Review Article

Bacteriophage treatment as an alternative therapy for multidrug-resistant bacteria

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ABSTRACT

تشكل البكتيريا المقاومة للأدوية المتعددة (MDR) واحدة من أخطر التهديدات الصحية العالمية. إن تزايد معدل الإصابة بالعدوى البكتيرية الناجمة عن سلالات MDR وانخفاض عدد المضادات الحيوية المطورة حديثًا دفع المجتمع العلمي إلى البحث عن بدائل. أحد هذه البدائل هو استخدام العاثيات. في هذه المراجعة، نناقش كائنات MDR الحية الأكثر خطورة، بما في ذلك Acinetobacter baumanni، و Pseudomonas aeruginosa، و Staphylococcus aureus المقاومة للميثيسيلين. وتناقش أيضًا فعالية العلاج بالعاثية ضد بكتيريا MDR. قمنا بتضمين دراسات من 10 سنوات ماضية فحصت فعالية العلاج بالعاثية ضد مسببات أمراض MDR. بالإضافة إلى ذلك، تسلط هذه المراجعة الضوء على تأثير العاثيات ضد الأغشية الحيوية البكتيرية. تشير المعرفة الحالية إلى أن العلاج بالعاثية هو استراتيجية علاجية محتملة ضد بكتيريا MDR. ومع ذلك، فإن الآثار الضارة للعلاج بالعاثية، مثل السُّمية وظهور مقاومة العاثيات لم يتم حلها بعد.

Multidrug-resistant (MDR) bacteria constitute one of the most serious global health threats. The increasing incidence rate of bacterial infections caused by MDR strains and the decrease in the number of newly developed antibiotics have prompted the scientific community to search for alternatives. One such alternative is the use of bacteriophages. In this review, we discuss the most critical MDR organisms, including *Acinetobacter baumanni*, *Pseudomonas aeruginosa*, and methicillin-resistant *Staphylococcus aureus*. The efficacy of phage therapy against MDR bacteria is also discussed. We included studies from the last 10 years that examined the efficacy of phage therapy against MDR pathogens. In addition, this review highlights the effect of bacteriophages against bacterial biofilms. The existing knowledge indicates that phage therapy is a potential therapeutic strategy against MDR bacteria. However, the adverse effects of phage therapy, such as toxicity, and the emergence of phage resistance have not yet been resolved.

Keywords: multidrug-resistant (MDR) bacteria, bacterial infections, bacteriophages, phage therapy, biofilms

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Multidrug-resistant (MDR) bacteria, sometimes referred to as antimicrobial-resistant (AMR) bacteria, are a serious public health threat worldwide. Multidrug-resistant bacteria possess resistance to antibiotics either as a natural trait or through the acquisition of resistance over time. In other words, MDR bacteria are immune to the toxic effects of antibiotics. Therefore, infections caused by MDR organisms result in worse outcomes for patients. In 2019, MDR bacterial infections caused almost 5 million deaths globally, as estimated using a systematic analysis.[1] In addition to being associated with a high mortality rate, MDR bacteria impose a significant financial burden on health-care systems. For instance, in the United States, the annual treatment cost of MDR bacterial infections was estimated to be 21–34 billion dollars.[2]

A large number of bacterial strains are becoming less susceptible or non-susceptible to commercially available antibiotics. The World Health Organization (WHO) has recently issued a list of critical pathogens that exhibit high resistance to antibiotics. The list is classified into 3 priority levels based on critical demand for new antibiotics, as follows: critical, high, and medium (Table 1; WHO). The decrease in the number of newly discovered antibiotics and the increasing number of resistant bacteria have urge the scientific community to search for alternatives to resolve this public health threat. Bacteriophages exhibit therapeutic potential

against bacterial infections, including those caused by MDR bacteria.[3] Numerous bacteriophages have been examined for their antibacterial effects against MDR bacteria in animals and humans.[4]

This review focuses on MDR strains, including gram-negative and gram-positive bacteria, and their mechanisms of antibiotic resistance. It then examines the therapeutic potential of bacteriophages against infections caused by MDR bacterial strains. It includes *in vitro*, *in vivo*, and human studies published in the last decade. In addition, it investigates the antibiofilm effect of lytic phages against biofilms produced by MDR strains.

