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Safety and Efficacy Assessment of Bacteriophages Isolated from Kibera, Kenya Wastewater Plant, against Multidrug-Resistant *Pseudomonas aeruginosa* Infection in BALB/c Mice

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Abstract *Pseudomonas aeruginosa* is a pathogen of great clinical importance to both humans and animals. It causes pneumonia in cystic fibrosis patients, and it is responsible for the infections of blood and lungs during surgery. Increased antibiotic use has led to the emergence of multidrug-resistant strains of *P. aeruginosa*. Recently, phage therapy has attracted much attention as a promising alternative against the increasing antimicrobial resistance. This study determined the safety and efficacy of phage therapy against virulent *P. aeruginosa* in a murine model. Phage PaCIKb2 was isolated from sewage water. Morphological characterization by transmission electron microscope was done. We assessed the phages' antimicrobial effect in vitro and in biofilms, its growth kinetics, host ranges, temperature and pH stability. Therapeutic safety and efficacy were observed 24 hours post-infection with virulent *P. aeruginosa* in a murine model. Transmission Electron Microscopy revealed phage PaCIKb2 to belong to the family myoviridae. The phage was found to be high temperature tolerant (up to 50°C). It was active between pH ranges (5 and 11), had a latent period of 15 minutes with a burst size of 316 viral particles, and exhibited a narrow host range. After intravenous phage administration dose (2×10^9 PFU/ml) post-infection with virulent *P. aeruginosa*, the presence of phages *in vivo* and reduction of bacterial loads in mice was observed. A reduction in mice tissue inflammation suggested the effectiveness of phage PaCIKb2 phage therapy. This research gives data that supports the use of phage therapy against multidrug-resistant *P. aeruginosa*.

Keywords: *phage therapy, MDR-PA, Bacteriophage (Phage), Pseudomonas aeruginosa, antimicrobial resistance, multi-drug resistance*

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1. Introduction

Pseudomonas aeruginosa is a motile Gram-negative bacterium found in water, soil, and other moist environments. The bacterium is pathogenic to animals and humans, causing wound infections, urinary tract infections, and various systemic infections [1]. It is responsible for pneumonia in cystic fibrosis patients, leading to continuous mortality [2].The treatment of diseases caused by *P. aeruginosa* is dependent on the extensive use of antibiotics. The antibiotics are used to kill the bacteria on target but also disrupt the host's normal flora. This antimicrobial agent's continuous use has led to the emergence of multidrug-resistant (MDR) *P. aeruginosa* strains [3]. Findings by Zhao & Hu [4] show that *P. aeruginosa* uses mechanisms such as the formation of biofilms, production of lactamases, and efflux pumps to gain resistance to antibiotics. These mechanisms makes the treatment of bacterial infections difficult. Thus, to address this challenge, new alternative therapies should be developed urgently. Bacteriophages, viruses that devour bacteria, are emerging with lots of promises as one of the potential alternatives. Phages attack specific bacteria, replicate inside the bacteria cells, produce progeny, and make the cell burst to release progeny; hence the bacteria cell is lysed [5]. The use of bacteriophages in treating infections is considered better based on either low or no side effects associated with antibiotics. Phages have desirable qualities such as host specificity, zero toxicity, and a drop in numbers when host bacteria declines [6]. A previous study in Kenya by Ochieng'Oduor et al. [7] showed that phages against MDR – *Staphylococcus aureus,* intraperitoneally administered into BALB/c mice spread to all organs, and the mortality of mice inoculated

with MDR –*S. aureus* decreased. Phage therapy has also shown promising findings in cases of infections caused by *P. aeruginosa.* However, only specific phages make for viable therapeutic agents. Phages with a vast host range, stable in physiological environments with large bursts and short latent periods, are necessary. In this study, we isolated and characterized two phages specific to *P. aeruginosa* clinical isolate. We further investigated the potential of phages in inhibiting and removing biofilms and performed an experiment investigating the safety and efficacy of the phages in treating MDR-*P. aeruginosa* –infected BALB/c mice.

2. Materials and Methods

2.1. Bacterial Strain

Clinical isolates of *P.aeruginosa* were obtained from archived bacterial samples at Kenya Medical Research Institute (KEMRI). Stocks of the *P. aeruginosa* strain were stored at -80°C in Tryptic Soy Broth (Tryptone 17g, Peptic digest 3g, Glucose 2.5g, NaCl 5g, Dipotassium phosphate 2.5g, in 1L of distilled water) containing 50% glycerol.

2.2. Enrichment, Isolation, and Purification of Phages

P. aeruginosa clinical isolate was used as a host bacterium for phage isolation. Water samples collected from the Kibera sewage plant in Nairobi, Kenya, and archived at IPR were utilized for the Isolation. Sixty ml of the sewage sample was centrifuged at 10,000g for 10 minutes to eliminate the debris. Two ml of the host bacteria and ten ml of the supernatant were placed in a sterile centrifuge tube. An equivalent volume of the double strength TSB supplemented with $CaCl_2$ (1mmol L^{-1}) was added to the tube. The constituents were then incubated for 24 hours at 37°C with shaking (120rpm). After 24 hours, the contents were centrifuged at 10,000g for 10 minutes. The supernatant was then ultra-filtered using a 0.22mm filter (ChmLab). Spot assay test was used to confirm phage presence in the filtrate. A plaque assay was performed to determine the plaque-forming units (PFU/ml). For phage purification, single plaques were sub-cultured five times. The phage obtained was designated Pa/CI/Kb2. The pure phage was resuspended in SM buffer (NaCl 5.8g, MgSO4 .7H2O 2g, Tris-Cl 1ml/L^{-1}, and 2% gelatin 5ml in 1L of distilled water, pH 7.5). The purified phages were then stored at 4°C for further use.

2.3. Characterization of Phage PaCIKb2

The Transmission electron microscope of the University of Leicester Core Biotechnology services facility was used to scan the images of the PaCIKb2 phage. Briefly, the phage samples were stained with 1% aqueous uranyl acetate and then viewed on a JOEL JEM-1400 TEM with an acceleration voltage of 120kV and images taken by an EMSIS Xarosa digital camera with radius software NSA. Thermal and pH stability tests were performed. A phage with a high titer (2×10^9 pfu/ml) was incubated for 2 hours at 4°C in pH ranges (3, 5, 7, 11, 13). Another was incubated for sixty minutes at pH7 in temperatures (4°C, 37°C, 40°C, 50°C, and 80°C) after the phage titers were determined by performing plaque assays

A single-step growth experiment was conducted according to a method described by Dydecka et al., [8] with slight modifications. First, an overnight growth culture of host bacteria (PA) was added with phages to attain an MOI (Multiplicity of Infection) of 0.1. The sample was then incubated on ice for 10 minutes to promote phage-bacterium adsorption. The mixture was then centrifuged at 11,000g for 5 minutes. (Cence[R] TGl-16M) to remove any unbound phage. Next, the pellet was resuspended in Tryptic soy Broth by adding 10 ml TSB. One ml aliquots were placed in Eppendorf tubes labeled different time points 0s, 30s, 1min, 5min, 10min, 15min, 30min, 60min, 90min, and 120 min. The phage titer for each time point was determined by a plaque assay method.

