Preprints.org

Review

Not peer-reviewed version

Antimicrobial Resistance in *Pseudomonas aeruginosa* and the Emerging Role of Phage Therapy

Rediet Guta Mideksa , Alazar Amare Amdiyee * , Alemayehu Godana Birhanu

Posted Date: 10 March 2026

doi: 10.20944/preprints202603.0722.v1

Keywords: antimicrobial resistance; *P. aeruginosa*; phage; phage therapy; phage cocktail

Preprints.org is a free multidisciplinary platform providing preprint service that is dedicated to making early versions of research outputs permanently available and citable. Preprints posted at Preprints.org appear in Web of Science, Crossref, Google Scholar, Scilit, Europe PMC.

Copyright: This open access article is published under a Creative Commons CC BY 4.0 license, which permit the free download, distribution, and reuse, provided that the author and preprint are cited in any reuse.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 March 2026 doi:10.20944/preprints202603.0722.v1

Disclaimer/Publisher's Note: The statements, opinions, and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions, or products referred to in the content.

Review

Antimicrobial Resistance in *Pseudomonas aeruginosa* and the Emerging Role of Phage Therapy

Rediet Guta Mideksa [1], Alazar Amare Amdiyee [1,2,*] and Alemayehu Godana Birhanu [1]

[1] Biotechnology research center, Addis Ababa University, Addis Ababa, Ethiopia.

[2] Dilla University, College of Medicine and Health Science, Department of Medical Laboratory Sciences.

* Correspondence: Alazar Amare Amidyee; email address: amarealazar633@gmail.com.

Abstract

Antimicrobial resistance has emerged as a significant global issue in combating bacterial diseases. *Pseudomonas aeruginosa* is one of the major opportunistic bacteria that cause acute, chronic, and nosocomial infections. The WHO has indicated *P. aeruginosa* as a member of ESKAPE group due to its high resistance rate to multiple existing treatments. The rapid rises in bacterial strains that are extensively drug-resistant (XDR), pan-drug-resistant (PDR), and multidrug-resistant (MDR) significantly increases the morbidity and mortality rates. In response to the escalating challenge of antimicrobial resistance (AMR), phage therapy has emerged as a promising alternative to the regular antibiotics. Lytic phages are specific viruses that infect and lyse bacterial cells, offering targeted antibacterial activity while minimizing disruption of normal microbiota. Recent progresses in specific bacteriophage isolation, optimized phage cocktail formulation, and combination therapy with antibiotics have demonstrated significant therapeutic potential in both laboratory and clinical studies. This review provides an overview of the current molecular mechanisms of antimicrobial resistance in *P. aeruginosa* and discusses the therapeutic potential of bacteriophages, highlighting their advantages, limitations, and future perspectives as an alternative therapy.

Keywords: antimicrobial resistance; *P. aeruginosa*; phage; phage therapy; phage cocktail

1. Introduction

Pseudomonas aeruginosa is a rod-shaped, motile, gram-negative facultative aerobe bacterium grows at 37°C, but can also tolerate temperatures ranging from 4°C to 42. Gersard isolated it for the first time from wound pus in 1882. It is an opportunistic microbe that causes a variety of infection including systematic infections, gastrointestinal infections, respiratory infections, urinary tract infections, soft tissue infections, and joint infections in immunocompromised patients[1–3]. Immunocompromised individual with additional predisposing conditions such as indwelling devices, burns, ocular injuries, or chronic diabetic wounds are particularly vulnerable to *P. aeruginosa* infections[2]. *P. aeruginosa* belongs to the class of bacteria known as ESKAPE, which are distinguished by their multidrug resistance and important role in nosocomial infections. The ESKAPE pathogens includes *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp [4,5]. Infections caused by *P. aeruginosa* pose significant public health challenges because of the escalating prevalence of antibiotic resistance. According to a WHO report, *P. aeruginosa* is one of the primary antibiotic-resistant bacteria for which the development of new antibiotics is critically needed [4,6,7].

1.1. Global Challenges of Pseudomonas aeruginosa Infection

P. aeruginosa is one of the common bacteria that cause acute, chronic, and nosocomial infections. It is prevalent in a clinical settings due to its ability to colonize moist environments, and it accounts for around 10% of hospital-acquired infections [8]. In various regions of the world, including North

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 March 2026 doi:10.20944/preprints202603.0722.v1

America, Europe, Asia-Pacific, Latin America, and Africa, *P. aeruginosa* is frequently isolated from pneumonia patients. In hospitalized patients, *P. aeruginosa* is most frequently linked to ventilator-associated pneumonia (44.6%), bloodstream infections (27.9%), and surgical site infections (19.1%). It is also responsible for 12% of hospital-acquired urinary tract infections (UTIs), most commonly associated with urinary catheterization, instrumentation, or surgical procedures, and is a frequent cause of infections among patients in intensive care units, accounting for 16.2% infection rate[9–12]. Mortality rates for serious infections, such as bloodstream infections and ventilator-associated pneumonia, range from 32% to 58.8%. Chronic *P. aeruginosa* infections are a major cause of morbidity and mortality among cystic fibrosis (CF) patients, with prevalence rates up to as 49.6%[13,14]. The potential for *P. aeruginosa* to cause a wide range of diseases and the rapid emergence of antibiotic resistance pose a serious threat to global health; treatment failure is being reported more frequently globally [15,16].

1.2. Antibiotic Resistance

Antimicrobial resistance (AMR) is characterized by microorganisms' capacity to withstand exposure to antimicrobial agents intended to eradicate them. The WHO has listed AMR as one of the major global health threats and a leading cause of mortality, accounting for approximately 9% of all deaths globally; In 2019 , it was estimated that antimicrobial-resistant infection caused 1.27 million fatalities globally, estimation suggests that, without a change in current therapy trends, antimicrobial resistance could result in a minimum of 10 million deaths annually and economic losses of approximately one trillion US dollars per year by 2050 [8,17–19]. The inappropriate antimicrobial agents use, coupled with the several resistance mechanisms of bacteria, has led to the development of drug-resistant strain. Additionally, poor regulation of antimicrobial drugs, insufficient monitoring of AMR emergence, misuse of antibiotics in healthcare facilities, and deficient quality assessments for available antibiotics are key contributing factors to the spread of AMR in developing nations[20,21].

1.3. Antibiotic Resistance in Pseudomonas aeruginosa

P. aeruginosa is a significant multidrug-resistant (MDR) pathogen responsible for high morbidity and mortality rates. The rising prevalence of healthcare associated infections caused by MDR *P. aeruginosa* strains significantly complicates the selection of effective treatment. MDR defined as the ability to withstand various antimicrobial agents from three or more classes of antibiotics, whereas XDR is the ability to withstand nearly all antimicrobial agents from multiple classes of antibiotics while still being susceptible to only one or two categories. In *P. aeruginosa* MDR described as the resistance to three or more antibiotic categories, including penicillin/cephalosporin/monobactam, carbapenems, aminoglycosides, and fluoroquinolones [22,23].

Recent global systematic reviews and meta-analyses indicate that MDR *P. aeruginosa* remains a major clinical challenge worldwide. In clinical isolates of *P. aeruginosa*, resistance rates to third-generation cephalosporins such as ceftazidime were approximately 66.8 %, and resistance to ceftriaxone reached as high as 98.7 % in meta-analysis data[24,25].