Multidrug-resistant bacteria. One of the critical (priority 1) organisms is *Acinetobacter baumannii* (*A. baumannii)* (**Table 1**). *Acinetobacter baumannii* is a gram-negative bacillus primarily associated with health care-acquired infections.[5] In recent years, MDR *A. baumannii* has attracted global attention owing to its ability to resist most first-line antibiotics.[5] *Acinetobacter baumannii* is defined as MDR when the pathogen resists at least 3 antibiotic classes (penicillins and cephalosporins, including inhibitor combinations, fluroquinolones, and aminoglycosides) and as extensively drug resistant (XDR) when it is resistant to more than 3 classes of antibiotics and to carbapenems. It is defined as pan drug-resistant (PDR) when it is resistant to all the above mentioned antibiotics, polymyxin, and tigecycline.[6,7]

Pseudomonas aeruginosa (*P. aeruginosa*) is another organism classified as critical (priority 1) by the WHO (**Table 1**). It is an opportunistic human pathogen that normally does not cause infection in healthy individuals. However, it can cause life-threatening infections in immunocompromised people such as those with severe burns, organ transplants, cystic fibrosis, and cancer.[8] The management of *P. aeruginosa* infections has become difficult due to the rise in the quantity of isolates that are resistant to antibiotics.[9] Several studies have reported that clinical isolates of *P. aeruginosa* exhibit resistance to a substantial number of commercially available antibiotics.[10-13] Extensive and pan drug resistance have also been reported.[14,15] For instance, Fernandes et al[16] reported several MDR *P. aeruginosa* isolates, including one that was resistant to all commercially available antibiotics.

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is a gram-positive coccus that is resistant to most available antibiotics.[17] Most MRSA infections are health-care-associated MRSA (HA-MRSA) infections. In other words, people who have been hospitalized are at risk of MRSA infections.[17] However, they can also manifest within the broader community, in which case they are referred to as community-associated MRSA.[16] Community-associated MRSA infections usually spread through skin-to-skin contact in crowded places such as schools, daycare centers, and gyms.[18]

Mechanisms of antibiotic resistance. The mechanisms of antibiotic resistance vary among resistant bacteria. For instance, *A. baumannii* avoid the toxic effects of antibiotics through several resistance mechanisms (**Figure 1**). First, it prevents antibiotic access into bacterial cells by reducing outer membrane permeability by down regulating the expression of porins, which results in a less permeable membrane. Second, using efflux pumps, *A. baumannii* pumps antibiotics out of their cells, thus preventing the toxic effects of antibiotics. Third, *A. baumannii* has genetic plasticity that allows rapid genetic mutations. Fourth, *A. baumannii* is capable of forming biofilms, which increases antibiotic tolerance.[20]

Bacteriophages. Bacteriophages, often referred to as phages, are viruses that exclusively target and kill bacteria without harming human cells. For this reason, scientists have studied bacteriophages as antibacterial agents since their discovery in 1915.[4,20] Bacteriophages were discovered by an English physician, Frederick Twort, when he observed for the first time cleared spots on his bacterial plates. Those clear spots were, in fact, dead bacteria lysed by the killing effects of phages. Two

Table 1 - Classification of priority pathogens based on their urgent need for new antibiotics (World Health Organization, 2017).

Bacteria	Resistance
Priority 1: Critical	
Acinetobacter baumannii	Carbapenem
Pseudomonas aeruginosa	Carbapenem
Enterobacteriaceae	Carbapenem
Priority 2: High	
Enterococcus faecium	Vancomycin
Streptococcus pneumoniae	Methicillin, vancomycin
Helicobacter pylori	Clarithromycin
Campylobacter spp.	Fluoroquinolone
Salmonella spp.	Fluoroquinolone
Neisseria gonorrhoeae	Cephalosporin, fluoroquinolone
Priority 3: Medium	
Streptococcus pneumoniae	Penicillin
Haemophilus influenzae	Ampicillin
Shigella spp	fluoroquinolone

