Evaluate phage single and cocktail as therapy against bedsores infection causes by some MDR pathogenic bacteria

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Abstract

Background: Multiple drug resistance (MDR) and extensive drug resistance (XDR) strains, in addition to the fact that there is no overarching strategy for the discovery and manufacturing of novel, All of these variables contribute to the development of antibiotic-resistant bacteria and the illnesses they cause. The need for alternative therapies has become more clear as a result of antibiotics. Methods In this investigation, certain phages that influence XDR Pseudomonas aeruginosa strains were identified and PDR Acinetobacter baumanii were separated out from sewage water at a hospital, and their lab properties as well as their lysis effect on 10 MDR,XDR strains of P. aeruginosa and 4 PDRand XDR A. baumanii strains were investigated .

Results: Virulent phages towards bacterial isolates were isolated from wastewater of hospitals and oil refinery from Karbala and identified by spot lysis method,and double layer agar plaque assay .Four phages were isolated, one phage against XDR P.aeruginosa and three phages against PDR A.baumanii.All the phages that isolated and characterized by Transmission electron microscope the phages belonged to the order of those with tails (Caudovirales) and family of Siphoviridae and Podoviridae. Each phage is Extensively resistant - Pseudomonas aeruginosa were found to lyse P.aeruginosa 20- 24%.While the same species phage cocktail that represents (P-AP3) and mixed with species(P-AA5) the phage cocktail two or three of phages that represents all phages were found to lyse, 70-80%. Conclusion: The findings of this study indicate that these phages have a significant potential for therapeutic use and prophylaxis of infections caused by this bacterium.

Keyword: *Bacteriophage; Pseudomonas aeruginosa; Antibiotic resistance*.

INTRODUCTION

Bed sores (BSs) are recognized as pressure ulcers (PUs), a prevalent clinical problem sometimes reported by patients with mobility constraints; it can be fatal, and its treatment puts financial obligations on the patient's family and society. (1). The most common organisms identified in BSs were Staphylococcus aureus (S. aureus), Proteus mirabilis, Pseudomonas aeruginosa (P. aeruginosa), and Enterococcus faecalis. (2). Pseudomonas aeruginosa Being an opportunistic bacteria, it is capable of causing severe infections in individuals with compromised immune systems. Patients with severe Bedsores, cystic fibrosis, malignancy, and acquired immunodeficiency syndrome are included in this category of patients. (3). P. aeruginosa causes a wide range of illnesses, from self-limiting skin infections to life-threatening pneumonia and septicemia, and can be particularly challenging to treat. Many resistance genes, including multidrug efflux

pumps, are encoded in the genome (4). And One of the most problematic bacteria in hospitals is Acinetobacter baumannii (A. baumannii), can lead to bloodstream infections (bacteremia), lung infections (pneumonia, cephalomeningitis), and skin, urinary tract, and soft tissue infections (cystitis, cellulitis), especially in patients with weakened immune. (5). The World Health Organization has categorised A. baumannii as one of the most dangerous ESKAPE pathogens. (6. A. baumannii-infected skin and soft tissues initially exhibit a peau d'orange look (similar to the skin of an orange).Researchers are turning to alternative medicines due to the rising prevalence of multidrug-resistant (MDR) P. aeruginosa and A.baumanii infections and the lack of a comprehensive plan for the development of new antibiotics to treat infections caused by these bacteria. Several researchers have proposed using bacteriophages as a complementary therapy for treating bacterial illnesses. (7) Bacteriophages, often known as phages, were independently discovered and introduced in 1915 and 1917 by two scientists, Frederick Twort and Felix d,Herell. Phages, which serve as natural bacteria controllers, are among the most abundant and widespread organisms on Earth. Phages are classified into two types based on their life cycle: lytic phages and lysogenic phages. Phage therapy, It employs the use of virulent phages (lytic phages) as an antibacterial agent, was first used more than 80 years ago (8). Because lytic phages are very selective and attack and destroy only specific bacterial species regardless of antibiotic susceptibility, the risk of them wreaking havoc on human microbial flora and causing other drug-related side effects is reduced. Phage therapy is acceptable for use in humans because phages cannot infect eukaryotic cells; however, it must be defined for some laboratory properties such as ; morphological properties, host range.Thus, this study aimed to estimate evaluate phage single and cocktail as therapy against bedsores infection causes by some common MDR pathogenic bacteria.

MATERIALS AND METHODS

Water samples: Eleven hospitals sewage water and sewage from an oil refinery in Karbala were sampled for sewage water. The samples were 0.22 m membrane filtered to exclude bacteria other than phages (9).

Phage Isolation: To obtain phages, target phages were enriched with indicator bacterial pathogens. 40 ml of membrane-filtered (0.22 m) sewage water, 5 ml of Brain heart infusion (BHI), and 5 ml of overnight indicator broth culture are combined (host) Bacteria (108 cfu/ml) were combined and cultured for 18 hours at 37 C0 with shaking. filtered (0.22 m) and examined for phage lytic activity against the identical bacteria. Initial enrichment indicator microorganisms (10).