Carbapenem (imipenem, meropenem) once considered as a last-resort antibiotics, is now have widespread resistance, with global prevalence reported around 30–40 %, driven by efflux pump overexpression, porin loss, and carbapenemase production [26]. High resistance rates are also observed for fluoroquinolones 64.9% and 41.8% aminoglycosides, further limiting empirical treatment options. The rising prevalence of MDR and XDR *P. aeruginosa* strains results the increased clinical severity and poorer clinical outcomes. For example, the death rate for MDR *P. aeruginosa* bloodstream infections is 58.8%, while that of non-MDR strains is 43.2%. All of these patterns indicate the depleting therapeutic options available to combat MDR *P. aeruginosa* and emphasize the need for improved alternative therapy [27–29].

1.3.1. Antibiotic Resistance Mechanisms of *Pseudomonas aeruginosa*

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 March 2026 doi:10.20944/preprints202603.0722.v1

The main resistance mechanisms of *P. aeruginosa* can be divided into three categories: acquired, intrinsic, and adaptive [23,30] .

1.3.1.1. Intrinsic Antibiotic Resistance Mechanism

This resistance mechanism is based on the Bacteria's inherent capacity to reduce the effectiveness of a specific antibiotic as a result of its structural or functional traits. *P. aeruginosa* possess natural resistant to several antibiotics, such as aminoglycosides, beta-lactams, and fluoroquinolones. This resistance mechanism involves restricted outer membrane permeability, an efflux system that expels drugs from the cell, and the synthesis of enzymes like β-lactamases that deactivate antibiotics [31–33].

1.3.1.2. Outer Membrane Permeability

Most antibiotics needs to break the cell membrane to reach their targets site within the cell to effectively target *P. aeruginosa* infections. The outer membrane of *P. aeruginosa* consists an asymmetric bilayer of phospholipid and LPS porins embedded in it which limits the penetration of small hydrophilic compounds and blocking larger ones, which prevents the entry of antibiotics[23]. *P. aeruginosa* possesses various specialized porins such as OprB, OprD, OprP, and OprO allows the transport of specific molecules including antibiotics across the outer membrane. Antibiotic entry depends on porin expression, channel structure, and molecular compatibility. When porins are downregulated, lost, or structurally altered, drug influx is reduced leading to resistance. For instance, OprD mutation or deletion is one of the primary causes of carbapenem resistance. Altered porin in conjunction with efflux pumps and β-lactamase synthesis significantly contributes to the rise of multidrug-resistant *P. aeruginosa* [34–36].

1.3.1.3. Efflux Systems

Antimicrobial agents produce toxic substances inside bacterial cells, which threaten bacterial cell survival. To encounter this, bacteria uses efflux pumps that actively expel these harmful substances from the cell by using ATP or proton motive force[8]. These efflux pumps are categorized into different families, including the resistance nodulation division (RND) family, proteobacterial antimicrobial compound efflux (PACE) family, major facilitator superfamily (MFS), ATP binding cassette (ABC) superfamily, small multidrug resistance (SMR) family, and multidrug and toxic compound extrusion (MATE) family[23,37].

The RND family efflux pump proteins are crucial for antibiotic resistance in *P. aeruginosa*. Twelve RND efflux pumps are present in this bacteria, including four distinct ones (MexAB-OprM, MexEF-OprN, MexCD-OprJ, and MexXY-OprM)[23]. MexAB-OprM is responsible for β-lactam and quinolone efflux, while MexXY-OprM pump out aminoglycosides, MexEF-OprN extrudes quinolones, and MexCD-OprJ may expel β-lactams [23,38]. The excessive production of multiple efflux pumps has been observed in certain *P. aeruginosa* strains leading to increased resistance to antibiotics and playing role in the emergence of MDR. MexAB OprM and MexXY overexpression is prevalent in strains, accounting for up to 30% of cases [23,39].

Additionally, efflux pump families that contribute to its multidrug-resistant (MDR) *Pseudomonas aeruginosa* includes major facilitator superfamily (MFS) pumps export a limited range of substrates, such as tetracyclines and chloramphenicol, while the smaller small multidrug resistance (SMR) family pumps remove cationic compounds such as quaternary ammonium compounds and dyes. ATP binding cassette (ABC) transporters superfamily, primarily involved in nutrient uptake, can potentially lead to the resistance to antibiotics. Furthermore, the proteobacterial antimicrobial compound efflux (PACE) family expels particular small antimicrobial compounds, such as cationic biocides and structurally related agents, while the multidrug and toxic compound extrusion (MATE) family, which includes PmpM, uses proton or sodium gradients to extrude fluoroquinolones and

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 March 2026 doi:10.20944/preprints202603.0722.v1

other cationic compounds. The synergistic activity of these efflux systems enhances drug resistance in *P. aeruginosa [40–43]*.

1.3.2. Acquired Antibiotic Resistance Mechanism of *P. aeruginosa*

Antibiotic resistance in *P. aeruginosa* can be acquired by horizontal gene transfer, such as transformation, transduction, and conjugation, or by mutational alterations. Mutation is one of the main mechanisms of acquired resistance, which results in altered antibiotic targets, decreased antibiotic uptake, overexpression of efflux pumps, and the synthesis of antibiotic-inactivating enzymes. These modifications allow the bacteria to withstand the impacts of antibiotic compounds[38,39,44]. One of well-studied example is OprD gene mutations, which cause OprD expression to be down-regulated and results in resistance to imipenem (IMP). Additionally, meropenem (MEM) susceptibility varies based on the type of OprD gene mutations [36,39,44]. Furthermore, mutations in certain gene loci for lipid A synthesis, which would modify lipid A by 4-amino-4-deoxy-L-arabinose (L-Ara4N), as well as the up-regulation of the LPS modification pathway, are the causes of *P. aeruginosa's* resistance to colistin. Colistin heterogeneous resistance is also mediated by two-component regulatory systems (TCS), including PhoPQ, ParRS, and CprRS [45,46]

1.3.2.1. Acquired β-Lactamases Production

β-lactamases are enzymes found at periplasmic region of the bacterium that hydrolyzed the β-lactam ring of antibiotics which degrades the antibiotic's active site and prevent its potency. In *P. aeruginosa*, two types of β-lactamases are recognized Extended-spectrum β-lactamases (ESBLs) and AmpC β-lactamase: ESBLs are typically plasmid-encoded and acquired through horizontal gene transfer, confer resistance to penicillins and cephalosporins. In contrast, AmpC β-lactamase is chromosomally encoded and intrinsic to *P. aeruginosa*; it is inducible by β-lactam exposure and hydrolyzes penicillins and cephalosporins[47,48]. Overall, antibiotic resistance genes can be found on plasmids, transposons, integrons and prophages. Bacteria can obtain these genes through horizontal gene transfer methods including transduction, transformation and conjugation [44,49,50].

1.3.3. Adaptive Antibiotic Resistance Mechanism of *P. aeruginosa*

Adaptive resistance is the ability of the bacteria to develop resistance when exposed to a range of environmental factors by temporarily modifying their gene and/or protein expression in various ways. These reversible mechanisms allow *P. aeruginosa* to withstand exposure to environmental or antimicrobial distress. In *P. aeruginosa* the most well-studied adaptive resistance mechanisms includes biofilm formation and the production of persister cells [23,51–53].

