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Bacteriophage therapy for *acinetobacter baumannii*-induced superficial wounds in experimental mice

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ABSTRACT

Antibiotic resistance is one of the most greatest hazards to human health in the modern era. Incredibly even more concerning is how quickly antibiotic-resistant bacteria can spread throughout the global community and in healthcare settings. In this study, locally isolated bacteriophages with the ability to lyse multidrug-resistant bacteria were isolated from wastewater as one strategy to combat multidrug-resistant bacteria. Examination of phage morphology by electron microscope showed three-tailed phages related to three families, in which ΦEEP belonged to the family *Siphoviridae*. ΦEPP phage belonged to *Podoviridae* and ΦEAP belonged to *Myoviridae*. The ΦEAP was specific to acinetobacter baumannii subsequently was chosen to applied on our study. The therapeutic efficacy of ΦEAP was evaluated in a mouse model of a complete wound that acquired an infection of *A.baumannii*. Phage-treated wounds inoculated with ΦEAP demonstrated full regeneration; high-quality wound healing, and elimination of bacteria with infection. All of those characteristics combine to ΦEAP phage a potential treatment for infections of skin wounds by MDR *A.baumannii*. Nevertheless, additional investigation and clinical research studies on the phage combinations that are needed to be applied topically in wound infections must be conducted.

1. Introduction

Bacteriophages could be used as an antibiotic for plants, animals and humans [1]. The clinical application of bacteriophages for the biological controls of pathogenic bacterial illnesses is known as phage treatment [2]. It have been used between the 1920s and 1940s until the first major development of antibiotic penicillin in 1944 [3]. After that, phage therapy still used in some parts of the world, although phage therapy research stopped because of widespread antibiotics, then will increase because antibiotic-resistant bacteria (ARBs) have appeared around the surrounding environment [4]. Bacteria resist antibiotics via horizontal gene transfer (HGT) and spontaneous mutations in their chromosomal genes, bacteria can acquire or evolve antibiotic resistance [5]. In addition, bacteria can resist β –lactam, where, since about 80 years ago, β -lactam antibiotics have been used extensively. These substances are bactericidal and prevent the creation of cell walls in bacteria alone [6]. Bacterial lactamases, which hydrolyze the lactam ring of antibiotics and make bacteria resistant to them, have grown excessively as a result of the prolonged usage of this family of antibiotics [7]. Biofilm is another method can bacteria used it to resists antibiotics, where, biofilm is known as a complicated microbial partnership that are fixed to abiotic or biotic surfaces. Single or multiple microbial species may form this structure [8]. Investigations have shown that efflux pumps play a significant role in the enlargement of bacterial biofilms.The majority of these investigations have demonstrated that the expression of these pumps is elevated in biofilms, increasing antibiotic resistance [9]. Performed a crucial study highlighting the significance of efflux pumps in bacterial biofilms; this highlights the probable of EPIs by way of anti-biofilm mediators. *S. aureus, K. pneumonia, P. aeruginosa*, and *E. coli* were investigated for their susceptibilities to three recognised EPIs, PAN, thioridazine, and NMP. Each bacterial species has a unique role for efflux pumps in the creation and upkeep of biofilms. However, four categories have been established to categorise the mechanisms behind their contribution: the production and release of poisons, such as antibiotics and waste metabolites. Influencing adherence to both cells and other surfaces, which promotes aggregation [10]. Three major methods are castoff by bacteria headed for obtaining external genetic material: transformation (the insertion of bare DNA), transduction (mediated by phages), and conjugation (bacterial "sex"). The simplest form of horizontal gene transfer is transformation, however, only a few clinically significant bacterial species have the capacity to "naturally" assimilate bare DNA to create resistance. Conjugation is a particularly effective way of gene transfer that includes cell-to-cell

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contact and is anticipated to happen at high rates in the gastrointestinal tract of individuals receiving antibiotic therapy [11]. Conjugation frequently occurs in the hospital environment. Antimicrobial resistance mutations change the way antibiotics work by one of the following methods, I) alterations to the antibacterial target (lowering drug affinities); Activation of efflux mechanisms to expel the toxic chemical, a decrease in drug absorption, or IV) broad modifications to crucial metabolic processes by means of regulating networks. As a result, the complexity of resistance resulting from acquired mutational alterations varies and is variable [12].

