ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 07, Issue, 12, pp.17989-17997, December, 2017

ORIGINAL RESEARCH ARTICLE OPEN ACCESS

ISOLATION AND CHARACTERIZATION OF BACTERIO PHAGESAGAINST MULTIPLE DRUG RESISTANT *PSEUDOMONAS AERUGINOSA* WITH USING THE BACTERIOPHAGE AS A THERPY IN THE MICE MODEL

[1]Mays B. Jalil, [1,]*Hayder A. Al-Hmudi, [2]Labeed Abdallah Al-Alsaad and [1]Zeinab R. Abdul-Hussein

[1]College of Science, University of Basra, Basra, Iraq
[2]College of Agriculture, University of Basra, Basra, Iraq

ARTICLE INFO

Article History:
Received 29th September, 2017
Received in revised form
17th October, 2017
Accepted 26th November, 2017
Published online 30th December, 2017

Key Words:
Burn wound,
MDR
Pseudomonas aeruginosa,
Bacteriophages.

ABSTRACT

One of the most common pathogens that are multidrug resistant and colonize burns wounds is *Pseudomonas aeruginosa*. This led researchers to find alternative treatment, so bacteriophages were alternative treatment methods used against bacterial infections. The phages were isolated from sewage and urine. Morphology of purified phages was examined by using the transmission electron microscopy. The titer of phage was determined by using plaque assay by serial dilutions (10^{-1} to 10^{-12}).The therapeutic effectiveness of phage for treatment of mice infected with MDR *P. aeruginosa* was determined. Four types of phages were isolated from sewage and urine. The our results showed that giving of the dose phage injection and orally was very effective after the bacterial challenge, this has helped in the speed of recovery, so that reduced the death rates. Blood culture of mice treated with phage dose appeared no viable *P. aeruginosa* cells in their blood compared to the positive groups. Our study present evidence in murine models that animals infected with MDRs *P. aeruginosa* can be successfully treated with specific bacteriophages that target these MDRs microbe.

Citation: Mays B. Jalil, Hayder A. Al-Hmudi, Labeed Abdallah Al-Alsaad and Zeinab R. Abdul-Hussein, 2017. "Isolation and characterization of Bacterio phagesagainst multiple drug resistant *Pseudomonas aeruginosa* with using the bacteriophage as a therpy in the mice model", *International Journal of Development Research*, 7, (12), 17989-17997.

INTRODUCTION

Large and open burn wounds and damage tissues because of the burning, prolonged patient stay in the hospital make it more susceptible to microbial infections easily because its surface is rich with protein, and after exposure to bacterial invasion and skin surface contamination (Lister *et al*., 2009).The presence of these microbes in burns wound with the development of infection post-burn to a systemic infection may cause serious complications and death (Church *et al*., 2006). Resistance of these organisms called Multiple Drugs Resistance (MDR) which has become increasingly important as a health problem (Gad *et al*., 2007). One of the most common pathogens that are multidrug resistant and colonize burns wounds is *Pseudomonas aeruginosa*, they are found everywhere in water, soil and moist environment and have the ability to adapt to different environmental conditions (Singh *et al*., 2010). This led researchers to find alternative treatment works with these strains and managed to eliminate them, so bacteriophages were alternative treatment methods used against bacterial infections (Gorski *et al*., 2009). For this global issue on public health, we undertook this study in order toisolation, purification and quantifications of bacteriophages against MDR *P. aeruginosa* from different sources, also characterization of these bacteriophages with animal experiments using a bacteriophage as a treatment for mice infected with MDR*P. aeruginosa*.

***Corresponding author:* Hayder A. Al-Hmudi**
College of Science, University of Basra, Basra, Iraq.

MATERIALS AND METHODS

Isolation and identification of MDR *Pseudomonas aeruginosa* and antibiotic sensitivity testing were performed according to our published article (Jalil *et al*., 2017).

Collection of samples

Bacteriophages samples were collected from urine including 69 and 12 years old male suffering from severe UTI patients in

the kidney transplant unit of Al-Basrah general hospital,also from a raw sewage obtained from different regions in Basrah governorate.

Isolation and purification of bacteriophages

The phage was enriched by mixing 200 ml of fresh samples (sewage or urine) with 20 ml of bacteriophage broth, 20 ml of a mixture of a five MDR *P. aeruginosa* and 20 ml of BHI broth to 1L sterile flask and incubated at 37°C for 48 h with shaking at 120 rpm. After incubation, the mixture was centrifuged at 10000 xg for 10 min to remove solid matters, then the supernatant was filtered by using 0.45 µm pore size millipore filter (Kumari *et al*., 2009; Golkar *et al*., 2013).

Determination of phage titer

The titer of phage was determined according to (Kropinski *et al*., 2009) by using plaque assay by serial dilutions (10^{-1} to 10^{-12}) according to agarose overlay method. The dilution factor that gave the best countable number of plaques was selected and used for all other experiments.

Determination of phage host range

The efficacy of phage was determined against MDR*P. aeruginosa* isolations by using spot assay according to (Karumidze *et al*., 2012). The presence of a clear zone on the bacterial lawn was recorded as a complete lysis.

Transmission electron microscopy (TEM)

Bacteriophage filtrate was centrifuged at 25000 xg for 1h., where one drop of phage filtrate (10^9) was placed on carbon-coated copper grid and stained negatively with 2% uranyl acetate, then morphology of purified phages was examined by using the transmission electron microscopy.

Molecular characterization of Phages

Phage DNA was extracted by using the QIAprep Spin M13 kit (QIAGEN, Germany) according to the manufacturers' instructions.By using a thermal Cycler (Gene Amp, PCR system 9700, Applied Pioneer, Germany). Random Amplified Polymorphic DNA (RAPD) of Purified phage DNA was performed according to (Gutierrez *et al*, 2011) by using the four primers.

