Phage infection reinstates antibiotic sensitivity in MDR *Pseudomonas aeruginosa:* A study on phage and bacterial evolution

Ph.D. Thesis

Sarshad Koderi Valappil

Supervisor:
Dr. Gábor Rákhely, PhD
Associate Professor,
Head of Department of Biotechnology
University of Szeged

Doctoral School of Biology

Department of Biotechnology
Faculty of Science and Informatics
University of Szeged
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1.0. ABBREVIATIONS

Abi	Abortive infection
BREX	Bacteriophage exclusion system
BSI	Blood stream infection
CDC	Centers for Disease Control and Prevention
CF	Cystic fibrosis
CFTR	CF transmembrane conductance regulator
cfu	Colony forming unit
CLSM	Confocal light scanning microscopy
CRISPR	Clustered regularly interspaced short palindromic repeat
cUTIs	Complicated urinary tract infection
DISARM	Defense island system associated with restriction modification
E-PIAS	Evolved PIAS phage
ECDC	European Centre for Disease Prevention and Control
EPS	Extracellular polymeric substances
ESBLs	Extended spectrum-β-lactamases
HAUTIs	Healthcare-associated UTIs
HCAIs	Healthcare-associated infections
ICU	Intensive care unit
LPS	Lipopolysaccharide
MATE	Multidrug and toxic compound extrusion
MBLs	Metallo beta-lactamases
MDR	Multidrug resistance
MFS	Major facilitator superfamily
MIC	Minimum inhibitory concentration
MOI	Mutiplicity of infection
OE	Otitis externa
OMVs	Outer membrane vehicles
PBS	Phosphate-buffered saline
PEG	Polyethylene glycol
pfu	Plague forming unit
PICI	Phage inducible chromosomal island
RBP	Receptor-binding protein

RM	Restriction-modification
RND	Resistance-nodulation-division
Sies	Superinfection exclusions
SMR	Small multidrug resistance
SNP	Single nucleotide polymorphism
UTIs	Urinary tract infection
WHO	World Health Organization
WTA	Wall teichoic acid
XDR	Extensively drug-resistant

2.0. PREFACE

This thesis was prepared at the University of Szeged, in the Department of Biotechnology. The Department of Biotechnology first began to explore the use of phages in the agricultural sector to overcome bacterial diseases in plants. The first collaborative study was conducted on the plant pathogen *Erwinia amylovora*. In that study, PhiEaH2 phage was used to control *E. amylovora* infection in the laboratory and in field experiments (Dömötör et al., 2012; Meczker et al., 2014). Later, we isolated 24 phages against *Xanthomonas arboricola* pv. *juglandis* (Xaj), which causes diseases in walnut. This study demonstrated the first two complete Xaj phage sequences, enabling an insight into the genomics of Xaj phages (Dömötör et al., 2016). After this, the Department collaborated with a Serbian laboratory in isolating 29 phages against *Bordetella bronchiseptica*, a well-known etiological agent of kennel cough in dogs and cats (Petrovic et al., 2017). In this project, phages against *Pseudomonas aeruoginosa* were also isolated and characterized. The Department also took part in isolating lytic phage (ΦIK1) against *E. coli* strain K1, which is a common causative agent of neonatal sepsis and meningitis. Additionally, an *in vivo* phage rescue model was successfully conducted, resulting in a high level of protection against bacterial infection (Schneider et al., 2018). In 2019, the Department took part in an international collaborative project that isolated lytic phages against *Xanthomonas oryzae* pv. *oryzae*, which causes leaf blight in rice plants. The project led to 10 newly isolated phages (Kovács et al., 2019). In 2020, we collaborated on characterization of lytic phages against drug-resistant *Klebsiella pneumoniae:* we studied the efficiency of lytic phages in *in vitro* biofilm assay (Horváth et al., 2020).

The study described in this thesis was carried out between December 2015 and October 2021. The first part of the thesis is published in Frontiers in Microbiology (Koderi Valappil et al., 2021), and one is under preparation. To fulfill the Doctoral school criteria to obtain Ph.D. degree, a coauthored article (Horváth et al., 2020) published in Scientific Report was used. The Ph.D. work was supported by the Tempus Public Foundation and the European Union within the Széchenyi 2020 Programmes EFOP-3.6.1-16-2016-00008 and EFOP-3.6.2-16-2017-00010 grants.

3.0. ABSTRACT

The emergence of antibiotic resistance among bacterial pathogens is a significant public health threat affecting humans worldwide. In Europe, *Pseudomonas aeruginosa* contributes to almost 9% of overall multi-drug-resistant (MDR) infections. Alternative methods for controlling MDR pathogens have been explored for several decades. Bacteriophage therapy is one of the oldest and most efficient alternative solutions. The study described in this thesis began with the isolation and characterization of 25 MDR *P. aeruginosa* clinical strains and eigth novel lytic phages. The investigation disclosed the infection with two phage isolates, PIAS and PAPSZ1, led to the sequential appearance of phage-resistant colonies with two phenotypes (green and brown). We examined the evolutionary basis for the two types of mutants and uncovered phage mutants capable of infecting green mutants. Simultaneously we also learned that PIAS phage infected the host via the OrpM-MexXY system involved in drug efflux. Thus, the PIAS-resistant mutants decreased the minimum inhibitory concentrations (MIC) for several non-effective antibiotics. After this new insight into the evolutionary arms race between hosts and phages, we decided to use this window to comprehensively eradicate mutants by treating MDR strain with previously resistant antibiotics combined with PIAS phage. The *in vitro* study with PIAS phage-antibiotic combination completely prevented the formation and growth of mutants. We tested the same strategy in an *in vivo* rescue experiment in the mouse lung infection model, when combined with PIAS phage and fosfomycin. The combination therapy saved 75% of the animals. Later, we used PAPSZ1 phage to investigate whether phage mutants can suppress bacterial resistance. We isolated multiple PAPSZ1 mutants after a continuous infection cycle, which can block or suppress bacterial resistance and mutant formation and broaden the host specificity of the phages. Phages like PIAS and PAPSZ1 offer a unique window that can exploit to eradicate MDR bacteria. This study highlights the importance of preliminary and detailed examinations of phage-host bacterium interactions preceding the application of a given phage. The experimental data in this thesis shows that studying phage-host bacterium interactions and coevolution will help to utilize phage therapy's full potential when treating MDR infections.

4.0. INTRODUCTION

4.1 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a ubiquitous Gram-negative bacterium occurring almost everywhere in the environment. The pervasion of *P. aeruginosa* in various environments is due to its ability to colonize multiple environmental niches and to utilize countless environmental compounds as energy sources (Frimmersdorf et al., 2010). This bacterium can infect a wide range of hosts, including humans, animals and plants (Eklöf et al., 2020; Haenni et al., 2015; Walker et al., 2004). This omnipresent microbe can act as an opportunistic pathogen under several circumstances. In humans, it is one of the leading pathogens that cause infections in vulnerable patients. These infections include cystic fibrosis (CF) and obstructive pulmonary diseases in patients who have permanent bladder catheters or who have been intubated for a long period of time (Eklöf et al., 2020; Modi & Kovacs, 2020). Similarly, *P. aeruginosa* is a frequently occuring pathogen in burn wounds, diabetic foot ulcers, otitis media and keratitis.

4.2. History

The first *P. aeruginosa* infections in humans were likely reported in 1862 by Luke, who observed rod-shaped particles in the blue-green pus of an infected wound (Lyczak et al., 2000). Dr Sedillot had previously reported a similar colouration on surgical dressings. This colouration was later determined to be caused by the pigment pyocyanin produced by *P. aeruginosa* (Moradali et al., 2017). *P. aeruginosa* can grow on a wide variety of media. This kind of bacterium grows well at 37°C, and it can survive at a wide range of temperatures from 4-42°C (LaBauve & Wargo, 2012). It is also capable of producing various pigments and of forming distinct colony morphologies. Colonies are usually spread flat with a serrated edge, but other morphologies also exist, including mucoid and small colony variants. They typically have a grape-like odour, and they are capable of producing hydrosoluble pigments, such as pyocyanin (blue), pyorubin (red-brown), pyomelanin (brown-black) and pyoverdin (yellow-green or yellow-brown). In fact, the name *aeruginosa* (from Latin *aerūgō*, 'copper') stems from the bacterial colonies' greenish-blue colour, which occurs when pyocyanin and pyoverdin pigments are coproduced (Howarth And & Dedman, 1964).

4.3. Current epidemiology

After the revolutionary discovery of penicillin in 1928, and particularly after the beginning of the 1940s, western medicine encouraged the widespread use of drugs (Tan & Tatsumura, 2015). Sir Alexander Fleming himself warned that the misuse of drugs could result in antibiotic-resistant bacteria (Rosenblatt-Farrell, 2009). As he predicted, the first resistant *Mycobacterium tuberculosis* was reported in 1948 (Gillespie, 2002). Nevertheless, the discovery of antibiotics has saved millions of lives throughout history. In the twentieth-twenty first century, however, the rate of new antibiotic discoveries has decelerated, and an alarming increase in the number of antibiotic-resistant strains has apparently led to the demise of the golden age of antibiotics. Recent reports by the World Health Organisation (WHO) on the antibiotic pipeline (WHO, 2020) revealed a trend for many new drugs to be weak and ineffective compared to the last three antibiotic classes: diarylquinolines, malacidins and teixobactin (Hover et al., 2018; Ling et al., 2015; Samson, 2005). The report considered 50 antibiotics under development. The data even showed that the new class of drugs has led to the potential evolution of drug-resistant clones. In 2017, the WHO warned of a new wave of Gram-negative pathogens that are resistant to multiple antibiotics (WHO, 2018). A recent survey by the European Antimicrobial Resistance Surveillance Network (EARS-Net) raised the alarm about eight increasingly prevalent drug-resistant bacteria species in the EU (Ecdc, 2019). Another recent review (WHO, 2021) analysed 43 antibiotics currently in development and found that none of them addressed extensively drug-resistant (XDR) or MDR Gram-negative bacteria. Novel drugs targeting WHO-priority pathogens, such as carbapenem-resistant *Acinetobacter baumannii* and *P. aeruginosa*, were lacking.

Nearly all clinical cases of *P. aeruginosa* infection can be correlated with the compromise of host defenses. *P. aeruginosa* infection has acquired a special status because it has become the leading cause of morbidity and mortality in CF patients (Kosorok et al., 2001; Osmon et al., 2004; Mulcahy et al., 2010; Vicente et al., 2013). *P. aeruginosa* has also become the leading nosocomial pathogen affecting hospitalized patients (Moradali et al., 2017). It is intrinsically resistant to a wide range of antibiotics, and it has been reported as one of the most lethal causative organisms of bacteraemia. One study has shown that patients with *Pseudomonas* bloodstream infections (BSIs) had higher mortality rates than those with infections caused by the family of *Enterobacteriaceae* (Shi et al., 2019). A recent study revealed a great prevalence of *P. aeruginosa* infections in ICU (Intensive Care Unit) patients in Europe. That study showed a nearly threefold increase of *P. aeruginosa* infections compared to a 2011 study. Among the

P. aeruginosa clinical isolates, 40% and 66.7% were MDR in 2011 and 2019, respectively (Litwin et al., 2021). Beyond the striking virulence, it has intrinsically evolved resistance to 'drugs of last resort,' resulting in emergent strains that are pan-drug-resistant. The WHO's global priority pathogens list includes MDR bacteria, thus prioritizing *P. aeruginosa* as a critical worldwide threat to public health (WHO, 2018). Infections caused by MDR strains of bacteria are challenging to treat because of the strain's physical adaption to its host and its genomic plasticity (Casadevall & Pirofski, 2000; Dubern et al., 2015; Faure et al., 2018). These assets control and contribute intrinsic resistance to different classes of antimicrobials. Designing and developing new alternative solutions is the only way to stop the rising crises related to drug-resistant bacteria.

4.4. Clinical manifestations

The human body's flora is open to different types of bacteria, sometimes including *P. aeruginosa*. This natural flora is always kept in check by the immune defense system of our body. Inhabiting a clinical environment or a hospital increases the likelihood of finding the bacteria on intact skin, however. A sizeable inoculum of the bacteria can overwhelm normal defenses and lead to infection. When people with other clinical histories undergo hospitalization, *P. aeruginosa* can colonize them and lead to severe infection. The most frequent *P. aeruginosa* infections target patients with cystic fibrosis (CF). *P. aeruginosa* can also cause infections in the urinary tract, eyes, ears and skin.

4.4.1. The opportunistic infections and cystic fibrosis

With a frequency of about one in 2,500 live births, CF is the most common autosomal recessive disorder in white people (Ratjen et al., 2009). CF patients inherit genetic defects in the CF transmembrane conductance regulator (CFTR) gene, which causes them to suffer from a multisystem disease. The CFTR regulator controls and maintains habitual mucus and homeostasis in the epithelial membrane. It plays a significant role in regulating the transport of electrolytes and chloride across cell membranes. The mutation in CFTR protein results in abnormally thick, dehydrated sticky mucus layers in the lungs (Flume & van Devanter, 2012). This causes CF patients to be especially susceptible to respiratory infections. Studies have shown that newborn infants with CF have a 30% chance to acquire initial *P. aeruginosa* strains from the environment, thus leading to acute infections. This rate increases to 50% from the age of three. Mucoid phenotypes causing chronic infections are widely reported from the ages of

3–16 (Vicente et al., 2013). These infections can significantly harm CF patients, increasing morbidity and mortality (Malhotra et al., 2019). Primary infection in CF patients requires frequent antibiotic treatments to control the disease. The extensive use of antibiotics to treat *P. aeruginosa* has resulted in the formation of antibiotic-resistant strains in many patients (Litwin et al., 2021). Recently CF has become a significant focus area in current research, particularly on CF airway infections – which seeks to understand the frequent failure of antibiotics. Despite severe antibiotic therapy after diagnosis, resistance development is rare during the first years after *P. aeruginosa* infection in CF airways. The reason for this relative lack of resistance development remains a mystery, but several explanations have been offered. One common explanation cites the biofilm mode of life of *P. aeruginosa* in CF airways. This mode is usually associated with increased antibiotic tolerance.

4.1.2. Biofilm lifestyle of *P. aeruginosa* in cystic fibrosis patients

During acute infection, the relationship between *P. aeruginosa* and host can become reciprocally destructive. This destruction is mainly triggered by different virulent cytotoxic molecules, which can impair the host's cellular processes (do Vale et al., 2016; Brito et al., 2019). On the other hand, the host can respond by producing antimicrobial compounds and reactive oxygen species, as well as by implementing enhanced phagocytosis (Chaplin, 2010; Lim et al., 2017). The host's immune system can easily detect a single freely motile cell, which triggers inflammatory responses and phagocytosis by macrophages. Switching to a protective and stationary lifestyle and to lower virulence is the most widespread survival strategy of many pathogenic bacteria, including *P. aeruginosa* (Amiel et al., 2010). They choose to lose motility, attach to surfaces and form microcolonies embedded in extracellular polymeric substances (EPS) to protect themselves from the surrounding environment. These surface associated microbial aggregates are called biofilms. Biofilms have shown an extreme capacity for persistence against phagocytosis, oxidative stresses, nutrient restriction, metabolic waste accumulation, interspecies competitions and antimicrobial agents (Lee et al., 2014; Olsen, 2015; Rasamiravaka et al., 2015; Wolska et al., 2016; Thi et al., 2020). The biofilm mode of life is a vital means of ensuring the persistence of *P. aeruginosa* during the long-term colonization of CF respiratory tracts (Ciofu, et al., 2010; Chadha, 2014; Høiby et al., 2017; Thi et al., 2020). Studies have also demonstrated that *P. aeruginosa* increases the expression of genes involved in the production of alginate, exopolysaccharide and other biofilm determinants in CF airways (Ciofu et al., 2015; Kordes et al., 2019). This finding aligns with numerous direct

observations from different biopsies, which have shown that *P. aeruginosa* grows in small aggregates resembling biofilms (Høiby et al., 2017; Wu et al., 2019). An MDR *P. aeruginosa* in cystic fibrosis patients can grow within chronic biofilm lung infections, making it extremely challenging to eliminate. Failure to eradicate the infection can be fatal.

4.1.3. Biofilm-specific antibiotic tolerance and resistance

Biofilm is a complex microbiome structure, sometimes featuring multispecies strains or a single type of cells encased in a polysaccharide matrix attached to a surface. This multifaceted structure is highly hydrated. It is comprised 97% of water, together with polysaccharides, proteins, lipids, nucleic acids and insoluble components such as amyloids, fimbriae, pili and flagella (Flemming et al., 2016). Biofilm development is a regulated multistep process beginning with cell adhesion, EPS production and the detachment of microorganisms from the mature biofilm (Fig. 1) (Grande et al., 2020). Infection-caused biofilm is more resistant to antibiotics than planktonic bacteria (Høiby, Bjarnsholt, et al., 2010; Høiby, Ciofu, et al., 2010; Ciofu et al., 2015; Grande et al., 2020; Olivares et al., 2020). For instance, biofilms can tolerate antimicrobial agents at concentrations 10–1,000 times higher than those that are needed to deactivate genetically equivalent planktonic bacteria of the same number (Chadha, 2014; Donlan & Costerton, 2002). One of the common barriers of biofilm begins with its impermeable matrix. Positively charged antibiotics – such as aminoglycosides and polypeptides, which can bind to a negatively charged biofilm matrix – can delay the penetration through biofilm (Olsen, 2015). The EPS matrix can also protect bacteria from antibiotics by decreasing permeability in bactericidal concentrations, thereby promoting resistance (Fig. 2) (Pinto et al., 2020). The nature of biofilm structures and other physiological changes, such as a slow growth rate, helps them to resist antimicrobial agents (Chadha, 2014; Donlan & Costerton, 2002).

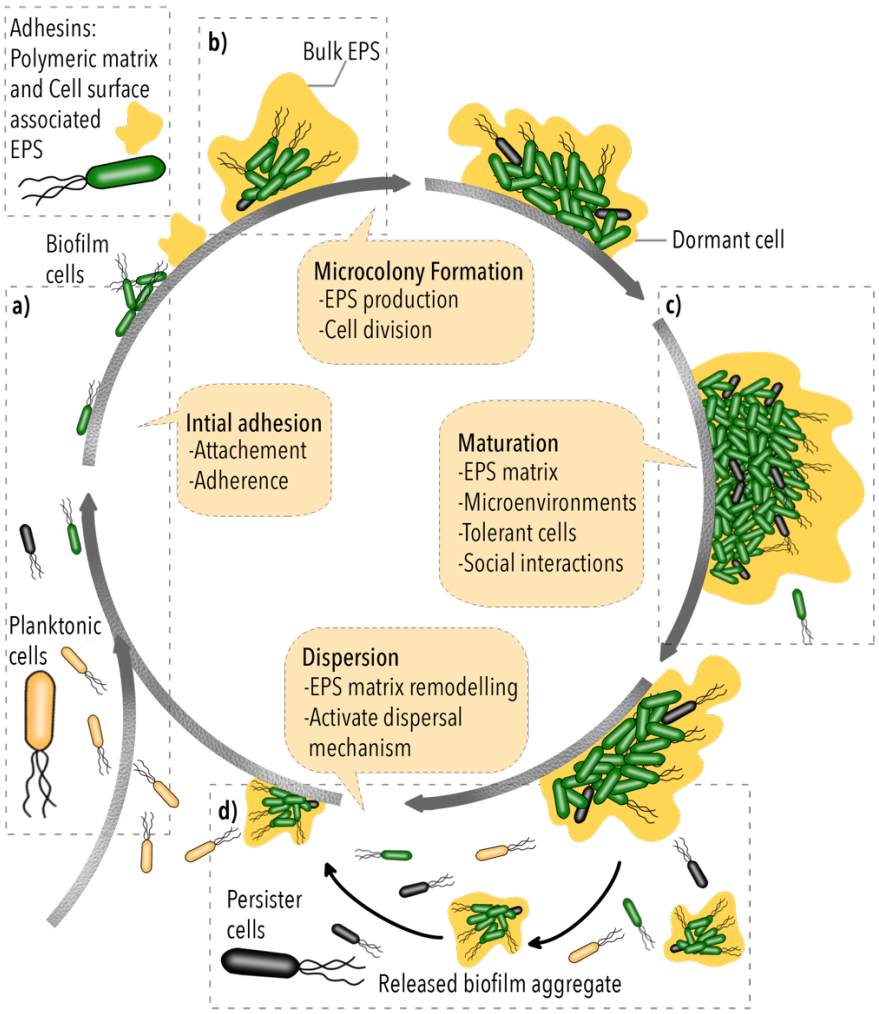


Figure 1. Stages of biofilm formation. a) Initial adhesion: *P. aeruginosa* adheres to a host or any surfaces through cell-surface-associated adhesins. b) Early biofilm formation: *P. aeruginosa* begins to divide and produce EPS, enhancing adhesion while forming the matrix that embeds the cells. c) Biofilm maturation: whereby 3D biofilm structures are developed in which the EPS matrix provides a multifunctional and protective scaffold. d) Dispersal: whereby cells leave the biofilm to re-enter the planktonic phase. The figure concept was adapted from a published article (Koo et al., 2017) and redrawn by the author.

Antibiotic resistance can generally be defined as an increase in the minimum inhibitory concentration (MIC) value of an antibiotic due to a change in the bacteria caused by genomic modification or to resistance acquired through horizontal gene transfer. These resistance mechanisms are further reinforced by additional factors, such as a polymeric matrix that can restrict diffusion; the ability of biofilm to alter metabolic activity inside the biofilm by slowing the growth rate; and masking or hiding the target sites (Fig. 2). The density of bacteria in a biofilm can also play a critical role in spreading antibiotic resistance. In a dense population, one of the most common mechanisms of virulence exchange takes place via horizontal transfer of

resistance genes (Abe et al., 2021). When the microorganisms within the matrix are too dense, due to close contacts, the resistant genes throughout the flora can be easily exchanged. This multicellular environment can also promote the transfer of conjugative plasmids due to the close proximity of cells (Lécuyer et al., 2018). Overall antibiotic resistance in biofilm depends on two main factors: physiological and gene-based makeover (Ciofu et al., 2015). Combining these two factors plays a crucial role in biofilm maturity and drug tolerance, which imposes a great challenge for the use of conventional antimicrobials. To conclude, bacterial biofilm plays a vital part in the development of antimicrobial resistance.

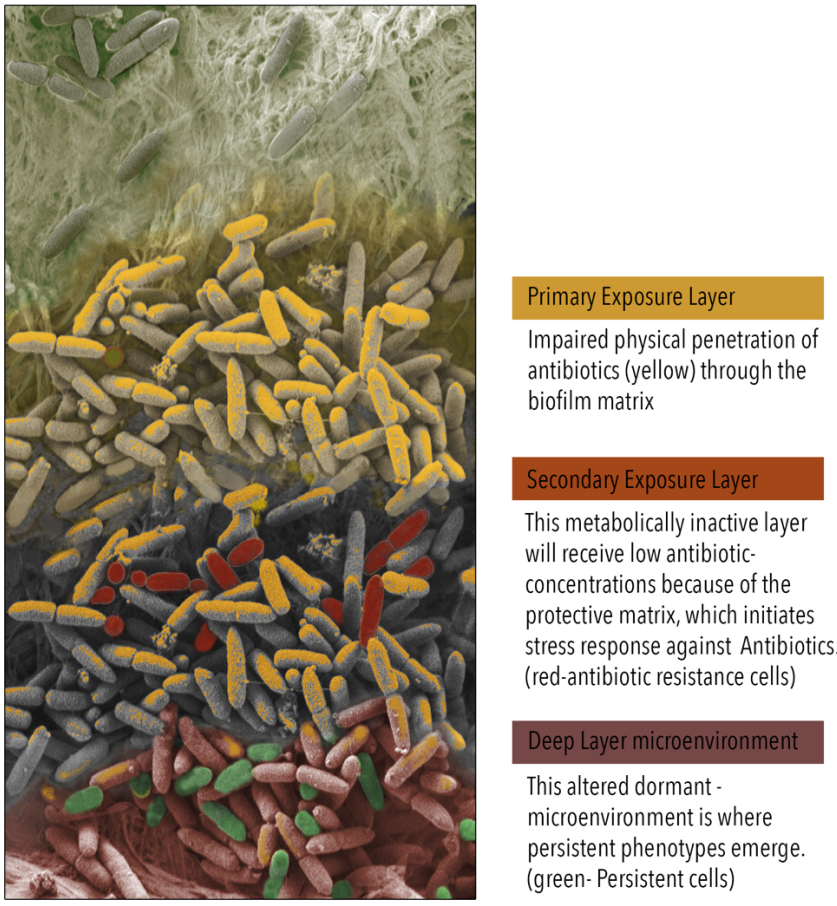


Figure 2. Mechanism of the antibiotic resistance in different layers of biofilm. The figure concept was adapted from a published article (Stewart & William, 2001) and redrawn by the author.

4.1.4. *P. aeruginosa* bloodstream infection or bacteraemia

P. aeruginosa is one of the common nosocomial infections that contributes to a higher mortality rate in immunocompromised patients (Cheol-In et al., 2003; Thaden et al., 2017). Recent studies have reported that BSI also contributes to this event (Fabre et al., n.d.; Pilmis et al., 2020). BSI is defined as the presence of the microorganism in a blood culture, along with clinical evidence of infection. The clinical outcomes of BSI are strongly associated with multiple factors, such as the host's underlying diseases or conditions, the severity of the infection and antibiotic appropriateness (Pilmis et al., 2020). BSI caused by *P. aeruginosa* has shown a higher mortality rate than an infection caused by other dominant MDR bacteria. In haematological patients, *P. aeruginosa* BSI has also been reported to represent an increased risk of death compared to other Gram-negative bacteria (Cattaneo et al., 2012).

Infections caused by *P. aeruginosa* are typically difficult to treat due to intrinsic antibiotic resistance and to the strain's remarkable ability to acquire resistance to multiple groups of antimicrobial agents. BSIs are often reported in ICU patients (Shi et al., 2019). This is primarily due to prolonged hospitalization, invasive medical procedures and the long-term use of antibiotics. In most cases, a BSI is a secondary infection that surfaces after a primary infection in areas like the respiratory or the urinary tract (Cheol-In et al., 2003; Enoch et al., 2013).

4.1.5. Other *P. aeruginosa* infections

4.1.5.1. Urinary tract infection

Urinary tract infections (UTIs) are among the most common kinds of bacterial infection. They cause severe health problems in millions of people each year (Tandogdu et al., 2014; Wagenlehner et al., 2020). UTIs are the second most prevalent form of infection in the human body. The last European point-prevalence survey of healthcare-associated infections (HCAIs) in acute care hospitals estimated that 3% of patients admitted to the ICU for more than two days were reported to have UTIs (ECDC, 2019). *P. aeruginosa* UTIs are frequently linked to healthcare-associated UTIs (HAUTIs) (Wagenlehner et al., 2020). HAUTIs are typically caused by the catheterization of the urinary tract (Johansen et al., 2006). They occur mainly in immunocompromised patients or in patients with structural or functional urinary tract abnormalities. A recent cohort study has shown that *P. aeruginosa* complicated UTIs were more frequently polymicrobial than UTIs caused by other microorganisms (Gomila et al., 2018).

4.1.5.2. Eye and ear infection

P. aeruginosa is one of the most common bacterial infections associated with contaminated contact lens solutions (Hue et al., 2009; Pachigolla et al., 2007; Kugadas et al., 2016; Reedy & Wood, 2000). The ability of *P. aeruginosa* to adhere to contact lens surfaces increases the chance of infection. In addition, *P. aeruginosa* can form biofilm on contact lenses within a short period of time (Boyle et al., 2001).

There are two major ear inflammations caused by *P. aeruginosa* infections, namely perichondritis and otitis externa (OE). Perichondritis is triggered by the wear and tear of ear ornaments or piercings (Sandhu et al., 2017; Sosin et al., 2015). The inflammation begins with the swelling of the pinna, which becomes red and tender. The infection progresses to necrosis of the cartilage. Perichondritis of the auricle due to *P. aeruginosa* infections has become more frequent (Klug et al., 2019). OE is an inflammatory process of the external auditory canal that primarily affects children, persons with diabetes and immunocompromised patients.

4.1.5.3. Soft tissue infection

There are many different types of soft tissue infections caused by *P. aeruginosa*. These include folliculitis, puncture wounds, osteomyelitis, necrotizing fasciitis, green nail syndrome and burn wound infections. Of these, the most common are burn wound infections and the green nail syndrome.

4.1.5.4. Green nail syndrome

Green nail syndrome (chromonychia) is a nail disorder characterized by onycholysis and the green-black discolouration of the nailbed. This condition is often associated with chronic paronychia (Klug et al., 2019). *P. aeruginosa* is the most frequently identified organism in the affected area. Despite the various treatment options featuring essential antipseudomonal drugs, it is still necessary to remove the nail in many cases (Bae et al., 2014; Müller et al., 2014).

4.1.5.5. Burn infection

Burns are one of the most common and devastating forms of trauma. Patients with severe thermal injuries require immediate specialized care. Burn wound sepsis is currently the leading cause of morbidity and mortality after burn trauma (Church et al., 2006). Burns can be infected with many pathogens, including *P. aeruginosa*. Among the burn wound pathogens, *P. aeruginosa* has

shown the highest incidence, and it plays a predominant role in developing infections (Azzopardi et al., 2014; Fournier et al., 2016; Gonzalez et al., 2016; Safaei et al., 2017). The immunosuppression state triggered by burn trauma and the wound's local microenvironment produce favourable elements for microbial colonization (Licheng et al., 2005). Many recent studies have reported MDR *P. aeruginosa* strain infection in burn patients (Fournier et al., 2016; Safaei et al., 2017).

4.5. Genome size and flexibility of *P. aeruginosa*

P. aeruginosa strains have a large genome size of ~5–7 Mbp. According to a recent large-scale study, the species' essential functions are encoded in its core genome, which contains approximately 665 genes (Freschi et al., 2019). These essential genes facilitate the ecological flexibility needed to adapt and grow in CF-affected lungs (Turner et al., 2015). The *P. aeruginosa* pan-genome consists of 54,272 genes. Of these, 665 are core genes, 26,420 are flexible genes and 27,187 are unique genes. Core genes represent only ~1% (665) of the *P. aeruginosa* pan-genome. The expanded repertoire of genes of the PAO1 strain comprises ~521 regulatory genes, ~150 code for outer membranes, ~300 cytoplasmic membrane proteins and many genes involved in the catabolism, multiple paths of transport/efflux of organic compounds (Stover et al., 2000). Statistical analysis of the repertoire of significantly conserved genes suggests the highest proportion of regulatory genes and networks that benefit from responding and adapting to distinct environments (Mathee et al., 2007; Frimmersdorf et al., 2010). The increased number of genes also allows *P. aeruginosa* to develop resistance to nearly all available antipseudomonal agents through chromosomal mutations (López-Causapé, Cabot, et al., 2018). The ubiquitous presence of *P. aeruginosa* – as well as its prevalence and persistence in clinical settings, a phenomenon that includes its intrinsic resistance to therapeutics – is attributable to its extraordinary capability for survival.

4.6. Current epidemiology of MDR *Pseudomonas*

The emergence of antibiotic resistance among bacterial pathogens is a significant public health threat affecting humans worldwide. MDR organisms have not only emerged in hospital environments; they are also often identified in common environmental flora. This suggests that a pool of antibiotic-resistant bacteria is present outside of the hospital. Many studies have characterized *P. aeruginosa* isolates as MDR and XDR according to the standardized international terminology presented in 2011 by the European Centre for Disease Prevention and

Control and the Centers for Disease Control and Prevention (CDC). MDR was defined as acquired nonsusceptibility to at least 1 agent in ≥3 antimicrobial categories, while XDR was described as nonsusceptibility to at least 1 agent in ≥6 antimicrobial categories. Recent years have witnessed an increasing prevalence of MDR and XDR *P. aeruginosa* strains, with rates between 15–30% in many countries (Eklöf et al., 2020; Mirzaei et al., 2020; Pappa et al., 2020; Karruli et al., 2021).

According to the CDC, 2.8 million cases of antibiotic resistance are reported each year in the US. These cases produce an annual mortality rate of approximately 35,000 people/year/US. The MDR *P. aeruginosa* causes 32,600 severe HCAIs and 2,700 estimated deaths yearly (CDC, 2019). The European Centre for Disease Prevention and Control's Prevalence Survey for HCAIs found that *P. aeruginosa* contributed to almost 9% of overall infections. It is the third most common pathogen in European hospitals (ECDC, 2019). According to this report, 31.8% of the *P. aeruginosa* isolates in the EU were reported to be resistant to at least one of the antimicrobial groups under surveillance. Resistance to two or more antimicrobial groups was common; it was seen in 17.6% of all tested isolates. In the last five years, many studies have reported the increasing incidence of nosocomial infections derived from MDR or XDR *P. aeruginosa* throughout the world (Table 1). The lack of new antibiotics and the increasing failure of conventional antibiotics have exacerbated the problem.

Table 1. **Studies showing recent *P. aeruginosa* drug resistance epidemiology**.

Study Design/ Date	Source	Cases	Outcomes no/percentge	Reference
Retrospective Cohort:2020	Tertiary care; Greece Hospital-acquired infections	71	58 (82%) MDR	Pappa et al., 2020
Retrospective Cohort:2020	Tertiary care; Iran Wound infections, bacteremia and burn injuries	75	48 (15.5%) XDR 51 (16.5%) MDR	Mirzaei et al., 2020
Retrospective Cohort:2020	Tertiary care; Pakisthan Wound infections, bacteremia and burn injuries	88	16 (18.1%) XDR 32 (36%) MDR	Saleem & Bokhari, 2020
Retrospective Cohort:2020	Tertiary care; Nigeria Wound infection	69	19 (28.1%) XDR 69 (100-%) MDR	Oluseye et al., 2021
Retrospective Cohort:2020	Tertiary care; Poland Clinical isolates	202	97 (48%) MDR	Brzozowski et al., 2020
Retrospective Cohort:2020	Tertiary care; Greece Respiratory tract, ventilator-associated pneumonia and bacteremia	504	141 (28.1%) XDR 142 (20.6 %) MDR	George et al., 2020;
Retrospective Cohort:2019	Tertiary care; Greece, Italy and Spain Ventilator-associated pneumonia	53	19 (35%) XDR 30 (16%) MDR	Pérez et al., 2019
Retrospective Cohort:2019	Tertiary care; USA Cystic fibrosis	32	9 (28.1%) XDR 32(100%) MDR	Atkin et al., 2018
Retrospective Cohort:2018	Tertiary care; Thailand Clinical isolates	255	56 (22%) XDR 32 (12.5%) MDR	Palavutitotai et al., 2018

4.7. Drug resistance mechanisms

Bacterial conflict with antibiotics is a prime example of bacterial adaptation and evolution. This process of survival of the fittest is driven by the immense genetic plasticity of bacterial pathogens. Plasticity is meticulously triggered and controlled by distinct responses that result in mutational adaptations, acquisition of genetic material or alteration of gene expression. Understanding the virtuosity that enables resistance is vital to prevent the spread of MDR organisms. Rates of antibiotic resistance in *P. aeruginosa* are increasing in many parts of the world. Drug resistance in *P. aeruginosa* is facilitated by several mechanisms, including multidrug efflux systems, neutralizing enzyme production, outer membrane protein loss and target mutations (Fig. 3).

4.7.1. Intrinsic antibiotic resistance

The innate ability of bacterial species to moderate the efficacy of an antibiotic through inherent functional characteristics is called intrinsic antibiotic resistance (Blair et al., 2015). For example, *P. aeruginosa* has shown remarkable resistance against many antibiotics due to its restricted outer membrane permeability, efflux systems that pump antibiotics out of the cell and enzymes inactivating or degrading antibiotics (Breidenstein et al., 2011).