MDR: multidrug-resistant, spp: species

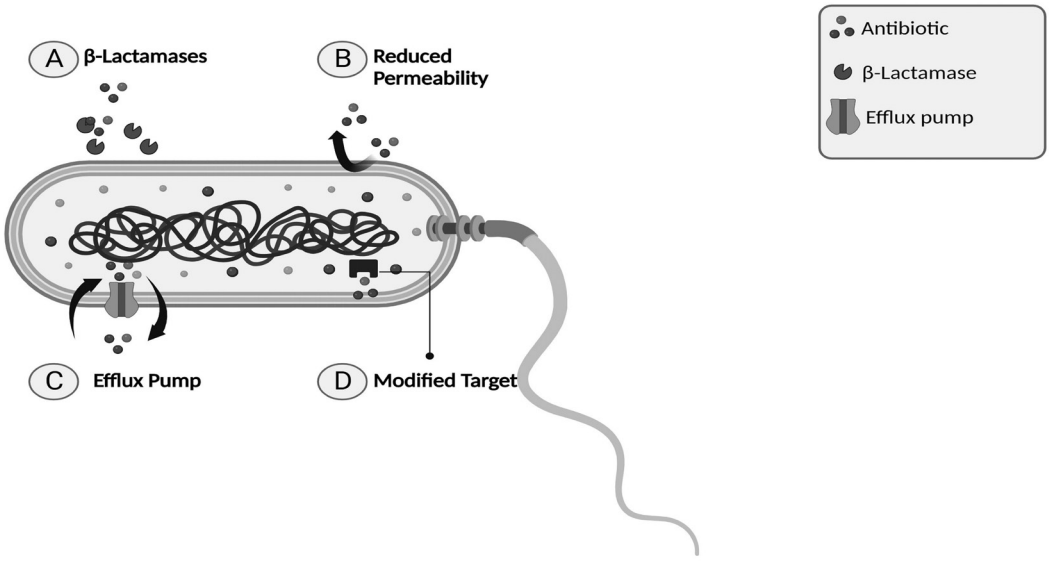

Figure 1 - Mechanisms of antibiotic resistance in *Acinetobacter (A. baumannii)*. Resistance can be conferred in *A. baumannii* through four main mechanisms; **A**) producing β-Lactamases that degrade the β-lactam ring; thus, inactivating β-lactam antibiotics. **B**) Preventing access to antibiotics into the bacterial cell through reducing the outer membrane permeability. **C**) Pumping antibiotics out of the bacterial cell using efflux pumps. **D**) Modifying antibiotic's target via genetic mutations; thus, antibiotic is no longer able to bind to its target.

years later, in 1917, Felix d'Herelle published a similar observation. He suggested that these cleared spots were due to the lytic effect of phages.[20]

In 1919, for the first time, 4 pediatric patients with bacterial dysentery were successfully treated with bacteriophages at des Enfants-Malades hopital in Paris. Since then, phage therapy has been widely utilized, mainly in the Soviet Union and Eastern Europe.[20]

However, once antibiotics were discovered in the 1940s, the western world overlooked phage therapy.[3] The newly discovered drug (antibiotics) was an ideal antibacterial agent until the rise of antibiotic-resistant pathogens during the 1980s.[3] Since then, phages have attracted attention from the scientific community and have been rediscovered by western medicine as an alternative agent to combat antibiotic-resistant pathogens.[3]

Efficacy of bacteriophages against MDR bacteria. In vitro/in vivo studies. Owing to the increasing number of MDR bacteria, researchers have reconsidered the use of phage therapy to overcome MDR organisms.[1,3] The efficacy of phage therapy against resistant bacteria to most, if not all, commercially available antibiotics has been extensively studied not only *in vitro* and *in vivo* but also in humans.[4,21,22]

Numerous *in vitro* and *in vivo* studies have shown a potent antibacterial effect of bacteriophages against MDR bacteria, as summarized in Tables 2 and Tables 3. The efficacy of phage therapy was evaluated against various genera of MDR bacteria, including *A. baumannii*, which is significantly difficult to treat using standard antibiotics.[5] Studies have shown that phage therapy is highly effective against MDR *A. baumannii*, including carbapenem- and colistin-resistant isolates.[23,24] Zhou et al[24] compared the therapeutic effect of 2 bacteriophages and polymyxin B against carbapenem-resistant *A. baumannii*. Using the *Galleria mellonella larva* model, the study demonstrated that phage therapy increased the survival of larvae infected with *A. baumannii* by up to 75%.[24] By contrast, polymyxin B increased the survival of larvae infected with *A. baumannii* by only 25%.[24] Another study assessed the killing effect of bacteriophages against colistin-resistant *A. baumannii*.[23] The study reported a substantial decrease in the number of colistin-resistant *A. baumannii* after only 40 minutes of a single phage treatment.[23] Phage treatment was also effective against XDR *A. baumannii* strains. Wang et al[25] assessed the killing efficacy of ϕkm18p phage against XDR *A. baumannii*. The study showed that mono-phage treatment of mice infected with XDR *A. baumannii* significantly increased survival by up to nearly 100%. The antibacterial activity of bacteriophages against MDR *P. aeruginosa* was also evaluated in *in vitro*

Table 2 - *In vitro* studies using bacteriophages or its derived enzymes against MDR bacteria.

Organism	Bacteriophage	Outcome	Reference
Colistin-resistant MDR *A. baumannii*	IsfAB78 phage	IsfAB78 phage significantly lysed MDR *A. baumannii*	(23)
Colistin-resistant *P. aeroginosa*	Phage cocktails (Psu1, Psu2, and Psu3)	Phage cocktail effectively lysed MDR *P. aeruginosa*	(26)
MDR *A. baumannii, P. aeruginosa* and *K. pneumoniae*	Endolysin ElyA1	ElyA1 was effective against all 25 tested strains of *A. baumannii* and *P. aeruginosa*, showing susceptibility Out of the 17 *K. pneumoniae* isolates, 13 of them were susceptible to ElyA1 led to a reduction of bacterial load by ≥2 $\log_{10}$	(34)
MDR K .pneumoniae	ZCKP1		(35)
MRSA, VRE, and *E. coli*	Vb_saum_LM12, vb_efas_ LM99 vb_ecom_JB75	The three phages showed significant inhibitory effects against MDR *S. aureus* , *E. coli*, and *E. faecalis*	(28)
MDR *P. aeruginosa*	Bacteriophage vb_paem_ LS1	Bacteriophage vb_paem_LS1 exhibited antibacterial activity against several *P. aeruginosa* isolates including MDR isolates	(27)