2.4. Phage PaCIKb2 Bactericidal *in vitro* Determination

The bactericidal effect of phage Pa/CI/Kb2 was analyzed by a method described by Cao et al., [9] with slight modifications. First, overnight cultures of *P.aeruginosa* were added into a new media ratio of 1:100 and incubated at 37°C with shaking (150rpm) until OD600 of 0.3 (1×10^8 CFU/ml) was attained. Next, different phage concentrations were added to other tubes containing host bacteria culture to achieve different MOIs (0, 0.1, 0.5 and 1). The tubes were then further incubated for 24 hours at 37°C with shaking (rpm). OD_{600} measurements of the bacterial culture were measured at the end of the 24 hours.

2.5. Phage PaCIKb2 Antibiofilm Activity to MDR-*P. aeruginosa* Biofilms

The procedure was done according to [10] for antibiofilm assay with slight modifications. MDR-*P. aeruginosa* were grown in TSB media; a 1:10 dilution of overnight bacterial culture was incubated at 37°C up to an OD600 =0.1. Twelve well plates were prepared in 4 replicates, and 1ml of the growing bacteria was added into six wells of each plate. One ml of the TSB broth was then added into the other three wells of each plate as a negative control. Two of the plates were incubated for 24 hours and the other two for 48 hours at 37°C and 100rpm. After the incubation period, the specimen was discarded. The wells were washed three times with Phosphate-Buffered Saline (PBS) to remove all the planktonic cultures. Five hundred ml of the phage Pa/CI/Kb2 (1×10^8 PFU/ml) were added into three wells of each 12 plate well plate. The control samples got TSB broth media (500ml) without bacteriophages. The plates were then incubated for further 24 hours with shaking 100rpm. After incubation, the samples were discarded, and the wells were washed three times using Phosphate –Buffered Saline. PBS was added for CFU counts of the biofilm. Scrubbing the bottom of the wells was done using a sterile loop to detach the biofilms and resuspend them in PBS. A ten-fold serial dilution was conducted, and 100ml of the dilutions were plated on TSA media and incubated at 37°C for 24 h. After growth, the plates containing colonies between 30 and 300 were

selected for CFU quantification. They were counted and expressed as colony-forming units per ml (CFU/ml).

2.6. Study Animals

BALB/c mice aged 6-8 weeks were purchased and maintained in the Institute of Primate Research (IPR) animal facility in Nairobi, Kenya. All animal procedures were conducted according to the guidelines approved by the Institutional Review Committee of IPR (SERC 02/21).

2.6.1. Study Animal Sample Size Determination

According to Arifin & Zahiruddin, [11] animal sample size calculation was done by Group comparison one-way ANOVA. For one-way ANOVA, the acceptable range of degrees of freedom (DF) is between 10 and 20, and the number of subjects per group is calculated as **n = DF/k + 1**, where n = number of subjects per group, k = number of groups. For this study, five groups of mice were formed, phage-infected group, Non-phage infected group, MDR-*P. aeruginosa* infected group, MDR-*P. aeruginosa* infected, and antibiotic-treated group, MDR-*P. aeruginosa* infected, and phage treated group. Based on the acceptable range of DF, we used DF (20) to obtain the maximum number of mice per group. Therefore to get mice to be used, **n=20/5+1 =5 mice.** Therefore, five mice per group were used, making the total number of mice used to be twenty five (25).

2.6.2. Experimental Design

The experimental mice were assigned into three groups: The MDR-*P. aeruginosa* infected group (n=15), The bacteria non-infected group(n=5), and the phage infected group (=5). The 15 mice in the MDR-*P. aeruginosa* infected group were induced with *P. aeruginosa* bacteria intravenously. The group was then divided into three sub-groups; Phage treated group (10^8 pfu/ml) (n=5), Clindamycin treated group (8mg/kg body weight) (n=5), and a non treated group (n=5). Treatment with the phage was done after 24 hours of bacterial inoculation. Each mouse received a single dose of the mentioned treatments. The mice were monitored for ten days. The health status of the mice was monitored daily and scored on a scale of 0-5 according to [12], as shown in Table 1. At the end of the ten days, the mice were euthanized, blood and organs (Brain, kidney, liver, and lungs) were obtained aseptically. The blood and some tissue homogenates were used in determining colony-forming units and plaque-forming units. Tissue homogenates were diluted with sterile saline in the ratio of 1:20. The diluted homogenized tissues were then plated on Cetrimide agar to select for MDR- *P aeruginosa* and incubated at 37°C for 18 hours. Other tissues were kept in formalin for histo- pathological analysis.

Table 1. Health status scores of experimental mice

Scores	Clinical signs
5	Normal health state
4	Slight sickness; ruffled hair, and lethargy
3	Moderate sickness; ruffled hairs, lethargy, and hunched back
2	Severe sickness; ruffled hair, lethargy, hunched back, and exudate at the eye
1	Near-death state
0	Death state

2.7. Histopathological Analysis

Histological analysis was done according to [13] with slight modifications. First, tissues from the organs harvested were fixed in 10% formalin for one week. After fixation, the specimens were processed in paraffin wax and sectioned at 6μm thickness), then stained with hematoxylin and eosin stains, and were mounted and finally viewed under a microscope.

2.8. Statistical Analysis

The levels of blood bacteria (CFU/ml) and Phage (PFU/ml) and the physical appearance scores were collected. The collected data were entered into Microsoft Office Excel 2010 software. Analysis was done by GraphPad Prism 9.0.0 (Graphpad software version 2020). The differences between and within the groups and physical appearance scores were analyzed by the two–way analysis of variance (ANOVA) with Bonferroni post-hoc tests. A p-value $p<0.05$ was considered statistically significant.

3. Results

3.1. Morphological Study of the Isolated Phage PaCIKb2

The isolated phage formed clear plaques on a lawn of MDR –*P aeruginosa* cultured on a Tryptic soy Agar plate. (Figure 1c). In addition, when viewed under Transmission Electron Microscope, the phage was identified as a virion with an approximate length of 200nm, with an icosahedral head approximately 50nm long and a tail about 100nm long. The phage tail had a collar and short spikes, indicating that the phage PaCIKb2 belongs to Myoviridae. (Figure 1a, Figure 1b).

3.2. Phage PaCIKb2 Characterization

In this study, Phage Pa/CI/Kb2 showed resilience at temperature +4°C to 50°C, but at 60°C and 80°C, the phage was completely inactivated ($p<0.05$) (Figure 2a). In addition, Phage Pa/CI/Kb2 was active within (pH 5 and pH 11) but was inactivated at extreme pH (2 and 13) (Figure 2b). The One-step growth curve of phage Pa/CI/Kb2 revealed a latent period of 15 minutes and a burst size of 316 viral particles per infected cell. (Figure 3a)

3.3. The Bactericidal Effect of Phage Pa/CI/Kb2 against *P. aeruginosa* In Vitro

To determine the bactericidal ability of the phage to *P. aeruginosa in vitro*, The optical density (OD) at 600nm of bacteria interacting with phage Pa/CI/kb2 for 24 hours was observed. The control group (MOI=0) had the highest OD, but then the others had lower optical densities depending on their MOIs ($p<0.05$) (Figure 3b). Since the optical density of the control group (MOI=0) was higher than that of the phage+ bacteria groups, we concluded that phage Pa/CI/Kb2 is lytic to *P. aeruginosa* In vitro (Figure 2b).