Notably, the antimicrobial activity of lytic phages has effects similar to antibiotics. On the other hand, phages are more effective than antibiotics at curing infectious disorders in both humans and animals [13, 14]. For instance, patients with deadly, infectious illnesses of the pleura and lungs caused by Staphylococcus aureus were utilized as a treatment sample in one study. No negative effects were noticed in subjects administered bacteriophage, and 82% of subjects recovered completely in the phage group and 64% in the drug group. As throughout the case of antibiotics, treatment with bacteriophages also carries a minimal risk of developing resistance [13].

Several advantages characterized bacteriophages over all the conventional methods of controlling pathogenic bacteria such as the ability to self-reproduce, host specificity and ability to develop with their hosts. However further research is necessary to improve the parameters of bacteriophage applications, comprising the impact of environmental conditions on decomposition & contagion performance as well as significantly decrease the production resistance of particular bacteria to bacteriophage [15]. Bacteriophages have many properties that over antibiotics, giving them advantages [16]. Phages are self-limiting and self-replicating. Also, act on particular pathogens by avoiding the destruction of normal flora often seen after treatment with broad-spectrum antibiotics. Prolonged usage phages to cure Eastern Europe's infection rates with little cruel properties proposes that bacteriophages are safe and could be used without the repeated production of sensitive responses. Animal experiments showed that oral and parenteral phages do not cause side effects in histopathological modifications, complications, or death [17]. Therefore, the aim of this study was to show, the efficacy of Bacteriophage therapy for *Acinetobacter baumannii*-induced superficial wounds in experimental mice.

2. Materials and methods

2.1 Isolation of bacterial isolates:

This study was conducted during the period from February 2021 to December 2022 at the Bacteriology Lab Department of Botany, Faculty of Science, Fayoum Univresity, Al-Azhar University, Egypt's University and Regional Centre for Mycology and Biotechnology Al-Azhar University. Pathogenic bacteria isolates were collected from Fayoum University and Elnabawy Elmohands hospitals, Fayoum Governorate, Egypt. At the same time of collection, many sub-cultures of bacterial isolates were prepared for further investigation. Isolates 1:70 from urine samples, 71: 140 from pus samples, 141: 210 from blood samples, and 211: 280 from sputum samples.

2.2 Antibiotic sensitivity testing :

Among 280 bacteria isolates, the MDR only were selected for further examination. The disk diffusion technique of the scientist Bauer [18] for studying the susceptibility of bacteria isolates to antibiotics. Then, make characterization of the physicochemical most resistant bacterial isolates against antibiotics, then identification of bacterial isolates using VITEK II automated system [19].

2.3 Isolation of Phage:

Phage isolation was done by two methods, Adam [20] and Cerveny [21]. The bacterial strains used as a host were *E. coli, P. aeruginosa*, and *A. baumannii* then, make purification and concentration of isolated phages:

Adam method summarized as follow: To remove debrides, sewage samples were first treated with buffer before being passed through a centrifuge at 5000 rpm for 15 min at 4°C. Each of the 250 Erlenmeyer flasks was inoculated with 5 mL of the overnight liquid containing the culture of the tested bacteria and around 0.5 g of sterilised calcium carbonate using 5 mL of this mixture and 50 mL of nutritional broth medium. Flasks were shaken (250 rpm/min) for a 48-hour incubation period at 37°C. The cultures were spun in a centrifuge at 6000 rpm for 15 minutes after incubation, and the liquid that resulted was collected into sterile flasks. To get rid of any little contaminated germs, chloroform was added at a rate of 1:10 and forcefully shaken for five minutes. The mixture was stored into the refrigerator for 2 h at 4°C then, the upper phases were transferred into sterile tubes as crude phage suspensions and discarded the pellets(lower phase).The obtained crude lysate from the phages was tested for the presence of bacteriophages qualitatively and quantitatively. Cerveny method summarized as follow: Sewage prepared by mixing SM buffer (NaCl, 5.8 g; $MgSO_4 7H_2O$, 2 g; 1 M Tris-HCl ‘pH 7.5, 50 ml; 2 % gelatin, 5 ml; add ddH2O to 1,000 ml) with sewage sample. Twenty millilitres of Fayoum University Hospital sewage sample was centrifuged 10,000g for 10 min at 4^{O}C to remove dirt and germs. The supernatant has been placed in a new tube and maintained at 4^{O}C. To amplify the phage, 20 ml of new LB media (containing 10 mg/l $CaCl_2$) and 20 ml of samples of water were combined with one millilitre of an overnight culture of bacterial suspension. The culture media was then centrifuged at 8,000 g for 10 min at 4^{O}C after having been incubated for 10 h at 37^{O}C with agitation at 160 rpm. Through Millipore filters with a pore size of 0.22 lm, the supernatant was filtered. Three times this amplification process was carried out.
0.1 ml of final filtered culture media was combined with 0.3 ml of bacterial suspension to test for the presence of phage, which was then incubated at 37^{O}C for 20 min. The mixture was then mixed with 3 ml of LB top agar that had been heated to 47^{O}C (containing 0.4% agar) and spread over a 1.5% LB agar plate. For six hours, the plate was kept warm at 37^{O} C, or until lysis zones developed. Isolated strains bacteriophage particles were purified and concentrated by ultracentrifugation method as follows, Firstly: phages lysates of different isolated strains phages were centrifuged at low-speed rpm for 15 min at 4°C to remove and slow bacteria and the cell debris. Then, results liquids were centrifuged in Back-man L7-35 at a high speed After chilling for 90 minutes at 30000 rpm, the pellets were carefully re-suspended in the supernatants. a small amount of SM buffer(NaCl, 5.8 g; $MgSO_4 7H_2O$, 2 g; 1 M Tris-HCl ‘pH 7.5, 50 ml; 2 % gelatin, 5 ml; add ddH_2O to 1,000 ml), transferred into sterilized tubes and the process (alternative low and high-speed centrifugation) was repeated two cycles according to Figrski and Christensen[22]. After that, make characterization of the isolated phages by examination of phage morphology by Transmission Electron Microscopy [23].

