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CHARACTERIZATION OF LYTIC BACTERIOPHAGES SPECIFIC TO KLEBSIELLA PNEUMONIAE: A STEP TOWARDS PHAGE THERAPY

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ABSTRACT

UTIs, bacteremia, multidrug-resistant pneumonia, and other systemic infections are frequently caused by *Klebsiella pneumoniae*. In blood cultures from patients with sepsis, *K. pneumoniae* is the third most often found pathogen. It can cause serious epidemic and endemic nosocomial infections that can spread quickly across the population. Because of the emergence of antibiotic resistance, treating *K. pneumoniae* infections with antibiotics is becoming more difficult, necessitating the development of a novel alternative approach. A kind of virus called a bacteriophage was once employed to cure human illnesses caused on by a variety of bacteria. With an estimated 10^{31} particles, bacteriophages are the most common biological entity on Earth. They invade bacterial cells as required intracellular parasites. They can multiply and lyse bacterial cells, and they have a high host specificity. Sewage samples were taken from hospital trash, while clinical samples of *K. pneumoniae* were obtained from the Khyber Teaching Hospital in Peshawar. Two new native bacteriophages against *K. pneumoniae* strain kp9 were identified and described during this study; they were assigned the scientific names KPP1 and. Since KPP2 was able to lyse four out of eight but was unable to infect bacteria from other species, and KPP1 was able to lyse 3 out of 8 distinct *K. pneumoniae* clinical isolates, it was determined that both of these phages had a particular host range for *K. pneumoniae*. The separated phages demonstrated viability at pH values between 3 and 9 and up to 50°C. KPP1 prevented bacterial growth for 18 hours, but KPP2 demonstrated superior bacterial reduction capabilities by preventing and reducing the initial bacterial inoculum count during 24 hours of observation. Both KPP2 and KPP1 may be viable options for treating *K. pneumonia*, given their superior bacterial growth reduction, phage titer, pH, thermal stability, and host range. Future genetic, physiological, and clinical studies are required to fully characterize these two phages (KPP1 and KPP2).

INTRODUCTION

Carl Friedlander first described *K. pneumoniae* in 1882. He identified it as an encapsulated bacillus after separating the bacterium from the lungs of pneumonia victims. Before being renamed in 1886, *K. pneumoniae* was known as Friedlander's bacillus. This non-motile, gram-negative bacteria is present in the environment and has been connected to pneumonia in hospitalized patients with weakened immune systems, diabetics, and alcohol addicts (Jondle *et al.,* 2018). In people suffering from sepsis The third most frequent infection identified in

blood cultures, *K. pneumoniae*, can result in serious endemic and epidemic hospital-acquired diseases that can quickly spread across the population (Wu and Li, 2015). *K. pneumoniae* is an opportunistic infection that mostly affects immunocompromised people, including the elderly and babies. Patients in hospitals are more vulnerable to *K. pneumoniae* infections, and the longer they stay there, the higher the risk of colonization. These bacteria mostly colonize the mucosal surfaces of the human oropharynx and gastrointestinal (GI) tract. Bacteria may exhibit significant degrees of pathogenicity and drug resistance after entering the body. *K. pneumoniae* is the most frequent cause of nosocomial pneumonia, which makes about 3% to 8% of all nosocomial bacterial infections in the US (Jondle *et al.,* 2018, Aghamohammad *et al.,* 2020). Biofilm constituents such adhesion factors, ureases, lipopolysaccharide (LPS), and capsular polysaccharide (CPS) are among the virulence factors of *K. pneumonia*. Numerous factors influence a bacterium's capacity for virulence, which can result in infection and drug resistance (Wu and Li, 2015). According to the nosocomial infection monitoring system, *K. pneumoniae* is the third most common cause of primary bacteremia and all lower respiratory tract infections. Pneumonia is frequently caused by *K. pneumoniae* in both the community and long-term care institutions. Infections caused by Klebsiella in hospitalized patients include meningitis, urinary tract infections, pneumonia, primary and secondary bacteremia, and surgical wound infections (Jondle *et al.,* 2018, Aghamohammad *et al.,* 2020).

Because of a genetic mutation, *K. pneumoniae* is one of the few bacteria that has currently achieved a significant level of antibiotic resistance. The resistance of gram-negative bacteria to beta-lactam medicines was identified by Alexander Fleming in 1929. *K. pneumoniae* has since been found to contain a beta lactamase that hydrolyzes the beta-lactam ring in medications. An extended-spectrum beta-lactamase (ESBL) was found in *K. pneumoniae* in the United States in 1989 and Europe in 1983 (Hegreness *et al.,* 2008, Sanchez *et al.,* 2013).