MDR: Multidrug-resistant, *A. baumannii: Acinetobacter baumannii*, *P. aeruginosa: Pseudomonas aeruginosa*, *K. pneumoniae: Klebsiella pneumoniae, E. coli: Escherichia coli, E. faecalis: Enterococcus faecalis,* MRSA: Methicillin-resistant *Staphylococcus aureus,* VRE: Vancomycin-resistant enterococci, *S. aureus: Staphylococcus aureus*

Table 3 - *In vivo* studies using phage therapy against MDR bacteria.

Organism	Model	Bacteriophage	Outcome	Reference
Carbapenem resistant *A. baumannii*	*Galleria mellonella larva*	2 lytic phages (WCHABP1 and WCHABP12)	Either phage WCHABP1 or WCHABP12 protected the larvae from a leathal dose of *A. baumannii*	(24)
MDR *A .baumannii*	*Galleria mellonella larva* Murine skin and murine lung infection models	Endolysin ElyA1 and colistin	The combination of ElyA1 and colistin increased survival rate of the treated larvae The combination of ElyA1 and colistin decreased bacterial load in the skin wounds of the treated mice The combination of ElyA1 and colistin reduced bacterial load in the lungs of the treated mice	(34)
MRSA and VISA	Mice	AB-SA01	Resulted in a substantial decrease in bacterial burden within the lungs of mice subjected to treatment	(36)
MRSA	Mouse model of lung-derived septicemia	S130'	Led to a significant increased in survival rate of treated mice	(29)
MRSA	Nude mice	Phage JD007	Prevented *S. aureus* from dermal abscesses formation Phage JD007 did not cause a robust immune responses in treated mice	(30)
Carbapenem-resistant *A .baumannii*	Mice	Phage SH-Ab15519	Enhanced the survival rate among the mice that received treatment	(37)
XDR *A. baumannii*	Mice	ϕkm18p	Enhanced the survival rate among the mice that received treatment and decreased bacterial load within them	(25)
MDR *K .pneumoniae*	Mice	1513	Resulted in a higher survival rate among the mice that received treatment	(38)

A. baumannii: Acinetobacter baumannii, MDR: Multidrug-resistant, MRSA: Methicillin-resistant *Staphylococcus aureus,* VISA: Vancomycin-intermediate *Staphylococcus aureus*, *K. pneumoniae: Klebsiella pneumoniae,* XDR: extensively drug resistant, *S. aureus: Staphylococcus aureus*

and *in vivo* studies.[26] On the basis of these studies, bacteriophages have a potent killing activity against MDR *P. aeruginosa*. For instance, studies have tested phage cocktail against colistin-resistant *P. aeruginosa*.[26,27] Three phages (Psu1, Psu2, and Psu3) completely lysed the bacterial cells of colistin-resistant *P. aeruginosa*.[26] Another study investigated the antibacterial activity a lytic phage named as vB_PaeM_LS1 against clinical *P. aeruginosa* isolates, including MDR strains. Phage vB_PaeM_LS1 exhibited potent antibacterial activities against MDR and non-MDR *P. aeruginosa* isolates.[27]

Phage therapy is effective not only against infections caused by gram-negative bacteria but also against those caused by gram-positive bacteria, such as MRSA.[28-31] Numerous studies have demonstrated the therapeutic effect of bacteriophages against MRSA infections.[28,30] For instance, Takemura-Uchiyama et al[29] carried out a preclinical study using a mouse model of lung-derived

septicemia to evaluate the efficacy of phage S13¢ against hospital-acquired MRSA isolates. In mice infected with MRSA, phage S13¢ was intraperitoneally administered 6 hours (h) after infection.[29] The study showed that phage S13¢ rescued the mice infected with a lethal dose of MRSA.[29] The phage-treated mice had significantly higher survival rates than the untreated mice on day 5 (67% vs. 10%).[29] These results suggest that S13¢ is capable of rescuing mice from a lethal dose of MRSA.[29] However, phage S13¢ was administrated at an early stage of lung infection in mice, 6 h post infection. This raises a question whether phage administration at a later stage of infection would obtain a similar outcome. More studies are needed to address this question.

