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ORIGINAL ARTICLE

Infected burn wound healing using Hydroxy-propyl-methyl cellulose gel containing bacteriophages against *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*

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ABSTRACT

Background and Objectives: *Pseudomonas aeruginosa (P. aeruginosa)* and *Klebsiella pneumoniae (K. pneumoniae)* are the two leading bacterial strains involved in wound infections. These bacteria have developed broad resistance to antibiotics, which has complicated their eradication. Additionally, the formation of a polymicrobial infection poses additional problems. Among alternative or complementary options, bacteriophages, viruses that parasitize bacterial hosts, have been promising.

Materials and Methods: In this research work, bacteriophages' therapeutic effects against *P. aeruginosa*- and *K. pneumoniae*-infected burn wounds were studied. The infectious burn wound model was performed on Balb/C male mice, aged six weeks and weighing 25 ± 5 gr. The effects of the Hydroxy-propyl-methyl cellulose (HPMC) gel containing phage were investigated compared to gentamicin. All of these actions were performed in separate groups for each bacteria and mixed group of bacteria (to test multi-bacterial infections treating) and the result were compared.

Results: Phages appear to be effective in gel forms. Pathologic samples of different groups confirmed therapeutic results of phages. These results at the microscopic level indicated the recovery of the tissue and the removal of the infection.

Conclusion: The results of this study indicate that lytic phages are powerful biological tools for the treatment of bacterial infections in burn wounds, which can be considered as one of the alternatives for drug-resistant bacterial species and the high costs of antibiotics; though further animal and trial studies are needed. Meanwhile, the complications due to their widespread use in humans should be investigated in more details.

Keywords: Bacteriophages; Antibiotic resistance; Hydroxy-propyl-methyl cellulose

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INTRODUCTION

Burn injuries are one of the major public health concerns, occurring in people of all ages and sometimes resulting in complication, including disability and death (1). The skin barrier function is compromised due to these injuries, which weakens the immunity of the affected individual to fight against any infection or systemic disorder. Various microorganisms are the usual causes of burn wound infection, which has a high fatality rate, indicating the urgency of better treatment methods (2, 3). Patients with burn injuries are particularly susceptible to sepsis due to several factors, such as wound colonization by pathogens, loss of immune cells and proteins, insertion of medical devices, and a hypermetabolic state (3). These challenges emphasize the importance of developing targeted therapies that can address the unique complexities of burn wound infections (3). Burn wound infections caused by multidrug-resistant (MDR) bacteria have become a significant challenge in clinical settings due to their high resistance to conventional antibiotics and ability to form biofilms (4, 5). Polymicrobial infections, in particular, pose an additional challenge due to the involvement of multiple bacterial species and the formation of complex microbial communities (6, 7). These challenges emphasize the importance of developing targeted therapies that can address the unique complexities of burn wound infections.

Antibiotics have long been the cornerstone of treating bacterial infections, significantly reducing the morbidity and mortality associated with these infections. However, the widespread use and misuse of antibiotics have contributed to the emergence of antibiotic resistance, a growing global health concern (8, 9). Antibiotic resistance occurs when bacteria develop mechanisms to counteract the effects of antibiotics, leading to less effective or even ineffective infection treatments (10, 11). Some of the key factors contributing to antibiotic resistance include over-prescription, misuse, and overuse of antibiotics, as well as lack of new antibiotic development (8, 12). In the search for alternative methods of fighting antibiotic resistance, bacteriophages -or phages- have emerged as one of the most viable strategies to offset the drawbacks of conventional antibiotics (13, 14). Phages are viruses that infect bacterial hosts and, compared to traditional antibiotics, they have some advantages, including specificity, self-replication, and the capability of targeting even antibiotic-resistant strains (15, 16). Additionally, phage therapy can be used in combination with antibiotics to enhance their effectiveness and potentially reduce the development of resistance (17). As research into phage therapy continues to grow, it is crucial to investigate its potential applications in treating burn wound infections and other challenging clinical contexts (18-20). By exploring the therapeutic potential of phages, we can work towards developing more effective and targeted treatments for patients affected by drug-resistant infections. This study investigated the infected burn wound healing using Hydroxy-propyl-methyl cellulose gel containing bacteriophages against *P. aeruginosa* and *K. pneumoniae*.