Establishing of MDRs *P. aeruginosa* infection in mouse model

MDR *P. aeruginosa* was grown in BHI broth and incubated at 37°C for 24 h. Serial dilution was performed, then calculated of CFU by Spectrometer at OD_{600} nm measurement (3×10^4 and 3×10^8CFU/ml), then centrifuged at 8000 rpm for 10 min., 4°C. The pellet was washed and suspended in 10 ml normal saline (pH 7.4). The suspension stored at 4°C until use. The present animal experiment was performed according to (Golkar *et al*., 2013; Kumar *et al*., 2015) with modified. The lytic phage of *Siphoviridae* family was used only in the current experiment. A 7 week's old BALB/c male mice (n=21, in four groups) with weighing 20 to 25 gm obtained from the animal house at the College of Education for Pure Sciences/University of Basrah. After confirming that the animals were free of any pathogens, they transferred to the Animal House at the College of Science/Department of Biology immediately and placed them in the cages (30×25×20 cm) and left for 48 hours to adapt at controlled room temperature. The hairs on the mice's backs were shaved by use of depilatory cream with 7-10 mm area. The skin was washed with normal saline (pH 7.0) and left for 24 h. and distributed in cages where each group consists of three animals. After 24 h., the animals were anesthetized with chloroform and burned using spatula of 90°C by placed it on the skin for 10 sec. to make superficial burns with stage II wound. In acute infections, the first group of animals are an intraperitoneal (i.p) injection group, and the second group is local swab group which consist two subgroups, one of which challenged by local infection of MDR *P. aeruginosa* with (~3×10^4 CFU/ml) while the other was challenged with (~3×10^8 CFU/ml). After bacterial challenge, each animal in subgroups was treated with injections, about 1 ml of phage (26×103 PFU/ml), administered for intraperitoneal (in an (i.p) injection group) and local swap (in local swap group) at 30 min and 24 hours, then given a daily oral dose of phage (26×10^3 PFU/ml). The third group served as positive control without phage therapy, but infected with MDR *P. aeruginosa* and the forth group served as the negative control, which only administered intraperitoneal (26×10^3 PFU/ml) of phage without bacterial infection. Infected mice and controls were observed under sterile condition for one week, also follow up to the wounds and recorded by photography. The number of deaths was counted every 24 h. In chronic infections, one week after infections with bacterial infection, the chronically infected mice (positive control group) were treated with two doses of phage (26×10^3 PFU/ml) administered (i.p) injection. The second dose was administered 24 hours after the first infection, and then given daily oral doses of phage (26×10^3 PFU/ml). Infected mice were observed for one week and photographed.

Bacteremia

After 7 days post-infection, blood samples were collected from the heart of each animal after dissection it by using a clean pair of sharp surgical scissors and collect approximately 1mL of blood and cultured on *Pseudomonas* base agar and incubated for 24 h. at 37°C and colonies on each plate were counted.

RESULTS

Isolation of Bacteriophages and Transmission electron microscopy

The phage was isolated successfully from sewage and urine (Table, 1), Only four types of phages appeared to have lytic activity against isolates of MDR *P. aeruginosa*. Morphology of purified phages showed two types of sewage phages under Order: *Caudovirales* belong to *Siphoviridae* and *Podoviridae* families (Figure, 1), and showed two types of urine phages belong to *Inoviridae* and *Corticoviridae* families (Figure, 2).

Host range and Titration of Bacteriophages

The ability of each lytic phage of sewage and urine was tested against MDRs *P. aeruginosa* by spot assay on BHI agar (Figure, 3). By using plaque assay (Figures, 4), the high titrations (PFU/ml) were 26×10^3 and 3×10^4 for *Siphoviridae* and *Podovirdiae* phages respectively (Table, 2). This result revealed that dilution factor 10^{-2} was the best countable number of plaques.

Table 1. Dimensions of *Siphoviridae*, *Podooviridae*, *Inoviridae* and *Corticoviridae*

Source of phages	Oder	Family	Head diameter (nm)		Tail or Filamentousdiameter (nm)	
			length	width	Length	width
Sewage	*Caudovirales*	*Siphoviridae*	326	332	470	66
Sewage	*Caudovirales*	*Podoviridae*	189	191	14	18
Urine	Unassigned	*Inoviridae*	-	-	2879	61
Urine	Unassigned	*Corticoviridae*	107	117	-	-

Table 2. Titration of sewage bacteriophages

Volume of Phage Plated (ml)	Dilution Factor (DF)	Plaque per plate (*Siphovirida*e)	Plaque forming unit (PFU/ml)	Plaque per plate (*Podovirdiae*)	Plaque forming unit (PFU/ml)
1	10^{-1}	Complete lysis	-	Complete lysis	-
1	10^{-2}	260	26×10^{3}	300	3×10^{4}
1	10^{-3}	150	15×10^{4}	117	117×10^{3}
1	10^{-4}	60	6×10^{5}	73	73×10^{4}
1	10^{-5}	20	2×10^{6}	37	37×10^{5}
1	10^{-6}	17	17×10^{6}	30	3×10^{7}
1	10^{-7}	13	13×10^{7}	25	25×10^{7}
1	10^{-8}	12	12×10^{8}	21	21×10^{8}
1	10^{-9}	11	11×10^{9}	18	18×10^{9}
1	10^{-10}	9	9×10^{10}	10	1×10^{11}
1	10^{-11}	8	8×10^{11}	9	9×10^{11}
1	10^{-12}	6	6×10^{12}	5	5×10^{12}