Although bacteriophages are effective for killing MDR strains, bacteriophages are large structures that are likely to induce harmful immune responses.[32] To overcome this challenge, instead of using intact phages, part of the phage components can be utilized as an antibacterial agent. For instance, endolysins are phage-encoded enzymes that digest the cell wall of either gram-positive or gram-negative bacteria.[33] However, the killing efficacy of endolysins is more effective in gram-positive bacteria because the peptidoglycan layer is exposed.[33] In gram-negative bacteria, the peptidoglycan layer is surrounded by an outer membrane layer, which reduces the accessibility of the targets of many endolysins.[33] One approach to increase the therapeutic effect of endolysins is by using them in combination with antibiotics. Blasco et al[33] assessed the therapeutic effects of endolysin ElyA1 and colistin against MDR *A. baumannii*. The researchers demonstrated that the combination of endolycin ElyA1 and colistin increased the survival of larvae infected with MDR *A. baumannii* and decreased the bacterial load in the skin and lungs of mice infected with MDR *A. baumannii*.[33] However, the combination therapy of endolycin ElyA1 and colistin had no antimicrobial effects on colistin-resistant *A. baumanni* isolates. This is probably due to the enzyme's failure to reach the peptidoglycan layer.[34] Thus, endolysins alone may not be the suitable choice to tackle MDR bacteria.

Human studies. In addition to the *in vitro* and *in vivo* studies of the efficacy of bacteriophages against bacterial isolates, studies have also been carried out to test the effectiveness of phage therapy in humans. Over the last decade, numerous case reports and clinical trials of phage therapy have been carried out, as summarized by Liu et al.[22] Most clinical studies of phage therapy utilized a combination treatment of bacteriophages and antibiotics to target various pathogens, including MDR isolates.[22] The available literature data have shown that bacteriophages are potent therapeutics in treating various bacterial infections such as bacteremia, urinary tract infections (UTIs), surgical site infections (SSIs), diabetic foot ulcers, brain infections, corneal abscesses, lung transplant-related infections, aortic graft infections, pancreatitis, otitis, burn wounds, and diarrheal diseases.[22,39]

The most important question is whether the potent efficacy of bacteriophages against MDR bacteria that been shown in *in vitro* and *in vivo* models applies in humans. According to the published data, the use of bacteriophages in humans indicated a potent therapeutic effect against infections with various MDR isolates, including but not limited to, *A. baumannii*, *P. aeruginosa*, MRSA, and *Klebsiella pneumoniae* (*K. pneumoniae)* (**Table 4**). A phage cocktail approach seems to be the most effective way to overcome MDR pathogens, as shown by several human studies (**Table 4**). For instance, LaVergne et al[40] reported the efficacy of phage therapy in treating MDR *A. baumannii*. A 77-year-old patient who had undergone craniotomy had SSI with MDR *A. baumannii*. The isolate was resistant to all tested antibiotics. Initially, the patient was treated with a combination of 3 antibiotics: colistin, azithromycin, and rifampin. However, no clinical improvement was observed. Therefore, a phage cocktail was administered intravenously. After a total of 98 doses of bacteriophages, no signs of infection at the cranioctomy site were observed. In this study, there was no microbiological data after phage administration; thus, it cannot be concluded that phage therapy was successful.[40] Further research is necessary to more thoroughly assess the effectiveness of phages.

In another case study, Khawaldeh et al[41] reported the use of phage therapy to treat a patient with UTI infection caused by *P. aeruginosa*. When the antibiotic treatment failed to cure the *P. aeruginosa* infection, a combination therapy with 6-lytic phages and antibiotics was administered. The patient was treated with the phage cocktail for 5 days, followed by meropenem and colistin for 2 days. Microbiological studies have shown that phage treatment caused a tenfold reduction of bacteria in urine. When antibiotic treatment was involved, no bacteria were detected in the urine samples. These results indicate that antibiotic treatment combined with phages completely cured UTI caused by *P. aeruginosa*.[41]

On the other hand, a few studies have reported negative outcomes associated with phage therapy.[42-44] For instance, a 15-year-old diagnosed with cystic fibrosis and infected by drug-resistant *mycobacterium abscessus* underwent treatment with an intravenous

Table 4 - Case reports using phage therapy against MDR bacteria.

Organism	Bacteriophage	Outcome	Reference
A .baumannii	Phage cocktail	The patient seemed to be more alert but still unresponsive No sign of infection at the craniotomy site was observed The patient's fever and leckocytosis were persisted	(40)
UTI infection with *P. aeruginosa*	Phage cocktail	The combination of phage and antibiotics (colistin and meropenem) cured the UTI Led to a significant reduction of *P. aeruginosa* cells in the urine	(41)
MDR *A. baumannii*	Phage cocktail	The patient's health significantly improved Phage cocktail led to clearance of the *A. baumannii* infection	(45)
Multidrug-resistant *P. aeruginosa* infection	Phage cocktail	Bacteriophage cocktail treatment led to better clinical outcomes Blood cultures were negative	(46)
P. aeruginosa infection	A single dose of phage OMKO1 and ceftazidime	The combination of phage OMKO1 and ceftazidime cured I infection	(47)
Drug-resistant *Mycobacterium abscessus*	3 phage cocktail	Phage cocktail led to improved clinical outcomes including improved liver function and wound closure	(43)
Chronic methicillin-resistant *S. aureus* prosthetic joint infection	3 IV doses of phage	Led to clearance of the severe chronic infection	(48)
Multidrug-resistant, carbapenemase (KPC-3)-producing *K. pneumoniae*	A custom-made, lytic bacteriophage	Eradicated the infection caused by *K. pneumoniae* and no adverse effects were observed	(49)