K. pneumoniae is quickly developing resistance to antibiotics, particularly lactams and broad-spectrum cephalosporins. There are several causes of antimicrobial resistance. The emergence of resistance is mostly caused by inadequate antibiotic stewardship, which results in misuse of antibiotics, inadequate empirical coverage, delays in precise diagnosis, and therapeutic de-escalation (Wu and Li, 2015). Antimicrobial resistance is increasing in the microbial community, most likely as a result of antibiotic abuse in people, animals, and fowl, which has accelerated the establishment of resistance, including strains that are resistant to several drugs. Globally, the variety of illnesses resistant to antibiotics is growing (Hegreness et al., 2008, Sanchez *et al.,* 2013). Bacteriophages consist of a tail and/or a capsid (head). Encased and protected by the capsid is genetic material like as DNA or RNA. Bacteriophages

are between 24 and 400 nm in size (Ivanovska *et al.,* 2004). Typically, in the form of a triangle, the capsid is made up of two or more different proteins. Bacteriophages come in two varieties: filamentous (some) and icosahedral (20 facets). The capsid is essential because without it, enzymes cannot use the genetic material (Orlova, 2012). Bacteriophages are believed to be the most common biological organism on Earth, with a population of at least 1030. In a range of environmental settings, including hot springs with acidic water, phages have been identified. Marine water is one of the primary sources of bacteriophage resources. Seawater is one of the primary reservoir (O'flynn *et al.,* 2006). Bacteriophages can have a wide range of functions in the biology of bacteria and archaea because of their varied life cycles. The two primary stages of a phage's life cycle are lytic and lysogenic. Both life cycles comprise two common phases: i) Absorbance of Phage ii) Leakage of genetic material (Salmond and Fineran, 2015). Despite decades of intensive research on phages for medical applications in the US and the EU, neither area currently has any phage products that have regulatory agency approval or that are entering phase-III clinical trials for human use. Even though these studies showed no adverse effects on people, researchers from all over the world need carefully planned controlled trials to validate the safety and efficacy of phages (Viertel *et al.,* 2014).

Methodology

Study area: The study was conducted at Hazara University Mansehra's Microbiology Research Laboratory. The Khyber Teaching Hospital in Peshawar supplied the bacterial samples, while THQ Poran Shangla, K.T.H. Peshawar, DHQ Shangla, DHQ Peshawar, LRH Peshawar, and Butt Pull Sewage Drain in Mansehra provided the bacteriophage samples.

Bacterial culture: *K. pneumoniae* clinical samples were gathered from Khyber Teaching Hospital in Peshawar. Fresh nutrient agar and the MacConkey agar plate were streaked with bacterial samples, and they were then incubated for the whole night at 37°C. The bacterium was identified using microscopy and Gram staining. Before every experiment, new bacterial samples were subcultured, and cultures were utilized for eight hours. Regular subculturing helped to preserve the culture's viability and purity. Additionally, reference strains KP9, KP4, and KP5 from the Abbottabad University of science and technology Department of Microbiology were used.

Isolation of bacteriophages from sewage: Sewage samples were taken from hospital waste at the Butt Pull Water Canal in Mansehra, the DHQ hospital in District Shangla, the THQ hospital in Poran Shangla, the LRH hospital in Peshawar, and the Khyber Teaching Hospital in Peshawar. To isolate bacteriophages, the samples were taken to the Hazara University

Microbiology Laboratory in Mansehra. A previously described methodology was used to extract bacteriophages from a sewage sample (Asif *et al.,* 2018). The water samples were agitated for two minutes before being centrifuged for ten minutes at 10,000 rpm to get rid of pathogens and sediment. In a conical flask, 40 mL of the clear sewage supernatant was added to 10 mL of sterile 5 X nutritional broth. A 200 L overnight culture of *K. pneumoniae* strain KP9 was used to inoculate the flask. Overnight incubation of the infected flask was conducted at 37°C while being shaken at 120 rpm. Following incubation, the flask's contents were centrifuged for five minutes at 10,000 rpm. The clear supernatant was collected in a fresh, clean falcon tube and stored at 4°C for later use after passing through a 0.22 syringe filter. By using a spot test, the presence of bacteriophages in the filtrate was identified (Asif *et al.,* 2018).

Detection of bacteriophages in the filtrate: Following bacteriophage enrichment, a spot test was used to identify the presence of a *K. pneumoniae*-specific phage. In the spot test, a nutrient agar plate was covered with 100 L of an overnight-grown *K. pneumoniae* culture. After applying 5 L of the filtrate, the plates were allowed to dry for nearly 10 minutes. The plates were then incubated for the whole night at 37°C. After that, the plates were examined for a distinct bacteriophage-induced lysis zone. The presence of a particular bacteriophage is indicated by the presence of the clear zone, or plaque (Asif *et al.,* 2018).

