at room temperature. Samples were taken at 5 minute intervals for 90 minutes and latent time (which is defined as the time between absorption and the beginning of the first burst) and burst size was determined by DLA method, all experiments were repeated in triplicate.
11. Thermal stability
To determine the temperature stability of the isolated phages, aliquots (500 µl) of high concentrated phage (1 - 2 × 10^9 pfu mL^-1) were incubated for one hour at 4°C, 25°C, 37°C, 50°C, 60°C, 70°C, 80°C and 90°C (pH 7) and titrated afterward [15].

12. pH stability
To measure the pH tolerance range of isolated phages, 100 µl of phage suspension (10^9 pfu mL^-1) was mixed with 900 µl of SM buffer at different pH (3 - 11) and incubated for 10 hours at 37°C. After incubation, the samples were titrated by DLA method (1 M HCl and 1 M NaOH were used to prepare SM buffer with different pH values) [15].

13. In vitro bactericidal effect of isolated phages
To investigate bactericidal effect of extracted phages against XDR- *Pseudomonas* (host bacteria) an aliquot of overnight culture of host bacteria was added into fresh LB broth and incubated for 6 h at 37 °C with shaking (150 RPM). Pelleted bacteria resuspended in Phosphate-buffered saline (PBS), to an optical density of 0.15 at 600 nm (∼2 × 10^8 CFU mL^-1). Isolated phages with different concentration (2 × 10^7, 2 × 10^8 and 2 × 10^9 pfu mL^-1) and PBS (control) were added to the prepared bacterial suspension and incubated at 37°C with shaking (150 RPM). For 12 h period, bacterial growth was monitored by measuring the OD 600 at a 30 min interval [15].

RESULTS

1. Bacteria and Bacteriophage isolation
*P. aeruginosa* XDR strain (resistant to GM, AN, CAZ, FEP, IPM, MEN, CIP and PTZ) isolated from burn patient was used as host to isolate lytic phage. Extracted phages were screened for lytic activity based on clear plaque formation. Three phages with the ability to form clear plaques (1 - 3 mm in diameter) were characterized and named as PaBa1, PaBa2, PaBa3 (*P. aeruginosa* bacteriophage 1, 2 and 3).

2. Characterization of isolated phages
Adsorption time, Latent period and Burst size of isolated phages were investigated after purification. Adsorption time (the time required for bacteriophage to be absorbed into host bacterial cells as first stage of phage infection) varied from 8 - 10 minutes as shown in Table 1. According to results obtained from DLA test More than 98.0% of phages (all three phages) were absorbed to host bacteria cell before 10 minutes (Fig. 1A).

Based on one step growth curve test, latent period which is defined as the time between absorption and the beginning of the first burst was determined as 35 - 40 minutes. The burst size of three phages was different and determined the minimum for PaBa3 and the maximum for PaBa2 (Fig. 1B). The differences in these characteristics confirm that these three phages are different.

| Phages | Adsorption time (min) | Latent period (min) | Burst size (pfu per bacterial cell) |
|--------|-----------------------|---------------------|------------------------------------|
| PaBa1  | 9.00 ± 1.00           | 38.3 ± 2.9          | 238 ± 6.0                           |
| PaBa2  | 8.66 ± 1.15           | 36.7 ± 2.9          | 246 ± 7.5                           |
| PaBa3  | 8.33 ± 0.57           | 38.3 ± 2.9          | 217 ± 6.0                           |

The values are meant ± standard deviation (n = 3).
XDR, extensive drug resistance.
3. Host range analysis
Phage host range was determined using 40 XDR strains isolated from burn patients as well as standard strains of *A. baumannii* (ATCC 19606), *E. coli* (ATCC25922), *S. aureus* (ATCC2392), *E. faecalis* (ATCC29212) and *S. flexneri* (ATCC12022) alone and in combination of three Phage (cocktail). Of 40 *P. aeruginosa* isolates 25 (62.5%), 14 (35.0%) and 11 (27.5%) isolates were sensitive to PaBa1, PaBa2 and PaBa3 respectively, and showed clear zones in the spot test. Phage cocktail was more effective than alone phages and lysed 67.5% of the isolates (Supplementary Table 1). None of the phages as well as phage cocktail was effective on mentioned different used bacteria including *A. baumannii, E. coli, S. aureus, E. faecalis* and *S. flexneri*.

4. Bacteriophage titer determination
DLA method was used for determination of Phages titration. Plaques were counted after overnight incubation at 37 °C and titer of the isolated phages were determined to be (1.5 ± 0.5 × 10**14** pfu mL**−1**), (2 ± 0.3 × 10**16** pfu mL**−1**) and (2.3 ± 0.6 × 10**16** pfu mL**−1**) for PaBa1, PaBa2, PaBa3 respectively.

5. Phage morphology
Based on electron microscope images (Fig. 2), the PaBa1 and PaBa2 phages are belonging to the Myoviridae family and the phage PaBa3 belongs to the Podoviridae family (Table 2). According to International Committee on Taxonomy of Viruses (ICTV) these families are belong to caudovirales order and include viruses with linear double-stranded DNA [16].