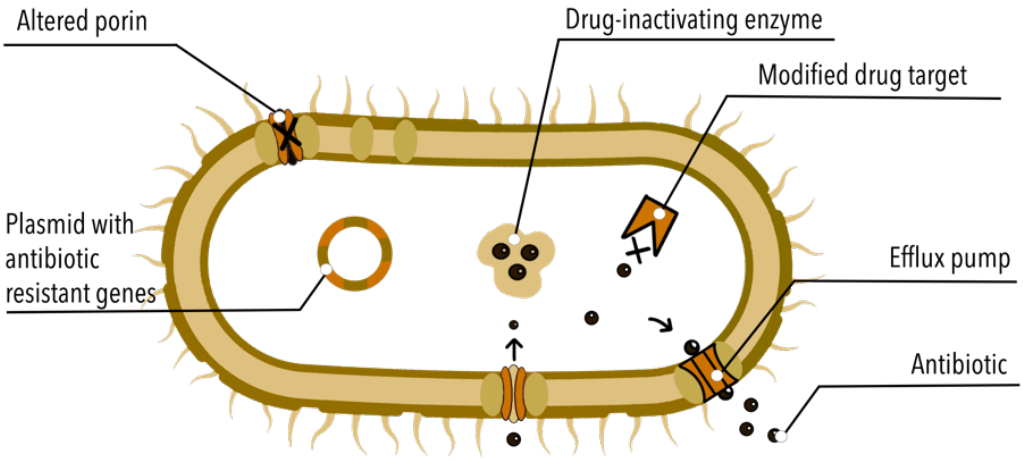


Figure 3. **A schematic representation of antibiotic resistance in *P. aeruginosa*.** The antibiotic resistance mechanisms possessed by *P. aeruginosa* include restricted outer membrane permeability by porin, pumping out antibiotics by efflux pump, production of drug inactivating enzymes, a plasmid with an antibiotic resistance gene, or modifying drug binding targets. The figure was designed and illustrated by the author.

The impermeable outer membrane

The impermeable defence mechanism is mostly effected by the class of antibiotics that hampers bacterial growth by degrading the outer cell wall. These antibiotics can block bacterial cell wall biosynthesis by conceding the penicillin-binding proteins involved in peptidoglycan synthesis. For example, β-lactams and quinolones, these antibiotics penetrate cell membranes through porin channels (Poole, 2004). Antibiotics like aminoglycosides and polymyxins initiate their uptake by binding to lipopolysaccharides (LPS) on the outer membrane of *P. aeruginosa* (Lambert, 2002). *P. aeruginosa* can counteract this entry by screening antibiotics with the help of phospholipids and LPS. This layer is embedded with porins that form β-barrel protein channels as a selective barrier to prevent antibiotic penetration (Delcour, 2009). The family of porins can be divided into four classes: the non-specific porins, which allow the slow diffusion of most hydrophilic molecules (Eg: OprF); specific porins, which possess specific sites to bind a particular set of molecules (Eg: OprB, OprD, OprE, OprO and OprP); gated porins, which are responsible for the uptake of ion complexes (Eg: OprC and OprH); and efflux porins, which are essential components of efflux pumps (OprM, OprN and OprJ) (Hancock & Brinkman, 2002). Among these porins, many antibiotics use OprD as a door. For example, the class of β-lactam-carbapenem type antibiotics uses OprD as a binding site to penetrate the cell. The absence of OprD can increase antibiotic resistance (Fang et al., 2014; Xian-Zhi & Hiroshi, 2009).

Drug efflux systems

Bacterial efflux pumps play a vital role in discharging toxic compounds from the cell. These efflux pumps belong to five families: The resistance-nodulation-division (RND) family, the major facilitator superfamily (MFS), the ATP-binding cassette (ABC) superfamily, the small multidrug resistance (SMR) family and the multidrug and toxic compound extrusion (MATE) family. In particular, the RND family proteins MexAB-OprM, MexCD-OprJ, MexEF-OprN and MexXY-OprM play a crucial role in antibiotic resistance in *P. aeruginosa* (Dreier & Ruggerone, 2015). The efflux pumps MexAB-OprM and MexCD-OprJ are able to pump out fluroquinolones and β-lactams (Masuda et al., 2000; Dupont et al., 2005). MexAB-OprM and MexEF-OprN extrude quinolones, while MexXY-OprM expels aminoglycosides (Masuda et al., 2000; Vogne et al., 2004). Thus, it is certain that the overexpression of multiple pumps can induce antibiotic resistance. This has been found in many clinical *P. aeruginosa* strains that possess a broad range of antibiotic resistance and that have contributed to the development of multidrug resistance (Kievit et al., 2001; Singh et al., 2017).

Antibiotic-inactivating enzymes

The production of antibiotic-degrading enzymes capable of breaking down or modifying antibiotics is one of the most effective mechanisms of intrinsic resistance in *P. aeruginosa*. Many antibiotics have chemical bonds such as amides and esters, which are vulnerable to hydrolysis by enzymes produced by *P. aeruginosa*. Such enzymes include β-lactamases and aminoglycosidases (Poole, 2004; Wolter & Lister, 2013). *P. aeruginosa* controls the production of the former by an inducible *ampC* gene that encodes the hydrolytic enzyme β-lactamase. This enzyme will break the amide bond of the β-lactam ring, leading to the inactivation of β-lactam antibiotics (Wright, 2005). Indeed, considering certain other Gram-negative bacteria, *P. aeruginosa* can produce extended-spectrum-β-lactamases (ESBLs), which confer a high degree of resistance to the majority of β-lactam antibiotics, including penicillins, cephalosporins and aztreonam (Paterson & Bonomo, 2005; Rawat & Nair, 2010). Aminoglycoside resistance in *P. aeruginosa* arises due to multiple factors, such as reduced cell membrane permeability, increased efflux, ribosomal changes and enzyme modification.

4.7.2. Acquired antibiotic resistance

P. aeruginosa can gain antibiotic resistance through mutational changes or the acquisition of resistance genes via horizontal gene transfer (Jose & Cesar, 2016). Spontaneous mutations that trigger or inhibit any function of the above systems can play a vital role in resistance. A single mutational change can alter antibiotic uptake, modify antibiotic targets and overexpress efflux pumps (Fernández & Hancock, 2012). An impulsive mutation in the porin gene can affect the function, thereby reducing permeability and increasing antibiotic resistance. For instance, a deficiency in OprD in *P. aeruginosa* confers a high level of resistance to carbapenems, especially imipenem (H. Li et al., 2012; Fang et al., 2014;).

Acquisition of resistance genes

Antibiotic resistance genes can be exchanged via horizontal gene transfer from the same bacterial species or from different ones, including plasmids, transposons, integrons and prophages (Breidenstein et al., 2011). For example, *P. aeruginosa* has the highest number of metallo beta-lactamases genes. These include Verona integron-encoded metallo-β-lactamase, Germany imipenemase, Sao Paulo metallo-β-lactamase, New Delhi metallo-β-lactamase, Florence imipenemase and imipenemase (Hong et al., 2015), which are able to hydrolyze most available β-lactam-based antibiotics. The acquisition of aminoglycoside and β-lactam

resistance genes is a common scenario in *P. aeruginosa* clinical isolates (Poirel et al., 2001). Genetic elements, including integrons and plasmids, have been detected carrying many genes responsible for the resistance.

4.7.3. Biofilm-associated resistance

Biofilm is astonishingly widespread, and it appears very early in the fossil record (Birger, 2000; Frances et al., 2001). It represents a protected mode of growth that allows cells to survive in hostile environments and to colonize new niches by dispersing microorganisms from microbial clusters. Many free-growing bacteria that lack intrinsic resistance or genomic mutation can become less susceptible to antibiotics when they grow in biofilm (Stewart, 2002). Additionally, sensitivity is rapidly reinstated when bacteria lose biofilm protection, which suggests that the many hostile-mediated resistance mechanisms are independent of genetic mutations (Walters et al., 2003). Common mechanisms of biofilm-mediated resistance include the prevention of antibiotic infiltration, the reform of the microenvironment for slow growth of cells, the induction of a versatile stress response and the differentiation of persister cells (Stewart, 2002) (Fig. 1).

P. aeruginosa can cause chronic biofilm infections in CF patients (Høiby, Bjarnsholt, et al., 2010; Høiby, Ciofu, et al., 2010; Ciofu et al., 2015; Høiby et al., 2017). This intimidating environment is regulated by multifactorial systems referred to as quorum sensing. Quorum sensing is a network of cell-to-cell communication that regulates gene expression profiles in response to changes in cell-population density (Miller & Bassler, 2001). The biofilm matrix also contains eDNA (extracellullar DNA), facilitating initial cell-cell adhesion and aggregation on surfaces (Das et al., 2010). Studies have shown that *P. aeruginosa* can acidify the biofilm environment and induce gene expression, a process that can increase aminoglycoside resistance (Wilton et al., 2016). *P. aeruginosa* also has unique genes – for example, *ndvB*, *PA14_40260-40230* and *tssC1* – that control antibiotic resistance in biofilm environments (Mah et al., 2003; Zhang & Mah, 2008; Zhang et al., 2011) . Biofilm is also capable of producing persister cells (Lewis, 2010). These are protected seedlings that grow slowly. They are usually metabolically suppressed or inactive as well as highly tolerant to antibiotics (Maisonneuve & Gerdes, 2014; Wood et al., 2013; Mlynarcik & Kolar, 2017). Persister cells comprise about 1% of biofilm cells. Antibiotics can kill the general flora of *P. aeruginosa*; persisters, however, can spawn dormant states and repopulate later (Fig. 2). This process can promote the recalcitrance of chronic infections in CF patients. Such infections resist common treatments. Studies have shown that CF patients often have high levels of persister cells compared to those with typical

infections (Mulcahy et al., 2010). This attribute even pushes *P. aeruginosa* to a higher class of multidrug-tolerant strains.

4.8. Alternative therapies

Alternative methods for the control of MDR pathogens have been explored for several decades. Presently, adopting alternative strategies that promote the elimination of resistant microbial strains from the environment is of the utmost importance. Many research groups pursue promising alternatives, such as antimicrobial peptides, lysin therapy, bacteriocins and antibacterial antibodies. One of the oldest and most efficient alternative solutions is the use of bacteriophage (or phage) therapy. Here, we will discuss and analyse the pros and cons of phage therapy.

4.9. Phage therapy

In recent years, phage therapy has been one of the most debated alternative therapies. Viruses that infect bacteria, or bacteriophages, were discovered in 1915 by Frederick Twort (Twort, 1915). Phage inception was discovered by Félix d'Hérelle in 1917, thus revolutionizing the world with a new therapy against bacterial infection (Ackermann, 2011). Phage treatment was considered to be a possible therapy around a century ago. Nevertheless, it was mostly suppressed by the antibiotic boom in 1929. Bacteriophage-based therapy exploits the ability of phages to selectively infect compatible bacterial hosts. Phages have been classified based on their morphology since before the existence of modern-day molecular tools or sequencing. David Bradley accomplished one of the first classifications of tailed phages. He classified these phages into three groups – long-contractile tail, short-contractile tail and non-contractile tail – based on electron microscopy (Bradley, 1967). In 1971, the International Committee on Nomenclature of Viruses (ICNV) formally adopted this method. Subsequently, the ICNV renamed the classification to *Myoviridae, Podoviridae* and *Siphoviridae* (Ackermann & Eisenstark, 1974).

Certain other phage classes have equally ancient histories. The families *Inoviridae, Microviridae, Tectiviridae, Corticoviridae, Plasmaviridae, Leviviridae* and *Cystoviridae* were all formalized by the ICNV in 1978 (Ackermann, 2004). Due to the rise of the genomics era, the sequencing of phage genomes has uncovered a considerably higher degree of genomic diversity by the 2000s. This was especially true in relation to phages belonging to the order

Caudovirales, which led to the invention of the first subfamilies within the three existing families of *Podoviridae*, *Myoviridae* and *Siphoviridae* (Ackermann, 2001; 2004). Recently, three new families of myoviruses have been officially approved: *Ackermannviridae*, *Chaseviridae Herelleviridae*. Two families have been approved for the siphoviruses: *Demerecviridae* and *Drexlerviridae*. One has been approved for podoviruses: *Autographiviridae* (Turner et al., 2021). Phages can conduct precise bactericidal activity by killing only a particular host with low inherent toxicity. They are the only auto-dosing antibacterial agent capable of increasing their numbers in the specific site where the host is located (Loc-Carrillo & Abedon, 2011). Compared to other therapeutic agents, a single infection can produce an exponential pattern, showing a continuous killing effect. The rise of antimicrobial resistance and the paucity of new antibiotics led to the rediscovery of phage therapy.

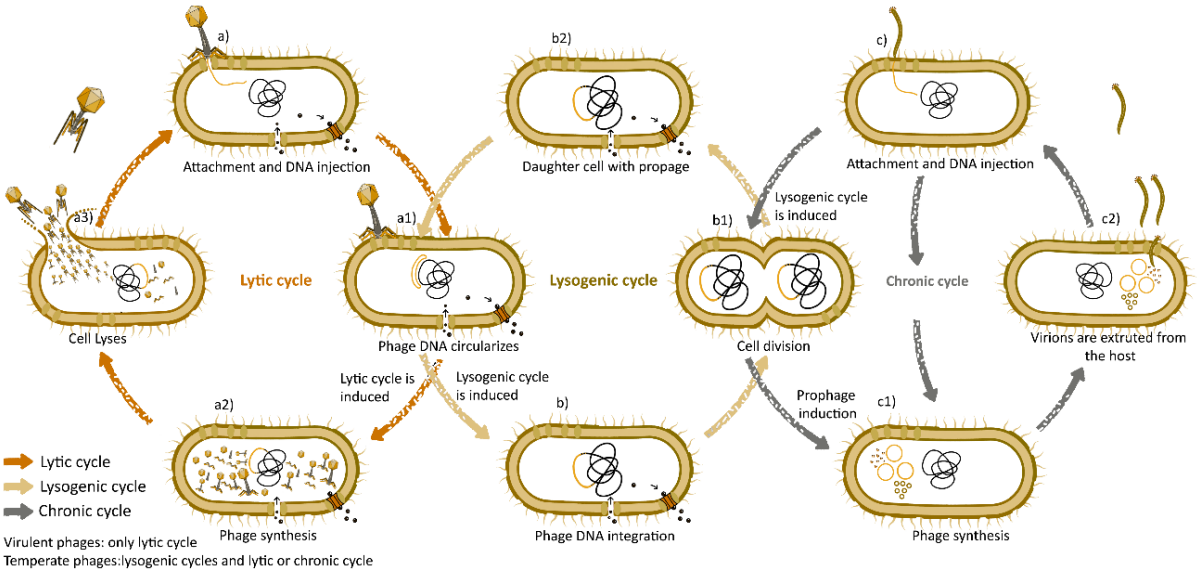


Figure 4. Bacteriophage replication cycles. The phage recognizes the receptor and initiates the attachment by tail fiber protein followed by injection of the DNA. After the internalization of the genome, the phage initiates the cycle either as lysogenic cycle and/or lytic or chronic replication cycles. The figure was designed and illustrated by the author.

The production of new virions is realized either through a reproductive lytic cycle in the case of *Caudovirales* and *Microviridae* phages or through a reproductive non-lytic life cycle in the case of filamentous phages or *Inoviridae* (Fig. 4). Both cycles start with the recognition and infection of the targeted bacteria (Fig.4a, 4c), a process that is followed by phage DNA replication and the synthesis of new virions (Fig. 4a1, 4a2 and 4c2). In lytic cycles, new virions

are released through bacterial lysis (Fig.4a3). Without bacterial lysis, new virions of filamentous phages exit bacteria through a dedicated secretion apparatus (Fig. 4c2). Phages that reproduce only through lytic cycles are called virulent. Temperate phages can perform either cycle; they can enter prophage state in the infected bacteria (Fig. 4b1, 4b). The prophage is either integrated within the bacterial genome, or it inhabits an episomal state that can replicate with the bacterial chromosome for as long as the bacteria divide (Fig. 4b1). When the host bacterium is exposed to stress, the prophage is cleaved off, and the phage enters a lytic or a chronic cycle.

In addition to the processes described above, the lysogenic phage can employ distinct repressor genes that act as a genetic switch to control the balance between lysis and lysogeny (Ptashne, 2004). This repressor gene can also inhibit the integration of any new, incoming phage genomes, thus conferring immunity to superinfection. The lysogenic life cycle of phages can profoundly affect the bacterial life cycle. Many studies have reported that bacteria use prophage as a beneficial tool to adapt and improve their genomes. For example, prophages often encode 'morons' that are not directly involved in viral replication and that can confer a benefit to their bacterial host (Juhala et al., 2000). Morons can comprise genes that improve the virulence of their bacterial host. For example, phage-encoded toxins can enhance bacterial ecological fitness during infection(Hacker & Carniel, 2001). Prophage has also been reported to promote biofilm-associated bacterial infection. The filamentous phage Pf4 is widespread in many clinical *P. aeruginosa* isolates, and it can influence several stages of biofilm maturation. Pf4 can switch to a superinfective form within mature biofilms by administering dispersal and promoting virulence (Rice et al., 2009). Prophage in *P. aeruginosa* has also been shown to convert non-mucoid strains to mucoidy, a phenotype characterized by the overproduction of the polysaccharide alginate. This phenotype nourishes bacteria with a physical protectant that helps them become resistant to many antibiotic treatments (Hentzer et al., 2001). In addition, prophage is well known to carry antimicrobial resistance genes. For example, salmonellae phages carry the blaCMY-2 gene encoding resistance to third generation cephalosporins (Zhang & LeJeune, 2008), and the staphylococcal phage, TEM123 (isolated from food) was shown to confer beta-lactam resistance via a metallo-β-lactamase gene (Lee & Park, 2015).

4.9.1. Biotechnological applications of phages

Many recent studies have explored phages as efficient molecular tools in therapeutics. Some well-known methods include phage display, phage typing and gene delivery (Ul Haq et al., 2012). The phage display technique helps researchers synthesize novel polypeptides with distinct properties. The DNA that encodes an oligopeptide population is fused to phage coat protein genes, and the desired protein is expressed on the surface of the phage particle. For example, phage M13 and the T7 phage of *E. coli* have been extensively used in phage display and phage typing (Sidhu, 2000).

Phage display libraries can be screened to isolate peptides that are highly specific to a target protein. These peptides can be used in drug design and as model agents to understand the molecular mechanism behind receptors (Sidhu, 2000). The phages' ability to display foreign polypeptides on their surfaces enables the technique to identify specific motifs targeting particular cell types, which is necessary for successful gene therapy (Benhar, 2001).

In agriculture, phages are renowned for their properties as biocontrol agents. For example, *Pectobacterium* spp. are pathogens associated with potato blackleg disease. Two *Myoviridae* phages were used as control agents for blackleg in potatoes (Adriaenssens et al., 2012). *Pseudomonas syringae* pathovars are responsible for many plant diseases in agriculture. Two parallel field trials with 6-phages have been attempted in three locations to control *P. syringae* pv. *porri* infections (Rombouts et al., 2016).

Phages have also been used in bioprocessing to reduce the bacterial load in foods, particularly in minimally processed foods, and thereby to avoid cooking-associated flavours or textures (García et al., 2010). Eliminating the pathogens of fruits and vegetables is of much concern because these foods cannot be further processed to kill any pathogens that might be present. The eradication of pathogens by phages is a nonthermal intervention to substantially reduce the CFU of *Salmonella enteritidis* in cheese (Modi et al., 2001) as well as *Listeria monocytogenes* in milk and meat products (Dykes & Moorhead, 2002) and fresh-cut fruit (Leverentz et al., 2003).

4.9.2. Significant advantages of phage therapy over antibiotics

The advantages of phage therapy over chemical antibiotics can be outlined in terms of phage properties. These properties highlight the use of phages as an effective alternative therapy against MDR bacteria.

Auto dosing therapy

The bacteriophage is the only bactericidal agent that can manifold its inhibition capability with each encounter (Abedon & Thomas-Abedon, 2010). Phages are capable of increasing their numbers in the specific sites where hosts are located. This ability confers a great advantage on the treatment period and frequency. Applying a single dose replicates itself and thereby achieves a continuously active therapy.

Bactericidal agents

If a bacterium was successfully infected by obligately lytic phage, it would be unable to regain its viability. By contrast, certain bacteriostatic antimicrobials have hormetic, dose-dependent effects in low concentrations, which can stimulate cell growth and conjugative gene transfer in addition to promoting bacteria to evolve against antibiotics (Stratton, 2003).

Minimal disruption of normal flora

The widespread use of antibiotics over the years has killed an incalculable number of microbes, both pathogenic and beneficial. Human-gut-associated microbes play a vital role in our lives. Killing this commensal flora can influence the function of the immune system, our ability to resist infection, and our capacity to digest different foods (Becattini et al., 2016). In contrast to antibiotics, phages possess a property called host specificity, which controls their ability to infect only a few particular strains of a bacterial species. Rarely, phages can infect members of closely related bacterial genera (Federici et al., 2021). This property protects commensal flora, thereby reducing the impact on health.

Biofilm eradication and clearance

The bacterial lifestyle in so-called biofilm has been recognized as one of the major causes of increasing antibiotic resistance (Walters et al., 2003; Stewart & J William, 2001; Ciofu et al., 2015; Philip G & MPhi, 2018; Ciofu & Tolker-Nielsen, 2019). Bacteriophages, however, seem to have a remarkable ability to target adaptation. The high numbers of bacteria present within biofilms facilitate rapid and efficient infection, consequently amplifying the bacteriophages. In addition, phages can produce enzymes that degrade the extracellular matrix (K Chan & T

Abedon, 2015). They can also infect persister cells, remaining dormant within them and reactivating when they become metabolically active (Harper et al., 2014).

Lack of cross-resistance

Phage infection processes are entirely different from antibiotics. Each phage might have a unique mechanism of infection that substantially decreases the chance of cross-resistance among phages (Rakhuba et al., 2010). In contrast, particular classes of antibiotic resistance can also affect other classes of antibiotics (Blair et al., 2015; Jose & Cesar, 2016).

4.9.3. Potential disadvantages of phage therapy

Phage therapy's cons can be grouped under three headings: (1) inappropriate phage selection, (2) phage host range limitation and (3) phage resistance.

Phage selection

The primary criterion for a potential phage candidate is its ability to exclusively undergo a lytic cycle. In addition, it is essential to determine properties and application conditions such as genome sequences, host specificity, burst size, optimum temperatures, titer, efficacy and safety studies (Abedon & Thomas-Abedon, 2010). It is essential to determine the phage sequence to confirm the absence of undesirable genes (Skurnik et al., 2007). The improper selection of phages for use in therapy can result in superinfection immunity (Hyman & Abedon, 2010; Sousa & Rocha, 2019), which can induce bacterial virulence and toxin production (Skurnik & Strauch, 2006).

Phage host range limitation

Phages' narrow host range can also be a disadvantage when these phages can only infect a few strains or a single species of bacteria (Abedon & Thomas-Abedon, 2010). This property of phages makes it important to identify and screen phage infection efficacy for a given pathogen to determine their susceptibility prior to treatment. Nevertheless, a narrow host specificity can be overcome by combining phage and other antibacterial agents or phage cocktails. In addition, the lytic spectra of phage cocktails can be much broader than the spectra of activity among individual phage types (Gu et al., 2012; Chan et al., 2013; Forti et al., 2018;).

Phage resistance

Phages are the obligate intracellular parasites of bacteria. They can regulate and control bacterial populations in the environment. Constant attacks by phages have influenced bacteria to evolve diverse mechanisms to defend themselves. Fig. 5 summarizes the most recently discovered and most common phage resistance mechanisms.

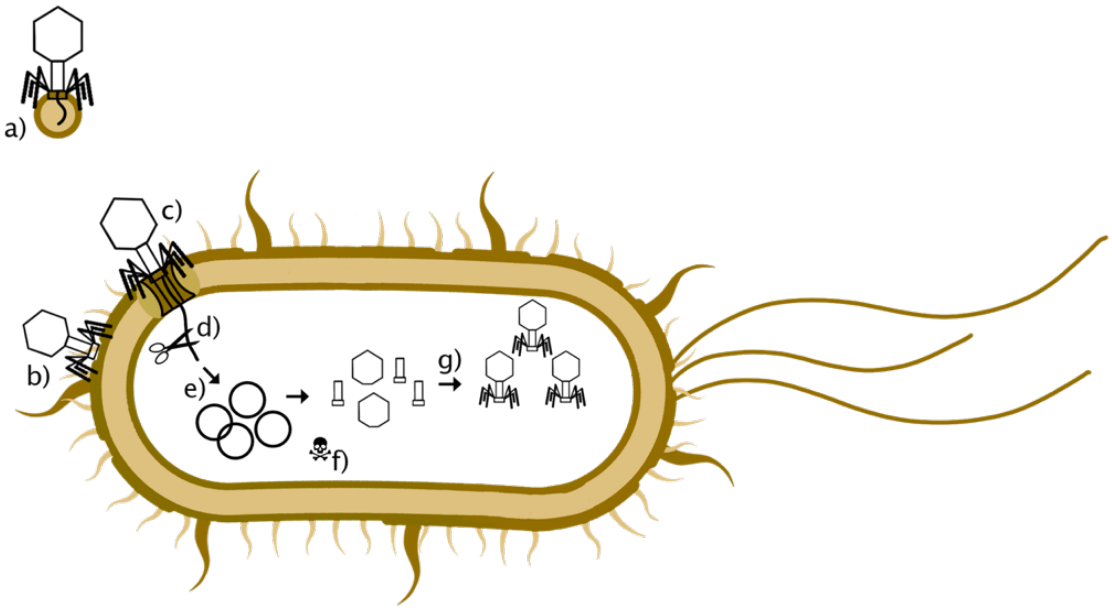


Figure 5. Summary of phage resistance mechanisms. a) Outer membrane vehicles as decoys are produced, b) Inhibition of phage adsorption, c) Blocking phage DNA injection, d) Cleavage of injected phage DNA e) Inhibition of phage DNA replication, f) Interference of phage assembly g) Suicide of bacteria. The figure concept was adapted from a published article (Azam & Tanji, 2019) and redrawn by the author.

Outer membrane vehicles as an anti-phage mechanism

Outer membrane vehicles (OMVs) are non-replicating, globular nanostructures composed of outer membrane lipid, outer membrane protein and periplasmic components produced during bacterial growth (Schwechheimer & Kuehn, 2015). OMVs have the same surface structure as the bacterial cells that produce them. Bacteria often use them as natural decoys to defend against harmful substances. Recent studies have reported that bacteria could use OMVs as a decoy against phages by tricking the phages into binding with and injecting their DNA into the vehicles, thus allowing the bacteria to escape infection (Fig. 5a) (Reyes-Robles et al., 2018).

Inhibiting phage adsorption by compromising phage receptors

Phages initiate infection by binding the phage receptor-binding protein (RBP) to the bacterial host's surface (Dowah & Clokie, 2018). Most bacteria instigate their first line of defence against phages by modifying their binding receptors. The infection begins with adsorption to the cell surface by attaching the phage protein to a receptor. This process is followed by the injection of the phage genome into bacteria. Phage infection can be inhibited either at the stage of adsorption or after it. To prevent the initial infection process, bacteria use several strategies, including alteration or deletion of the receptor. Generally, Gram-positive bacteria phages use peptidoglycan or teichoic acid as their receptors (Bertozzi Silva et al., 2016). LPS moieties present on the outer membrane are the most-studied phage receptor in Gram-negative bacteria. Some phages have also been reported to use pilus, LPS, pill flagella, porins and efflux pumps (Fig. 6; Hyman & Abedon, 2010; Bertozzi Silva et al., 2016)

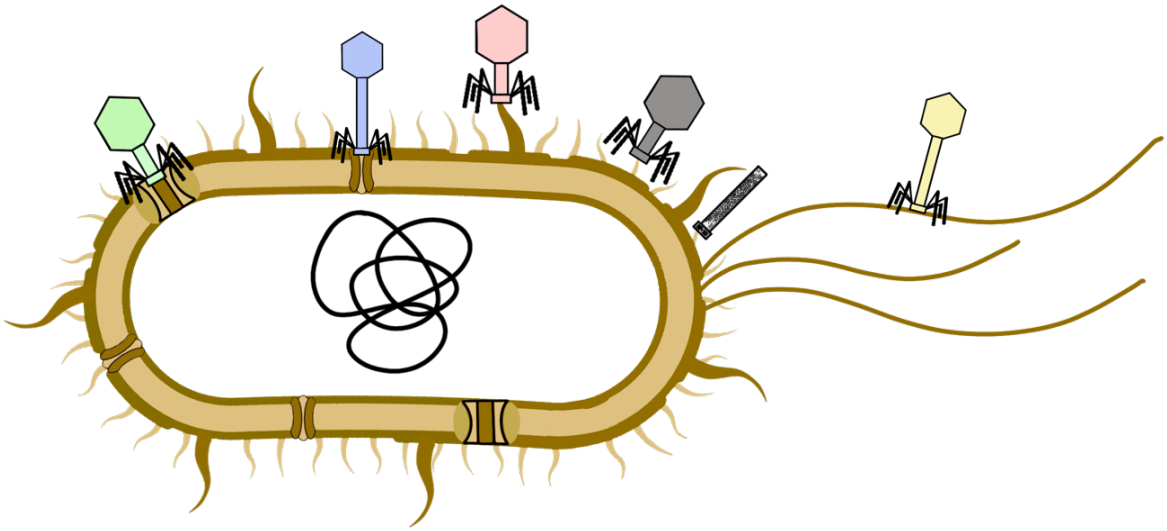


Figure 6. Example of bacterial receptors for phage binding. Phage DNA encode binding proteins that recognize and attach to the sites on the surface of the bacterial cell. Many phages bind to protein structures on the bacteria, such as LPS (black), pill (red), flagella (yellow), porins (blue) or efflux pumps (green)**.** The figure concept was adapted from a published article (Kortright et al., 2019) and redrawn by the author.

The most-reported defence mechanism is the induction of single nucleotide polymorphisms (SNPs) in receptor genes, which alters the function or expression of a particular receptor (Pan et al., 2016; Hesse et al., 2020). Phages might overcome this defence by inducing mutation in their genomes (Pan et al., 2016; Azam et al., 2018; Li et al., 2018). Bacteria can also delete the

phage binding receptor – such as OmpC protein or wall teichoic acid – to prevent adsorption (Parent et al., 2014; Hesse et al., 2020). Phages can counter this evolutionary upgrade by adapting to new receptors (Habusha et al., 2019). For example, a recent study reported that the *P. aeruginosa* phage could use the surface-exposed OprM of the MexAB and MexXY as a 'novel' receptor (Chan et al., 2016; Gurney et al., 2020). The second defence strategy involves masking the receptor by overproducing capsular polysaccharides or overproducing cell-wall-anchored proteins (Labrie et al., 2010). Phages can counter this strategy by expressing enzymes capable of degrading the physical barriers produced by bacteria (Samson et al., 2013).

Inhibiting phage infection by hindering phage DNA

Bacteria can apply various strategies to interfere with phage DNA function. These methods include blocking phage DNA injection, cleaving injected phage DNA and hindering phage DNA replication. One mechanism is known as superinfection exclusions (Sies), a phenomenon that blocks the entry of phage DNA (Fig. 5c,d). The Sies controlled by various proteins encoded in the prophage genomes block the entry of phage DNA into the host bacteria (Labrie et al., 201 0; Azam et al., 2018). When DNA injection is inevitable, bacteria can employ other defence mechanisms by cutting the injected phage DNA. The injected DNA can be cleaved by the host's innate or adaptive defence systems. The innate defence system consists of a defence island system associated with restriction-modification (Ofir et al., 2018), restriction-modification system (Oliveira et al., 2014) and prokaryote argonaute proteins (Swarts et al., 2014). The adaptive defence system only includes a clustered, regularly interspaced and short palindromic repeat (CRISPR)-Cas system (Godde & Bickerton, 2006) and bacteriophage exclusion system (BREX) (Goldfarb et al., 2015).

Preventing phage assembly

Many bacteria contain a chromosomal region called the phage-inducible chromosomal island (PICI) (Penadés & Christie, 2015; Fillol-Salom et al., 2018), which can interfere with the reproduction of specific phage (Fig. 5e). Upon infection by phage, the PICI is excised, and then it circularizes, replicates, and packages itself. Therefore, host bacteria can alter the expression of a gene involved in the PICI region and the PICI can interfere with phage reproduction (Fillol-Salom et al., 2018).

Abortive infection

Abortive infection (Abi) is a phage resistance mechanism in which phages cannot release themselves from bacteria, causing them to perish. This is a suicidal defence strategy wherein

the host bacteria die to protect surrounding bacteria from phages (Fig. 5g) (Dy et al., 2014). The Abi systems can arrest and interrupt phage development at different stages of the infection cycle. Unlike RM and BREX systems, which target any phage DNA regardless of origin, Abi systems are relatively specific to certain phages.

4.9.4. Phage therapy in practice

Growing concerns about antibiotic-resistant pathogens have reignited interest in phage research and even patient trials. The recent PhagoBurn project is one example. This project was the first prospective multicentric, randomized, single-blind and controlled clinical trial of phage therapy ever performed in the world (CORDIS, 2017; Jault et al., 2019). Many studies have reported the successful treatment of bacterial infections with phages as a first-line therapy; successes have been particularly evident among infections caused by MDR pathogens. Two compassionate phage treatments conducted in Europe and the United States validated this approach's safety and efficacy (Jennes et al., 2017; Schooley et al., 2017). Advances in molecular biology have also opened a wide range of genetic-modification techniques, thereby revolutionizing the scientific view of phage therapy. A clinical trial that deployed an engineered phage cocktail against MDR *Mycobacterium abscessus* in cystic fibrosis patients is one excellent example (Dedrick et al., 2019). Another prominent trial treated MDR *P. aeruginosa* infections by administering a spray-dried phage formulation (Chang et al., 2018). In addition to single-phage therapy, cocktails with a broad host range have also been developed. A six-phage cocktail of multiple, broad-range *P. aeruginosa* phages was much more efficient than single-phage therapy (Forti et al., 2018). This treatment model demonstrated that phages can enter the biofilm, destroy the biomass and reach the bacteria embedded inside.

4.9.5. *P. aeruginosa* phages and their clinical applications

Phages that explicitly target the *Pseudomonas* genus were first encountered in the middle of the twentieth century (Kellenberger & Kellenberger, 1957). To date, numerous *P. aeruginosa* phages have been isolated. All of them can be classified into four categories based on their morphologies and genome compositions (Fig. 7). The majority of these phages fall into the double-stranded DNA phage families, namely *Myoviridae* (Ackermann et al., 1988; Campbell et al., 2021) as well as *Siphoviridae* and *Podoviridae* (Ojeniyi et al., 1991; Namonyo et al., 2022). Some phages belong to the single-stranded DNA phage family, *Inoviridae* (Bradley, 1973; Zeng et al., 2021). A few phages with a tri-segmented dsRNA genome belong to

Cystoviridae (Mäntynen et al., 2018). Some with a single-stranded RNA genome fall into the *Leviviridae* family (Weppelman & Brinton, 1971; Ruokoranta et al., 2006).