Purification of bacteriophages using a double layer agar assay: Double layer agar overlay method was used to quantify and purify bacteriophage from the lysate (filtrate that caused lysis) (Alvi *et al.,* 2020). Before the experiment began, 100 mL of semisolid nutritional agar was autoclaved and put in a water bath at 48°C in a conical flask. Initially, 900 L of nutritional broths were used to serially dilute the lysate (1:9) up to 1/1012 in microtubes. The chosen dilutions were then supplemented with 100 L of a new *K. pneumoniae* culture. For five minutes, the bacterial culture and phage lysate were incubated to enable the phages to adhere to the bacterial cells. After pouring the chosen dilutions onto nutritional agar plates, 3 mL of semisolid agar was added, and the plates were incubated at 37°C for the whole night. Swirling motion was used to disperse the mixture and semisolid agar on the plate. We looked for countable plaques (30 pfu-200 pfu) on the plates. The chosen plate's plaque forming units (pfu) were counted (Vinogradov *et al.,* 2002). Plates with unique plaque were chosen for phage purification. With the use of a sterile micropipette tip, phage was removed from a plaque by gently tapping its surface. For phage propagation, the tip was placed in a test tube with 10 mL of nutritional broth and 1 mL of fresh *K. pneumoniae* strain. It was then cultured for 24 hours at 37°C, after which plaque was visible and purified (Di Martino *et al.,* 2003,

Fader *et al.,* 1979). The purifying process was carried out up to ten times. The following formula was utilized to determine the titer of lysate solutions:

Titter (*pfu*/mL) = plaque (*pfu*) dilution number x phage (mL) volume added to plate.

Characterization of bacteriophages

Determination of host range: An established procedure was used to analyze the host range utilizing bacterial cultures from several strains of *K. pneumoniae* as well as other species (Alvi *et al.,* 2021). On a plate, a bacterial lawn was created and spotted using 5 L of phage lysate. The plates were incubated at 37°C for 24 hours before bacterial lysis was observed.

Determination of thermal stability of bacteriophages: Determining the bacteriophages' temperature stability is crucial since it offers a hint for phage transportation and storage. Aliquots of known titers of KPP2 and KPP1 were placed at 4, 25, 37, 50, and 60°C for an hour in order to assess the thermal stability of the isolated bacteriophages. The two layer agar overlay technique was used to determine the bacteriophage titer following incubation (Alvi *et al.*, 2021).

Determination of pH stability of bacteriophages: HCl and NaOH were used to change the medium's pH. A pH paper was used to measure the pH. To find the pH stability of the isolated bacteriophages, aliquots of known titers of KPP2 and KPP1 were placed at pH 2–10 for an hour. The two layer agar overlay technique was used to determine the bacteriophage titer following incubation (Alvi *et al.,* 2020).

Long term storage stability: For six month, bacteriophages were maintained in LB broth at 4, 25, and 37°C. Prior to storage, bacteriophages KPP2 and KPP1 had respective titers of 7×10^{9}pfu/mL and 8×10^{10}pfu/mL. The two layer agar method was used to measure each bacteriophage's titer both before and after storage (Alvi *et al.,* 2018).

Bacterial growth reduction: Phage-mediated inhibition of bacterial growth is frequently referred to as in-vitro phage treatment. The test determines the bacteriophages' capacity to inhibit bacterial growth. The viruses KPP2 and KPP1 decrease in bacterial growth was assessed using a previously developed procedure (Alvi *et al.,* 2021). In brief, an 8-hour-old culture of *K. pneumoniae* strain KP9 (3×108 CFU/mL, 1mL) was injected into two flasks holding 50 mL of broth. Bacteriophage KPP2/KPP1 (3×107pfu/mL, 1mL) was introduced to the second flask, which served as a control (MOI, 001). Optical density at 600 nm wavelength (O. D600) was used to measure the growth decrease. The growth decline was shown on a graph and contrasted with the control. The experiment was carried out in duplicate and independently (Du *et al.,* 2009).

Results

Bacterial growth on different media

K. *pneumoniae* is a rod-shaped, facultatively anaerobic, encapsulated, lactose-fermenting, gram-negative bacteria that is not mobile. Lactose fermentation caused the *K. pneumoniae* colonies to become pink on MacConkey agar medium. On nutrient agar, however, colonies were large and pale. Colonies were mucoid on both of these media.

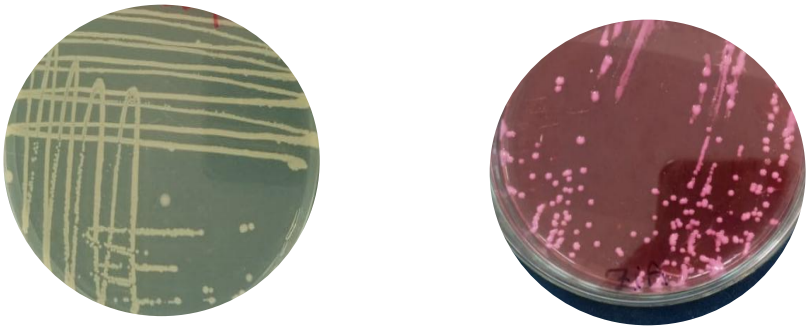


Figure 1: Growth of *K. pneumoniae* on nutrient agar (A) and MacConkey agar (B)

Spot test for the detection of *K. pneumoniae* specific bacteriophages

Bacteriophages against *K. pneumoniae* strain KP9 were detected in two of five sewage samples. KPP2 was the name of the bacteriophage that was acquired from DHQ Shangla, whereas KPP1 was the name of the bacteriophage that was acquired from THQ Shangla.