MATERIALS AND METHODS

Materials. Polyethylene glycol (PEG) 6000 Solution (207 g PEG 6000, 49.9 g NaCl, in 350 ml distilled water), Sodium Magnesium (SM) solution (5.8 g NaCl, 2.0 g MgSo4.7H2O, 5 ml of 2% gelatin in 1 liter), MgSo4.7H2O 0.1 M, CaCl2 0.1 M, Luria Bertani broth medium, Luria Bertani agar medium. *P. aeruginosa* (ATCC 27853) and *K. pneumoniae* (ATCC 13833) were purchased from the Pasteur Center of Iran.

Isolation of phages. The method described by Carvery et al. (21) was used to isolate active phages from sewage samples collected at Vali Asr Hospital in Fasa County against *P. aeruginosa* and *K. pneumoniae*. To do this, equal volumes of sewage samples and bacteria grown in LB broth supplemented with 5 mM CaCl2 were mixed and incubated overnight at 37°C at 120 rpm in a shaker incubator (Chest-Type GYROMAX 777, Amerex Instruments, Inc.). Following the incubation, the samples were centrifuged at 2800 RCF for 7 minutes, and the supernatant was collected and passed through 0.45 and then 0.22 µm filters (Orange, UK).

Spot assay. The lysate was then analyzed for phage activity using the spot assay described by Chang et al. (22). To perform the spot assay, a mixture of 3 mL of soft agar (0.7% LB agar) and 500 µL of fresh bacterial suspension was prepared. The plates were left at room temperature for 30 minutes for solidification, after which 10 µL of phage solution was poured onto three

different sections of the plates. Clear zones were observed by the naked eye after 24 hours of incubation at 37°C. Phage titration was carried out by the double agar overlay method (23). For each serial dilution, 200 µL was mixed with 500 µL of fresh bacterial culture in separate sterile tubes. The tubes were incubated for 20 minutes at 37°C, then combined with 3 mL of soft agar (0.7% LB agar supplemented with 0.1 M magnesium sulfate maintained at 48°C) and poured onto solid agar plates (1.5% LB agar). Following incubation for 24 hours at 37°C, the plaques were counted in order to estimate the phage titer.

Phage purification. Phage purification was carried out by plaque picking technique, as described by Peters (24). The plaques formed on the surface of spot plates. Isolated plaques were selected and removed by a Pasteur pipette by removing both superficial and deep layers of agar plates. Then they were solved in 1.5 ml SM and 200 uL chloroform (to eliminate bacteria). With this solution, plate loading (Plaque assay) was performed again. About 10^5 to 10^6 phages were expected. This process should be repeated two to three times in order to obtain pure phages.

Amplification of phage. The method described by Skaradzińska et al. (25) was used to amplification of phage by plate lysate method. To perform the amplification of phage, 500 uL of bacteria in the exponential growth phase, with 200 uL of purified phage were mixed. After mixing with 3 ml soft agar, it was spread on the plate LB 1.5% agar. After 4 hours of incubation at 37°C, about 3 ml SM was poured on the plate surface and was kept for 16 hours at 4°C. If agar absorbed SM, 3 ml SM throw to the plate surface, and left for 4 hours at 4°C. Entire SM removed by syringe. Obtained phages had concentrations of about 10^9 Pfu. Then it was precipitated by PEG 6000.

Large lysate. 10 ml bacteria inoculated in 500 ml LB broth. It was incubated at 37°C for 18 hours. Then was centrifuged at 5000 rpm for 7 minutes and the supernatant was removed for the next step. Liquid was passed through the filter 0.45 um. Phages were precipitated by PEG 6000.

Precipitate phages. Phage precipitation was performed following the previous methods (26). Polyethylene glycol (PEG, 5X) was mixed with a ratio of 1:4 with the phage (in the sterile 50 cc Falcon and with a gentle ten times upside down). Then were put on ice for one hour. Samples were centrifuged at 9000 rpm for 15 min at 4°C (with constant temperature conditions at 4°C without shake). The supernatant was discarded and the Falcons were put on absorbent paper. After taking excess fluid pellet was dissolved in 10 ml SM (If it is not solved in the solution, 1M KCl can be used).

Morphology of bacteriophages. The morphology of each of the four bacteriophage was analyzed using TEM) transmission electron microscopy, EM10c model, Zeiss) and a modified method (27). A drop of purified phage suspension was placed on formvar carbon-coated copper grids for 5 min. Susequently, the purified phage suspension was removed using a pipette and negatively stained with uranyl acetate 2% (TAAB Laboratory, UK). The liquid was removed using a filter paper after 10 min. The grids were examined using the TEM.