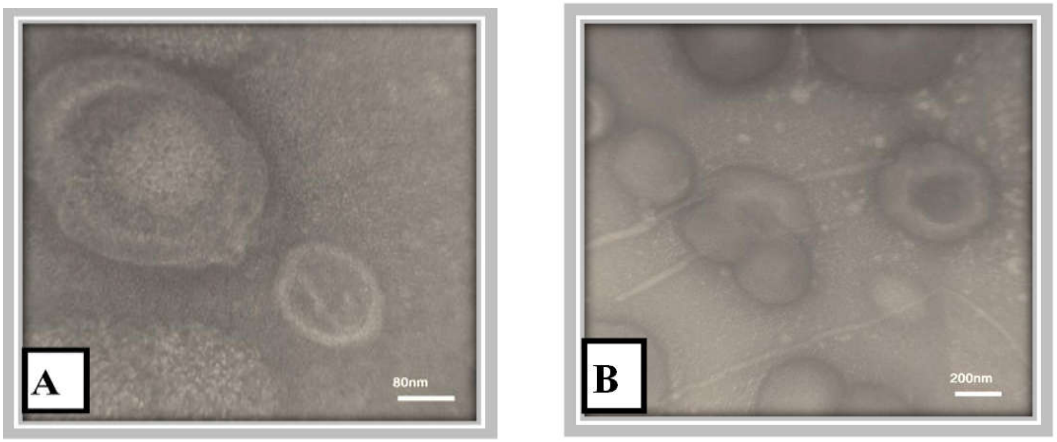


Figure 1. TEM of negatively stained from sewage sample (A) *Podoviridae*, phages hasshort non contractile flexible tails. Bar =80 nm (B) *Siphoviridae*, phages has long non contractile flexible tails, Bar =200 nm

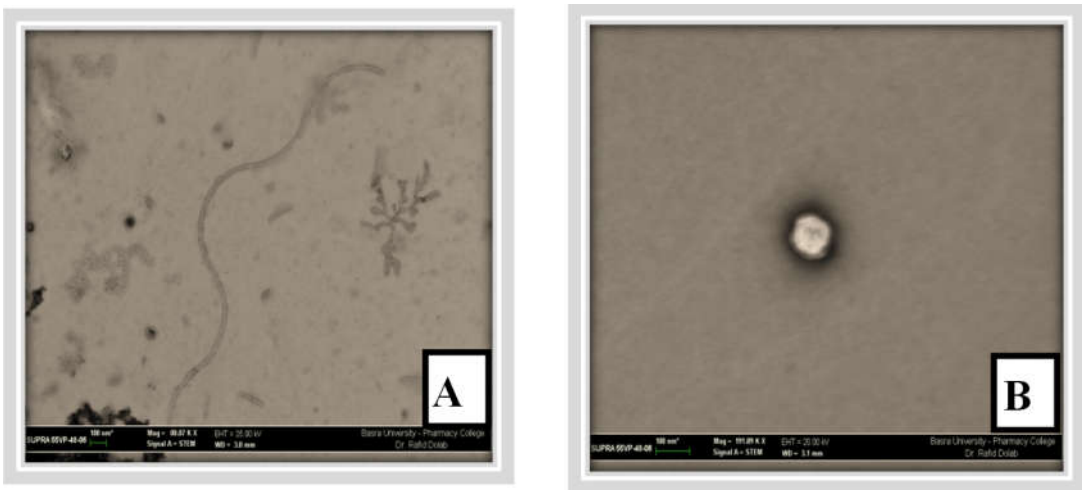


Figure 2. TEM of negatively stained from urine sample (A) *Inoviridae*, Bar =100 nm (B) *Corticoviridae*, Bar =100 nm

Table 3. Amplified fragments size of RAPD-PCR products of sewage sample

Family	Primers	Lanes	Bands				
			B1	B2	B3	B4	B5
Podoviridae	P1	L1	239.216	458.678	700.00	1161.702	N.P
	P2	L2	207.843	475.207	598.272	1800.00	2108.108
	P3	L3	360.784	512.00	642.262	1774.814	
	P4	L4	368.627	480.00	615.868	1680.500	
Siphoviridae	P1	L1	247.725	402.235			
	P2	L2	231.210	388.827			
	P3	L3	168.820	22.035	391.061		
	P4	L4	244.055				

