The combination of bacteriophage therapy and antibiotic therapy

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Declaration

It is hereby declared that

1. The paper does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.

2. The paper does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.

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Ethics Statement

Ethics is the discipline dealing with what is good and bad and with moral duty and obligations. In this review research work, ethical approval was not necessary.

Abstract

Antibiotic resistance has become a major problem of current time. A lot of deadly bacterial pathogens have become resistant to antibiotics. In addition, pathogens like P.aeruginosa, E.coli, Acinetobacter baumannii and many other bacteria has strains which are resistant to multiple antibiotics. This has made these pathogens even more deadly. Also the overuse of antibiotics is harmful for human health. Thus this indicates to choose an alternative. However, the bacteriophage therapy could be a great and effective alternative. This review paper contains the effectiveness of the combined treatment of bacteriophage and antibiotics against deadly pathogens which have also become resistant to multiple drugs as well.

Bacteriophages are viruses which infect and replicates within the specific bacterial cell. The phage virus is highly specific. This indicates that the phage virus will only infect the targeted bacterial pathogen and would not harm the surroundings.

The combination of phage therapy with antibiotic therapy has showed positive results in many cases. Multiple experiments have showed significant success by using the combined therapy. The success rate of this combined treatment against pathogens like P.aeruginosa, E.coli or Acinetobacter baumannii and their multiple resistant strains is very promising. Although a lot of experiments should be done to achieve better results in this field.

Acknowledgement

First and foremost we would like to thank Almighty Allah for gifting us our knowledge, capability and the strength to complete our project in the best way possible. We are thankful to **Dr. A F M Yusuf Haider**, (Chairperson Department of Mathematics and Natural Sciences, BRAC University) for looking after all the students and teachers under his department and always providing his helping hands whenever needed.

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We really appreciate all the teachers that taught us throughout our undergraduate level and helped us understand the theories as well as lab works very well. From the knowledge that we gained from their teaching helped us better understand each and every aspect of our research that we did during our project.

Table of Content

Introduction:

Bacteriophage, also known as phage, is a virus that only infects and replicates within bacterial cells.Frederick W. Twort in the United Kingdom and Félix d'Hérelle in France were the first to find bacteriophages.The name "bacteriophage" comes from two words: "bacteria" and "phagein," which all mean "to devour. Félix d'Hérelle coined the phrase. They are the most abundant biological particles in water and the second most abundant portion of biomass on land after prokaryotes. They can be found in a various environments around the world. Depending on the type of bacteria they infect, bacteriophages vary in form, scale, and genome organization however their basic composition remains the same. A nucleic acid genome is enclosed within a shell of phage-encoded capsid proteins in all bacteriophages.Different phages have different head structures, and their sizes vary from 24-200 nm in length. Their shapes, sizes, and structures varies based on the type of bacteriophage. The ability of phages to infect and potentially destroy infectious bacterial agents refers them as an alternative to antibiotics. Bacteriophage invasion follows an almost identical pattern: they first bind to the host cell and then insert their genome into the host cell, suspending the host's cellular machinery. Bacteriophages have a significant level of species specificity when it comes to their host cell. They only infect a particular bacterial species or even particular strains within that species. This highly specificity character of bacteriophage has made itself a strong alternative of antibiotics. This means that the phage will infect the targeted bacterial host cell only and letting no harm to any other cells of the system.

Life Cycles of Bacteriophage

Bacteriophages, like all viruses, replicate in the bacterial host cell. There are two kinds of viral lifecycles, each with its own DNA replication process. In one, the viral DNA replicates independently of the host DNA (Lytic Cycle) and in the other, the viral DNA is inserted into the host DNA (Lysogenic Cycle). In various types of bacteriophages, these lifecycles can occur individually or alternately.

Lytic Cycle:

During the lytic cycle, viral DNA exists as a free-floating molecule that replicates independently of bacterial DNA. This cycle is most often seen in virulent phages, which cause the infected cell membrane to be destroyed during the release of viral particles. Bacteriophage destroys the host bacteria in this cycle.
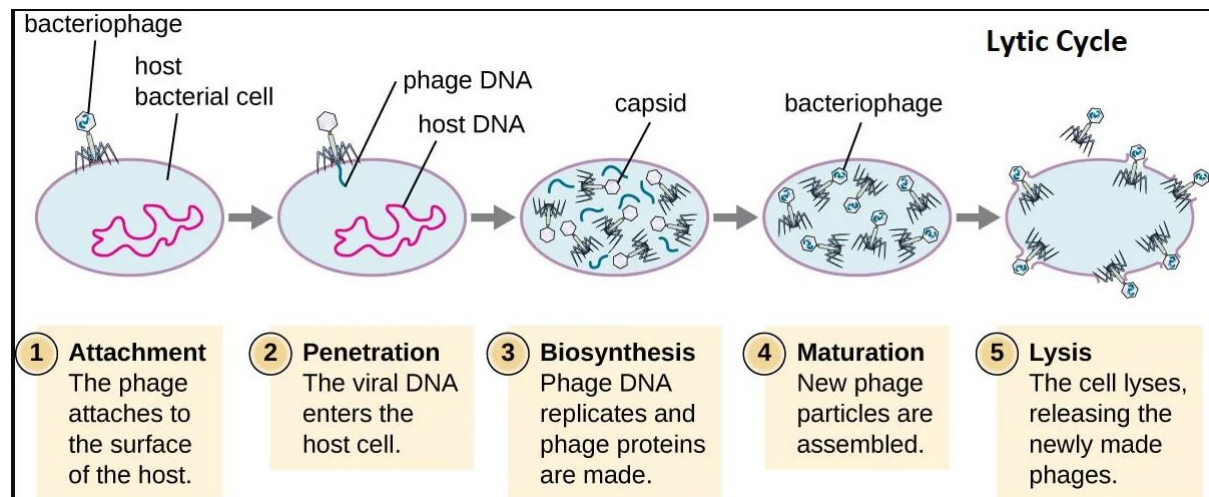
Figure: Lytic Cycle of Bacteriophage

Lysogenic Cycle:

During the lysogenic lifecycle, the host bacteria continue to survive and replicate naturally after bacteriophage reproduction. Here in the stage called "prophage" bacteriophage genetic material is inserted into bacterial DNA during the lysogenic lifecycle and can be transferred to daughter cells during bacterial cell division. Since the bacteriophage does not destroy the host cell, the lysogenic period is a mild and non-virulent infection.

Figure: Lysogenic Cycle of Bacteriophage

Phage Therapy:

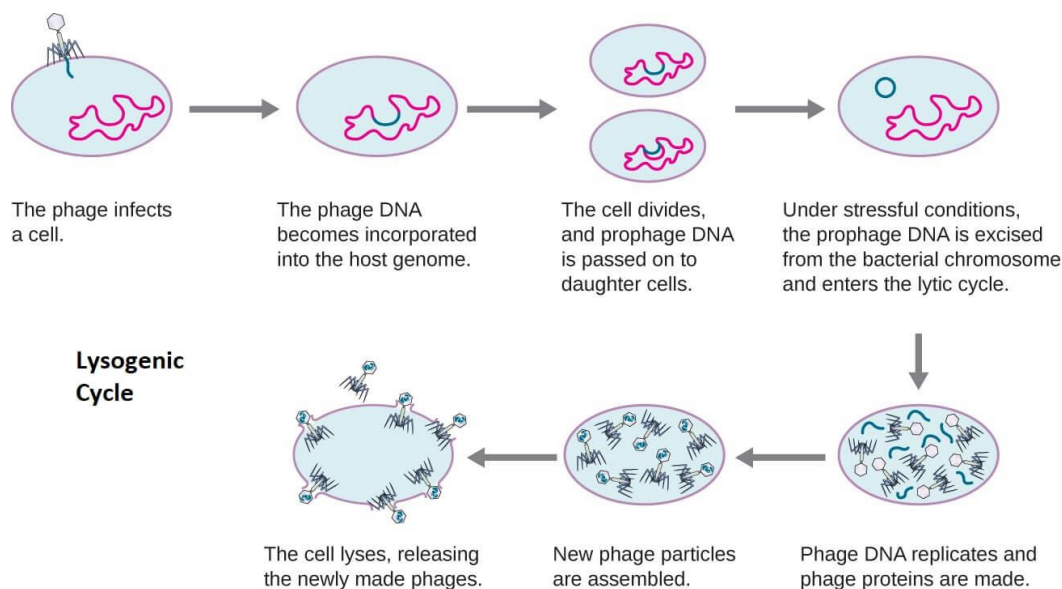The use of bacteriophages to cure multiple bacterial infections is known as phage therapy or virus therapy. The origin of phage therapy dates back to Twort and d'Herelie's discovery of bacteriophages in 1917. Phage therapy was first used in humans over a century ago, and it is now used to treat various bacterial infections such as Staphylococcus aureus, Enterococcus, Proteus, and Pseudomonas aeruginosa. Antibiotics are often related to phage therapy, and it has been recognized that phage therapy has many benefits over antibiotics. Phage therapy has less to no side effects, and it is also selective against the bacterial population contained in biofilms. The use of bacteriophages to kill bacterial cells involved in infections is the fundamental concept of phage therapy as a potential means of treatment and prevention of bacterial infection. The administration of phages is the first step in using phages as a method of therapy. Within 2-4 hours after an oral injection, the phages enter the bloodstream, and after 10 hours, they are present in the internal organs. The phages' bactericidal behavior is the product of viruses replicating via the lytic cycle within the host cell.

Antibiotic Therapy:

Molds and plants were used to cure infection by the ancient Chinese, Greeks, and Egyptians. Infectious diseases have been treated with a number of herbal medicines throughout history, including quinine, which has long been used to cure malaria. Ernest Duchesme described the antibacterial properties of Penicillium spp. in 1897, making it the first modern discovery. In 1928, Fleming's dissertation was published. Salvarsan, a Syphilis medication developed by Paul Ehrlich after his experiments on arsenic and other metallic compounds in Germany in 1909, was the first genuine antibiotic.

Antibiotics are pharmacological agents that selectively kill or hinder bacterial cell growth while having little or no effect on their mammalian hosts. Bacteriostatic antibiotics stop bacteria from reproducing, relying on a strong immune system to remove the infection, while bactericidal antibiotics destroy bacteria. Since bacteria are shielded from host immune functions inside valve vegetations, the use of a bactericidal agent is required when treating infective endocarditis.

A rational approach to phage therapy has many potential benefits that antibiotics alone cannot achieve. However, in comparison to traditional antibiotics, there are also

limitations to phage therapy. While many of these differences historically have been considered limitations to using phage therapy, the perceived drawbacks may instead be leveraged as benefits in some circumstances. In the past, both types of therapies have typically been investigated alone; however, with many identified distinctions, a combination approach utilizing both therapies may prove to be the most efficacious in the long run. (Kaitlyn E. Kortright, 2019)

Realistically, administration of chemical antibiotics may never be completely replaced by therapeutic use of phage and may be inappropriate under some clinical conditions, suggesting that adjuvant approaches should be closely studied. A combined therapy of phage and antibiotics could be ideal capitalizing on each treatment's differing strengths. Bedi et al. (2009) observed an additive effect when phage and antibiotics were used to treat a Klebsiella pneumonia biofilm. Knezevic et al. (2013) investigated the potential synergism between phage and antibiotics and observed synergy between P. aeruginosa phage and subinhibitory concentrations of ceftriaxone, but not with gentamicin, ciprofloxacin, or polymyxin B. They proposed that, the mechanism of action of the antibiotic must not interfere with critical processes in phage replication to see a synergistic effect. While not all combinations of phage and antibiotic appear to be synergistic, and the mechanisms behind synergism are still being explored, precision medicine is currently in vogue, and phage therapy shows promise as a ''personalized'' approach for at least some clinical cases. As with any drug, the ideal circumstance is that phage therapy should be developed to reduce off-target effects and to minimize disruption of helpful microbiome communities to the extent possible. (Kaitlyn E. Kortright, 2019)

Antibiotic and Phage in Combination; with many in vivo studies on the efficacy of phage therapy, not many recent studies have compared the in vivo efficacy of phage therapy to that of antibiotics or even phage in combination with antibiotic treatment.

The combination of bateriophage therapy and antibiotic therapy against _Pseudomonas aeruginosa_:

Among the most widespread drug-resistant bacteria are PRSP (penicillin-resistant Streptococcus pneumoniae), VRE (vancomycin-resistant Enterococcus), MRSA (methicillin-resistant Staphylococcus aureus), MDR-Pa (multiple drugresistant Pseudomonas aeruginosa), VRSA (vancomycin-resistant Staphylococcus aureus),

VRSA (vancomycin-resistant Staphyloc Despite the fact that new antibiotics are continuously being produced, bacteria immune to these antibiotics will appear sooner or later, reducing the effectiveness of traditional antibiotic chemotherapy. However, to fight bacterial infections and treat a wide range of infectious diseases, alternative remedial therapies, such as biologic therapeutics or other novel therapeutics, must be created. The use of phages as biological agents for the prevention of bacterial infectious diseases could be a great option in this purpose which is known as phage therapy.

Pseudomonas aeruginosa (P. aeruginosa) is a common human pathogen that causes serious clinical infections like fatal sepsis and nosocomial infections like pneumonia and urinary tract infection, as well as severe complications and mortality. According to the Chinese National Pathogen Resistance Surveillance (CNPRS) study, P. aeruginosa susceptibility to the 11 major antibiotics is decreasing, with susceptibility to imipenem and ceftazidime falling from 96 percent and 92 percent, respectively, to 75 percent and 79 percent from 1994 to 2001. Imipenem is the most common antibiotic used to treat Gram-negative bacterial infections, and once bacteria become immune to it, there are no other options. Furthermore, when P. aeruginosa is immune to an antibiotic like imipenem in the laboratory, it is also resistant to other antibiotics. As a result, infection with imipenem-resistant P. aeruginosa (IMPR-Pa) is one of the most difficult therapeutic challenges to solve.

According to the study of JING WANG(2005), the mixture of phage ØA392 and Ø1093 is shows positive result in order to treat imipenem-resistant P. aeruginosa cases. The use of a phage mixture yielded positive results. The aim of this study was to observe if mixed phage was effective against IMPR-Pa infections. Mice were injected intraperitoneally with one of four therapeutic IMPR-Pa strains: 9747, 9915, 9994, or 1613. For this experiment, the phage mixture was propagated once for each subsequent IMPR-Pa strain to see whether it could form plaques on them. In comparison to the >90 percent mortality in the phage-untreated mice (Fig. 01), three classes of mice infected with these phage mixture lived for 7 days after IMPR-Pa injection. This means that phage mixture might well be beneficial for human IMPR-Pa infections. Just 10% of the other group's mice (9994) survived (Fig. 01), indicating that phage rescue is dependent on lytic activity.
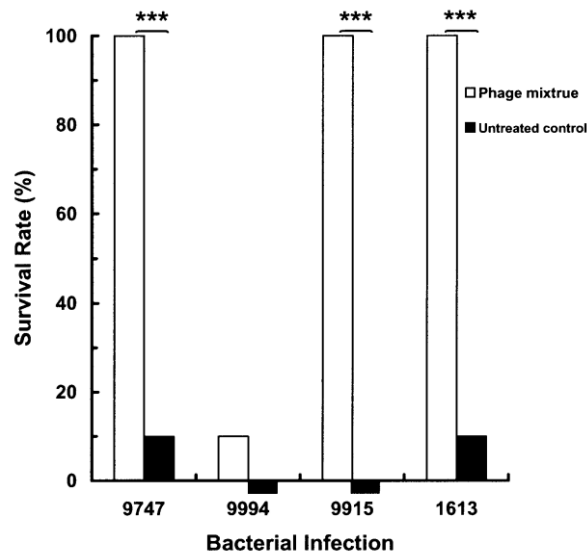
Figure 01. Effect of mixed phage on imipenem-resistant P. aeruginosa (IMPR-Pa) infections in mice

Using newly isolated lytic phages, we created an animal model to test if the phage could be used to treat IMPR-Pa bacteremia. The findings of the current studies are encouraging: after injecting MLD (3×10^7 CFU) doses of IMPR-Pa bacterial strains into one side of the abdomen, injecting phages at MOI 0.01 into the other side of the abdomen saved 100% of the bacteremic mice. Even when phage therapy was postponed by up to 3 hours, nearly 40% of the animals were rescued and fully recovered. In comparison to the therapeutic role of active phages, heat-inactivated phages had little effect in the studies. The phage ØA392 and phage Ø1093 'cocktail' saved bacteremic mice from IMPR-Pa 9747, 1613, and 9915 infection, but not from IMPR-Pa 9994 infection. In our mouse model of IMPR-Pa bacteremia, these studies show that phages have a strong curative role.