The hydrogel preparation was selected for this study since bacteriophages retain their antibacterial properties in various pharmaceutical forms. According to the method described by (28, 29), bacteriophages were encapsulated by hydroxypropyl methylcellulose (HPMC) gel. HPMC powder was dispersed in water and then stirred at 112 RCF for 2 h at a temperature of 85°C. Firstly, the HPMC was prepared as a 4% gel; after reaching a homogenous consistency, it was subjected to a vacuum oven at 25°C. Subsequently, the obtained hydrogel was sterilized for 30 minutes by autoclaving. Preparation of phage stocks was performed at concentration of 10^9 PFU/mL. The HPMC gel at a ratio of 1:1 was mixed with the phage cocktail solution to a final concentration of 2%. This was followed by the addition of 0.4 volumes of 1-octanol, after which shaking at 25°C for 1 hour took place. It was centrifuged at 4000 × g for 10 minutes and the aqueous phase was collected. Centrifugation at 20,000 × g for 1.5 hours removed the residual organic solvent. This was followed by a buffer change to fresh SM buffer.

Animals. Animal studies were performed according to a modified technique that was described earlier by Björn et al. This approach was basic for developing our experimental method and only needed some modification to suit the requirements of the present study (30).

Balb/C male mice, aged 6 weeks and weighing 25

± 5 were prepared. The mice were kept in the lab for up to 1 week to maintain a stable physical condition for testing. The surface of the back of the mice neck was carved and shaved. The area was cleaned with gauze and normal saline, and then it was impregnated with Iodine. Burning was performed using a standard brass rod (Dimensions 10 * 10 * 100 mm) at 100°C for 45 seconds. The rod persists for 5 minutes in boiling water. Then it was immediately placed behind the neck of the anesthetized mice. First, mice were inflicted with burn wounds, after which various infectious agents were introduced at the wound sites. An hour post-infection, mice were treated with hydrogel-based bacteriophages and antibiotics.

Mice were categorized in eleven groups, each consisting of seven mice. The groups were as follows: Group 1: Negative control, negative control with HPMC gel without bacteriophage, Group 2: *P. aeruginosa* group: Positive control, Antibiotic treatment and Phage therapy, Group 3: *K. pneumoniae* group: Positive control, Antibiotic treatment and Phage therapy and Group 4: Mixed group of bacteria: Positive control, Antibiotic treatment and Phage therapy.

The conditions were kept as sterile as possible. Sterile conditions were followed for all stages of the animal experiment, using a modification of the technique of Björn et al. (30). The following precautions were undertaken to avoid contamination: Animal preparation: The Balb/C male mice were acclimatized for one week in the laboratory in order to keep them in good physical condition. Their necks were cleaned by shaving that part with gauze, normal saline, and iodine. Wound infliction: Sterile burn wounds were inflicted on anesthetized mice using a standard brass rod (10 × 10 × 100 mm) that had been boiled 5 minutes before each use. Infection creation: Introduction of infectious agents at the wound sites was performed under a Class II biosafety cabinet, maintaining a sterile environment with filtered air and laminar airflow. Treatment application: Mice were treated with hydrogel-based bacteriophages and antibiotics one hour post-infection. Mice within each treatment group received the same dosage, with phage therapy administered as a single dose and antibiotic therapy repeated every 24 hours. Sterilization of resources: Water and food were sterilized by autoclaving, whereas the storage environment was prepared using UV lamps. Phage dosage was administered as a single dose, taking advantage of the self-replicating or auto-dosing nature of bacteriophages. In contrast, antibiotic therapy was administered repeatedly every 24 hours.

Evaluation of the health score of mice. A daily scoring system was utilized daily, ranging from 0 to 5 to monitor the health status of mice. This approach paralleled that of other studies, albeit with some modifications, using mouse models (31). In all groups, mice were observed and scored for overall condition over the course of the treatment. Skin pathology samples were collected from both deceased mice after 12 days of storage and from surviving mice at the end of the study for further analysis.

Histopathologic studies. Wounds and surrounded skins were removed and were placed in formalin 10% to evaluate the histopathologic changes during mice' treatment. Then they were dehydrated in a rising series of alcohol (70-100%). The tissues were embedded in paraffin wax, sectioned, and stained with hematoxylin and eosin (32).