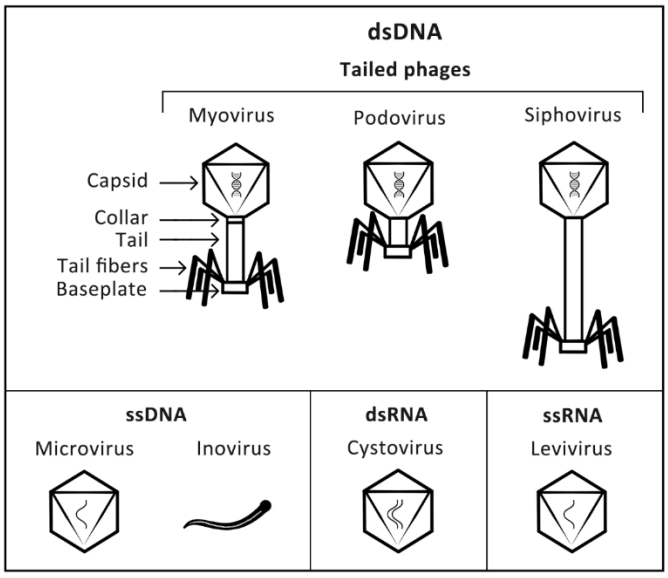


Figure 7. ***P. aeruginosa*** **phage taxonomy based on morphology and type of the genome.** The figure concept was adapted from a published article (Ofir & Sorek, 2018) and redrawn by the author.

There are currently 1,047 complete *Pseudomonas* bacteriophage genomes in public databases. Of these, approximately 95% are members of the tailed *Caudovirales*, and 5% belong to other classes. The antimicrobial efficacies of many isolated phages have been investigated in several applications including experiments against *P. aeruginosa* infections, prevention of biofilm formation, and phage therapy in clinical trials. The first published, quality-controlled clinical trials applying a *P. aeruginosa* phage cocktail (BFC-1) to treat burn wound infections were reported in 2009 (Merabishvili et al., 2009). A thoroughly controlled clinical study involving 24 patients was also attempted in the same year to treat chronic otitis arising from an antibiotic-resistant *P. aeruginosa* infection. Both treatments were well-conducted, and they were executed without any adverse side effects. In 2011, a six-phage cocktail was used to treat MDR *P. aeruginosa* urinary tract infections under the control of the Australia Therapeutic Goods Administration of Public Health Institute, which regulates and controls phage therapy (Khawaldeh et al., 2011). Many clinical trials are currently underway. A private enterprise BiomX has developed BX004, a quite promising cocktail that targets a broad range of *P. aeruginosa* strains focused primarily on CF patients.

5.0. GOALS AND OBJECTIVES

As antibacterial agents, phages have several advantages that make them convincing alternatives to antibiotics. Nevertheless, not all phages make for promising therapeutics. A decent therapeutic phage should have a high potential to reach and overcome bacterial virulence, the bacterial resistance or defence shields discussed above. The existence of these characteristics can be reasonably ascertained by a detailed study on bacteria and phages.

The main goals of this project are as follows:

1) To isolate and characterize drug-resistant *P. aeruginosa* from clinical samples.
2) To isolate and characterize potential phages that target MDR *P. aeruginosa.*
3) To evaluate the efficacy of isolated phages against *P. aeruginosa* biofilm.
4) To isolate and characterize phage-resistant strains.
5) To reveal the relationship between phage resistance and antibiotic sensitivity in *P. aeruginosa.*
6) To disclose the molecular background and evolutionary reasons for the formation of two types of mutants.
7) To assess the efficacy of phage and antibiotic combinational therapy *in vitro*.
8) To ascertain the efficacy of combinational therapy *in vivo* using a murine lung infection model.
9) To establish the role of PAK-like phages in bacterial genome deletions.
10) To isolate and characterize PAPSZ1 mutants that can block/suppress bacterial resistance.

6.0. MATERIALS AND METHODS

6.1. Bacterial strains, growth conditions, and antibiotic susceptibility

Bacterial isolates were acquired from the Clinical Microbiology Laboratory, University of Szeged over the course of a year. The strains were identified by MALDI-TOF MS and 16S ribosomal RNA gene sequencing using universal 16S rDNA bacterial primers: 27F (5'-AGAGTTTGATCATGGCTCA-3') and 1492R (5'-TACGGTTACCTTGTTACGACTT-3'). All bacterial strains were grown aerobically at 37°C in either Luria Broth (LB) or agar (LA). We screened all the strains for serotype according to the BioRad 16 monovalent sera kit. Primary antibiotic resistances of the wild-type and mutant strains were determined by the EUCAST disk diffusion antibiotic susceptibility tests (BioRad). An overnight grown bacterial culture 300µL (~2×10^8 CFU/ml) was spread onto an LA plate and allowed to dry for 10 minutes, then antibiotic-containing disks were applied to the agar. Plates were incubated at 37 °C for 12 h and susceptibility was estimated based on the size of the inhibition zone. Antibiotics (gentamicin, fosfomycin, ceftazidime and tetracycline (Sigma)) were chosen, and the EUCAST broth microdilution method was used to establish minimal inhibitory concentrations (MIC) (EUCAST, 2020). Four antibiotic stocks were diluted to different test concentrations in LB medium. The overnight grew culture was diluted to ~2×10^5 cfu/mL in LB. MICs for the above antibiotics were determined by dispensing 100 µL of a given antibiotic concentration and 100 µL of diluted bacteria into the wells of a flat bottom 96-well plate and incubating at 37 °C with 180 rpm for 12 h. Bacterial growth was monitored by a spectrophotometer (Thermo Multiskan Ascent Plate Reader) at 600nm.

6.2. Bacteriophage isolation and stock preparation

We assayed different clinical sources, including swabs from an infected wound and blood samples, for *P. aeruginosa*–specific bacteriophages. We homogenized solid and liquid samples in 5 ml of phage suspension buffer (10 mM Tris–HCl, pH 7.6, NaCl 0.4%, gelatin 0.1%), centrifuged the homogenate, and filtered it through 0.22 µm pore-size PTFE filters (Sigma). The filtrate was added to 25 ml LB broth containing 1 ml overnight culture of various *P. aeruginosa* MDR strains and incubated at 37°C for 24 h. We added chloroform (0.1% (v/v), final concentration) to the culture then incubated at 37°C with gentle shaking for 5 min. After removing bacterial cell debris by 10 min centrifugation at 9,000 × g at 4°C, we filtered the

suspension through a 0.22 μm pore-size filter. We determined the presence of phages in the filtrates by spot assay, as described previously (Kropinskiet al., 2009).

6.3. Phage purification and Transmission Electron Microscopy (TEM)

Amplified high-titer phage stocks were obtained through liquid broth amplification (LB medium). We adapted the protocol from Cui et al. (Cui et al., 2017), with minor modifications. Single plaques from a plate containing phage pregrown on the individual host inoculum (1 ml for 10 ml fresh broth) were incubated at 37°C for 12 hours. We prepared the phages used in the experiments from continuous consecutive passages: each passage was centrifuged, filtered, and combined with fresh inoculum every 12 h and incubated at 37°C for 48 h to minimize the risk of host coevolution. We treated the lysed liquid culture obtained after incubation with chloroform (final concentration of 0.2% (v/v)) for 30 min with gentle shaking to kill the remaining bacteria, and we then added NaCl (0.5 M) directely to the solution and incubated the culture for 1 h at 4°C. We removed bacterial debris by 10 min centrifugation at 8,000 × g and 4°C. We coagulated the phages in the supernatant overnight using polyethylene glycol PEG8000 (100 g/L) at 4°C. After 30 min centrifugation at 12,000 × g and 4°C, the collected pellet was dissolved in phage suspension buffer (1 mL phage suspension buffer per 100 mL original volume) and vortexed. We added an equal volume of chloroform to remove PEG8000 and other debris and carried out 15 min centrifugation at 5,000 × g and 4°C. The aquatic phase (upper phase) with a high titer of phages was collected and stored at 4°C. We viewed samples of the purified phage under a JEM-1400 Flash transmission electron microscope (JEOL; Tokyo, Japan) at the Biological Research Centre, Szeged, Hungary. We deposited 5 μl of the sample onto formvar-coated 150 mesh copper grids (Electron Microscopy Sciences). We used the edge of a filter paper to remove the excess fluid after 30 sec, and then samples were contrasted by adding 20 μl 1% (v/v) uranyl acetate (Electron Microscopy Sciences) in 50% (v/v) ethanol (Molar) for 3 min (three times). After removing the superfluous staining solution, samples were dried at room temperature in a Petri dish for 2 h before taking the electron microscopic picture. We screened samples systematically at 10,000× magnification to localize the presence of phages on the grid. We then recorded healthy, unbroken phages at 80,000–100,000× magnification with a 16 MP Matataki Flash sCMOS camera (JEOL).

6.4. Phage genome sequencing and phylogenetics

Phage DNA isolation was carried out using the High Pure Viral Nucleic Acid Large Volume Kit (Roche). We sequenced the phage genomes on the Illumina MiSeq platform and achieved coverages of at least 50×. The sequences were filtered by BBduk (http://jgi.doe.gov/data-and-tools/bb-tools/) and assembled using Spades (v.3.13.1) with default settings and the "careful" option (Nurk et al., 2013). From the output, we used the "contigs.fasta" files for annotation with Prokka (v.1.14.6) (Seemann, 2014). We downloaded all other *Pseudomonas* phage gene sequences from Uniprot (Chen et al., 2016) and used them as trusted sequences for annotation. Phage phylogeny was carried out using VICTOR (Meier-Kolthoff & Göker, 2017) a comparison was performed by Brig (Alikhan et al., 2011). The phage sequences were deposited in the NCBI database (Bioproject: PRJNA720536).

6.5. Biofilm inhibition assay

In the light microscopy and confocal light scanning microscopy (CLSM) study, the biofilm grew on glass slide pieces (1 x 1 cm) placed in 24-well polystyrene plates containing LB broth. After 24 h, 37°C static incubation, the control slides were analysed for biofilm formation. Next, the pre-formed biofilm was infected with phages and further incubated for 24 h. The glass slide pieces were then washed with PBS and stained with 0.1% (v/v) acridine orange (Sigma). The depth of phage infection on the biofilm was studied with an Olympus confocal laser-scanning biological microscope FV1000. The 24-h phage-post-infected samples and control biofilms without phage infection were analysed separately. The stained cells were detected using a 488 nm argon laser and a 500-640 nm bandpass excitation filter. Z stack analysis (surface topography and three-dimensional architecture) was performed for all triplicates. The biofilm stack images were then analysed using COMSTAT software (Heydorn et al., 2000). The thickness (mm) of the biofilms and the biovolume (mm^3) of bacteria per mm^2 of the surface were determined by COMSTAT.

6.6. Phage cocktail

Virulent phages with a broad-host range spectrum and biofilm inhibition were mixed to make the cocktail. Four *Myoviridae* (PAPSZ2, PAPSZ3, PAPSZ6 and PAPSZ7) phages and two *Siphoviridae* (PAPSZ4 and PAPSZ5) phage were selected based on biofilm inhibition assay result. Each phage lysate was grown and purified as described above (MM 6.3). A phage

cocktail was generated by mixing six phage preparations at the same PFU/ml immediately before each experiment to ensure accurate phage titres.

6.7. Determination of PIAS phage infection efficiency.

The PIAS phage infection efficiency was assayed in 96-well-flat bottom plates (VWR) for 24 h. All samples were run in triplicate in three independent experiments. We added 500 µl of overnight test strain culture to 10 ml of LB in a 250 mL flask and incubated it at 37°C, shaking it at 200 rpm, until an OD_{600nm} = 0.6 (~1×10^8 CFU/ml) was reached. Once the required culture density was obtained, we put 90 µL of bacteria into the wells. We treated bacterial samples with 10 µL of phage stock with different multiplicity of infection (MOI) (0.1, 1.0, and 10.0). The control was mock-treated with phage suspension buffer. We measured the OD_{600nm} every 3 h for 24 h using a Thermo Multiskan Ascent Plate Reader. The latent period and burst size were determined according to the protocol of the Jeffrey Barrick laboratory (Heineman and Bull, 2007). To determine the time required for PIAS adsorption to the wild-type and transposon mutants, an adsorption assay was performed following the protocol of Kropinski et al. (Kropinski, 2009). We determined the mutant frequency via the previously described method of Shen et al. (Shen et al., 2018).

6.8. Bacterial genome sequencing and genome comparison

Bacterial genomic DNA was extracted from mutant and parental strains using GenElute Bacterial Genomic DNA kits (Sigma). We sequenced five wild-type strains and 15 mutants. We first prepared libraries using the Nextra XT kit (Illumina) and sequenced them in a MiSeq sequencer (Illumina Inc.). Paired-end sequencing with 150 bp read length was used to generate reads. Quality assessment was carried out with FastQ (Wingett & Andrews, 2018). We trimmed raw reads to remove adapter sequences and PhiX174 contamination using BBduk (Bushnell B, 2017). Sequence reads were assembled using Unicycler (version v0.4.7) (Wick et al., 2017). The assembled sequences were annotated with Prokka (version 1.14.6) (Seemann, 2014) against all *P. aeruginosa* proteins. We downloaded 6,661,531 protein sequences belonging to *P. aeruginosa* from Uniprot (Chen et al., 2016) and used these as trusted proteins for annotating assembled contigs in Prokka. BBMap (Bushnell B, 2017) utilities were applied to obtain assembly metrics' statistics. To identify genome deletions, all reads were mapped back to the reference *P. aeruginosa* PAO1 strain (Stover et al., 2000). Mapped reads were analyzed using Samtools (H. Li et al., 2009) and visualized using an Integrated Genome Viewer (Robinson et

al., 2011). The annotated genomes were compared to identify the missing genes across the strain and mutants using Roary (Page et al., 2015). We identified SNPs in the green mutants by mapping the reads to their respective reference strain using Breseq (version: 0.35.4) (Deatherage & Barrick, 2015).

6.9. Membrane integrity measurements

Membrane integrity can be measured using the membrane-damaging agent bile acid (Lázár et al., 2018). We studied the membrane integrity of the mutant strains by measuring their sensitivity to bile acid. The experiments were carried out in 96 well plates for 24 h (37°C) at 160 rpm. We added 10 µL of overnight test strain culture to 1 mL of LB supplemented with 1%–7% bile acid. We plated 200 µL from each concentration. The mock-treated control contained a sterile buffer without bile acid. We measured the OD_{600nm} in every hour over 24 h (Thermo Multiskan Ascent Plate Reader).

6.10. Screening the knock-out mutants for PIAS sensitivity – drop assay

Based on the genome sequences of the green and brown mutants, we purchased 12 transposon knock-out mutants from the Transposon Mutant Collection of the University of Washington (Genome Sciences, Manoil Lab) (Table S1). For the drop assay, we added 200 µL of each mutant overnight culture to 5 mL of molten LB soft agar (0.6% (m/v) agar) and overlaid the mixture onto a 1.5% (m/v) LB agar plate to generate a bacterial lawn. After solidification, we spotted 5 µL volumes of serially diluted (10-fold dilution) PIAS phage suspensions and then incubated the plates at 37°C for 24 h. We determined the PIAS susceptibility of tested bacteria based on the formation of a single clear plaque or a bacterial growth inhibition zone on the plates.

6.11. Isolation of phage mutants (E-PIAS and PAPSZ1 derivatives) - plaque assay

We used the previously published double agar plaque assay protocol with slight modifications (Kropinski, 2009). The host strains were grown at 37°C with agitation until reaching ~2×10^8 CFU/ml. We then mixed 200 µl cultures with the same number of PIAS (MOI 1.0) in the presence of 5 mM of $CaCl_2$. After 20 min incubation at room temperature, the suspension was mixed with 5 mL warm, soft agar (4 g/L) and poured onto LB agar. Following 64 h incubation

at 37°C, the plate's soft agar was scraped and homogenized in 5 mL of phage suspension buffer. This suspension was centrifuged and filtered through 0.22 µm pore-size PTFE filters (Sigma). Finally, we diluted phage in the suspension buffer in tenfold increments and plated them with overnight bacterial mutant culture (SNP mutant). After 24 h, we isolated single plaques and carried out purification and genome sequencing as described above. For isolation PAPSZ1 mutants, the same protocol was followed as mentioned above with minor modification. Phage lysate from 64 h incubated (37°C) plate was used to infect a fresh host and plated again for 12 continuous cycles of infection with 64-hour-long incubation. Lysate from each cycle was screened by plague assay on PA22 mutants (CR-PA22M and PR-PA22M), and a single plague was collected and later sequenced.

6.12. Isolation of PAPSZ1 phage-resistant bacterial mutants

To isolate phage-resistant bacterial mutants, 1ml of mid-log (~0.6 OD_{600}, ~1 × 10^8 CFU/ml) PA22 host grown at 37°C in LB medium was infected with the wt PAPSZ1 (~10^8 pfu/ml) and incubated at 37°C. The culture was reseeded every 24 h for 3 days into a new LB medium with a fresh PAPSZ1 phage to check the phage resistance of the bacteria obtained. During the incubation, 100 μl of broth was collected, serially diluted and spread over LB plate every 3 h beginning from the twelfth hour of incubation. The single colonies from overnight plates were sub-cultured and subjected to drop assay with wild type PAPSZ1 phage to determine host sensitivity.

6.13. Elimination of bacteria with combined PIAS phage and antibiotic therapy *in vitro*

Following a spot test, we mixed 100 µl from an overnight-grown sensitive strain (2 × 10^8 ± 0.2 × 10^8 cfu/mL) with an equal number of PIAS (MOI 1.0) and 3 mL of the molten soft agar (0.5% (m/v)) and overlaid it on the surface of the solidified LB agar (1.5% (m/v)). We allowed each overlay to solidify for 20 min and incubated them at 37°C for 48 h. To investigate the combination therapy, we plated the above mixture on solid antibiotic agar with soft agar containing various antibiotics (FSF, GMN, TET and CAZ). In addition, we spread culture without phage as control onto the antibiotic-containing agar plates. The broth susceptibility assay was carried out in 96 well plates. We ran all samples in triplicate with three independent experiments. We added 500 µl of overnight culture to 10 mL of LB in a 250 mL flask and

incubated it at 37°C, shaking it at 200 rpm, until reaching an OD_{600nm} 0.6 (~1×10^8 cfu/ml). After the required culture density was attained, we placed 180 µL of it in the wells. Bacterial samples were mixed with 10 µL of phage (MOI 1.0) and 10 µL antibiotic stock solutions in various concentrations, ranging from 10 µg/mL to 100 µg/mL (two controls were also prepared for testing the phage and antibiotic sensitivities, separately). We measured the OD_{600nm} every 3 h for 24 h using a Thermo Multiskan Ascent Plate Reader.

6.14. *In vivo* rescue experiments in mouse lung infection model

P. aeruginosa clinical strain PA16 was used as the host bacterium. We used PIAS and FSF as therapeutic agents. (Table 8, Page 66) shows the basic experimental setup**.** We assayed the potential therapies in a lung infection model using 8–10-week-old BL67 black female mice (C57/BL67 weight: 18–25 g; Charles River Laboratories). The animals were maintained in room with standard temperature (23°C ± 1°C) using 12-h dark/12-h light cycles for the bacterial challenge. PA16 grew up to OD_{600nm} ≈0.5 in 50 mL of LB broth at 37°C with shaking at 120 rpm. The bacterial suspension in the log phase was centrifuged (for 1 min at 12,000 rpm), washed with PBS, and the OD_{600nm} was set to 1.0. The suspension was diluted to 5-fold and 15-fold diluted suspension was administered through the nostrils of intraperitoneally anesthetized mice. The anesthetic used was one dose of 70 µl Combo solution (10 ml PBS, 10 ml Calypso (50 mg/ml injection; Richter Gedeon) and 150 µl primazin. Five days after the bacterial challenge, we administered PBS, FSF, PIAS phage, and their combinations for both the control and the treatment groups once intraperitoneally. All mouse experiments were conducted according to the guidelines of the European Federation for Laboratory Animal Science Associations. The Animal Welfare Committee approved all protocols and procedures involving animals of the Enviroinvest Co. (Permit Number: BAI/35/867-6/2019). To investigate whether any phage-resistant mutant colonies were formed during the *in vivo* phage treatment, we removed the organs after the end of the post-infection treatments. The lung, spleen, and brain were aseptically homogenized in sterile PBS using a tissue homogenizer. We plated homogenized tissue aliquots onto a *P. aeruginosa*–selective nutrient agar (BioRad) and incubated them at 37°C for 48 h. We selected 20 random colonies and screened them against PIAS, FSF, and E-PIASs sensitivities. In addition, we screened homogenized aliquots for phages using the double-layer agar method (Kropinski, 2009) with wild-type PA16 bacteria.

6.15. Statistical analysis

Statistical analysis was performed using one-way analysis variance (ANOVA) with dunnett's multiple comparisons test for the line chart and two-way (ANOVA) was performed with Tukey's multiple comparison test for bar graph in GraphPad Prism Version 8.4.2 (GraphPad Software). Differences were considered statistically significant with a P-value if $P<0.001$ ***, if $P<0.01$**, if $P<0.05$ *. *ns*= not significant.

7.0. RESULTS

7.1. Bacterial host and antibiogram

Strains were collected from diverse clinical samples and typed using serotyping and antibiograms. We isolated and identified 25 strains resistant to at least one antibiotic class (Fig. 8). Previously, *P. aeruginosa* isolates had been classified into three major groups. Nonetheless, a more recent revision has defined five groups of *P. aeruginosa* isolates (Freschi et al., 2019). The first linage strains are closely related to Group 1, and the second linage is closer to Group 2. Thus, most clinical isolates belong to Group 1 or Group 2.

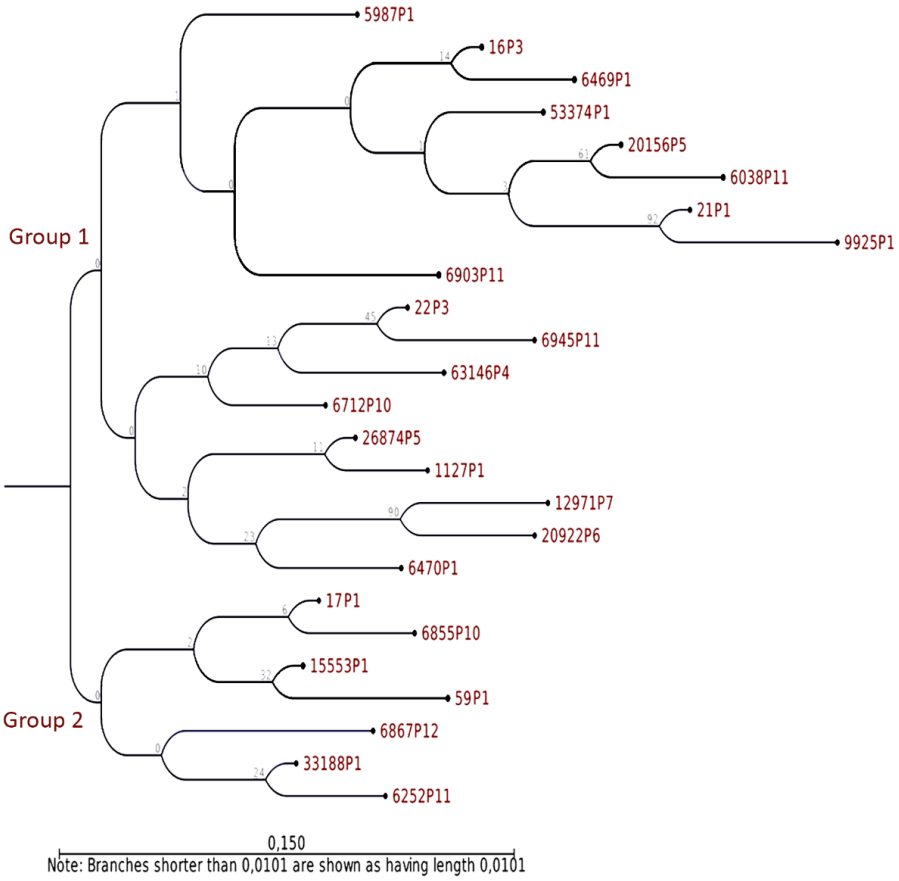


Figure 8. The Neighbor-Joining Phylogenetic tree of *P. aeruginosa* hosts isolated. The evolutionary distance was computed using the Jukes-Cantor (JC) method. Evolutionary analyses were conducted using the CLC genomics workbench 21 (version 21.0.3).

The prevalences of various serotypes were: P1 (2.5%); P10 (0.75%); P11 (1%); P3 and P5 (0.5%); and P12, P4, P6 and P7 (0.25%). Although the prevalence of *P. aeruginosa* serotype

varies from one hospital to another, P1 and P11 are often the most prevalent serotypes reported. Table 2 shows the antimicrobial susceptibility of *P. aeruginosa* isolates against 16 agents out of seven antimicrobial categories is shown in (Table 2). The highest susceptibility values pertained to polymyxins. The lowest susceptibilities belonged to meropenem (3%) and fosfomycin (5%).

Finally, we have chosen five MDR strains (PA16, PA17, PA21, PA22 and PA59) for further studies. The PA16 and PA22 strains were the primary model hosts, but many experiments were performed with all five strains.

Table 2. Drug sensitivity for twenty-five clinical isolates was determined. Drug sensitivity is shown in gray and dark-red shows drug resistance.

Sample Names	Sero-type	Amino-glycosides		Carba-penems		Cephalo-sporins		Penicillins		Monobactams & Phosponic acid		Poly-myxins		Fluoro-quinolones	
	-	GMN	AKN	MEM	DOR	CZD	FEP	TCC	PTZ	ATM	FSF	CST	PXB	CIP	LVX
PA59	P1														
PA33	P1														
PA17	P1														
PA21	P1														
PA15	P1														
PA59	P1														
PA11	P1														
PA64	P1														
PA53	P1														
PA64	P1														
PA22	P3														
PA16	P3														
PA63	P4														
PA20	P5														
PA26	P5														
PA09	P6														
PA12	P7														
PA99	P10														
PA68	P10														
PA67	P10														
PA60	P11														
PA62	P11														
PA69	P11														
PA65	P11														
PA87	P12														

7.2. Phage isolation and characterization

We isolated eight strictly lytic phages from different clinical sources (Fig. 9). Ten different *P. aeruginosa* strains of various serotypes were used for phage isolation. All of the phages were characterized by electron microscopy and biofilm inhibition assay. The phage isolates were classified based on TEM and genomic data. Six isolates (PIAS, PAPSZ1, PAPSZ2, PAPSZ3, PAPSZ6 and PAPSZ7) belonged to the family of *Myoviridae* and two isolates (PAPSZ4, PAPSZ5) belonged to *Siphoviridae* family.

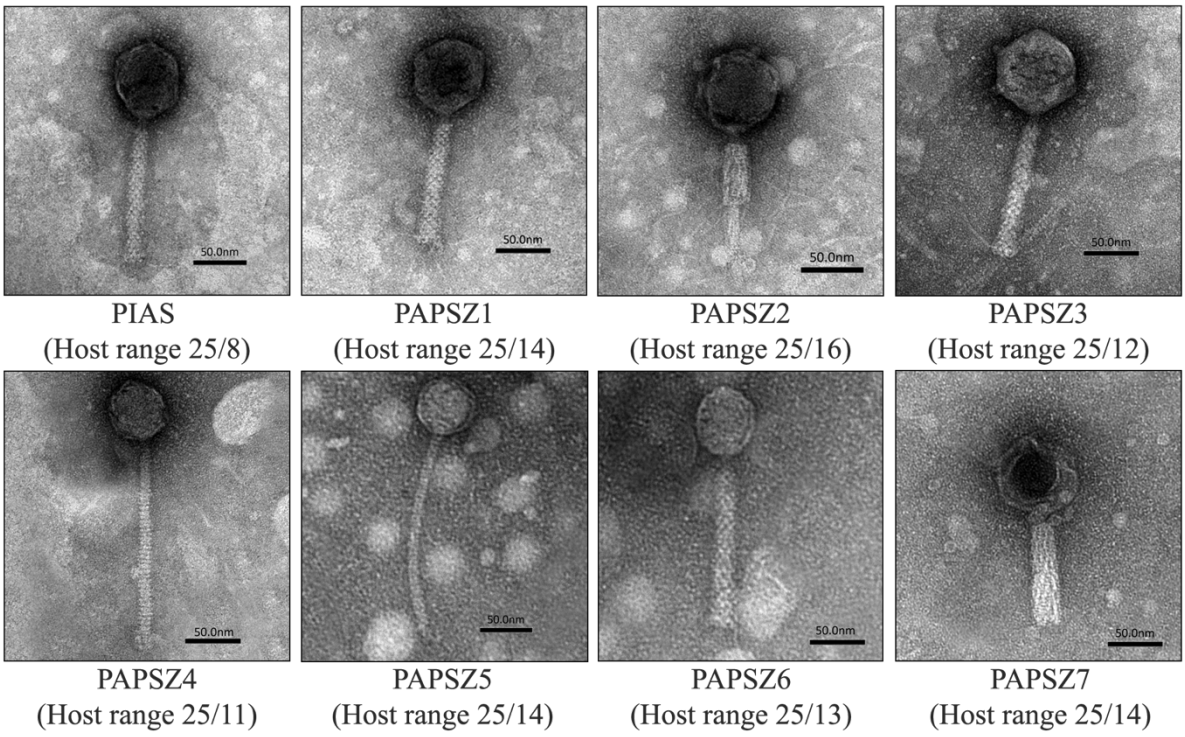


Figure 9. Transmission electron microscopy images of isolated phages. The scale bar represents 50 nm. Phage was scanned under a JEM-1400 Flash transmission electron Microscopy.

The host range was established by screening 25 isolates against phages. All isolates were shown to form clear transparent plaques in many clinical strains. The isolate PAPSZ2 showed a broad spectrum by infecting 16 strains. Meanwhile, the PIAS phage could infect only eight bacterial isolates. The PIAS and PAPSZ1 made a halo surrounding the plaques. It became larger after a few days of incubation. This may be the result of the lysis function of endolysin (Huang et al., 2013).

7.3. Biofilm inhibition assay

Research on surface-attached bacteria began almost a century ago. Nevertheless, scientists still work on understanding the significance of biofilm communities in depth. The discovery of confocal scanning laser microscopes (CLSM) has helped researchers to acquire deeper insights into the three-dimensional structure and the function of biofilms. In this study, we used light microscopy as the primary tool to visualize biofilm, and CLSM for in-depth study (Fig. 10).

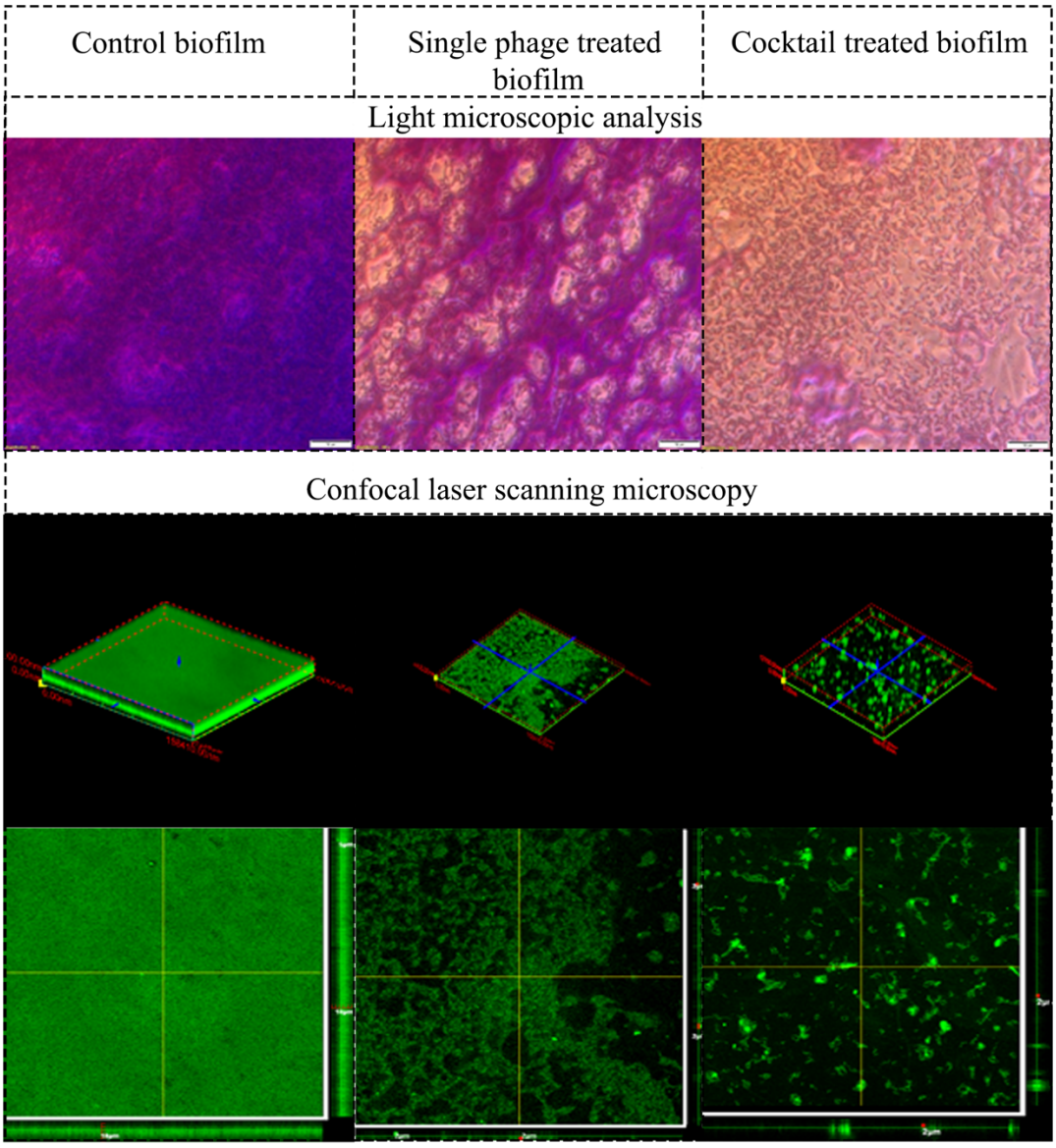


Figure 10. The light microscopy and CLSM micrograph of *P. aeruginosa* biofilm. The first column is the control biofilm (non-treated) after 48 hours, and the second shows the bacterial biofilm column treated with a single phage PAPSZ2 (~2 $\times 10^8$ pfu), and the third row displays the bacterial biofilm treated with a cocktail of six phages (PAPSZ2-7).

PA16 host was used to study the biofilm assay. The biomass and thickness of biofilm architecture were measured against phage-treated biofilms. Light microscopy and confocal micrographs showed that the non-infected (control) group displayed a more highly structured matrix formation than the treated group (Table 3).

Table 3. COMSTAT analysis of biofilm. The biomass, average thickness, and maximum thickness of biofilm architecture of *P. aeruginosa* PA16 infected with various bacteriophages. The values represent the mean of six independent scan data.

Parameters			
Phages	**Biomass (um^3/ um^2)**	**Average Thickness (um)**	**Maximum thickness (um)**
Biofilm Control	16.02 ± 2.50	20.02 ± 2.50	26.02 ± 5.00
PIAS	12.02 ± 2.50	16.02 ± 2.00	19.02 ± 3.00
PAPSZ1	12.02 ± 2.50	16.02 ± 2.50	20.02 ± 3.00
PAPSZ2	8.02 ± 2.00	10.02 ± 2.00	16.02 ± 3.00
PAPSZ3	12.02 ± 2.50	16.02 ± 2.02	21.02 ± 3.02
PAPSZ4	10.02 ± 2.50	12.02 ± 2.50	20.02 ± 2.50
PAPSZ5	14.02 ± 2.50	14.02 ± 2.02	20.02 ± 3.00
PAPSZ6	13.02 ± 2.50	15.02 ± 2.00	23.00 ± 3.00
PAPSZ7	11.02 ± 2.50	14.02 ± 2.02	20.02 ± 2.00
Phage Cocktail	4.02 ± 2.00	6.02 ± 2.00	12.02 ± 2.00

The phage PAPSZ2 disrupted biofilm most effectively. Six of the most efficient phages (PAPSZ2–PAPSZ7) were mixed to prepare a phage cocktail. The cocktail-treated biofilm contained disintegrated clumps with scattered microflora, which resulted in the collapse of thick biofilm. Table 3 shows the results of further analyses with COMSTAT-definite CLSM

micrographs. The phage PAPSZ2 showed high antibiofilm activity. The structural difference between the biofilm and the phage-treated biofilm indicates the phages' biofilm-disrupting efficacy. The decrease in biomass, average thickness and maximum thickness in treated micrographs confirm the ability of phages to break down the biofilm architecture.