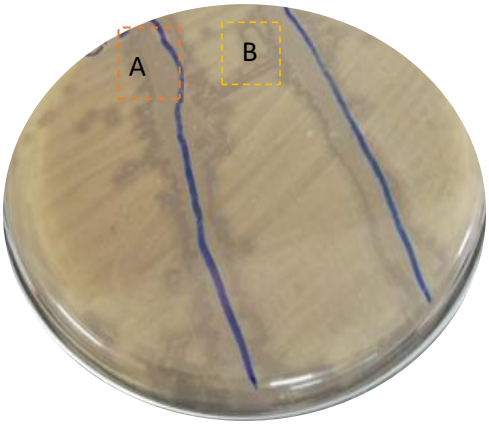


Figure: 2 Detection of bacteriophages through spot test KPP1 (A) and KPP2 (B) spot test

Isolated bacteriophages produced clear transparent plaques

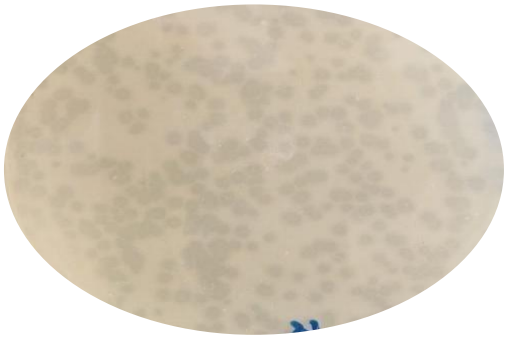
On a double layer agar plate, the isolated phage produced a circular, clear plaque that was antagonistic to *K. pneumoniae* strain KP9. The plaque is composed of two circular layers, with an outer circle around the inner, completely transparent center. The existence of this hazy layer surrounding plaque is a sign that bacteriophages are producing the depolymerase enzyme. KPP2 had a diameter of 2 mm while KPP1 had a diameter of 3 mm. The halo

surrounding the plaque indicates that the bacterial host cell was decapsulated by soluble enzymes such depolymerase, which was produced by phages KPP2 and KPP1.

A B

Figure: 3 Isolated bacteriophages have transparent plaque KPP1 (A) and KPP2 (B)

Isolated phages have high titer: The isolated phages' lytic behavior and promise as a therapeutic agent are demonstrated by the translucent, clear plaques. KPP1 and KPP2 titers were reported to be 4×10^{9} pfu/mL and 2×10^{11} pfu/mL, respectively, following a 24-hour phage propagation period in liquid culture.



B

Figure 4. Titer of purified bacteriophage titer of KPP2 (A) phage titer of KPP1 (B) phage

Isolated bacteriophages were found to have narrow spectrum

It was discovered that the isolated *K. pneumoniae* KPP1 and KPP2 phages were very strain-specific. While no infectivity was seen for the other examined genera, KPP1 and KPP2 bacteriophages were able to infect and create a lytic zone against isolates of *K. pneumoniae*. According to the findings of the host range specificity test, the KPP1 and KPP2 were able to infect four out of eight *K. pneumoniae* and three out of eight *K. pneumoniae*, respectively, but not bacteria from other genera (*E. coli*, *S. typhi*, *Enterobacter, P. aeruginosa*, and *S. aureus*).

Table 1: Host range spectrum of KPP1 and KPP2 bacteriophages against various bacterial cultures

S.No	Bacterial culture	Spot test KPP1	Spot test KPP2	Source
1	*Klebsiella pneumoniae 1*	+	+	Clinical source
2	*Klebsiella pneumoniae 2*	-	-	Clinical source
3	*Klebsiella pneumonia 3*	+	+	Clinical source
4	*Klebsiella pneumoniae 4*	-	+	Clinical source
5	*Klebsiella pneumoniae 5*	+	-	Clinical source
6	*Klebsiella pneumoniae 6*	-	+	Clinical source
7	*Klebsiella pneumoniae 7*	-	-	Clinical source
8	*Klebsiella pneumoniae 10*	-	-	Clinical source
9	*E. coli* isolate	-	-	Clinical source
10	*Salmonella* isolate	-	-	Clinical source
11	*Enterobacter* isolate	-	-	Clinical source
12	*Pseudomonas* isolate	-	-	Clinical source
13	*Staphylococcus aureus*	-	-	Clinical source