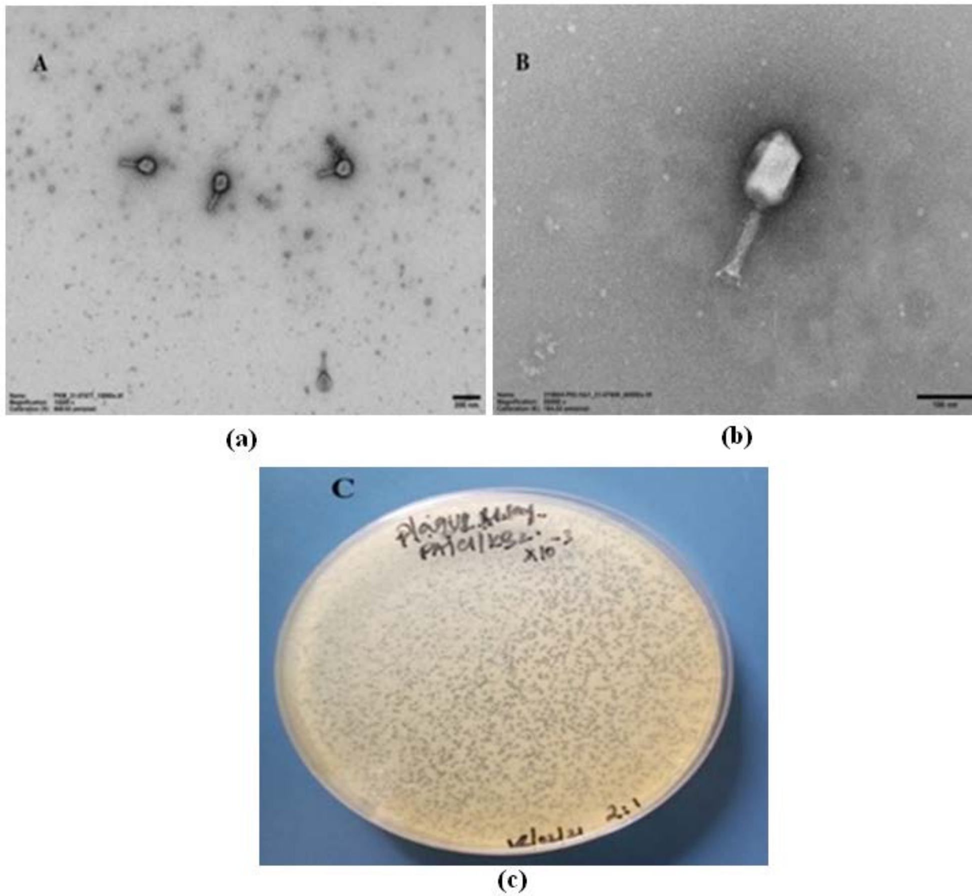

Figure 1. Morphology of *P aeruginosa* phage PaCIKb2: **(a)** Electron micrographs of phage PaCIKb2 at 1500× magnification. **(b)** Electron micrograph of phage Pa/CI/Kb2 at 60000× magnification. **(c)** Plaque morphology of phage Pa/CI/Kb2

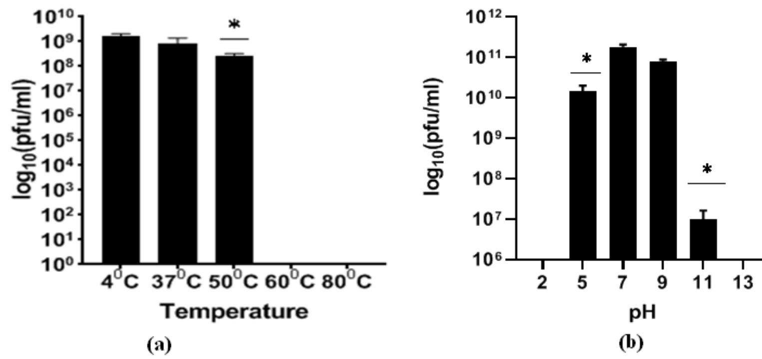

Figure 2. The Stability of phage PaCIKb2 at different (a) Temperatures (b) pH. Error bars indicate the standard deviation of three independent experiments. **p*<0.05 statistically significant between test assays and controls (+4°C or pH 7)

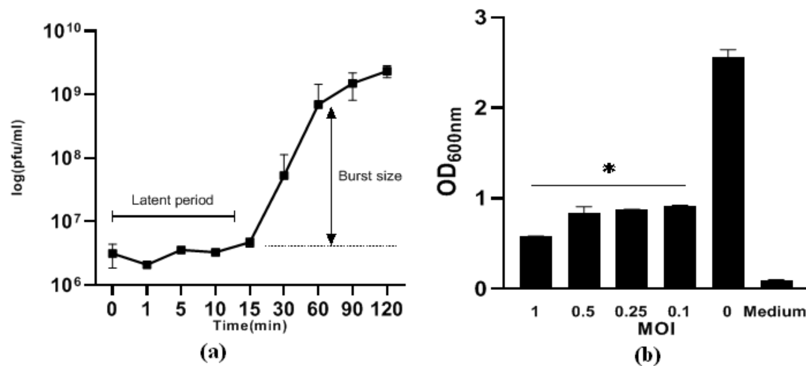

Figure 3. (a) Phage PaCIKb2 one-step growth curve. Bacteriophages showed a latent period of about 15 min and a burst size of 316 viral particles per infected cell. **(b)** The lytic ability of phage PaCIKb2 *in vitro*. *P. aeruginosa* (2×10^8CFU mL^{-1}) was infected with the phage at different multiplicities of infection. Error bars indicate the standard deviation of three independent experiments. **p*<0.05 statistically significant between test assays and control (MOI=0)

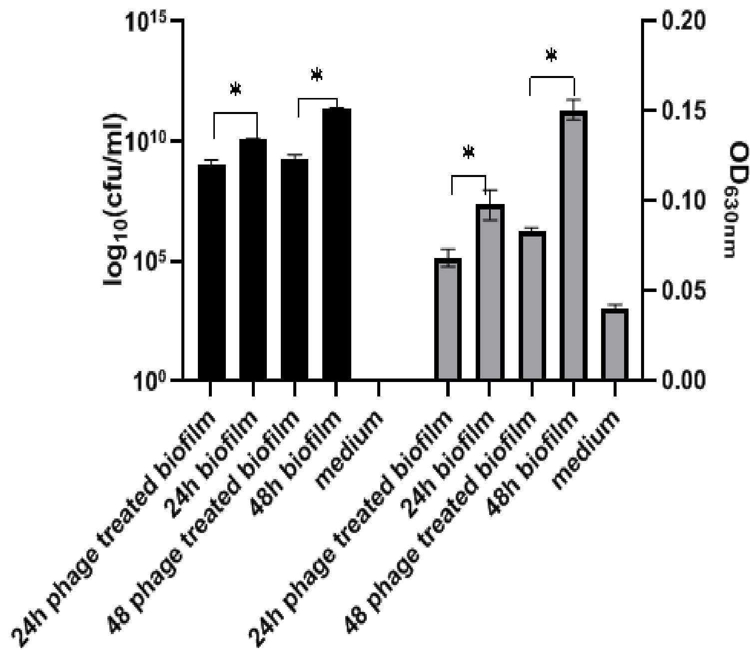

Figure 4. Effect of phage PaCIKb2 on 24h and 48h biofilm formed by MDR-*Pseudomonas aeruginosa*, after 24h of interaction between bacteria and the phage. The anti-biofilm activity of the phages on biofilm viability was expressed in $\log_{10}$ CFU/ml (left y-axis), and the metabolic activity was expressed in absorbance at OD_{630nm}(right y-axis). The error bar indicates experiments done in triplicates. * Significance level (p<0.05) between test assays and controls (24h biofilms without phage or 48h biofilms without phage)