7.4. Phage PIAS and PAPSZ1

The phages PIAS and PAPSZ1 were selected for further study based on their ability to induce phenotypic changes within target bacteria. The growth of phage-resistant colonies after prolonged incubation (24–64 h) was observed for both phages. Nonetheless, the number of these colonies varied in terms of number-based host and phage number. Both phages were further investigated in one-step growth experiments and genome sequencing. PIAS and PAPSZ1 phages belong to the *Myoviridae* phage family. Both phages formed massive plaques on all sensitive clinical strains surrounded by haloes (Fig. 11).

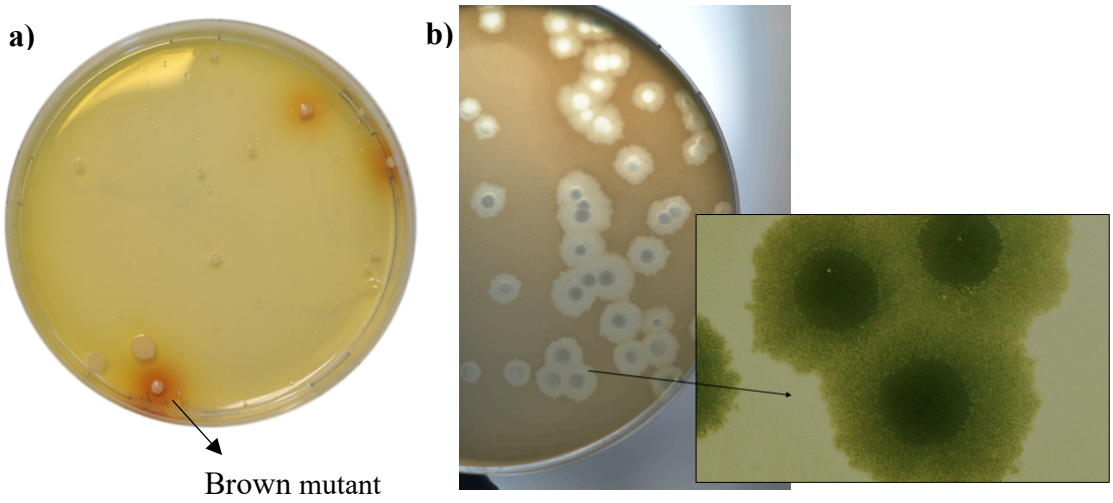


Figure 11. Phage PIAS and PAPSZ1 infection a) Phage resistant PA16 red colonies after PIAS phage infection. **b)** The massive plague of PIAS and PAPSZ1 phages with surrounding haloes.

The phage PIAS has a narrow host range, which infected 8 of 25 clinical strains. PAPSZ1 infected 16 isolates. No host serotype specificity could be observed for either of these phages. Using the PA16 strain, the latent periods of PIAS and PAPSZ1 were between 55–60 min. The burst size of PIAS and PAPSZ1 phages were approximately ~110 and ~80 PFU per infected cell, respectively. Fig. 12 shows that approximately 90% of the phages were adsorbed to host

cells within 30 min. The PIAS phage had a larger bust size compared to that of PAPSZ1. PIAS also induced more brown mutants than PAPSZ1.

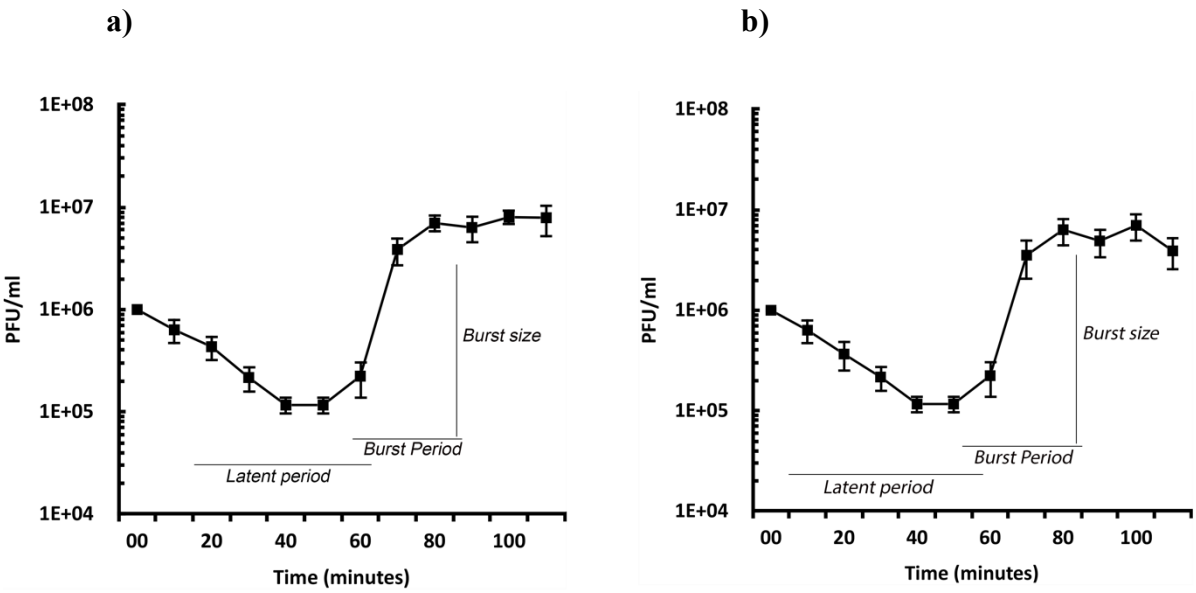


Figure 12. One-step growth curves of phage PIAS and PAPSZ1. The plot represents the values of three independent experiments. The latent phase of PIAS and PAPSZ1 takes approximately 55-60 min; PIAS phage can produce about ~110 phages and PAPSZ1 ~80 progeny per infected cell. Each experiment was done in triplicate. Error bars represent SD for three replicates.

To assess the PIAS and PAPSZ1 phage's host killing efficiency, culture was grown in LB broth and infected with PIAS and PAPSZ1 using different MOI values (0.1, 1.0, and 10.0). Bacterial growth was monitored by measuring optical densities at OD_{600} nm. The phage infection inhibited the bacterial growth at MOI ≥ 1.0, a result that grew progressively more prominent as MOI increased. The optical density of the culture decreased at approximately 3 h post-infection for all three MOI values (Fig. 13). At a higher phage titre (MOI 10), bacterial growth utterly ceased for both phages. At a lower phage titre (MOI 0.1), bacterial growth was higher than observed when the cells were infected at both MOI 1.0 and MOI 10, but it was still lower than in the case of non-infected cells.

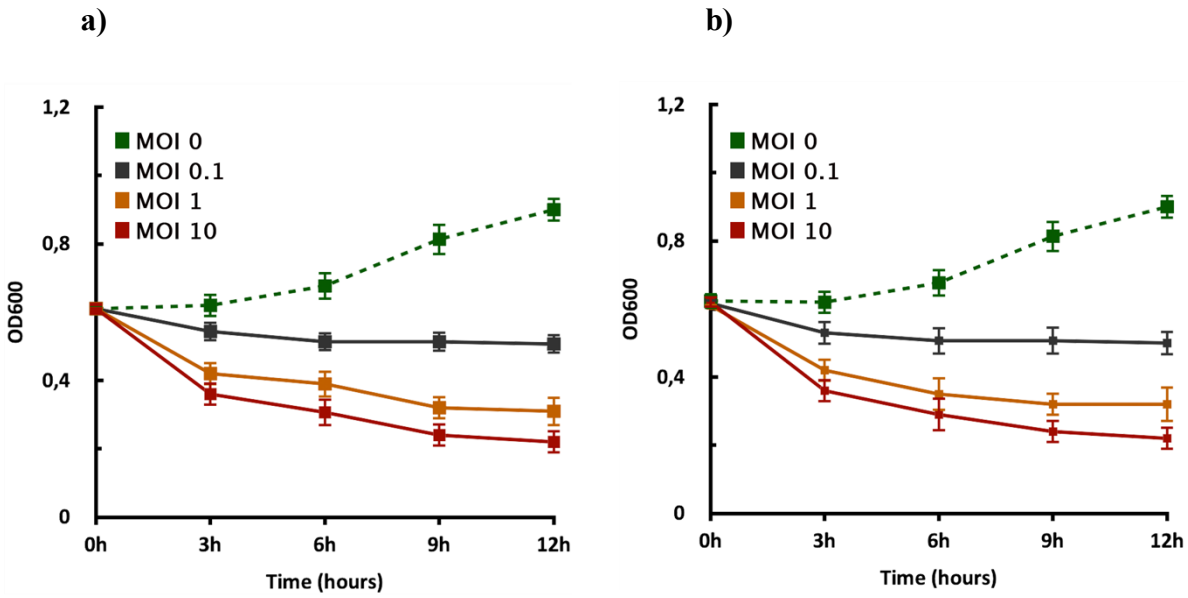


Figure 13. Growth curves of PA16 host infected by the phage PIAS and PAPSZ1 at different MOI. At a high phage titer (MOI 10), bacterial growth was inhibited by phage infection (black). When an equal ratio of phage/bacteria was used (MOI 1), a growth increase was observed (mutant growth) after 16 hours (red). At lower phage titer (MOI 0.1), the bacterial growth inhibition could hardly be observed. The non-infected sample was used as a control (green). Each experiment was done in triplicate, and OD_{600nm} values were measured, averaged, and plotted. Error bars represent SD for three replicates.

7.5. Phage genome sequencing and comparison

The complete genomes for PIAS and PAPSZ1 were sequenced, assembled and annotated (Bioproject: PRJNA722489). Phage annotation against phage sequences in Uniprot provided hits for only 15 out of 181 predicted CDS regions. Phage phylogenetic analysis revealed that PIAS was closely related to the phages PaP1 (Lu et al., 2013) and PaoP5 (Shen et al., 2016) (Fig. 14a)

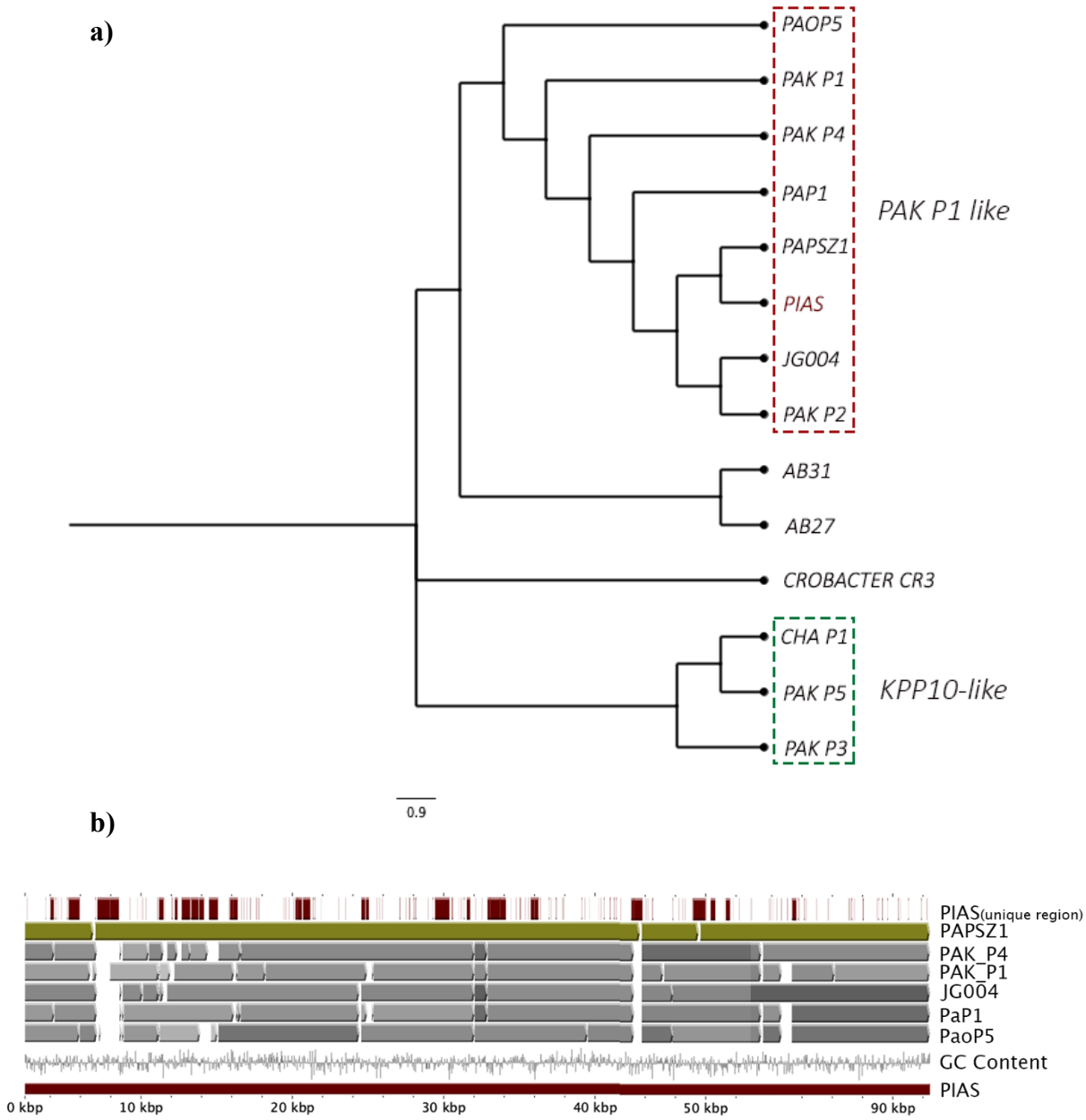


Figure 14. Genome-wide comparison of PIAS and PAPSZ1 to other selected *P. aeruginosa* phages. a) Phylogenetic analysis of PIAS and few related phages. **b)** Whole genome comparison of PIAS to closely related phages.

Table 4. Characteristics of the genomes of PIAS, PAPSZ1 and other *P. aeruginosa* PAK_P1-like and KPP10-like phages

Clade	Name	Genome length	ORFs predicted	GC content	tRNA
PAK_P1-like	PAK_P1	93398	181	49.50%	13
	PAK_P2	92495	176	49.30%	11
	PAK_P4	93147	174	49.30%	13
	JG004	93017	161	49.30%	12
	PaP1	91715	157	49.40%	13
	PIAS	92 397	181	49.29%	15
	PAPSZ1	92 261	181	49.30%	15
KPP10-like	PAK_P3	88097	165	54.80%	3
	PAK_P5	88789	164	54.70%	3
	CHA_P1	88255	166	54.60%	3

Previous studies have identified these phages as a separate group called PAK-P1 like phages (Henry et al., 2015). This group of phages clusters separately from other *P. aeruginosa* phages. Multiple whole genome comparisons of PIAS and PAPSZ1 against other Pak-P1 phages suggests the phages share close synteny (Fig. 14b). Phage annotation with closely related phage sequences present in Uniprot provided annotations only for 15 CDS regions out of 181 predicted CDS regions (Table. 4).

7.6. Formation of PIAS and PAPSZ1 phage resistant strains

PIAS and PAPSZ1 phage infections led to the sequential appearance of colonies with two phenotypes (Fig. 15). The initial set of colonies were green/pale, then these colonies turned to brown in the presence of phages.

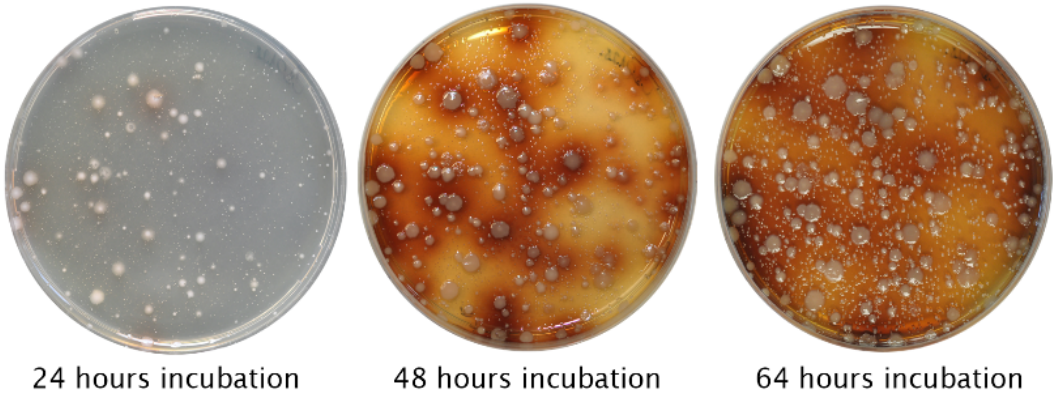


Figure 15. Appearance of resistant colonies after phage infection. The color of mutant colonies shifted from green or pale to brown over a period.

Colonies of these two phenotypes could be observed with the other four strains, as well. However, the time elapsed till the appearance of the differently colored variants depended on strains and MOI values. In a double-layer soft agar plate, primarily green or pale. Notably, no green or brown colonies appeared in the absence of phage infection after several replating of the hosts, therefore the phenotypic (and genotyping see below) changes were evidently phage-induced or phage-provoked. At higher MOI, there were fewer phage-resistant colonies for many clinical strains (Fig. 16). The average frequencies of PIAS mutant in the five studied strains were: at MOI 0.1: ~311 ± 20 × 10^{-8} for green and ~183 ± 25 × 10^{-8} for brown colonies; at MOI 1: ~120 ± 22 × 10^{-8} for green and ~238 ± 24 × 10^{-8} for brown; at MOI 10: ~43 ±10 × 10^{-8} for green and ~53 ± 10 × 10^{-8} for brown colonies (Fig. 16a).

The average mutant frequencies of the five studied strains were lower in the case of PAPSZ1 than PIAS infections: at MOI 0.1: ~263 ± 28 × 10^{-8} for green and ~160 ± 20 × 10^{-8} for brown colonies; at MOI 1: ~60 ± 20 × 10^{-8} for green and ~73 ± 23 × 10^{-8} for brown; at MOI 10: ~23 ±15 × 10^{-8} for green and ~30 ± 10 × 10^{-8} for brown colonies (Fig. 16b). A higher MOI always generated a lower incidence of phage-resistant colonies. Therefore, we used MOI 1 as a standard for further experiments.

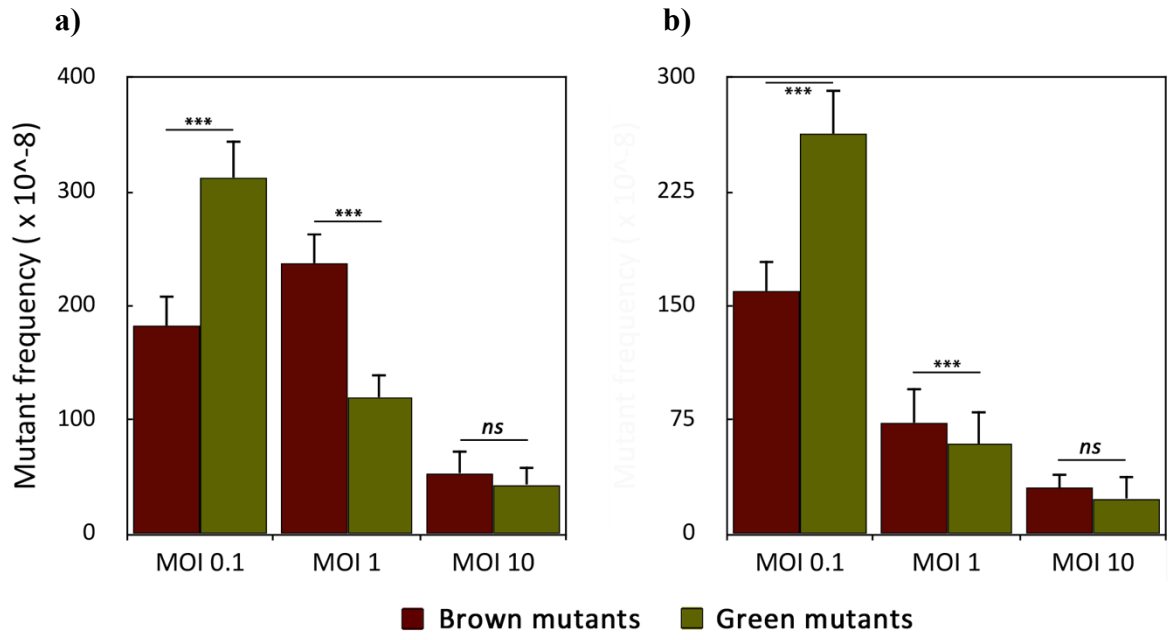


Figure 16. Bacterial mutant frequencies. MOI dependence of the frequencies of the green and brown colony formation of a) PIAS and b) PAPSZ1. For statistical analysis, two-way ANOVA was performed with Tukey's multiple comparison test. *** if P<0.001 and ns= not significant.

7.7. PIAS phage and its mutual evolutionary effect on the bacterial hosts

7.7.1. Genome characterization of PIAS phage resistant mutants

Phage resistant colonies were cultivated for isolating and sequencing their genomes. Again, we used the PA01 genome as the reference genome. As shown in Table 5 and Fig. 17, the genomes of all green colonies harbored SNPs and relatively small deletions (20–80 kbp**)**, while we observed large deletions (274–417 kb) in all the brown colonies. Most importantly, the mutated genomic regions of the green and the brown mutants were very similar (from 2,000 to 2,500 kbp).

Table 5. Single nucleotide genetic variations in the *mexY* gene of the green/pale mutant of various *P. aeruginosa* strains. All strains were resistant against PIAS but could be infected by E-PIASs (see below).

Mutated Gene	Strain	Mutation	Annotation
MexY-Multidrug efflux RND transporter subunit (MexXY-OrpM)	G16	C→A	T408N (AC<u>C</u>→A<u>A</u>C)
	G21	829bp	MC
	G22	G→A	Intergenic (–/+520)
	G17	G→T	E658 (A<u>G</u>G→A<u>T</u>G)
	G59	80bp	MC
	IVA16	C→A	T408N (AC<u>C</u>→A<u>A</u>C)
	IVB16	C→A	T408N (AC<u>C</u>→A<u>A</u>C)
	IVC16	C→A	T408N (AC<u>C</u>→A<u>A</u>C)
	IVD16	C→A	T408N (AC<u>C</u>→A<u>A</u>C)

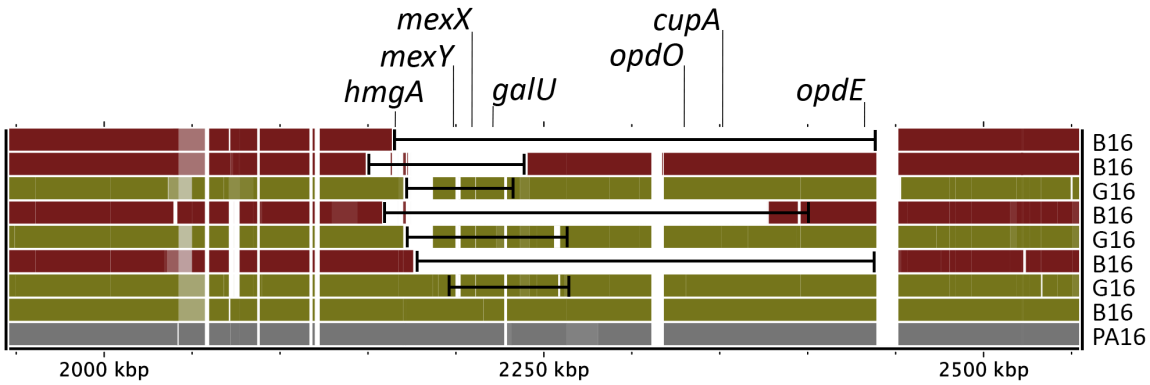


Figure 17. Mutated genomic regions. Alignment of the genomic deletions of PA16 green and brown mutants in the region of 2000 kbp-2500 kbp.

Deeper analysis of the mutations in the green colonies disclosed that common SNPs occurred in the *mexY* gene (Table 5), which might hinder the wild type PIAS phage infection of the green mutants. On the other hand, the larger deletion in the brown mutants occurring in the genome's 2000–2500 kb region removed the *mexY* gene with many genes involved in membrane integrity, transport, *quorum sensing*, pigmentation, and antibiotic resistance (Table S2).

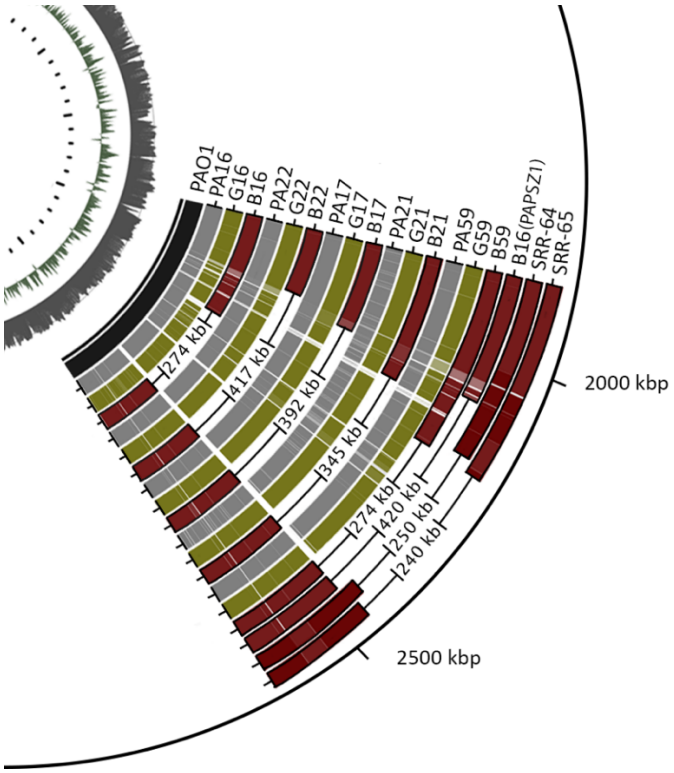


Figure 18. Genomic deletions. The mutated genomic regions of the wild type, green (G) and brown (B) PA16, PA17, PA21, PA22, PA59 mutants induced by either PIAS or PAPSZ1.

We compared the mutated genomic regions of the brown mutants to those presented in a previous study (Shen et al., 2018) (Figure 18, SRR64, SRR65). Despite of using different phages and hosts, the deleted regions in the brown mutants were similar in both studies. We note that Shen et al. (date?) used the PaoP5 phage, which is also a member of PAK-P1-like phage family (Fig. 14). The pattern of smaller deletions in various PA16 green mutants (Fig. 17) suggested that the formation of the large deletions might have occurred via a continuous multistep evolutionary process that began with the emergence of SNPs, followed by smaller than larger deletions.

7.7.2. Antibiotics'MIC assay for PIAS phage resistance mutant

As mentioned above, five MDR *P. aeruginosa* clinical strains were selected for detailed study (PA16, PA17, PA21, PA22, and PA59). The antibiotic sensitivities of these strains were screened by the disk diffusion method, followed by a minimum inhibitory concentration (MIC) assay. In the case of four strains, the MIC of gentamicin (aminoglycosides) was ≥45 μg/mL; for two strains, the MIC of ceftazidime (antipseudomonal cephalosporins) was ≥45 μg/mL. For all five strains, the MICs of fosfomycin (phosphonic acid) and tetracycline (polyketide) were ≥180 μg/mL and ≥93 μg/mL, respectively (Table 6).

Table 6. Antibiotic's MIC values in the case the wild-type and phage-provoked green and brown mutants of various MDR *P. aeruginosa* clinical isolates.

Antibiotic	Strain	Antibiotic's MIC		
		Wild-type	Green mutant	Brown mutant
Tetracycline	PA16	173.3±11.1	19.3±1.5	16.6±1.5
	PA21	126.6±11.5	16.6±1.5	12.6±1.5
	PA22	93.3±11.5	18.6±1.5	14.6±1.5
	PA17	126.7±11.5	21.3±1.5	18.6±1.5
	PA59	130.0±10.0	18.7±1.5	18.7±1.5
Fosfomycin	PA16	201.6±2.8	29.3±3.0	20.3±2.5
	PA21	180.0±0.0	28.0±0.0	21.3±4.1
	PA22	200.3±20.0	31.6±2.8	27.6±2.5
	PA17	193.3±11.5	26.6±1.5	31.6±2.8
	PA59	193.3±11.5	40.0±5.0	35.6±5.0
Ceftazidime	PA16	30.0±2.0	8.6±1.5	5.3±1.1
	PA21	45.3±1.1	7.3±1.5	6.6±1.2
	PA22	30.6±1.1	9.3±1.2	5.3±1.2
	PA17	46.7±1.1	10.0±2.0	6.6±1.1
	PA59	46.7±1.1	17.6±2.5	11.6±2.5
Gentamycin	PA16	51.6±2.8	21.6±2.5	15.0±2.5
	PA21	61.7±2.8	21.6±2.5	15.0±2.5
	PA22	51.6±2.8	12.0±2.5	10.0±2.5
	PA17	45.0±5.0	12.0±2.5	10.0±2.5

The MIC of the above antibiotics were significantly changed - dominantly dropped – for the mutants (Table 6). The MICs of gentamicin for green and brown mutants were ≤ 21 μg/mL and ≤ 15 μg/mL, respectively; the MICs of ceftazidime for green and brown mutants were ≤ 10

µg/mL and ≤ 6 µg/mL, respectively. The MICs of fosfomycin and tetracycline for green mutant and brown mutants were ≤ 40 µg/mL and ≤ 35 µg/mL, respectively.

7.7.3. Membrane integrity

The membrane integrity of bacterial cells is a well-accepted criterion for characterizing their virulence. A break in the integrity of the cell membrane immediately compromises its essential role as a barrier, which can seriously endanger the cell. Whole-genome analyses of the mutant bacteria disclosed that they lost many membrane-related genes (Table S2). Furthermore, examination of the deleted regions indicated that the brown bacteria lost many genes of proteins having membrane-related functions such as cell adhesion, cell surface receptor, pilus organization, genes responsible for regulating biosynthesis of important organic molecules, and cell wall organization.

To investigate the impact of these gene deletions, we measured the membrane sensitivity to bile acid (Fig. 19). All five brown mutants exhibited higher sensitivity to bile acid than wild-type bacteria did. The average MIC concentrations of bile acid for wild type, green and brown mutants were 6 % (±0.5%), 4% (±1%) and 2.5 % (± 0.5%), respectively. Conversely, the bile acid sensitivity of the green mutants was almost similar to that of the wild type.

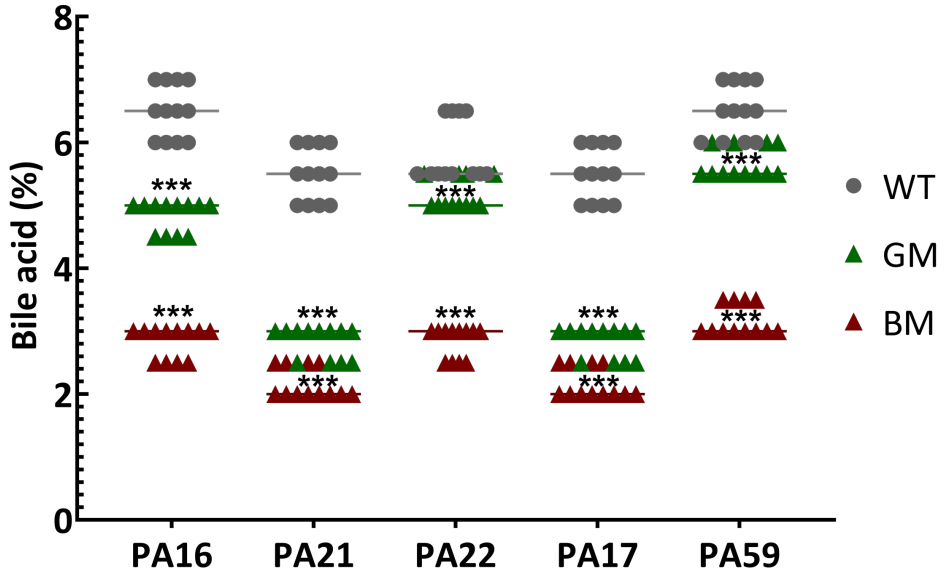


Figure 19. The MIC concentrations of bile acid for the wild type and mutant strains. GM: green mutants, BM: brown mutants. For statistical analysis, two-way ANOVA was performed with Tukey's multiple comparison test. *** if P<0.001.

7.7.4. Screening receptor-based knock-out library for phage sensitivity and adsorption

Bacteria evolve phage resistance by alerting, losing, or masking phage receptors from binding (Dowah & Clokie, 2018). In such a mutant, the phage can not be adsorbed and multiply. The genomic analysis of the mutants identified a few potential targets as phage receptors. We screened 12 knock-out mutants (Fig. 20 and Table S2) for PIAS sensitivity.

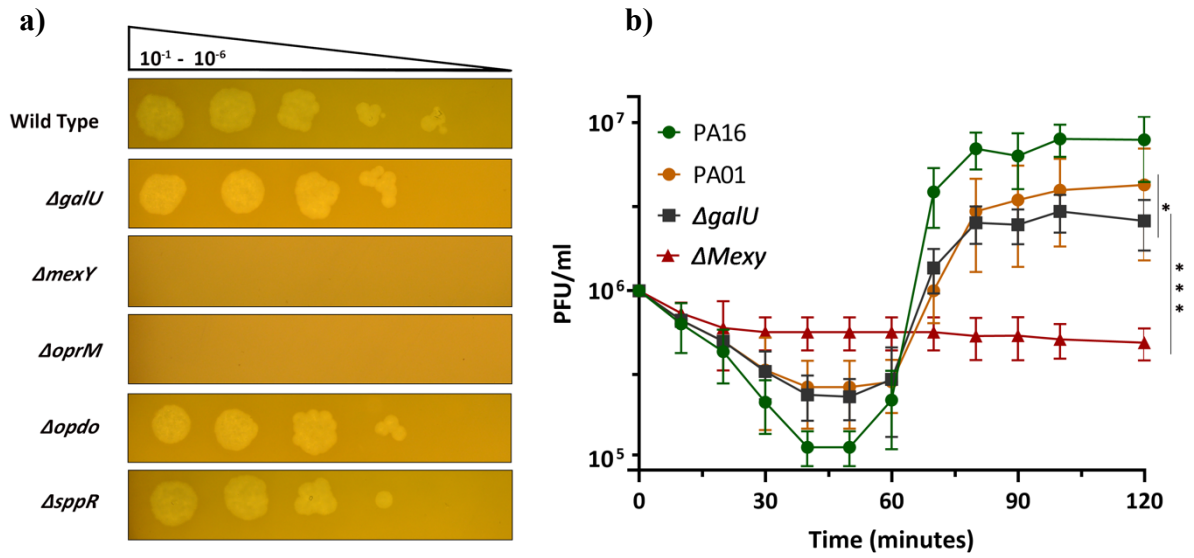


Figure 20. PIAS phage sensitivity of the PAO1 transposon mutant strains. a) PIAS plaque spot assay on various PAO1 transposon mutants (Table S2). **b)** PIAS adsorption assay with the PA16 and PAO1 wild-type strains as well as selected PAO1 transposon mutants. Each experiment was done in triplicate. Error bars represent SD for three replicates. For statistical analysis, one-way ANOVA was performed with Tukey's multiple comparison test. *** if $P<0.001$, * if $P<0.05$.