1.3.3.1. Biofilm-Mediated Resistance

A biofilm refers to a collection of microorganisms that stick together on surfaces. These microorganisms are surrounded by a matrix made up of substances they produce, such as exopolysaccharides, proteins, metabolites, and extracellular DNA [15,23,54]. The biofilm exhibits a heterogeneous cell population, comprising both rapidly growing cells and those slower growth rate. Certain cells display resistance by producing enzymes and operating efflux pumps, whereas others lack these resistance mechanisms. Biofilm cells are similar to free-living planktonic cells and can detect the presence of other cells through a process known as quorum sensing. This allows them to adjust their characteristics based on the population density of cells around them. Quorum sensing serves as a means for bacteria to communicate with one another and it influences gene expression, in response to shifts in cell population density [38,55,56]. When *P. aeruginosa* forms biofilms, it goes through a number of physiological and appearance changes. For example, in cases of CF chronic infection, *P. aeruginosa* strains forms clusters of cells called microcolonies and a mucoid form characterized by increased alginate production. This adaptation facilitates the establishment of

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 March 2026 doi:10.20944/preprints202603.0722.v1

biofilm colonies which avoids antibiotic penetration into the bacterial cell and makes it approximately 10 to 1000 times resistant to antibiotics than planktonic cells [15,38,56,57].

1.3.1.2. Persister Cells Formation

Persister cells are a small subpopulation of bacteria that enter a dormant or metabolically inactive state. During extended exposure to antibiotics, persister cells embedded in biofilms help to stimulate adaptive resistance[58]. Despite lacking genetic resistance determinants, these cells can withstand exposure to high antibiotic doses by shifting almost into physiological inactive state. These cells are extremely resistant to high antibiotic doses due to their metabolic inactivity and the inhibition of the synthesis of compounds that act with antibiotics. Persister cells can restart active proliferation and repopulate the infection site after antibiotic pressure is removed and suitable environmental condition are established [59].The rising prevalence of MDR and XDR *P. aeruginosa* strains by the above common resistance mechanisms leave the clinicians with limited treatment options. Several genes contribute to the emergence of AMR in *P. aeruginosa* (Table). The resistant *P. aeruginosa strains* have a potential to cause more complex and prolonged infections in clinical settings. Therefore, the development of innovative alternative antimicrobial therapies that can eradicate antibiotic-resistant *P. aeruginosa* is urgently needed [45,46].

Table 1. Common genes associated with AMR in P. aeruginosa.

Categories	Genes	Resistance mechanism	Drugs affected	Ref.
Chromosomal β-Lactamase	*ampC*	Hydrolysis of carbapenems is caused by overexpression of the chromosomal β-lactamase ampC, which is coupled with the oprD mutation.	Penicillins cephalosporins	[60,61]
Serine carbapenemases	*blaOXA*	Encode class D serine β-lactamases (oxacillinases), which break down the β-lactam ring enzymatically and provide resistance to carbapenem.	Imipenem (IMP). Meropenem (MEM). Doripenem	[62,63]
Metallo-β-lactamases	*blaVIM, blaIMP, blaNDM*	Develop resistance to carbapenems by encoding zinc-dependent metallo-β-lactamases, which break down the β-lactam ring and render carbapenems inactive.	Imipenem (IMP). Meropenem (MEM). Doripenem	[64]
pmrAB (PhoPQ) Two Component System	*pmrAB, phoPQ*	causes changes to lipid A in the LPS layer, usually by adding L-Ara4N, which reduces the binding of colistin.	polymyxin antibiotics, including colistin and polymyxin B	[65]

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 March 2026 doi:10.20944/preprints202603.0722.v1

Lipid A Biosynthesis Genes	*lpxA, lpxC, and lpxD*	Resulting in altered LPS production and the loss of the antibiotic's main target, which causes colistin resistance.	Polymyxins antibiotics	[66,67]
Efflux Pump Genes	*oprD, mexX, mexY*	Encodes multidrug efflux pumps and expel a variety of antibiotics.	Imipenem, Meropenem, other β-lactams	[68]
Quinolone Resistance Genes	*qnr*	Encodes proteins that provide quinolone resistance by shielding DNA gyrase and topoisomerase IV from quinolone inhibition.	Ciprofloxacin Levofloxacin Ofloxacin	[69]
Aminoglycoside Resistance Genes	*aac, aph, and aad*	encode the enzymes necessary to change aminoglycosides and make them ineffective.	Gentamicin, Tobramycin, Amikacin, Netilmicin, Streptomycin	[28,70]

2. Novel Therapeutic Strategies

2.1. Bacteriophages as Antimicrobial Agents

Bacteriophage is a type of virus that targets and eradicates bacteria. They consist protein capsid-bounded double-stranded or single-stranded DNA or RNA. Approximately 10^{31}-10^{32} phages are present globally. Phages are classified by their size, shape, genetic makeup, structure and life cycle (Table). They have a key role in controlling bacterial populations and contribute to the mortality of around 20% - 40% of marine surface bacteria daily [71–74]. They are entirely depending on their host bacteria for replication and survival. Based on their developmental cycles, phages are classified as virulent or temperate [74]. Virulent phages have a lytic cycle, which destroys the bacterial cell. On the other side, temperate phages undergo a lysogenic cycle, which integrate their genetic material into the bacterial DNA and confer immunity against infection by identical phages. Virulent phages are relevant for therapeutic applications, as they can exclusively perform the lytic cycle [72,75].

Phage infections start when the virus attaches to specific bacterial cell surface receptors. Following a successful binding, the lytic phages inject their genetic material into the host bacterium. Followed by viral transcription by using the host's RNA polymerase. Subsequently, the synthesis of structural and enzymatic proteins occurs, which enables the assembly of new phage particles. Following the production of a dense amount of phages, which can range from a few to over a thousand, viral genes are expressed to produce viral proteins including holin and endolysin, which damage the bacterial cell wall and rupture the cytoplasmic membrane. This process leads to cell lysis and the release of newly formed viral particles, which can then infect other bacterial cells[74–76]. On the other hand, Tempered viruses integrate their genome into the host chromosome or plasmid, the incorporated genome is called a prophage. The phage replicates its DNA together with that of the bacteria and during cell division this genetic material is inherited between daughter cells. This Phage

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 March 2026 doi:10.20944/preprints202603.0722.v1

is remained in a latent state and occasionally can enter the lytic cycle. Although the exact causes of the temperate phage lytic cycle's activation are not well described, it is recognized that substances that stress the host cell or damage its DNA can trigger the phage lytic cycle[15,72,74,77].

Table 2. Classification of Bacteriophages Based on Biological and Structural Characteristics.

Classification	Category	Description	Phages Example	Ref
Size	Small Phages	20–60nm diameter, simple genome organization	MS2	[78–88]
	Large Phages	Large capsid size (>200 nm) and complex genomes	Pseudomonas phage phiKZ	
Morphology	Icosahedral (non-tailed)	Capsid symmetry lacking tail structure	PhiX174	
	Tailed phages	Head–tail architecture	T4 phages	
	Filamentous phages	An extended and flexible sort of filamentous structure	M13 bacteriophage	
Genetic Material	Double-stranded DNA (dsDNA)	This group includes the majority of bacteriophages	Bacteriophage lambda	
	Single-stranded DNA (ssDNA)	reduced genome size	PhiX174, M13	
	Single-stranded RNA (ssRNA)	RNA-dependent RNA polymerase is required	MS2	
	Double-stranded RNA (dsRNA)	A rare set of bacteriophages	Phi6 phages	
Structural complexity	Simple phages	Capsid with no tail structures	PhiX174	
	Complex phages	Head, tail, base plate, tail fibers	T4 Phages	
Life Cycle	Lytic phages	reproduce within the host and induce lysis of cells	T7 Phages	
	Lysogenic	Integrate viral genome into host chromosome	phage lambda	

2.2. Phage Cocktail

The host specificity of phages is highly variable, some can infect a broad range of bacterial species, while others are restricted to a particular strain. The phage specificity is mostly determined by the capacity of phages recognition and bind to the specific host cell receptor as well as the ability

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 March 2026 doi:10.20944/preprints202603.0722.v1

to recognize the host's antiviral defense mechanism[89,90]. In phage therapy, phage cocktails are often used to expand the range of hosts that kill and improve the effectiveness. These cocktails are formulated by combining a few selected phages that are either already known or recently discovered into a therapeutic mixture [91]. Phage cocktails have the key advantage of attacking a broader spectrum of bacterial strains compared to a single phage. By combining phages that have different host ranges, the cocktail can address the issue of bacterial resistance that could arise against a specific type of phage[92]. Phage cocktail also facilitates the easy penetration of *P. aeruginosa* biofilm, destroying its structure by triggering the production of enzymes like polysaccharide depolymerase, which reduces bacterial density and increases phage effectiveness[15,93,94].