Statistical analyses. Prism software (GraphPad v7, San Diego, CA, USA) was used to perform statistical analysis. All values are presented as mean ± standard deviation (SD). Data were analyzed using one-way analysis of variance (ANOVA) and Tukey's multiple comparisons test. Statistically significant was considered a P-value below 0.05.

RESULTS

Isolation and morphology of bacteriophages. The initial results obtained in the laboratory were visible on the plate. Separated phages were observed as uniform plaques after several stages of purification (Figs. 1 and 2).

After the purification of phages, the samples were prepared for electron microscopy. Observations indicated that they most probably belonged to the family *Siphoviridae* according to the ICTV classification. Both phages had a hexagonal head shape, with a size of about 50 ± 5 nm. The lengths of the tail were about 180 ± 5 nm for phage KPP1 and 170 ± 5 nm for phage SAP1 (Figs. 3 and 4).

To capture the process by which phages bind to their specific bacteria, the phages were exposed to the target bacteria for 20 minutes. Photo 5 illustrates the phages attacking the bacteria (Figs. 5 and 6).

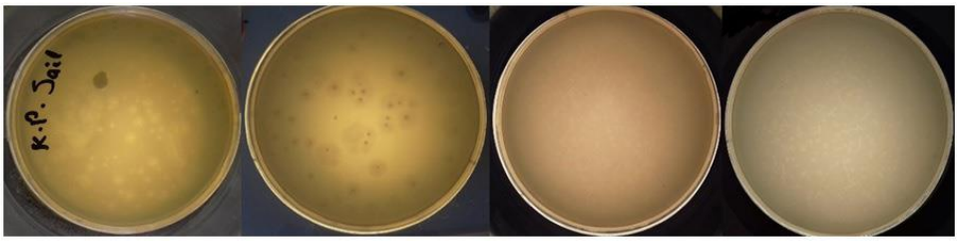

Fig. 1. Different stages of *K. pneumoniae* bacteriophage isolation. KPP1 from early stages of detection (left) to higher levels of purification (right). Processes of concentration and uniformity of plaques are visible for host bacteria.

Fig. 2. Different stages of *P. aeruginosa* bacteriophage isolation. SAP1 from early stages of detection (left) to higher levels of purification (right). Processes of concentration and uniformity of plaques are visible for host bacteria.

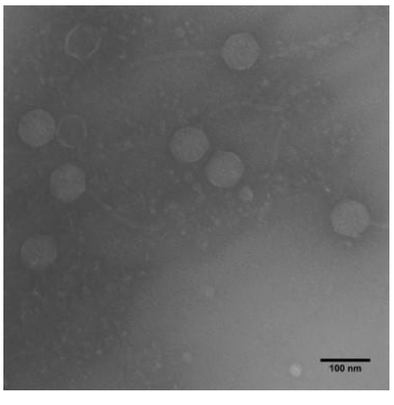

Fig. 3. Transmission electron microscopic image of phage SAP1.

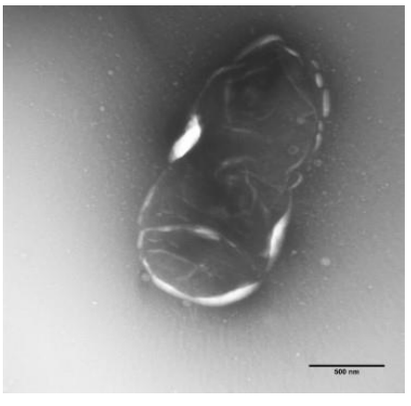

Fig. 5. Transmission electron microscopic images show SAP1 attacking *P. aeruginosa* bacteria.

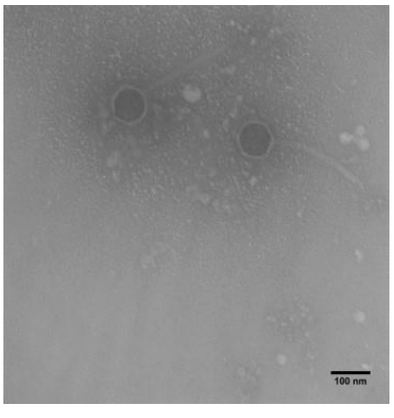

Fig. 4. Transmission electron microscopic image of phage KPP1.