3.4. Efficacy of Phage PaCIKb2 to Control Biofilms of MDR-*Pseudomonas aeruginosa*

In this experiment, the previously isolated virulent phage Pa/CI/Kb2 was investigated on how effective it is in controlling 24 hours and 48 hours biofilms of MDR-*Pseudomonas aeruginosa* formed on well plates. The CFU and OD_{630} were used to quantify the assay. The bacteriophage was able to inhibit the growth of biofilms (p<0.001). It reduced the 24-hour biofilm by 1.5 log $CFUml^{-1}$ and by OD_{630}=0.04 (p<0.05). (Figure 4) The phage also reduced the biofilm of the 48-hour biofilm by 3 log CFUml-1 and by OD630=0.09 (p<0.05) (Figure 4).

3.5. Phage Safety

The health scores showed that phage Pa/CI/Kb2 are not pathogenic or toxic to mice since the phage-treated group maintained normal health throughout the experiment. The health of mice in groups (non-infected and phage infected) was significantly different from MDR-PA infected mice groups (*p*< 0.001). The mice in MDR-PA phage treated and MDR-PA antibiotic-treated groups improved their health scores (*p*<0.05). The mice in group MDR-PA non-treated groups' health scores declined to the minimum (*p*<0.05) (Figure 5). Mice in groups; non-infected, phage infected, and MDR-PA-Phage treated had 100% survivorship, unlike groups; MDR-PA non-treated and MDR-PA antibiotic-treated whose percentage survival dropped (*p*<0.001) (Figure 6).

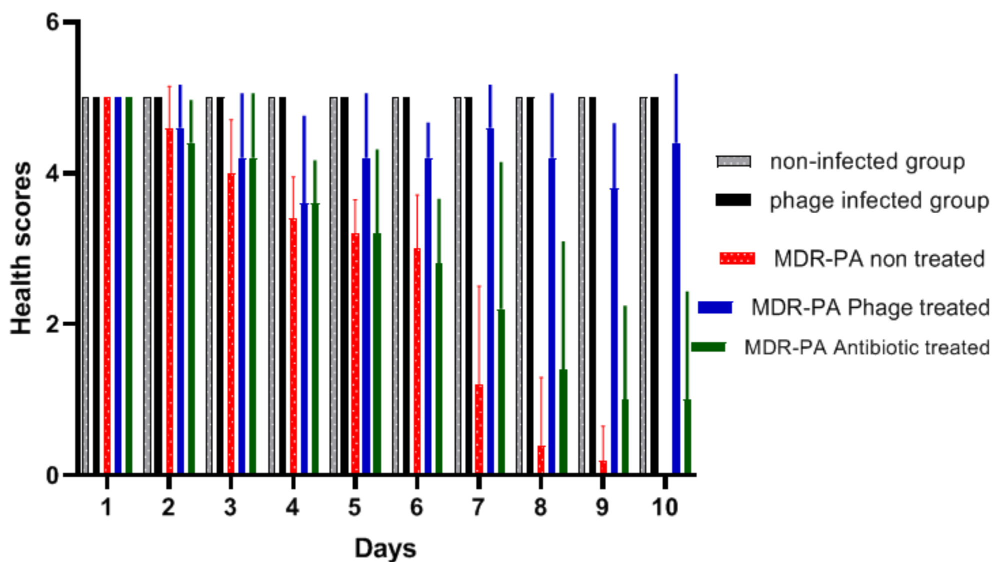

Figure 5. Health scores of mice treated after 12 hours of bacteria inoculation

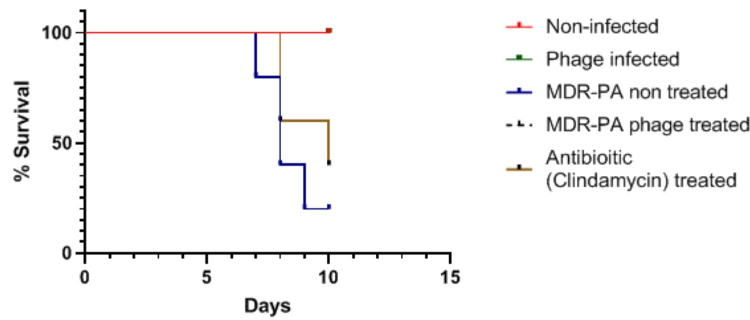

Figure 6. Survival rates of mice treated after 12 hours of bacterial inoculation ($p<0.001$)

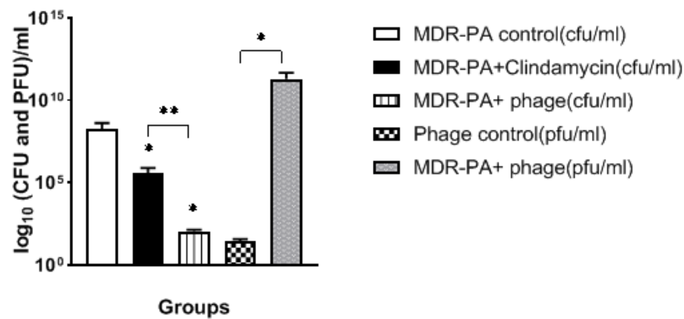

Figure 7. Bacteremia and viremia levels from infected mice, MDR-PA received no treatment, and phage control had no bacteria. Each group had five mice. Significance levels* $p < 0.05$ and ** $p<0.001$

3.6. The Therapeutic Effects of Phage PaCIKb2 in Mice

The bacteremia and viremia levels were used to assess the therapeutic potential of Phage Pa/CI/Kb2. The bacteremia level of the MDR-PA infected, the non-treated group was highest compared to MDR-PA infected groups ($p<0.05$), and its viremia level was the lowest compared to infected phage groups ($p<0.05$). The bacteremia level was significantly lower in the group of mice MDR-PA, phage treated compared to MDR-PA, infected non treated, and MDR-PA antibiotic-treated groups ($p<0.05$). The differences in bacteremia level between MDR-PA antibiotic-treated and MDR-PA phage treated were also statistically significant ($p<0.001$) (Figure 7).