2.3. Antipseudomonal Bacteriophages and Their Clinical Potential

Pseudomonas-specific phages were initially identified in mid-20th century. Currently, genomic databases have approximately 137 sequenced phages that target *P. aeruginosa*, most of which are members of the order Caudovirales [95,96]. The first controlled clinical trial of a therapeutic bacteriophage preparation was conducted in 2009, revealing that it was safe and effective in treating antibiotic-resistant *P. aeruginosa* chronic otitis. Due to the prevalence of the bacterium in hospital-acquired infections and its rising antibiotic resistance, there has been considerable interest in employing phages to combat *P. aeruginosa*. The efficacy of phage treatment for *P. aeruginosa* infection and its compatibility with eukaryotic cells in both vitro and vivo have been demonstrated [97,98]. In a chronic lung infection murine model infected with *P. aeruginosa*, phage PELP20 was proved to be effective against a 9-day established lung infection by totally eradicating all bacteria from 70% of the mice's lungs [99]. Furthermore, various studies have demonstrated that phage PEV20 works synergistically with ciprofloxacin to enhance the elimination of *P. aeruginosa* biofilm in CF and wound patients [100].

Jeon *et al*., conducted a study to evaluate the effectiveness of two newly identified bacteriophages, BΦ-R656 and BΦ-R1836, in treating acute pneumonia in mouse models infected with XDR *P. aeruginosa.* The study involved testing the phages in laboratory settings, computer simulations, and live animal experiments. Both phages demonstrated strong activity against XDR *P. aeruginosa* strains isolated from pneumonia patients. Additionally, these phages showed a significant ability to remove biofilms formed by XDR *P. aeruginosa*[15]. Furthermore, Phage cocktails have proved high efficacy in treating antibiotic-resistant *P. aeruginosa* infections of the gastrointestinal tract, skin, and lungs in animal models [74,101,102]. A study conducted in 2017 demonstrated that phage cocktails have a potential therapeutic effect against 97.9% of the 96 *P. aeruginosa* isolates tested, which included 94 MDR, 63 XDR, and 1 PDR strain[97,103].

Researchers also investigated different ways of phages delivery in animal models for *P. aeruginosa* infection treatment. In a comparison of intramuscular, intraperitoneal and subcutaneous injections of a phage mixture, intraperitoneal injection proved to be the most successful method. This effectiveness can be due to its ability to deliver a larger quantity of phages more quickly and consistently over time [104]. After Successful experiment on animal model, phage therapy has been conducted on clinical patients. As an example, in 2016 a patient who had a persistent aortic graft infection that had not improved after several surgeries and vigorous antibiotic treatment have been treated by bacteriophage. Additionally, two cystic fibrosis patients with s antibiotic resistant infection were recently treated using phages [98,105]. (Table) summarizes successful Phage therapies targeting *MDR P. aeruginosa in human patients.*

Table 3. Example of Successful Phage Therapies Against P. aeruginosa.

Case	Phage(s)	Phage Dosage (PFU/mL)	Route of Administration	Clinical Outcome	Ref

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 March 2026 doi:10.20944/preprints202603.0722.v1

Chronic lung infection	phiYY	1×10^9	Nebulized	The patient's symptoms relief and they were discharged.	[106]
lung transplant patient developing pneumonia	AB-PA01 (Pa193, Pa204, Pa222, and Pa223)	4×10^9	i.v.	Clinical cure	[107]
Probable aortic graft infection and recurrent bacteremia	PPM2 (3 phages)	2.6×10^6	i.v.	When administering phage treatment and ciprofloxacin, blood cultures reported negative.	[108]
Infection from a ventricular assisted device	APIs (PNM, 14/1, PT07)	1.9×10^7	i.v. and direct application	Clinical cure	[109]
Pneumonia and empyema	AB-PA01 (Pa 193, Pa 204, Pa 222, Pa 223)	1×10^9	i.v. and nebulized	Clinical cure	[110]
Periprosthetic joint infection	PP1450, PP1777, and PP1792	3×10^{10}	Direct application	Resolution	[111]
Osteomyelitis	1777, 1792, 1797, 1450	$(1.2–9.7) \times 10^8$	Direct application	Recovered.	[112]
Cardiothoracic surgery	PA5, PA10	4×10^{10}	locally	Recovery was achieved	[113]
Urinary tract	Bacteriophage cocktail BFC1	50 mL of BFC1 bacteriophage cocktail	i.v.	The wound recovered.	[114]

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 March 2026 doi:10.20944/preprints202603.0722.v1

	PNMbacteriophages, Bacteriophage 14/1				

2.4. Bacterial Resistance Mechanisms Against Phages

Bacteria have developed various resistant mechanisms to lytic phages, by changing or losing of phage binding receptors or by integrating phage DNA into the CRISPR/Cas system of prokaryotic immune mechanism that targets and eliminate foreign genetic material. In response, phages have also evolved likewise to bypass these defenses by recognize receptors and some even produce anti-CRISPR proteins to counteract CRISPR/Cas system of the bacteria [74,115–117].

2.5. Advantages of Phage Therapy over Antibiotics

The comparative benefits of phage therapy over conventional antibiotics in managing bacterial infections are summarized in Table .

Table 4. The main advantages of phage therapy over antibiotics.

Biofilm penetration	Phages penetrate deep biofilm layers, while antibiotics mainly affect surface bacteria.	Ref
Destroy persister cells	Phages can infect and eliminate persister cells that contribute to recurrent infections	[15,118]
Host specificity	Phages selectively target pathogenic bacteria, preserving beneficial microbiota	[119]
Reduced dosing requirement	Due to their capability to self-amplify at the site of infection, phage therapy may require fewer doses compared to repeated antibiotic administration.	[120,121]
Lower incidence of side effects	Fewer adverse effects; phages are safely cleared by the immune system without toxic by-products.	[118]
Resistance considerations	Phages can be tailored to target antibiotic-resistant strains, offering a promising alternative to conventional antibiotics.	[89,122]
Minimal impact on the environment	Phages are naturally occurring particles of the environment.	[123,124]

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 March 2026 doi:10.20944/preprints202603.0722.v1

comparatively low costs of manufacturing and discovery	The expenses required in the process of discovering phage and purification are minimal.	[123,124]

2.6. Challenges and Limitations of Phage Therapy

The major limitation of phage therapy includes lack of well-established pharmacokinetic data, susceptible to degradation by human metabolism and the immune system, with some pharmacokinetic research indicating that a substantial portion of phage administrations are cleared within 36 hours, and the effective concentration is diluted by bodily fluids [125,126]. Moreover, Policies and regulations governing the clinical use of phage therapy are also not available, which can delay awareness and opportunities for this promising treatment. Furthermore, the absence of clear standards for phage isolation and purification leads to variable efficiency in isolated phage preparations[127].The overall challenges and Proposed solutions for phage therapy are summarized in the Table .