2.4 Application of bacteriophage to control multidrug-resistant bacteria in Skin wounds of laboratory animals:

For the investigation, inbred adult Swiss albino mice approximately 30-35 g were employed. The animals were bought from Cairo University's cancer research center (NCI) animal house after a permission animal ethical approval certificate. They were kept separately in aseptic cages, given regular food and drink in a separate room, then disinfected daily. At the beginning and end of the trial, the animals were frequently weighed, and the bedding and bowl were changed everyday. Prior to and following the infliction of laboratory wounds on mice, anesthesia was administered. The skin excision procedures were carried out in sterile settings while being anaesthetized with a 50 mg/kg and 8 mg/kg total body weight combination of ketamine & xylazine. To keep an eye on the animals' health, thorough observation was made of them. A subcutaneous injection of the doses of ketamine plus xylazine was used to put mice to sleep. The back of their hair was then clipped using an electric razor, and their outer layer was cleaned with 10% the povidone- solution. Each animal had a circular wound made on its back by removing skin via a surgical blade after the region of the wound was marked with a marker utilising a circular metal stencil that was 20 mm in diameter. The injury was not bandaged. Each wound was covered with a small piece of gauze prior being administered with a 100-L suspension comprising 2.5 × 108 CFU of A. baumannii. A skin clip was used to seal the wound. After 24 hours, the operation produced local abscesses. The animals were each given a single wound, put back in their own cages, and daily studied in detail. The wounds of the controller animals had been opened and the dressing of gauze remained taken off after 24 hours. The treatment was initiated after infected granulation tissue was taken in lieu of microbial quantification (CFU/mg of diseased tissue). To the various animal groups, doses of phage stock solution were topically applied.

2.4.1 Design of the experiment:

Five groups of six experimental animals each were separated into the groups. Group 1: Animals in G.1 (control) who are uninfected and non-treated (Positive control). Group 2: Animals in G. 2 (infected control) were infected by 2.5 ×10^8 CFU of the A.baumannii bacteria and left untreated (negative control) (Negative control). Group 3: Animals in G. 3 (an infected wound and phage treatment) received a daily dosage of phage (ΦEAP: specific for *A.baumannii*) by topical application and were infected with 2.5 ×108 CFU of the *A. baumannii* strain. Group 4: Animals in Group 4 (an infected wounds and Fusidic acid 2% treated) had 2.5 × 10^8 CFU of the A. baumannii strain and were given a daily dose of 200 mg daily dose of the Fusidic acid 2%(as it is available in a topical form and very effective in wound healing) (Market name Fuci-Top-C). Group 5: (an infected wound administered with phage and fusidic acid 2%): animals treated with 200 mg of fusidic acid 2% and phage daily after contracting 2.5 ×10^8 CFU of the *A.baumannii* strain.The treatment groups received daily treatments until they reached the point of full re-epithelialization up until the 24th day of the trial, and all animals were regularly observed. The study was carried out until the 28th day after treatment, and the rate of healing of the wound was determined by outlining the region around the wound once on the 4th, 8th, 16th, 18th, and 24th days. All experimental animals from various groups had their wounds traced using paper for tracing and a permanent marker. Graph paper was used to measure the wound regions that were documented on tracing paper. Re-epithelialization was defined as the point whereby the eschar flaked off without leaving any trace of the raw wound. The experiment was repeated twice and the three parameters of wound healing rate, bacterial load (bacterial cultures & turbidity), and mice survival were recorded in line with Shetru, Gan, and Tan [24, 25, 26].