In cystic fibrosis (CF) patients, Pseudomonas aeruginosa has been one of the most common causes of respiratory infection. Chronic infections are also caused by this pathogen in patients with obstructive pulmonary disease. The US Food and Drug Administration (FDA) has approved five inhalable antibiotics for the treatment of respiratory infections: ciprofloxacin, tobramycin, colistin, aztreonam, and amikacin.

Phage therapy is gaining popularity as a therapeutic alternative for multidrug-resistant bacteria (MDR bacteria). In contrast to antibiotics, phages are highly selective to their target pathogen and do not damage commensal bacteria. Since bacteria may develop resistance to phages, the use of bacteriophage mixes with or without antibiotics has been used to combat this. Combining the two is much more sensible and effective

option. In vitro and in vivo experiments have shown that phage can generate synergistic antimicrobial effects when combined with antibiotics.

Yu Lin (2018) find that, PEV20 is a podovirus phage that has antibacterial activity in vitro and in vivo against P.aeruginosa. At 24 hours after intratracheal administration, spray dried PEV20 powder decreased bacterial load in the lungs of P. aeruginosa-infected mice by 5.3 log10.

PEV20 was shown to have synergistic antibacterial action against FADD1-PA001 when combined with ciprofloxacin, amikacin, or colistin. During a 24-hour incubation period, the bacterial density of ciprofloxacin or amikacin in combination with phage PEV20 remained consistently low and did not show any apparent development.

PEV20 was shown to have synergistic antibacterial efficacy against JIP865 when combined with ciprofloxacin, amikacin, or tobramycin. Just ciprofloxacin totally stopped bacteria from growing while being combined with the phage PEV20 (Figure 02).
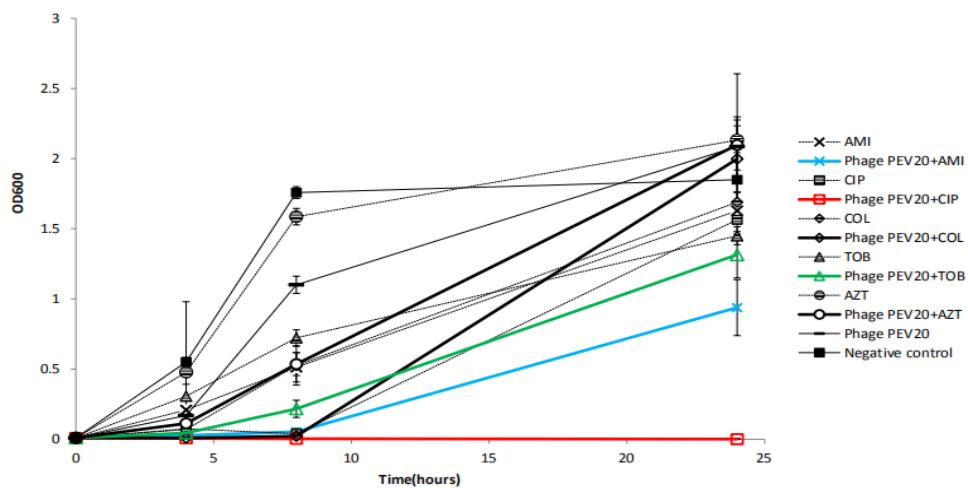
Figure 02. Antibacterial activities of phage PEV20 (MOI=100) against P. aeruginosa in the presence of 1/2 MIC of ciprofloxacin (CIP), amikacin (AMI), colistin (COL), tobramycin (TOB), and aztreonam (AZT)

On FADD1-PA001 or JIP865, aztreonam showed no synergy. There was no synergy found for bacterial strain 20844n/m(s) for any of the phage-antibiotic combinations.

Against P. aeruginosa FADD1-PA001 and JIP865, the mixture of PEV20 and ciprofloxacin had a synergistic antibacterial impact.

	FADD1-PA001		JIP865		20844n/m(s)	
	Calculated	Observed	Calculated	Observed	Calculated	Observed
CIP +PEV20	0.06±0.02	0.005±0.001*	1.0±0.2	0.001±0.002*	0.2±0.09	0.2±0.02
AMI+PEV20	0.3±0.04	0.01±0.01*	1.0±0.1	0.5±0.1*	0.6±0.2	0.8±0.3
COL+PEV20	0.5±0.07	0.2±0.1*	1.0±0.1	1.0±0.2	0.4±0.2	0.3±0.1
TOB+PEV20	0.4±0.07	0.3±0.1	0.9±0.1	0.7±0.1*	0.7±0.3	0.6±0.3
AZT+PEV20	0.3±0.04	0.3±0.09	1.3±0.2	1.1±0.2	0.8±0.3	0.6±0.3

Note: Ciprofloxacin (CIP), Tobramycin (TOB), Aztreonam (AZT), Colistin (COL), Amikacin (AMI)

Table01. Calculated and observed bacterial survival after 24 h treatment of phage PEV20 and antibiotic combinations (n=5).

Ciprofloxacin was shown to have the greatest synergistic activity with PEV20 against two therapeutic P. aeruginosa strains, FADD1-PA001 and JIP865, of the five FDA-approved inhalable antibiotics, and this result is consistent with recent studies on other phage-antibiotic combinations against P. aeruginosa. For two clinical strains, another antibiotic candidate, amikacin, demonstrated synergy with PEV20, but the result was less than ciprofloxacin for strain JIP865. On FADD1-PA001 and JIP865, synergy with PEV20 was observed for colistin and tobramycin, respectively. However, the growth of bacteria was not totally stopped. While there was no synergy between aztreonam and PEV20, there were additive effects.

According to this in vitro study, phage PEV20 in combination with ciprofloxacin prevents clinical P. aeruginosa strains from regrowing 24 hours after therapy.

Urinary Tract Infection Case Report

Khawaldeh et al. (2011) reported treatment of a urinary tract infection caused by P. aeruginosa associated with a bilateral ureteral stent. The infection consistently recurred within a week, following cessation of antibiotic therapy. A suitable commercial phage product was identified as libraries of phage from the Eliava Institute were screened against the bacterial isolate. This phage mixture contained phage with activity against Streptococcus pyogenes, S. aureus, E. coli, P. aeruginosa, Proteus vulgaris, and Proteus mirabilis. On day 6 of the treatment, antibiotic therapy with meropenem and colistin was initiated. After 5 days of phage treatment, 10-fold reduction of bacteria in the urine was reported by Khawaldeh et al. (2011). Subsequent antibiotic treatment for two days resulted in apparent clearance of the infection, at which point culturable P. aeruginosa was below the limit of detection. With the 30-day course of meropenem being completed, both stents were removed, and one was replaced. After the treatment, urine samples remained sterile for a year; at which point observations were concluded.

Case 2: Aortic Graft Infection Case Report

A P. aeruginosa infection that was refractory to standard treatment was caused by a surgical intervention for repairing an aortic aneurysm with a Dacron graft (Chan et al., 2018). This chronic infection resulted in an aorto-cutaneous fistula with purulent discharge to form. An attempt to control the infection with intravenous ceftazidime followed by oral ciprofloxacin was considered. Resistance to ciprofloxacin evolved during the course of treatment, debridement and irrigation were unsuccessful in resolving the infection and surgical replacement of the graft was not an option. Other options for infection control were considered, after 3 years of suppressive antibiotic therapy which failed to eradicate the infection. A recent report of P. aeruginosa phage OMKO1 which had demonstrated synergy when combined with ceftazidime (Chan et al., 2016) was screened for lytic activity against the strain. While continuing the existing therapy of intravenous ceftazidime, instillation of a single dose of ceftazidime and phage OMKO1 was applied topically at the site of fistular discharge. Partial graft excision and replacement was required following bleeding from the fistula, four weeks after the administration of phage. At the time of surgery, acquired cultures were negative for P. aeruginosa, as a result the course of ceftazidime was discontinued. There was no recurrence of the infection in the absence of any antibiotic therapy, two years after phage treatment. The favorable outcome of this case emphasizes the rational choice of phage and route of administration for this particular infection; thoughtful

selection of a phage that had previously demonstrated synergy with the clinically relevant antibiotics, applied in proximity to the source of infection undoubtedly contributed to the positive outcome.

A study from Chang et al. (2019) showed synergistic antibacterial activities using a combination of PEV20 and ciprofloxacin against biofilms from clinical Pseudomonas aeruginosa strains isolated from wounds and sputum of CF (cystic fibrosis) patients. Biofilm eradication can be enhanced and at the same time, host cell growth can be facilitated by phage and antibiotic combination formulation. The antibiotic concentration required to treat P. aeruginosa infections in CF and wound patients could potentially be lowered by the addition of phage. This indicates the potential for implementing lower dosage regiment to help circumvent the side effects often associated with administration of high doses of antibiotics (Chang et al., 2019). However, to avoid antagonistic effect, it is essential to select phages that are highly effective against the target bacteria (Chang et al., 2019).
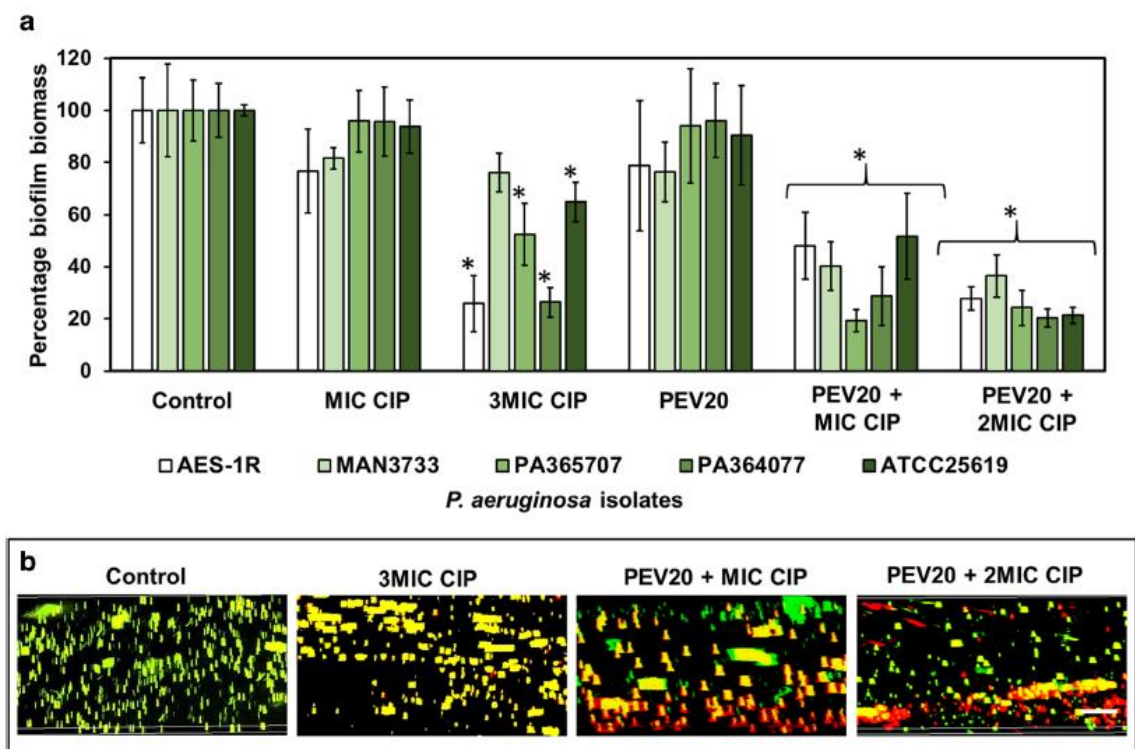

Fig. 03. a Percentage biofilm biomass after 24-h treatment with ciprofloxacin (CIP) alone minimum inhibitory concentrations (MIC) (MIC and 3MIC), PEV20 alone (108 PFU/mL), or antibiotics (MIC and 2MIC) combined with PEV20 (108 PFU/mL). To measure the biofilm biomass of P. aeruginosa isolates, crystal violet assay was used. Standard deviations from multiple cultures (n = 4) are represented by error bars. Statistically significant differences are indicated by asterisks (P < 0.05) in percentage biofilm biomass of the treated groups in comparison with non-treated control. b Representative images showing the effect of ciprofloxacin and PEV20 on PA365707

biofilm architecture. Marked disruption of biofilm architecture and increased dead biofilm after 24 h treatment with combination formulation containing PEV20 and ciprofloxacin was showed by confocal microscopy in conjugation with Live/Dead bacterial viability kit. Scale bar = 50 μm. Red, dead cells; green, live cells; yellow, mix of live and dead cells. The experiment was conducted in biological replicates (n = 3).
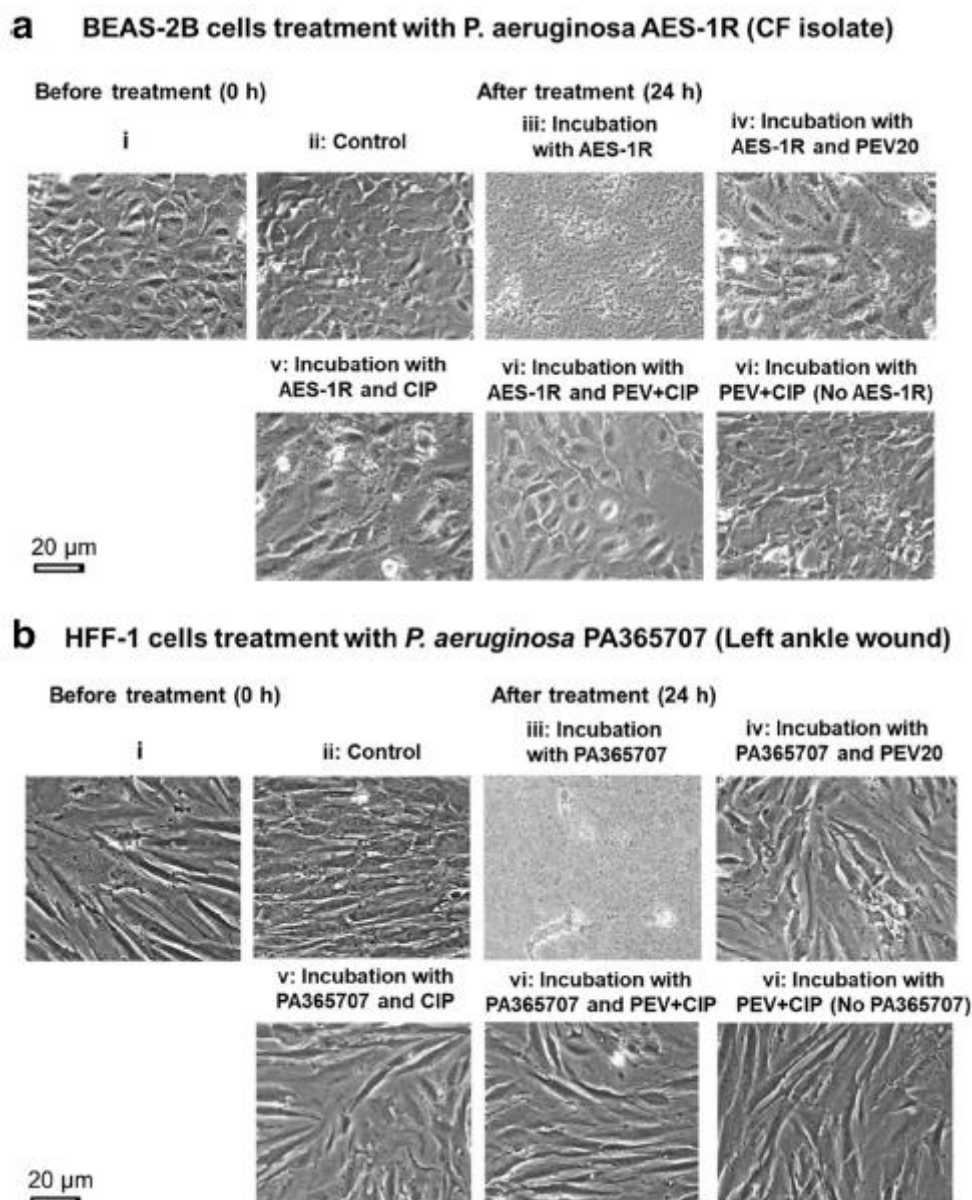
Fig. 04. a Lung epithelial cells BEAS-2B treated with P. aeruginosa Australian CF isolate (AES-1R): i: 72 h (100% confluence) before treatment. ii: 24 h (control/no bacterial treatment, 100% confluence). iii: 24 h incubation with bacteria (no adherent BEAS-2B found, complete AES-1R colonization). iv: 24 h incubation with bacteria and PEV20 (BEAS-2B cells adhered with AES-1R colonization). v: 24 h incubation with bacteria and ciprofloxacin (10 μg/mL) (BEAS-2B cells adhered with AES-1R