Table 5. The Challenge and proposed Solutions for Phage Therapy.

Challenge	**Proposed solutions**	**Ref.**
Pharmacokinetics	✓ Encapsulation of phages in nanoparticles or hydrogels to improve stability, circulation time, and targeted delivery ✓ Genetic engineering of phages to modify surface properties and enhance pharmacokinetic profiles.	[89,125,126]
Bacterial resistance	✓ Combination therapy using phages and antibiotics to prevent/delay resistance development. ✓ Exploration of phage-derived enzymes like endolysin to overcome resistance mechanisms.	[89,125,126]
Phage specificity	✓ Development of phage cocktails or “phage libraries” targeting a wider range of bacterial strains/species. ✓ Utilization of engineered or synthetic phages with expanded host ranges.	[89,126]

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 March 2026 doi:10.20944/preprints202603.0722.v1

Regulator and manufacturing	✓ Establishment of standardized guidelines and regulatory frameworks for phage-based therapeutics. ✓ Optimization of large-scale phage production and purification methods to ensure consistent quality and safety.	[89,126]
Immune response and safety	✓ Screening and selection of phages with low immunogenicity ✓ Exploration of methods for regulating the immunological response, such as encapsulation or use of immunosuppressive agents	[89,126]

3. Future Perspective of Phage Therapy

Phage therapy has gained interest as a potential substitute for traditional antibiotics due to the rising incidence of bacterial infections that are resistant to several drugs. Recent advances in phage genetic manipulation employing CRISPR-based technologies allow the removal of lysogenic or virulence-associated genes, resulting in the generation of lytic phages with extended host range and enhanced bactericidal efficiency. Furthermore, phage stability, pharmacokinetics, and site-specific targeting have been enhanced by advancements in delivery technologies, including encapsulation methods, inhalable aerosols for pulmonary infections, and biofilm-penetrating formulations. In order to improve dosage requirement, delivery method, treatment combinations, as well as to establish clear regulatory pathways that guarantee safety, efficacy, and quality, further research and rigorous clinical evaluation are needed. Phage therapy could play a significant role in infection control plans with further scientific advancements and regulatory developments, providing a viable solution for the growing worldwide problem posed by antimicrobial-resistant *P. aeruginosa* infections. The continuous collaboration of various fields, including clinical medicine, molecular biology, synthetic biology, pharmacology and microbiology will be crucial to the development and advancement of phage-based treatments.

4. Conclusion

AMR has become a significant public health concern of the modern era. The continuous rise in resistance has led to limited options for treatment and increases the morbidity and mortality rates. *P. aeruginosa* is one of the leading causes of hospital-acquired infections. The ineffectiveness of antibiotics that were once successful against *P. aeruginosa* indicates the urgent need for novel and more effective strategies to treat infections caused by MDR *P. aeruginosa*. Phage therapy has demonstrated potential in the treatment of *P. aeruginosa* infections in both humans and animals. Since antibiotic resistance remains a major threat to global health, further research, investment, and development in phage therapy are strongly recommended to enhance its effectiveness, ensure safety, and expand its role in improving future healthcare outcomes.

Data availability statement: Not applicable.

Conflicts of Interests: The authors declare no conflict of interest.

Funding: This research received no external funding.

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 March 2026 doi:10.20944/preprints202603.0722.v1

Abbreviations

AMR – Antimicrobial resistance
MDR - Multidrug-resistant
XDR-Extensive Drug Resistance
WHO - World Health Organization
UTI - Hospital-acquired urinary tract infections
ICU - Intensive care units
PDR- Pandrug-Resistant

References

1. Karruli, A., et al., *Evidence-based treatment of Pseudomonas aeruginosa infections: a critical reappraisal.* Antibiotics, 2023. **12**(2): p. 399.
2. Diggle, S.P. and M. Whiteley, *Microbe Profile: Pseudomonas aeruginosa: opportunistic pathogen and lab rat.* Microbiology, 2020. **166**(1): p. 30-33.
3. Amankwah, S., et al., *Assessment of phage-mediated inhibition and removal of multidrug-resistant Pseudomonas aeruginosa biofilm on medical implants.* Infection and Drug Resistance, 2022: p. 2797-2811.
4. Organization, W.H., *WHO publishes list of bacteria for which new antibiotics are urgently needed.* 2017.
5. Miller, W.R. and C.A. Arias, *ESKAPE pathogens: antimicrobial resistance, epidemiology, clinical impact and therapeutics.* Nature Reviews Microbiology, 2024. **22**(10): p. 598-616.
6. Sathe, N., et al., *Pseudomonas aeruginosa: Infections and novel approaches to treatment "Knowing the enemy" the threat of Pseudomonas aeruginosa and exploring novel approaches to treatment.* Infectious medicine, 2023. **2**(3): p. 178-194.
7. Li, N., et al., *Characterization of phage resistance and their impacts on bacterial fitness in Pseudomonas aeruginosa.* Microbiology spectrum, 2022. **10**(5): p. e02072-22.
8. Qin, S., et al., *Pseudomonas aeruginosa: pathogenesis, virulence factors, antibiotic resistance, interaction with host, technology advances and emerging therapeutics.* Signal transduction and targeted therapy, 2022. **7**(1): p. 199.
9. Reyes, J., et al., *Global epidemiology and clinical outcomes of carbapenem-resistant Pseudomonas aeruginosa and associated carbapenemases (POP): a prospective cohort study.* The Lancet Microbe, 2023. **4**(3): p. e159-e170.
10. Hailemichael, E.B., et al., *Prevalence, microbiological profiles, and determinants of hospital-acquired pneumonia in Addis Ababa: A focus on Pseudomonas aeruginosa and its antimicrobial resistance patterns in three hospitals.* PloS one, 2026. **21**(1): p. e0340680.
11. Karlowsky, J.A., et al., *Carbapenem-resistant Enterobacterales and Pseudomonas aeruginosa causing infection in Africa and the Middle East: a surveillance study from the ATLAS programme (2018–20).* JAC-Antimicrobial Resistance, 2022. **4**(3): p. dlac060.
12. Vincent, J.-L., et al., *Prevalence and outcomes of infection among patients in intensive care units in 2017.* Jama, 2020. **323**(15): p. 1478-1488.
13. Reynolds, D. and M. Kollef, *The Epidemiology and Pathogenesis and Treatment of Pseudomonas aeruginosa Infections: An Update.* Drugs, 2021. **81**(18): p. 2117-2131.
14. Salsgiver, E.L., et al., *Changing epidemiology of the respiratory bacteriology of patients with cystic fibrosis.* Chest, 2016. **149**(2): p. 390-400.
15. Chegini, Z., et al., *Bacteriophage therapy against Pseudomonas aeruginosa biofilms: a review.* Annals of clinical microbiology and antimicrobials, 2020. **19**(1): p. 45.
16. Blomquist, K.C. and D.E. Nix, *A critical evaluation of newer β-lactam antibiotics for treatment of Pseudomonas aeruginosa infections.* Annals of Pharmacotherapy, 2021. **55**(8): p. 1010-1024.
17. O'Neill, J., *Tackling drug-resistant infections globally: final report and recommendations.* 2016.
18. Aslam, B., et al., *Antibiotic resistance: a rundown of a global crisis.* Infection and drug resistance, 2018: p. 1645-1658.
19. O'Neill, J., *Antimicrobial resistance: tackling a crisis for the health and wealth of nations/the Review on Antimicrobial Resistance chaired by Jim O'Neill.* Wellcome Collection, 2014.