2.5 Statistical analysis:

The findings of each experiment were performed in triplicate and are shown as mean values. The analysis of variance in two ways was conducted by means of the statistical Infostat (version 2019) software bundle [27].The multiple range test of Duncan was used to distinguish the mean value of treatments when significant differences at $p \leq 0.05$ were detected [28].

3. Results and discussion

3.1 Collection of bacterial isolates.

There were 280 isolates of bacteria collected during February 2021 to December 2022 at Fayoum Governorate, Egypt from Fayoum University Hospital and Elnabawy Elmohands General hospital and grouped as follows: Seventy bacterial isolates came from urine samples and take numbers from 1-70 with codes EU1-EU70, seventy isolates of bacteria were isolated from pus samples with codes EP71-EP140, seventy bacteria isolates was possible to separate from blood samples with codes EB141-EB210, and finally, seventy bacterial isolates were obtained from sputum samples with codes ES211-ES280.

3.2 Antibiotic sensitivity of the bacterial isolates.

Based on their antibiotic resistance only 60 isolates were chosen from 280 bacterial samples. Fifteen bacterial isolates of urine samples and take numbers from 1-15 with codes EU1-EU15, fifteen bacterial isolates of pus samples with codes EP1-EP15, fifteen bacterial isolates of blood samples with codes EB1-EB15, and finally, fifteen bacterial isolates of sputum samples with codes ES1-ES15. The inhibition zone of antibiotics obtained from 60 bacterial isolates was given in (Fig. 1). The Agar plate showing antibiotic sensitivity test for EP15 (A.baumannii), EP11 (P. aeruginosa), and EP5 (E.coli) isolate showed multidrug-resistant pattern in figures (2) & (3) & (4).

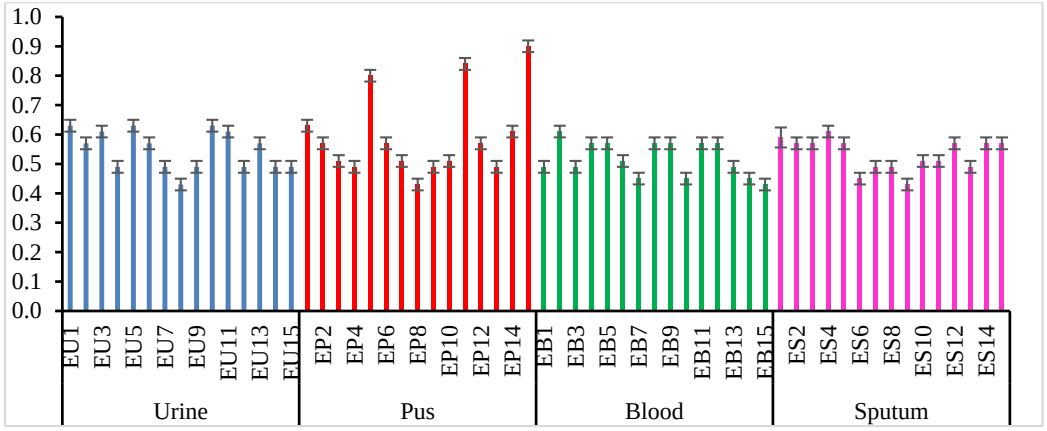

Fig. 1: Histogram showing the bacterial isolates across the different sources significantly differed regarding resistance ratios (The resistance ratio was calculated by dividing the sensitive antibiotics to the resistant one). The isolates of EP5, EP11, and EP15, which were identified as E. coli, P. aeruginosa, and A. baumannii exhibited the best resistance ratios of 0.80, 0.84, and 0.90, respectively. Also, show antibiotic sensitivity test for screening the most multi-drug resistant isolate, showing the mean of inhibition zone ratios. A, b, c, d, f and g: statically analysis. E: Essam, P: pus, U: urine, B: blood, and S: sputum.