The *ΔoprM* and *ΔmexY* mutants were completely resistant to PIAS, but the *ΔgalU* strain (GalU is responsible for the biosynthesis of LPS core region) was still sensitive (Fig. 20a,b). We then quantified unadsorbed phages after a brief incubation period, revealing defects in binding. Adsorption was lower to both the wild-type PAO1 strains and transposon knock-out mutants than to our wild-type clinical strains. At a set point, adsorption to the PAO1 and *ΔgalU* PAO1 strains was ~40% less than to the wild type clinical strain (PA16). No PIAS adsorption to the *ΔmexY* and *ΔoprM* PAO1 mutants could be observed.

7.7.5. Isolation of co-evolved PIAS phage mutant E-PIASs

To study the (evolutionary) reason of the two types of mutants (pale and brown), we first isolated early pale mutants of PA16, PA22, PA21 and PA17. We used whole-genome sequencing to confirm the SNPs. Then, we were able to isolate phages capable of infecting these green mutants from previously infected plates. Phage lysates were collected and plated with these green mutants. Next, we purified, propagated phages from single plaque(s) capable of infecting green mutants from various hosts. Their genetic materials were purified and sequenced (Bioproject: PRJNA722489). Comparison of their genomes to that of the wild-type PIAS revealed that phages had SNPs in the phage tail fiber protein (Table 7). These mutations enabled the phages to infect the green bacterial mutant.

Table 7. Single nucleotide genetic variations in the phage tail fiber protein of various E-PIAS phages multiplied on various green hosts.

Mutated Gene	E-PIAS	Mutation	Annotation
Tail fiber protein	E-PIAS H16	T→G	I639S (AT<u>C</u>→AG<u>C</u>)
	E-PIAS H22	T→G	I639S (AT<u>C</u>→AG<u>C</u>)
	E-PIAS H21	G→A	H635Y (T<u>G</u>A→T<u>A</u>A)

7.7.6. Frequency of the formation off E-PIAS phage cocktail resistance mutants

Mutation frequencies were also examined with E-PIAS phage cocktail (E-PIAS H17+ E-PIAS H21+ E-PIAS H16) and first-line SNP bacterial mutants. At higher MOI, there were fewer phage-resistant colonies relative to wild type PIAS phage. The average mutation frequencies of the wild type hosts infected with E-PIAS cocktail for the five studied strains were: at MOI 0.1: ~25 ± 5 × 10^{-8} for green and ~68 ± 10 × 10^{-8} for brown colonies; at MOI 1: ~3 ± 1 × 10^{-8} for green and ~8 ± 5 × 10^{-8} for brown; at MOI 10 there was no growth observed (Fig. 21). The average mutation frequencies of the five PIAS resistant green mutant strains infected with E-PIAS were: at MOI 0.1: ~9 ± 5 × 10^{-8} for green and ~50 ± 10 × 10^{-8} for brown colonies; at MOI 1: ~4 ± 2 × 10^{-8} for brown and no green mutant were observed. At a higher MOI (MOI 10), E-PIAS cocktail completely eradicated the bacterial growth.

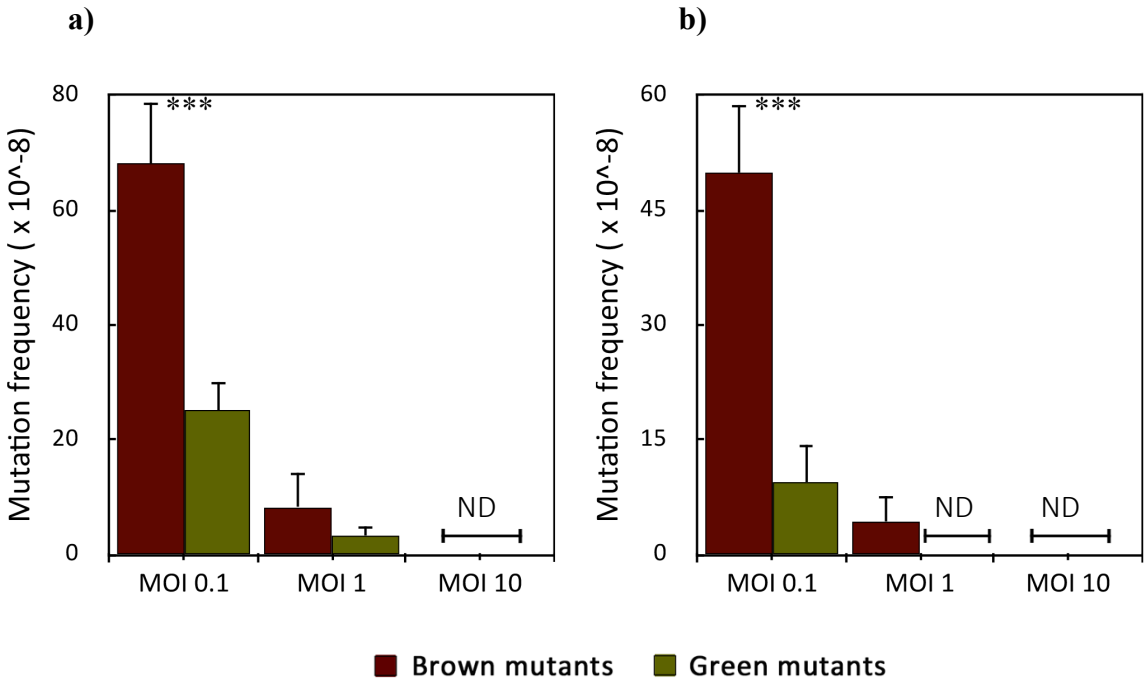


Figure 21. Mutation frequencies of various hosts infected with E-PIAS phage cocktail. a) Bar graph represented the green and red/brown mutant frequencies when wild type *P. aeruginosa was* infected with E-PIAS phage cocktail. **b)** Bar graph represents the frequency of green and brown mutant formations when SNP green mutant hosts were infected with E-PIAS phage cocktail. Each experiment was done in triplicate, ND - Not detected. Error bars represent SD for three replicates. For statistical analysis, two-way ANOVA was performed with Tukey's multiple comparison test. *** if $P<0.001$.

7.7.7. Challenging bacteria with phage and phage antibiotic combinational therapy

One of the mostly discussed potential drawbacks of phage therapy is the possible rise of phage-resistant bacterial mutants that evolve and proliferate during treatment. Genome analysis of the evolved bacterial mutants revealed that the bacteria had an altered membrane-related gene product MexY (of MexXY-OprM system) for self-defense against PIAS. This protein plays a vital role in the RND drug efflux system involved in antibiotic resistance (Masuda et al., 2000). We first determined the MIC values of four antibiotics for all five clinical strains and their mutants for (Table 6). In all cases, the phage-provoked mutants were substantially more sensitive to all antibiotics. This means that the formation of phage resistance comes at the cost of increased drug sensitivity. We used this window as a strategy for comprehensively eradicating mutants by treating the Pa16 wild-type strain with an antibiotic (fosfomycin, gentamycin, tetracycline and ceftazidime) combined with PIAS. A control plate with

gentamycin but without phage had visible colonies after 24 h (Fig. 22a). Upon phage treatment, the mutant formation described above could also be observed (Fig. 22b). The PIAS-antibiotic combination completely prevented the formation and growth of mutants after 48 h incubation (Fig. 22c).

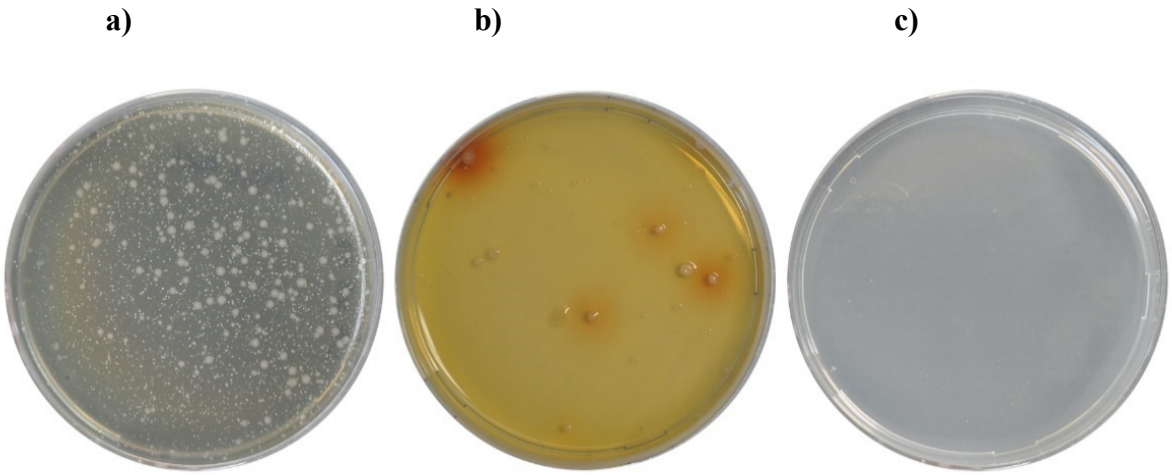


Figure 22. Combination therapy on solid agar. PA16 plated with **a)** antibiotic (30 µg/ml GMN), **b)** with PIAS (~2 × 10^8 pfu) and **c)** with PIAS (~2 ×10^8 pfu) and antibiotic (30 µg/ml GMN).

We confirmed this in a planktonic assay using four antibiotics (Fig. 23 a,b,c,d). We found that combination therapy outperforms either phage or antibiotic treatments alone. Notably, the same strategy can be achieved by many other antibiotics against which bacterial resistance is based on the RND system.

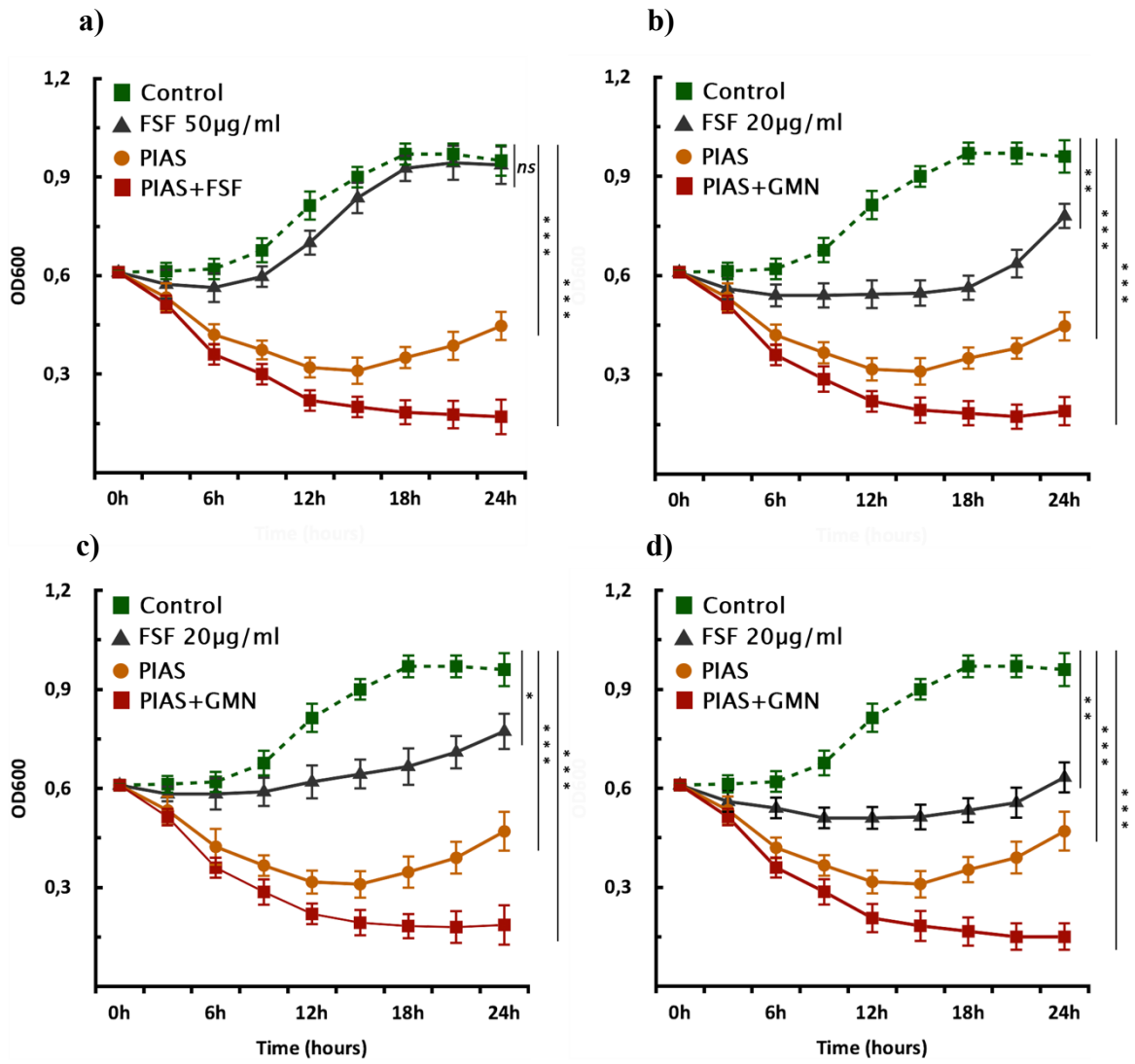


Figure 23. Combination therapy in liquid broth (LB). The single and combined effects of phages and antibiotics on cell growth as compared to the non-treated cells. The PIAS (~2 ×10^8 pfu) and the following antibiotics: **a)** FSF 50µg/ml, **b)** GMN 30 µg/ml, **c)** TET 30 µg/ml and **d)** CAZ 30 µg/ml were used for the combinational therapy. For statistical analysis, one-way ANOVA was performed with Tukey's multiple comparison test. *** if P<0.001, ** if P<0.01, * if P<0.05, ns=not significant.

7.7.8. *In vivo* rescue experiment in the mouse lung infection model

Fig. 24 illustrates the scheme of the experiments and Table 8 summarizes our experimental setup and results. We first tested the effects of controls - phosphate-buffered saline (PBS), high dose of phage, and FSF - on the mice. None of these had a drastic effect on the animal during the 10-day monitoring period (Table 8, Group 1-3).

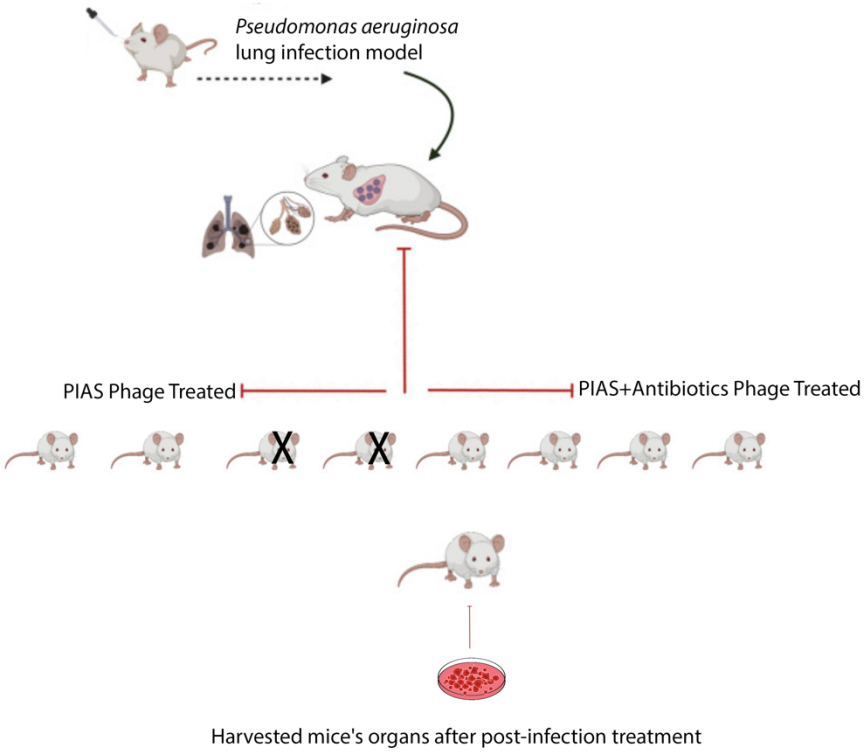


Figure 24. Schematic representation of *in vivo* experiment.

Infection doses in the applied bacterial lung model adapted to PA16 were established in preliminary studies. The administration of 5×10^6 cfu/mouse was fatal and killed all experimental animals in two days. Conversely, administration of one-third of this bacterial load [15× dilution (15×)] ensured the survival of 75% of the animals (Table 8, Group 4). Therefore, the experiments with 5×10^6 cfu/mouse doses will be discussed here. In the case of the 5× bacterial dilution, the survival ratios were modified minimally if either PIAS - at any applied MOI (0.2, 1, and 5) - or 1 mg FSF was administered intraperitoneally to the challenged groups. However, this situation changed drastically if 1 mg FSF and PIAS were used in combination as therapeutic agents. In the case of PIAS (MOIs 1 and 2) combined with FSF, 25% of the animals

survived, and their lifespans were elongated in some cases. In the case of MOI 5 applied with FSF, 75% of the animals survived (Table 4, Group 7). Combination therapy ensured 100% survival of all mice in groups in which the bacterial challenge was performed with the 15× diluted bacterial inoculum.

Table 8. Treatments and survival rates in the in *vivo* rescue experiments in the mouse lung infection model.

Group	Bacterium dilution	Bacterium cfu/mouse	FSF mg/mouse	Phage MOI	Phage pfu/mouse	Survival (%)
						10 days
1	**A. PBS control**					
	-	-	-	-	-	100%
2	**B. PIAS control**					
	-	-	-	-	25,000,000	100%
3	**C. fosfomycin control**					
	-	-	1.0	-	-	100%)
4	**D. Bacterium PA16 dilution controls**					
	5x	5,000,000	-	-	-	0%
	15x	1,500,000	-	-	-	75%
5	**E. Bacterium PA16 + Phage $PIAS_{PA16g}$**					
	5x	5,000,000	-	0.2	1,000,000	0%
	5x	5,000,000	-	1.0	5,000,000	0%
	5x	5,000,000	-	5.0	25,000,000	25%
6	**F. Bacterium PA16 + fosfomycin**					
	5x	5,000,000	1.0	-	-	0%
7	**G. Bacterium PA16 + fosfomycin + Phage $PIASPA_{16g}$**					
	5x	5,000,000	1.0	0,2	1,000,000	25%
	5x	5,000,000	1.0	1.0	5,000,000	25%
	5x	5,000,000	1.0	5.0	25,000,000	75%

Infection doses in the applied bacterial lung model adapted to PA16 were established in preliminary studies. The administration of 5×10^6 cfu/mouse was fatal and killed all experimental animals in two days. Conversely, administration of one-third of this bacterial load [15× dilution (15×)] ensured the survival of 75% of the animals (Table 8, Group 4). Therefore, the experiments with 5×10^6 cfu/mouse doses will be discussed here. In the case of the 5× bacterial dilution, the survival ratios were modified minimally if either PIAS - at any applied

MOI (0.2, 1, and 5) - or 1 mg FSF was administered intraperitoneally to the challenged groups. However, this situation changed drastically if 1 mg FSF and PIAS were used in combination as therapeutic agents. In the case of PIAS (MOIs 1 and 2) combined with FSF, 25% of the animals survived, and their lifespans were elongated in some cases. In the case of MOI 5 applied with FSF, 75% of the animals survived (Table 4, Group 7). Combination therapy ensured 100% survival of all mice in groups in which the bacterial challenge was performed with the 15× diluted bacterial inoculum.

We recovered PA16 bacterial colonies from many organs, including the brain, spleen, and lung (Fig. 24). We screened 20 isolates from each organ against FSF, phage PIAS, and E-PIASs. We recovered fewer bacteria from the brain than from either spleen or lung. Conversely, we found a higher number of bacterial mutants in lung than in other organs. Approximately 4% of the isolated colonies from lung and 2% of isolates from spleen were mutants. We isolated one brown colony and two green colonies from the lung. The brown colony was sensitive to FSF (> 50 μg/ml), and the two green colonies displayed decreased MIC for FSF and were sensitive to E-PIAS. Two mutant colonies in spleen had decreased MIC for FSF and were sensitive to E-PIAS. We found no mutant colonies in brain isolates. We also screened the homogenized lung solution for phages. We selected multiple plaques and screened them against PA16g mutants and wild-type strains. Phage isolates were able to infect the wild-type PA16 but not the PA16g mutant strains. We could not detect any phages in the other organs.

7.8. PAPSZ1 phage and its coevolution with the host

7.8.1. Isolation of *P. aeruginosa* PAPSZ1 phage-resistant mutants

To get a deeper insight into bacterial phage resistance, we infected PA22, with PAPSZ1 (Fig. 25a). Next, we tested PA22 phage resistance by reseeding the PA22-C1 (infection cycle 1) sampled at 24 h into a fresh LB medium with a fresh PAPSZ1 phage to generate more mutations. This cycle repeated for 64 h until three continuous infection cycles had been reached (PA22-C1,-C2,-C3).

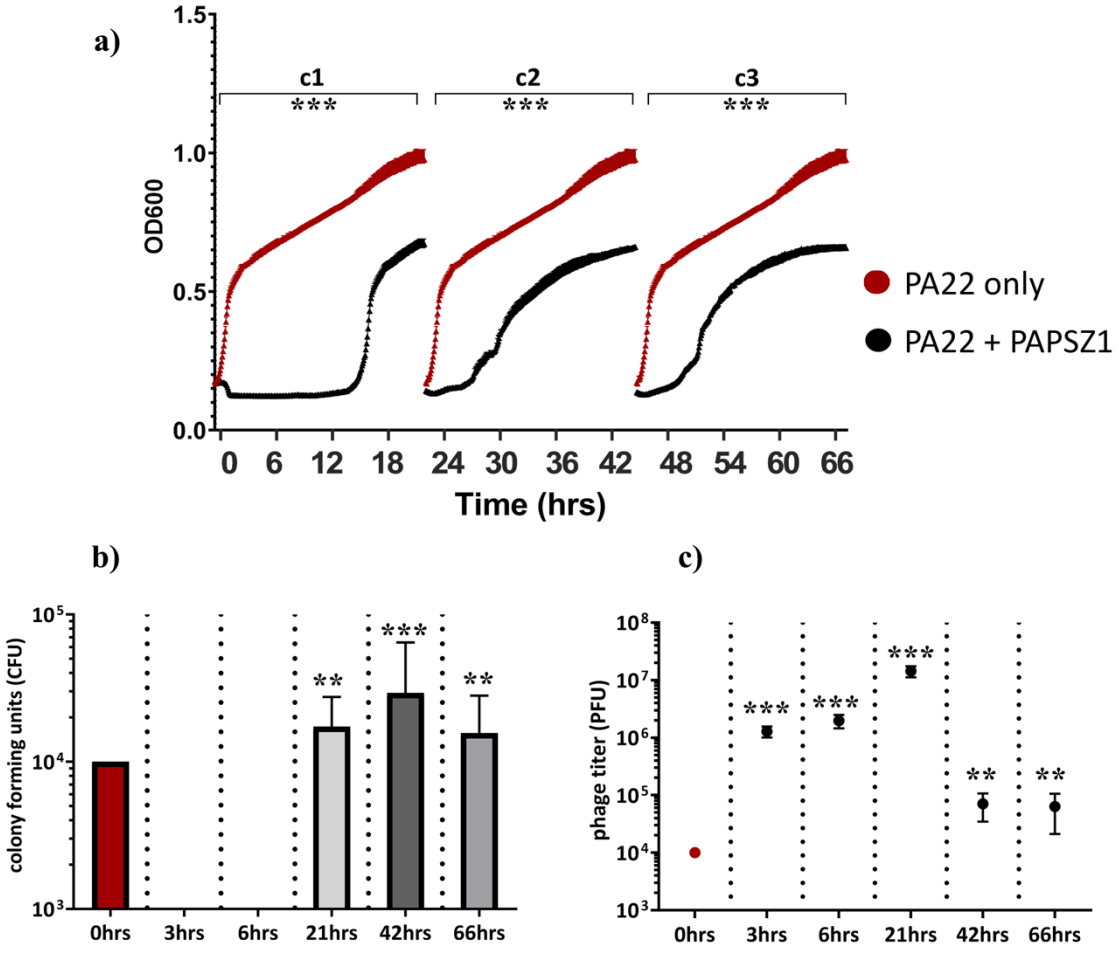


Figure 25. a) Bacterial kinetic growth curve of PA22 (red) and PA22 (c1, c2, c3) infected with PAPSZ1 (black). PA22 cultures were infected with PAPSZ1 and reseeded into a fresh LB medium every 24 h for 64 h. Data are shown as the mean ± SD from three experiments. **b)** Bacterial titre at the given time points. **c)** Phage titres of the evolved PAPSZ1 lysates on the actual host. The results are presented as mean values; error bars are ± SD from three experiments. For statistical analysis, one-way ANOVA was performed with Tukey's multiple comparison test. *** if P<0.001, ** if P<0.01.

We isolated multiple bacterial colonies during growth kinetic assay. These colonies were once again screened for phage resistance by drop assay. Many colonies exhibited a variety of PAPSZ1 resistance. Some displayed complete resistance, while others showed partial phenotypes. The partial phenotype strains were dominant among the first infections (C1), but they became less prevalent in the second cycle (C2). The C3 cycle only had the complete resistance phenotype. Plaque- and colony-forming assays from samples taken at different time points also confirmed the formation of phage-resistant mutants and their multiplication after 15 h (Fig. 25 b,c).

Table 9. Single nucleotide genetic variations in PAPSZ1-resistant colonies of the PA22 host.

Strain	Gene	Mutation	Annotation
PR-PA22	Hypothetical protein	C→T	Intergenic (–/+320)
	Hypothetical protein	C→G	A5G (GCC→GGC)
	CRISPR associated endonuclease Cas1	N-JE	intergenic (–/-891)
CR-PA22	Hypothetical protein	C→T	Intergenic (–/+320)
	Hypothetical protein	C→G	A5G (GCC→GGC)
	Glycoprotein	C→A	Intergenic (–/-8)
	CRISPR associated endonuclease Cas1	N-JE	intergenic (–/-891)
CRR-PA22	280 kb genome	Deletion	2,000kbp→2,500kbp
	CRISPR associated endonuclease Cas1	N-JE	intergenic (–/-891)

Furthermore, genome analyses of (PR-PA22M partial-resistant colonies, CR-PA22M completely resistant green colonies and CRR-PA22M completely resistant brown colonies) had several mutations (Table 9). In the PR-PA22M genomes, SNPs in a hypothetical protein-coding gene were identified. CR-PA22M had the same mutations, followed by missing nucleotides in the glycoprotein and WYL domain. CRR-PA22M had a significant (280kb±20kb) genomic deletion. The phenotypes of PR-PA22M and CR-PA22M were distinguishable in 16–20 h, and the brown phenotype was noticeable after 24–48 h of infection.

7.8.2. Isolation and characterization of PAPSZ1 phage mutants

Because CRR-PA22M had genomic deletion that caused permanent phage resistance, PR-PA22M and CR-PA22M colonies were used for mutant phage screening. Notably, the CR-PA22M strain was resistant to the wildtype PAPSZ1. Therefore, it was used only in the mutant phage assays. Phage filtrate from different infection cycles (see 'Materials and Methods') was used to test host sensitivity. The preliminary plaque assay on the above strains resulted in numerous plaques. The whole-genome analyses of these phages revealed multiple mutations in many genes and intergenic regions (Table 10).

Table 10: Summary of the single nucleotide genetic variations in all PAPSZ1 mutants.

Position	Mutation	Annotation	Description
5,912	+A	intergenic (+56/-179)	DNA primase/helicase protein
7,966	+G	intergenic (+13/-47)	Primase/helicase/DNA polymerase I
20,183	+A	intergenic (+81/-31)	Thymidylate synthase
21,558	+A	intergenic (+35/-59)	Ribonucleotide reductase
28,662	+A	coding (311/315 nt)	SprT-like domain-containing protein
40,481	+G	intergenic (+73/-504)	Endonuclease/hypothetical protein
65,482	+A	intergenic (+2/-60)	tRNA-Gly(tcc)/tRNA-Phe(gaa)
71,612	+C	coding (609/639 nt)	Major head protein
79,667	+A	coding (2165/2271 nt)	Tail length tape-measure protein
83,089	+A	coding (+68/-131)	Baseplate protein
86,126	T→G	L294R (C<u>T</u>T→C<u>G</u>T)	Tail fiber protein
86,717	A→G	H491R (C<u>A</u>C→C<u>G</u>C)	Tail fiber protein
86,733	C→A	S496R (AG<u>C</u>→AG<u>A</u>)	Tail fiber protein
87,161	T→G	I639S (A<u>T</u>C→A<u>G</u>C)	Tail fiber protein
89,068	3 bp→CAT	coding (1385/1500 nt)	Tail fiber protein

The phage mutants were classified into 4 categories based on the nature of their mutations (Fig. 26). The PAPSZ1/M1 mutant category had single SNP change in the tail fiber protein (TFP) gene (*tfp*). The PAPSZ1/M2 mutant contained two mutations in *tfp* as well as SNP changes in phage tail length tap-measure protein (TLTMP) *tltmp*; PAPSZ1/M3 had 4 SNPs in *tfp*, one SNP in *tltmp* and 1-1 insertion in head protein (HP) and base plate protein (BPP) gene. Compared to

PAPSZ1/M3, PAPSZ1/M4 harboured two extra mutation in the *tfp* gene. Apart from these genes, all mutants contained SNPs in the genes of SprT-like domain-containing protein, DNA primase/helicase proteins and a tRNA-Gly/tRNA-Phe.

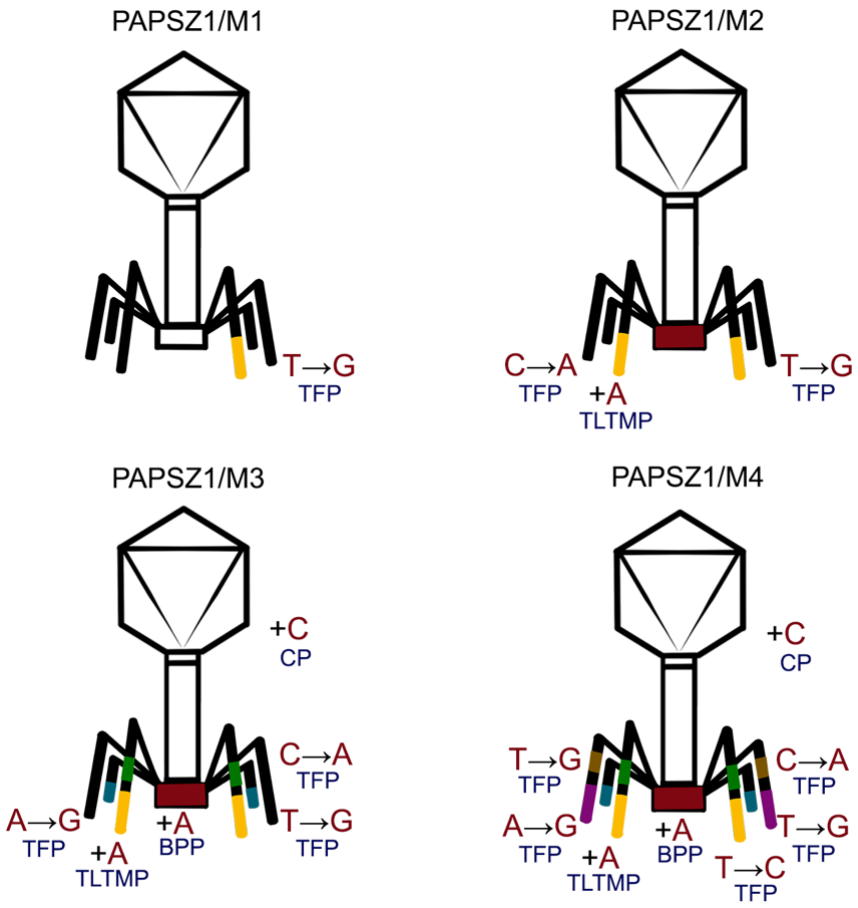


Figure 26. PAPSZ1 mutants capable of infecting PA22 green mutants. Four categories of mutants and the corresponding typical single-nucleotide genetic variations of PAPSZ1 mutants.

7.8.3. PAPSZ1 mutants gained a broader host range and suppressed bacterial mutation

At this point, we had a PA22 wild type and mutant collection as well as a set of PAPSZ1/M phage mutants. We were interested in how the host spectrum of the mutant phages had changed. Therefore, we tested mutant phages from all categories on the wild-type and mutant PA22 hosts. The PAPSZ1/M4 category of phage mutants had a higher infection spectrum than the other phages. The PAPSZ1/M3 and PAPSZ1/M4 phages could infect both the PRPA22 and the CRPA22 mutants, which were utterly resistant to wtPAPSZ1. PAPSZ1/M2 could infect

PRPA22 and CRPA22, but it was not so efficient as PAPSZ1/M3 or M4. Meanwhile, PAPSZ1/M1 was only able to infect PRPA22 (Fig. 27).

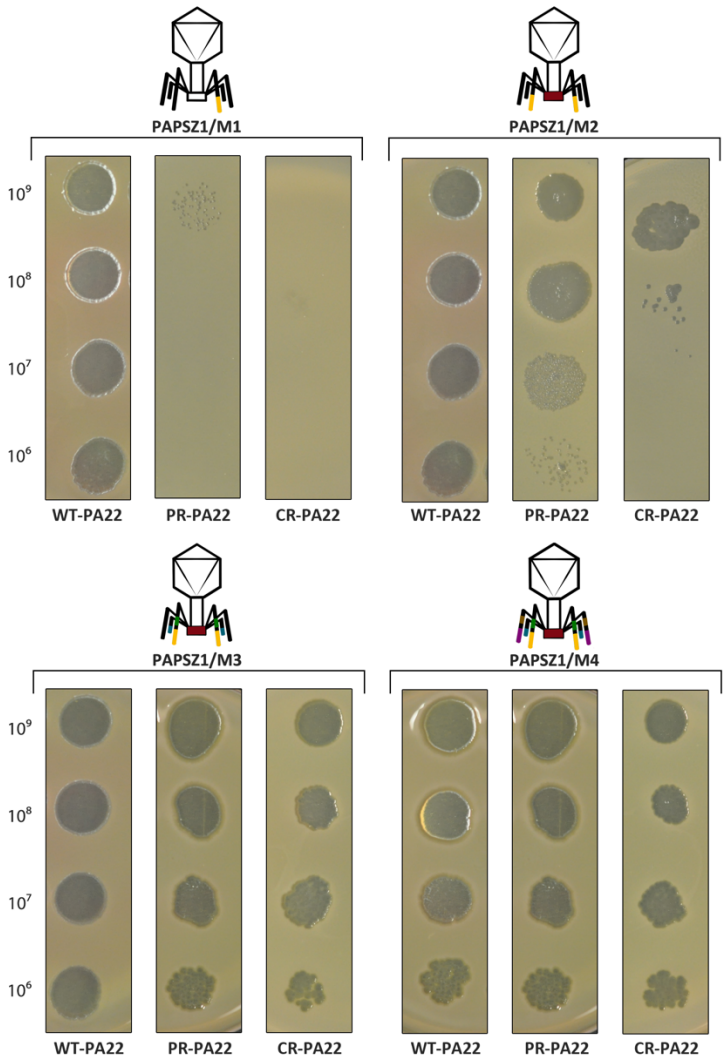


Figure 27. Plaque assay using PAPSZ1/M phage mutants as well as wild-type and mutant PA22 hosts.

These mutant phages were then used to study the broth infection assay on wt-PA22. To study bacterial resistance, we infected WT-PA22 with PAPSZ1/M1, PAPSZ1/M2, PAPSZ1/M3 and PAPSZ1/M4 for a 24-h broth assay. The phage mutants PAPSZ1/M3 and PAPSZ1/M4 overcame the bacterial phage resistance (Fig. 28, d,e) derived from various genetic changes (Table 9). PAPSZ1/M2 was able to delay the time required for the formation of bacterial resistance (Fig. 28,c). The PAPSZ1/M1 phage was unable to overcome the formation of bacterial resistance (Fig. 28,b). We then used PAPSZ1/M3 and PAPSZ1/M4 mix as a cocktail

to infect the PA22 host. These cocktails were more efficient than the single-mutant phage (Fig. R28, f).