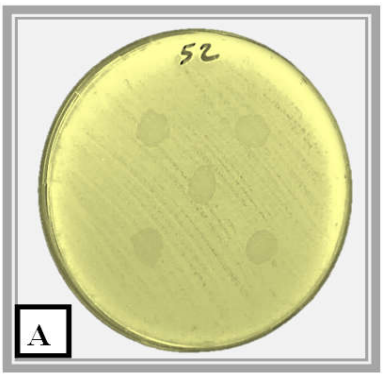


Figure 3. Spot test assay (A) for urine sample (B) for sewage sample

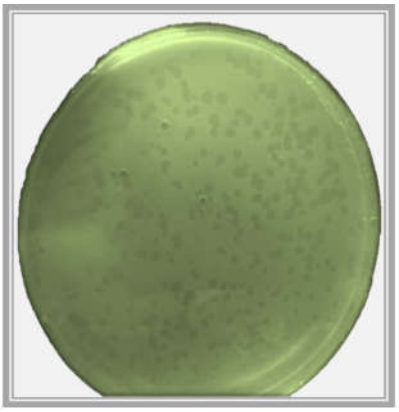
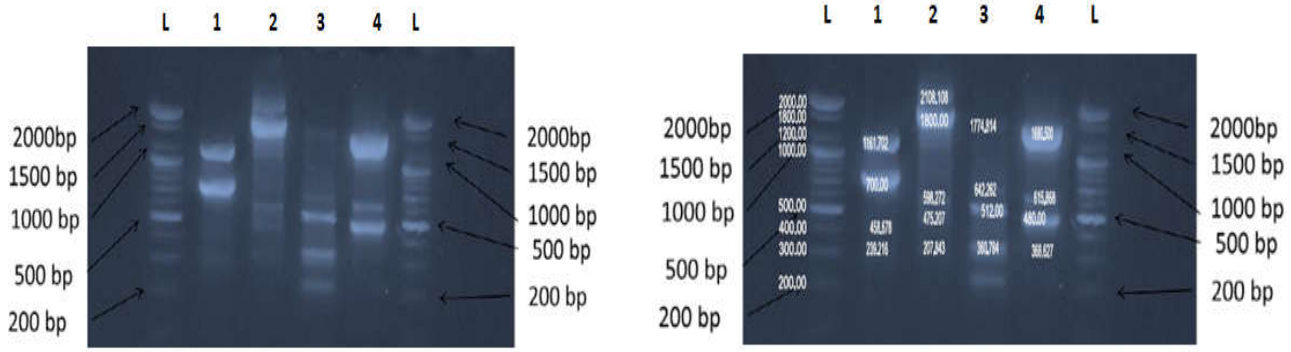
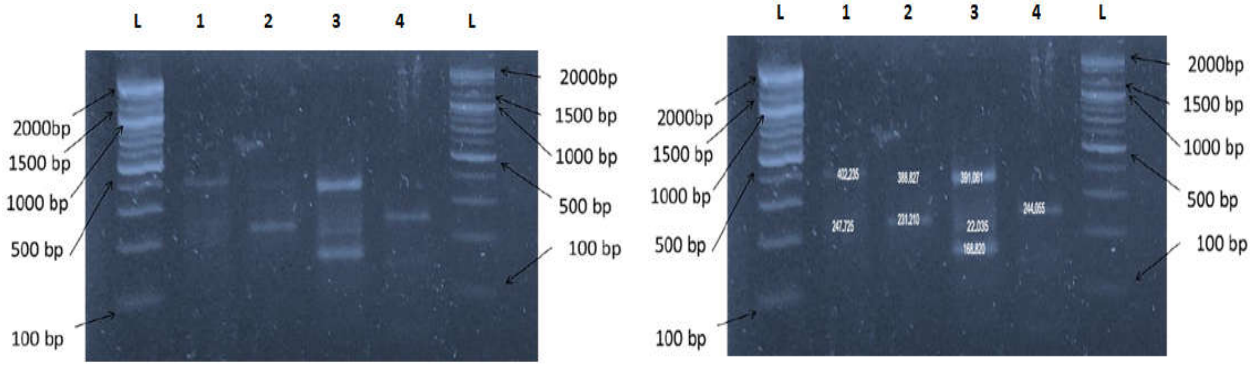


Figure 4. Plaque test assay of sewage phage



(A) **(B)**

Figure 5. RAPD-PCR products of *Podoviridae* (A) Orginal bands (B) bands with size L= DNA Ladder



(A) **(B)**

Figure 6. RAPD-PCR products of *Siphoviridae* (A) Orginal bands (B) bands with size