3.3 Phenotypic and biochemical characterization of the most resistant pathogenic bacteria:

Numerous biochemical and morphological investigations were conducted, then to ensure the identification using the VITEK II system where The isolate EP5 showed resistance for all antibiotics except for Ceftriaxone (CRO), Ofloxacin(OFX), and Colistin(CT) with a ratio 82% (14/17) . The isolate EP11 showed resistance for all antibiotics except for Ciprofloxacin(CIP) and Colistin(CT) with ratio 88 %.(15/17).The isolate EP15 showed resistance for all antibiotics except Colistin (CT) with ratio 94 %(16/17) . Results in figure 1 revealed that the bacterial isolates across the different sources significantly differed regarding resistance ratios. The isolates of EP5, EP11, and EP15, which were identified as E. coli, P. aeruginosa, and A. baumannii exhibited the best resistance ratios of 0.80, 0.84, and 0.90, respectively.

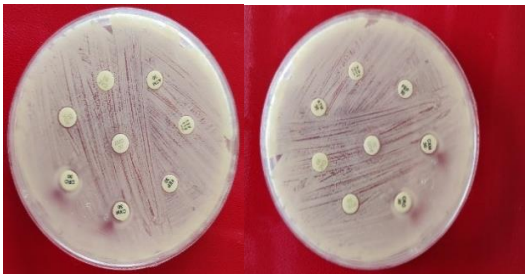

Fig.2: Agar plate showing antibiotic sensitivity test for EP15 (*A.baumannii*) isolate showed multidrug-resistant pattern.

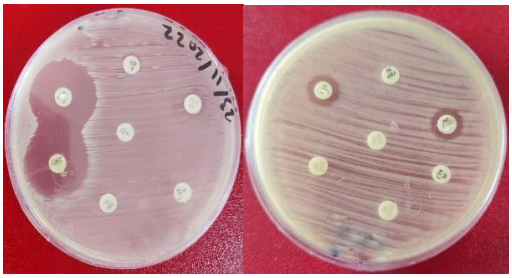

Fig. 3: Agar plate showing antibiotic sensitivity test for EP11 (*P. aeruginosa*) isolate showed Multidrug-resistant pattern.

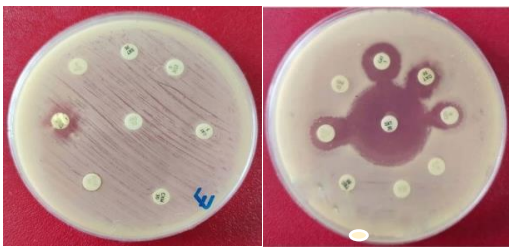

Fig.4: Agar plate showing antibiotic sensitivity test for EP5 (*E.coli*) isolate showed Multidrug-resistant pattern.

3.4 Isolation and morphology of phage:

Three distinct morphological phages in total, plaques were isolated from different collected sewage water. Isolated one plaque was repeated three times to obtain biologically purified phages. Plaques produced by the phage ΦEEP, which attacks *E.coli*, were circular, turbid, without center and without hallo. The plaques produced by the phage ΦEPP, which attacks *Pseudomonas aeruginosa*, were circular, clear, without a center, and without hallo. The plaques produced by the phage ΦEAP, which attacks *Acinetobacter baumannii*, were Circular, clear, without a center, and without hallo Table 1.

Table 1: Plaques morphology of isolated phage: ΦEAP (E: Essam, A: Acinetobacter, and P: phage); ΦEEP, (E: Essam, E: e.coli, and P: phage); and phage ΦEPP (E: Essam, P: pseudomons, and P: phage)

Phage isolates	Plaque morphology	Plaque Diameter(mm)
ΦEAP	Circular, clear, without centre, without hallo	2.2 - 3.2
ΦEEP	Circular, Turbid, without centre, without hallo	3.5 -5.5
ΦEPP	Circular, clear, without centre, without hallo	1.5 - 2.5

3.5 Examination of phage morphology by electron microscope:

Phage lysate in pure suspension was negatively stained for transmission electron microscopy (TEM) examination. Electron micrographs (Fig. 5) showed three-tailed phages related to three families (Table 2), in which ΦEEP belonged to the family Siphoviridae. ΦEPP phage belonged to Podoviridae and ΦEAP belonged to Myoviridae.