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 March 2026 doi:10.20944/preprints202603.0722.v1

20. Bekele, T., et al., *Pseudomonas aeruginosa isolates and their antimicrobial susceptibility pattern among catheterized patients at Jimma University Teaching Hospital, Jimma, Ethiopia.* BMC research notes, 2015. **8**(1): p. 488.
21. Chokshi, A., et al., *Global contributors to antibiotic resistance.* Journal of global infectious diseases, 2019. **11**(1): p. 36-42.
22. Rezk, N., et al., *Bacteriophage as a potential therapy to control antibiotic-resistant Pseudomonas aeruginosa infection through topical application onto a full-thickness wound in a rat model.* Journal of Genetic Engineering and Biotechnology, 2022. **20**(1): p. 133.
23. Pang, Z., et al., *Antibiotic resistance in Pseudomonas aeruginosa: mechanisms and alternative therapeutic strategies.* Biotechnology advances, 2019. **37**(1): p. 177-192.
24. Asmare, Z., et al., *Antimicrobial resistance profile of Pseudomonas aeruginosa clinical isolates from healthcare-associated infections in Ethiopia: A systematic review and meta-analysis.* PLoS One, 2024. **19**(8): p. e0308946.
25. Horcajada, J.P., et al., *Epidemiology and treatment of multidrug-resistant and extensively drug-resistant Pseudomonas aeruginosa infections.* Clinical microbiology reviews, 2019. **32**(4): p. 10.1128/cmr. 00031-19.
26. Ramatla, T., et al., *Global prevalence and antibiotic resistance profiles of carbapenem-resistant Pseudomonas aeruginosa reported from 2014 to 2024: A systematic review and meta-analysis.* Frontiers in microbiology, 2025. **16**: p. 1599070.
27. Ahmadi, M.H., Z.F. Aghmiyuni, and S. Bakhti, *Worldwide threatening prevalence of carbapenem-resistant Pseudomonas aeruginosa.* Epidemiology & Infection, 2025. **153**: p. e114.
28. Panahi, T., L. Asadpour, and N. Ranji, *Distribution of aminoglycoside resistance genes in clinical isolates of Pseudomonas aeruginosa in north of Iran.* Gene Reports, 2020. **21**: p. 100929.
29. Rahimzadeh, M., et al., *Antibiotic resistance and genetic diversity among Pseudomonas aeruginosa isolated from urinary tract infections in Iran.* Future Microbiology, 2023. **18**(16): p. 1171-1183.
30. Kunz Coyne, A.J., et al., *Therapeutic strategies for emerging multidrug-resistant Pseudomonas aeruginosa.* Infectious diseases and therapy, 2022. **11**(2): p. 661-682.
31. Moubareck, C.A., et al., *Role of outer membrane permeability, efflux mechanism, and carbapenemases in carbapenem-nonsusceptible Pseudomonas aeruginosa from Dubai hospitals: Results of the first cross-sectional survey.* International Journal of Infectious Diseases, 2019. **84**: p. 143-150.
32. Breidenstein, E.B., C. de la Fuente-Núñez, and R.E. Hancock, *Pseudomonas aeruginosa: all roads lead to resistance.* Trends in microbiology, 2011. **19**(8): p. 419-426.
33. Haidar, A., et al., *Biofilm formation and antibiotic resistance in Pseudomonas aeruginosa.* The Microbe, 2024. **3**: p. 100078.
34. Hu, M. and S.L. Chua, *Antibiotic-Resistant Pseudomonas aeruginosa: Current Challenges and Emerging Alternative Therapies.* Microorganisms, 2025. **13**(4): p. 913.
35. Glen, K.A. and I.L. Lamont, *β-lactam resistance in Pseudomonas aeruginosa: current status, future prospects.* Pathogens, 2021. **10**(12): p. 1638.
36. Yano, H., et al., *Nationwide genome surveillance of carbapenem-resistant Pseudomonas aeruginosa in Japan.* Antimicrobial Agents and Chemotherapy, 2024. **68**(5): p. e01669-23.
37. Hassan, K.A., et al., *Pacing across the membrane: the novel PACE family of efflux pumps is widespread in Gram-negative pathogens.* Research in microbiology, 2018. **169**(7-8): p. 450-454.
38. Lambert, P., *Mechanisms of antibiotic resistance in Pseudomonas aeruginosa.* Journal of the royal society of medicine, 2002. **95**(Suppl 41): p. 22.
39. Giovagnorio, F., et al., *Resistance in Pseudomonas aeruginosa: a narrative review of antibiogram interpretation and emerging treatments.* Antibiotics, 2023. **12**(11): p. 1621.
40. Pasqua, M., et al., *Host-bacterial pathogen communication: the wily role of the multidrug efflux pumps of the MFS family.* Frontiers in Molecular Biosciences, 2021. **8**: p. 723274.
41. Lorusso, A.B., et al., *Role of Efflux Pumps on Antimicrobial Resistance in Pseudomonas aeruginosa.* Int J Mol Sci, 2022. **23**(24).
42. He, G.-X., et al., *EmmdR, a new member of the MATE family of multidrug transporters, extrudes quinolones from Enterobacter cloacae.* Archives of microbiology, 2011. **193**(10): p. 759-765.
43. Huang, L., et al., *Bacterial multidrug efflux pumps at the frontline of antimicrobial resistance: an overview.* Antibiotics, 2022. **11**(4): p. 520.

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 March 2026 doi:10.20944/preprints202603.0722.v1