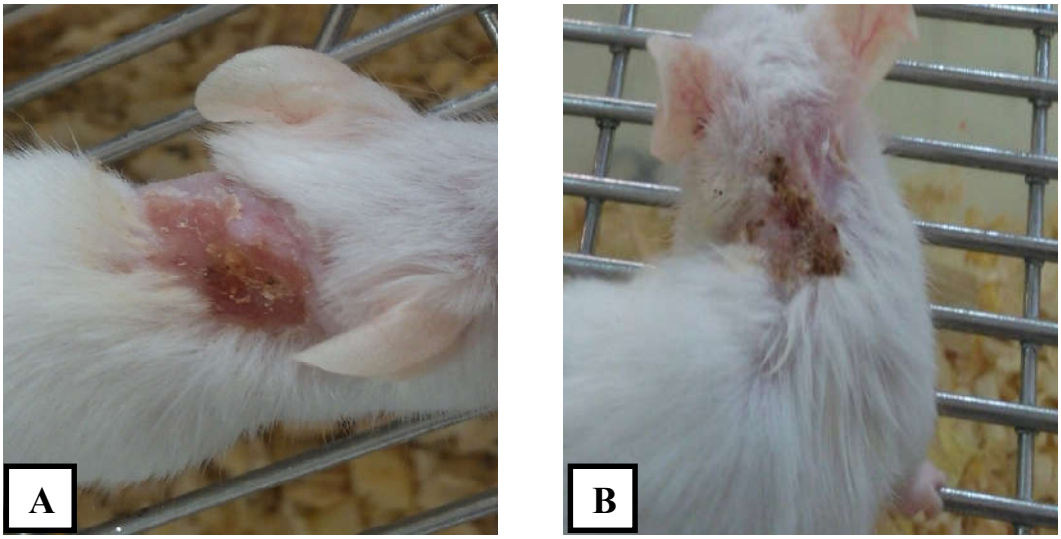


Figure 7. Local group(A) In two days after infection (B) In seven day

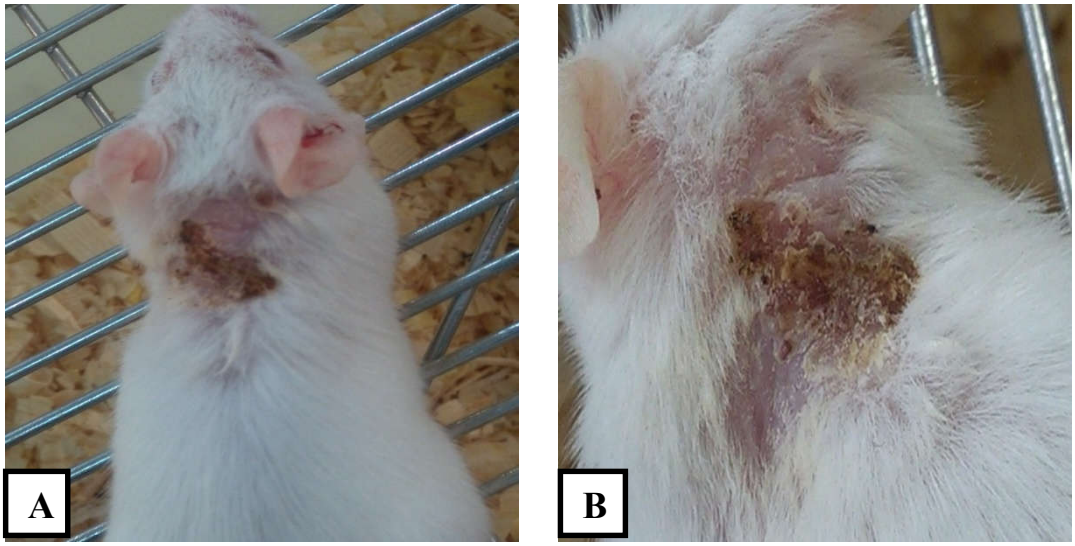


Figure 8. I.P. group(A) In two days after infection (B) In seven day

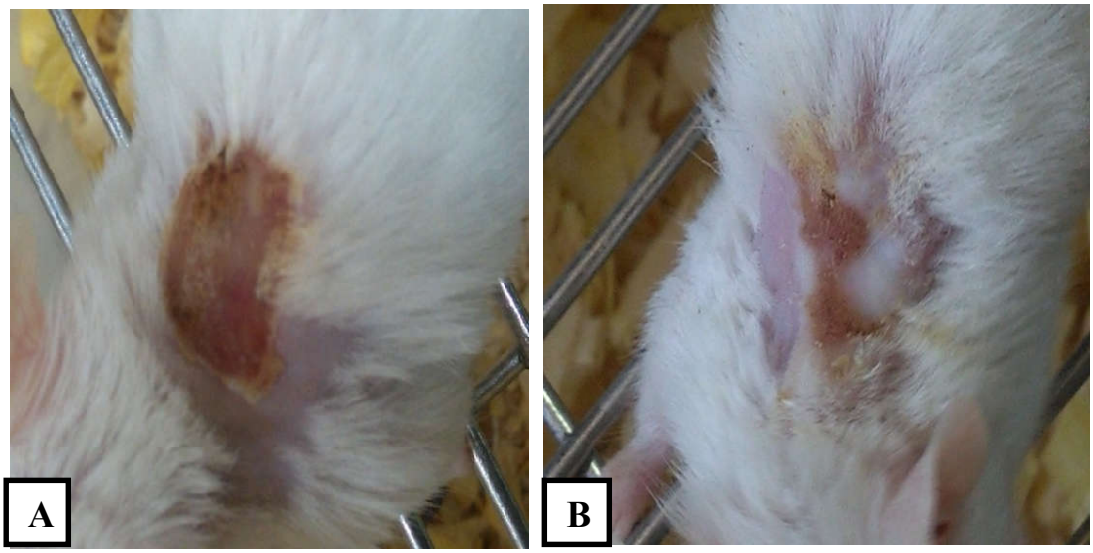


Figure 9. Positive control(A) In two days after infection (B) In fifthday

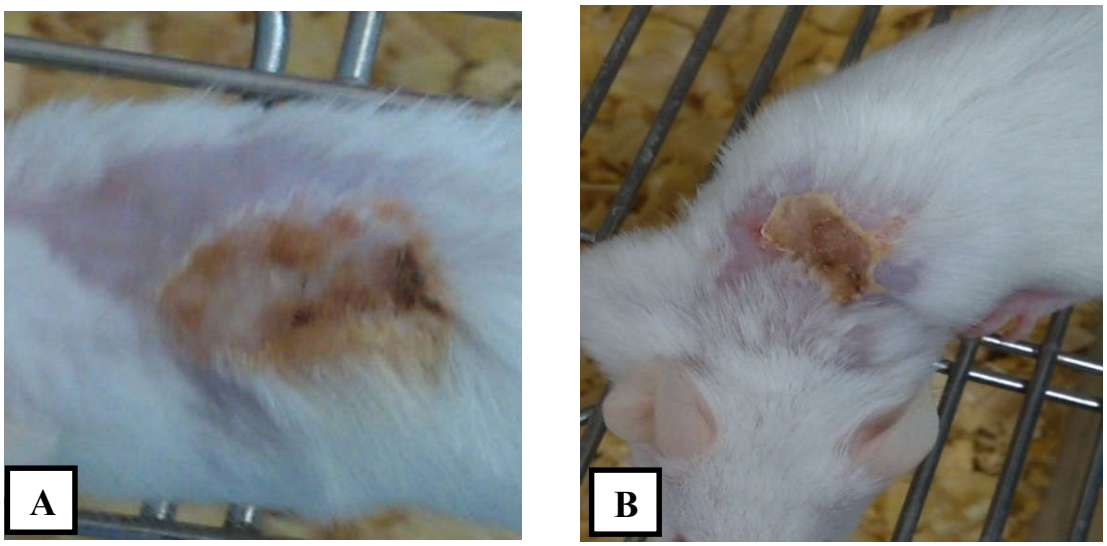


Figure 10. Negative control (A) In two days after infection (B) In seven day

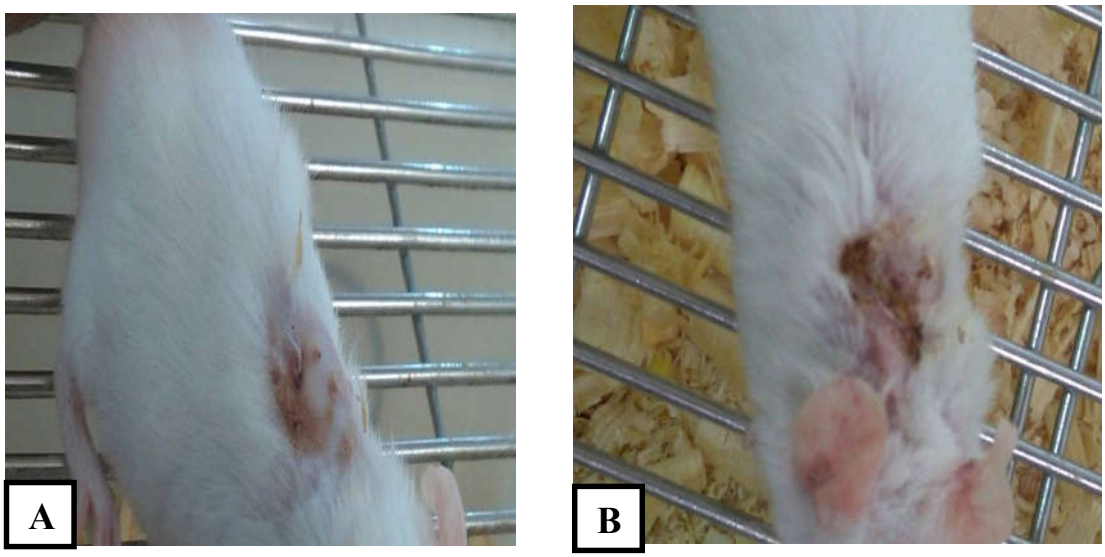


Figure 11. Chronic group (A) In two days after infection (B) In seven day

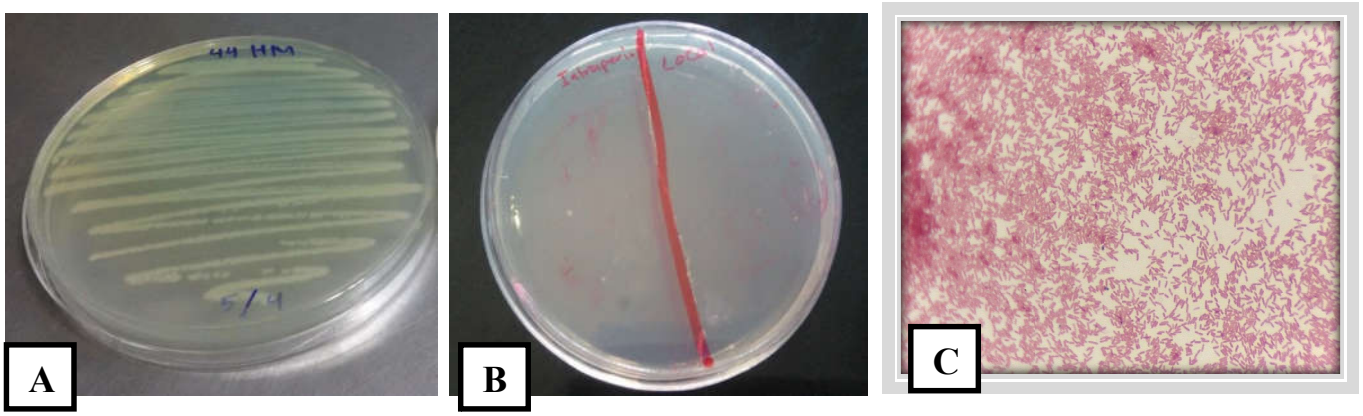


Figure 12. Blood culture of the animal (A) Infected with bacteria. (B) After treated with phage therapy. (C) Gram stain of mice infected with bacteremia (100x)

RAPD-PCR analysis

DNA bands of the RAPD-PCR products of *Podoviridae* and *Siphoviridae* (Figures 5 and 6) were performed by using four different primers. Each primer gave several bands (Table 3) on 0.8% agarose gel when compared to DNA ladder (2000 bp).

The therapeutic effectiveness of *Siphoviridae* phage

In acute infections, the results were very encouraging as it was found in response to treatment strongly in the intraperitoneal (i.p) injection group in deferent doses than the treatment with local swap group in which one animal died (in the dose 3×10^4 CFU/ml) on the first day after the injection and was also suffering from sleep and red eyes in each animal in subgroups (Figure, 7). While the i.p injection group was all animals from the first day after the injection intact and active, however eventually all the animals were cured (Figure 8). Furthermore, in the positive group there was death one animal after the first day of injection and the death of the rest on the fifth day (Figure 9). Also all animals in the negative group were intact and active (Figure 10). In chronic infections, the positive group was retested due to the death of all animals. The animals were challenged by local infection of MDR *P. aeruginosa* with (3×10^4 CFU/ml), and treated with doses of lytic phage via i.p injection and daily oral dose after three days after the injection