colonization). vi: 24 h incubation with bacteria and PEV20 + ciprofloxacin (5 µg/mL) (BEAS-2B cells adhered with 75% confluence with lower AES-1R colonization) vii: BEAS-2B cells with addition of PEV20 + ciprofloxacin (5 µg/mL) showed complete confluence. b Human foreskin Fibroblast cells HFF-1 treated with P. aeruginosa wound isolate (PA365707): i: 72 h (100% confluence) before treatment. ii: 24 h (control/No bacterial treatment, 100% confluence). iii: 24 h incubation with bacteria (No adherent HFF-1 found, 100% PA365707 colonization). iv: 24 h incubation with bacteria and PEV20 (HFF-1 cells adhered and no PA365707 colonization). v: 24 h incubation with bacteria and ciprofloxacin (0 .5µg/mL) (HFF-1 cells adhered and no PA365707 colonization) vi: HFF-1 cells with PEV20 + ciprofloxacin (0.25 µg/mL) (HFF-1 cells adhered and no PA365707 colonization) vii: HFF-1 cells with addition of PEV20 + ciprofloxacin (0.25 µg/mL) showed complete confluence. Four independent biological replicates were performed. Scale bar = 20 µm

For CF and wound isolates, MIC of ciprofloxacin ranged from 0.25–5 µg/mL (Table I). Compared with ciprofloxacin (MIC range, 2–5 µg/mL), CF isolates were less susceptible to tobramycin (MIC range, 15–20 µg/mL). Whereas wound infection isolates were susceptible to both tobramycin and ciprofloxacin at low concentration with MIC of 0.25 µg/mL.

	Minimum inhibitory concentration (µg/mL)						
	AES-1R	LESB58	MANC3733	PA365707	PA364077	AES-2	ATCC25619
Ciprofloxacin	5	5	2	0.25	0.25	2	1
Tobramycin	20	20	15	0.25	0.25	20	1
PEV20	Susceptible	Resistant	Partially resistant	Susceptible	Susceptible	Susceptible	Susceptible

Table 2. Minimum Inhibitory Concentrations of Ciprofloxacin and Tobramycin, and Phage PEV20 Susceptibility Against Seven P. aeruginosa Isolates

Across all seven isolates, the minimum biofilm inhibitory concentrations of ciprofloxacin (1–5 µg/mL) were similar to MICs (Table II). For tobramycin, intermediate resistance at 60 µg/mL (3MIC) was exhibited by AES-1R and LESB58; all other isolates were resistant at 5MIC. Five P. aeruginosa isolates, including AES-1R, PA364077, PA365707, AES-2, and ATCC25619 were highly susceptible to PEV20; MANC3733 was partially and LESB58 was completely resistant (Table I). For antibiotic combination treatment and anti-biofilm activity of phage, all seven isolates were assessed.

Minimum Biofilm Inhibitory Concentration (µg/mL)							
	AES-1R	LESB58	MANC3733	PA365707	PA364077	AES-2	ATCC25619
Ciprofloxacin	5 (S)	5 (S)	2 (S)	1 (S)	1 (S)	5 (S)	1 (S)
Tobramycin	60 (I)	60 (I)	45 (R)	1.5 (R)	1.5 (R)	60 (R)	45 (R)

Note: S, susceptible with ≥ 90% decrease in biofilm biomass; I, intermediate 25–50% decrease in biofilm biomass. R, resistance > 50% decrease in biofilm biomass compare with untreated biofilm/control. Antibiotic concentrations of up to five times the MIC were used to assess biofilm inhibitory concentration

Table 3. Minimum Biofilm Inhibitory Concentrations of Ciprofloxacin and Tobramycin Against Seven P. aeruginosa Isolates

Furthermore, bacterial killing within the biofilm as compared with ciprofloxacin treatment alone at 3MIC or untreated control biofilms was enhanced by the combination formulation. A study by Walters et al. showed that ciprofloxacin action is limited to areas adjacent to the airbiofilm interface and not the interior of the biofilm (Walters et al., 2003). Filamentation of bacteria was observed on the air-biofilm interface of ciprofloxacin-treated biofilm, while those residing in the interior were spared. Antibiotic tolerance in the midlayer of the biofilm is likely due to lack of oxygen, which decreases bacterial metabolic activity. The presence of phage could help reduce the integrity of extracellular matrix, thereby exposing the metabolically inactive bacteria to surrounding nutrients in the media (Glonti et al., 2010). Both ciprofloxacin and phage could induce antimicrobial effect once these bacteria become metabolically active,. Furthermore, phages can diffuse across biofilm and amplify and remain viable within the complex biofilm matrix ((González et al., 2018), (Briandet et al., 2008)). In fact, within the biofilm, close proximity of bacterial cells is favorable for the phages to multiply resulting in high local titres and rapid spread of phage infections (Taylor et al., 2014).

It has also been suggested that for physiological and ecological reasons, in terms of killing bacteria within biofilms, bacteriophage are likely to be more effective than antibiotics: (i) The polysaccharide depolymerase enzymes produced by phage are able to break down the extracellular matrix of biofilms; antibiotics are not. (ii) Lytic phages expose cells within these structures to exogenous nutrients by lysing the bacteria in the exterior of biofilms and thereby make the cells in the interior of the biofilm more metabolically active therefore more susceptible to killing by antibiotics [±(Abedon, 2015)]. Chan and colleagues observed what might be called `evolutionary' synergy between antibiotics and phage [(Chan et al., 2016)]: resistance to a phage that uses an outer membrane porin as a receptor site led to increases in the susceptibility to

antibiotics of different classes because resistance engendered a modification of the efflux pump responsible for resistance to these drugs. In short, both pathways of resistance evolution were reciprocally blocked by the combination of drug and phage.

In an investigation from Chaudhry et al. (2017), for the clinical potential of using combinations of antibiotics and phage additional support was provided to treat biofilm infections with P. aeruginosa using two newly isolated lytic phages and P. aeruginosa PA14, and showed that the efficacy of several antibiotics commonly employed for treating P. aeruginosa biofilms can be increased by these viruses. The effect of phages in limiting the ascent of minority populations resistant to the treating antibiotic is also considered by the study.

Synergy between some drugs and phages:

The effect of phages plus drugs was synergistic for ciprofloxacin (1X MIC only) and for ceftazidime (at 1X and 8X MIC). For ciprofloxacin (8X MIC) and for tobramycin (1X MIC), the combination of phages plus drug was facilitative. No facilitation or synergy was apparent between phages and the other two drugs (gentamicin, colistin).

Staggered phage and antibiotic treatment:

Antibiotics can be antagonistic to phage because they reduce the density of the bacteria and thus the capacity of these viruses to replicate[(PAYNE & JANSEN, 2001)]. Worse, antibiotics may even reduce phage numbers by interfering with phage replication within the cell (Alonso et al., 1981). The effects of this possible antagonism can be tested by treating with phage first; subsequently treating with the antibiotic, comparing the outcome with the case of simultaneous treatment. Here, delays of 4 and 24 hours are used. Results show substantial effects of delayed treatment with phage for some antibiotics (Fig 05). The only statistically significant effects of delay are for the 24 hours delay using tobramycin and gentamicin, but the magnitude of the effect is profound. These are two of the three drugs for which simultaneous treatment suppressed phage replication (Fig 5B). The third such drug suppressing phage replication with simultaneous treatment (ciprofloxacin) also exhibited greater kill with phage-first treatment, but the statistics fail to reject the null hypothesis of no effect of delay. Further investigation is warranted.
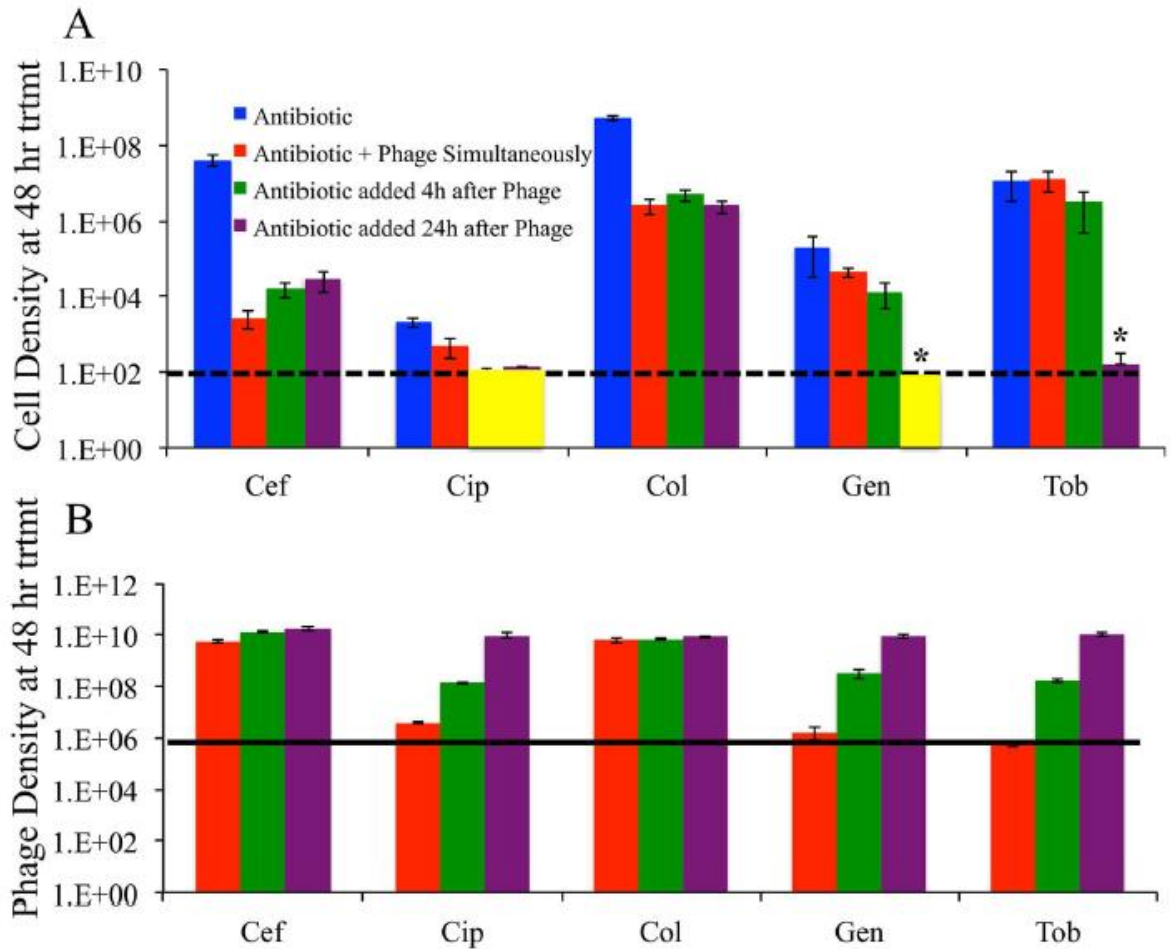
Fig 05. Effect of treatment order in killing P. aeruginosa PA14 on plastic. Bacteria was treated with the two phages (N) for 0, 4 or 24 hours after 48 hours of growth . Using the drug (8X MIC, abbreviations as in Fig 3), they were then treated . With the longer phage pretreatments, the duration of treatment following antibiotic addition was shorter as the culture was grown 48 hours.

(A) Densities of viable P. aeruginosa PA14 at the end of treatment. The horizontal dashed line represents the limit of detection (102/mL), and yellow boxes indicate that estimates were below the limit of detection.

(B) Densities of phage at the end of treatment. The bold black line in (B) indicates the initial density of introduced phage. * specifies that the 24 hours delay of gentamycin and tobramycin each have statistically significant effects on cell density compared to simultaneous treatment (P< 0.04, when correcting for multiple comparisons; tests of significance were equivalent for a Mann-Whitney U test and a median test using a Fisher's exact test calculation parametric tests were not possible because of some censored data). Standard errors and means from data obtained from two experiments, with a combined total of 5 replicate cultures.

There has been a resurgence of interest in theoretical and experimental studies exploring the efficacy of bacteriophage in combination with antibiotics to treat bacterial infections with increasing frequencies of pathogens resistant to multiple antibiotics [(Torres-Barceló et al., 2014)± (Lu & Collins, 2009),]. Taken at large, the results of these studies support the proposition that phage can increase the efficacy of antibiotics to treat these infections and facilitate the breaking up the of biofilms that commonly thwart antibiotic therapy [(Danis-Wlodarczyk et al., 2016)]. There is evidence for evolutionary synergy by phage treatment increasing the susceptibility to antibiotics in addition to this pharmacodynamics synergy, [(Chan et al., 2016)]. The results of our study provides additional evidence for both pharmacodynamic synergy of bactericidal antibiotics and phage in treating biofilm populations of P. aeruginosa PA14 and the prevention of treatment failure due to the ascent of minority populations resistant to the treating antibiotic.

In this study, we focused on the potential practical application of combinations of phage and antibiotics for treating biofilm infection. We have not explored the pharmaco population and evolutionary dynamic processes responsible for the observed results. Clarifying these processes, the aid of computer simulation and mathematical models is certainly required to understand and predict the conditions under which combinations of antibiotics and phage will be more effective than antibiotics alone.

A 48-h biofilm of PA14 was treated by Chaudhry et al. with the two phages NP1 (Siphoviridae, NP1Virus) and NP3 (Myoviridae) together or both in combination with five antibiotics (Chaudhry et al., 2017). Only moderate anti-biofilm efficacy was shown by each antimicrobial, however, true synergistic effects were observed between phages and ceftazidime at 1x MIC and 8x MIC and for ciprofloxacin at 1x MIC when applied simultaneously. For ciprofloxacin at 8x MIC and for tobramycin at 1x MIC, an improved effect by way of facilitation was also achieved, but interestingly not at 8x MIC (Chaudhry et al., 2017). The dose dependency of simultaneous applications with higher antibiotic concentrations likely eliminating the minimum bacterial density

required for optimal phage replication is indicated by these findings. Although this antibiotic belongs to the same class as tobramycin, no improvement was observed with colistin and gentamicin. With time-delayed use of phages and antibiotics, the therapeutic outcome differed. 24 h after phage application, the addition of tobramycin or gentamicin led to a significant synergistic effect. Conversely, compared to the simultaneous application, successive addition of ciprofloxacin or ceftazidime did not lead to a better outcome. Thus, critical to a successful combined application is the time point of antibiotic addition and the dosage. The antibacterial outcome may also be impacted by variations in the phage dosage, which was not further evaluated in this study.