The isolated bacteriophages were found thermally stable

The stability of bacteriophages is significantly impacted by temperature. It impacts adhesion, penetration, and proliferation in all aspects of phage replication. It was discovered that the KPP2 and KPP1 phages remained stable at 50ºC without experiencing any titer changes. For KPP1, there was no drop in phage titer at 4 or 37ºC, however there was a 2-fold drop at 25 and 50ºC (Figure 4). At 60ºC, the KPP1 titer decreased 6 times (Figure 4). At 4ºC, there was no titer drop for phage KPP2, but at 25, 37, 50, and 60ºC, there was a decrease of 1, 2, 3, and 5 folds, respectively (Figure 5). Since KPP2and KPP1 phages were stable at 4°C, 25°C and 37°C, respectively, and since the average human body temperature is 37°C, these phages may be readily used if they were to be utilized as a therapeutic agent. Moreover, these phages may be transported without the need for specific heating conditions.

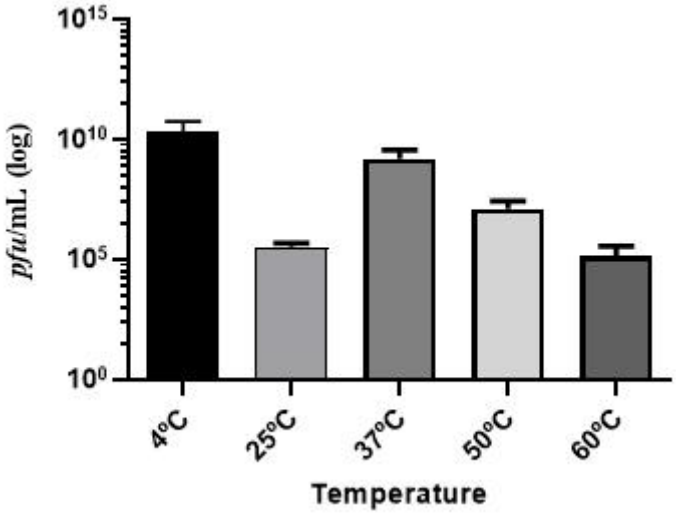


Figure 5. Thermal stability of KPP1 bacteriophage at different temperatures

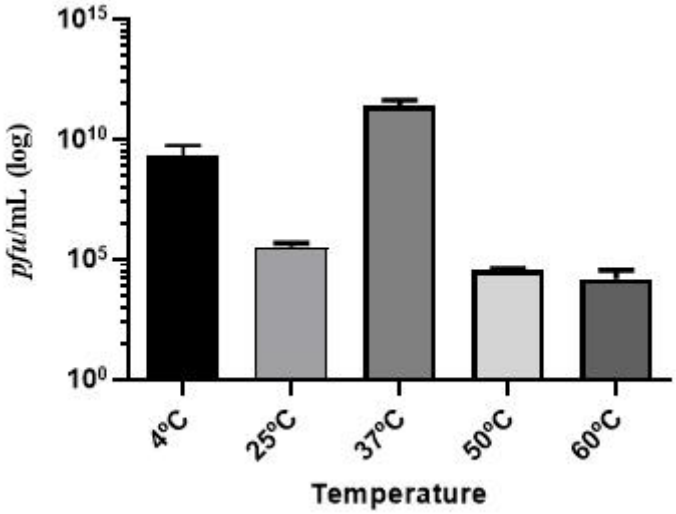


Figure 6. Thermal stability of KPP2 bacteriophage at different temperatures

PH stability of bacteriophages

The environment's acidity and alkalinity have a crucial role in phage stability. After an hour, the KPP2 and KPP1 phages showed resistance to the pH range of 3.0 to 9.0. It was discovered that the ideal pH range for phage KPP1 was 3–9 (Figure 7). Additionally, at pH 9, 8, 6, 5, 4 and 3, a drop of 2, 3, 2, 2, 3 and 4 log were noted, respectively (Figure 7). The ideal pH for phage KPP2 was discovered to be -3-9 (Figure 8). At pH 9, 8, 6, 5, 4 and 3, a 1 log drop of 3, 2, 1, 1, 2 log and 4 log drop, respectively, was noted at pH7 no viability was observed (Figure 8). Very few phages can withstand such a wide pH range.