Table 2: Morphological properties and predicted family of phage isolates by TEM

Phages	Head (nm)		Tail (nm)		predicted family
	size	shape	Size	shape	
ΦEAP	94.4	icosahedral	302	contractile	Myoviridae
ΦEEP	55.4	Oval	70.7	Non-contractile	Siphoviridae
ΦEPP	56.2	icosahedral	77.2	Non-contractile	Podoviridae

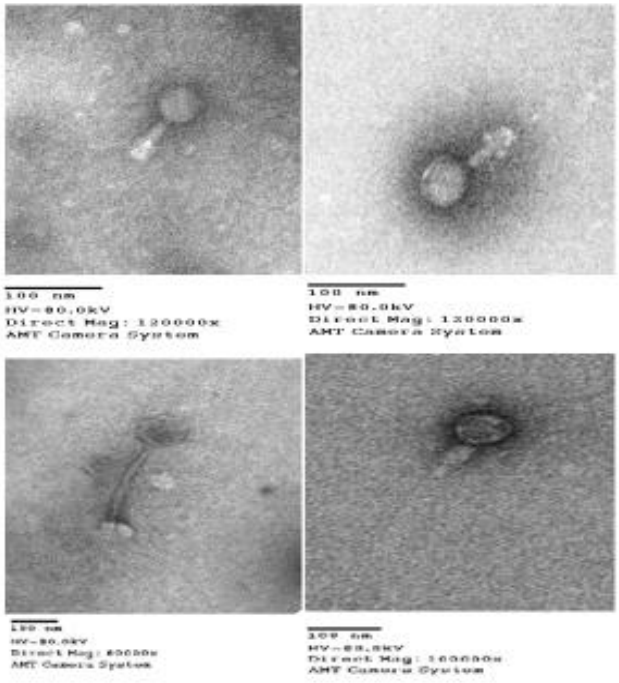

Fig.5: Transmission electron micrographs of bacteriophages, used in this study (A) Phage ΦEPP, (B) phage ΦEAP, and (C) phage ΦEEP**.**

3.6 Application of bacteriophage to control pathogenic bacteria in Skin wounds of experimental animals:

Fig. 6. Model of a mouse wound infected with skin. Five groups of test animals were created, and they are as following Group1: Wound control (Positive control); Group 2: Infected wound control (Negative control); G3: Infected Wound + Phage; G4: Infected Wound + Fusidic acid; G5: Infected Wound + Fusidic + Phage. Every group had an infection with 2.5 × 108 CFU of A.baumannii administered to the wound to close it. Standard methods were used to measure and determine the healing of the wound rates.

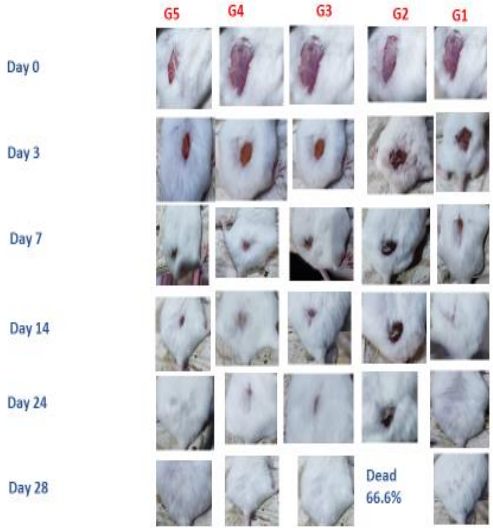

Fig. 6: Model of a mouse wound infected with skin. Group 1: non-infected & untreated animals (positive control). Group 2: animals infected and untreated (negative control). Group 3: mice infected & treated with phage Group 4: mice infected & treated with fusidic acid antibiotic. Group 5: animals infected and treated with phage and fusidic acid antibiotic.

Within each experimental group, the various stages of the process of wound curative (swelling, proliferation, inflammation, and remodelling) were noted and documented. In comparison to the further groups, those with the infection wound in the control group (G2) displayed slowed wound healing processes. More rapid remodelling was observed in the phage (G3) and phage in combination with Fusidic acid than in the G2 infected control group (G5) treated groups. In the phases of inflammation, proliferation, and remodelling, Group G3 excelled. Re-epithelialization, cell proliferation, and deposits of collagen appeared to occur more quickly and effectively in the G3 group.

Fig.7, displays photos of the development of healing of wounds in the G1–G5 skin wound model mouse groups during the 28-day trial. Mice exposed to phage (G3) and fusidic acid (G4 & G5) displayed a reduction in wound size, leading to full healing by day 28. At the conclusion of the study period, the G1 control group, which was free of infection, also displayed full healing.However, in the infected control group (G2), healing was not noticeable. The wound area shrank less than days 4 to 24 is depicted in. Fig. 7. From day 4 to day 24, all groups displayed a significant difference in the wound area (P 0.05). The decline was seen in the G3 group but it didn't happen in the G4 group. The group that received both fusidic acid and phages (G5) experienced the most reduction. The G2 group experienced a lesser drop, though (Fig. 6). When compared to group G2 on the day 24, the wound zone in groups Group3, Group4, and G5 was substantially less (P 0.05).