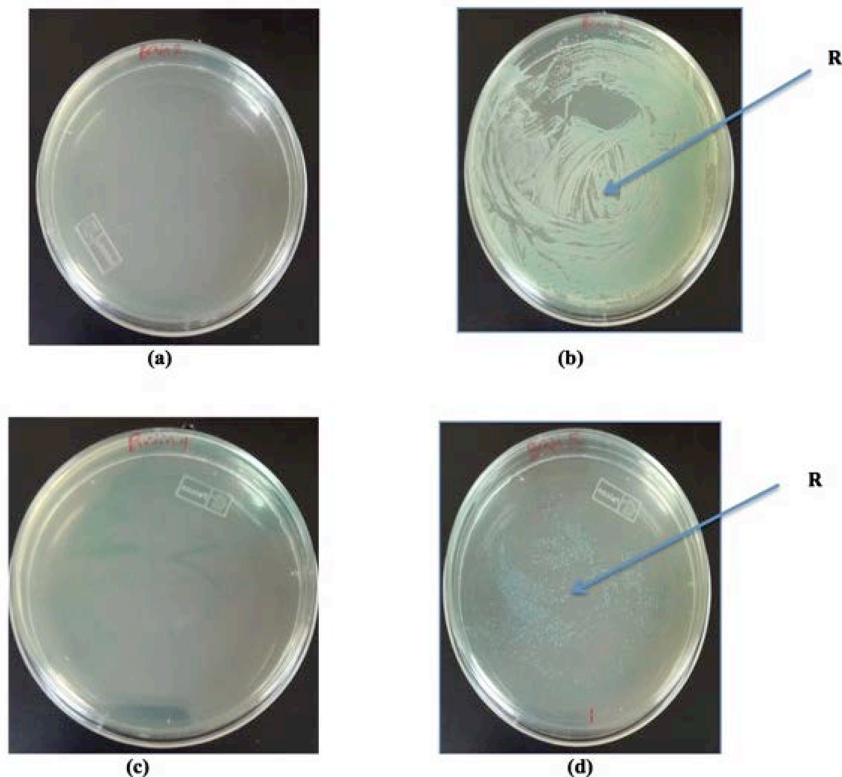

Figure 8. Brain homogenate plated on Cetrimide Agar; **(a)** Homogenates from MDR-PA non-infected groups. (**b**) MDR-PA non-treated group (**R**- *P aeruginosa* bacteria colonies). (**c**) Culture from MDR -PA infected, phage treated group (**d**) Antibiotic treated MDR-PA infected group homogenate. (**R**- bacteria colonies)

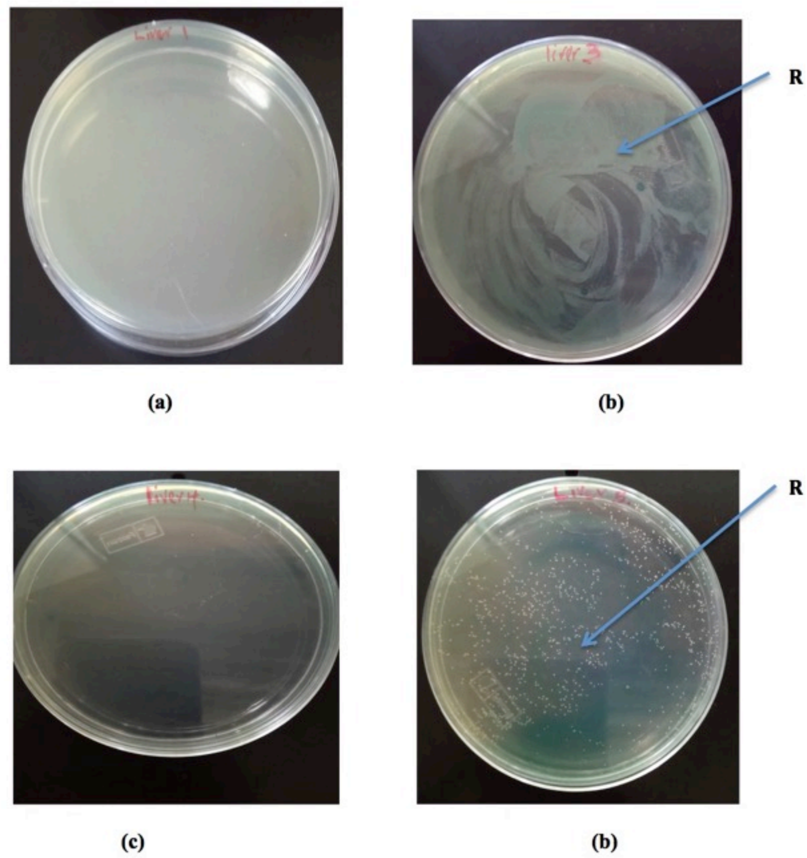

Figure 9. Liver homogenate plated on Cetrimide Agar; (a) Homogenates from MDR-PA non-infected groups. (b) MDR-PA non treated group (R- *P aeruginosa* bacteria colonies). (c) Culture from MDR -PA phage treated group (d) Antibiotic (clindamycin) treated MDR-PA infected group homogenate. (R- bacteria colonies)

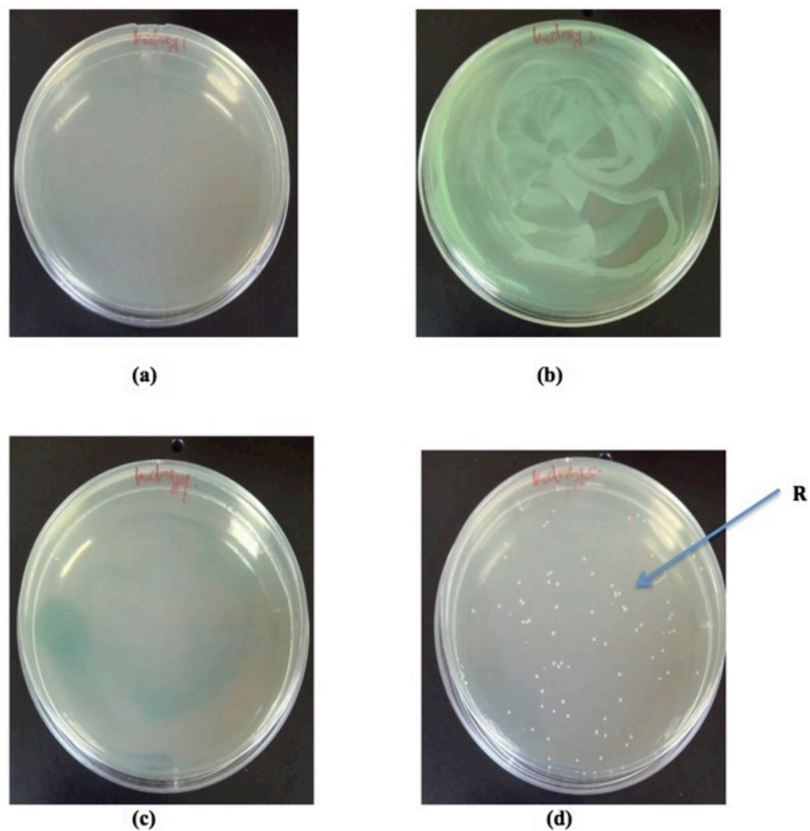

Figure 10. Kidney homogenate plated on Cetrimide Agar; (a) Homogenates from MDR-PA non-infected groups. (b) MDR-PA non treated group (R- *P aeruginosa* bacteria colonies). (c) Culture from MDR -PA phage treated group (d) Antibiotic (clindamycin) treated MDR-PA infected group homogenate. (R- bacteria colonies)