Purification of Phages:

The procedure described by Sambrook et al.,(1989)(11) was used to purify isolated phages. Based on different morphology, a single plaque was chosen and re-suspended in 500lµl of SM buffer (5.8 g NaCl, 2.0 g MgSO4•7H2O, 50 ml 1 M Tris-HCl pH 7.4, dissolved in 1L dH2O). The solution was centrifuged after 60 minutes of incubation at 37 Co, and the supernatant was filtered through 0.22 µ mfilters(12). To create a phage stock that is homogeneous.

Phage spot lysis assay (PSA):

To determine the existence of lytic activity (virulent phages) against the target bacterium, spot tests were performed on the membrane-filtered supernatant of phages enriched with the target bacterium. Plaque formation rather than spot formation indicates the existence of virulent phages against the target bacterium. A sterile loop was used to gather up plate agar within the area of a plaque.transported in 1.5

ml of sterile SM buffer. Transported in 1.5 ml of sterile SM buffer (Sigma-Aldrich) in a 1.5 ml Eppendorf tube, shaken by hand for 5-7 minutes, then centrifuged at 10,000 rpm. rpm for 5 minutes The supernatant was thought to be a transitory(13).

Phage titration

The double agar test with agarose overlay method was used to detect phage titration, and the results showed that dilution, as a result of infection and bacteriophage multiplication inside the cells, produced the highest number of spots in the plaque assay. This causes cell death and further infection by appearing as clear spots and is expressed by Pfu/ml to the research Jiang et al.,(2012)(14).

Determination of host range

To investigate the host range of isolated phages, 10MDR P. aeruginosa clinical isolates (all resistant to GEN, AMK, CAZ, TRI, CEF, AMC, and DOX) acquired from Pressure ulcers(kindly provided from AL-Yarmouk teaching holspital and Ibn-quff hospital, Baghdad,Iraq) were employed. The sensitivity of bacterial strains was determined using a spot test. In summary, 2 - 3 colonies of isolated strains were dissolved in Brain heart infusion broth (5 mL) and cultured overnight at 37 °C with shaking (120 RPM), following which 100 µl ol of bacterial culture was added to 5 mL of soft BH agar (45°C, 0.7% agar) and overlaid onto plates containing 1.5% NB agar. After 10 minutes of solidification, 10 µl of isolated phages were spotted on the top layer. The plates were incubated overnight at 37°C until the formation of lysis zones. Lytic activity of extracted phages was also tested on A. baumannii, Escherichia coli , Staphylococcus aureus .

TEM examination

A drop of concentrated phage (106pfu mL-1) was put on a carbon-coated copper acid grid for 4 minutes to look at the shape of the isolated phages. An extra amount was then blotted with filter paper and stained negatively with uranyl acetate at a concentration of 2.0% (w/v) (pH 7.0). The grid was looked at with a 90 kV transmission electron microscope (Zeiss LEO 906 TEM, Tabriz, Iran) and AL-Nahrain University.

Influence of temperature on phage viability

The experiment was conducted to determine the viability of isolated phages in various temperature media. Several aliquots of phage lysates were incubated at various temperatures for one hour, namely 4Co,37Co, 40Co, 50 Co, 70Coand 80Co. Nine hindered microliters of sterile distilled water were pre-heated to temperatures ranging from 40Co,50Co,to 80Co, then 100 ml of phage sample (106PFU/mL) was added to these pre-heated tubes (pH 7) and titrated (15).

PH stability

Isolated phages were put through a stability test similar to that published by Wang et al.,2019 (16). This investigation examined the phages' ability to adapt to different pH settings by testing their viability in various pH-modified media.rate was determined using a spot test on double-layer agar (17).

Preparation of Phage cocktail

By combining phages at a concentration of 106 PFU/ml in SM buffer, we were able to create a cocktail that is effective against all isolates of the same species.

RESULTS

Isolation and purification of phages

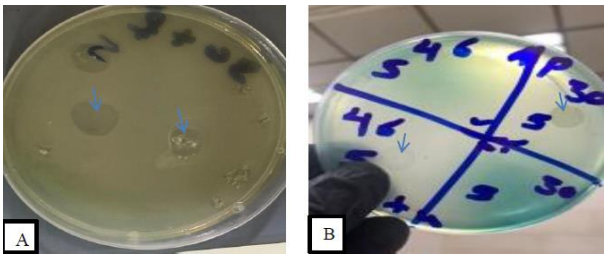
Clear plaque formation evaluated extracted phages for lytic activity. Four clear plaque-forming phages (2–6 mm in diameter) were named. P-AP3 (Phage- Ashwaq P.aeruginosa 3) and P-AA5,P-AA7,P-AA10 (Phage - Ashwaq A.baumanii 5,7,10) respectively, as shown in Fig(1).