According to new research(Oechslin et al., 2016) evaluated the in vitro activity of the whole phage cocktail and its individual phage components against a group of independent *P. aeruginosa* isolates in test tubes and in fibrin clots. This exposed the presence of bacterial strains with opposite susceptibility profiles, either resistant to all of the cocktail's phages (eg, strain PA7) or susceptible to all of them (eg, strainCHA). The rate of spontaneous phage resistance mutations of the susceptible strain CHA was found to be ca 10^{-7}. They expected that phage resistance would appear in infected fibrin clots, which contained ≥ 108 CFUs/g. This was certainly the case. On the other hand, these approach experiments highlighted 2 further significant facts. Initially, phages could readily spread into clots, kill the over powering bulk of phage-susceptible bacteria in situ, and protect the fibrin medium from bacterial-induced degeneration. Finally, combining phages with low concentrations of ciprofloxacin or meropenem (2.5 × the MIC) inhibited there growth of phage-resistant mutants, signifying possible success of in vivo therapy.

The in vivo experiments provided further critical information. Regarding PK/PD parameters, phages were relatively stable in plasma (elimination half-life of ca 2.3 hours following bolus administration) and persisted longer in organs (half-life up to 9 hours). These values were consistent with those observed in previous work (Merril et al., 1996) and confirmed that phages, whose sizes vary from ca 50 nm to 200 nm, can diffuse into various body compartments [5, 6, (Przerwa et al., 2006)]. As a result, phages were able to kill bacteria inside valve vegetations and multiply by up to 3 log PFUs/g within 6 hours locally. Phage-induced killing corresponded a burst of IL-1β and IL-6. The data did not permit extrapolating the dynamics of cytokine responses over

time, as cytokine levels were measured only at a single time point. However, as compared with rats treated with ciprofloxacin—the significant increase in IL-1β and IL-6 levels in rats treated with phages—most likely reflected the release of cell debris by phage-mediated lysis. Accordingly, it is known that both cytokines are inducible by LPS (Bont et al., 2006) and that similar results were obtained in EE using a bactericidal phage lysin (Entenza et al., 2005).
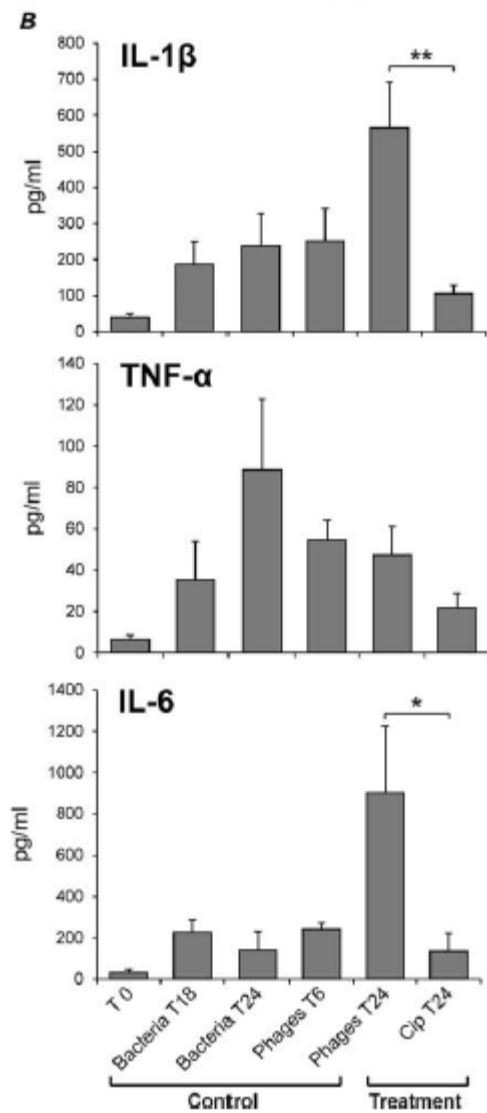

Figure 06. Cytokine quantification in rat plasma during experimental endocarditis. *B*, Levels of interleukin 1β (IL-1β), interleukin 6 (IL-6), and tumor necrosis factor α (TNF-α) measured after 6 hours of phage or antibiotherapy (treatment). Controls included rats 24 hours after surgery (inoculum, T0), untreated but infected rats for 18 hours or 24 hours (bacteria T18 and T24), and uninfected rats receiving phage for 6 hours (Phages T6). Each value represents the mean ± SEM from 4–10 individual animals (*, $P = .03$; **, $P = .005$ using the Mann–Whitney test). Abbreviation: cip, ciprofloxacin.(Oechslin et al., 2016)

The combination of bateriophage therapy and antibiotic therapy against *Escherichia coli***:**

The efficacy of phage treatment and traditional antibiotics, or a combination of both in a head-to-head trial in an E. coli challenge in broiler chickens have been investigated by Huff et al. (2004). Enrofloxacin (fluoroquinolone), the standard of care treatment, reduced mortality in untreated bird from 68% to 3%, while phage treatment alone reduced mortality to 15%. A therapy of phage in combination with enrofloxacin resulted in no mortality. Correspondingly, a combination therapy of phage and ciprofloxacin resulted in a 10,000-fold greater reduction in bacterial load as compared to phage or ciprofloxacin treatment alone in rats with experimental endocarditis due to P. aeruginosa, as observed by Oechslin et al. (2017). Furthermore, it is noted that, synergistic killing of P. aeruginosa both in vivo and in vitro was resulted by this particular combination of antibiotics and phage (Oechslin et al., 2017). Additional studies examining potential synergy between phage and antibiotics both in vitro and in vivo are required, as the future of phage therapy will likely be that of combined therapy with chemical antibiotics, In contrast with phage therapy studies in vivo animal models, there have been relatively few reports on the clinical use of phage and even fewer controlled clinical trials. As summarized in

Table 4, below some notable case studies and clinical trials that have been performed are described; the lists are not exhaustive, and other examples can be found in the literature (e.g., Jennes et al., 2017; Hoyle et al., 2018).

Table 1. Case Reports and Clinical Trials

Case Reports

	Infection	Complicating Conditions	Antibiotic Courses	Antibiotic Resistance or Allergies	Phage Dose and Application	Duration of Phage Treatment	Outcome
Case 1 (Duplessis et al., 2017)	*Pseudomonas aeruginosa* bacteremia	DiGeorge syndrome and congenital heart disease with pacemaker	Meropenem, tobramycin, aztreonam, polymyxin B, and colistin	Meropenem, tobramycin, aztreonam, polymyxin B, colistin, Cephalosporins, and fluoroquinolones	3.5×10^5 PFU delivered intravenously every 6 h	Initial treatment for 36 hours (six doses total), treatment resumed 11 days later	Blood cultures negative after phage treatment; reverted to positive following cessation of phage administration
Case 2. (Khawaldeh et al., 2011)	*Pseudomonas aeruginosa* urinary tract infection (2 years)	Intra-abdominal resection and irradiation for adenocarcinoma, bilateral ureteral stent placement	Gentamicin, ceftazidime, ciprofloxacin, and meropenem	None reported	2×10^7 PFU directly instilled into the bladder every 12 h	10 days (meropenem and colistin initiated on day 6)	Urine samples sterile following phage therapy and a 30-day course of meropenem
Case 3 (LaVergne et al., 2018)	*Acinetobacter baumanii* surgical site infection	Craniectomy	Combination of colistin, azithromycin, and rifampin	Intermediate sensitivity to colistin, with resistance to all other tested antibiotics	8.56×10^7 PFU delivered intravenously every 2 h	8 days (98 doses total)	Initial improvements observed; bacterial load not measured
Case 4 (Schooley et al., 2017)	*Acinetobacter baumanii* infected pseudocyst (3 months)	Necrotizing pancreatitis	Azithromycin, colistin, and rifampin	Cephalosporins, meropenem, gentamicin, amikacin, trimethoprim-sulfamethoxazole, tetracycline, ciprofloxacin, and colistin	5×10^9 PFU delivered intravenously every 6 h	84 days (336 doses total), minocycline added on day 2	Clinical improvement and resolution of infection
Case 5 (Chan et al., 2018)	*Pseudomonas aeruginosa* infected aortic graft (3 years)	Aorto-cutaneous fistula	Ceftazidime and ciprofloxacin	Ciprofloxacin	1×10^8 PFU delivered topically on fistula	Single dose	Cultures negative four weeks post treatment; no recurrence of infection after >2 years

Clinical Trials

	Infection	Trial	Treatment Group	Placebo Group	Phage Dose and Application	Outcome
Trial 1 (Wright et al., 2009)	*Pseudomonas aeruginosa* otitis	Placebo controlled, double blind for safety and preliminary effectiveness	12 individuals received phage cocktail	12 individuals received a single dose of glycerol-PBS buffer	10^9 PFU delivered intra-aurally (single dose)	Three individuals from each group had undetectable levels of *P. aeruginosa* at the end of the trial
Trial 2 (Sarker et al., 2016)	*Escherichia coli* diarrheal diseases	Placebo controlled, double blind for safety and efficacy	40 individuals received phage cocktail M, 39 individuals received phage cocktail T	41 individuals received oral rehydration solution	1.4×10^9 PFU cocktail M or 3.6×10^8 PFU cocktail T delivered orally in oral rehydration solution three times per day for 4 day (12 doses)	No significant difference between phage treatment group and placebo group
Trial 3 (Jault et al., 2018)	*Pseudomonas aeruginosa* burn wound infection	Placebo controlled, blinded trial for safety and efficacy	12 individuals received a phage cocktail	13 individuals received standard of care 1% sulfadiazine silver	2×10^7 PFU (expected) 200–2,000 PFU (actual) applied topically one time per day for 7 days (seven doses)	Trial halted due to insufficient efficacy; this was likely due to significantly lower applied dose of phage than expected

Details and summaries are provided for each of the case reports and clinical trials discussed in the main text.

At present, bacteriophages (bacterial viruses) are considered to be a potential bio-control agent against antibiotic-resistant strains. Major biotechnological applications, such as controlling bacterial infections in both humans and animals worldwide are caused by bacteriophages or simply phages (Sagona et al., 2016; Sarker et al., 2016; Woolston et al., 2013). Phage therapy is also being advanced as an alternative to antibiotics and to improve the quality of food and water (Burrowes et al., 2011; Nobrega et al., 2015; Villegas et al., 2009; Zhang et al., 2013). More specifically, against pathogenic E. coli in chickens, turkeys, calves, pigs and humans, phage therapy was successfully effective (Lau et al., 2012; Niu et al., 2009; Skaradzinska et al., 2017), and since 1920, phage therapy has been applied against bacterial diseases in swine (Zhang et al.,2015).

However, resistance characters to their specific phages by nature or by phage therapy treatments by the pathogenic strains could be shown (Projan, 2004). Therefore, the therapy combined of a bacteriophage and antibiotics has been used as a novel effective tool as opposed to a single treatment, as it showed several advantages such as effectively penetrate into biofilms, increased the bacterial growth suppression, and lowering the chances of emergence of phage resistance (Comeau et al., 2007, Ryun et

al., 2012, Easwaran et al., 2015, Uchiyama et al., 2018, Tagliaferri et al., 2019). For instance, Comeau et al., reported the phage-antibiotic synergy (PAS), demonstrating that when sub-inhibitory concentrations of antibiotics used, it enhanced the phage productivity, and thereby phage-mediated bacteria is declined (Comeau et al., 2007). Kamal and his colleague reported that, the propagation of phages, plaque size and their efficacy can be increased by antibiotics (Kamal & Dennis, 2015). However, not only the beneficial effects of phage-antibiotic combinations, but the negative or neutral effects have also still been reported (Gelman et al., 2018; Tagliaferri et al., 2019). Hence, for the development of effective phage therapy against multidrug resistant bacteria, it is still important to investigate the type of phages and antibiotics with their combined ratios. (Easwaran et al., 2020)

In a research from Easwaran et al. (2020) the antibiotic-resistant E. coli Sw1 was significantly inhibited by the combination of ΦEcSw and ampicillin. The results highlight the advantages of a phage-antibiotic combination to prevent the antibiotic-resistant strain E. coli Sw1. The most important finding of this study was that ampicillin has the potential to induce phage numbers in combined therapy. This study provides useful information to overcome the challenges of antibiotic and phage resistance. Moreover, Easwaran and the team have shown that ΦEcSw should have clinical relevance because of its in vivo effectiveness in retrieving infected zebrafish and mice. The vital role of antibiotics to induce phage propagation, and the role of ΦEcSw to rescue animals from antibiotic-resistant strains have been highlighted by our results. Additional knowledge is required to improve phage-based therapy and effective phage therapy trials in the future.

A study (Ryan et al., 2012) categorizes antimicrobial synergy between bacteriophage T4 and a usual antibiotic, cefotaxime, using standard plaque assay and, importantly, in the in vitro eradication of biofilms of the T4 host strain Escherichia coli 11303. Phage-antibiotic synergy (PAS) is when sub-lethal concentrations of specific antibiotics can extensively stimulate the host bacteria's production of virulent phage. Increasing sub-lethal concentrations of cefotaxime resulted in a practical increase in T4 plaque size and T4 concentration. The application of PAS in this research (Ryan et al., 2012) to the T4 one-step growth curve resulted in an increased burst size including the reduced latent period. The eradication of bacterial biofilms was significantly enhanced when treatment of combinations of T4 bacteriophage and cefotaxime was given compared to treatment with cefotaxime alone. The addition of medium (104 PFU mL

$^{-1}$) and high (107 PFU mL $^{-1}$) phage titres reduced the minimum biofilm eradication concentration value of cefotaxime against E. coli ATCC 11303 biofilms from 256 to 128 and 32 lg mL $^{-1}$, respectively. Even though further study is needed to confirm PAS, but this study shows that synergy between bacteriophage and conventional antibiotics can significantly improve biofilm control in vitro.
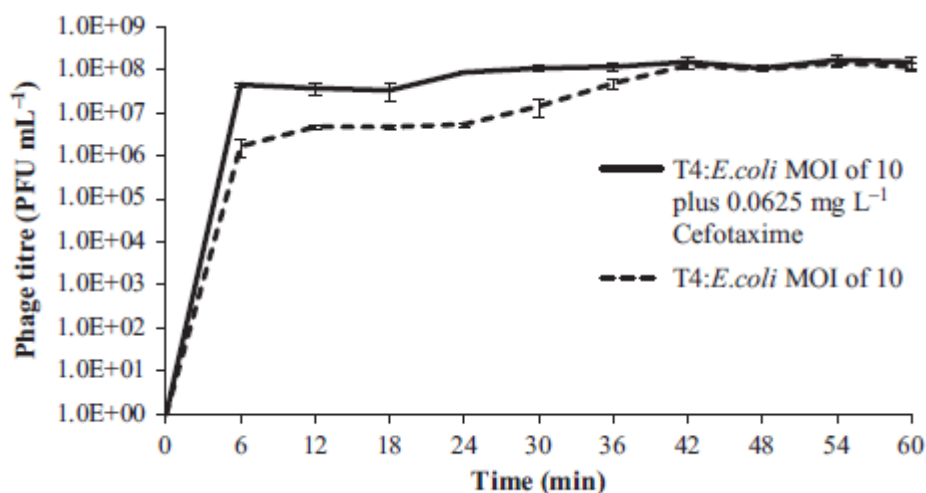

Fig. 07. Effect of cefotaxime addition on the one-step growth curve of T4 phage.