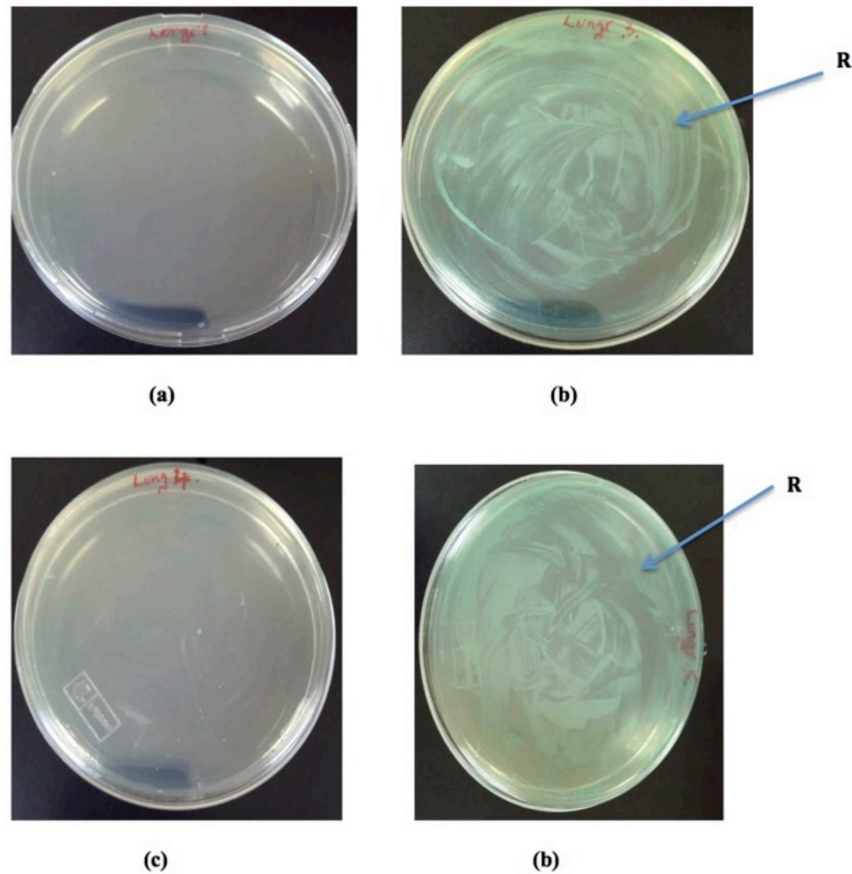

Figure 11. Lungs homogenate plated on Cetrimide Agar; (a) Homogenates from MDR-PA non-infected groups. (b) MDR-PA non treated group (R- *P aeruginosa* bacteria colonies). (c) Culture from MDR -PA phage treated group (d) Antibiotic (clindamycin) treated MDR-PA infected group homogenate. (R- bacteria colonies)

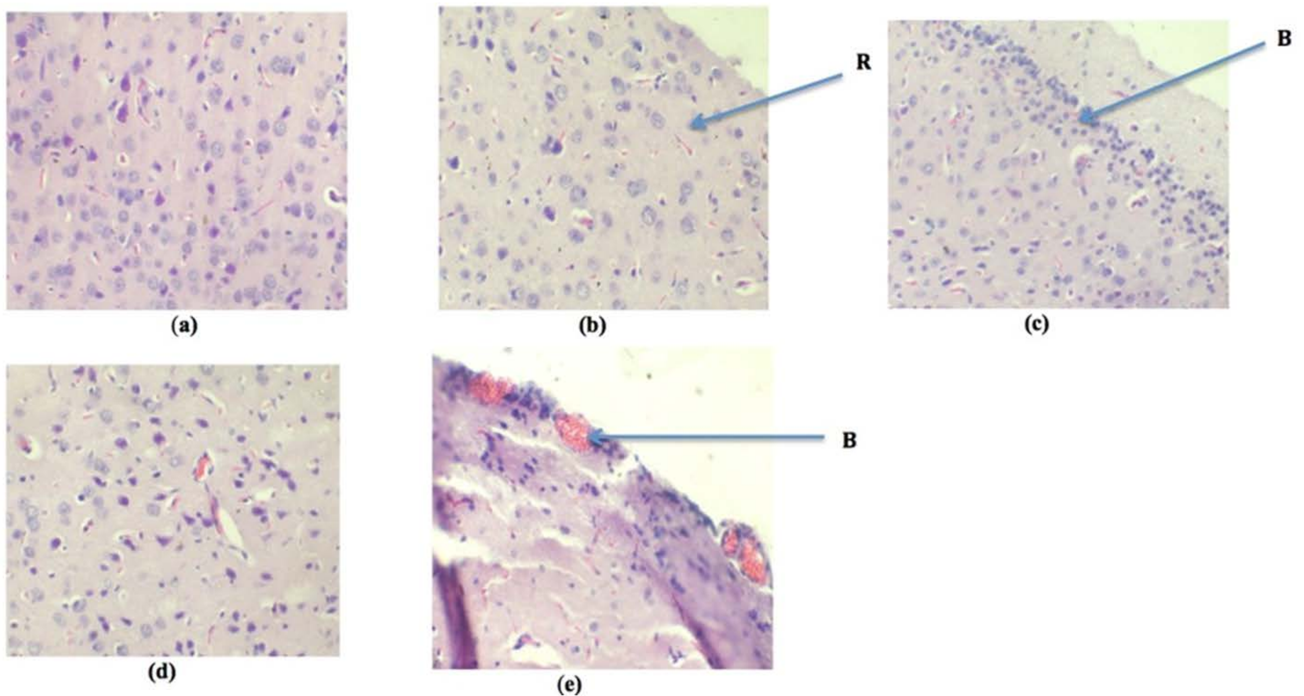

Figure 12. Hematoxylin and eosin-stained Brain tissues; (a) Non-infected group, (b) Phage infected, (c) MDR-PA non treated group B- Lymphocytic infiltration (inflammation), (d) MDR-PA Phage treated group (e) MDR-PA antibiotic (Clindamycin) treated group B- Lymphocytic infiltration. Magnification= ×400

The MDR-PA isolated from tissues homogenate after the end of the ten days showed colonies on selective agar for *Pseudomonas aeruginosa,* Cetrimide Agar. The MDR-PA, phage treated formed no colonies on cetrimide agar. MDR-PA

non-treated had more colonies than MDR-PA antibiotic treated in many of the tissue homogenates. Groups without Bacteria were used as controls. (Figure 8, Figure 9, Figure 10, Figure 11).