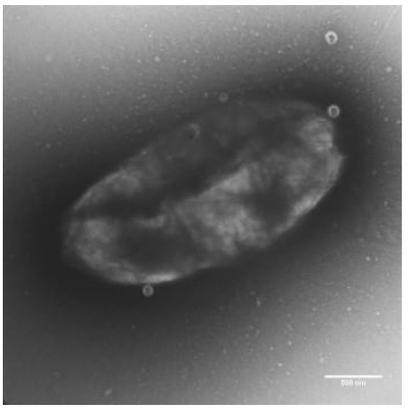

Fig. 6. Transmission electron microscopic images show KPP1 attacking *K. pneumoniae* bacteria.

Evaluation of the health score of mice. A glance at the results of the survival of mice shows different results between treatments. The shape of the wound and the general condition of the mice during treatment indicated a better treatment with phage. In all groups, mice were infected, and the mice from positive control group died between approximately 48-72 hours. Comparison between phage and antibiotic treatment groups shows a stronger and better treatment with phage (Figs. 7-9). As shown in these charts, phages were very successful in controlling the mortality of mice.

Histopathologic studies. Pathologic samples of different groups confirmed the therapeutic results of phages. These results at the microscopic level indicated the recovery of the tissue and the removal of the infection (Fig. 10). General and common symptoms are explained to make the understanding and the comparison easier.

Fig. 10A and B illustrate pathology of the positive control group. In Fig. 10A, edema and loss of fatty tissue and blood vessels can be seen. Fig. 10B shows extensive infiltration of inflammatory cells in the tissue, including neutrophils, lymphocytes, and macrophages. All the mentioned symptoms indicate early and acute stages of burns, severe inflammation, and lack of tissue repair. Fig. 10C (pathology figure of the negative control group) connective tissue, hair follicles, sebaceous glands, and healthy vessels (signs of complete recovery of skin and tissue) can be seen. According to several photographs, large edema was seen in all cases. Extensive infiltration of inflammatory

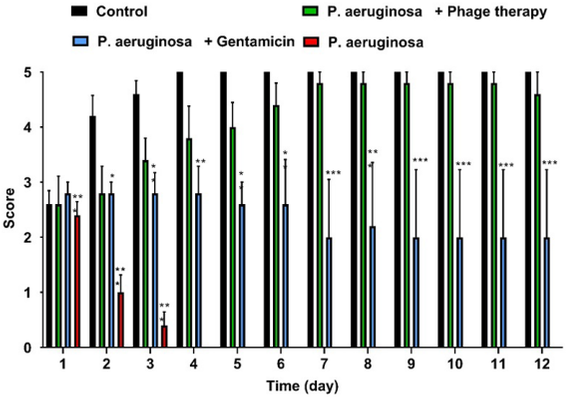

Fig. 7. Mortality of mice by 10^7 CFU/ml *P. aeruginosa* on the burn wound. Rank 5 means general health, rank 4 means mild disease state, rank 3 means more severe weakness, body hair creeping and hunchback, rank 2 in addition to other symptoms contributes to the accumulation of secretions around the eyes that lead to partial eye closure. The values are based on the mean ± SD. **, p<0.01. ***p<0.001 *p<0.05 compared to the control group.

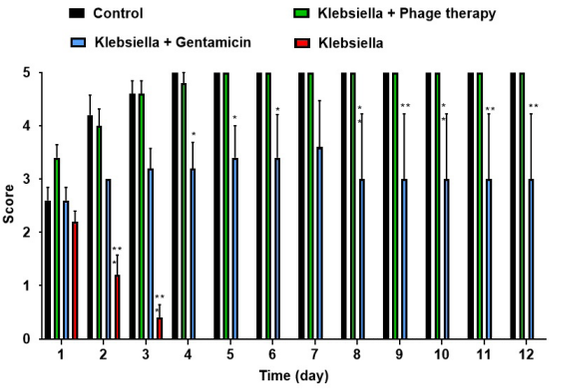

Fig. 8. Mortality of mice by 10^7 CFU/ml *K. pneumoniae* on the burn wound. Rank 5 means general health, rank 4 means mild disease state, rank 3 means more severe weakness, body hair creeping and hunchback, rank 2 in addition to other symptoms contributes to the accumulation of secretions around the eyes, leading to the partial closure of the eyes. Rank 1 and rank 0 are contributed to dying state and death respectively. Values are based on mean ± SD. **, p<0.01. ***p<0.001 *p<0.05 compared to the control group.