As anticipated, the MBEC for cefotaxime against 24-h E. coli biofilm (256 lg mL^{-1}, in agreement with previously published MBEC data (Ceri et al., 1999)) was significantly higher (approximately 10 000 times) than the measured MIC value (0.0156 lg mL $^{-1}$). T4 phage has already proven to exert a synergistic effect when combined with cefotaxime against E. coli in its planktonic mode of growth, the effect of adding T4 bacteriophage on the antibiofilm activity of cefotaxime, by measuring reduction in MBEC value, was examined in the presence of low (102 PFU mL^{-1}), medium (104 PFU mL^{-1}) and high (107 PFU mL^{-1}) T4 phage titres. Table 2 shows, while the addition of low phage concentration to cefotaxime resulted in no change in the MBEC, medium and high phage titres resulted in a reduction in MBEC from 256 lg mL $^{-1}$ (as previously determined for cefotaxime alone) to 128 and 32 lg mL $^{-1}$, correspondingly. When used without cefotaxime, none of the phage titres could result in complete biofilm eradication. In fact, even the challenge with the highest phage titre (107 PFU mL^{-1}) resulted in no more than 0.9-log reduction in biofilm surviving cells.

T4 phage concentration (PFU mL^{-1})	Cefotaxime MBEC (µg mL^{-1})
0	256
10^2	256
10^4	128
10^7	32

Table 5. Effect of T4 phage addition, at three different titres, on MBEC of cefotaxime against Escherchia coli biofilm

It was proven for the first time that the additive effects of phage and antibiotics can also be functional in treating bacteria within the biofilm matrix and that such combinations used in synergy significantly improve biofilm eradication (Ryan et al., 2012).

Abdominal infections, urinary tract infections (UTI), enteric infections, pneumonia, bacteremia, and meningitis are all typical bacterial infections caused by Escherichia coli. This bacterium is the most common cause of both community-acquired and nosocomial UTI, making it a public health problem. Approximately about 50% of females would have at least one episode of UTI at any point in their lives. E coli is responsible for 12 to 50% of nosocomial infections and 4% of diarrheal disease outbreaks. Antibiotic overuse has resulted in a substantial rise in the proliferation of antimicrobial multidrug-resistant bacteria over time. Many E. coli strains gained resistance to a wide range of antibiotics, including those with various modes of action. To combat bacterial infections, phage therapy may be used as an alternative. The threat of phage-resistant mutants is a big concern when using phages to combat infections. Resistance may arise from changes in or lack of bacterial cell surface receptors, inhibition of phage DNA penetration, development of restriction endonucleases that degrade phage DNA, and the CRISPR (clustered regularly interspaced short palindromic repeats) mechanism, which is a widespread microbial response to bypass the selective pressure imposed by phage infection. By combining the use of phages and antibiotics, this restriction can be overcome. Antibiotics and phages used together have been seen to have a synergetic impact in several experiments such as the experiment done by Nádia Valério (2017).

In this experiment, antibiotics Ciprofloxacin, tetracycline, and chloramphenicol were all effective against E. coli, but ampicillin, kanamycin, and piperacillin were immune.

After 2 hours of therapy, the maximum bacterium inactivation with phage ECA2 was 3.90.3 - 4.60.1 log CFU/mL, according to the bacterial control. The bacterial inactivation was already elevated (2.70.4 – 4.00.3 log CFU/mL) after 8 hours. After 8 hours of incubation, bacterial density in the BC rose by 3.60.4 – 3.90.1 log CFU/mL (ANOVA, p 0.05).

After 2 hours of therapy, bacterial inactivation with a combination of the phage ECA2 and the antibiotics ampicillin, kanamycin, and piperacillin was close to that seen with the phage alone, with reductions of 4.10.1 - 4.70.1 log CFU/mL compared to the bacterial control. After 8 hours of incubation, the bacterial density in the BC, AmpC, PipC, and KanC increased by around 3.70.3 log CFU/mL (ANOVA, p 0.05). This means that combining the phage with antibiotics that the bacteria were immune to had little effect.

After 2 hours of therapy, the bacterial inactivation with the combination of phage ECA2 and ciprofloxacin at MIC (B+P+Cip0.5) was slightly higher than when the phage was measured alone (Fig. 08), with a reduction of 5.10.3 log CFU/mL compared to the bacterial control (Table 06). The rate of inactivation was already 3.60.7 log CFU/mL after 1 hour of incubation. After 2 hours of incubation, almost all bacterial cells were inactivated, and after 30 minutes of incubation, no new phages were formed (Fig.08). When phage ECA2 and ciprofloxacin at a sub-lethal concentration were used to study bacterial inactivation, a substantially higher reduction was found than when the phage was measured alone (Fig. 09). After 8 hours of therapy, there was a reduction in CFU/mL of 8.40.6 log CFU/mL compared to the BC. (Table 06). After 2 hours of incubation, bacterial inactivation has already reached a significant level (a reduction of 5.60.3 log CFU/mL compared to the BC) (Fig. 09).

However, when phage ECA2 was combined with the antibiotics tetracycline (B+P+Tetra) and chloramphenicol (B+P+Chl), bacterial inactivation was close to that seen when the phage was studied alone.

Furthermore, when the phage ECA2 was combined with ciprofloxacin at a sub-lethal concentration (0.05 mg/mL) to inactivate E. coli in urine, the treatment efficacy was slightly higher than when the phage was used alone. After 8 hours of therapy with a combination of phage ECA2 and ciprofloxacin (B+P+Cip0.05, Fig. 10), the bacterium was inactivated to the detection limit (reduction of 7.80.1 log CFU/mL) (Fig. 10).

Antibiotics, phage ECA2, and a combination of the phage ECA2 and antibiotics all demonstrated varying rates of development of resistant mutants in E. coli. The growth of antibiotic-resistant mutants of E. coli against ampicillin, kanamycin, piperacillin, and ciprofloxacin was higher than that observed with phage ECA2 and phage ECA2 in the presence of antibiotics. The prevalence of resistant mutants of E. coli against the phage ECA2 and the phage ECA2 in the presence of the antibiotics piperacillin, kanamycin, and ampicillin, on the other hand, was comparable. The phage ECA2 in combination with ciprofloxacin resulted in a lower prevalence of resistant mutants.

It has been demonstrated that combining antibiotics with phages is a viable solution that not only reduces bacterial numbers but also helps control resistance levels. Antibiotics added to phage therapy may also regulate the development of phage-mutants, according to our findings. The level of development of phage-mutants was lower when ciprofloxacin was applied at a sub-lethal concentration (concentration 10 times lower than MIC) with the phage than when the antibiotic was not added.
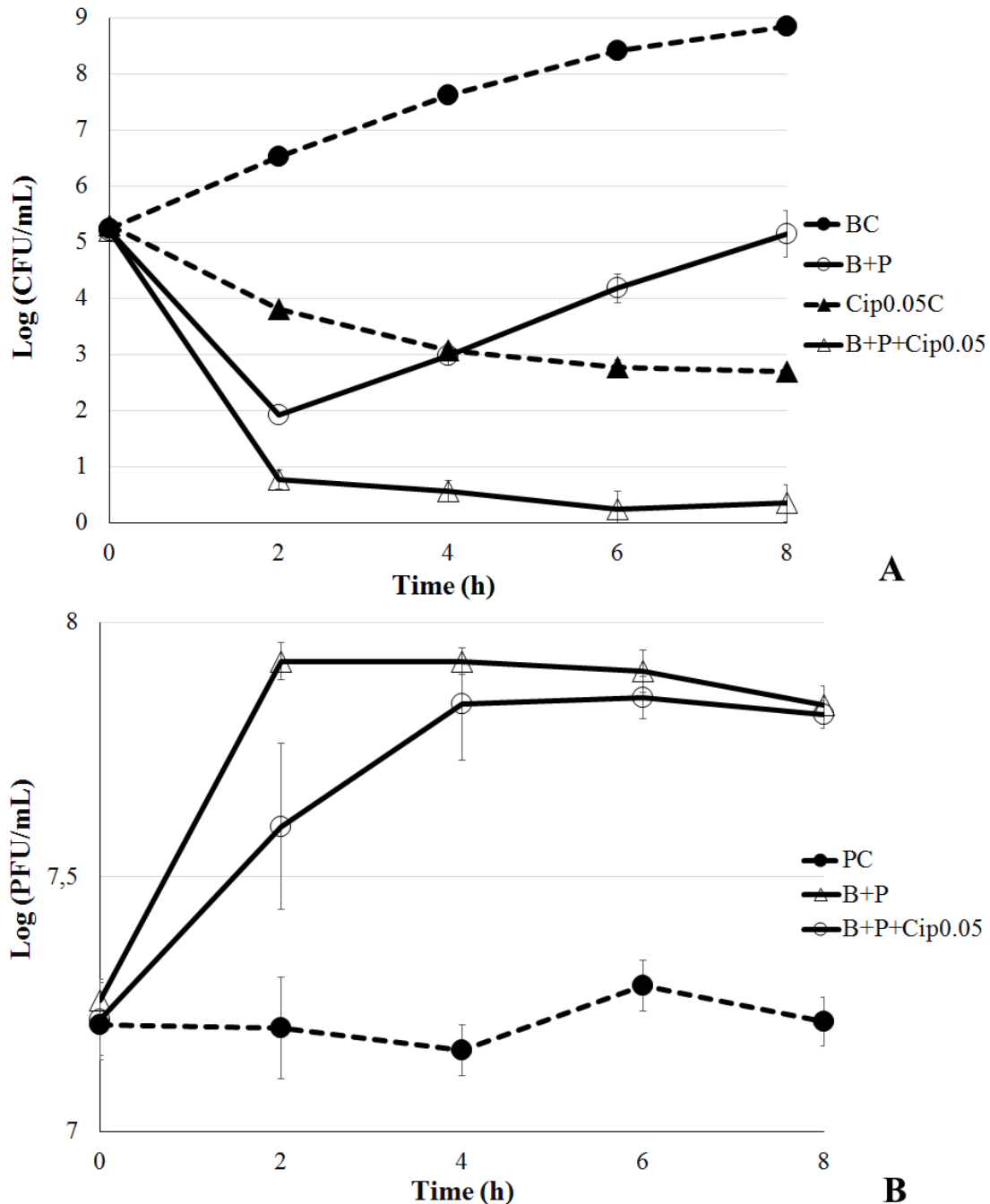

Fig. 08. Inactivation of E. coli with phage ECA2 alone and ciprofloxacin at MIC (0.5 mg/L) in PBS during 2 h. (A) - Bacterial concentration: BC- bacterial control; Cip0.5C - ciprofloxacin control; B+P- bacteria plus phage, B+P+Cip0.5- bacteria plus phage plus ciprofloxacin at MIC.
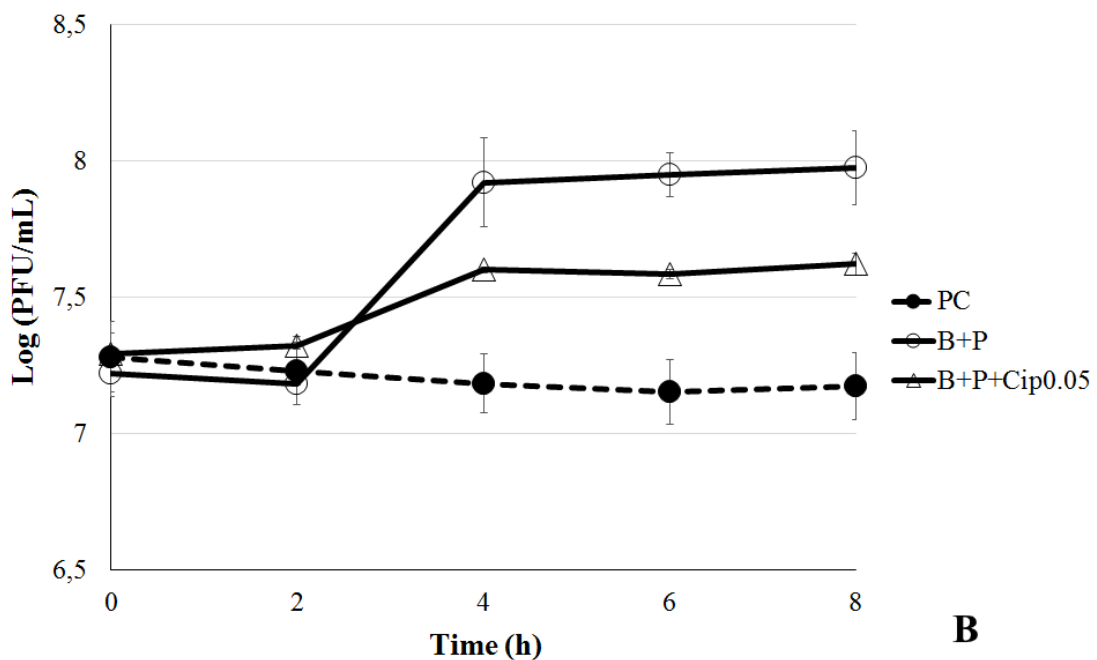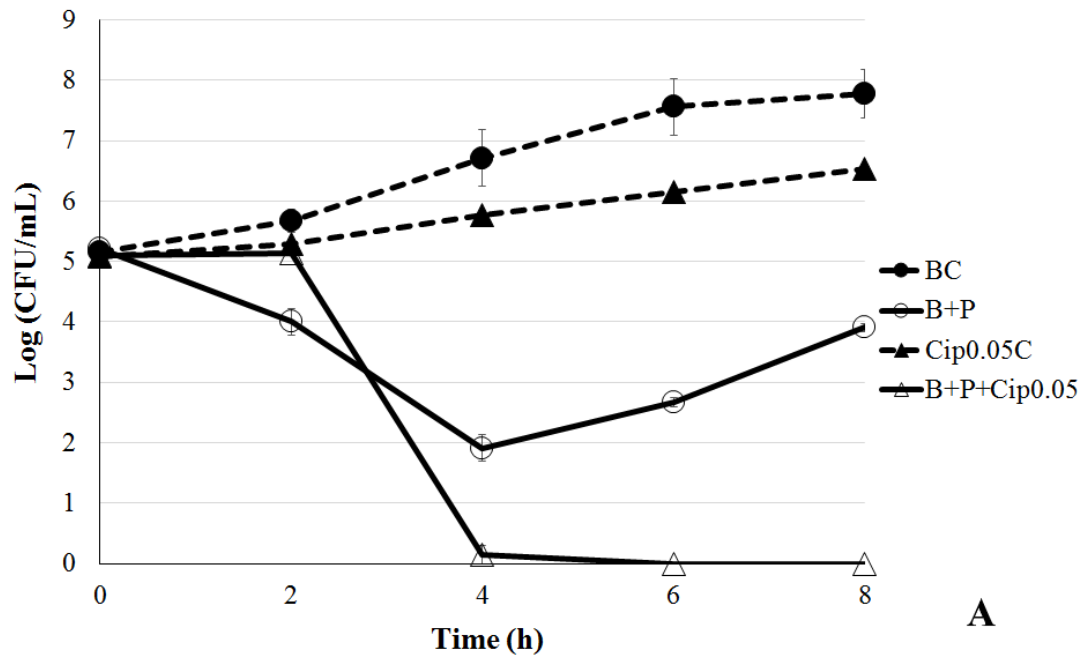
Fig. 09. Inactivation of E. coli with phage ECA2 alone and ciprofloxacin (1/10 MIC = 0.05 mg/L) in PBS during 8 h. A) - Bacterial concentration: BC- bacterial control; Cip0.05C - ciprofloxacin (1/10 MIC = 0.05 mg/L) control; B+P - bacteria plus phage, B+P+Cip0.05 - bacteria plus phage plus ciprofloxacin (1/10 MIC =0.05 mg/L).