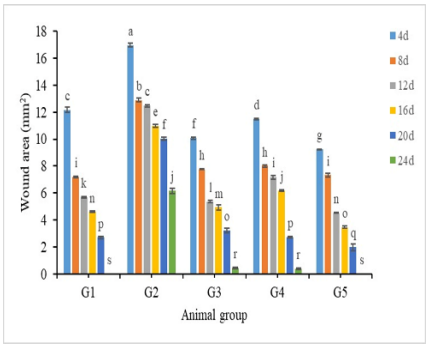

Fig. 7: Impact of various therapies happening the wound region during the experimental epoch on the rate of wound healing in experimental animals. The wound area assessed on each mouse in the several experimental groups is expressed as a mean standard deviation in values stated in mm2. Results in Figure 7 revealed that the wound area significantly differed in the phage G3 group than the control. G1: Positive control, G2: Negative control, G3: phage group, G4: Fusidic acid group, and G5: phage-fusidic group.

3.7 Bacterial impose in the infected Wound:

All groups had a significant reduction in bacterial load (mean standard deviation of CFU/mg at day 16) over the course of the trial (P 0.05) (Fig. 8). Notably, in the G3 phage-treated group, the first bacterial load decreased by day 12 and was no longer detected by day 16 (P<0.05). On the other hand, In the G4 fusidic acid-treated G., the bacterial impose decreased significantly on day 8. Similarly, the bacterial load decreased in-group G5 in which phage treatment was supplemented with fusidic on day 8. On day, 12 or day 16 for the G4 - and G5 groups no bacterial growth was observed. No aberrant behavioural patterns, such as weariness, tension, or aggression, were ever noticed in the phage-treated mice until the conclusion of the experiment.

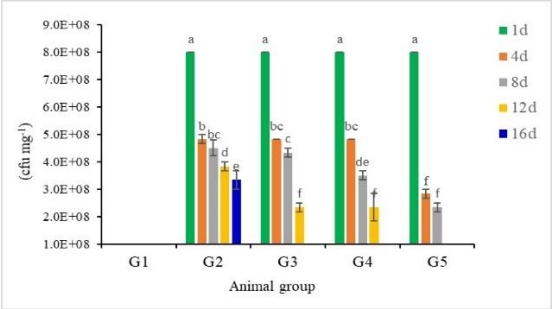

Fig.8: Assessment of bacterial load in wound tissues of infected groups (i.e., Group2, Group3, G4, and G5) compared to the uninfected positive control group (i.e., G1) throughout the experimental period. A. baumannii was injected into each site at a dosage of 2.5 × 108 CFU to begin the infection experiment. Biopsies of tissue from excisional wounds were analysed for microorganisms using culture. The mean standard errors of the number of bacterial cultures discovered in each mouse among the various experimental groups are values represented as log CFU/mg. Bars for several experimental mouse groups having the same letters for the same sampling day do not differ statistically (p≤ 0.05).

3.8 Mortality of mice across multiple treatment groups:

Mice in the control group with uninfected wounds (G1), the fusidic acid-treated animals (G5) and the phage-treated group (G3) Zero mortality rate up to day 28 after treatment. (Fig.9). In difference, only 34% of the animals survived on day 9 in the Group2 group and no survivors were discovered on day 22. The mortality rate in the G2 group differed meaningfully from all further groups (p ≤ 0.001). A survival rate of 84 and 83% was observed in the G3 group and in the G5 group (fusidic acid + phage), respectively.

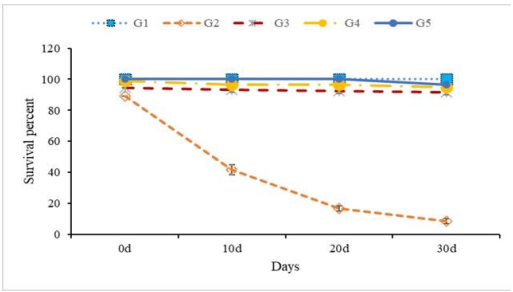

Fig.9: Effect of bacteriophage therapy on mice infected with A. baumannii's survival. Animals in the contaminated wound control group G2, which did not get phage therapy, passed away on day 24. The remaining animals all made it to day 28 and displayed full wound healing. In comparison to all other groups, G2 had a considerably lower survival rate (p< 0.05).