before it death after being stressed and almost died. The results showed that all animals were cured after a week of treatment with no side effects were recorded, indicating the safety of phage as a treatment (Figure 11). The culture of animal blood proved that there were no bacteria in it, while blood culture of the positive groups showed infected with bacteremia (Figure 12).

DISCUSSION

Isolation and morphology of Bacteriophages

The bacteriophage therapy has been widely used in Eastern Europe and the Soviet Union for many decades. Its remains one of the important ways to kill and lysis pathogenic bacteria and eliminate its high virulence (Lu and Koeris, 2011). In present study four types of lytic bacteriophages specific for *P. aeruginosa* were isolated from sewage and urine, where it showed high virulence and lytic capacity against clinical isolates. In other studies, *Podoviridae* has isolated against pathogens *Vibrio cholera* and *P. aeruginosa* (Mitra and Ghosh, 2007; Kumari *et al*., 2009). About 96% of all diagnostic phages to the previous years belong to a family *Siphoviridae*, the *Podoviridae* or the *Myoviridae* (Sung-Sik *et al*., 2007). *Pseudomonas* phages are diverse belong to *Siphoviridae* with 59% frequency and *Podoviridae* with 19%, *Myoviridae* with 18%, and *Leviviridae* 4% (Sepúlveda-Robles *et al*., 2012). In bacteriophages from sewage, the study showed that the length of the icosahedral head and tail of *Podoviridae* were equal to 189 nm and 14 nm, respectively. As well as the length of the head of *Siphoviridae* was 326 nm and the tail was long 470 nm. In study of Ceyssens *et al*., 2006 were isolated *Podoviridae* against *P. aeruginosa* with the head diameter 60 nm and length of the tail was 8-10 nm, but study of Han *et al*., 2014 were recorded the head diameter of *Podoviridae* against *P. aeruginosa* about 50 nm. While Azizian *et al*., 2015 were isolated phages belongs to family *Siphoviridae* with head diameter 120 nm. In study of Yang *et al*., 2011 were isolated *Siphoviridae* phages against *Acinetobacter baumannii* with icosahedral head 50 nm in diameter and an 80 nm length of the tail.