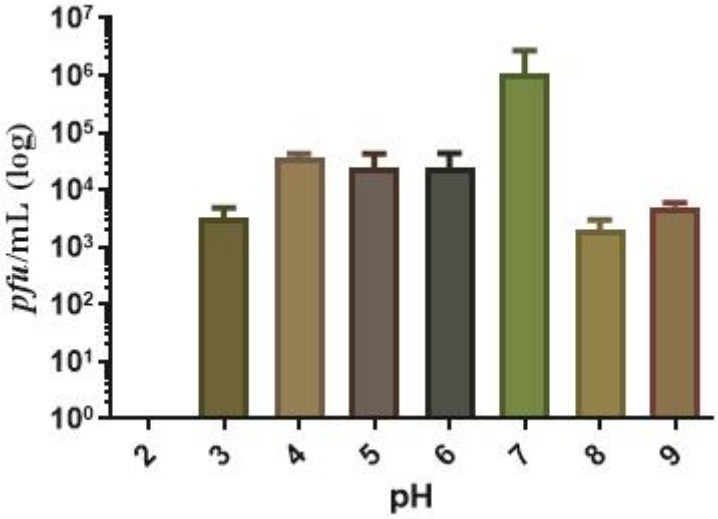


Figure 7. Effect of various pH on the viability of KPP1 bacteriophage

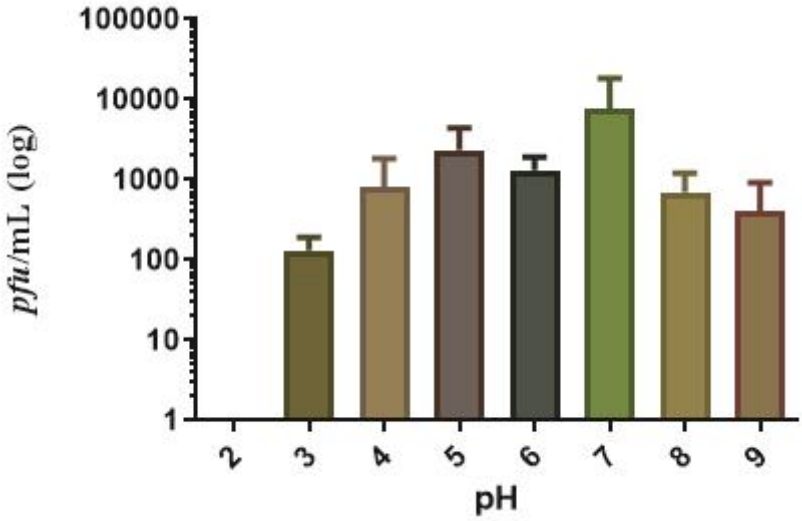


Figure 8. Effect of various pH on the viability of KPP2 bacteriophage

Phages storage at 4°C

After three months of storage, the phage KPP2 and KPP1 titer was determined to be steady. For phage KPP1, there was no titer drop at 4°C, but at 25°C and 37°C, there was a decrease of 1 and 2 log, respectively (Table 2). For phage KPP2, there was no drop in titer at 4°C or 25°C, but after three months of storage, there was a 1 log drop in titer at 37°C.

Table 2. Storage stability of bacteriophage at different temperature

Phage	Titer Before Storage	Titer After Storage		
		4°C	25°C	37°C
KPP1	8×10^{9} *pfu*/mL	5×10^{9}	7×10^{8}	4×10^{7}

KPP2	7×10^{10} *pfu*/mL	5×10^{10}	7×10^{10}	4×10^{9}

KPP2 and KPP1 reduced *K. pneumoniae* growth

The bacterial growth reduction test was used to evaluate the antibacterial activity of the KPP2 and KPP1. Using a spectrophotometer to record the OD600 every two hours for the following twenty-four hours, the capacity of both phages to inhibit bacterial growth was tracked and contrasted with the growth of the control (Figure 9). Up to 18 hours, KPP2 prevented any increase in bacterial growth; but, beyond that time, a little rise in the number of bacteria was noted. The emergence of phage-resistant bacterial mutants may be the cause of this rise in the number of bacteria. Until 24 hours of observation, KPP1 inhibited and reduced the original bacterial inoculum, demonstrating remarkable bacterial reduction abilities (Figure 10). Since these phages are extremely uncommon and no resistant mutants appeared during KPP1 infection, it is possible that the phage uses a variety of bacterial receptors to attach. Although the effectiveness of bacteriophages in reducing bacteria has not been demonstrated, bacteriophages in cocktails have the potential to hinder or limit bacterial development. KPP2 and KPP1 are proposed as a possible phage treatment option based on the suppression of bacterial growth, provided that the required clinical and animal model investigations are conducted.