(B) - Phage concentration: PC - phage control; B+P bacteria plus phage; B+P+Cip0.05 - bacteria plus phage plus ciprofloxacin (1/10 MIC = 0.05 mg/L).
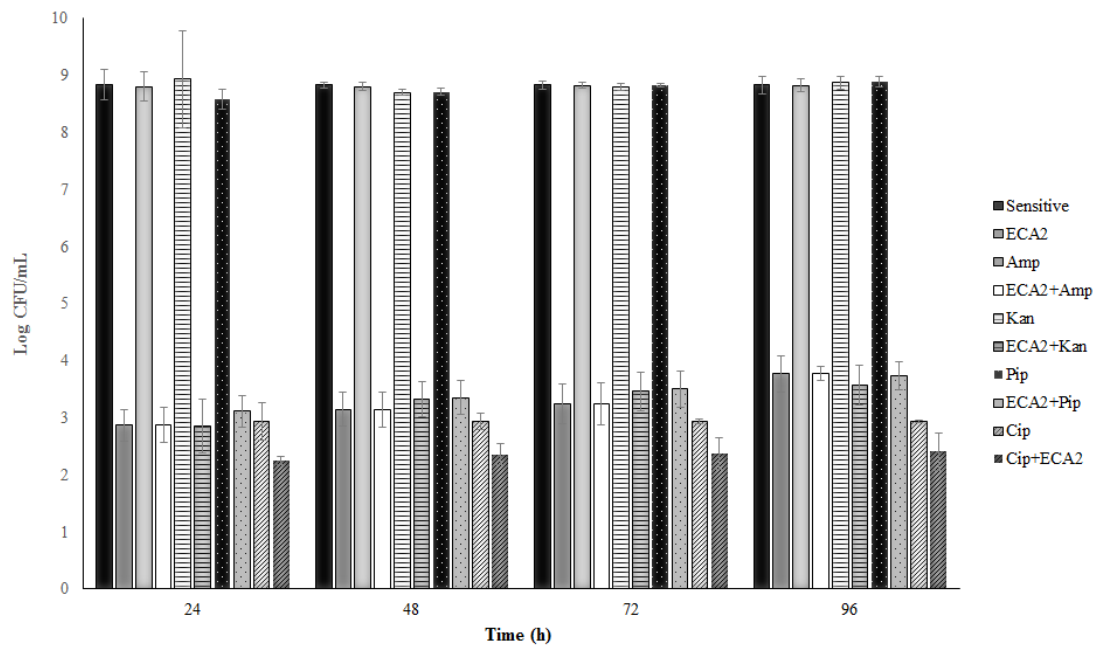
Fig. 10. Inactivation of E. coli with phage ECA2 and ciprofloxacin (1/10 MIC =0.05 mg/L) in urine during 8 h. A) - Bacterial concentration: BC - bacterial control; Cip0.05-, C - ciprofloxacin (1/10 MIC=0.05 mg/L) control; B+P - bacteria plus phage, B+P+Cip0.05 - bacteria plus phage plus ciprofloxacin (1/10 MIC =0.05 mg/L). Phage concentration: PC - phage control; B+P - bacteria plus phage; B+P+Cip0.05 - bacteria plus phage plus ciprofloxacin (1/10 MIC =0.05 mg/L).

Table 2. Maximum reduction of the *E. coli* obtained after treatment with phage ECA2 and phage ECA2 with antibiotics relatively bacteria control and antibiotics control.

Parameter	Maximum reduction relatively to the bacteria control		Time (h)	Maximum reduction relatively to the antibiotic control		Time (h)
	Log	%		Log	%	
Phage ECA2	4.2	66.9	2			
Phage ECA2 + ampicillin	4.7	74.1	2	4.4	72.9	2
Phage ECA2 + kamanycin	4.6	72.3	2	4.6	72.3%	2
Phage ECA2 + piperacillin	4.2	55.8	4	4.1	55.5	4
Phage ECA2 + tetracycline	3.8	43.8	8	0.1	2.6	8
Phage ECA2 + chloramphenicol	3.9	55.1	8	0.1	2.3	4
Phage ECA2 + ciprofloxacin 0.05 mg/mL	8.4	96.0	8	3.0	79.8	2
Phage ECA2 + ciprofloxacin 0.5 mg/mL	5.1	78.2	2	0.25	14.9	2

Table 06: Maximum reduction of *E.coli* after treatment with phage ECA2 and combination of antibiotics with phage ECA2

The combination of bateriophage therapy and antibiotic therapy against *Acinetobacter baumannii*:

According to a new research… (Grygorcewicz et al., 2020), a promising approach enhancing the phage therapy effectiveness is presented by a combination of the bacteriophages and antibiotics. Here, the bacteriophage vB_AbaP_AGC01 posessing antibacterial activity against clinical *Acinetobacter baumannii* strains was characterized. The phage AGC01 efficiently adsorbs to *A. baumannii* cells and possesses a bacterio-lytic lifecycle resulting in high production of progeny phages (317 ± 20 PFU $\times$ cell$^{-}1$). The vast host range (50.27%, 93 out of 185 strains) against A. baumannii isolates and the ineffectiveness of AGC01 to infect other bacterial species show its high specificity. A high resemblance of the AGC01 genome sequence with that of the Friunavirus genus from an Autographivirinae subfamily was revealed through genomic analysis. The A. baumannii cell count in a human heat-inactivated plasma blood model (HIP-B) can significantly be reduced by the AGC01 alone and also in combination with antibiotics: (gentamicin (GEN), ciprofloxacin (CIP), and meropenem (MER). When a combination of phage treatment with CIP or MER was used, the synergistic action was observed. Using an in vivo larva model, the antimicrobial activity of AGC01 and phage-antibiotic combinations was confirmed. This study presents the greatest increase in survival of G. mellonella larvae; when the combination of MER and phage (MOI = 1) was used, larval survival was increased by 42% (from 35% to 77%). Therefore, AGC01 constitutes a novel candidate for phage therapy. Our study also suggests that antibiotics and phages can show synergy for higher antimicrobial effect when used as combination therapy.

Bacteriophage Isolation and Host Range

A clear plaque surrounded by a halo zone was formed by the isolated phage (size: 1.6 ± 0.3mmin diameter). The plaque size and halo zone increased to 4.6 ± 0.4 mm over prolonged incubation (Figure 11). 50.27% (93 of 185) of the A. baumannii strains, including reference strains and clinical isolates (Table S1) were infected by the phage AGC01. The AGC01 only infects A. baumannii and does not infect other species tested during a host range analysis (including Escherichia coli, Enterobacter spp., Klebsiella spp., and Pseudomonas spp. strains) (Grygorcewicz et al., 2020).

Bacteriophage Growth and Stability Characterization

Under various conditions, the virulence of AGC01 phage was initially characterized in terms of stability and growth. Approximately 99% of virions adsorb to host within 5 min as shown in the adsorption analysis (Figure 11). With the latent period of AGC01 being 20 min long, the burst size comprised approximately 317 ± 20 progeny phages per infected cell (Figure 11). In A. baumannii liquid culture, absolute clearance of the bacterial optical density was observed and attributed to efficient lysis of cells by AGC01 at all the multiplicities of infection (MOIs) used (Figure 11). Against the used strain The AGC01 was therefore found to possess strong and concentration-dependent lytic activity (Grygorcewicz et al., 2020).

In order to assess the stability of the phage under various physicochemical conditions, the temperature changes and resistance of phage AGC01 to pH was investigated. Remaining active at pH values ranging from 5 to 7; AGC01 loses activity at pH 3. Within 2 hours, only 37% of virus particles remained active and able to infect the host when incubated at pH 9, which further dropped to 10% active virus particles after exposure to pH 11 (Figure 11). Analysis of thermal stability of bacteriophage showed that AGC01 retains activity at 30 ˚ C throughout the duration of this analysis, while time-dependent decreases in phage activity were observed at temperatures 40 ˚ C and 50 ˚ C, and temperatures higher than 60 ˚ C resulted in immediate loss of phage activity (Figure 11). Additionally, storage of AGC01 phage stock at 4 ˚ C resulted in loss of only 13% of active virions after 14 months (Grygorcewicz et al., 2020).
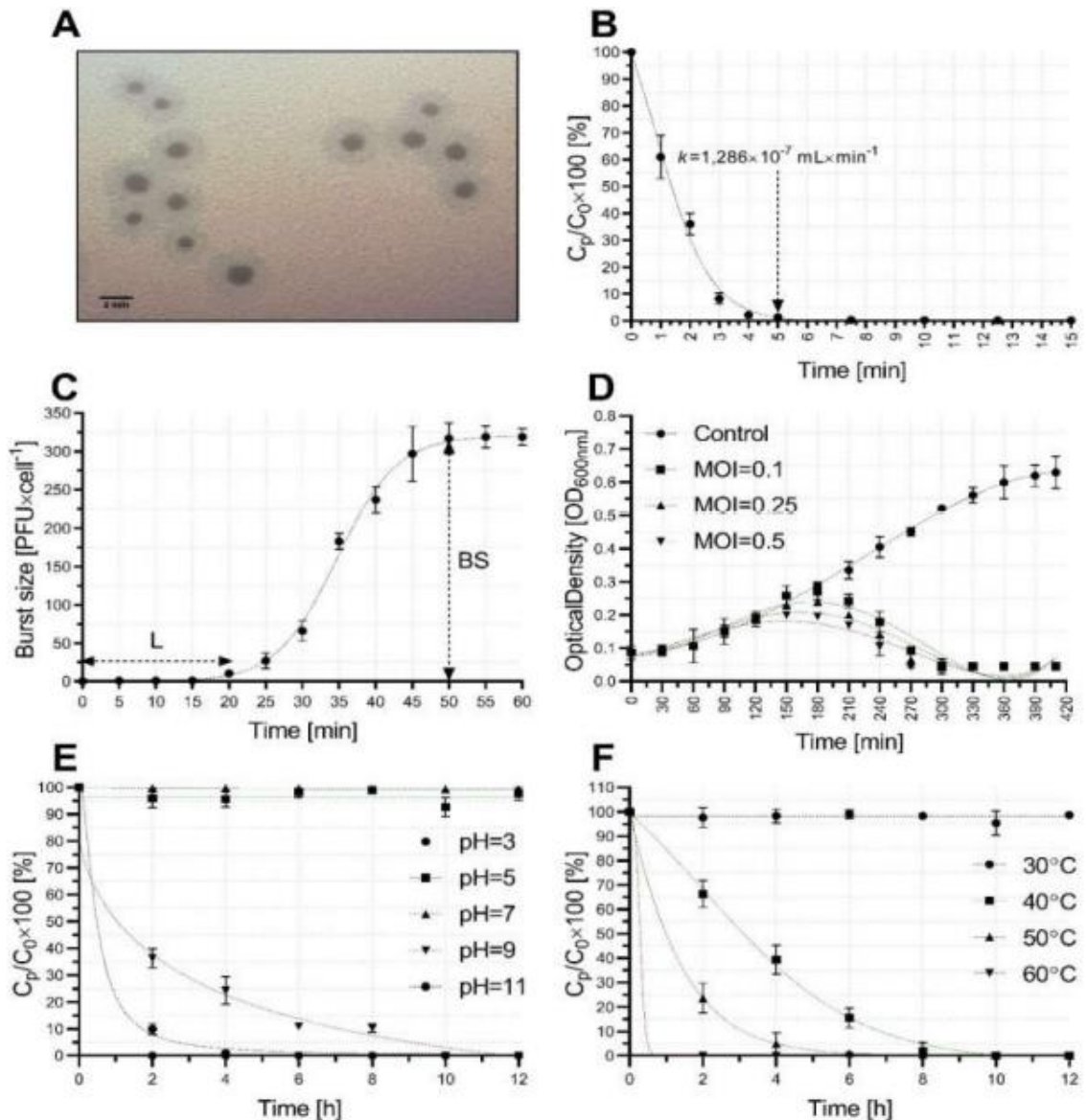
Figure 11. Characterization of bacteriophage vB_AbaP_AGC01 growth and stability. (A) After 18 h incubation plaques formed by bacteriophage vb_AbaP_AGC01 at 37 °C. (B) Kinetics of the phage adsorption to host at a multiplicity of infection (MOI) of 0.1. (C) One-step growth curve indicating burst size (BS = 317 PFU × cell⁻1) and the latent period (L = 20 min) . (D) Lytic activity of bacteriophage. (E) Stability of bacteriophages at different pH values. With triplicate biological replication, all experiments were technically repeated three times. (F) Susceptibility of isolated bacteriophage to increases in temperature (Grygorcewicz et al., 2020).

Activity of vB_AbaP_AGC01 on Biofilm, HIP-B, and G. Mellonella Larva Models

The research first analyzed the antibiofilm activity of AGC01 to evaluate the activity and suitability of bacteriophage as an antibacterial agent. After incubation with AGC01 phage, the biofilm biomass of infection-susceptible A. baumannii was reduced to 71.57% and 84.76% ($p < 0.05$) (Figure 12). Therefore, AGC01 possesses a high ability to reduce biofilm production as indicated by these data.
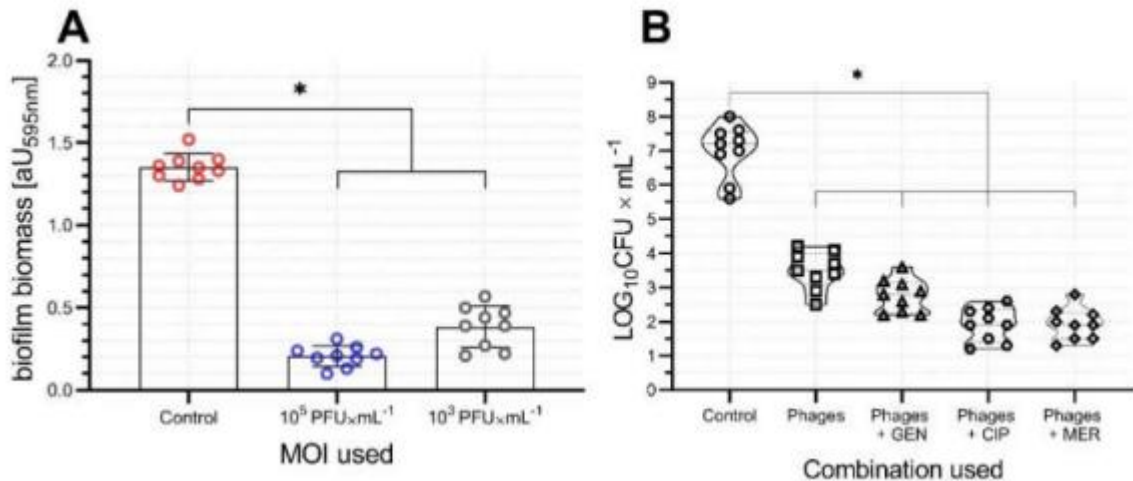
Figure 12. Activity of vB_AbaP_AGC01 with the use of the selected models. (A) Antibiofilm activity of isolated phage. (B) In human heat-inactivated plasma blood model antibacterial activity of AGC01 alone and in combination with the antibiotics ciprofloxacin (CIP), meropenem (MER) and gentamicin (GEN). The asterisk (*) indicates data that is statistically significant ($p < 0.05$). With triplicate biological replication, all experiments were technically repeated three times.

A heat-inactivated plasma blood model (HIP-B) was used to assess the ex vivo activity of AGC01 in blood. The MDRAB cell count was significantly reduced by phage AGC01 activity when used alone and in combination with antibiotics in the HIP-B model (Figure 12B). Using phage in combination with CIP and MER, the highest reduction of bacterial cell count was observed; both of which resulted in an approximately 4 log reduction ($p < 0.05$, compared to phage used alone). Using the phage and GEN combination, the poorest efficiency was observed where only a slight reduction of bacterial cell count was achieved which was found to be insignificant ($p = 0.0667$).