Table (1) This study examines the properties of four phage isolates that were the most virulent to their bacterial host isolate, as measured by the size and clarity of their plaques.

Table (1) Phage name for XDR-P. aeruginosa and PDR-A.baumanii and the size of pluqes of phage.

Phage name	Meaning	Size of Spot (mm)	Degree of size
P-AP3	Phage- Ashwaq *P.aeruginosa* 3	5	Medium
P- AA5	Phage -Ashwaq *A.*baumanii 5	4	Medium
P-AA7	Phage - Ashwaq *A.baumanii* 7	2	Small
P- AA10	Phage - Ashwaq *A.baumanii* 10	6	Medium

Figure (1) (A) plaques result of PDR A.baumaniiin Spot Lysis Assay(B). spot assay for XDR P.aeruginosa



Determination of the host range of isolated phages

Table (2) that showed the assess the host ranges of the four phages isolated in this study, they were tested on PDR A.baumanii and XDR P.aeruginosa strains. The host range of each phage was tested at the temperature 37C0 at which it was isolated. Different host ranges were seen for the individual phages. This may be because the isolated phages were originating from different sewage samples from different hospital The phage P-AA5, P-AA7 and P-AA10 to lysis the strain PDR A.baumanii. and the phage P-AP3 make lysis to strain XDR P. aeruginosa.

Table (2) phage host range against XDR pseudomonas aeruginosa and PDR Acinetobacter baumanii

NO	Phage isolate	Host bacterium	Plaque size (mm)	Plaque shape	Margin cut	Plaques clarity
1	P-AP3	*p.aeruginosa*XDR	7	Circular	Irregular	Turbid
2	P-AA5	*A.baumanii* PDR	5	Circular	Irregular	Clear
3	P-AA7	*A.baumanii* PDR	4	Oval	Regular	Turbid
4	P-AA10	*A.baumanii* PDR	5	Oval	Regular	Clear

Bacteriophage titer determination

DLA method was used for determination of Phages titration. Plaques were counted after overnight incubation at 37 C° and titer of the isolated phages were determined to be were (5 ×108), (8.9×107), (3.2X107), and (1×105) PFU/ ml to P-AP3, PAA5, P-AA7 and P-AA10 respectively respectively.

Transmission electron microscopy (TEM) of phages

All phages from the collection belonged to the order of tailed phages, Caudovirales. In multiphage samples (P-AP3,P-AA5,7 and 10), Each of the four isolated phages was examined by transmission electron microscopy following negative staining. All phages were

identified to be members of the Caudovirales order and of the Siphoviridae family with long non-contractile tails (18)(Figure 2).

phageAA5 and phageAA 7, belonged to the family Siphoviridae, characterized by long non-contractile tails. Most of them had isometric heads. Phages from the family Podoviridae, characterized by short tails, were the least abundant like phageAP3 and phage AA10. Head and tail measurements were found to range between15-142 nm and12–200 nm respectively (Table3) based on measurements taken from four individual phage particles and reported as a mean value ± standard deviation., similar in range to previously reported(19).

Figure (2) Representative Transmission electron microscopy images(A) of A. baumanii phages AA 5 isolated from sewage from oil refinery,karbala related to siphoviridae family of bacteriophage (B)P.aeruginosa phages AP3 isolated at 37C° from sewage of hospital,(C) A. baumanii phages AA 7 isolated at 37C° and (D) P- AA 10 isolated from fall water and belong to podoviride family.

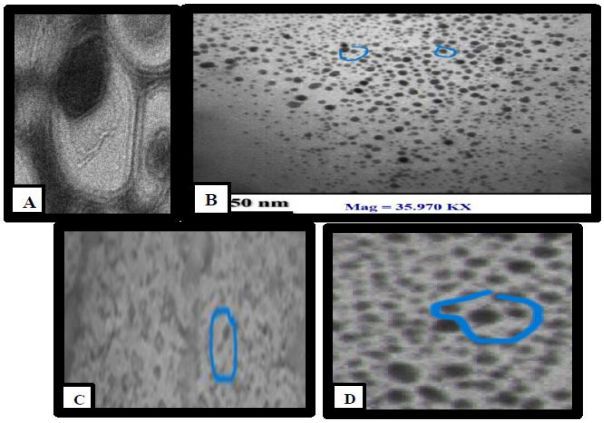


Table (3) Phages characteristic under TEM.

Phage	Head Capsid (nm) Diameter (mean) ±SD*	Tail length (nm) (mean) ±SD*	Family	Order
P-APa3	Icosahedral 15±1	12.5±3	Podoviridae	Caudovirales
P-AA5	Icosahedral 140±2	200±1	Siphoviridae	Caudovirales
P-AA7	Icosahedral 120±3	30±1	Siphoviridae	Caudovirales
P-AA10	Icosahedral 26±1	12±1	Podoviridae	Caudovirales

* SD Standard deviation. The values in the table represent the mean of 10 measurements.