RAPD-PCR analysis

In RAPD PCR analysis, the results showed different bands indicating that phages were genetically different.In some studies RAPD PCR analysis was used to make a fingerprint of 10 isolated phages against *Escherichia coli* (ETEC), but in other studies used to differentiate between six *Leuconostoc fallax* bacteriophages isolated from industrial sauerkraut fermentation and revealed that bacteriophages closely related (Jothikumar *et al*., 2000; Barrangou *et al*., 2002).

The therapeutic effectiveness of *Siphoviridae* phage

Many experiments and studies have been conducted on humans and animals to assess the efficiency and effectiveness of the phage, especially against *P. aeruginosa*. The phage was used to treating human cancer, wound infection and opportunistic bacterial treatment in mice and recorded that the survival rate of mice infected with bacteria alive after giving the phage ranged from 80-100% (Rhoads *et al*., 2009; Dąbrowska *et al*., 2010; Zimecki *et al*., 2010). The our study present evidence in "murine models" that animals infected with MDRs *P. aeruginosa* can be successfully treated with specific bacteriophages that target these MDRs these MDRs microbe and the culture of animal blood proved that there were no bacteria in it, while blood culture of the positive groups showed infected with bacteremia (Figure 12). The present results showed that giving of the dose phage via i.p injection and orally were very effective than the local dose after the bacterial challenge. One animal of the positive group dead after the first day of injection and other animals died on the fifth day, whereas all animals in the negative group were intact and active after administering the dose of phage. Interestingly, the results showed that all animals were cured after a week of treatment with no side effects were recorded, indicating the safety of phage as a treatment (Figure, 4-13). In a study included the treatment of 24 patients who had chronic Otitis infection caused by *P. aeruginosa* to assess the efficacy of phage safety (called Biophage-PA) with a single dose showed significant clinical improvements (Wright et al., 2009). The method of giving the phage dose varies according to the location of the infection; burns and skin infection are applied directly also phage can be given orally, locally, or systemically. Some studies have shown that phage can overcome the acidity of the stomach to pass into the bloodstream, and in other experiment was conducted by given the phage in blood circulation of mice and was isolated from the blood after 7 hours of injection (Weber-Dabrowska *et al*., 1987; Merril *et al*., 1996; Ahmad, 2002). The intraperitonial injection and oral administration of phage is the better method to understand the effects of phage in vivo, this method makes murine safe from bacteria and mortality and few numbers of *P. aeruginosa* cells in their blood, liver, and spleen after blood culture. Another study showing the phage in the bloodstream in the first hour and even after 3-4 h. after the injection, but the number of phages was low at 24 h. and completely absents within 36 h. in the body of mice (Harjai and Chhibber, 2009; Abengaña *et al.,* 2012). Likewise, Effectiveness of phage on chronic infection appeared in 6 days (Golkar *et al*., 2013). Matsuzaki *et al*., 2003 explained if bacteria acquire resistant to phage, the phage produced a new mutant that kills and lysis the MDR bacteria, therefore may be a prepared mixture of

different strains of phages effective against resistant strains through the administration of phage therapy. by spontaneous mutation during the produce of anti-phage antibodies by phage-treated hosts, the bacteria may become resistant to phages. these results interact with treatments with the same phage, so may be used high titer of phages produced immediately after bacterial infection (Cerveny *et al*., 2002).

REFERENCES

Ahmad, S.I. 2002. "Treatment of post-burns bacterial infections by bacteriophages, specifically ubiquitous *Pseudomonas* spp. notoriously resistant to antibiotics." *Medical Hypotheses*, 58(4): 327-331.

Azizian, R., Nasser, A, Askari, H., Taheri Kalani, M., Sadeghifard, N. Pakzad, I., Amini, R., Mozaffari Nejad, A.S. and Azizi Jalilian, F. 2015. Sewage as a rich source of phage study against *Pseudomonas aeruginosa* PAO. *Biologicals*. 43(4):238-41.

Barrangou, R., Yoon, S.S., Jr., Breidt, F., Fleming, H.P. and Klenhammer. T.R. 2002. Characterization of six *Leuconostoc fallax* bacteriophages isolated from an industrial sauerkraut fermentation. *Appl Environ Microbiol*., 68(11), 5452-5458.

Cerveny E. K., DePaola A., Duckworth H. D. and Gulig A. P. 2002. Phage Therapy of Local and Systemic Disease Caused by *Vibrio vulnificus* in Iron-Dextran-Treated Mice. *Infectionand Immunity*, p. 6251–6262. Vol. 70, No. 11.

Ceyssens P-J. and Lavigne, R. 2010. Bacteriophages of *Pseudomonas*. *Future Microbiol*., 5:1041–1055.

Church, D., Elsayed S., Reid, O., Winsto, N B. and Lindsay, R. 2006. Burn wound infections. *Clin Microbiol Rev*., 19: 403-434.

Dąbrowska, K., Skaradziński, G., Kurzępa, A., Owczarek, B., Zaczek, M., Weber- Dąbrowska, B. *et al*. 2010. The effects of staphylococcal bacteriophage lysates on cancer cells in vitro. *Clin Exp Med*., 10:81-5.

Gad, G.F., El-Domany, R.A., Zaki, S. and Ashour, H.M. 2007. Characterization of *Pseudomonas Aeruginosa* Isolated from Clinical and Environmental Samples in Minia, Egypt: Prevalence, Antibiogram and Resistance Mechanisms. *Journal ofAntimicrobial Chemotherapy*, (60) 1010– 1017.

Golkar, Z., Bagasra, O. and Jamil, N. 2013. Experimental Phage Therapy on Multiple Drug Resistant *Pseudomonas aeruginosa* Infection in Mice. Golkar *et al*., J Antivir Antiretrovir, S10.

Gorski A, Miedzybrodzki R, Borysowski J, et al. 2009. Bacteriophage therapy for the treatment of infections. *Curr Opin Investig Drugs*, 10:766-774.

Gutierrez, D., Martın-Platero, A.M., Rodrıguez, A., Martınez-Bueno, M., Garcıa, P. and Martınez, B. 2011. Typing of bacteriophages by randomlyampli¢ed polymorphicDNA (RAPD)-PCRto assess geneticdiversity. *FEMS Microbiol Lett*., 322: 90–97.