44. Pina-Sanchez, M., M. Rua, and J.L. Del Pozo, *Present and future of resistance in Pseudomonas aeruginosa: implications for treatment.* Revista Española De Quimioterapia, 2023. **36**(Suppl 1): p. 54.
45. Erdmann, M.B., P.P. Gardner, and I.L. Lamont, *The PitA protein contributes to colistin susceptibility in Pseudomonas aeruginosa.* PLoS One, 2023. **18**(10): p. e0292818.
46. Tierney, A.R. and P.N. Rather, *Roles of two-component regulatory systems in antibiotic resistance.* Future microbiology, 2019. **14**(6): p. 533-552.
47. Gómez Álvarez, C.A., et al., *Resistance mechanisms in Pseudomonas aeruginosa: understanding a dangerous enemy.* Revista de la Facultad de Medicina, 2005. **53**(1): p. 27-34.
48. Flores-Velázquez, V.J. and P. Rocío, *Pseudomonas aeruginosa: Mechanisms of resistance to antibiotics and case analysis.* GSC Biol. Pharm. Sci, 2021. **14**: p. 179-188.
49. Villa, T.G., et al., *Horizontal gene transfer in bacteria, an overview of the mechanisms involved.* Horizontal gene transfer: Breaking borders between living kingdoms, 2019: p. 3-76.
50. Khedkar, S., et al., *Landscape of mobile genetic elements and their antibiotic resistance cargo in prokaryotic genomes.* Nucleic acids research, 2022. **50**(6): p. 3155-3168.
51. Motta, S.S., P. Cluzel, and M. Aldana, *Adaptive resistance in bacteria requires epigenetic inheritance, genetic noise, and cost of efflux pumps.* PloS one, 2015. **10**(3): p. e0118464.
52. Organization, W.H. and W.H. Organization, *Antibiotic resistance. 2020*. 2020.
53. Yang, J., J.-F. Xu, and S. Liang, *Antibiotic resistance in Pseudomonas aeruginosa: mechanisms and emerging treatment.* Critical Reviews in Microbiology, 2025. **51**(5): p. 841-859.
54. Kanematsu, H. and D.M. Barry, *Fundamentals for biofilms*, in *Formation and control of biofilm in various environments*. 2020, Springer. p. 13-37.
55. Mah, T.-F.C. and G.A. O'Toole, *Mechanisms of biofilm resistance to antimicrobial agents.* Trends in microbiology, 2001. **9**(1): p. 34-39.
56. Sousa, A.M. and M.O. Pereira, *Pseudomonas aeruginosa diversification during infection development in cystic fibrosis lungs—a review.* Pathogens, 2014. **3**(3): p. 680-703.
57. Tuon, F.F., et al., *Pathogenesis of the Pseudomonas aeruginosa biofilm: a review.* Pathogens, 2022. **11**(3): p. 300.
58. Song, S. and T.K. Wood, *Are we really studying persister cells?* Environmental Microbiology Reports, 2021. **13**(1): p. 3-7.
59. Van den Bergh, B., M. Fauvart, and J. Michiels, *Formation, physiology, ecology, evolution and clinical importance of bacterial persisters.* FEMS microbiology reviews, 2017. **41**(3): p. 219-251.
60. Jacoby, G.A., *AmpC β-lactamases.* Clinical microbiology reviews, 2009. **22**(1): p. 161-182.
61. Mack, A.R., et al., *β-Lactamase diversity in Pseudomonas aeruginosa.* Antimicrobial Agents and Chemotherapy, 2025. **69**(3): p. e00785-24.
62. Yoon, E.-J. and S.H. Jeong, *Mobile carbapenemase genes in Pseudomonas aeruginosa.* Frontiers in microbiology, 2021. **12**: p. 614058.
63. Tenover, F.C., D.P. Nicolau, and C.M. Gill, *Carbapenemase-producing Pseudomonas aeruginosa–an emerging challenge.* Emerging microbes & infections, 2022. **11**(1): p. 811-814.
64. Di Pilato, V., et al., *Identification of a novel plasmid lineage associated with the dissemination of metallo-β-lactamase genes among pseudomonads.* Frontiers in Microbiology, 2019. **10**: p. 1504.
65. Huang, J., et al., *Regulating polymyxin resistance in Gram-negative bacteria: roles of two-component systems PhoPQ and PmrAB.* Future microbiology, 2020. **15**(6): p. 445-459.
66. Raetz, C.R. and C. Whitfield, *Lipopolysaccharide endotoxins.* Annual review of biochemistry, 2002. **71**(1): p. 635-700.
67. Raetz, C.R., *Biosynthesis of lipid A*, in *Bacterial endotoxic lipopolysaccharides*. 2024, CRC Press. p. 67-80.
68. Varadarajan, A.R., et al., *An integrated model system to gain mechanistic insights into biofilm-associated antimicrobial resistance in Pseudomonas aeruginosa MPAO1.* npj Biofilms and Microbiomes, 2020. **6**(1): p. 46.
69. Cayci, Y.T., A. Coban, and M. Gunaydin, *Investigation of plasmid-mediated quinolone resistance in Pseudomonas aeruginosa clinical isolates.* Indian journal of medical microbiology, 2014. **32**(3): p. 285-289.
70. Wang, C., J. Wang, and Z. Mi, *Pseudomonas aeruginosa producing VIM-2 metallo-β-lactamases and carrying two aminoglycoside-modifying enzymes in China.* Journal of Hospital Infection, 2006. **62**(4): p. 522-524.

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 March 2026 doi:10.20944/preprints202603.0722.v1

71. Kakasis, A. and G. Panitsa, *Bacteriophage therapy as an alternative treatment for human infections. A comprehensive review.* International journal of antimicrobial agents, 2019. **53**(1): p. 16-21.
72. Brives, C. and J. Pourraz, *Phage therapy as a potential solution in the fight against AMR: obstacles and possible futures.* Palgrave Communications, 2020. **6**(1): p. 1-11.
73. Gordillo Altamirano, F.L. and J.J. Barr, *Phage therapy in the postantibiotic era.* Clinical microbiology reviews, 2019. **32**(2): p. 10.1128/cmr. 00066-18.
74. Lin, D.M., B. Koskella, and H.C. Lin, *Phage therapy: An alternative to antibiotics in the age of multi-drug resistance.* World journal of gastrointestinal pharmacology and therapeutics, 2017. **8**(3): p. 162.
75. Abedon, S.T., et al., *Phage treatment of human infections.* Bacteriophage, 2011. **1**(2): p. 66-85.
76. De Smet, J., et al., *Pseudomonas predators: understanding and exploiting phage-host interactions.* Nat Rev Microbiol, 2017. **15**(9): p. 517-530.
77. Weigele, P. and E.A. Raleigh, *Biosynthesis and Function of Modified Bases in Bacteria and Their Viruses.* Chem Rev, 2016. **116**(20): p. 12655-12687.
78. Ackermann, H.-W., *5500 Phages examined in the electron microscope.* Archives of virology, 2007. **152**(2): p. 227-243.
79. Krylov, V., et al., *Phage phiKZ-The First of Giants.* Viruses, 2021. **13**(2).
80. Abuelizz, H.A., et al., *Biological Investigation of 2-Thioxo-benzo [g] quinazolines against Adenovirus Type 7 and Bacteriophage Phi X174: An In Vitro Study.* Current Issues in Molecular Biology, 2023. **45**(5): p. 3787-3800.
81. Arisaka, F., et al., *Molecular assembly and structure of the bacteriophage T4 tail.* Biophysical reviews, 2016. **8**(4): p. 385-396.
82. Rakonjac, J., et al., *Structure, biology, and applications of filamentous bacteriophages.* Cold Spring Harbor Protocols, 2024. **2024**(8): p. pdb. over107754.
83. Chase, J.D., *Structural and Biochemical Investigations of the Initiation of DNA Replication in Bacteriophage Lambda and Escherichia coli*. 2022, City University of New York.
84. Kirchberger, P.C. and H. Ochman, *Microviruses: A World Beyond phi X174.* Annual Review of Virology, 2023. **10**(1): p. 99-118.
85. Rūmnieks, J. and K. Tārs, *Protein-RNA interactions in the single-stranded RNA bacteriophages.* Virus protein and nucleoprotein complexes, 2018: p. 281-303.
86. Levanova, A.A. and M.M. Poranen, *Utilization of bacteriophage phi6 for the production of high-quality double-stranded RNA molecules.* Viruses, 2024. **16**(1): p. 166.
87. Luque, A., et al., *The missing tailed phages: prediction of small capsid candidates.* Microorganisms, 2020. **8**(12): p. 1944.
88. Dennehy, J.J. and S.T. Abedon, *Phage infection and lysis*, in *Bacteriophages: biology, technology, therapy*. 2021, Springer. p. 341-383.
89. Romero-Calle, D., et al., *Bacteriophages as alternatives to antibiotics in clinical care.* Antibiotics, 2019. **8**(3): p. 138.
90. Strathdee, S.A., et al., *Phage therapy: From biological mechanisms to future directions.* Cell, 2023. **186**(1): p. 17-31.
91. Hyman, P., *Phages for phage therapy: isolation, characterization, and host range breadth.* Pharmaceuticals, 2019. **12**(1): p. 35.
92. Malik, D.J., et al., *Formulation, stabilisation and encapsulation of bacteriophage for phage therapy.* Advances in colloid and interface science, 2017. **249**: p. 100-133.
93. Kunisch, F., et al., *Targeting Pseudomonas aeruginosa biofilm with an evolutionary trained bacteriophage cocktail exploiting phage resistance trade-offs.* Nature communications, 2024. **15**(1): p. 8572.
94. Harper, D. and M. Enright, *Bacteriophages for the treatment of Pseudomonas aeruginosa infections.* Journal of applied microbiology, 2011. **111**(1): p. 1-7.
95. Pires, D.P., et al., *Phage therapy: a step forward in the treatment of Pseudomonas aeruginosa infections.* Journal of virology, 2015. **89**(15): p. 7449-7456.
96. Fu, W., et al., *Bacteriophage cocktail for the prevention of biofilm formation by Pseudomonas aeruginosa on catheters in an in vitro model system.* Antimicrobial agents and chemotherapy, 2010. **54**(1): p. 397-404.