Bacterial sensitivity against phage and antibiotic

The relationship of the isolates between the sensitivity to bacteriophage and antibiotics because bacteriophages are often not effective against all strains, even within a single bacterial species(20).On the depending of table(4) which by linking the resistance of isolates to antibiotics and susceptibility to the phage shows that there is no relationship between isolates susceptibility to bacteriophage and resistance to phage we find the isolate No 1 was the more resistant of isolates to antibiotic as it was resistant to 8 out

of 13 antibiotic and it is sensitive to phageAA5,AP3,AA7,AA10.

Table (4) Comparison between sensitivity strain XDR P.aeruginosa and PDR A.baumanii to antibiotic and to phage.

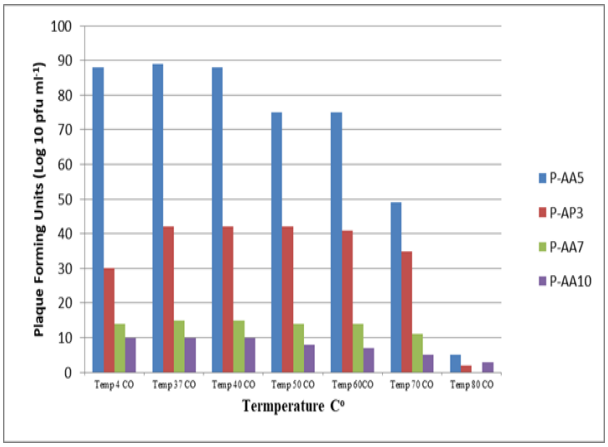
The strains	Sensitive to antibiotic	Sensitive to phage
*p.aeruginosa*XDR1	AZM	P-AA5,P-AP3,PAA7,P-AA10
*p.aeruginosa*XDR2	AZM,AK,GEN,IPM	P-AA5,P-AP3
p.aeruginosa XDR3	CAZ,ATM,IPM,AK,GEN,LEV	P-AA5,P-AP3,PAA7,P-AA10
p.aeruginosa MDR 4	IPM,ATM	P-AA5,P-AP3
p.aeruginosa MDR 5	CAZ,LEV,GEN,ATM	P-AA5,,P-AA7,P-AA10
p.aeruginosa MDR6	AK,GEN	P-AA5,,P-AA7,P-AA10
p.aeruginosa MDR 7	CAZ,ATM,IPM,AZM	P-AP3,P-AA7,P-AA10
p.aeruginosa MDR 8	AZM,	P-AA5,P-AA10
p.aeruginosa MDR 9	CAZ,AK,ATM,IPM,GEN,AZM,LEV	P-AA5,P-AA10
p.aeruginosa MDR10	AZM,ATM,IPM	P-AA7,P-AA10
A.baumanii XDR1	AZM,DO	P-AA5,P-AP3,P-AA10
A.baumanii PDR2	0	P-AA5,P-AA10
A.baumanii XDR3	AZM,DO	P-AA5,P-AA10
A.baumanii PDR4	0	P-AA5,P-AP3,P-AA7,P-AA10

phage (+)=sensitive to phage (-)= resistance to phage.And the antibiotic mean in table is:CAZ= Ceftazidime - AZM= Azithromycin –DO= Doxycycline ATM= Azetreonam – GEN=Gentamicin – AK=Amikacin– IMP=Imipenem LEV= Levofloxacin.

Stability of phages to temperature and pH

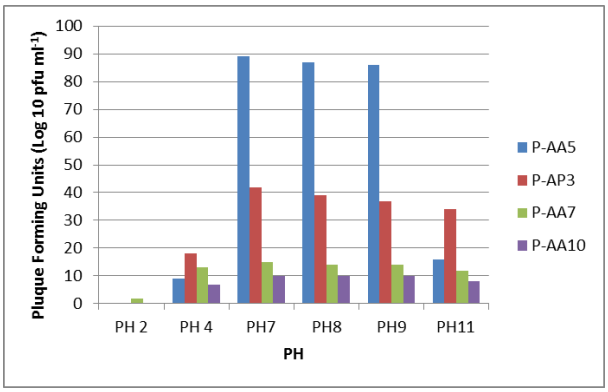
The temperature to phage stability was studied at seven different temperatures 4 CO, 37 CO, 40 CO, 50 CO, 60CO,70CO, and 80 CO. Storage at 4 CO was considered as the control for the temperature stability experiments figure(3)The measured the MOI of 103 to 105 for their bacteriophages.we showed that phages were stable against the temperatures of 37C0 and 50C0 for 60 min but were inactivated at 80Co after 60 min of incubation.