4. Discussion

Numerous transgenic varieties and models were produced to assist researchers in their investigation of the molecular mechanisms involved in wound repair and regeneration. The laboratory mice continues to be the standard research animal tool [29]. Skin is the body's first line of resistance against pathogenic infections; any damage to the skin permits pathogens to enter, which can cause sepsis and death [30]. Chemical, mental agents and illnesses like diabetes can induce wounds and skin damage [31]. One of the most prevalent surgical contaminations having a high fatality rate is wound infection [32]. Skin infections frequently start out as localized induration, erythema, discomfort, or soreness at the bacterial infection site but can develop into the potentially fatal necrotizing fasciitis. The most prevalent species in wound infections is A. baumannii [33, 34, and 35]. Among the few studies to test the efficacy of phage therapy against Acinetobacter baumannii infections used a skin excision wound prototypical in mice. Testing the effectiveness of locally isolated phages against A.baumannii was the main goal of this investigation. We showed that the Myoviridae family phage against A.baumannii, EAP was very efficient in curing the bacterial infection within 4 weeks. Our findings demonstrated that the progressive decrease of bacterial impose in the wounds was related with quicker wound healing in phage-treated wounds. We used a tissue excision approach to count the bacteria in the infected wounds in order to monitor the bacterial burden during the study period. Swab cultures are frequently utilised, however the technique has come under heavy fire because it estimates bacterial counts from the surface of the wound rather than from deeper tissue [36]. Numerous earlier research on both people and animals claimed that healing wounds was connected to a concurrent decrease in exposure to the contagious bacterial strain [37, 38]. In order for phage therapy to be effective, wound healing, which has a complex aetiology, must also be prevented in addition to bacterial development. Importantly, phages do not obstruct wound healing and, according to some, may even aid in it. In light of this concept, the excision wound model was used in the current investigation to assess a phage's potential effectiveness ΦEAP against A.baumannii infected wounds and wound healing activity in mouse through topical usages. Rodents are the favoured model for the majority of researchers since they are less expensive, easier to handle, take up less room, and produce faster findings than humans because their wounds heal more quickly [39]. The most thorough evaluation of wound healing mechanisms, including B. the process of re granulation, and blood vessel development, is provided by the excision wound model [40]. In phage therapy, it is crucial that phages are unique to the bacterial strain that is infected. Strain specificity securely prevents unintended negative effects on a person's typical flora's beneficial bacteria. However, a multi-phage cocktail may be an alternative because it is hard in the clinical situation to identify the precise strain identity prior to delivering phage therapy. There is evidence in the literature that using numerous phages in therapy increases success rates [41, 42]. Other important aspects of phage therapy are dosage and application timing due to the host's fast elimination of phages [43, 44]. After infecting the wound with the bacterial strain for 24 hours, we decided to apply the phages right away. Our results on wound healing and bacterial burden during the trial period show that this strategy was successful. Phages are recognised not to be human infectious, yet they might also have immunogenic properties.

The phage dose in our investigation led to a quicker decline in bacterial load and higher survival rates. Higher doses may result in increased efficacy, according to existing research in the literature [45, 46].However; The likelihood of problems from treatment cannot be disregarded [45].Few contemporary researches have examined the in vivo efficacy of phage treatments to that of antibiotics, or even combined phage and antibiotic treatment, despite the fact that there have been several in vivo investigations on the effectiveness of phage therapy [47]. Group (combination phage and fusidic acid) in our study concurred with the studies of Sarker et al; Schooley et al., and LaVergne et al. [48,49, 50].For the treatment of multidrug-resistant A.baumannii diseases, the use of phage or phage lysine, either alone or in conjunction with traditional antibiotics, may provide alternative therapeutic choices.

5.Conclusion

Because bacteriophages are unique, they are remarkably effective therapeutic agents. In addition, due to their high level of specificity, bacteriophages are particularly successful at lysing a particular pathogenic disease. They have demonstrated to be extremely safe when used therapeutically, and they can quickly adapt to compete with newly mutated bacterial species. However, more in-depth study of bacteriophages is still required in order to develop new methods for combating bacteria with fast evolving, resistant, and altered strains. In the present investigation, we demonstrated that phages could be successfully applied topically to treat skin wound infections using an in vivo skin excisional model. The findings highlight the significance of selectively isolated, tailored to needs phages as an achievable antimicrobial treatment substitute for antibiotics. More studies on unknown mechanisms of bacteriophages and their application in humans must be conducted.

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Author Contributions

All authors contributed to this work. Essam Saad Mahmoud prepared the samples, completed the experimental measurements, statistics, write and submission the manuiscript. Reda M. Taha, Rasha H. Bassyouni, Fatma A. Ahmed and Farag A.Samhan contribute in the revision of the manuscript for publication.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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