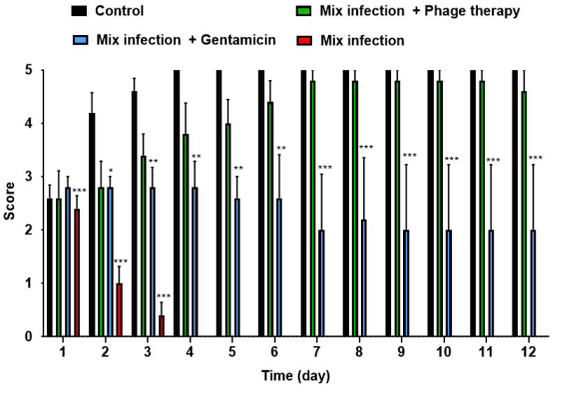

Fig. 9. Comparison of mortality of mice exposed to 10^7 CFU/ml *K. pneumonia* and *P. aeruginosa* on the burn wound. Rank 5 means general health, rank 4 means mild disease state, rank 3 means more severe weakness, body hair creeping and hunchback, rank 2 In addition to other symptoms contributes to accumulation of secretions around the eyes, leading to the partial closure of the eyes. Rank 1 and rank 0 are contributed to dying state and death respectively. Values are based on mean ± SD. **, p<0.01. ***p<0.001 *p<0.05 compared to the control group.

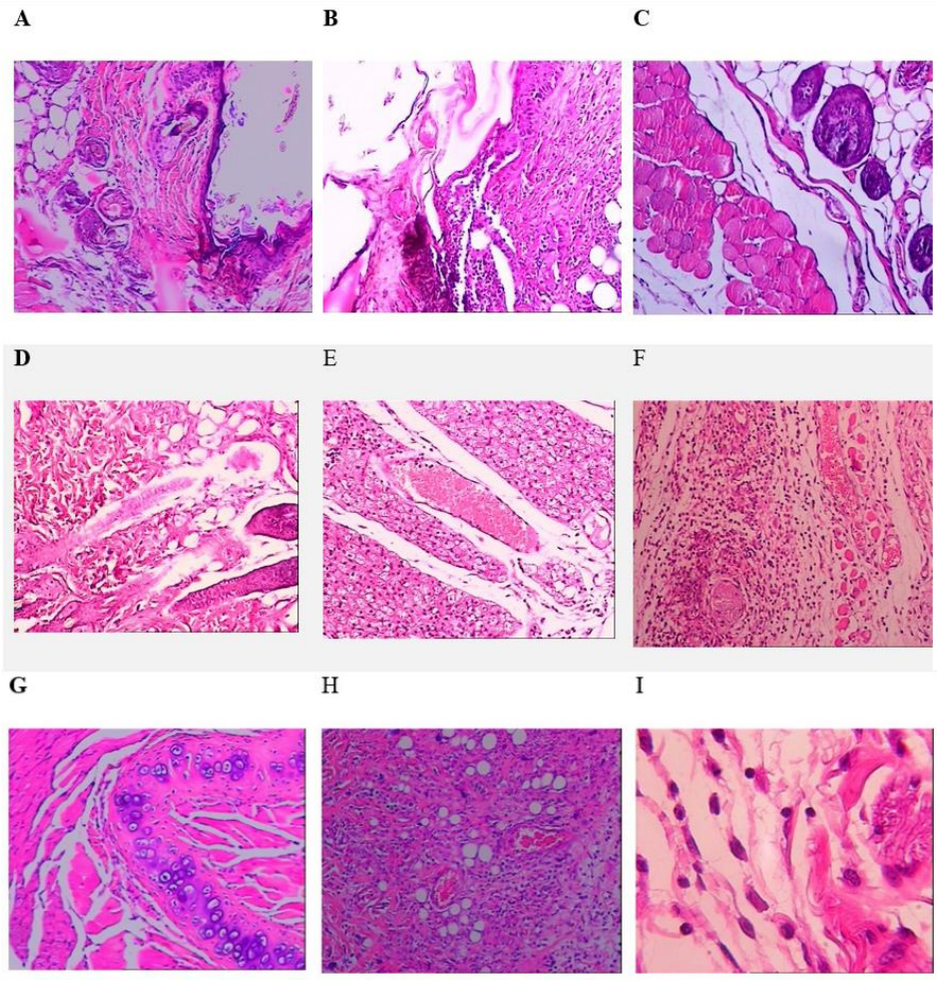

Fig. 10. Wound pathology evaluation of mice burn wound after staining with hematoxylin and eosin