Figure (3) Stability of phages under different thermal conditions. a Graph showing efects of different temperature conditions on the stability phages. Phages aliquots were incubated at diferent temperatures, 4Co, 37Co, 40Co, 50 C°,60 C°,70Co and 80Co for 60 min, followed by enumeration of viable phages by standard double-layer plaque assay. Stability of phage stored at 4 °C was considered as control for comparison



The effects of high and low pH on phage virion stability were also studied. Almost all phages were stable at pH 8,9just the phage AA5 no survival was observed when phages at ph of 2 and the titer of only 4 phages dropped after of incubation 24h under these conditions in ph (figure 6). Their phages were stable between pH 7,8, 9 but were sensitive to acidic PH.

Figure (4) Graph showing effects of different pH conditions on the stability of phages. Aliquots of phage were added to buffers adjusted to various pH conditions (2–11), incubated at room temperature for 18-24 h, followed by enumeration of viable phages by standard double-layer plaque assay.



Phage cocktail

Table (5) showed eight phages were formulated in a cocktail and tested versus each of the following single phage of P-AA5, P-AP3, P-AA7 and p-AA10 in growth in double agar assay Results of lysis for activity of each one of the 4 single phages and phage cocktail against the one isolate of XDR p. aeruginosa and PDR A.baumanii have showed the zone of single phages is small than phage cocktail .

Table (5): Phage plaque characteristics of 8 phage cocktail lytic on isolates of XDRP. aeruginosa and PDR A.baumanii.

Ph Cok	Bacteriophage isolate	Host bacterium	Plaque size (mm)	Plaque shape	Margin cut	Plaqus clarity
1	P-AP3+P-AA5	*A.baumanii*	11	Circular	Regular	Clear
2	P-AP3+P-AA10	*A.baumanii*	10	Circular	Regular	Clear
3	P-AP3+P-AA7	*A.baumanii*	8	Circular	Regular	Clear
4	P-AA5+P-AA7	*A.baumanii*	13	Circular	Regular	Clear

5	P-AA5+P-AA10	*A.baumanii*	10	Circular	Regular	Clear
6	P-AA5+P-AA7+P-AA10	*A.baumanii*	12	Circular	Regular	Clear
7	P-AA5+P-AP3+P-AA7	*A.baumanii*	10	Circular	Regular	Clear
8	P-AA5+P-AP3+P-AA7+P-AA10	*A.baumanii*	12	Circular	Regul	Clear

Figure (5) A spot method for growth inhibition assay of a 8 phage cocktail against XDRA. baumanii isolates

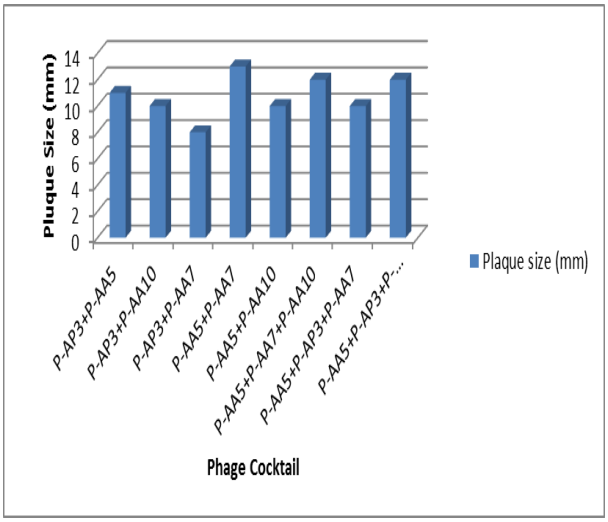
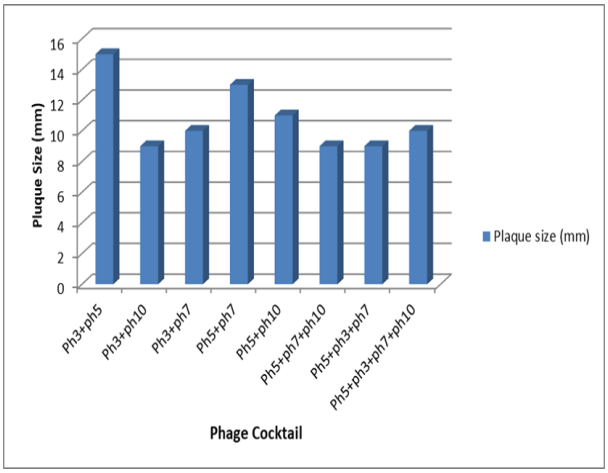


Figure (6) A spot method for growth inhibition assay of a 8 phage cocktail against MDR P. aeruginosa isolates.