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 March 2026 doi:10.20944/preprints202603.0722.v1

97. Vaitekenas, A., et al., *Pseudomonas aeruginosa resistance to bacteriophages and its prevention by strategic therapeutic cocktail formulation.* Antibiotics, 2021. **10**(2): p. 145.
98. Chan, B.K., et al., *Phage treatment of an aortic graft infected with Pseudomonas aeruginosa.* Evolution, medicine, and public health, 2018. **2018**(1): p. 60-66.
99. Ashworth, E.A., et al., *Exploiting lung adaptation and phage steering to clear pan-resistant Pseudomonas aeruginosa infections in vivo.* Nature Communications, 2024. **15**(1): p. 1547.
100. Santamaría-Corral, G., et al., *Bacteriophage–antibiotic combination therapy against Pseudomonas aeruginosa.* Antibiotics, 2023. **12**(7): p. 1089.
101. Jaiswal, A., et al., *Efficacy of cocktail phage therapy in treating Vibrio cholerae infection in rabbit model.* Microbes and Infection, 2013. **15**(2): p. 152-156.
102. Gu, J., et al., *A method for generation phage cocktail with great therapeutic potential.* PloS one, 2012. **7**(3): p. e31698.
103. Shokri, D., A. Soleimani-Delfan, and S.M. Fatemi, *Assessment of phage cocktails with extended host range activity against antibiotic resistant strains of Pseudomonas aeruginosa.* Comparative Clinical Pathology, 2017. **26**(2): p. 417-422.
104. McVay, C.S., M. Velásquez, and J.A. Fralick, *Phage therapy of Pseudomonas aeruginosa infection in a mouse burn wound model.* Antimicrobial agents and chemotherapy, 2007. **51**(6): p. 1934-1938.
105. Chan, B., et al., *Phage selection restores antibiotic sensitivity in MDR Pseudomonas aeruginosa. Sci Rep 6: 26717*. 2016.
106. Li, L., et al., *First-in-human application of double-stranded RNA bacteriophage in the treatment of pulmonary Pseudomonas aeruginosa infection.* Microbial Biotechnology, 2023. **16**(4): p. 862-867.
107. Aslam, S., et al., *Early clinical experience of bacteriophage therapy in 3 lung transplant recipients.* American Journal of Transplantation, 2019. **19**(9): p. 2631-2639.
108. Aslam, S., et al. *Lessons learned from the first 10 consecutive cases of intravenous bacteriophage therapy to treat multidrug-resistant bacterial infections at a single center in the United States*. in *Open forum infectious diseases*. 2020. Oxford University Press US.
109. Tkhilaishvili, T., et al., *Successful case of adjunctive intravenous bacteriophage therapy to treat left ventricular assist device infection.* 2021.
110. Maddocks, S., et al., *Bacteriophage therapy of ventilator-associated pneumonia and empyema caused by Pseudomonas aeruginosa.* American journal of respiratory and critical care medicine, 2019. **200**(9): p. 1179-1181.
111. Ferry, T., et al., *Case report: arthroscopic "debridement antibiotics and implant retention" with local injection of personalized phage therapy to salvage a relapsing Pseudomonas aeruginosa prosthetic knee infection.* Frontiers in medicine, 2021. **8**: p. 569159.
112. Ferry, T., et al., *Innovations for the treatment of a complex bone and joint infection due to XDR Pseudomonas aeruginosa including local application of a selected cocktail of bacteriophages.* Journal of Antimicrobial Chemotherapy, 2018. **73**(10): p. 2901-2903.
113. Rubalskii, E., et al., *Bacteriophage therapy for critical infections related to cardiothoracic surgery.* Antibiotics, 2020. **9**(5): p. 232.
114. Jennes, S., et al., *Use of bacteriophages in the treatment of colistin-only-sensitive Pseudomonas aeruginosa septicaemia in a patient with acute kidney injury—a case report.* Critical Care, 2017. **21**(1): p. 129.
115. Labrie, S.J., J.E. Samson, and S. Moineau, *Bacteriophage resistance mechanisms.* Nature Reviews Microbiology, 2010. **8**(5): p. 317-327.
116. Barrangou, R. and L.A. Marraffini, *CRISPR-Cas systems: prokaryotes upgrade to adaptive immunity.* Molecular cell, 2014. **54**(2): p. 234-244.
117. Bondy-Denomy, J., et al., *Bacteriophage genes that inactivate the CRISPR/Cas bacterial immune system.* Nature, 2013. **493**(7432): p. 429-432.
118. Pires, D.P., et al., *Genetically engineered phages: a review of advances over the last decade.* Microbiology and Molecular Biology Reviews, 2016. **80**(3): p. 523-543.
119. Wittebole, X., S. De Roock, and S.M. Opal, *A historical overview of bacteriophage therapy as an alternative to antibiotics for the treatment of bacterial pathogens.* Virulence, 2014. **5**(1): p. 226-235.

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 March 2026 doi:10.20944/preprints202603.0722.v1

120. Grabowski, Ł., et al., *Highly different effects of phage therapy and antibiotic therapy on immunological responses of chickens infected with Salmonella enterica serovar Typhimurium.* Frontiers in immunology, 2022. **13**: p. 956833.
121. Secor, P.R. and A.A. Dandekar, *More than simple parasites: the sociobiology of bacteriophages and their bacterial hosts.* Mbio, 2020. **11**(2): p. 10.1128/mbio. 00041-20.
122. Abdelkader, K., et al., *The preclinical and clinical progress of bacteriophages and their lytic enzymes: the parts are easier than the whole.* Viruses, 2019. **11**(2): p. 96.
123. Principi, N., E. Silvestri, and S. Esposito, *Advantages and limitations of bacteriophages for the treatment of bacterial infections.* Frontiers in pharmacology, 2019. **10**: p. 457104.
124. Silva, C., et al., *The history and applications of phage therapy in Pseudomonas aeruginosa.* Microbiology Research, 2021. **13**(1): p. 14-37.
125. Lin, J., et al., *Limitations of phage therapy and corresponding optimization strategies: a review.* Molecules, 2022. **27**(6): p. 1857.
126. Caflisch, K.M., G.A. Suh, and R. Patel, *Biological challenges of phage therapy and proposed solutions: a literature review.* Expert review of anti-infective therapy, 2019. **17**(12): p. 1011-1041.
127. Fauconnier, A., *Phage therapy regulation: from night to dawn.* Viruses, 2019. **11**(4): p. 352.

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.