MDR: Multidrug-resistant, *A. baumannii: Acinetobacter baumannii*, *P. aeruginosa: Pseudomonas aeruginosa*, *S. aureus: Staphylococcus aureus, K. pneumoniae: Klebsiella pneumoniae,* IV: intravenous, UTI: urinary tract infection

mixture of 3 phages. On the second day of treatment, the patient exhibited diaphoresis and cutaneous flushing; nevertheless, there were no indications of fever or modifications in the results of the physical examination.[43] Another study reported adverse events related to bacteriophages. Nine patients with UTI were treated with an adapted Pyo bacteriophage. All phage-treated patients had no phage-associated adverse effects except for one patient with *P. aeruginosa* infection. The patient had a sudden fever (38.5°C) and chills on the third day of phage therapy. Thus, the phage therapy was stopped, and cephalosporin was prescribed. The authors attributed the sudden increase in the patient's temperature to the release of endotoxins during *P. aeruginosa* lysis.[42] A recent study showed that of 9 patients with chronic rhinosinusitis caused by *Staphylococcus aureus (S. aureus)*, 6 developed mild adverse effects after phage treatment, which were classified as treatment-emergent adverse effects such as diarrhea, epistaxis, oropharyngeal pain, cough, rhinalgia, and decreased blood bicarbonate levels.[44]

Efficacy of bacteriophages against biofilms. Another major obstacle in treating bacterial infections is the ability of most bacteria genera, including MDR, to form biofilm structures. A biofilm is a population of microorganisms residing within a self-generated matrix composed of extracellular polymeric substances (EPSs). Extracellular polymeric substances are mainly composed of polysaccharides and proteins, lipids, and extracellular deoxyribonucleic acid. Within a biofilm, bacteria demonstrate greater protection from antibiotics and host immune defenses.[50,51] A biofilm increases antibiotic resistance by up to 1000-fold.[52] The significant increase in antibiotic resistance among biofilm cells is attributed to 2 factors. One factor is biofilm reducing antibiotic accessibility into biofilm cells. The biofilm matrix of EPS as a physical barrier prevents antibiotics from penetrating the EPS matrix.[53] The other factor is biofilm cells that grow slowly and exhibit reduced metabolic activity; thus, the slow-growing cells are immune against antibiotics whose killing mechanisms require metabolically active cells.[54]

Treatment of biofilm-associated infections requires not only the inhibition of causative agents and bacteria but also the disruption of biofilm structures. One potential candidate for disrupting biofilms is bacteriophages. Bacteriophages exhibit potent activities against biofilm structures. As shown in **Figure 2**, bacteriophages can destroy bacterial biofilms through 2 strategies. First, bacteriophages penetrate bacterial biofilms and eradicate them. Second, endolysins and depolymerases, which are phage-derived enzymes, possess enzymatic activity against bacterial biofilms.[55]

Several studies have assessed the antibiofilm activity of phages against biofilm formed by MDR isolates (**Table 5**). Phages have shown antibiofilm effects against biofilms formed by various MDR isolates, including but not limited to MDR *P. aeruginosa*, MDR *A. baumannii*, MDR *K. pneumoniae*, MDR *Escherichia coli*, MDR *S. gallinarum*, and MRSA.[56-61] For instance, a recent study by Adnan et al[56] examined the efficacy of a bacteriophage (MA-1) against MDR *P. aeruginosa*