DISSCUSION

Many infections caused by P. aeruginosa strains are highly resistant to antibiotics. One of the reasons is bacterial innate resistance, but another key cause of this resistance is connected to other variables such as inadequate antibiotic use (21). With the rapid expansion of antibiotic-resistant bacteria and the pharmaceutical industry's desire to invest in the production of new antibiotics, particularly against Gram-negative bacteria, due to increased research costs, finding effective and inexpensive therapy approaches for controlling bacterial infections has become a priority in recent years(22).The present study findings indicate there are high prevalence of bedsores among patients admitted to intensive care unit in IRAQ, with P.aeruginosa as the most prevalent isolate bacterium in the bedsore patients. Phage therapy has been investigated as one of the novel biologic techniques to combating multi-drug resistant bacteria in therapeutic fields .Sewage Water is known to be a rich source of microorganisms such bacteriophage which can be used to control harmful bacterial infections Lytic phages are superior to lysogenic phages for phage treatments due to their faster proliferation in host bacteria(23). In this regard, the isolation and characterisation of lytic bacteriophages against clinical antibiotic-resistant isolates of XDR P.aeruginosa and PDR A.baumanii from Bed sores should be highly useful in combating these diseases that have recently threatened human healthcare. Then, as a candidate for phage therapy, we identified and studied a lytic bacteriophage with antibacterial activity against P.

aeruginosa isolates from bedsores in Iraq. The clinical isolates of XDR P. aeruginosa from BSs were infected with the lytic phage AP3 recovered from wastewater from hospital (24).Bacteriophages isolated from sewage were tested against MDR-bacterial isolates from patients with pressure ulcers .in another study these phages exhibited perfect lytic activity against MDR bacteria that cause septic wounds. As a result, the authors proposed phages as therapeutic possibilities for treating septic wounds (25). strong lytic efficiency and also broad spectrum of its lytic effect on clinical isolates can select it as an appropriate candidate to phage therapy. Selecting a potentiated bacteriophage for phage therapy needs to be regarded to different factors not just one characteristic. In TEM morphological analysis, All phages from the collection belonged to the order of tailed phages, Caudovirales order and of the Siphoviridae family with long non-contractile tails, Most of the phages, from the collection phageAA5 and phage AA7, belonged to the family Siphoviridae, characterized by long non-contractile tails. Most of them had isometric heads. Phages from the family Podoviridae, characterized by short tails, were the least abundant like phageAP3 and phageAA10. The sensitivity of bacterial to the phage in our study the resistance of isolates to antibiotics and susceptibility to the phage shows that there is no relationship between isolates susceptibility to bacteriophage and resistance to antibiotic we find the isolate No1 of XDR P.aeruginosa was the more resistant of isolates to antibiotic as it was resistant to 8 out of 13 antibiotic and it is sensitive to phage AA3,AP5,AA7 and AA10.And this consistent with thesis of Raghda 2018(26);that results of its that sensitive to A. baumanii 28.6%and this is consisten withJin et al.,(2012) (27) that found 3 isolates of strains sensitive from 23 strains, and the results of Yang et al,2010 (28). The phages as candidates for phage therapy should be more stable in different environmental conditions(29),that showed that phages were stable against the temperatures of 4C0,37CO and 50CO for 60 min but were inactivated at 80C0 after 30 min of incubation, moreover phage AA7 not survive at 80CO.However, after 30 minutes of incubation at 70 CO, a significant reduction in phage titer was observed and this consistent with Mocé-Llivina (30) the thermal inactivation of phages occurring in dewatered sludge and raw sewage has rarely been investigated. However, in such studies, significant reduction of the phage titer was observed after 60 min of incubation at 60C° Sonika Sharma et al.,(2021) results demonstrated that the phage was substantially stable at 4 C0, 37 Co ,40 Co temperatures, while moderately stable at 50Co ,60 Co and 70 Co temperatures. However, at a temperature of 80 Co and above, phage stability decreased specifically, as compared to control. The results is consistent with Based on the studies devoted to obtaining thermo stable phage particles for biotechnological purposes, it was suggested that formation of disulfide cross-links within phage capsid proteins could play a role in stabilization of the phage against thermal denaturation (31)

Whether such stabilization occurs in p-AA5,p-AP3and p-AA10 remains to be verified of it.The effects of high and low pH on phage virion stability were also studied. Almost all phages were stable at pH 8,9 just the phage AA5 no survival was observed when phages at pH of 2 and the titer of only 4 phages dropped after of incubation 24hr under these conditions in pH Their phages were stable between pH 7,8 and 9 but were sensitive to acidic pH and this consistent with Sonika Sharma (31) and Ghasemi, (32) and the Phage cocktail The eight phages were mixed together and evaluated in a two fold agar assay against each of the following single phages: P-AA5, P-AP3, P-AA7, and P-AA10. The results of lysis for activity of each of the four single phages and phage cocktail against a single isolate of

P. aeruginosa and A.baumanii revealed that the zone of single phages is smaller than the zone of phage cocktail. All phages used in the phage cocktail have a lytic lifecycle as represented by their leaving clear plaques when plated with P. aeruginosa and A.baumanii. In the results of this study so suggest incorporating two phages and three phages in the treatment of bed sores and the phage cocktail not only efficiently kills P.aeruginosa ,A.baumanii; but also prevent the emergence of phage -resistant mutants in vitro Figure(5)and Figure(6).