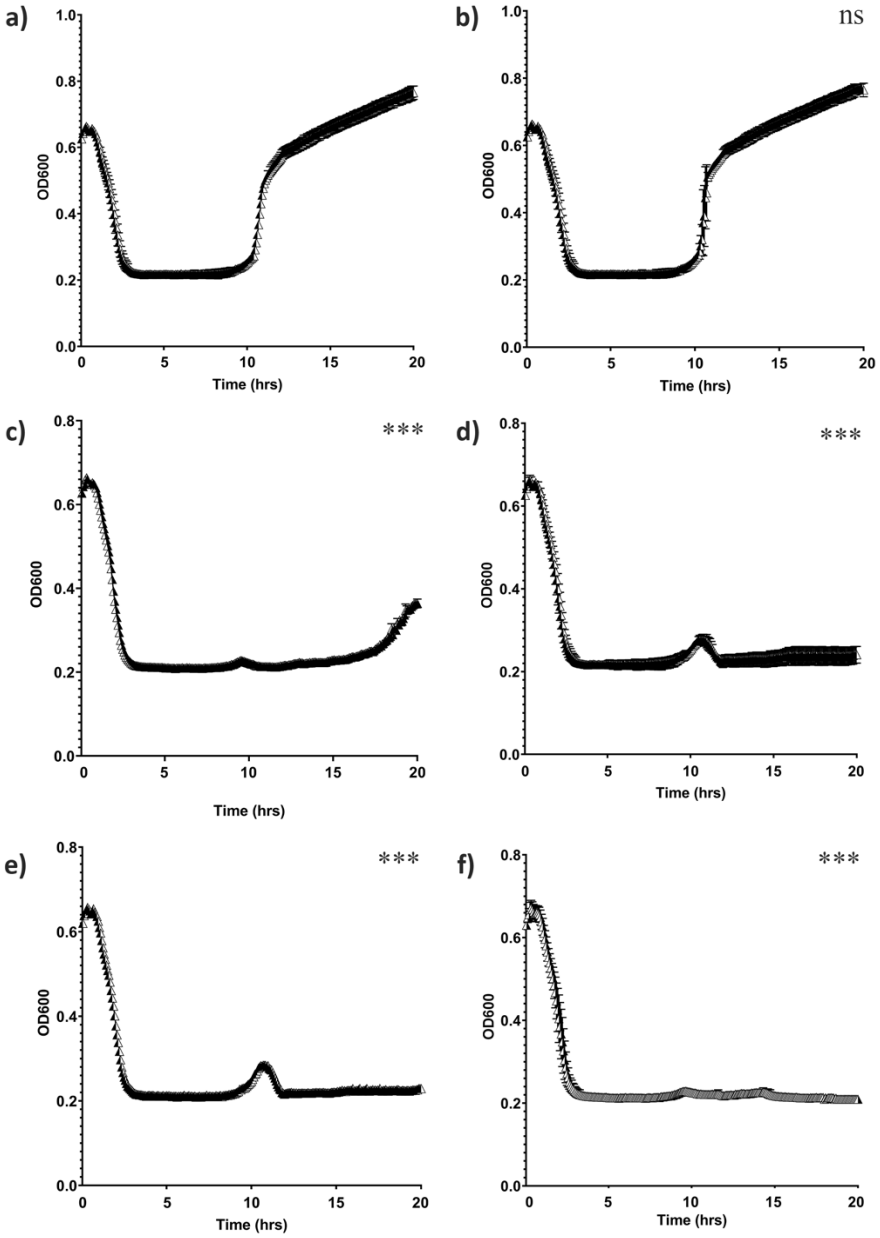


Figure 28. Kinetic plots showing growth curves of WT-PA22 bacterial cultures that were infected with PAPSZ1 and PAPSZ1 mutants. WT-PA22 was infected with **a)** PAPSZ1 (control); **b)** PAPSZ1/M1; **c)** PAPSZ1/M2; **d)** PAPSZ1/M3; **e)** PAPSZ1/M4; **f)** a cocktail composed of PAPSZ1/M3 and PAPSZ1/M4 (1:1). MOI 1.0 was applied, and the bacterial growth was monitored by measuring optical density at 600 nm. Each plot consists of three replicates from three independent experiments and is shown as the mean ± SD. For statistical analysis, one-way ANOVA was performed with Tukey's multiple comparison test. *** if $P<0.001$ and ns= not significant.

8.0. DISCUSSION

Bacteriophages are the only antimicrobial entities that show precision in choosing a host and self-replicate by continuously co-evolving with their host. The idea of using phages to combat bacteria is not new. The PhagoBurn study of burn patients infected with *Escherichia coli* and *P. aeruginosa* are some recent examples of such application (Jault et al., 2019). However, the idea of using phage therapy to its full potential by utilizing an evolutionary window is unique. Many interactions occur during the phage infection cycle. These interactions happen in different stages, beginning with the early reversible binding of the virion and continuing till the end of the viral release (Thomassen et al., 2003). In many cases, the *O*-antigene of the LPS layer serves as a receptor for phage adsorption in *P. aeruginosa* (Bertozzi Silva et al., 2016). One of the most striking features of *P. aeruginosa* is its remarkable capacity to develop fast resistance via chromosomal mutations; this helps it combat antimicrobial treatments and/or phage infection. For example, an SNP in the phage receptor is sufficient to stop phage infection in *P. aeruginosa* and *Klebsiella pneumonia* (Hesse et al., 2020; G. Li et al., 2018). The phage genome can also be evolved to counteract the bacterial defense mechanisms by inducing mutation in its genome, as it was described for *Bacillus subtilis* (Habusha et al., 2019).

The PIAS described in this study could inhibit and eradicate many tested clinical strains at high MOI values. At low MOIs, hosts undergo multistep evolution by generating changes in their genomes to block phage infection. This is a critical issue to review, as the uneven distribution of the bacteria and phages in real applications will result in different MOI values in different organs. The first line of bacterial defense caused the growth of green colonies containing SNPs and small genomic deletions. These mutations appeared shortly after single plating. A comparative study of multiple green mutants from a single clinical strain revealed small deletions with some variations (Fig. 17). Nevertheless, the patterns of the more minor genomic deletions in the green mutants suggest a multistep bacterial evolution process, which begins with SNPs followed by smaller to larger deletions that interfere with phage infection. Apart from observing random and more minor deletions in the 2000–2500 kb region, we could not isolate a mutant with single-gene deletion responsible for phage resistance. All these green mutants had either SNPs in the *mexY* gene and/or smaller deletions in that genomic region. Our experiments clearly showed that these mutations defended the bacterial lineages from phage infection. PIAS counteracted this through evolving mutations against the first lineage of bacterial mutants. The genomic study of E-PIASs and PAPSZ1 phage sequences identified a mutation in the tail-fiber protein that restored the phage's virulence against the green mutants

(Table 7). Ultimately, the bacteria shielded itself against this evolved phage by generating a stable brown mutant with a larger genomic deletion containing many genes responsible for the membrane function and integrity, including *mexY* but not the *orpM*. MexY, as a part of the MexXY-OprM efflux system, plays a key role in PIAS adsorption. We confirmed this using knock-out mutants derived from *P. aeruginosa* strain PA01 in spotting and adsorption assays. Moreover, the *orpM* knock-out mutant was also resistant to phage infection, indicating the essential role of OrpM in phage-host interaction. In contrast, the disruption of the *galU* gene coding for a protein involved in the LPS core region biosynthesis had no apparent effect on the phage infection process. Consequently, PIAS phage uses the MexXY with OprM as a receptor to initiate infection. Previously studied OMKO1 phage (*Myoviridae* family) has also been reported using MexXY-OprM as a receptor for adsorption (Gurney et al., 2020). The brown color in the mutants is attributed to the deletion of the *hmgA* gene, causing accumulation of homogentisic acid (Rodríguez-Rojas et al., 2009). The deleted region also has many other genes necessary for bacterial defense, pathogenesis membrane integrity, transport, *quorum sensing*, and antibiotic resistance (Table S2). Chromosomal DNA deletion-producing pigment mutants have been studied (Shen et al., 2018; Tanji et al., 2008). According to these studies, random brown mutants with chromosomal deletion and a white mutant with a single nucleotide variation in the LPS gene prevented phage adsorption after phage infection. In (Le et al., 2014), the authors also stated that the brown mutants formed spontaneously before phage infection. However, we found that no pigment mutant appeared without phage infection. Large genome deletions (50-600 kb) have also been found when *P. aeruginosa* is exposed to certain antibiotics such as ceftazidime and meropenem (Cabot et al., 2016; Sanz-garcía et al., 2018).

In a previous study, a single *galU* mutant was shown to have increased ceftazidime MIC value, linked to a decreased membrane permeability (Alvarez-Ortega et al., 2010). However, in phage provoked brown mutant the same *galU* gene is missing with a more significant genome deletion including many membrane-related genes (Table S2, GO:0006011). Our bile acid permeability study showed that the brown mutants significantly increased the membrane permeability compared to the wild-type strain (Fig. 19). However, no point mutation could be identified in the *galU* gene in our green mutants. Thus, the decreased MIC of ceftazidime in the mutants might have multiple reasons, including the mutation/elimination of genes responsible for the membrane integrity. Notably, no antibiotics were used in our study when large genomic deletions took place. The other difference in this study from previous findings is that the PIAS uses a different receptor - MexXY-OprM - to initiate the infection.

Interestingly, previous studies with phages PaP1 and PaoP5 revealed similar genomic deletion patterns in the bacterial host (Shen et al., 2018). Our study compared the genomic deletions in their host mutants generated by PIAS and PaoP5 (Fig. 18a). We found that the mutants had similar deleted regions that clogged phage infection. One important difference in our study was that PaoP5 was adsorbed to the LPS, whereas PIAS interacted with MexXY-OprM as the phage receptor of bacteria. It is also important to note that phage-provoked chromosomal deletions are not limited solely to PAK-P1-like phages. Another group of *Podoviridae* phages, Ab31, Ab27 and Ab09 also gives rise to mutants with genomic deletions (Latino et al., 2014, 2019). However, the genomes of these phages (~45kb) are much smaller than are those of PAK-P1-like phages (~93kb). Thus, further studies are required to identify the genera to which both Ab31 and Ab27 belong and any underlying functional similarities in how these phages infect their hosts and induce deletions. Our study also observed phage-provoked chromosomal deletion by PAPSZ1, which could also provoke the formation of brown mutants from various clinical strains. However, after PAPSZ1 infection, the green mutant did not show any significant drug sensitivity compared to the wild type. Thus, we believe that PAPSZ1 uses an alternative receptor to initiate infection. The formation of bacterial mutants with different colony morphology following phage infection has been studied (see above). However, there have been no published studies concerning the interaction between genomic deletion and restoration of antibiotic resistance. Furthermore, this thesis provides insight into the short-term bacterial and phage coevolution both *in vitro* and *in vivo*.

8.1. Compromised RND multidrug efflux system

P. aeruginosa mainly neutralizes the action of antibiotics by reducing the permeability of its outer membrane and efficiently excluding drug molecules through efflux pumps (Lambert, 2002; Riou et al., 2016; Cunrath et al., 2019;). Most common clinical resistance in *P. aeruginosa* is associated with mutations affecting efflux pumps (Kievit et al., 2001; Laohavaleeson et al., 2008; Ohene-Agyei et al., 2012; Ozer et al., 2012; Singh et al., 2017; Guénard et al., 2014; Serra et al., 2019). Shreds of evidence support that the membrane-associated peptidoglycan and a major facilitator superfamily (MFS) transporter can also contribute to antibiotic resistance (Borisova et al., 2014; Sharma et al., 2017). Efflux pumps are vital for bacteria to overcome the current era of antibiotics. This significance has attracted them as novel drug targets, and many new efflux pump inhibitors have been developed (Lamut et al., 2019). Meanwhile, PIAS can directly utilize the components of an efflux pump for infection.

This will induce a race between bacterial fitness/antibiotic sensitivity and phage resistance. Of the various RND type efflux pumps, MexAB-OprM, MexCD-OprJ, MexEF-OprN, and MexXY-OprM are extremely important. The MexXY-OprM RND comprises an outer membrane porin (OprM), a cytoplasmic-membrane antiporter (MexY), and a periplasmic membrane fusion protein that joins the membrane-associated components together (MexX) (Daury et al., 2016). This system, together with OprM, contributes to the intrinsic drug-resistance mechanism in MDR *P. aeruginosa*. In our study, the mutant colonies displayed changes (SNPs or deletions) in the *mexY* gene, potentially changing the function of the efflux system. The brown mutant showed more severe mutations, with-complete loss of genes such as the *mexY* efflux transporter gene, the *mexX* precursor gene, and the regulatory gene *mexZ*. Previous studies have shown that loss of *mexY* caused sensitivity to drugs of different classes (Masuda et al., 2000). PIAS-provoked mutation and subsequent deletion may interfere with the function of the efflux pump, making previous MDR clinical strains sensitive to different drug classes. Aminoglycosides are an important class of drugs in treating *P. aeruginosa* infections. Aminoglycoside resistance is widespread among CF patients. Overexpression and mutation in *mexXY* have been reported in *P. aeruginosa* isolated from CF patients (Laohavaleeson et al., 2008; Singh et al., 2017; López-Causapé, Rubio, et al., 2018). Noticeably, in the PIAS provoked green and brown mutants, the MIC of gentamicin decreased.

The larger genomic region was deleted during prolonged exposure of ceftazidime (Sanz-García et al., 2018). However, followed by this deletion, SNPs in MexAB-OprM and MexR genes were also reported. Interestingly, an elevated MIC of ceftazidime for these mutants could be measured. In contrast to this study, in our case, the phage provoked mutant had lowered MIC for ceftazidime, which might have multiple genetic reasons. An earlier study has shown that the outer peptidoglycan layer can also contribute to resistance (Borisova et al., 2014). In *P. aeruginosa, glpT* transporter mutation can trigger FSF resistance (Castañeda-García et al., 2009). This mutation was absent in studied wild-type strains. For the PIAS-provoked bacterial mutants, we found the MIC of FSF to be lower than for the mother strains. Phage-provoked green mutants of PA16 had multiple minor deletions and SNPs in membrane-related genes that might affect the mutants' FSF resistance. The exact mechanism underlying the FSF resistance remains unclear. We selected FSF for combinational therapy, as all the mutants became at least 5-times more sensitive to FSF than were the clinical wild-type cell lines. The ability of PIAS to utilize efflux pumps as a receptor and induce deletions will qualify it as a novel phage. Thus,

phage selection will be critical in using the full potential of such PIAS-like phages to combat MDR bacteria.

8.2. *In vivo* and *in vitro* antibiotic and phage combination therapy

Though there have been ground-breaking *in vivo* studies on the efficiency of phage therapy, few recent studies have evaluated the efficiency of combinational treatment with antibiotics and phages in treating bacterial infection. A recent study of *in vivo* combination therapy with OMKO1 phage (which also utilizes efflux pumps for infection) and erythromycin showed increased sensitivity in a wax moth (*Galleria mellonella*) larvae model (Burmeister et al., 2020). For PIAS, it is vital to study how *P. aeruginosa* will respond in an environment where both the phage and antibiotics are present. During infection and the subsequent phage treatment, the actual MOI values depend strongly on the infection level, the applied phages doses, the method of administration, and the quantitative tissue distribution. We used combined phage and antibiotic therapy to challenge clinical strains of *P. aeruginosa*. The selected clinical strains were infected *in vitro* with MOI 1 PIAS and with different antibiotics against which the strains were resistant. Combination treatment eliminated the MDR-resistant strains, and we observed no growth after 48 h incubation. This elimination may be provoked by the combined effect of the antibiotics and the phages, with the antibiotics blocking the growth of mutants and the phages killing the non-mutants. Though the way in which these gene mutations and subsequent deletions were induced at the molecular level remains unclear, the phages clearly play an active role in the process. From the *in vivo* experiments it could be concluded that PIAS in combination with antibiotics could fight *P. aeruginosa* infection in mice. Several studies have recently shown that phage therapy can treat *P. aeruginosa* infection in animal models (Beeton et al., 2015; Cafora et al., 2019). We have also isolated bacteriophages capable of killing a range of *P. aeruginosa* clinical isolates without the formation of phage resistant strains. Our study points out the importance of preliminary, detailed examinations of the phage-host bacterium interactions preceding the application of a given phage. This will determine the further strategy for treating the infection.

Nevertheless, the phage mutants isolated from the animal organs confirmed that phage–bacterial coevolution could also happen *in vivo*. As described above, we found a positive interaction between phage and antibiotic therapy, as lethality was reduced when phages and FSF were administered in combination. The combined action of phages and antibiotics has been efficient in controlling MDR strains (Oechslin et al., 2017). As an antibacterial agent, PIAS has

several properties that make it an undeniable alternative for treating MDR *P. aeruginosa*; the ability of PIAS to compromise the drug efflux mechanism, the fitness, and the pathogenicity of the target cells will add further advantages to the promising combination therapy. Combination treatment may be able to alleviate some concerns associated with phage therapeutics. This study shows that phages such as PIAS offer a unique window that can be exploited to eradicate MDR bacteria.

8.3 PAPSZ1 mutant phages gained a broader host range and suppressed the formation of bacterial resistance

Phages are the only known abundant bacterial parasite on the planet. Furthermore, they are possibly the most diverse entity in existence. The diversity in phages is driven primarily by their dynamic adaptation when facing selective pressures such as phage resistance, which is widespread in bacterial hosts. In nature, this evolutionary upgrade can take a sustained period of time to develop. Nonetheless, a study on PIAS phages has revealed that PAK-like phages can undergo rapid mutation to overcome temporary bacterial resistance (Koderi Valappil et al., 2021). When we compared the bacterial mutant frequency (PA22 green and brown mutant) induced by PIAS and PAPSZ1, the PAPSZ1 phage provoked less mutants than the PIAS (Fig.16). It is also important to note that PAPSZ1 infection resulted in more green than brown PA22 mutants. Additionally, PAPSZ1 had broader host specificity than PIAS. Considering these properties, we selected PAPSZ1 over PIAS to investigate whether phage mutants can block/suppress bacterial resistance.

In this study, PA22 mutant hosts were generated against PAPSZ1. We confirmed PA22M's phage resistances by reseeding the strains. The colonies exhibited variations in resistance. When we correlated resistance with genomic data, the colonies displaying complete resistance in 24 h had an added mutation in a glycoprotein gene. This gene may play a significant role in phage resistance and, consequently, infection cycles. In addition to the genes of this glycoprotein and the WYL domain protein, the bacteria also caused a change in the CRISPR-associated endonuclease gene (Table 9). Previously, it was assumed that *P. aeruginosa* blocked the infection of PAK-like phages either by a membrane-based defence strategy or by an advanced approach of genomic deletion. The comparative genome analysis of host and phage mutants revealed a more complex mutation pattern. In PAPSZ1 mutants, genomic changes in the DNA primase/helicase gene and in many other genes code for proteins likely playing functional roles in overcoming resistance. It is also important to consider the new junction evidence in CRISPR-

associated endonuclease in resistant bacteria. These comparative data show that PAPSZ1 has evolved to overcome multiple defence strategies in host bacteria. As described in the 'Results' section, PAPSZ1/M3 and PAPSZ1/M4 utterly prevented the formation of PA22 resistance. The selection of such mutants will increase the efficiency of phage therapy. This study also established the hotspot region of mutation, which suppresses bacterial resistance.

9.0. SUMMARY

This thesis was written based on three projects carried out at the University of Szeged, Department of Biotechnology. The study began with the isolation and characterization of novel phages against MDR *Pseudomonas aeruginosa*. This process was followed by an investigation of efficacy of these phages against *in vitro P. aeruginosa* biofilm. The isolated phages were later characterized based on their morphologies, genomes and infection spectra. During this study, we isolated two novel PAK-like phages capable of provoking single mutations and genomic deletion in MDR *P. aeruginosa.* Further study on these two phages has uncovered new insights into phage and bacterial coevolution. This window was used to explore the possibilities of treating MDR resistance infection in an *in vivo* mouse model.

The following points summarize the primary findings of my research:

1) We isolated and identified 25 clinical *P. aeruginosa* strains. The highest antimicrobial susceptibility, which was 100%, pertained to polymyxins. The lowest susceptibilities belonged to meropenem (3%) and fosfomycin (5%). The prevalences of various serotypes were: P1 (2.5%); P10 (0.75%); P11 (1%); P3 and P5 (0.5%); and P12, P4, P6 and P7 (0.25%).
2) We isolated eight lytic phages (PIAS, PAPSZ1, PAPSZ2, PAPSZ3, PAPSZ4, PAPSZ5, PAPSZ6 and PAPSZ7). The host range was further established by screening 25 isolates against phages. The isolate PAPSZ2 showed a broad spectrum by infecting 16 strains. Meanwhile, PIAS phage exhibited a narrower host spectrum; it infected only eight isolates. Six isolates (PIAS, PAPSZ1, PAPSZ2, PAPSZ3, PAPSZ6 and PAPSZ7) belonged to the family of *Myoviridae*. Two isolates (PAPSZ4 and PAPSZ5) belonged to the *Siphoviridae* family.
3) The phages' biofilm infection efficiency was studied using confocal laser-scanning microscopy. *In vitro* biofilm study showed that PAPSZ2 could disrupt biofilm most effectively. A six-phage cocktail (PAPSZ2-PAPSZ7) was more efficient in disintegrating the biofilm, however.
4) Further study on PIAS and PAPSZ1 phage infections led to the sequential appearance of phage-resistant colonies with two phenotypes. The initial set of colonies was green (pale). These colonies turned brown in the presence of phages. The colonies were isolated and screened against phages and antibiotic sensitivity. Both isolated mutants

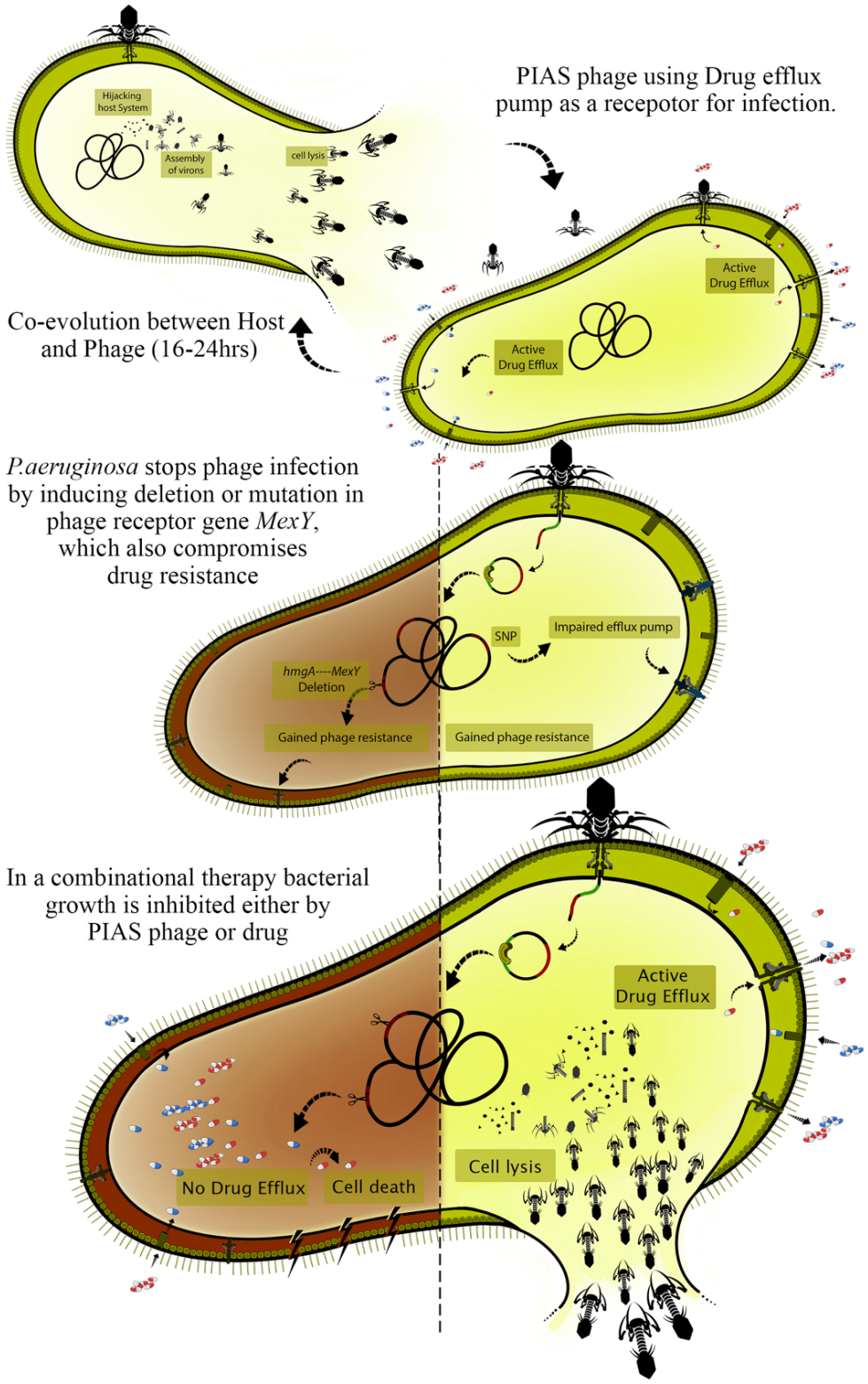
exhibited resistance to phages, but PIAS mutant had decreased MIC for gentamicin, ceftazidime, tetracycline and fosfomycin.

5) Genome analysis of these mutants revealed they had undergone significant mutations. The PIAS-phage infection resulted in green colonies harbouring SNPs and relatively small deletions (20–80 kbp**)**. A closer investigation of several genomic data revealed that SNPs change in the *mexY* gene is responsible for phage resistance and antibiotic sensitivity. The PAPSZ1 phage provoked only SNPs. Both Phage-provoked brown mutants had larger genome deletions (274–417 kb).
6) We investigated the evolutionary explanation for the two types of mutants: green (pale) and brown. We uncovered phage mutants capable of infecting green mutants from previously infected plates. Comparison of their genomes and that of the wild-type PIAS revealed that phages had SNPs in the phage tail fiber protein, which enabled them to infect the green bacterial mutant.
7) After achieving this new insight into the evolutionary arm race between hosts and phages, we comprehensively eradicated mutants by treating MDR strain with an antibiotic (fosfomycin, gentamycin, tetracycline and ceftazidime) combined with PIAS. The *in vitro* study with PIAS-antibiotic combination completely prevented the formation and growth of mutants after a 48-h incubation period. We confirmed this finding in a broth susceptibility assay, and we found that combination therapy outperforms either phage or antibiotic treatments alone.
8) After a successful *in vitro* experiment, we experimented with same strategy in an *in vivo* rescue mouse lung infection model. Combinational therapy, combined PIAS (MOI 1 and 2) with FSF, saved 5% of the animals. In the case of MOI 5 applied with FSF, 75% of the animals survived. Combination therapy ensured a 100% survival rate among mice in groups in which the bacterial challenge was performed with the 15-time diluted bacterial inoculum.
9) The study on PIAS phages revealed that PAK-like phages can undergo rapid mutation to overcome temporary bacterial resistance. When we compared the frequencies of the green and brown mutants formed after PIAS and PAPSZ1 infection. The PAPSZ1 infection yielded lower mutant frequency than PIAS. Additionally, PAPSZ1 had a broader host specificity compared to PIAS. Considering these properties, we selected PAPSZ1 over PIAS to investigate whether phage mutants can prevent/suppress the formation of bacterial resistance. We intently isolated multiple PAPSZ1 mutants after a continuous evolutionary infection cycle.

10) The phage mutants were classified into four categories based on the nature of their mutations. The PAPSZ1/M1 mutant category had single SNP change in the *tfp* (tail fiber protein). The PAPSZ1/M2 mutant contained two mutations in *tfp* as well as an SNP in phage tail length tap-measure protein, *tltmp* gene. PAPSZ1/M3 had 4 SNPs *tfp*, one SNP in *tltmp* and 1-1 insertion in the HP and the BPP gene. Compared to PAPSZ1/M3, PAPSZ1/M4 harboured two extra mutation in the *tfp* gene. Further study showed that the phage mutants PAPSZ1/M3 and PAPSZ1/M4 overcame bacterial phage resistance.

Although there have been groundbreaking *in vivo* studies on phage therapy's efficiency, few recent studies have compared the combinational efficiency of antibiotics and phage infection. This *in vitro* and *in vivo* combinational study with PIAS phage and previously resistant antibiotics eliminated bacterial growth. As an antibacterial agent, PIAS has several properties that make it an undeniable alternative to treat MDR *P. aeruginosa*. A combinational treatment could overcome some concerns associated with phage therapeutics. As shown in this thesis, phages such as PIAS and PAPSZ1 offer a unique window that can be exploited to eradicate MDR bacteria. An assortment of phage mutants such as PAPSZ1/M3 and PAPSZ1/M4 could suppress/block the formation of host resistance. Selecting such mutants will increase the efficiency of phage therapy.

Summary Diagram



Overview of the phage-induced coevolutionary process leading to phage resistance and antibiotic sensitivity.

10.0. ÖSSZEFOGLALÁS

A PhD tézis 3, az SZTE TTIK Biotechnológiai Tanszékén végzett, projekten alapul. A tanulmány első lépése: MDR *Pseudomonas aeruginosa* ellenes új bakteriofágok izolálása és jellemzése. Ezt követte az izolált fágok hatékonyságának vizsgálata *in vitro P. aeruginosa* biofilmen, a fágok morfológiai jellemzése, genomjuk szekvencia analízise és a fertőzési spektrumuk megállapítása. Ezek eredményeként sikeresen azonosítottunk két új, PAK-szerű fágot, melyek képesek genomi deléciót kiváltani a gazda MDR *P. aeruginosa* törzsekben. Ennek a két fágnak a további részletes vizsgálata újfajta betekintést engedett a bakteriofág-baktérium ko-evolúcióba, amely alapján a kezelés terápiás potenciálját kívántuk kiaknázni az MDR fertőzés *in vivo* egérmodelljén keresztül.

Kutatásaim legfontosabb eredményeit a következő pontokban foglalhatjuk össze:

1) Izoláltunk és azonosítottunk 25 klinikai törzset, melyek legnagyobb érzékenységet polymixinekre (100% gátlás) mutattak, meropenemre és foszfomicinre kevésbé voltak érzékenyek: 3% illetve 5% gátlást tapasztaltunk. A különböző szerotípusok gyakorisága a következő volt: P1 (2,5%); P10 (0,75%); P11 (1%); P3, P5 (0.5%) és P12, P4, P6, P7 (0,25%).
2) Izoláltunk nyolc litikus bakteriofág törzset (PIAS, PAPSZ1, PAPSZ2, PAPSZ3, PAPSZ4, PAPSZ5, PAPSZ6, és PAPSZ7), melyek gazda specificitását a 25 izolált klinikai baktérium törzsön teszteltük. A PAPSZ2 széles-spektrumú izolátum 16 törzset fertőzött, míg az alacsonyabb gazdaspektrumú PIAS 8 törzset fertőzött. A fágizolátumok közül 6 (PIAS, PAPSZ1, PAPSZ2, PAPSZ3, PAPSZ6, PAPSZ7) a *Myoviridae* családba, kettő (PAPSZ4, PAPSZ5) pedig a *Siphoviridae* családba tartozott.
3) A fágizolátumok biofilm-fertőző hatékonyságát konfokális pásztázó mikroszkópia segítségével vizsgáltuk, melyben a leghatékonyabb izolátumnak a PAPSZ2 bizonyult. Azonban 6 fágból álló koktél (PAPSZ2-PAPSZ7) hatékonyabban bontotta meg a biofilmet, mint az egyedül alkalmazott PAPSZ2.
4) A PIAS és PAPSZ1 izolátumokkal történő további fertőzési tesztek során megjelentek fágrezisztens baktérium kolóniák is, melyek kétféle fenotípust mutattak. Kezdetben zöldes vagy halvány színűek voltak, majd a fágok jelenlétében később rozsdabarnává váltak. Ezeket a kolóniákat izoláltuk és teszteltük fág és antibiotikum érzékenységüket.

Mindkét típusú mutáns megőrizte a fágrezisztenciáját, ellenben csökkent MIC értéket mutattak gentamicinre, ceftazidimre, tetraciklinre és foszfomicinre.

5) A fenti mutáns baktériumtörzsek genomanalízise számos mutációt tárt fel. A PIAS fágra rezisztens zöld kolóniákat SNP-k (single nucleotide polymorphism) és kisebb deléciók (20-80 kbp) jellemezték. A genomi adatok részletesebb vizsgálata alapján arra jutottunk, hogy PIAS fertőzés hatására a *mexY* génben megjelenő SNP-k felelősek a fágrezisztenciáért és az antibiotikum érzékenységért. A PAPSZ1 fágokkal való fertőzés csak SNP-k megjelenéséhez vezetett. A bármelyik fág fertőzése során megjelenő barna kolóniákban viszont nagyobb kiterjedésű deléciók (274-417 kbp) voltak azonosíthatók.
6) A kétfajta mutáns (zöld és barna) kialakulásának evolúciós okát próbáltuk megfejteni. A korábban fertőzött lemezekről visszanyertük a zöld mutánsokat fertőzni képes fágmutánsokat. Összehasonlító genom analízisükből fény derült arra, hogy a vad típusú PIAS-hoz hasonlítva ezek a mutánsok SNP-ket hordoztak a fág farokszál proteinjét (tail fiber protein) kódoló génben, mely lehetővé tette a zöld baktérium mutánsok megfertőzését.
7) Miután betekintést nyertünk a gazdabaktérium és fágok fej-fej mellett zajló evolúciós versenyébe, megkíséreltük a baktérium mutánsokat kiirtani úgy, hogy az eredeti MDR baktériumtörzseket együttesen kezeltük antibiotikumokkal (foszfomicin, gentamicin, tetraciklin és ceftazidim) és PIAS fággal. Ez a kombináció *in vitro* teljesen meggátolta a mutánsok megjelenését 48 óra inkubáció alatt. Szuszpenziós tesztben is hatékonyabbnak bizonyult a kombinációs terápia az egyedüli fág vagy antibiotikum terápiánál.
8) A sikeres *in vitro* kísérleteket követően ugyanazt a stratégiát alkalmaztuk *in vivo* egér tüdőfertőzéses modellben. A PIAS (MOI 1 és MOI 2) és foszfomicin (FSF) kombinációs terápiát kapó állatok 5%-a túlélte a fertőzést, a kombinációs terápiához fágot (MOI 5) és FSF-et használva pedig az állatok 75%-a élt túl. Abban a csoportban, ahol az állatok 15x hígított baktérium inokulummal lettek fertőzve, 100%-os túlélést tapasztaltunk.
9) A PIAS fágok tesztelése rámutatott, hogy a PAK-szerű fágok gyorsan mutálódnak, hogy áthidalják az ideiglenes baktérium rezisztenciát. A PAPSZ1 esetén zöld és barna mutánsok előfordulási gyakorisága alacsonyabb, mint PIAS esetén, a PAPSZ1 pedig a baktériumtörzsek szélesebb körét fertőzi, mint a PIAS. A fentieket figyelembe véve további vizsgálatokhoz a PAPSZ1-et választottuk, melyekben azt vizsgáltuk, a fág

képes-e elnyomni a baktérium rezisztenciát. Ehhez számos PAPSZ1 mutánst izoláltunk folyamatos evolúciót biztosító fertőzési ciklusokból.