Acinetobacter baumannii infecting phage vB_AbaP_AGC01 was isolated, deeply characterized (both genomically and phenotypically) and analyzed for antimicrobial activity in two infection models (HIP-B and G. mellonella larvae). In these models, the influence of three different antibiotics (CIP, GEN, and MER) on phage activity was also determined. In the in vivo model, CIP and MER appeared to improve therapeutic outcomes of phage therapy. The potential of vB_AbaP_AGC01 for therapeutic application alone or as a part of phage concoctions is supported by the cumulative evidence. However, to the best our knowledge this is the first study that shows an antibiotic enhancement of the fully characterized lytic phage-based therapy against A.

baumannii in human heat-inactivated plasma blood and Galleria mellonella (greater wax moth) model. The phages and antibiotics can act complementarily when administered together as suggested by the results. The vB_AbaP_AGC01 was classified (based on genome sequence similarity) as a member of the Friunavirus genus from a subfamily of Autographivirinae. Its potential is highlighted as a component of a phage mixture against A. baumannii or as a tool for phage therapy of aforementioned infections; a broad lytic spectrum and a high target specificity is possessed by isolated AGC01 phage (Łubowska et al., 2019). The genome of the AGC01 was characterized and annotated, so the requirements of deep genome analysis of phages candidates for therapeutic purposes are met (Szymczak et al., 2020). Additionally, a broad host range of isolated phage is suggested by the lytic spectrum of around 50%. In some reports, newly isolated phages infect only their propagation host while the host range of other Acinetobacter infecting phages varied between 2–68%, (Turner et al., 2017), (Yang et al., 2010). The host range of acinetobacter-infecting podoviruses mainly depended on pectate lyase depolymerase domains located on the tail fibers (Oliveira et al., 2017). It can be presumed that characterization and isolation of Acinetobacter phages that occur naturally is important and novel insights into their biology is settled. They could also be used as a source of capsule degrading enzymes possessing antimicrobial properties (Liu et al., 2019). Production of the depolymerases could be associated with the disruption of bacteriophage-mediated biofilm. Biofilm biomass can be reduced by isolated phage at all concentrations, and this suggests that AGC01 exhibits antibiofilm properties. During the phage-antibiotic combination therapy, antibiotic penetration and antibiofilm activity was increased by phage-mediated biofilm disruption (Bedi et al., 2009). Limited penetration of antibiotics throughout the biofilm is contributed to increased biofilm resistance to antibiotics.

In research from Nir-Paz et al. (2019) described a 42 year old patient with a trauma-related left tibial infection; bacterial osteomyelitis associated with extensively drug resistant XDR *Acinetobacter baumannii* (*Ab*) and multi-drug resistant MDR *Klebsiella pneumoniae* was treated with bacteriophages and antibiotics combination. A phage-resistant *Ab* mutant developed *in vitro*, but fortunately, not in the patient, and scientists could quickly isolate a new lytic phage to combat it. Tissue healing and eradication of positive cultures were rapidly observed which shows the potential flexibility of phage treatments. Osteomyelitis heals slowly and can relapse months or years after the initial infection. Eight months after completion of the combined

treatment with antibiotics and phage, the wound remains closed and dry. As a result, the patient's leg did not have to be amputated and he is undergoing rehabilitation.

Figure 13.
A. The progress of the patient wound prior to and after phage treatment. Before treatment, the flap edges did not heal well with dehiscence and evisceration. Left Panel). Two weeks after treatment the wound completely healed and no dehiscence and evisceration of flap was noted even with probing. (Middle panel) and 5 months after treatment complete healing of wound was observed (Right panel).

AbKT722 and KpKT1 mutuality

*Kp*KT1 could not grow in the presence of meropenem (Figure 13D). However, when mixed with *Ab*KT722 its growth recovered (Figure 13E), most probably due to the carbapenemase that *Ab*KT722 produces (Supplemental Table S2). Moreover, when they were cultured together, the 2 bacteria were relatively resistant to antibiotics. It was only when both of the phages and all 3 antibiotics were given together that growth of both bacteria ceased.
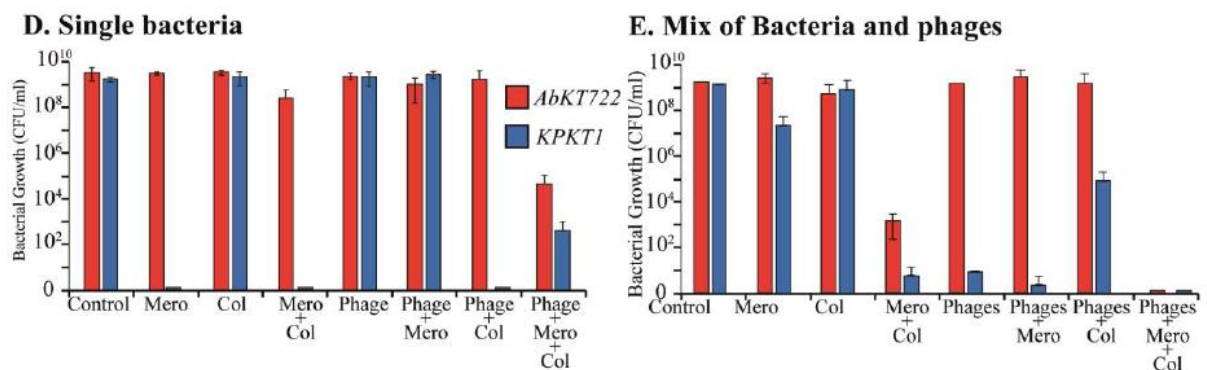

Fig: 13. CFU counts at the endpoint of the experiments, of the bacterial strains grown individually (D) and as a mixture (E), following treatment. Note that for the bacterial mixtures (E) both φAbKT21phi3 and φKpKT21phi1 were added. The mixed bacteria were plated on MacConkey agar plates which differentiate between *Ab* and *Kp* colonies.

The combination of bateriophage therapy and antibiotic therapy against *Enterococcus faecalis* and other pathogens:

A research model (Gelman et al., 2018) resembles the current clinical applications of bacteriophages given together with antibiotics in critical situations. This research also mimics cases of remaining untreatable antibiotic resistant bacterial strains, after prolonged antibiotic treatments, in different infectious conditions. Gelman and team were able to show a clear correlation between the Enterococcaland anti-Enterococcal bacteriophages concentrations, supporting the notion that bacteriophages are predisposed to auto-dosing.

Combined bacteriophage-antibiotic therapy leads to the most significant decrease in bacterial titer To verify whether the administration of phages inhibits bacterial proliferation, they determined the bacterial loads in mouse organs, including livers and hearts, representing intra- and extra abdominal organs. The samples were acquired from live animals that survived the peritonitis model after being treated with bacteriophages, antibiotics or with the combined therapy, when administered one hour after bacterial challenge. The samples were obtained at 96 h post infection, and compared to untreated mice at the point of death. Each of the treatments was sufficient to significantly reduce the bacterial load compared to untreated mice, indicating a correlation between survival chance and bacterial load reduction (Fig. 14). Although bacteriophage therapy alone manifested in a significant reduction of bacteria in the liver tissues compared with surviving mice receiving only antibiotic therapy, the most impressive reduction in the bacterial loads were resulted by the combined bacteriophage-antibiotic treatment, both in distal and proximal tissues, compared with both of the mono-therapy treatments.
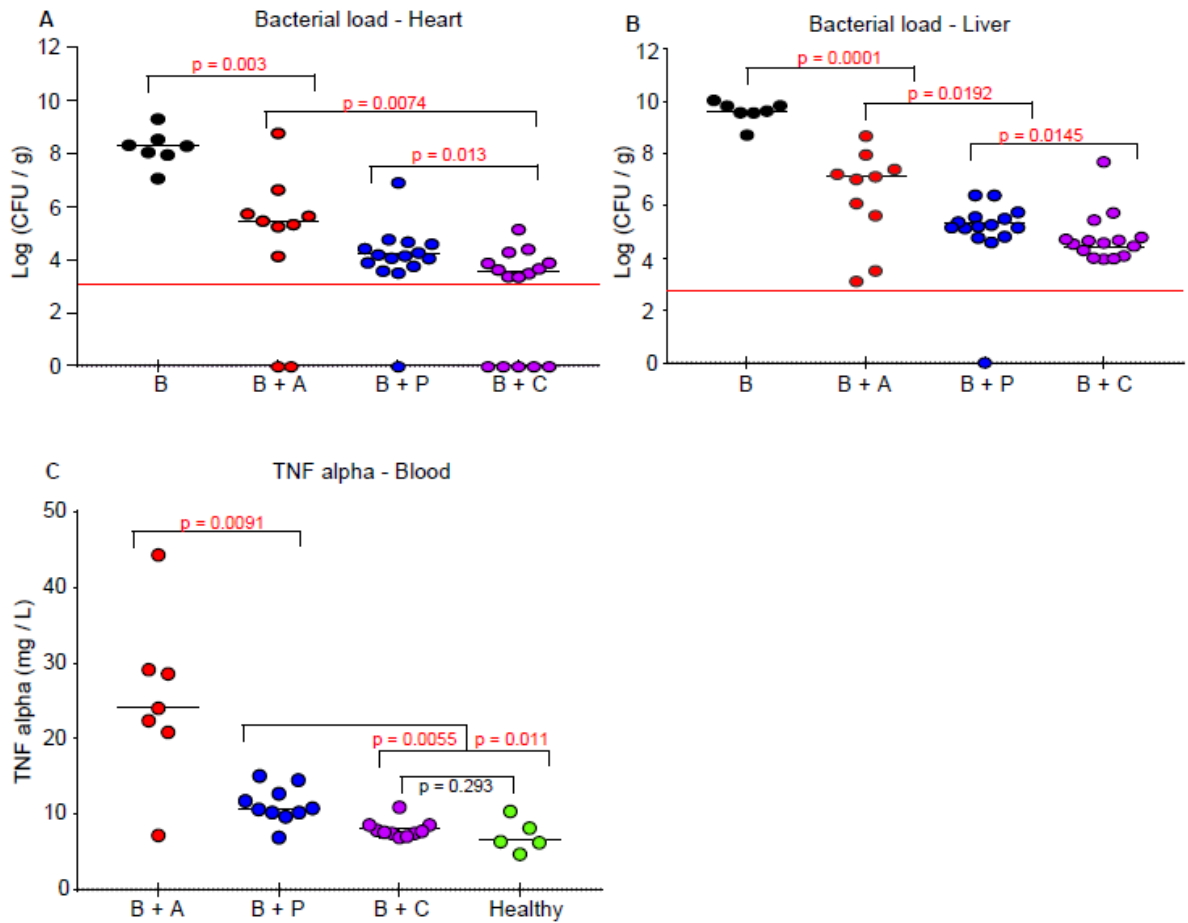
Fig. 14. The most significant reduction in the bacterial infiltration load and inflammatory level of infected mice is by combined bacteriophage antibiotic treatment. (A-B) The graphs show E. faecalis colony counts in liver and heart tissues, retrieved from the phage- (B + P - blue), antibiotic- (B + A - red), and combined phage-antibiotic- (B + C - purple) treated mice surviving the mouse peritonitis model, or infected mice receiving no further treatment (B - black).

One hour after the bacterial challenge, the treatments were administered and the organs were retrieved 96-h post-infection. The bacterial loads are represented by a logarithmic scale of CFU/g, collected from n = 10-15 mice in each treatment group, and from n = 7 mice for mice receiving only bacterial challenge. Each dot represents the bacterial load counts of a single mouse, obtained in duplicates, and each treatment group is comprised of two independent experiments. The median values for each treatment group are presented. The Mann Whitney and KruskaleWallis tests were used to compare the bacterial concentration data. According to the Holm-Bonferroni procedure, significant differences were determined and are indicated in red. The red line represents the bacterial detection level. (C) Levels of the inflammatory cytokine TNF-a in the sera of the mice, treated for the peritonitis model 1-h after the bacterial injection. The TNF-a

levels in the sera were measured at 96-h post-bacterial infection by enzyme-linked immunosorbent assay (ELISA). Seven to ten mice were sacrificed to collect serum, from phage- (B + P - blue), antibiotic- (B + A - red), and combined phage-antibiotic- (B + C - purple) treatment groups. Five samples from three untreated healthy mice were used as the negative control (healthy -green). The median values for every treatment group are presented. Each dot represents the TNF-a levels in every sample, obtained in duplicates.

The logic of applying the dual therapy, for example combining bacteriophages and antibiotics, emerges from a developmental methodology proposing that diverse selective pressures will be more effective than either alone, both in limiting the development of resistance and controlling bacterial growth. A few past papers have portrayed great expansions in antibacterial effectiveness created by such combinations (Oechslin et al., 2016); (Knezevic et al., 2013). Sometimes, it has been exhibited that this phenomenon is not influenced by the antibiotic resistance profile of the bacteria (Kamal & Dennis, 2014).

According to a research (Bao et al., 2020); a case of a 63-year-old female patient who developed a recurrent urinary tract infection (UTI) with extensively drug-resistant Klebsiella pneumoniae (ERKp) treated with phage and antibiotic synergism which is a major challenge in clinics. At first two rounds of phage therapy was given and phage resistant mutants developed within days. The combination of sulfamethoxazole-trimethoprim with the phage cocktail inhibited the emergence of phage resistant mutant in vitro, and the UTI of patient was successfully cured by this combination treatment even though ERKp strains were completely resistant to sulfamethoxazole-trimethoprim.

After this phage therapy combined with non-active antibiotics treatment, the pathogenic ERKp of the patient was completely eliminated and the recurrent UTI symptoms subsequently disappeared. The patient was finally discharged at the end of the month without having any adverse effect of phage therapy. This patient was under antibioticfree conditions during 6 months of follow-up and no signs of recurrence were observed (Bao et al., 2020).

Therefore, non-active antibiotic and bacteriophage synergism (NABS) might be an alternative strategy in personalized phage therapy was proposed (Bao et al., 2020).
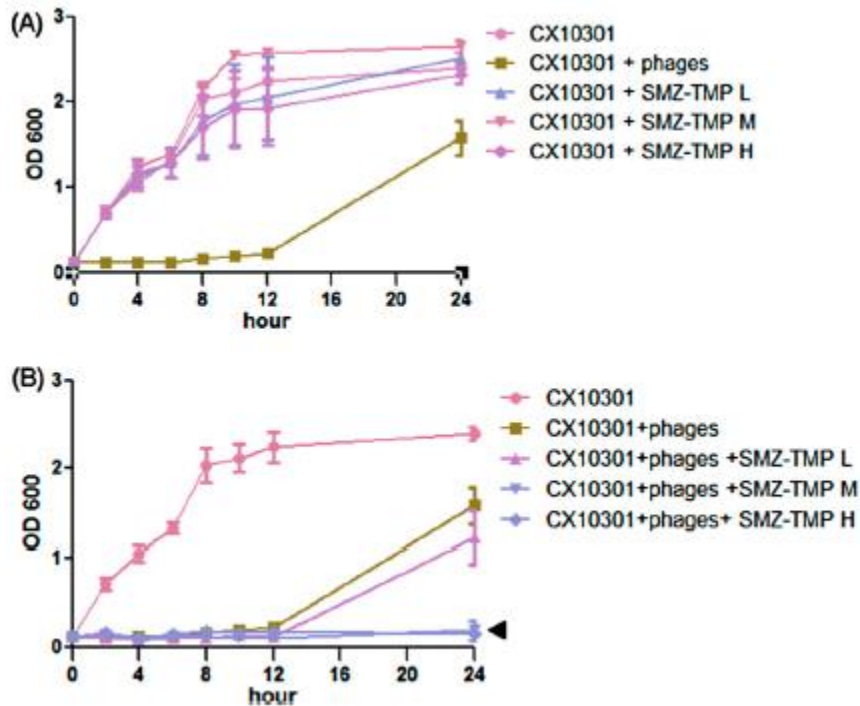
Figure 15. Growth curve of ERKp strain CX10301 under various treatments. (A) Six phages (Kp152, Kp154, Kp155, Kp164, Kp6377, and HD001, 5×108 pfu/mL for each phage) were equally mixed to make a phage cocktail III. 10 mL of bacterial culture (OD600 = 0.1) was mixed with 100 μL of phage cocktail III. Cocktail III inhibits the growth of CX10301 for 12 h, and the resistant mutants developed to a high density within 24 h. Trimethoprim- sulfamethoxazole (SMZ-TMP) cannot inhibit the growth of CX10301 at three concentrations. (H = 300 μg/mL SMZ, 100 μg/mL TMP; M = 150 μg/mL SMZ, 50 μg/mL TMP; L = 75 μg/mL SMZ, 25 μg/mL TMP). (B) The combination of higher concentrations of SMZ-TMP (M and H) and cocktail III could significantly inhibit the emergence of phage-resistant mutants. The in vitro experiments were performed in Luria-Bertani liquid medium, and each experiment was repeated three times.