cells were seen in neutrophils, lymphocytes and macrophages. Another indication was the loss of adipose tissue and vessels. All mice died after 48-72 hours. All specimens were taken immediately after death of mice. In the negative control (Fig. 10C) a healthy skin slide was seen. Connective tissue, hair follicles, fatty glands and healthy vessels are visible. Healthy skeletal muscle and tissue were seen. Samples of this group were taken after 12 days of storage. Infected mice (with *P. aeruginosa*, *K. pneumoniae* and Mix group) were treated by gentamicin (Fig. 10D-F).

Photographs of mice showing antibiotic treatment and recovery of skin burn wound infection following surgery. D: Pathology of *K. pneumoniae* group treated with gentamicin. E: Pathology of *P. aeruginosa* group treated with gentamicin. F: Pathology of mixed group of bacteria treated with gentamicin. Common symptoms include hyperemia (a large flow of blood or blood accumulation in tissues), as well as connective tissue and inflammatory cell infiltration. Typically, fibroblasts were seen in some cases. Bacteria were also observed. In some cases, edema also could be seen. All of these indicated the presence of inflammation and infection. Infected mice (with *P. aeruginosa*, *K. pneumoniae* and Mix group) were treated by phages (Fig. 10G-I).

Photographs of mice showing phage treatment and recovery of skin burn wound infection following surgery. G: Pathology of *K. pneumoniae* group treated with phage. H: Pathology of *P. aeruginosa* group treated with phage. I: Pathology photo of mixed group of bacteria treated with phage. Better treatment can be seen in phage therapy in comparison to antibiotic therapy. The final repair has been in progress. The tissue was more natural. Connective tissue and fibroblasts can be seen. In some slides, fibroblasts were observed. The main difference with antibiotic therapy was less inflammation and more tissue recovery in phage ther-

apy. The fibroblasts that formed fibrosis were seen in the formation and repair of the tissue. The structure of the body tissue is in progress. In mixed infection, as can be seen, muscle cells and subcutaneous tissue were forming. Complete recovery was observed. However, the final mortality rate was much lower in phage treatment than in antibiotic therapy.

DISCUSSION

Antimicrobial resistance (AMR) has become a challenge to human health worldwide and has caused serious threats to the successful elimination of infections. In spite of various actions taken in recent decades to tackle AMR, this issue has the potential to emerge as an important healthcare threat if left unchecked and could put into motion another pandemic (33, 34). AMR has been seen in burn wounds, leading to serious complications (35). In particular, a high prevalence of resistant bacteria such as *P. aeruginosa* and *K. pneumoniae* has been reported in burn wounds. Therefore, these two common bacteria were used in this study (35). *P. aeruginosa* and *K. pneumoniae* are opportunistic pathogens that commonly causes various hospital and opportunistic infections. *K. pneumoniae* and *P. aeruginosa* are pernicious bacterial pathogen that are difficult to treat due to the high prevalence of antibiotic resistance (36, 37).

One of these alternative approaches to control these bacteria includes the use of prokaryotic viruses or bacteriophages. Study on the role of bacteriophages has great value in the treatment of infections. The effect of phages on the polymicrobial burn injury is remarkable. Completely eliminating the infection and fully recovering from infection with two or more bacteria is difficult to attain for chemical antibiotics. In poly-microbial infections, bacteria form a contaminated layer that prevents antibiotics from reaching bacteria. This layer, called biofilm, is incapable of preventing phage access to bacteria and infections (38). This field of medicine has gained attention especially over the last two decades due to the lack of new antibiotic classes and the emergence of multidrug resistant strains (38).

In this study, bacteriophages with antimicrobial properties were obtained and their morphology was determined. Then, the antibacterial effects of bacteriophages in a specific drug form were investigated and histological burn wound parameters were evaluated in mice. The isolated phages are probably associated with the *Caudovirales* and the *Siphoviridae* family. This family of phages is lytic and destroys host bacteria. Lytic phages are similar to antibiotics in terms of their significant antibacterial activity. These phages are effective in the treatment of antibiotic-resistant infections in humans and animals.