A fágmutánsokat a mutációk alapján négy kategóriába soroltuk. A PAPSZ1/M1 mutánsokban csak egyetlen SNP található a TFP struktúrfehérjét kódoló *tfp* génben, a PAPSZ1/M2 mutánsokban 2 mutáció van a TLTMP (tail length tap-measure protein) fehérjét kódoló *tltmp* génben, a PAPSZ1/M3-ban pedig 4 SNP a *tfp* génben, 1 SNP a *tltmp* génben és 1-1 inszerció a HP (head protein), illetve BPP (base plate protein) fehérjét kódoló génben. A PAPSZ1/M4 a PAPSZ1/M3 mutációin felül még két extra *tfp* génmutációt is hordoz. A fenti kategóriák közül a PAPSZ1/M3 és a PAPSZ1/M4 típus tudott felülkerekedni a baktériumok fágrezisztenciáján.

A fágok *in vivo* terápiás hatékonyságát tekintve áttörő eredmények születtek az elmúlt években, azonban csak néhány tanulmány vizsgálta a fágok és az antibiotikumok kombinált terápiás hatékonyságát. Ebben a tézisben kimutattuk, hogy a PIAS fág és előzőleg rezisztencia miatt hatástalan antibiotikumok kombinálása *in vitro* és *in vivo* egyaránt hatékonyan gátolta a baktériumok növekedését. A PIAS fág, antibakteriális tulajdonságai alapján tagadhatatlan alternatívát jelent az MDR *P. aeruginosa* kezelésére. A kombinált kezelés megoldást adhat a fágterápia alkalmazásával kapcsolatos kételyekre. Ahogy a tézisben bemutattuk, a PIAS és PAPSZ1 fágok új távlatokat jelentenek az MDR baktériumok megsemmisítésére. Az olyan fágmutánsok, mint a PAPSZ1/M3 és PAPSZ1/M4 alkalmasak lehetnek a gazdában evolválódó fágrezisztencia kialakulásának megakadályozására/szuppressziójára és az ilyen mutánsok szelekciójával növelhető a fágterápia hatékonysága.

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12.0. REFERENCES

Abe, K., Nomura, N., & Suzuki, S. (2021). Biofilms: Hot spots of horizontal gene transfer (HGT) in aquatic environments, with a focus on a new HGT mechanism. *FEMS Microbiology Ecology*, *96*(5), 1–12. https://doi.org/10.1093/FEMSEC/FIAA031

Abedon, S., & Thomas-Abedon, C. (2010). Phage Therapy Pharmacology. *Current Pharmaceutical Biotechnology*, *11*(1), 28–47. https://doi.org/10.2174/138920110790725410

Ackermann, H. W. (2001). Frequency of morphological phage descriptions in the year 2000 Brief Review. In *Arch Virol* (Vol. 146). http://www.phage.org/names.htm

Ackermann, H. W. (2004). Bacteriophages. *Virologie*, *8*(6), 409–412.

Ackermann, H. W. (2011). Bacteriophage taxonomy. *Microbiology Australia*, *32*(2), 90. https://doi.org/10.1071/ma11090

Ackermann, H. W., Cartier, C., Slopek, S., & Vieu, J. F. (1988). Morphology of Pseudomonas aeruginosa typing phages of the Lindberg set. *Annales de l'Institut Pasteur. Virology*, *139*(4), 389–404. https://doi.org/10.1016/s0769-2617(88)80075-3

Ackermann, H. W., & Eisenstark, A. (1974). The Present State of Phage Taxonomy. *Intervirology*, *3*(4), 201–219. https://doi.org/10.1159/000149758

Adriaenssens, E. M., van Vaerenbergh, J., Vandenheuvel, D., Dunon, V., Ceyssens, P. J., de Proft, M., Kropinski, A. M., Noben, J. P., Maes, M., & Lavigne, R. (2012). T4-related bacteriophage LIMEstone isolates for the control of soft rot on potato caused by "Dickeya solani." *PLoS ONE*, *7*(3). https://doi.org/10.1371/journal.pone.0033227

Ajibola, O. A., Jose, O. A., Catherine, O. A., Benjamin, U. A., Olufunmilola, B. M., Olakunle, J. O., Adejoke, Y. O., & Olugbenga, A. O. (2021). Multidrug-Resistance Genes in Pseudomonas aeruginosa from Wound Infections in a Tertiary Health Institution in Osogbo, Nigeria. *Infectious Disorders - Drug Targets*, *21*(1). https://doi.org/10.2174/1871526520666200117112241

Alikhan, N. F., Petty, N. K., ben Zakour, N. L., & Beatson, S. A. (2011). BLAST Ring Image Generator (BRIG): Simple prokaryote genome comparisons. *BMC Genomics*, *12*(402). https://doi.org/10.1186/1471-2164-12-402

Alvarez-Ortega, C., Wiegand, I., Olivares, J., Hancock, R. E. W., & Martínez, J. L. (2010). Genetic determinants involved in the susceptibility of Pseudomonas aeruginosa to β-lactam antibiotics. *Antimicrobial Agents and Chemotherapy*, *54*(10), 4159–4167. https://doi.org/10.1128/AAC.00257-10

Amiel, E., Lovewell, R. R., O'Toole, G. A., Hogan, D. A., & Berwin, B. (2010). Pseudomonas aeruginosa evasion of phagocytosis is mediated by loss of swimming motility and is independent of flagellum expression. *Infection and Immunity*, *78*(7), 2937–2945. https://doi.org/10.1128/IAI.00144-10

Atkin, S. D., Abid, S., Foster, M., Bose, M., Keller, A., Hollaway, R., Sader, H. S., Greenberg, D. E., Finklea, J. D., Castanheira, M., & Jain, R. (2018). Multidrug-resistant Pseudomonas aeruginosa from sputum of patients with cystic fibrosis demonstrates a high rate of

susceptibility to ceftazidime–avibactam. *Infection and Drug Resistance*, *11*, 1499–1510. https://doi.org/10.2147/IDR.S173804

Azam, A. H., Hoshiga, F., Takeuchi, I., Miyanaga, K., & Tanji, Y. (2018). Analysis of phage resistance in Staphylococcus aureus SA003 reveals different binding mechanisms for the closely related Twort-like phages ϕSA012 and ϕSA039. *Applied Microbiology and Biotechnology*, *102*(20), 8963–8977. https://doi.org/10.1007/s00253-018-9269-x

Azam, A. H., & Tanji, Y. (2019). Bacteriophage-host arm race: an update on the mechanism of phage resistance in bacteria and revenge of the phage with the perspective for phage therapy. In *Applied Microbiology and Biotechnology* (Vol. 103, Issue 5, pp. 2121–2131). Springer Verlag. https://doi.org/10.1007/s00253-019-09629-x

Azzopardi, E. A., Azzopardi, E., Camilleri, L., Villapalos, J., Boyce, D. E., Dziewulski, P., Dickson, W. A., & Whitaker, I. S. (2014). Gram Negative Wound Infection in Hospitalised Adult Burn Patients-Systematic Review and Metanalysis-. *PLOS ONE*, *9*(4), 1–7. https://doi.org/10.1371/journal.pone.0095042

Bae, Y., Lee, G. M., Sim, J. H., Lee, S., Lee, S. Y., & Park, Y. L. (2014). Green nail syndrome treated with the application of tobramycin eye drop. *Annals of Dermatology*, *26*(4), 514–516. https://doi.org/10.5021/ad.2014.26.4.514

Becattini, S., Taur, Y., & Pamer, E. G. (2016). Antibiotic-Induced Changes in the Intestinal Microbiota and Disease. In *Trends in Molecular Medicine* (Vol. 22, Issue 6, pp. 458–478). Elsevier Ltd. https://doi.org/10.1016/j.molmed.2016.04.003

Beeton, M. L., Alves, D. R., Enright, M. C., & Jenkins, A. T. A. (2015). Assessing phage therapy against Pseudomonas aeruginosa using a Galleria mellonella infection model. *International Journal of Antimicrobial Agents*, *46*(2), 196–200. https://doi.org/10.1016/j.ijantimicag.2015.04.005

Benhar, I. (2001). Biotechnological applications of phage and cell display. *Biotechnology Advances*, *19*(1), 1–33. https://doi.org/10.1016/S0734-9750(00)00054-9

Bertozzi Silva, J., Storms, Z., & Sauvageau, D. (2016). Host receptors for bacteriophage adsorption. *FEMS Microbiology Letters*, *363*(4), 1–11. https://doi.org/10.1093/femsle/fnw002

Birger, R. (2000). Filamentous microfossils in a 3235 million year old volcanogenic massive sulphide deposit. *Nature*, *405*(8), 676–678. https://doi.org/10.1038/35015063

Blair, J. M. A., Webber, M. A., Baylay, A. J., Ogbolu, D. O., & Piddock, L. J. V. (2015). Molecular mechanisms of antibiotic resistance. In *Nature Reviews Microbiology* (Vol. 13, Issue 1, pp. 42–51). Nature Publishing Group. https://doi.org/10.1038/nrmicro3380

Borisova, M., Gisin, J., & Mayer, C. (2014). Blocking peptidoglycan recycling in pseudomonas aeruginosa attenuates intrinsic resistance to fosfomycin. *Microbial Drug Resistance*, *20*(3), 231–237. https://doi.org/10.1089/mdr.2014.0036

Boyle, E. M., Ainsworth, J. R., Levin, A. v., Campbell, A. N., & Watkinson, M. (2001). Ophthalmic Pseudomonas infection in infancy. *Archives of Disease in Childhood: Fetal and Neonatal Edition*, *85*(2). https://doi.org/10.1136/fn.85.2.f139

Bradley, D. E. (1967). Ultrastructure of bacteriophage and bacteriocins. *Bacteriological Reviews*, *31*(4), 230–314. https://doi.org/10.1128/br.31.4.230-314.1967

Bradley, D. E. (1973). The adsorption of the Pseudomonas aeruginosa filamentous bacteriophage Pf to its host. *Canadian Journal of Microbiology*, *19*(5), 623–631. https://doi.org/10.1139/m73-103

Breidenstein, E. B. M., de la Fuente-Núñez, C., & Hancock, R. E. W. (2011). Pseudomonas aeruginosa: All roads lead to resistance. *Trends in Microbiology*, *19*(8), 419–426. https://doi.org/10.1016/j.tim.2011.04.005

Brito, C., Cabanes, D., Sarmento Mesquita, F., & Sousa, S. (2019). Mechanisms protecting host cells against bacterial pore-forming toxins. In *Cellular and Molecular Life Sciences* (Vol. 76, Issue 7, pp. 1319–1339). Birkhauser Verlag AG. https://doi.org/10.1007/s00018-018-2992-8

Brzozowski, M., Krukowska, A., Galant, K., Jursa-Kulesza, J., & Kosik-Bogacka, D. (2020). Genotypic characterisation and antimicrobial resistance of Pseudomonas aeruginosa strains isolated from patients of different hospitals and medical centres in Poland. *BMC Infectious Diseases*, *20*(1). https://doi.org/10.1186/s12879-020-05404-w

Bushnell B. (2017). *BB Tools*. https://jgi.doe.gov/data-and-tools/software-tools/bbtools/

Burmeister, A. R., Fortier, A., Roush, C., Lessing, A. J., Bender, R. G., Barahman, R., Grant, R., Chan, B. K., & Turner, P. E. (2020). Pleiotropy complicates a trade-off between phage resistance and antibiotic resistance. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(21). https://doi.org/10.1073/pnas.1919888117

Cabot, G., Zamorano, L., Moyà, B., Juan, C., Navas, A., Blázquez, J., & Oliver, A. (2016). Evolution of Pseudomonas aeruginosa antimicrobial resistance and fitness under low and high mutation rates. *Antimicrobial Agents and Chemotherapy*, *60*(3), 1767–1778. https://doi.org/10.1128/AAC.02676-15

Cafora, M., Deflorian, G., Forti, F., Ferrari, L., Binelli, G., Briani, F., Ghisotti, D., & Pistocchi, A. (2019). Phage therapy against Pseudomonas aeruginosa infections in a cystic fibrosis zebrafish model. *Scientific Reports*, *9*(1), 1–10. https://doi.org/10.1038/s41598-018-37636-x

Campbell, R. A., Farlow, J., Freyberger, H. R., He, Y., Ward, A. M., Ellison, D. W., Getnet, D., Swierczewski, B. E., Nikolich, M. P., & Filippov, A. A. (2021). Genome Sequences of 17 Diverse Pseudomonas aeruginosa Phages. *Microbiology Resource Announcements*, *10*(19), 17–20. https://doi.org/10.1128/mra.00031-21

Casadevall, A., & Pirofski, L. A. (2000). Host-pathogen interactions: Basic concepts of microbial commensalism, colonization, infection, and disease. *Infection and Immunity*, *68*(12), 6511–6518. https://doi.org/10.1128/IAI.68.12.6511-6518.2000

Castañeda-García, A., Rodríguez-Rojas, A., Guelfo, J. R., & Blázquez, J. (2009). The glycerol-3-phosphate permease GlpT is the only fosfomycin transporter in Pseudomonas aeruginosa. *Journal of Bacteriology*, *191*(22), 6968–6974. https://doi.org/10.1128/JB.00748-09

Cattaneo, C., Antoniazzi, F., Casari, S., Ravizzola, G., Gelmi, M., Pagani, C., D'Adda, M., Morello, E., Re, A., Borlenghi, E., Manca, N., & Rossi, G. (2012). P. aeruginosa

bloodstream infections among hematological patients: An old or new question? *Annals of Hematology*, *91*(8), 1299–1304. https://doi.org/10.1007/s00277-012-1424-3

CDC. (2019). *MULTIDRUG-RESISTANT PSEUDOMONAS AERUGINOSA*. www.cdc.gov/hai/organisms/pseudomonas.html

Chadha, T. (2014). Bacterial Biofilms: Survival Mechanisms and Antibiotic Resistance. *Journal of Bacteriology & Parasitology*, *05*(03). https://doi.org/10.4172/2155-9597.1000190

Chan, B. K., Abedon, S. T., & Loc-Carrillo, C. (2013). Phage cocktails and the future of phage therapy. *Future Microbiology*, *8*(6), 769–783. https://doi.org/10.2217/fmb.13.47

Chan, B. K., Sistrom, M., Wertz, J. E., Kortright, K. E., Narayan, D., & Turner, P. E. (2016). Phage selection restores antibiotic sensitivity in MDR Pseudomonas aeruginosa. *Scientific Reports*, *6*(March), 1–8. https://doi.org/10.1038/srep26717

Chang, R. Y. K., Chen, K., Wang, J., Wallin, M., Britton, W., Morales, S., Kutter, E., Li, J., & Chana, H.-K. (2018). Proof-of-Principle Study in a Murine Lung Infection Model of Antipseudomonal Activity of Phage PEV20 in a Dry-Powder Formulation. *Antimicrob. Agents Chemother*, *62*(2), 1–8. https://doi.org/10.1128/AAC.01714-17

Chaplin, D. D. (2010). Overview of the immune response. *Journal of Allergy and Clinical Immunology*, *125*(2 SUPPL. 2). https://doi.org/10.1016/j.jaci.2009.12.980

Chen, C., Huang, H., Mazumder, R., Natale, D. A., Mcgarvey, P. B., Zhang, J., Polson, S. W., Wang, Y., Wu, C. H., & Consortium, U. (2016). Computational clustering for viral reference proteomes. *Bioinformatics*, *32*(13), 2041–2043. https://doi.org/10.1093/bioinformatics/btw110

Cheol-In, K., Sung-Han, K., Hong-Bin, K., Sang-Won, P., Young-Ju, C., Myoung-don, O., Eui-Chong, K., & Choe1, and K.-W. (2003). Pseudomonas aeruginosa Bacteremia: Risk Factors for Mortality and Influence of Delayed Receipt of Effective Antimicrobial Therapy on Clinical Outcome. In *Clinical Infectious Diseases* (Vol. 6). https://academic.oup.com/cid/article/37/6/745/298539

Church, D., Elsayed, S., Reid, O., Winston, B., & Lindsay, R. (2006). Burn wound infections. In *Clinical Microbiology Reviews* (Vol. 19, Issue 2, pp. 403–434). https://doi.org/10.1128/CMR.19.2.403-434.2006

Ciofu, O., & Tolker-Nielsen, T. (2019). Tolerance and resistance of Pseudomonas aeruginosa biofilms to antimicrobial agents -How P. aeruginosa can escape antibiotics. *Frontiers in Microbiology*, *10*(913). https://doi.org/10.3389/fmicb.2019.00913

Ciofu, O., Tolker-Nielsen, T., Jensen, P. Ø., Wang, H., & Høiby, N. (2015). Antimicrobial resistance, respiratory tract infections and role of biofilms in lung infections in cystic fibrosis patients. In *Advanced Drug Delivery Reviews* (Vol. 85, pp. 7–23). Elsevier. https://doi.org/10.1016/j.addr.2014.11.017

CORDIS. (2017). *Evaluation of phage therapy for the treatment of Escherichia coli and Pseudomonas aeruginosa burn wound infections (Phase I-II clinical trial)*. https://cordis.europa.eu/project/id/601857

Cui, Z., Feng, T., Gu, F., Li, Q., Dong, K., Zhang, Y., Zhu, Y., Han, L., Qin, J., & Guo, X. (2017). Characterization and complete genome of the virulent Myoviridae phage JD007 active against a variety of Staphylococcus aureus isolates from different hospitals in Shanghai, China. *Virology Journal*, *14*(1), 1–8. https://doi.org/10.1186/s12985-017-0701-0

Cunrath, O., Meinel, D. M., Maturana, P., Fanous, J., Buyck, J. M., saint Auguste, P., Seth-Smith, H. M. B., Körner, J., Dehio, C., Trebosc, V., Kemmer, C., Neher, R., Egli, A., & Bumann, D. (2019). Quantitative contribution of efflux to multi-drug resistance of clinical Escherichia coli and Pseudomonas aeruginosa strains. *EBioMedicine*, *41*, 479–487. https://doi.org/10.1016/j.ebiom.2019.02.061

Das, T., Sharma, P. K., Busscher, H. J., van der Mei, H. C., & Krom, B. P. (2010). Role of extracellular DNA in initial bacterial adhesion and surface aggregation. *Applied and Environmental Microbiology*, *76*(10), 3405–3408. https://doi.org/10.1128/AEM.03119-09

Daury, L., Orange, F., Taveau, J. C., Verchère, A., Monlezun, L., Gounou, C., Marreddy, R. K. R., Picard, M., Broutin, I., Pos, K. M., & Lambert, O. (2016). Tripartite assembly of RND multidrug efflux pumps. *Nature Communications*, *7*, 1–8. https://doi.org/10.1038/ncomms10731

Deatherage, D. E., & Jeffrey E. Barrick. (2015). Identification of Mutations in Laboratory-Evolved Microbes from Next-Generation Sequencing Data Using breseq. In *Engineering and Analyzing Multicellular Systems: Methods and Protocols* (Vol. 1151, Issue Ldi, pp. 1–8). Springer Science+Business Media New York 2014. https://doi.org/10.1007/978-1-4939-0554-6_126

Dedrick, R. M., Guerrero-Bustamante, C. A., Garlena, R. A., Russell, D. A., Ford, K., Harris, K., Gilmour, K. C., Soothill, J., Jacobs-Sera, D., Schooley, R. T., Hatfull, G. F., & Spencer, H. (2019). Engineered bacteriophages for treatment of a patient with a disseminated drug-resistant Mycobacterium abscessus. *Nature Medicine*, *25*(5), 730–733. https://doi.org/10.1038/s41591-019-0437-z

Delcour, A. H. (2009). Outer Membrane Permeability and Antibiotic Resistance. *Biochim Biophys Acta*, *1794*(5), 808–816. https://doi.org/10.1016/j.bbapap.2008.11.005.Outer

Dimopoulos, G., Akova, M., Rello, J., & Poulakou, G. (2020). Understanding resistance in Pseudomonas. In *Intensive Care Medicine* (Vol. 46, Issue 2, pp. 350–352). Springer. https://doi.org/10.1007/s00134-019-05905-6

do Vale, A., Cabanes, D., & Sousa, S. (2016). Bacterial toxins as pathogen weapons against phagocytes. In *Frontiers in Microbiology* (Vol. 7, Issue FEB). Frontiers Media S.A. https://doi.org/10.3389/fmicb.2016.00042

Dömötör, D., Becságh, P., Rákhely, G., Schneider, G., & Kovács, T. (2012). Complete Genomic Sequence of Erwinia amylovora Phage PhiEaH2. *Journal of Virology*, *86*(19), 10899–10899. https://doi.org/10.1128/jvi.01870-12

Dömötör, D., Frank, T., Rákhely, G., Doffkay, Z., Schneider, G., & Kovács, T. (2016). Comparative analysis of two bacteriophages of Xanthomonas arboricola pv. juglandis. *Infection, Genetics and Evolution*, *43*, 371–377. https://doi.org/https://doi.org/10.1016/j.meegid.2016.06.011

Donlan, R. M., & Costerton, J. W. (2002). Biofilms: Survival mechanisms of clinically relevant microorganisms. In *Clinical Microbiology Reviews* (Vol. 15, Issue 2, pp. 167–193). https://doi.org/10.1128/CMR.15.2.167-193.2002

Dowah, A. S. A., & Clokie, M. R. J. (2018). Review of the nature, diversity and structure of bacteriophage receptor binding proteins that target Gram-positive bacteria. *Biophysical Reviews*, *10*(2), 535–542. https://doi.org/10.1007/s12551-017-0382-3

Dreier, J., & Ruggerone, P. (2015). Interaction of antibacterial compounds with RND efflux pumps in Pseudomonas aeruginosa. In *Frontiers in Microbiology* (Vol. 6, Issue JUL). Frontiers Research Foundation. https://doi.org/10.3389/fmicb.2015.00660

Dubern, J. F., Cigana, C., de Simone, M., Lazenby, J., Juhas, M., Schwager, S., Bianconi, I., Döring, G., Eberl, L., Williams, P., Bragonzi, A., & Cámara, M. (2015). Integrated whole-genome screening for Pseudomonas aeruginosa virulence genes using multiple disease models reveals that pathogenicity is host specific. *Environmental Microbiology*, *17*(11), 4379–4393. https://doi.org/10.1111/1462-2920.12863

Dupont, P., Hocquet, D., Jeannot, K., Chavanet, P., & Plésiat, P. (2005). Bacteriostatic and bactericidal activities of eight fluoroquinolones against Mex-AB-OprM-overproducing clinical strains of Pseudomonas aeruginosa. *Journal of Antimicrobial Chemotherapy*, *55*(4), 518–522. https://doi.org/10.1093/jac/dki030

Dy, R. L., Richter, C., Salmond, G. P. C., & Fineran, P. C. (2014). Remarkable mechanisms in microbes to resist phage infections. *Annual Review of Virology*, *1*(1), 307–331. https://doi.org/10.1146/annurev-virology-031413-085500

Dykes, G. A., & Moorhead, S. M. (2002). Combined antimicrobial effect of nisin and a listeriophage against Listeria monocytogenes in broth but not in buffer or on raw beef. *International Journal of Food Microbiology*, *73*(1), 71–81. https://doi.org/10.1016/S0168-1605(01)00710-3

Ecdc. (2019). *Antimicrobial consumption in the EU and EEA: Annual Epidemiological Report 2019*.https://www.ecdc.europa.eu/sites/default/files/documents/Antimicrobial consumption-in-the-EU-Annual-Epidemiological-Report-2019.pdf

ECDC. (2019). *Point prevalence survey of healthcare-associated infections and antimicrobial use in European acute care hospitals ECDC PPS validation protocol version 3.1.2*. https://doi.org/10.2900/444036

Eklöf, J., Sørensen, R., Ingebrigtsen, T. S., Sivapalan, P., Achir, I., Boel, J. B., Bangsborg, J., Ostergaard, C., Dessau, R. B., Jensen, U. S., Browatzki, A., Lapperre, T. S., Janner, J., Weinreich, U. M., Armbruster, K., Wilcke, T., Seersholm, N., & Jensen, J. U. S. (2020). Pseudomonas aeruginosa and risk of death and exacerbations in patients with chronic obstructive pulmonary disease: an observational cohort study of 22 053 patients. *Clinical Microbiology and Infection*, *26*(2), 227–234. https://doi.org/10.1016/j.cmi.2019.06.011

EUCAST. (2020). *Pseudomonas aeruginosa: Calibration of zone diameter breakpoints to MIC values.Version:9.0*.https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Disk_criteria/Validation_2022/Pseudomonas_aeruginosa_v_9.0_January_2022.pdf

Enoch, D. A., Kuzhively, J., Sismey, A., Grynik, A., & Karas, J. A. (2013). Pseudomonas aeruginosa bacteraemia in two UK district hospitals. *Infectious Disease Reports*, *5*(1), 4. https://doi.org/10.4081/idr.2013.e4

Fabre, V., Amoah, J., Cosgrove, S. E., & Tamma, P. D. (n.d.). *Antibiotic Therapy for Pseudomonas aeruginosa Bloodstream Infections: How Long is Long Enough?* https://doi.org/10.1093/cid/ciz223/5382362

Fang, Z. li, Zhang, L. yan, Huang, Y. min, Qing, Y., Cao, K. yuan, Tian, G. bao, & Huang, X. (2014). OprD mutations and inactivation in imipenem-resistant Pseudomonas aeruginosa isolates from China. *Infection, Genetics and Evolution*, *21*, 124–128. https://doi.org/10.1016/j.meegid.2013.10.027

Faure, E., Kwong, K., & Nguyen, D. (2018). Pseudomonas aeruginosa in Chronic Lung Infections: How to Adapt Within the Host? *Frontiers in Immunology*, *9*(OCT), 1–10. https://doi.org/10.3389/fimmu.2018.02416

Federici, S., Nobs, S. P., & Elinav, E. (2021). Phages and their potential to modulate the microbiome and immunity. In *Cellular and Molecular Immunology* (Vol. 18, Issue 4, pp. 889–904). Springer Nature. https://doi.org/10.1038/s41423-020-00532-4

Fernández, L., & Hancock, R. E. W. (2012). Adaptive and mutational resistance: Role of porins and efflux pumps in drug resistance. *Clinical Microbiology Reviews*, *25*(4), 661–681. https://doi.org/10.1128/CMR.00043-12

Fillol-Salom, A., Martínez-Rubio, R., Abdulrahman, R. F., Chen, J., Davies, R., & Penadés, J. R. (2018). Phage-inducible chromosomal islands are ubiquitous within the bacterial universe. *ISME Journal*, *12*(9), 2114–2128. https://doi.org/10.1038/s41396-018-0156-3

Flemming, H. C., Wingender, J., Szewzyk, U., Steinberg, P., Rice, S. A., & Kjelleberg, S. (2016). Biofilms: An emergent form of bacterial life. In *Nature Reviews Microbiology* (Vol. 14, Issue 9, pp. 563–575). Nature Publishing Group. https://doi.org/10.1038/nrmicro.2016.94

Flume, P. A., & van Devanter, D. R. (2012). State of progress in treating cystic fibrosis respiratory disease. In *BMC Medicine* (Vol. 10). https://doi.org/10.1186/1741-7015-10-88

Forti, F., Roach, D. R., Cafora, M., Pasini, M. E., Horner, D. S., Fiscarelli, E. v., Rossitto, M., Cariani, L., Briani, F., Debarbieux, L., & Ghisotti, D. (2018). Design of a broad-range bacteriophage cocktail that reduces pseudomonas aeruginosa biofilms and treats acute infections in two animal models. *Antimicrobial Agents and Chemotherapy*, *62*(6), 1–13. https://doi.org/10.1128/AAC.02573-17

Fournier, A., Voirol, P., Krähenbühl, M., Bonnemain, C.-L., Fournier, C., Pantet, O., Pagani, J.-L., Revelly, J.-P., Dupuis-Lozeron, E., Sadeghipour, F., Pannatier, A., Eggimann, P., & Que, Y.-A. (2016). Antibiotic consumption to detect epidemics of Pseudomonas aeruginosa in a burn centre: A paradigm shift in the epidemiological surveillance of Pseudomonas aeruginosa nosocomial infections. *Burns*, *42*(3), 564–570. https://doi.org/https://doi.org/10.1016/j.burns.2015.10.030

Frances, W., de Wit, M. J., Dann, J., van der Gaast, S., de Ronde, C. E., & Gerneke, D. (2001). Early Archean fossil bacteria and biofilms in hydrothermally-influenced sediments from the Barberton greenstone belt, South Africa. In *Precambrian Research* (Vol. 106). www.elsevier.com/locate/precamres. https://doi.org/10.1016/S0301-9268(00)00127-3

Freschi, L., Vincent, A. T., Jeukens, J., Emond-Rheault, J. G., Kukavica-Ibrulj, I., Dupont, M. J., Charette, S. J., Boyle, B., & Levesque, R. C. (2019). The Pseudomonas aeruginosa Pan-Genome Provides New Insights on Its Population Structure, Horizontal Gene Transfer, and Pathogenicity. *Genome Biology and Evolution*, *11*(1), 109–120. https://doi.org/10.1093/gbe/evy259

Frimmersdorf, E., Horatzek, S., Pelnikevich, A., Wiehlmann, L., & Schomburg, D. (2010). How Pseudomonas aeruginosa adapts to various environments: A metabolomic approach. *Environmental Microbiology*, *12*(6), 1734–1747. https://doi.org/10.1111/j.1462-2920.2010.02253.x

García, P., Rodríguez, L., Rodríguez, A., & Martínez, B. (2010). Food biopreservation: Promising strategies using bacteriocins, bacteriophages and endolysins. *Trends in Food Science and Technology*, *21*(8), 373–382. https://doi.org/10.1016/j.tifs.2010.04.010

Gillespie, S. H. (2002). Evolution of drug resistance in Mycobacterium tuberculosis: Clinical and molecular perspective. In *Antimicrobial Agents and Chemotherapy* (Vol. 46, Issue 2, pp. 267–274). https://doi.org/10.1128/AAC.46.2.267-274.2002

Godde, J. S., & Bickerton, A. (2006). The repetitive DNA elements called CRISPRs and their associated genes: Evidence of horizontal transfer among prokaryotes. *Journal of Molecular Evolution*, *62*(6), 718–729. https://doi.org/10.1007/s00239-005-0223-z

Goldfarb, T., Sberro, H., Weinstock, E., Cohen, O., Doron, S., Charpak-Amikam, Y., Afik, S., Ofir, G., & Sorek, R. (2015). BREX is a novel phage resistance system widespread in microbial genomes. *The EMBO Journal*, *34*(2), 169–183. https://doi.org/10.15252/embj.201489455

Gomila, A., Carratalà, J., Eliakim-Raz, N., Shaw, E., Wiegand, I., Vallejo-Torres, L., Gorostiza, A., Vigo, J. M., Morris, S., Stoddart, M., Grier, S., Vank, C., Cuperus, N., van den Heuvel, L., Vuong, C., Macgowan, A., Leibovici, L., Addy, I., & Pujol, M. (2018). Risk factors and prognosis of complicated urinary tract infections caused by pseudomonas aeruginosa in hospitalized patients: A retrospective multicenter cohort study. *Infection and Drug Resistance*, *11*, 2571–2581. https://doi.org/10.2147/IDR.S185753

Gonzalez, M. R., Fleuchot, B., Lauciello, L., Jafari, P., Applegate, L. A., Raffoul, W., Que, Y.-A., & Perron, K. (2016). Effect of Human Burn Wound Exudate on Pseudomonas aeruginosa Virulence. *MSphere*, *1*(2). https://doi.org/10.1128/msphere.00111-15

Grande, R., Puca, V., & Muraro, R. (2020). Antibiotic resistance and bacterial biofilm. In *Expert Opinion on Therapeutic Patents* (Vol. 30, Issue 12, pp. 897–900). Taylor and Francis Ltd. https://doi.org/10.1080/13543776.2020.1830060

Gu, J., Liu, X., Li, Y., Han, W., Lei, L., Yang, Y., Zhao, H., Gao, Y., Song, J., Lu, R., Sun, C., & Feng, X. (2012). A method for generation phage cocktail with great therapeutic potential. *PLoS ONE*, *7*(3). https://doi.org/10.1371/journal.pone.0031698

Guénard, S., Muller, C., Monlezun, L., Benas, P., Broutin, I., Jeannot, K., & Plésiat, P. (2014). Multiple mutations lead to MexXY-OprM-dependent aminoglycoside resistance in clinical strains of pseudomonas aeruginosa. *Antimicrobial Agents and Chemotherapy*, *58*(1), 221–228. https://doi.org/10.1128/AAC.01252-13

Gurney, J., Pradier, L., Griffin, J. S., Gougat-Barbera, C., Chan, B. K., Turner, P. E., Kaltz, O., & Hochberg, M. E. (2020). Phage steering of antibiotic-resistance evolution in the

bacterial pathogen, Pseudomonas aeruginosa. *Evolution, Medicine and Public Health*, *2020*(1), 148–157. https://doi.org/10.1093/EMPH/EOAA026

Habusha, M., Tzipilevich, E., Fiyaksel, O., & Ben-Yehuda, S. (2019). A mutant bacteriophage evolved to infect resistant bacteria gained a broader host range. *Molecular Microbiology*, *111*(6), 1463–1475. https://doi.org/10.1111/mmi.14231

Hacker, J., & Carniel, E. (2001). Ecological fitness, genomic islands and bacterial pathogenicity. A Darwinian view of the evolution of microbes. *EMBO Reports*, *2*(5), 376–381. https://doi.org/10.1093/embo-reports/kve097

Haenni, M., Hocquet, D., Ponsin, C., Cholley, P., Guyeux, C., Madec, J. Y., & Bertrand, X. (2015). Population structure and antimicrobial susceptibility of Pseudomonas aeruginosa from animal infections in France. *BMC Veterinary Research*, *11*(1). https://doi.org/10.1186/s12917-015-0324-x

Hancock, R. E. W., & Brinkman, F. S. L. (2002). Function of Pseudomonas porins in uptake and efflux. In *Annual Review of Microbiology* (Vol. 56, pp. 17–38). https://doi.org/10.1146/annurev.micro.56.012302.160310

Harper, D. R., Parracho, H. M. R. T., Walker, J., Sharp, R., Hughes, G., Werthén, M., Lehman, S., & Morales, S. (2014). Bacteriophages and biofilms. *Antibiotics*, *3*(3), 270–284. https://doi.org/10.3390/antibiotics3030270

Henry, M., Bobay, L. M., Chevallereau, A., Saussereau, E., Ceyssens, P. J., & Debarbieux, L. (2015). The search for therapeutic bacteriophages uncovers one new subfamily and two new genera of Pseudomonas-infecting myoviridae. *PLoS ONE*, *10*(1), 1–16. https://doi.org/10.1371/journal.pone.0117163

Hentzer, M., Teitzel, G. M., Balzer, G. J., Heydorn, A., Molin, S., Givskov, M., & Parsek, M. R. (2001). Alginate overproduction affects pseudomonas aeruginosa biofilm structure and function. *Journal of Bacteriology*, *183*(18), 5395–5401. https://doi.org/10.1128/JB.183.18.5395-5401.2001

Hesse, S., Rajaure, M., Wall, E., Johnson, J., Bliskovsky, V., Gottesman, S., & Adhya, S. (2020). Phage Resistance in Multidrug-Resistant Klebsiella pneumoniae ST258 Evolves via Diverse Mutations That Culminate in Impaired Adsorption. *MBio*, *11*(1), e02530-19. https://doi.org/10.1128/mBio.02530-19.