CONCLUSION

1-Hospitals and oil refinery sewage water samples are the most reliable sources for isolating phages against XDR-P.aeruginosa and PDR -A.baumanii isolated from patients with BSs.

2- All the phages that isolated and diagnosed were belonged to the order of tailed phages (Caudovirales) and family of Siphoviridae and Podoviridae. Three primary phages against PDR -A.baumanii isolated and one phage against XDR-P.aeruginosa isolated, while no single phages were found to be specific against XDR-S.aureus isolates.

3- The phage cocktails were exceed on phage singles and it showed broad spectrum activity to lyse clinical MDR G+ve and G-ve bacterial strains of the same genus and species, in addition to other genus and species, also exceed when compared to highly sensitive antibiotic.

4. Stability of all phages performed best at pH (7–9), while susceptible to acidic circumstances (pH less than 4). In addition, phages were more stability at temperature ranged 40-60 Cº, while sensitive at 80C°.

Reference

1-Alzapir I. Ibrahim, Alsafi B. Mukhtar, Mahmoud H. Ahmed, Suliman M. Yahia and Alamin M. Ibrahim.Prevalence and Antimicrobial Susceptibility Pattern of Aerobic Bacteria Isolated from Patients with Bedsores Admitted to Intensive Care Units in Khartoum State. Int.J.Curr.Microbiol.App.Sci. (2021) 10(05): 759-767.

2-Dana A.N, and Bauman W.A. Bacteriology of pressure ulcers in individuals with spinal cord injury: What we know and what we should know. The Journal of Spinal Cord Medicine 2015 VOL. 38 NO. 2 147-160.

3-Merakou C, Schaefers MM, Priebe GP. Progress toward the elusive Pseudomonas aeruginosa vaccine. Surg Infect (Larchmt) 2018;19:757-68.

4- Poole K. Multidrug efflux pumps and antimicrobial resistance in Pseudomonas aeruginosa and related organisms. J Mol Microbiol Biotechnol. 2001; 3 (2):255–264.

5- Gong, Y., Shen, X., Huang, G., Zhang, C., Luo, X., Yin, S., et al. (2016). Epidemiology and resistance features of Acinetobacter baumannii isolates from the ward environment and patients in the burn ICU of a Chinese hospital. J. Microbiol. 54, 551–558.

6- Pereira, S. G., Domingues, V. S., Theriága, J., Chasqueira, M. J., and Paixão, P. (2018). Non-antimicrobial drugs: etodolac as a possible antimicrobial or adjuvant agent against ESKAPE pathogens. Open Microbiol. J. 12, 288–296. doi: 10.2174/1874285801812010288.

7- Krylov, V., Shaburova, O., Pleteneva, E., Krylov, S., Kaplan, A., Burkaltseva, M., et al. (2015). Selection of phages and conditions for the safe phage therapy against Pseudomonas aeruginosa infections. Virol. Sin. 30, 33–44. doi: 10.1007/ s12250-014-3546-3543.

8-K. Ho, Bacteriophage therapy for bacterial infections. Rekindling a memory from the pre-antibiotics era, Perspect. Biol. Med. 44 (2001) 1–16, https://doi.org/ 10.1353/pbm.2001.0006, 1.

9- S. Kumari, K. Harjai, S. Chhibber, Isolation and characterization of Klebsiella pneumoniae specific bacteriophages from sewage samples, Folia Microbiol. 55 (3) (2010) 221–227, https://doi.org/10.1007/s12223-010-0032-7.

10-G.H. Tan, M.S. Nordin, A.B. Napsiah, Isolation and characterization of lytic bacteriophages from sewage water (Pengasingan dan pencirian bakteriofaj daripada air kumbahan), J. Trop. Agric. Food Sci. 36 (2008) 1–5, 2.

11- Sambrook J, Fritsch ER, Maniatis T. Molecular cloning: a laboratory manual, 2nd ed. New York: Cold Spring Harbor Press; 1989.

12-Bonilla, N., Rojas, M.I., Cruz, G.N.F., Hung, S.-H., Rohwer, F., and Barr, J.J. (2016), 'Phage on tap-a rapid and efficient technique for the creation of bacteriophage laboratory stocks,' PeerJ, 4, e2261.

13- S.A. Jassim, A.S. Abdulamir, F. Abu Bakar, Novel phage-based bioprocessing of pathogenic Escherichia coli and its biofilms, World J. Microbiol. Biotechnol. 28 (2012) 47–60, https://doi.org/10.1007/s11274-011-0791-6, 1.

14-Jiang, M. J. ; Zhao, Sh. P. and Li, J. M. (2012) . Resistance of β-lactamase–producing Klebsiella pneumonia to Imipenem with Ompk36 loss. Afri. J. of Microbiology Research . 6(13): 3231-3236

15- Cao F, Wang X, Wang L, Li Z, Che J, Wang L, Li X, Cao Z, Zhang J, Jin L, Xu Y. Evaluation of the efficacy of a bacteriophage in the treatment of pneumonia induced by multidrug resistance Klebsiella pneumoniae in mice. BioMed Res Int 2015;2015:752930.