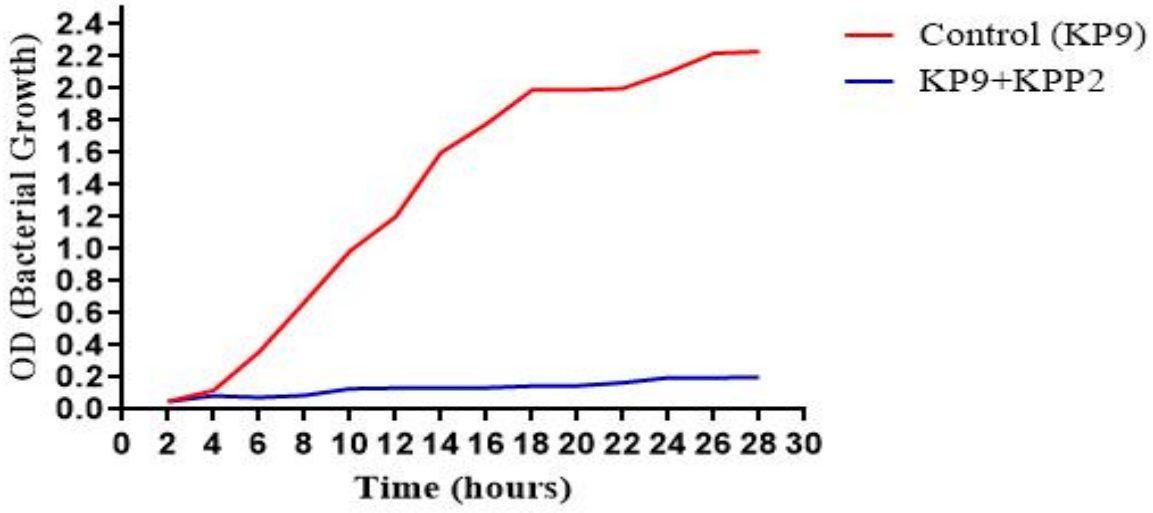


Figure 9. Bacterial growth reduction potential of KPP1 bacteriophage

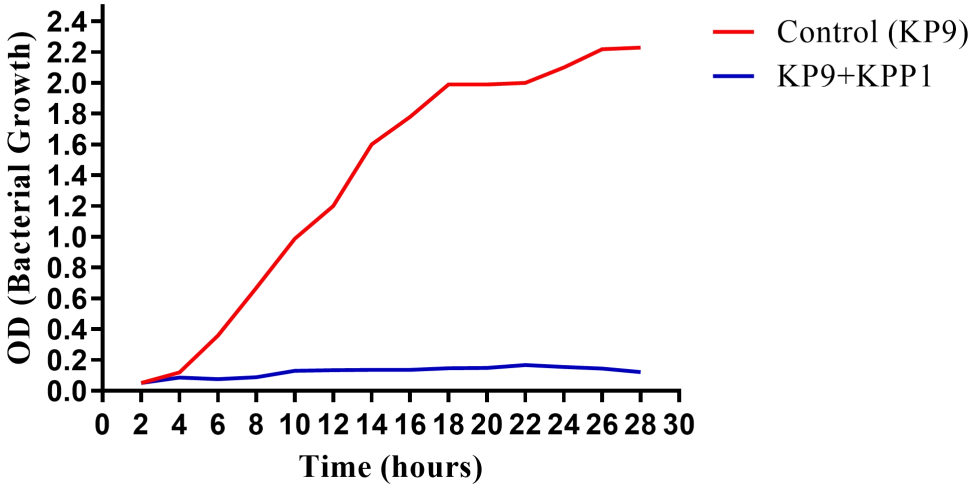


Figure 10. Bacterial growth reduction potential of KPP2 bacteriophage

Discussion

Klebsiella belongs to the family Enterobacteriaceae. The bacteria *Klebsiella pneumoniae* is non-motile, encapsulated, and facultatively anaerobic. Gram-negative rods feature a unique polysaccharide capsule (CPS) and can be organized singly, in pairs, or in short chains. They are 0.3-1 µm wide and 0.6-6 µm long. Between 3% and 5% of all instances of community-acquired pneumonia are believed to be caused by *K. pneumoniae* infections. Furthermore, this bacterium is responsible for a significant percentage of community-acquired diseases worldwide. High rates of morbidity and mortality, along with the potential for metastatic spread, are characteristics of these illnesses (Paczosa and Mecsas, 2016). The multidrug-resistant (MDR) strain of Klebsiella spp. is responsible for a large number of illnesses. However, it has been demonstrated that some Klebsiella species can develop genetic components and mutations that confer virulence traits and/or resistance to antibiotics, ultimately leading to the development of convergent clones called multidrug-resistant Klebsiella species. Bacteriophage treatment is one such tactic that can be used in replacement of antibiotics (Russo and Marr, 2019). Isolating and characterizing lytic bacteriophages against *Klebsiella pneumonia* strains is the aim of the current investigation. Sewage samples were gathered from THQ Poran Shangla, K.T.H. Peshawar, DHQ Shangla, DHQ Peshawar, LRH Peshawar, and Butt Pull Sewage Drain in Mansehra in order to isolate bacteriophages. Two out of five sewage samples had bacteriophages that were effective against K. pneumoniae strain KP9. The virus obtained from DHQ Shangla was known as KPP2, whereas the bacteriophage obtained from THQ Shangla was known as KPP1. Alike study by Rehman *et al.,* (2015) which shows bacteriophages against *K. pneumoniae* strains were