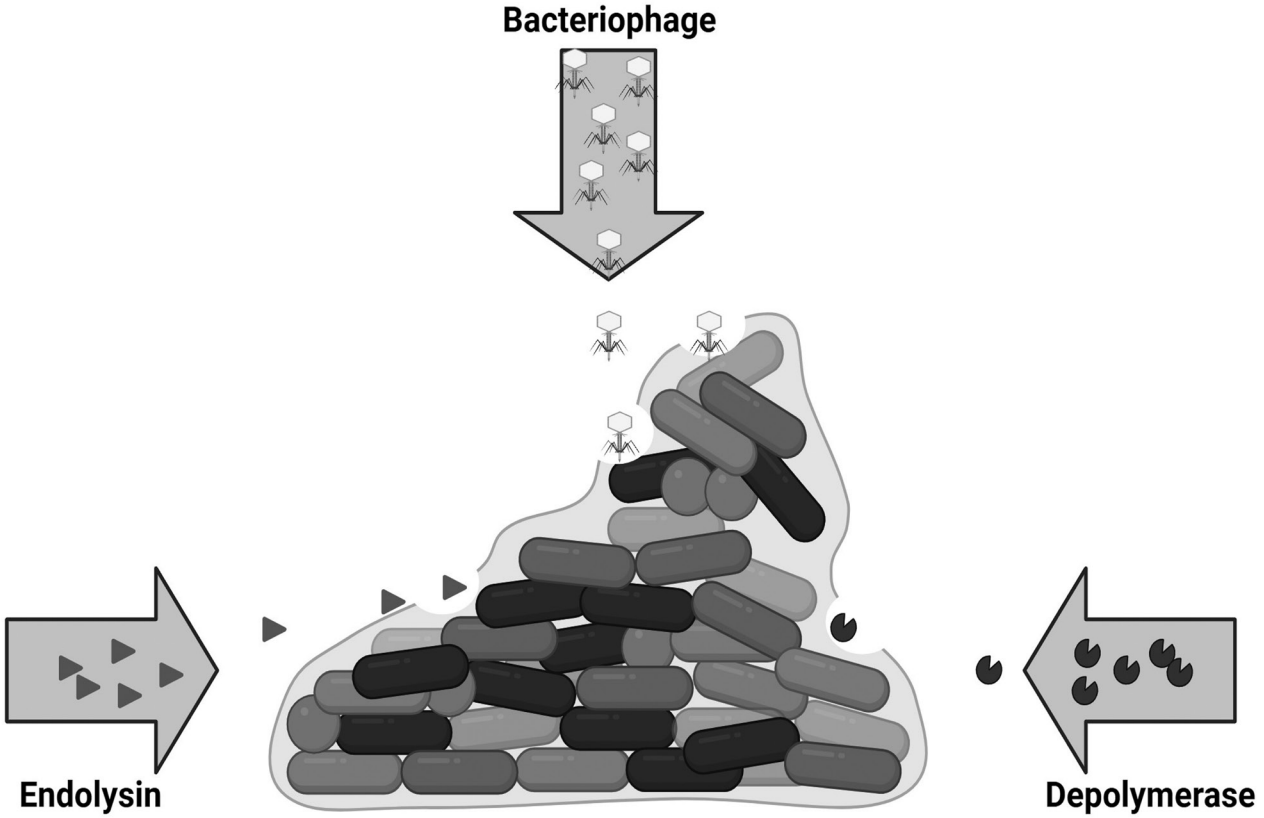

Figure 2 - Schematic representation of biofilm degradation by bacteriophages and its derived enzymes. Bacteriophages degrade bacterial biofilm by 3 mechanisms; (**i**) Phage-derived endolysins, which degrades bacterial cell walls. (**ii**) bacteriophages degrade bacterial cell walls and biofilm matrix. (**iii**) phage-derived depolymerases as free enzyme or tail spike protein degrade biofilm matrix.

Table 5 - Studies of antibiofilm activity of bacteriophages on biofilms formed by MDR isolates.

Organism	Phage treatment	Outcome	Reference
MDR *P. aeruginosa*	MA1-	Reduction of 2.1-fold in a 24 hour (h) old biofilm, 2.5-fold in a 48 h old biofilm, and 3.2-fold in a 72 h old biofilm	(56)
MDR *P. aeruginosa*	vB_PaeM_LS1	Eliminated preformed biofilm	(27)
MRSA	CSA13	Reduced the biofilm biomass in a 24h biofilm by 93%	(60)
MDR *E .cloacae*	MJ2	Reduction of 2.8-log in a 24 h old biofilm, 3-log in a 72 h old biofilm, and 3.5-log in 120 h old biofilm	(62)
MDR uropathogenic *E. coli*	vB_EcoP-EG1	The phage eliminated 60% of a 24 h old biofilm developed by MG1655 and 50% of a 24 h old biofilm developed by 390G7	(59)
MDR *K. pneumoniae*	Z	Reduction of 2-fold in a 24 h old biofilm and 3-fold in a 48 h old biofilm	(58)
MDR A .baumannii	Combination of bacteriophage cocktail and antibiotics	A synergistic effect between phage cocktail and some antibiotics caused a significant reduction in biofilm biomass formed by *A. baumannii*	(57)
MDR *P. stuartii*	Two lytic phages (PSTCR4 and PSTCR6)	Reduced *P. stuartii* biofilm cells by 2.86- and 2.46-log in latex and silicone catheters	(63)
MDR *K. pneumoniae*	Depolymerase (Dep42)	Dep42 significantly inhibited biofilm formation and degrade existed biofilms *K. pneumoniae* Dep42 enhanced polymyxin activity against biofilm formed by *K. pneumoniae* biofilms	(64)
MDR *A. baumannii*	Phage vABWU2101	Phage vABWU2101 exhibited activity against both existed biofilm and biofilm formation, accounting for 18.77 to 70.25% reduction in formation of biofilm biomass and 9.43 to 52.43% reduction in existed biofilm biomass The combination treatment of phage vABWU2101 and tigecycline showed synergistic antibiofilm activity against biofilm formed by MDR *A. baumanni*	(65)