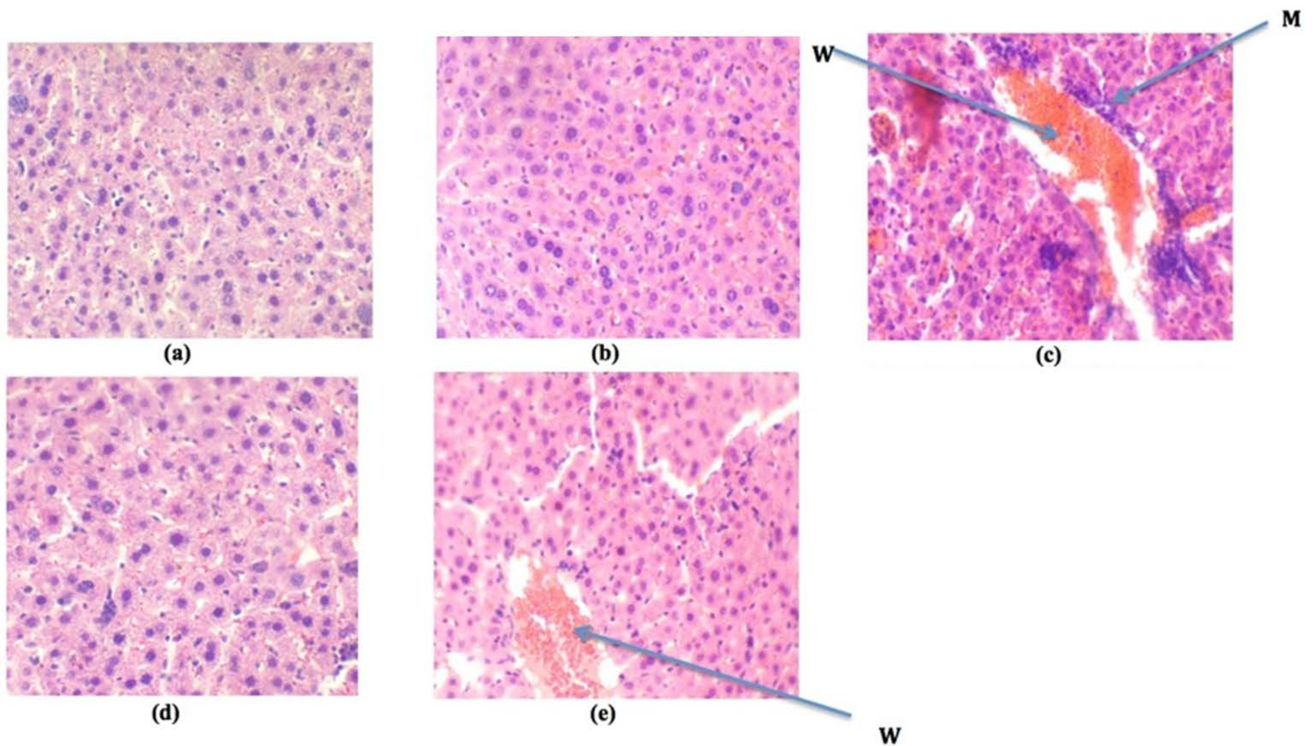

Figure 13. Hematoxylin and eosin-stained Liver tissues; (a) Non-infected group, (b) Phage infected, (c) MDR-PA non treated group M- Perivascular lymphocytic infiltration (inflammation), W- Severe congestion (d) MDR-PA Phage treated group (e) MDR-PA antibiotic (Clindamycin) treated group W- Severe congestion Magnification= ×400

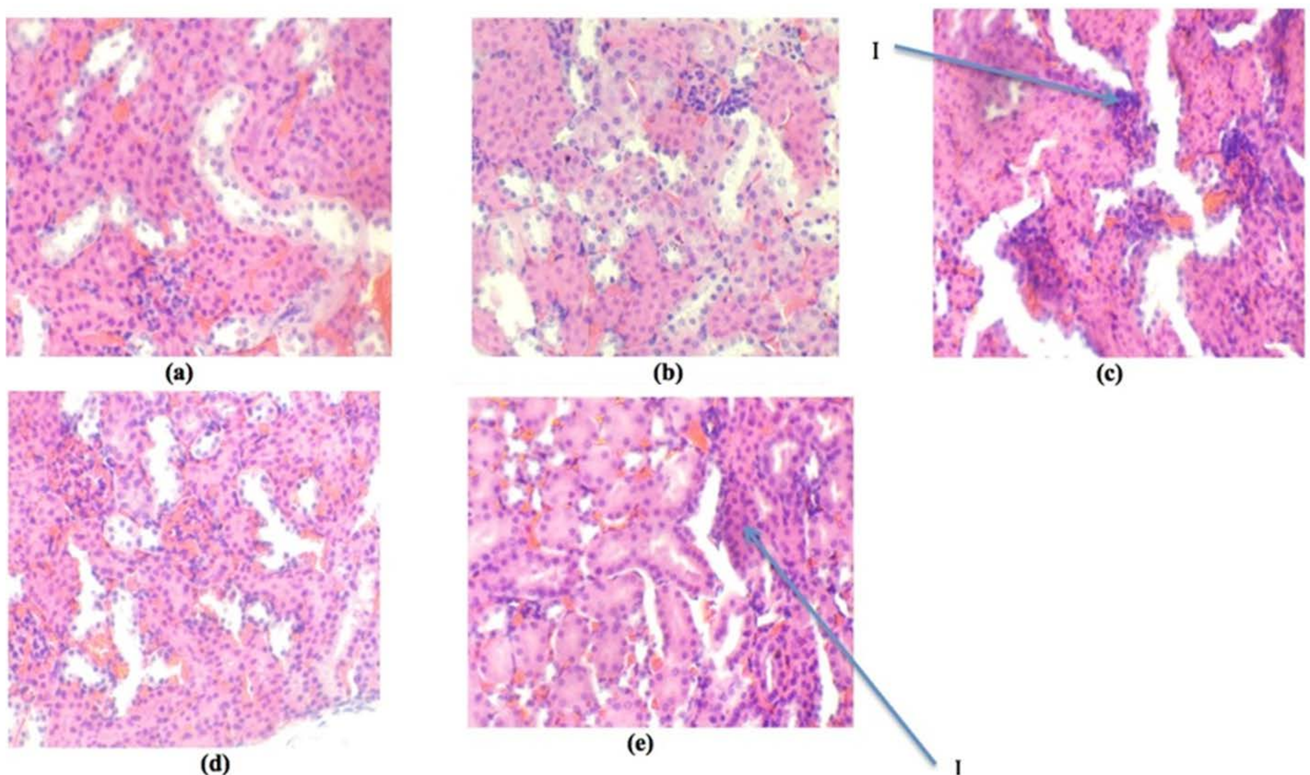

Figure 14. Hematoxylin and eosin-stained kidney tissues; (a) Non-infected group, (b) Phage infected, (c) MDR-PA non treated group I- Inflammatory cell infiltrate. (d) MDR-PA Phage treated group (e) MDR-PA antibiotic (Clindamycin) treated group I- mild inflammatory cell infiltrate. Magnification= ×400