16- WANG, J., ZHAO, F., SUN, H., WANG, Q., ZHANG, C., LIU, W., ZOU, L., PAN, Q. & REN, H. 2019. Isolation and characterization of the Staphylococcus aureus bacteriophage vB_SauS_SA2. AIMS microbiology, 5, 285].

17- Ahmadi, M., Karimi Torshizi, M.A., Rahimi, S. and Dennehy, J.J. 'Prophylactic bacteriophage administration more effective than post-infection administration in reducing Salmonella enterica serovar Enteritidis shedding in quail', Frontiers in microbiology. 2016, 7, 1253.

18- Fokine, M. Rossmann Published 21 February 2014 Biology Bacteriophage The tailed double-stranded DNA bacteriophages, or Caudovirales

19-Salifu, S.P. , Valero ‐ Rello, A. , Campbell, S.A. , Inglis, N.F. , Scortti, M. , Foley, S. and Vazquez ‐ Boland, J.A. Genome and proteome analysis of phage E3 infecting the soil ‐ borne actinomycete Rhodococcus equi . Environmental microbiology reports .2013,5, 170–178.

20-Hyman P, Abedon ST. Bacteriophage host range and bacterial resistance. Adv. Appl. Microbiol. 2010, 70, 217–248

21- Breidenstein EB, de la Fuente-Núñez C, Hancock RE. Pseudomonas aeruginosa: all roads lead to resistance. Trends Microbiol. 2011 Aug;19(8):419-26. doi: 10.1016/j.tim.2011.04.005. Epub 2011 Jun 12. PMID: 21664819.

22-Vieira A, Silva YJ, Cunha A, Gomes NC, Ackermann HW, Almeida A. Phage therapy to control multi- drug-resistant Pseudomonas aeruginosa skin infections: in vitro and ex vivo experiments. Eur J Clin Microbiol Infect Dis 2012; 31: 3241-3249

23-Goodridge LW. Phages, bacteria and food. In: bacteriophage ecology: population growth, evolution and impact of bacterial viruses. Ed, ST Abedon. Cam- bridge University Press, 1st ed, Cambridge, UK, pp. 2008, 302-305.

24-Yu X, Xu Y, Gu Y, Zhu Y, Liu X. Characterization and genomic study of "phiKMV-Like" phage PAXYB1 infecting Pseudomonas aeruginosa. Sci Rep 2017; 7: 13068.

25- Pallavali RR, Degati VL, Lomada D, Reddy MC, Durbaka VRP. Isolation and in vitro evaluation of bac- teriophages against MDR-bacterial isolates from septic wound infections. PLoS One 2017; 12(7): e0179245.

26- Raghda Ayad Taha. Genetic and Molecular study of Acintobacter baumannii isolated from different infection with relationship of Phage in Diyala province. A Thesis Submitted to the Department of Biology, College of Science, DiyalaUniversity .2018.

27-Jin,J; Li,Z, Wang,S, Wang,S. Huang, Li,Y et al.Isolation and characterization of ZZ1, a novel lytic phage that infects Acinetobacter baannii clinical isolates. BMC Microbiol; .2012.12: 156. doi: 10.1186/1471-2180- 12-156

28- Yang, H.; Liang, L.; Lin, S. and Jia, S..Isolationland Characterization of a Virulent Bacteriophage AB1 of Acinetobacter baumannii .BMC Microbiol. 2010;10: 131-141.

29-Ahmadi M, Karimi Torshizi MA, Rahimi S, Dennehy JJ. Prophylactic bacteriophage administration more effective than post-infection administration in reducing Salmonella enterica serovar Enteritidis shedding in Quail. Front Microbiol 2016; 7: 1253.

30- Moce-Llivina, L., Muniesa, M., Pimenta-Vale, H., Lucena, F. and Jofre, J. Survival of Bacterial Indicator Species and Bacteriophages after Thermal Treatment of Sludge and Sewage. Applied and Environmental Microbiology 2003; 69,1452-1456.

31-Sonika Sharma1,3, Sibnarayan Datta1,3, Soumya Chatterjee1 , Moumita Dutta2 , Jhuma Samanta1 , MohanG.Vairale1 , RajeevGupta1 , VijayVeer1 & Sanjai K. Dwivedi[Isolation and characterization of a lytic bacteriophage against Pseudomonas aeruginosa. 2021;11:19393

32- Ghasemi, S.M., Bouzari, M. & Emtiazi, G. Preliminary characterization of Lactococcus garvieae bacteriophage isolated from wastewater as a potential agent for biological control of lactococcosis in aquaculture. Aquacult Int 22, 1469–1480 (2014). https://doi.org/10.1007/s10499-014-9760-z