MDR: Multidrug-resistant, *P. aeruginosa: Pseudomonas aeruginosa*, MRSA: Methicillin-resistant *Staphylococcus aureus, E .cloacae: Enterobacter cloacae, E. coli: Escherichia coli, A. baumanni: K. pneumoniae: Klebsiella pneumoniae,* Z: bacteriophage Z, *P. stuartii: Providencia stuartii*

biofilm. MA-1 resulted in a significant reduction of 2-fold in 24-h-old biofilms, 2.5-fold in 48-h-old biofilms, and 3.2-fold in 74-h-old biofilms developed by *P. aeruginosa* However, MA-1 had no effect on other *P. aeruginosa* isolates due to the phage's limited host range.Further research is needed to assess the efficacy of a phage cocktail against biofilm developed by different isolates of *P. aeruginosa*.

Another study targeted biofilms formed by MRSA isolates with bacteriophage CSA13. A 24-h-old biofilm formed in a 96-well plate was treated with the bacteriophage CSA13. The phage eradicated 93.4% of S. aureus CCARM 3793 MRSA biofilms and 78.5% of S. aureus Newman (MSSR) biofilms. This result suggests that biofilm of MRSA strain is more susceptible to phages than that of developed by MSSR.[61] However, It is important to confirm this finding with more research by comparing the susceptibility of biofilms developed by MRSA isolates versus biofilms developed by MSSA isolates.

A recent study combined bacteriophage cocktails consisting of 5 bacteriophages and antibiotics against biofilms of MDR *A. baumannii*. The study reported a significantly greater reduction of biofilm biomass when 24 biofilms were treated with a combined treatment (antibiotic + bacteriophage cocktail). The highest antibiofilm effect was observed when the bacteriophage cocktail was combined with trimethoprim/sulfamethoxazole, accounting for a 98.6% reduction of the biofilm biomass of *A. baumannii*. The study concluded that the combination of bacteriophage cocktails and some antibiotics has a synergistic effect against biofilms of MDR *A. baumannii*.[57]

Challenges of phage therapy. Although phage therapy is a promising treatment for bacterial infections, particularly against MDR bacteria, it also comes with several challenges. One major challenge is selection of the right phage. There are 2 types of phages; lytic and temperate. Lytic phages immediately kill their bacterial hosts, while temperate, or lysogenic, phages integrate their genetic material into the host chromosome without killing their hosts. The concern of utilizing temperate phages in phage therapy is the possibility of transferring toxin or antibiotic resistant genes to their target bacterial.[66,67] Another obstacle of phage therapy is the emerging of phage resistance. Similar to antibiotic resistance, bacteria are capable of developing resistance to phages. This can be achieved by a modification on bacterial cell surface.[67,68] A study carried out by Le et al[69] showed *P. aeruginosa* exhibited a mutation in galU after exposure to lytic phage. GalU is a gene necessary for the production of LPS. Administration of phage is not straightforward as well. Unlike antibiotics, phages are self-replicating organisms; meaning the concentration of a phage mixture given to a patients may not be the exact concentration they actually receive.[67,68]

In conclusion, MDR bacteria are a terrifying major health issue worldwide. Infections caused by MDR bacteria are difficult to treat with conventional treatments (antibiotic therapies). The available antibiotics are becoming less or even ineffective in inhibiting MDR isolates. The golden age of antibiotics appears to have come to an end, and a post-antibiotic era is about to commence. Thus, the clinical and economic impacts of MDR bacteria necessitate the search for alternative antibacterial agents. One alternative is bacteriophages. They are the most ubiquitous organisms in the universe.[70] Scientific studies have demonstrated the potent efficiency of bacteriophages and bacteriophage-derived enzymes against MDR bacteria not only in *in vitro* models but also in *in vivo* models and humans, as described earlier. Besides their efficacy against MDR bacteria on planktonic form, bacteriophages have also demonstrated an antibiofilm effect against biofilms of MDR isolates (Table 5).

According to the existing literature, the most effective strategy to overcome antibiotic-resistant bacteria is by a combination treatment consisting of a phage cocktail and antibiotics (Tables 3 & 4). However, more investigations are required to evaluate the side effects of phage therapy, such as toxicity and unwanted immune response. In addition to conducting more studies on phage therapy, the scientific community should implement suitable regulations for the clinical use of phage therapy to accelerate the process of eradicating MDR bacteria.

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