![Figure 1. (A) Adsorption curve of extracted phages. More than 98.0% of phages (all three phages) were absorbed to host bacteria cell before 10 minutes. (B) One step growth curve of isolated phages. Latent period was determined for PaBa1 and PaBa3 as 38.3 ± 2.9 (min) and 36.7 ± 2.9 (min) for PaBa2. The burst size of three phages was determined 238 ± 6 (pfu mL**−1**), 246 ± 7.5 (pfu mL**−1**) and 217 ± 6 (pfu mL**−1**) for PaBa1, PaBa2, PaBa3 respectively.](https://icjournal.org)

![Figure 2. Electron micrograph of isolated phages (A) PaBa1 (B) PaBa2 (C) PaBa3.](https://icjournal.org)
6. Thermal and pH stability

Temperature tolerance test showed that all three isolated phages were 100.0% active in the temperature range of 4 - 50°C. At 70°C, approximately 50.0% (50 ± 5) of the phages formed clear plaques, and at 80°C no plaques formed (Fig. 3A).

pH stability test showed that the isolated phages were sensitive to highly acidic and alkaline conditions (pH ≤3 or pH >9). Best performance of phages was determined to be between pH: 6 - 8 (Fig. 3B).

7. In vitro bactericidal effect

Monitoring of host bacterial growth in presence of different concentration of isolated phages showed that the MOI: 10 was more effective (Fig. 4). The optical density at 600 nm was decreased to near zero in all treated (phage introduced) groups after four hours while it constantly raised for PBS (control) group. These differences indicate effective lytic activity of isolated phages against host bacteria in vitro.

DISCUSSION

The emergence of resistant bacteria and various infections caused by them is a challenging threat to public health, so that according to the United Nations, by 2050 we will have about 10 million deaths due to incurable infections caused by these bacteria [17]. The increasing prevalence of multidrug resistant (MDR) and XDR strains of \textit{P. aeruginosa} especially in developing countries, and the inclusion of these resistant strains in the WHO list of critical

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### Table 2. Phages electron microscopic characteristic

| Phages | Head Capsid | Diameter | Tail length | Family       | Order       |
|--------|-------------|----------|-------------|--------------|-------------|
| PaBa1  | Icosahedral | 109 ± 5  nm | 164 ± 5 nm  | Myoviridae   | Caudovirales |
| PaBa2  | Icosahedral | 97 ± 5  nm  | 98 ± 5 nm   | Myoviridae   | Caudovirales |
| PaBa3  | Icosahedral | 132 ± 5 nm  | 19 ± 2 nm   | Podoviridae  | Caudovirales |

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**Figure 3.** Stability of the isolated phages under different pH (A) and temperature (B).
priority pathogens for research and production of new antibacterial agents [18, 19], the use of lytic phages has been considered by researchers. Based on this, extraction and laboratory identification of phages will have a significant impact on the proper use of these natural antibacterial agents as a successful alternative therapy.

According to various studies, wastewater sources are the most suitable sites for the isolation of phages affecting pathogenic bacteria [20, 21]. Identifying the suitable sites for phage separation will make them more cost-effective with saving in time and initial cost of extraction. In this study, in spite of numerous sampling of wastewater sources of different hospitals as well as municipal wastewater, specific phages of the host strain were extracted from the wastewater source of the hospital related to the host strain. Therefore, the results of this study confirm the wastewater sources of host strains as the most likely place for specific phage extraction.

Transmission electron microscopy (TEM) images showed that the extracted phages were morphologically different and belonged to the myoviride and podoviride families. According to various studies, more than 94.0% of *Pseudomonas*-specific phages are from the caudovirales class and belong to three families: *Myoviridae*, *Siphoviridae* and *Podoviridae* [6, 21]. The *Caudovirales* class are double-stranded DNA viruses that is divided into three families based on tail morphology: *Podoviridae* (short and non-contractile), *Myoviridae* (long and contractile) and *Siphoviridae* (long, flexible but non-contractile) [22, 23].

In contrast to the results of most studies, Samadi et al. [24] extracted two specific phages effective on carbapenem-resistant *P. aeruginosa* belonging to the families *Inoviridae* and *Plasmaviridae*. In another study, Shokri et al. reported the phages isolated in their study, belonging to the families *Cystoviridae*, *Leviviridae* and *Inoviridae* [18]. These differences indicate the high diversity of phages affecting resistant strains of *P. aeruginosa* and the completion of the necessary information requires more and extensive studies in different geographical areas.

According to the results of various studies, phages ability to survive at relatively high temperatures and strong acidic and alkaline environmental conditions are unique features of bacteriophages as biological controllers of bacteria. This feature makes it possible to use of phages as a complementary to the physicochemical methods of wastewater treatment to reduce the number of pathogenic bacteria. whilst, the results of studies have shown the possibility of reusing wastewater containing bacteria after treatment with specific phages in irrigation of green space or release to nature [25, 26].
Based on the results of this part of the study and due to the widespread presence of host bacteria in different environments, it is possible to use these phages to reduce and eliminate host bacteria in sewage and various hospital locations in addition to clinical uses.