Han, F., Li, J., Lu, Y., Wen, J., Zhang, Z. and Sun, Y. 2014. Isolation and Characterization of a Virulent Bacteriophage φPA-HF17 of *Pseudomonas aeruginosa*. *Int.J. Bioautomation*, 18(3), 241-250.

Harjai K. and Chhibber S. 2009. Bacteriophage Treatment of Burn Wound Infection Caused by *Pseudomonas aeruginosa* PAO in BALB/c Mice. *Am. J. Biomed. Sci*., 1(4), 385-394.

Jalil, M.B., Abdul-Hussien, Z.R. and Al.Hmudi, H.A. 2017. Isolation and identification of multi drug resistant biofilm producer *Pseudomonas aeruginosa* from patients with burn wound infection in Basra province/Iraq. *International Journal of Development Research*, Vol. 7(11):17258-17262.

Jothikumar, N., Reddy, C.G., Sundari, R.B. and Saigopal, D.V.R. 2000. Isolation of coliphages specific to enterotoxigenic *E. Coli* (ETEC). *J Environ Monit*., 2, 372-374.

Justin Paolo B. Abengaña, Irni Mark C. Gemzon, Jonathan Mark S. Leung, John Carlo A. Mamauag, Jose C. Nolasco Jr., Ma. Sheila M. de Jesus and Donna May D.C. Papa. 2012. Comparative treament of *Pseudomonas aeruginosa* burn wound infection using bacteriophage MB08 and antibiotics. *Acta Manilana*, 60, pp. 77–81.

Karumidze, N., Kusradze, I., Rigvava, S., Goderdzishvili, M., Rajakumar, K. and Alavidze, Z. 2012. Isolation and Characterisation of Lytic Bacteriophages of Klebsiellapneumoniae and Klebsiellaoxytoca. Current Microbiology66(3):251-8

Kropinski, A.M., Mazzocco, A., Waddell, T.E., Lingohr, E. and Johnson, R.P. 2009. Enumeration of bacteriophages by double agar overlay plaque assay. *Methods Mol Biol*., 501:69–76.

Kumari, S., Harjai, K. and Chhibber, S. 2009. Bacteriophage Treatment of Burn Wound Infection Caused by *Pseudomonas aeruginosa* PAO in BALB/c Mice. *Am. J. Biomed. Sci*., 1(4), 385-394.

Kumari, S., Harjai, K. and Chhibber, S. 2015. Bacteriophage Treatment of Burn Wound Infection Caused by *Pseudomonas aeruginosa* PAO in BALB/c Mice. *Am. J. Biomed. Sci*., 1(4), 385-394.

Lister, P.D., Wolter, D.J. and Hanson, N.D. 2009. Antibacterial-resistant *Pseudomonas aeruginosa*: clinical impact and complex regulation of chromosomally encoded resistance mechanisms. *Clin Microbiol Rev*., 22(4):582-610.

Lu, T.K. and Koeris, M.S. 2011. The next generation of bacteriophage therapy. *Current Opinion in Microbiology*, 14:524–531.

Merril, C. R., Biswas, B., Carlton, R., Jensen, N. C., Creed, G. J., Zullo, S. and Adhya, S. 1996. "Long-circulating bacteriophage as antibacterial agents." Proceedings of the National Academy of Sciences of the USA 93(8): 3188-92.

Mitra, K. and Ghosh, A.N. 2007. Characterization of *Vibrio cholerae* O1 ElTor typing phage S5. *Arch Virol*., 152 (10): 1775-86.

Rhoads, D.D., Wolcott, R.D., Kuskowski, M.A., Wolcott, B.M., Ward, L.S. and Sulakvelidze, A. 2009. Bacteriophage therapy of venous leg ulcers in humans: results of a phase 1 safety trial. *J Wound Care*, 18:237-43.

Sepúlveda-Robles O., Kameyama L. and Guarneros G. 2012. High Diversity and Novel Species of *Pseudomonas aeruginosa* Bacteriophages. *Appl. Environ. Microbiol*., 78: 4510-5.

Singh, G., Wu, B., Baek, M.S., Camargo, A., Nguyen, A., Slusher, N.A., Srinivasan, R., Wiener-Kronish, J.P. and Lynch, S.V. 2010. Secretion of *Pseudomonas aeruginosa* type III cytotoxins is dependent on *pseudomonas* quinolone signal concentration. *Microb Pathog*., 49,196–203.

Sung-Sik, Y., Barrangou-Poueys, R., Breid, F. and jr, Fleming, H.P. 2007. Detection and characterization of a lytic Pediococcus bacteriophage from the fermenting cucumber brine. *J Microbiol Biotechnol*., 17(2), 262–270.

Weber-Dabrowska, B., Dabrowski, M. and Slopek, S. 1987. "Studies on bacteriophage penetration in patients subjected

to phage therapy." Archivum Immunologiae et Therapiae Experimentalis (Warsz) 35(5): 563-8.

Wright A, Hawkins CH, Anggård EE, Harper DR. 2009. A controlled clinical trial of a therapeutic bacteriophage preparation in chronic otitis due to antibiotic-resistant Pseudomonas aeruginosa; a preliminary report of efficacy. *Clin Otolaryngol.,* 34:349–357.

Yang L., Hu Y., Liu Y., Zhang J., Ulstrup J. and Molin S. 2011. Distinct roles of extracellular polymeric substances in *Pseudomonas aeruginosa* biofilm development. *Environ Microbiol*., 13:1705-17.

Zimecki, M., Artym, J., Kocieba, M., Weber-Dabrowska, B., Borysowski, J. and Górski, A. 2010. Prophylactic effect of bacteriophages on mice subjected to chemotherapy-induced immunosuppression and bone marrow transplant upon infection with *Staphylococcus aureus*. *Med Microbiol Immunol*., 199:71-9.

****