Biofilms are one of the most challenging infection modalities to treat (Shlezinger et al., 2017). Combinations of phage and vancomycin are highly efficient against vancomycin-resistant Enterococcus faecalis (VRE) as shown by the present study (Shlezinger et al., 2019). Vancomycin-phage EFLK1 (anti-E. faecalis phage) synergy was assessed against VRE planktonic and biofilm cultures. By evaluating the viable counts (Figure 16) and biomass and then visualized using scanning electron microscopy (SEM) (Figure 17), the effect of the combined treatment on VRE biofilms was determined. The combined treatment was synergistically effective both in biofilm and planktonic cultures. According to confocal microscopy and FACS analysis, a change in the peptidoglycan of the cell wall was suggested as fluorescence intensity of phage-treated bacteria increased eight-fold.
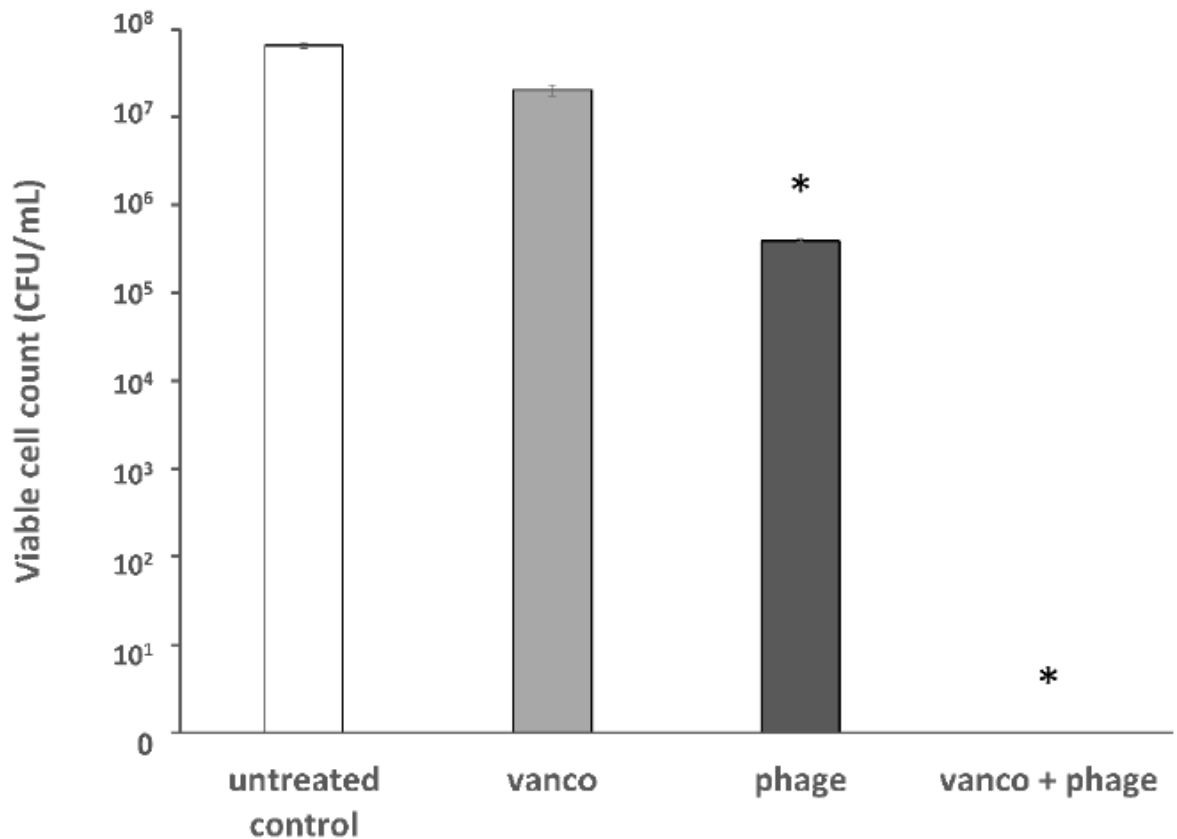
Figure 16. Viable counts of planktonic vancomycin-resistant E. faecalis following a combined treatment of phage EFLK1 and vancomycin. The colony forming units (CFU)/mL of VRE treated with 0.015 mg/mL vancomycin combined with phage EFLK1 1.2 ⁻ 108 PFU/well is presented. Bacteria were below the limit of detection after treating the cells by combining phage EFLK1 and vancomycin. Bacteria treated only with vancomycin showed survival scores like those of the untreated bacteria; cells treated with phage EFLK1 showed medium survival rates. Combining vancomycin and phage EFLK1 caused seven log reductions in CFU/mL. Light gray = vancomycin-treated bacteria, dark gray = phage EFLK1 treatment, black = phage EFLK1 + vancomycin. Statistically significant ($p < 0.01$) compared to the untreated control. The results are mean ± SD based on three independent biological replicates.

This study addresses two key healthcare concerns: the issue of antibiotic-resistant pathogens and the lack of effective biofilm treatment options. Specifically, they tested whether phage-antibiotic synergy (PAS) also occurs when bacteria are considered resistant to the antibiotic. The research demonstrated that the antibiotic alone had almost no effect induced phage lethality in a model of VRE faecalis treated with a combination of vancomycin and an E. faecalis-specific phage. Planktonic and biofilm associated VRE were effectively targeted by the combined treatment. As expected, only extremely high concentrations (>0.25 mg/mL) of the antibiotic eradicated the infection when used alone. However, much lower (0.015 mg/mL) antibiotic

concentrations demonstrated significant antibacterial effects when the phage was combined with the antibiotic. A last-resort antibiotic named Vancomycin, is used mainly in serious Gram-positive bacterial infections that do not respond to other antibiotics. This effective antibiotic inhibits cell wall synthesis in Gram-positive bacteria but is ineffective against VRE. The present study showed that less vancomycin was required in the presence of phage for the same amount of E. faecalis killing. Moreover, VRE growth and viability were minimized in both planktonic cultures and biofilm cultures following exposure to combinations of the two. A synergistic effect was produced by combining vancomycin with phage EFLK1, resulting in almost no resistant bacteria surviving in some of the treatment combinations. Even though the phage was previously shown to be effective against VRE (Khalifa et al., 2018), (Khalifa et al., 2015), especially as a phage cocktail (Khalifa et al., 2018), this effect was intensified when the treatment modality included vancomycin, an antibiotic to which the target bacteria are resistant. It was highly efficacious when targeting E. faecalis V583 although this effect was present in other E. faecalis strains.
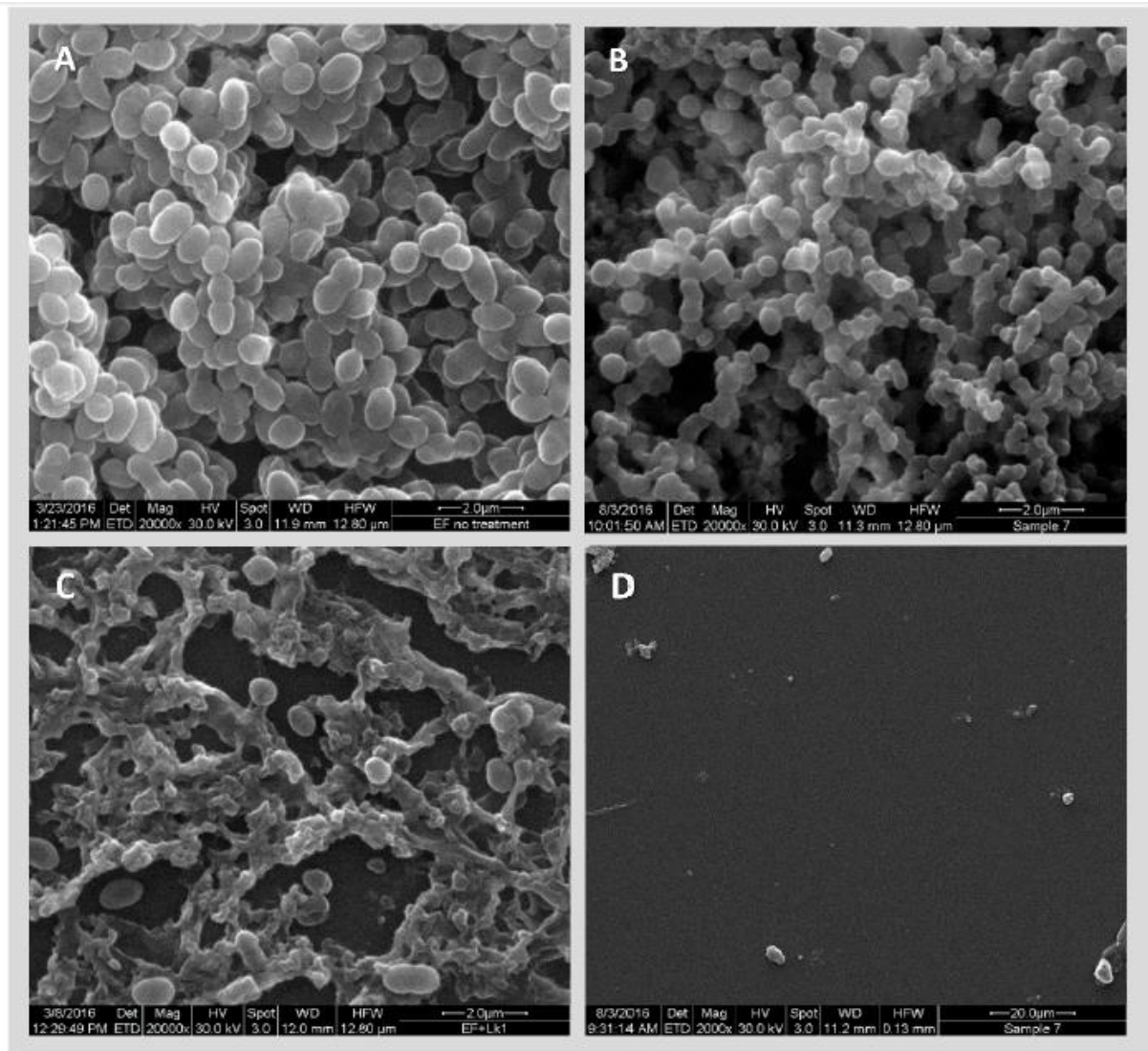
Figure 17. Combinations of phage EFLK1 and vancomycin target VRE. Scanning electron microscopy images (MAG: **A**–**C**: 20,000, **D**: 2000): (**A**) E. faecalis 72-h biofilm. (**B**) Biofilm exposed to vancomycin alone, showing no effect. (**C**) E. faecalis biofilm exposed to phage EFLK1 showing bacterial lysis, leaving mainly the extracellular matrix. (**D**) E. faecalis biofilm exposed to vancomycin and phage EFLK1 showing massive bacterial lysis, degradation and biofilm deformation, leaving almost no trace of biofilm.

The effects on biofilms when the phage therapy and antibiotic therapy are combined:

Treatment for multidrug-resistant organisms; phage therapy is an interesting approach. However, phage resistance is an alarming issue in phage therapy (Rohde et al., 2018). Phage resistance is quite common in vitro (Shen et al., 2018) and was observed very quickly in this patient. The quick development of phage-resistant mutants is likely due to the poor immunity of the patient was observed. The synergy between the immune system and the phages is essential for the clearance of bacterial infection (Roach et al., 2017). If the immunity is strong, the remaining minor number of immune-compromised

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patients is growing due to the growing number of transplant recipients, and patients with cancer and diabetes. As a result, phage resistance would be a frequent issue of phage therapy in the near future.

In this study, we focused on the potential practical application of combinations of phage and antibiotics for treating biofilm infection. We have not explored the pharmaco Đpopulation and evolutionary dynamic processes responsible for the observed results. Clarifying these processes, the aid of computer simulation and mathematical models is certainly required to understand and predict the conditions under which combinations of antibiotics and phage will be more effective than antibiotics alone.

A 48-h biofilm of PA14 was treated by Chaudhry et al. with the two phages NP1 (Siphoviridae, NP1Virus) and NP3 (Myoviridae) together or both in combination with five antibiotics (Chaudhry et al., 2017). Only moderate anti-biofilm efficacy was shown by each antimicrobial, however, true synergistic effects sensu stricto were observed between phages and ceftazidime at 1x MIC and 8x MIC and for ciprofloxacin at 1x MIC when applied simultaneously. For ciprofloxacin at 8x MIC and for tobramycin at 1x MIC, an improved effect by way of facilitation was also achieved, but interestingly not at 8x MIC (Chaudhry et al., 2017). The dose dependency of simultaneous applications with higher antibiotic concentrations likely eliminating the minimum bacterial density required for optimal phage replication is indicated by these findings. Although this antibiotic belongs to the same class as tobramycin, no improvement was observed with colistin and gentamicin. With time-delayed use of phages and antibiotics, the therapeutic outcome differed. 24 h after phage application, the addition of tobramycin or gentamicin led to a significant synergistic effect. Conversely, compared to the simultaneous application, successive addition of ciprofloxacin or ceftazidime did not lead to a better outcome. Thus, critical to a successful combined application is the time point of antibiotic addition and the dosage. The antibacterial outcome may also be impacted by variations in the phage dosage, which was not further evaluated in this study.

Conclusion

Antibiotics have been exceptionally effective at controlling bacterial pathogens (Torres-Barceló & Hochberg, 2016). But horizontal gene transfer or de novo mutations bringing about expanding quantities multidrug-resistant bacteria and the reduced disclosure of new antimicrobial particles lead to the unavoidable end that different methodologies are currently important to monitor the activity of existing molecules and keep up the high strength of future discoveries (Torres-Barceló & Hochberg, 2016). Six results of particular relevance to the clinical potential of combination phage and antibiotic therapy are present.

1. The combination's effectiveness is determined by the intended bacteria's antibiotic tolerance to the employed antibiotic as well as the antibiotic form (bactericide or bacteriostatic).

2. The combination of drugs and phages can kill more bacteria in biofilms than either agent alone. In some cases, the combination kills more bacteria than would be expected if the agents were acting independently.

3. When combined with phage, some antibiotics may be more effective at lower doses than higher.

4. The efficacy of some antibiotics for treating biofilm infections can be considerably augmented when the phages are administered before the antibiotic rather than if they are simultaneously administered [(Torres-Barceló et al., 2014), (Torres-Barceló & Hochberg, 2016)].

5. Phage can prevent treatment failure due to the ascent to high densities by minority populations of bacteria resistant to the treating antibiotic [(Torres-Barceló & Hochberg, 2016), (Chan et al., 2016), (Escobar-Páramo et al., 2012)].

6. Antibiotics added to phage therapy will prevent the development of phage mutants.

In research from Yehl et al. (2019)predicted that by the mutagenesis to regions of the phage tail fiber that are expected to be most dynamic, i.e., the host-range-determining regions (HRDRs) approach may facilitate the creation of next-generation antimicrobials that slow resistance development and could focus on producing viable phages with subtle host-range alterations to target resistant mutants and extended to other viral scaffolds for a broad range of applications. Treatment for multidrug-resistant organisms; phage therapy is an interesting approach. However,

phage resistance is an alarming issue in phage therapy (Rohde et al., 2018). Phage resistance is quite common in vitro (Shen et al., 2018) and was observed very quickly in this patient. The quick development of phage-resistant mutants is likely due to the poor immunity of the patient was observed. The synergy between the immune system and the phages is essential for the clearance of bacterial infection (Roach et al., 2017). If the immunity is strong, the remaining minor number of immune-compromised patients is growing due to the growing number of transplant recipients, and patients with cancer and diabetes. As a result, phage resistance would be a frequent issue of phage therapy in the near future.

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