Our study presents the successful isolation and purification of phages. The purified phages probably belong to the *Siphoviridae* family. Indeed, several other investigations also described the isolation and characterization of phages that can infect *P. aeruginosa* and *K. pneumoniae* thereby pointing out the possibility of using these agents in phage therapy for these infections (39, 40). During the study, the isolated bacteriophages were found to be members of *Caudovirales* order and *Siphoviridae* family, both of which are lytic and possess antibacterial activity. The effectiveness of these lytic phages against infection with antibiotic-resistant human and animal infections further supported bacteriophage therapy as an alternative or adjunctive therapy. In addition, bacteriophages display antimicrobial activity and the possible use of bacteriophages for infection treatment has been gaining much interest in recent years. The antibacterial feature of bacteriophages to permeate bacterial biofilms, a barrier often hampering the activities of antibiotics, makes an attractive approach for the treatment of polymicrobial burn wounds.

Also, the first results obtained confirms success of phages treatment on the burn wound infection. The infected and untreated mice (positive control group in each group), all died within 48 to72 hours. In the negative control group, all animals survived, indicating that environmental conditions did not affect the mortality of the mice. After 3-4 days mice treated with phages gained score, indicating improvement in the appearance of the infection symptoms. While in the treatment with antibiotics, this score was achieved in 8-10 days. The third-degree type of burn causes severe damage to the epidermis, dermis, and deeper tissues, including muscles and connective tissues and surface vessels. In early stages of burns, severe inflammation can be seen in damaged site. Hyperemia and increased inflammatory cells are present in the site. An increase in the number of fibroblastic cells is observed in the last inflammatory process. Ultimately, the reduction of primary inflammation and the onset of ultimate repair of the primary tissues is observed (41).

The fact that phages directly affect fibroblasts and myofibroblasts do need further research. The effect of phages on stimulating the secretion of inflammatory cytokines is significant (42). At the time of inflammation, some cytokines are increased such as IL-1β, TNF-α, IL-10 in the serum and lungs of the rats exposed to burn. Treatment with KPP1 reduces these cytokines. Since IL-1β and TNF-α are part of the anti-inflammatory cytokines, phage therapy not only helps eliminate bacteria from the environment, but also decreases inflammation by reducing inflammatory cytokines and accelerating the wound healing process (43). Many bacteria distorting the immune system from the burn site and the wound would be more susceptible to infection; for instance, *K. pneumoniae*, causes the migration of macrophages and neutrophils from the site and makes the wound more susceptible to infection by secretion of succinates at the site of wound (44, 45). This can be due to severe inflammation in treatment with antibiotics even after 12 days. According to our results, it can be seen that the survival rate and wound healing were higher in the case of phage-treated mice compared with the untreated group or those treated with antibiotics. The better efficacy of bacteriophages in controlling infection and enhancing tissue repair has also been addressed in some literature (46, 47).

In antibiotic-treated groups (D, E, and F) hyperemia, connective tissue infiltration, and inflammatory cells indicated ongoing inflammation and infection in alignment with previous literature reporting the disadvantages of antibiotics in the treatment of burn wound infections (48). The phage-treated groups (G, H, and I), on the contrary, showed reduced inflammation, more natural tissues, and better tissue regeneration with more fibroblasts and connective tissue. These findings also support the possible benefits of phage therapy for augmentation of tissue regeneration, reduction of inflammation in infected burn wound, as described in other studies (2, 49). Our work also shows the promise that bacteriophage therapy can potentially serve as a useful therapy for burn wound infections, especially for infections caused by drug-resistant bacteria such as *P. aeruginosa, K. pneumoniae*. The successful isolation and characterization of phages, an improved survival rate, and histopathological evidence of tissue repair, all indicated the therapeutic feature of phages. The beneficial effects observed in phage treated mice point the need to further investigate alternative treatments to address the increasing challenge of antibiotic resistance and the associated high costs.

CONCLUSION

The results of this study reveal that lytic phages are powerful biological tools for the treatment of bacterial infections in burn wounds, which can be considered as one of the alternatives for drug-resistant bacterial species and the high costs of antibiotics; though further animal and trial studies are needed. Meanwhile, the complications due to their widespread use in humans should be investigated in more details.

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