detected in six of fifteen samples from hospital sewage water. The isolated phage produced a distinct, circular plaque on a double-layer agar plate that was hostile to *K. pneumoniae* strain KP9. The plaque is made up of two circular layers, with the inner, fully transparent core surrounded by an outside circle. The presence of this murky layer around plaque indicates that the depolymerase enzyme is being produced by bacteriophages. The diameters of KPP2 and KPP1 were 2 and 3 mm, respectively. The halo around the plaque shows that soluble enzymes such depolymerase, which are produced by the phages KPP2 and KPP1, decapsulated the bacterial host cell. Alike study by Russo *et al.,* (2013) shows on double layer phage KP1 and KP5 produces transparent plaques against *K. pneumoniae* strains.

The transparent, clear plaques show the lytic nature of the isolated phages and their potential as a therapeutic agent. The KPP1 and KPP2 titers were found to be 4×109 pfu/mL and 2×1011 pfu/mL, respectively, after a 24-hour liquid culture phage propagation time. Similar study was conducted by Abedon (2011) which shows phage titer in this range for *K. pneumonia* phage Kp1 and Kp5was 4.1×10^{8}*pfu*/mL and 5.1×10^{8}*pfu*/ml. It was discovered that the isolated *K. pneumoniae* KPP1 and KPP2 phages were very strain-specific. While no infectivity was seen for the other examined genera, KPP1 and KPP2 bacteriophages were able to infect and create a lytic zone against isolates of *K. pneumoniae*. Similar study by (Roy, 2018) demonstrates that the Klpp1 bacteriophage exclusively lysed Klebsiella species and did not infect any other bacteria. The isolated phages' spectrum of infectivity was unique to *K. pneumoniae*, which can be useful in treating infections caused by the bacteria because these phages won't harm normal microflora. Up to 50ºC, the KPP2 and KPP1 phages were shown to remain stable with no change in titer. For KPP1, a 2-fold drop in phage titer was noted at 25 and 50ºC, while no decrease was seen at 4 or 37ºC. At 60ºC, the titer of KPP1 decreased nine times. Phage KPP2 showed no titer decline at 4ºC, but at 25, 37, 50, and 60ºC, the titer decreased by 1, 2, 3, and 9 twofold, respectively. Since KPP2 and KPP1 phages were stable at 25°C and 37°C, respectively, and since the average human body temperature is 37°C, these phages may be readily used if they were to be utilized as a therapeutic agent. Similar study was conducted by Gill and Abedon (2003) which shows *K. pneumonia* phage is active in temperatures ranging from 4 to 60 degrees Celsius. After an hour, the KPP2 and KPP1phages showed resistance to the pH range of 3.0 to 9.0. It was discovered that the ideal pH range for phage KPP1 was 6–8. Additionally, at pH 9, 5, and 4, respectively, a drop of 1, 2, and 3 log was seen. The ideal pH for phage KPP2 was discovered to be 7-8 (Figure 3.8). At pH 9 and 6, a 1 log drop was noted. Nevertheless, a 2 log and 7 log drop, respectively, was noted at pH 5

and pH 4. At pH 2 and pH 3, no viability was observed. Alike study by Abedon (2011) which shows *K. pneumonia* best work at pH. ranges from 5-9.

KPP2 antibacterial activity shown a remarkable capacity to suppress and reduce the initial bacterial inoculum after a 28-hour observation period. The phage may be able to attach itself to a large number of bacterial receptors since these phages are uncommon and there are no resistance mutations present during KPP2 infection. Bacteriophages may prevent or lessen bacterial development in mixtures, but their ability to limit bacterial growth on their own has not been shown. After the required clinical and animal model tests, it is proposed that KPP1 would be a suitable option for phage therapy based on the reduction in bacterial growth. To evaluate KPP2 and KPP1, the bacterial growth reduction test was employed. Using a spectrophotometer, the OD600 was measured every two hours for the next twenty-four hours in order to track both phages' capacity to inhibit bacterial growth and compare it with the control's growth. KPP1 stopped the growth of bacteria for sixteen hours. It has been demonstrated that phages that can prevent *K. pneumonia* from growing might stop the bacterial growth for up to 16 hours (Lin *et al.,* 2017).

Conclusion

Multidrug-resistant bacterial pathogen infections and their consequences are turning into a serious health emergency. It becomes challenging to treat the common infections. Although there are numerous options for alternative therapy techniques, bacteriophages are a powerful substitute. It is possible to separate the bacteriophages from the natural habitat of bacteria. Following the required animal model and clinical trials, bacteriophages KPP1 and KPP2 appear to be a strong contender for phage therapy due to them in vitro effectiveness in inhibiting bacterial growth, stability across a wide pH and temperature range, and long-term storage stability without the use of chemicals

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