The adsorption time assay determined that more than 98.0% of phages are adsorbed to their specific host in less than 10 minutes. The adsorption time directly affect the completion time of phage life cycle and the efficiency of phages in lysis of infectious bacteria and recovery of infection. Different results can be due to variables that can affect the adsorption time and rate of phages into the surface of host bacteria such as temperature, pH of environment, host bacteria density, concentration of MgSO4 in the growth medium, and the initial incubation time of phages with the host bacteria [27, 28]. Accordingly our results showed that isolated phages are suitable for this purpose.

No need to use repeated doses due to self-reproduction nature is another advantage of phage therapy compared to antibiotics. Accordingly, the use of phages with low latent time and high burst size due to creating the appropriate population required for phage therapy with less propagation makes it possible to use these specific phages in lower concentrations. Based on this, the properties of the extracted phages confirm the possibility of their clinical use.

Infection and specific lysis of host bacteria by lytic phages and no effect on other bacteria in the environment are other advantages of clinical use of phages compared to antibiotics, but, due to the existing regulations, all phages must undergo extensive clinical trials before being presented as antibacterial agents in humans [29], the use of broad-spectrum phages reduces the number of phages required to treat infections caused by pathogenic species, thereby reducing the cost of clinical trials. So, phages with a broad host range are ideal for clinical use in the treatment of bacterial infections. In this study, the PaBa1 was the most effective among the extracted phages by lysing 62.5% of the studied XDR strains. It is notable that above 50.0% mortality rate has been reported for patients infected with XDR pathogens [30], so the specificity of extracted phages in this study along with their wide range of effects on XDR strains shows the importance of preventive and therapeutic use of these phages.

Resistance of pathogenic bacteria to effective phages is one of the main problems of phage therapy. Bacteria become resistant to phages through various mechanisms [31]. Change in phage receptor through mutation and non-recognition of host is one of the most important mechanisms of resistance, accordingly, the use of phage cocktails is one of the simplest ways to prevent mutant strains [32], whilst, in contrast bacterial defense systems, antagonistic mechanisms in phages have developed [33, 34]. The use of phage cocktail due to the identification of different surface receptors of bacteria by the phages in the cocktail and the intolerance of bacteria to change all receptors, while expanding the lysis range of phages, prevents the spread of phage resistant mutants [30, 32]. The results of various studies confirm the greater efficiency of phage cocktails compared to single use of phages [18, 35, 36]. In this study, phage cocktail by lysing 67.5% of the studied strains had a wider effect compared to single phages. In the study of Arumugan et al. the infectivity of single phages, AP025 and AP006 was 39 and 30.0% respectively, while phage cocktail showed a significant increase in the infectivity rate, so that 52.9% and 62.7% of studied strains lysed by two and five-phage cocktails respectively [21].

The effectiveness of phages has been demonstrated in various studies in the treatment of specific infections in humans as well as in animal models compared to antibiotics [37, 38].
Various studies on phage therapy of burn wound infections caused by *P. aeruginosa* and other pathogens suggest the fact that phages have the potential to control burn wound infections [8, 36, 39]. In 2007, Catherine and colleagues demonstrated the therapeutic effect of a specific phage cocktail on burn wound infection caused by *P. aeruginosa*. In their study after burn wound in an animal model of a mouse and subcutaneous injection of a lethal dose of the *P. aeruginosa*, which led to sepsis and eventually death, injecting a single dose of phage cocktail showed that the survival rate in the phage-treated group increased from 6.0% to 87.0% compared to the control group [8]. In 2012, Fukuda et al. showed the therapeutic effects of *P. aeruginosa* specific phage in the treatment of ocular keratitis caused by this bacterium in an animal model. Keratitis caused by this bacterium made corneal perforation in all control group members, while no corneal perforation was observed in the phage group [40]. The results of these studies indicate the possibility of therapeutic use of lytic phages in human infections.

In conclusion, phage therapy has introduced a significant field in modern science; its application can be diverse and useful in various fields such as environmental, agricultural, industrial, pharmaceutical and medical. Phage therapy due to its unique advantages such as: specificity to host bacteria without affecting eukaryotic cells, possibility of rapid preparation against newly emerging resistant bacterial strains due to high diversity and presence in different environments, low extraction cost and rapid effect on host bacterium is a very suitable option for therapeutic use as well as preventing the appearance of superbugs. For this purpose, performing detailed *in vivo* studies and genetic identification on *in vitro* identified phages and the formation of more complete databases is necessary.

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**SUPPLEMENTARY MATERIAL**

**Supplementary Table 1**

Lytic spectrum of isolated phages and cocktail on the *Pseudomonas aeruginosa* panel

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