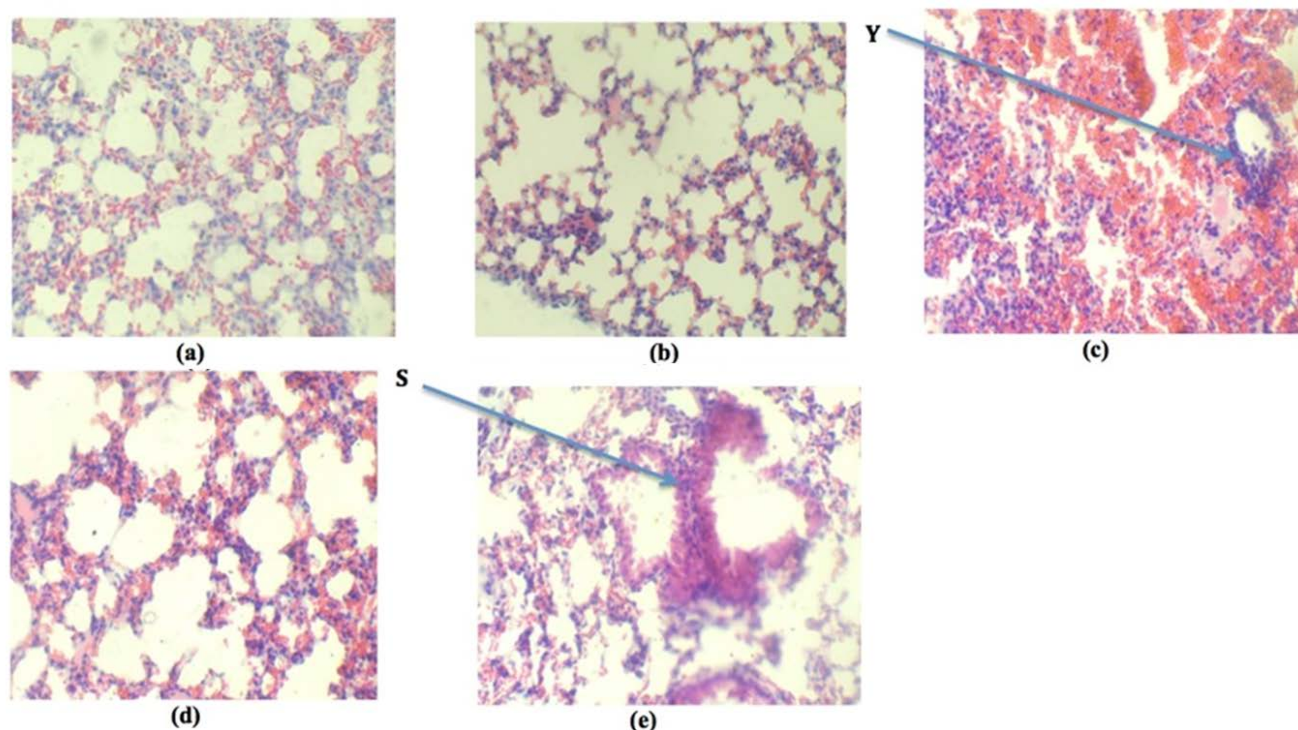

Figure 15. Hematoxylin and eosin-stained Lung tissues; (a) Non-infected group, (b) Phage infected, (c) MDR-PA non-treated group; Y- lymphocytic infiltrated septae. (d) MDR-PA Phage treated group (e) MDR-PA antibiotic (Clindamycin) treated group; **S-** Perivascular fibrosis. Magnification= ×400

3.7. Histopathology of Tissues

Haemotoxylin and eosin stain, histopathological analysis of tissues obtained (brain, liver, lungs, and kidney) showed that MDR- PA bacteria affected tissues. Organs of MDR-PA non-treated group and MDR-PA phage treated groups depicted severe inflammation while organs non-infected, Phage infected groups, and MDR-PA phage treated showed no inflammation. (Figure 12 - Figure 15).

3.8. Discussion

Nosocomial infections caused by P. aeruginosa are a severe public health problem [14]. Despite extensive research in antimicrobial therapy, the morbidity and mortality of these infections are still high and uncontrollable. In addition, the emergence of resistance of this bacterium to antibiotics worsens the situation. P. aeruginosa has been reported to be multidrug-resistant since it is resistant to at least three classes of antibiotics (ceftazidime, imipenem, ciprofloxacin and gentamicin) [3]. Now that the use of antibiotics is associated with resistance, Phage therapy is considered an alternative to treat these infections [15]. In this study, we isolated a novel phage Pa/CI/Kb2 to control infections of MDR-P. aeruginosa, A clinical isolate that is predominant in Kenya.

According to Ackermann [16], classification shows that tailed phages, the Caudovirales are the most abundant phages. They comprise phage families; Myoviradae, characterized by contractile tails; Podoviridae that contain short tails; and Siphoviradae that have long and non-contractile seats. As revealed by electron microscopy, phages PaCIKb2 contained contractile hence are Myoviridae.

The *in-vitro* study showed that phages PaCIKb2 are stable between temperature and pH ranges, supporting physiological processes. Its effectiveness against P. aeruginosa was ascertained with a short latent period and large burst size. The phage formed clear plaques on lawns of 3 clinical strains and one standard strain (ATCC 27583) of P. aeruginosa. It did not form plaques on the lawns *of staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumonia* bacteria, confirming previous studies showing phages are highly specific to the host target. [17].

In vivo study was done by an intravenous injection of 10^8 CFU/ml P. aeruginosa bacterial titer in 100ml suspension and treatment with 10^9 pfu/ml in 100ml phage PaCIKb2 suspension. Speck and Smithyman, [18] suggest a single intravenous route is safe and more effective in phage treatment. For phages to be sufficiently effective, it was found that their concentration should be way higher than that of bacterial titer inoculated to attain the most increased MOI. Higher phage doses ensure rapid bacteria-killing hence increasing the survival rate [19]. In vitro phage infection showed increased bacterial elimination in higher MOI than lower MOIs. The mice treated with phages alone after 12 hours survived and acted like the non-infected mice, and the bacteremia level was reduced drastically after ten days. This showed the safety and efficacy of the isolated phages. Phage alone infected groups had a 100% survival rate, unlike the MDR-PA infected and non-treated groups with a 20% survival rate indicating the phage were non-toxic or pathogenic in mice. Histopathological analyses revealed that tissues of mice from the non-infected group and MDR-PA-infected phage treated showed no pathological damages, showing that phage PaCIkb2 has therapeutic qualities against MDR-PA similar to study done by [20]. Tissues (brain, lungs, kidney, and liver) harvested from these groups had no pathological changes**.** Tissues from the MDR-PA, non-treated, and MDR-PA clindamycin treated groups showed inflammation.

The results show that a single treatment of phage PaCIKb2 (10^8 pfu/ml) eliminated bacteria from mice, unlike a single dose of Clindamycin (8mg/kg) that did not eliminate bacteria**.** The study, therefore, suggests that phage PaCIKb2 can be used as an alternative to antibiotics used in treating MDR-PA.

3.9. Conclusion

This study isolated a novel phage PaCIKb2 specific to MDR- *P. aeruginosa* from Kibera, Kenya sewage water. The phages isolated belong to the family myoviridae. They have a broad pH and thermal stability range, short latent period, and are effective in treating *P. aeruginosa* infection in mice. The study shows that phages can potentially treat MDR bacteria infections as an alternative to current antibiotics that have failed in treating these infections. Further research is needed to ensure that more phages are isolated, genetically characterized, and commercialized for therapeutic use.

Authors' Contributions

MTO performed all practical experiments and wrote the manuscript. AN contributed with his experiences in phage isolation and characterization and provided the lab to conduct investigations. JM and AKN supervised the work. The final manuscript was read and accepted by all co-authors.

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