Heydorn, A., Nielsen, A. T., Hentzer, M., Sternberg, C., Givskov, M., Ersboll, B. K., & Molin, S. (2000). Quantification of biofilm structures by the novel computer program COMSTAT. *Microbiology*, *146*(10), 2395–2407. https://doi.org/10.1099/00221287-146-10-2395

Heineman, R. H., & Bull, J. J. (2007). Testing optimality with experimental evolution: Lysis time in a bacteriophage. *Evolution*, *61*(7), 1695–1709. https://doi.org/10.1111/j.1558-5646.2007.00132.x

Høiby, N., Bjarnsholt, T., Givskov, M., Molin, S., & Ciofu, O. (2010). Antibiotic resistance of bacterial biofilms. In *International Journal of Antimicrobial Agents* (Vol. 35, Issue 4, pp. 322–332). https://doi.org/10.1016/j.ijantimicag.2009.12.011

Høiby, N., Bjarnsholt, T., Moser, C., Jensen, P. Ø., Kolpen, M., Qvist, T., Aanæs, K., Pressler, T., Skov, M., & Ciofu, O. (2017). Diagnosis of biofilm infections in cystic fibrosis

patients. In *APMIS* (Vol. 125, Issue 4, pp. 339–343). Blackwell Munksgaard. https://doi.org/10.1111/apm.12689

Høiby, N., Ciofu, O., & Bjarnsholt, T. (2010). Pseudomonas aeruginosa biofilms in cystic fibrosis. In *Future Microbiology* (Vol. 5, Issue 11, pp. 1663–1674). https://doi.org/10.2217/fmb.10.125

Hong, D. J., Bae, I. K., Jang, I. H., Jeong, S. H., Kang, H. K., & Lee, K. (2015). Epidemiology and characteristics of metallo-ß-lactamase-producing Pseudomonas aeruginosa. *Infection and Chemotherapy*, *47*(2), 81–97. https://doi.org/10.3947/ic.2015.47.2.81

Horváth, M., Kovács, T., Koderivalappil, S., Ábrahám, H., Rákhely, G., & Schneider, G. (2020). Identification of a newly isolated lytic bacteriophage against K24 capsular type, carbapenem resistant Klebsiella pneumoniae isolates. *Scientific Reports*, *10*(1). https://doi.org/10.1038/s41598-020-62691-8

Hover, B. M., Kim, S. H., Katz, M., Charlop-Powers, Z., Owen, J. G., Ternei, M. A., Maniko, J., Estrela, A. B., Molina, H., Park, S., Perlin, D. S., & Brady, S. F. (2018). Culture-independent discovery of the malacidins as calcium-dependent antibiotics with activity against multidrug-resistant Gram-positive pathogens. *Nature Microbiology*, *3*(4), 415–422. https://doi.org/10.1038/s41564-018-0110-1

Howarth And, S., & Dedman, M. D. (1964). Pigmentation variants of pseudomonas aeruginosa. In *journal of bacreriology* (Vol. 88, Issue 2). https://doi.org/10.1128/jb.88.2.273-278.1964

Huang, G., Le, S., Peng, Y., Zhao, Y., Yin, S., Zhang, L., Yao, X., Tan, Y., Li, M., & Hu, F. (2013). Characterization and genome sequencing of phage Abp1, a new phiKMV-like virus infecting multidrug-resistant acinetobacter baumannii. *Current Microbiology*, *66*(6), 535–543. https://doi.org/10.1007/s00284-013-0308-7

Hue, B., Doat, M., Renard, G., Brandely, M.-L., & Chast, F. (2009). Severe Keratitis Caused by Pseudomonas aeruginosa Successfully Treated with Ceftazidime Associated with Acetazolamide. *Journal of Ophthalmology*, *2009*, 1–3. https://doi.org/10.1155/2009/794935

Hyman, P., & Abedon, S. T. (2010). Bacteriophage Host Range and Bacterial Resistance. In *Advances in Applied Microbiology* (1st ed., Vol. 70, Issue 10). Elsevier Inc. https://doi.org/10.1016/S0065-2164(10)70007-1

J. Wolter, D., & D. Lister, P. (2013). Mechanisms of β -lactam Resistance Among Pseudomonas aeruginosa. *Current Pharmaceutical Design*, *19*(2). https://doi.org/10.2174/1381612811306020209

Jault, P., Leclerc, T., Jennes, S., Pirnay, J. P., Que, Y. A., Resch, G., Rousseau, A. F., Ravat, F., Carsin, H., le Floch, R., Schaal, J. V., Soler, C., Fevre, C., Arnaud, I., Bretaudeau, L., & Gabard, J. (2019). Efficacy and tolerability of a cocktail of bacteriophages to treat burn wounds infected by Pseudomonas aeruginosa (PhagoBurn): A randomised, controlled, double-blind phase 1/2 trial. *The Lancet Infectious Diseases*, *19*(1), 35–45. https://doi.org/10.1016/S1473-3099(18)30482-1

Jennes, S., Merabishvili, M., Soentjens, P., Pang, K. W., Rose, T., Keersebilck, E., Soete, O., François, P. M., Teodorescu, S., Verween, G., Verbeken, G., de Vos, D., & Pirnay, J. P. (2017). Use of bacteriophages in the treatment of colistin-only-sensitive Pseudomonas

aeruginosa septicaemia in a patient with acute kidney injury-a case report. In *Critical Care* (Vol. 21, Issue 1). BioMed Central Ltd. https://doi.org/10.1186/s13054-017-1709-y

Johansen, T. E. B., Çek, M., Naber, K. G., Stratchounski, L., Svendsen, M. v., & Tenke, P. (2006). Hospital acquired urinary tract infections in urology departments: pathogens, susceptibility and use of antibiotics. Data from the PEP and PEAP-studies. *International Journal of Antimicrobial Agents*, *28*(SUPPL. 1), 91–107. https://doi.org/10.1016/j.ijantimicag.2006.05.005

Jose, M. M., & Cesar, A. A. (2016). Mechanisms of Antibiotic Resistance. *Microbiol Spectr*, *4*(2). https://doi.org/10.1128/microbiolspec.vmbf-0016-2015

Juhala, R. J., Ford, M. E., Duda, R. L., Youlton, A., Hatfull, G. F., & Hendrix, R. W. (2000). Genomic sequences of bacteriophages HK97 and HK022: pervasive genetic mosaicism in the lambdoid bacteriophages11Edited by M. Gottesman. *Journal of Molecular Biology*, *299*(1), 27–51. https://doi.org/https://doi.org/10.1006/jmbi.2000.3729

K Chan, B., & T Abedon, S. (2015). Bacteriophages and their enzymes in biofilm control. *Current Pharmaceutical Design*, *21*(1), 85–99. http:// doi: 10.2174/1381612820666140905112311.

Karruli, A., Boccia, F., Gagliardi, M., Patauner, F., Ursi, M. P., Sommese, P., de Rosa, R., Murino, P., Ruocco, G., Corcione, A., Andini, R., Zampino, R., & Durante-Mangoni, E. (2021). Multidrug-Resistant Infections and Outcome of Critically Ill Patients with Coronavirus Disease 2019: A Single Center Experience. *Microbial Drug Resistance*, *27*(9), 1167–1175. https://doi.org/10.1089/mdr.2020.0489

Kellenberger, G., & Kellenberger, E. (1957). Electron microscopical studies of phage multiplication. III. Observation of single cell bursts. *Virology*, *3*(2), 275–285. https://doi.org/10.1016/0042-6822(57)90093-4

Khawaldeh, A., Morales, S., Dillon, B., Alavidze, Z., Ginn, A. N., Thomas, L., Chapman, S. J., Dublanchet, A., Smithyman, A., & Iredell, J. R. (2011). Bacteriophage therapy for refractory Pseudomonas aeruginosa urinary tract infection. *Journal of Medical Microbiology*, *60*(11), 1697–1700. https://doi.org/10.1099/jmm.0.029744-0

Kievit, T. de, Parkins, M., Gillis, R., Srikumar, R., Ceri, H., Poole, K., Iglewski, B. H., & Storey, D. (2001). Multidrug efflux pumps: Expression patterns and contribution to antibiotic resitatnce. *Antimicrobial Agents and Chemotherapy*, *45*(6), 1761–1770. https://doi.org/10.1128/AAC.45.6.1761

Klug, T. E., Holm, N., Greve, T., & Ovesen, T. (2019). Perichondritis of the auricle: bacterial findings and clinical evaluation of different antibiotic regimens. *European Archives of Oto-Rhino-Laryngology*, *276*(8), 2199–2203. https://doi.org/10.1007/s00405-019-05463-z

Koderi Valappil, S., Shetty, P., Deim, Z., Terhes, G., Urbán, E., Váczi, S., Patai, R., Polgár, T., Pertics, B. Z., Schneider, G., Kovács, T., & Rákhely, G. (2021). Survival Comes at a Cost: A Coevolution of Phage and Its Host Leads to Phage Resistance and Antibiotic Sensitivity of Pseudomonas aeruginosa Multidrug Resistant Strains. *Frontiers in Microbiology*, *12*(December), 1–17. https://doi.org/10.3389/fmicb.2021.783722

Koo, H., Allan, R. N., Howlin, R. P., Stoodley, P., & Hall-Stoodley, L. (2017). Targeting microbial biofilms: Current and prospective therapeutic strategies. In *Nature Reviews*

Microbiology (Vol. 15, Issue 12, pp. 740–755). Nature Publishing Group. https://doi.org/10.1038/nrmicro.2017.99

Kordes, A., Preusse, M., Willger, S. D., Braubach, P., Jonigk, D., Haverich, A., Warnecke, G., & Häussler, S. (2019). Genetically diverse Pseudomonas aeruginosa populations display similar transcriptomic profiles in a cystic fibrosis explanted lung. *Nature Communications*, *10*(1). https://doi.org/10.1038/s41467-019-11414-3

Kortright, K. E., Chan, B. K., Koff, J. L., & Turner, P. E. (2019). Phage Therapy: A Renewed Approach to Combat Antibiotic-Resistant Bacteria. *Cell Host and Microbe*, *25*(2), 219–232. https://doi.org/10.1016/j.chom.2019.01.014

Kosorok, M. R., Zeng, L., West, S. E. H., Rock, M. J., Splaingard, M. L., Laxova, A., Green, C. G., Collins, J., & Farrell, P. M. (2001). Acceleration of Lung Disease in Children With Cystic Fibrosis After Pseudomonas aeruginosa Acquisition. In *Pediatric Pulmonology* (Vol. 32). https://doi: 10.1002/ppul.2009.abs.

Kovács, T., Molnár, J., Varga, I., Nagy, I. K., Valappil, S. K., Papp, S., Vera Cruz, C. M., Oliva, R., Vizi, T., Schneider, G., & Rákhely, G. (2019). Complete Genome Sequences of 10 Xanthomonas oryzae pv. oryzae Bacteriophages. *Microbiology Resource Announcements*, *8*(27). https://doi.org/10.1128/mra.00334-19

Kropinski, A. M. (2009). Measurement of the rate of attachment of bacteriophage to cells. In *Bacteriophages: Methods and Protocols* (Vol. 501, pp. 151–155). Springer Science Business Media. https://doi.org/10.1007/978-1-60327-164-6_15

Kropinski, A. M., Mazzocco, A., Waddell, T. E., Lingohr, E., & Johnson, R. P. (2009). Enumeration of bacteriophages by double agar overlay plaque assay. In *Bacteriophages: Methods and Protocols* (Vol. 501, pp. 69–76). https://doi.org/10.1007/978-1-60327-164-6_7

Kugadas, A., Christiansen, S. H., Sankaranarayanan, S., Surana, N. K., Gauguet, S., Kunz, R., Fichorova, R., Vorup-Jensen, T., & Gadjeva, M. (2016). Impact of Microbiota on Resistance to Ocular Pseudomonas aeruginosa-Induced Keratitis. *PLoS Pathogens*, *12*(9). https://doi.org/10.1371/journal.ppat.1005855

LaBauve, A. E., & Wargo, M. J. (2012). Growth and laboratory maintenance of Pseudomonas aeruginosa. *Current Protocols in Microbiology*, *SUPPL.25*. https://doi.org/10.1002/9780471729259.mc06e01s25

Labrie, S. J., Samson, J. E., & Moineau, S. (2010). Bacteriophage resistance mechanisms. *Nature Reviews Microbiology*, *8*(5), 317–327. https://doi.org/10.1038/nrmicro2315

Lambert, P. A. (2002). Mechanisms of antibiotic resistance in Pseudomonas aeruginosa. *Journal of the Royal Society of Medicine, Supplement*, *95*(41), 22–26. https://doi.org/99.1002/215932

Lamut, A., Peterlin Mašič, L., Kikelj, D., & Tomašič, T. (2019). Efflux pump inhibitors of clinically relevant multidrug resistant bacteria. *Medicinal Research Reviews*, *39*(6), 2460–2504. https://doi.org/10.1002/med.21591

Laohavaleeson, S., Lolans, K., Quinn, J. P., Kuti, J. L., & Nicolau, D. P. (2008). Expression of the MexXY-OprM efflux system in Pseudomonas aeruginosa with discordant

cefepime/ceftazidime susceptibility profiles. *Infection and Drug Resistance*, *1*, 51–56. https://doi.org/10.2147/idr.s4418

Lázár, V., Martins, A., Spohn, R., Daruka, L., Grézal, G., Fekete, G., Számel, M., Jangir, P. K., Kintses, B., Csörgo, B., Nyerges, Á., Györkei, Á., Kincses, A., Dér, A., Walter, F. R., Deli, M. A., Urbán, E., Hegedus, Z., Olajos, G., … Pál, C. (2018). Antibiotic-resistant bacteria show widespread collateral sensitivity to antimicrobial peptides. *Nature Microbiology*, *3*(6), 718–731. https://doi.org/10.1038/s41564-018-0164-0

Le, S., Yao, X., Lu, S., Tan, Y., Rao, X., Li, M., Jin, X., Wang, J., Zhao, Y., Wu, N. C., Lux, R., He, X., Shi, W., & Hu, F. (2014). Chromosomal DNA deletion confers phage resistance to Pseudomonas aeruginosa. *Scientific Reports*, *4*, 1–8. https://doi.org/10.1038/srep04738

Lécuyer, F., Bourassa, J.-S., Gélinas, M., Charron-Lamoureux, V., Burrus, V., & Beauregard, P. B. (2018). Biofilm Formation Drives Transfer of the Conjugative Element ICE Bs1 in Bacillus subtilis . *MSphere*, *3*(5). https://doi.org/10.1128/msphere.00473-18

Lee, K. W. K., Periasamy, S., Mukherjee, M., Xie, C., Kjelleberg, S., & Rice, S. A. (2014). Biofilm development and enhanced stress resistance of a model, mixed-species community biofilm. *ISME Journal*, *8*(4), 894–907. https://doi.org/10.1038/ismej.2013.194

Lee, Y. D., & Park, J. H. (2015). Phage conversion for β-Lactam antibiotic resistance of staphylococcus aureus from foods. *Journal of Microbiology and Biotechnology*, *26*(2), 263–269. https://doi.org/10.4014/jmb.1508.08042

Leverentz, B., Conway, W. S., Camp, M. J., Janisiewicz, W. J., Abuladze, T., Yang, M., Saftner, R., & Sulakvelidze, A. (2003). Biocontrol of Listeria monocytogenes on fresh-cut produce by treatment with lytic bacteriophages and a bacteriocin. *Applied and Environmental Microbiology*, *69*(8), 4519–4526. https://doi.org/10.1128/AEM.69.8.4519-4526.2003

Lewis, K. (2010). Persister cells. In *Annual Review of Microbiology* (Vol. 64, pp. 357–372). https://doi.org/10.1146/annurev.micro.112408.134306

Li, G., Shen, M., Yang, Y., Le, S., Li, M., Wang, J., Zhao, Y., Tan, Y., Hu, F., & Lu, S. (2018). Adaptation of Pseudomonas aeruginosa to phage PaP1 predation via O-antigen polymerase mutation. *Frontiers in Microbiology*, *9*(JUN), 1–10. https://doi.org/10.3389/fmicb.2018.01170

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., & Durbin, R. (2009). The Sequence Alignment/Map format and SAMtools. *Bioinformatics*, *25*(16), 2078–2079. https://doi.org/10.1093/bioinformatics/btp352

Li, H., Luo, Y. F., Williams, B. J., Blackwell, T. S., & Xie, C. M. (2012). Structure and function of OprD protein in Pseudomonas aeruginosa: From antibiotic resistance to novel therapies. In *International Journal of Medical Microbiology* (Vol. 302, Issue 2, pp. 63–68). https://doi.org/10.1016/j.ijmm.2011.10.001

Licheng, W., Oscar, E., Olga, Z., Manjeet, B., Le, S., Jonathan, E. K., Nachiket, P., Mark, W. M., Eugene, B. C., Yang-Xin, F., Michael, A. J., Michael, I. N., Robert, E. W. H., Jerrold, R. T., & John, C. A. (2005). Recognition of Host Immune Activation by Pseudomonas aeruginosa. *Science*, *309*(5735), 771–774. https://doi.org/10.1126/science.1113988

Lim, J. J., Grinstein, S., & Roth, Z. (2017). Diversity and versatility of phagocytosis: Roles in innate immunity, tissue remodeling, and homeostasis. *Frontiers in Cellular and Infection Microbiology*, *7*(MAY). https://doi.org/10.3389/fcimb.2017.00191

Ling, L. L., Schneider, T., Peoples, A. J., Spoering, A. L., Engels, I., Conlon, B. P., Mueller, A., Schäberle, T. F., Hughes, D. E., Epstein, S., Jones, M., Lazarides, L., Steadman, V. A., Cohen, D. R., Felix, C. R., Fetterman, K. A., Millett, W. P., Nitti, A. G., Zullo, A. M., … Lewis, K. (2015). A new antibiotic kills pathogens without detectable resistance. *Nature*, *517*(7535), 455–459. https://doi.org/10.1038/nature14098

Litwin, A., Rojek, S., Gozdzik, W., & Duszynska, W. (2021). Pseudomonas aeruginosa device associated – healthcare associated infections and its multidrug resistance at intensive care unit of University Hospital: polish, 8.5-year, prospective, single-centre study. *BMC Infectious Diseases*, *21*(1). https://doi.org/10.1186/s12879-021-05883-5

Loc-Carrillo, C., & Abedon, S. T. (2011). Pros and cons of phage therapy. *Bacteriophage*, *1*(2), 111–114. https://doi.org/10.4161/bact.1.2.14590

López-Causapé, C., Cabot, G., del Barrio-Tofiño, E., & Oliver, A. (2018a). The versatile mutational resistome of Pseudomonas aeruginosa. *Frontiers in Microbiology*, *9*(APR), 1–9. https://doi.org/10.3389/fmicb.2018.00685

López-Causapé, C., Rubio, R., Cabot, G., & Oliver, A. (2018b). Evolution of the Pseudomonas aeruginosa Aminoglycoside Mutational Resistome In Vitro and in the Cystic Fibrosis Setting. *Antimicrob Agents Chemother*, *62:e02583-17.* https://doi.org/10.1128/AAC.02583-17.

Lu, S., Le, S., Tan, Y., Zhu, J., Li, M., Rao, X., Zou, L., Li, S., Wang, J., Jin, X., Huang, G., Zhang, L., Zhao, X., & Hu, F. (2013). Genomic and Proteomic Analyses of the Terminally Redundant Genome of the Pseudomonas aeruginosa Phage PaP1: Establishment of Genus PaP1-Like Phages. *PLoS ONE*, *8*(5). https://doi.org/10.1371/journal.pone.0062933

Lyczak, J. B., Cannon, C. L., & Pier, G. B. (2000). Establishment of Pseudomonas aeruginosa infection: lessons from a versatile opportunist. *Microbes and Infection*, *2*(9), 1051–1060. https://doi.org/10.1016/S1286-4579(00)01259-4

Mah, T.-F., Pitts, B., Pellock, B., Walker, G. C., Stewart, P. S., & O'Toole, G. A. (2003). A genetic basis for Pseudomonas aeruginosa biofilm antibiotic resistance. *Nature*, *426*(6964), 302–306. https://doi.org/10.1038/nature02090

Maisonneuve, E., & Gerdes, K. (2014). Molecular mechanisms underlying bacterial persisters. In *Cell* (Vol. 157, Issue 3, pp. 539–548). Elsevier B.V. https://doi.org/10.1016/j.cell.2014.02.050

Malhotra, S., Hayes, D., Jr., & Wozniak, D. J. (2019). Cystic Fibrosis and Pseudomonas aeruginosa: the Host-Microbe Interface. *Clin Microbiol Rev*, *32*(3), e00138-18. https://doi.org/10 .1128/CMR.00138-18

Mäntynen, S., Sundberg, L. R., & Poranen, M. M. (2018). Recognition of six additional cystoviruses: Pseudomonas virus phi6 is no longer the sole species of the family Cystoviridae. *Archives of Virology*, *163*(4), 1117–1124. https://doi.org/10.1007/s00705-017-3679-4

Masuda, N., Sakagawa, E., Ohya, S., Gotoh, N., Tsujimoto, H., & Nishino, T. (2000). Contribution of the MexX-MexY-OprM efflux system to intrinsic resistance in Pseudomonas aeruginosa. *Antimicrobial Agents and Chemotherapy*, *44*(9), 2242–2246. https://doi.org/10.1128/AAC.44.9.2242-2246.2000

Mathee, K., Narasimhan, G., Valdes, C., Qiu, X., Matewish, J. M., Koehrsen, M., Rokas, A., Yandava, C. N., Engels, R., Zeng, E., Olavarietta, R., Doud, M., Smith, R. S., Montgomery, P., White, J. R., Godfrey, P. A., Kodira, C., Birren, B., Galagan, J. E., & Lory, S. (2007). Dynamics of Pseudomonas aeruginosa genome evolution. In *Harvard MedicalSchool*. www.pnas.org/cgi/content/full/ https://doi.org/10.1073/pnas.0711982105

Meczker, K., Dömötör, D., Vass, J., Rákhely, G., Schneider, G., & Kovács, T. (2014). The genome of the Erwinia amylovora phage PhiEaH1 reveals greater diversity and broadens the applicability of phages for the treatment of fire blight. *FEMS Microbiology Letters*, *350*(1), 25–27. https://doi.org/10.1111/1574-6968.12319

Meier-Kolthoff, J. P., & Göker, M. (2017). VICTOR: genome-based phylogeny and classification of prokaryotic viruses. *Bioinformatics (Oxford, England)*, *33*(21), 3396–3404. https://doi.org/10.1093/bioinformatics/btx440

Merabishvili, M., Pirnay, J. P., Verbeken, G., Chanishvili, N., Tediashvili, M., Lashkhi, N., Glonti, T., Krylov, V., Mast, J., van Parys, L., Lavigne, R., Volckaert, G., Mattheus, W., Verween, G., de Corte, P., Rose, T., Jennes, S., Zizi, M., de Vos, D., & Vaneechoutte, M. (2009). Quality-controlled small-scale production of a well-defined bacteriophage cocktail for use in human clinical trials. *PLoS ONE*, *4*(3). https://doi.org/10.1371/journal.pone.0004944

Miller, M. B., & Bassler, B. L. (2001). *QUORUM SENSING IN BACTERIA* (Vol. 12). www.annualreviews.org. https://doi.org/10.1146/annurev.micro.55.1.165

Mirzaei, B., Bazgir, Z. N., Goli, H. R., Iranpour, F., Mohammadi, F., & Babaei, R. (2020). Prevalence of multi-drug resistant (MDR) and extensively drug-resistant (XDR) phenotypes of Pseudomonas aeruginosa and Acinetobacter baumannii isolated in clinical samples from Northeast of Iran. *BMC Research Notes*, *13*(1). https://doi.org/10.1186/s13104-020-05224-w

Mlynarcik, P., & Kolar, M. (2017). Starvation- and antibiotics-induced formation of persister cells in Pseudomonas aeruginosa. *Biomedical Papers*, *161*(1), 58–67. https://doi.org/10.5507/bp.2016.057

Modi, A. R., & Kovacs, C. S. (2020). Hospital-acquired and ventilator-associated pneumonia: Diagnosis, management, and prevention. In *Cleveland Clinic journal of medicine* (Vol. 87, Issue 10, pp. 633–639). NLM (Medline). https://doi.org/10.3949/ccjm.87a.19117

Modi, R., Hirvi, Y., Hill, A., & Griffiths, M. W. (2001). Effect of phage on survival of Salmonella Enteritidis during manufacture and storage of Cheddar cheese made from raw and pasteurized milk. *Journal of Food Protection*, *64*(7), 927–933. https://doi.org/10.4315/0362-028X-64.7.927

Moradali, M. F., Ghods, S., & Rehm, B. H. A. (2017). Pseudomonas aeruginosa lifestyle: A paradigm for adaptation, survival, and persistence. In *Frontiers in Cellular and Infection Microbiology* (Vol. 7, Issue FEB). Frontiers Research Foundation. https://doi.org/10.3389/fcimb.2017.00039

Mulcahy, L. R., Burns, J. L., Lory, S., & Lewis, K. (2010). Emergence of Pseudomonas aeruginosa strains producing high levels of persister cells in patients with cystic fibrosis. *Journal of Bacteriology*, *192*(23), 6191–6199. https://doi.org/10.1128/JB.01651-09

Müller, S., Ebnöther, M., & Itin, P. (2014). Green nail syndrome (pseudomonas aeruginosa nail infection): Two cases successfully treated with topical nadifloxacin, an acne medication. *Case Reports in Dermatology*, *6*(2), 180–184. https://doi.org/10.1159/000365863

Nurk, S., Bankevich, A., Antipov, D., Gurevich, A. A., Korobeynikov, A., Lapidus, A., Prjibelski, A. D., Pyshkin, A., Sirotkin, A., Sirotkin, Y., Stepanauskas, R., Clingenpeel, S. R., Woyke, T., McLean, J. S., Lasken, R., Tesler, G., Alekseyev, M. A., & Pevzner, P. A. (2013). Assembling single-cell genomes and mini-metagenomes from chimeric MDA products. *Journal of Computational Biology*, *20*(10), 714–737. https://doi.org/10.1089/cmb.2013.0084

Oechslin, F., Piccardi, P., Mancini, S., Gabard, J., Moreillon, P., Entenza, J. M., Resch, G., & Que, Y. A. (2017). Synergistic interaction between phage therapy and antibiotics clears Pseudomonas Aeruginosa infection in endocarditis and reduces virulence. *Journal of Infectious Diseases*, *215*(5), 703–712. https://doi.org/10.1093/infdis/jiw632

Ofir, G., Melamed, S., Sberro, H., Mukamel, Z., Silverman, S., Yaakov, G., Doron, S., & Sorek, R. (2018). DISARM is a widespread bacterial defence system with broad anti-phage activities. *Nature Microbiology*, *3*(1), 90–98. https://doi.org/10.1038/s41564-017-0051-0

Ofir, G., & Sorek, R. (2018). Contemporary Phage Biology: From Classic Models to New Insights. *Cell*, *172*(6), 1260–1270. https://doi.org/10.1016/j.cell.2017.10.045

Ohene-Agyei, T., Lea, J. D., & Venter, H. (2012). Mutations in MexB that affect the efflux of antibiotics with cytoplasmic targets. *FEMS Microbiology Letters*, *333*(1), 20–27. https://doi.org/10.1111/j.1574-6968.2012.02594.x

Olivares, E., Badel-Berchoux, S., Provot, C., Prévost, G., Bernardi, T., & Jehl, F. (2020). Clinical Impact of Antibiotics for the Treatment of Pseudomonas aeruginosa Biofilm Infections. In *Frontiers in Microbiology* (Vol. 10). Frontiers Media S.A. https://doi.org/10.3389/fmicb.2019.02894

Oliveira, P. H., Touchon, M., & Rocha, E. P. C. (2014). The interplay of restriction-modification systems with mobile genetic elements and their prokaryotic hosts. *Nucleic Acids Research*, *42*(16), 10618–10631. https://doi.org/10.1093/nar/gku734

Olsen, I. (2015). Biofilm-specific antibiotic tolerance and resistance. In *European Journal of Clinical Microbiology and Infectious Diseases* (Vol. 34, Issue 5, pp. 877–886). Springer Verlag. https://doi.org/10.1007/s10096-015-2323-z

Osmon, S., Ward, S., Fraser, V. J., & Kollef, M. H. (2004). Hospital Mortality for Patients with Bacteremia Due to Staphylococcus aureus or Pseudomonas aeruginosa. *Chest*, *125*(2), 607–616. https://doi.org/10.1378/chest.125.2.607

Ozer, B., Duran, N., Onlen, Y., & Savas, L. (2012). Efflux pump genes and antimicrobial resistance of Pseudomonas aeruginosa strains isolated from lower respiratory tract infections acquired in an intensive care unit. *Journal of Antibiotics*, *65*(1), 9–13. https://doi.org/10.1038/ja.2011.102

Pachigolla, G., Blomquist, P., & Cavanagh, H. D. (2007). Microbial keratitis pathogens and antibiotic susceptibilities: A 5-year review of cases at an urban county hospital in north Texas. In *Eye and Contact Lens* (Vol. 33, Issue 1, pp. 45–49). https://doi.org/10.1097/01.icl.0000234002.88643.d0

Page, A. J., Cummins, C. A., Hunt, M., Wong, V. K., Reuter, S., Holden, M. T. G., Fookes, M., Falush, D., Keane, J. A., & Parkhill, J. (2015). Roary: Rapid large-scale prokaryote pan genome analysis. *Bioinformatics*, *31*(22), 3691–3693. https://doi.org/10.1093/bioinformatics/btv421

Palavutitotai, N., Jitmuang, A., Tongsai, S., Kiratisin, P., & Angkasekwinai, N. (2018). Epidemiology and risk factors of extensively drug-resistant Pseudomonas aeruginosa infections. *PLoS ONE*, *13*(2). https://doi.org/10.1371/journal.pone.0193431

Pan, X., Cui, X., Zhang, F., He, Y., Li, L., & Yang, H. (2016). Genetic evidence for O-specific antigen as receptor of Pseudomonas aeruginosa phage K8 and its genomic analysis. *Frontiers in Microbiology*, *7*(MAR), 1–13. https://doi.org/10.3389/fmicb.2016.00252

Pappa, O., Kefala, A. M., Tryfinopoulou, K., Dimitriou, M., Kostoulas, K., Dioli, C., Moraitou, E., Panopoulou, M., Vogiatzakis, E., Mavridou, A., Galanis, A., & Beloukas, A. (2020). Molecular epidemiology of multi-drug resistant Pseudomonas aeruginosa isolates from hospitalized patients in Greece. *Microorganisms*, *8*(11), 1–16. https://doi.org/10.3390/microorganisms8111652

Parent, K. N., Erb, M. L., Cardone, G., Nguyen, K., Gilcrease, E. B., Porcek, N. B., Pogliano, J., Baker, T. S., & Casjens, S. R. (2014). OmpA and OmpC are critical host factors for bacteriophage Sf6 entry in Shigella. *Molecular Microbiology*, *92*(1), 47–60. https://doi.org/10.1111/mmi.12536

Paterson, D. L., & Bonomo, R. A. (2005). Extended-spectrum β-lactamases: A clinical update. In *Clinical Microbiology Reviews* (Vol. 18, Issue 4, pp. 657–686). https://doi.org/10.1128/CMR.18.4.657-686.2005

Penadés, J. R., & Christie, G. E. (2015). The Phage-Inducible Chromosomal Islands: A Family of Highly Evolved Molecular Parasites. In *Annual Review of Virology* (Vol. 2, pp. 181–201). Annual Reviews Inc. https://doi.org/10.1146/annurev-virology-031413-085446

Pérez, A., Gato, E., Pérez-Llarena, J., Fernández-Cuenca, F., Gude, M. J., Oviaño, M., Pachón, M. E., Garnacho, J., González, V., Pascual, Á., Cisneros, J. M., & Bou, G. (2019). High incidence of MDR and XDR Pseudomonas aeruginosa isolates obtained from patients with ventilator-associated pneumonia in Greece, Italy and Spain as part of the MagicBullet clinical trial. *Journal of Antimicrobial Chemotherapy*, *74*(5), 1244–1252. https://doi.org/10.1093/jac/dkz030

Petrovic, A., Kostanjsek, R., Rakhely, G., & Knezevic, P. (2017). The First Siphoviridae Family Bacteriophages Infecting Bordetella bronchiseptica Isolated from Environment. *Microbial Ecology*, *73*(2), 368–377. https://doi.org/10.1007/s00248-016-0847-0

Philip G, B., & MPhi. (2018). Antibiotic resistance and biofilm tolerance: A combined threat in the treatment of chronic infections. *JOURNAL OF WOUND CARE*, *27*(5), 273–276. https://doi.org/10.1038/nature21377

Pilmis, B., Alby-Laurent, F., Fasola, M. L., Seegers, V., Guery, R., Guet-Revillet, H., Postaire, M., Toubiana, J., Bille, E., Lortholary, O., & Zahar, J. R. (2020). Pseudomonas aeruginosa

bloodstream infections in children: a 9-year retrospective study. *European Journal of Pediatrics*, *179*(8), 1247–1254. https://doi.org/10.1007/s00431-020-03598-4

Pinto, R. M., Soares, F. A., Reis, S., Nunes, C., & van Dijck, P. (2020). Innovative strategies toward the disassembly of the EPS matrix in bacterial biofilms. In *Frontiers in Microbiology* (Vol. 11). Frontiers Media S.A. https://doi.org/10.3389/fmicb.2020.00952

Poirel, L., Lambert, T., Türkoglü, S., Ronco, E., Gaillard, J. L., & Nordmann, P. (2001). Characterization of class 1 integrons from Pseudomonas aeruginosa that contain the blaVIM-2 carbapenem-hydrolyzing β-lactamase gene and of two novel aminoglycoside resistance gene cassettes. *Antimicrobial Agents and Chemotherapy*, *45*(2), 546–552. https://doi.org/10.1128/AAC.45.2.546-552.2001

Poole, K. (2004). Resistance to β-lactam antibiotics. In *Cellular and Molecular Life Sciences* (Vol. 61, Issue 17, pp. 2200–2223). https://doi.org/10.1007/s00018-004-4060-9

Rakhuba, D. v., Kolomiets, E. I., Szwajcer Dey, E., & Novik, G. I. (2010). Bacteriophage receptors, mechanisms of phage adsorption and penetration into host cell. *Polish Journal of Microbiology*, *59*(3), 145–155. https://doi.org/10.33073/pjm-2010-023

Rasamiravaka, T., Labtani, Q., Duez, P., & el Jaziri, M. (2015). The formation of biofilms by pseudomonas aeruginosa: A review of the natural and synthetic compounds interfering with control mechanisms. In *BioMed Research International* (Vol. 2015). Hindawi Limited. https://doi.org/10.1155/2015/759348

Ratjen, F., Brockhaus, F., & Angyalosi, G. (2009). Aminoglycoside therapy against Pseudomonas aeruginosa in cystic fibrosis: A review. In *Journal of Cystic Fibrosis* (Vol. 8, Issue 6, pp. 361–369). https://doi.org/10.1016/j.jcf.2009.08.004

Rawat, D., & Nair, D. (2010). Extended-spectrum ß-lactamases in gram negative bacteria. *Journal of Global Infectious Diseases*, *2*(3), 263. https://doi.org/10.4103/0974-777x.68531

Reedy, J. S., & Wood, K. E. (2000). Endogenous Pseudomonas aeruginosa endophthalmitis: A case report and literature review. *Intensive Care Medicine*, *26*(9), 1386–1389. https://doi.org/10.1007/s001340000623

Reyes-Robles, T., Dillard, R. S., Cairns, L. S., Silva-Valenzuela, C. A., Housman, M., Ali, A., Wright, E. R., & Camilli, A. (2018). Vibrio cholerae outer membrane vesicles inhibit bacteriophage infection. *Journal of Bacteriology*, *200*(15). https://doi.org/10.1128/JB.00792-17

Rice, S. A., Tan, C. H., Mikkelsen, P. J., Kung, V., Woo, J., Tay, M., Hauser, A., McDougald, D., Webb, J. S., & Kjelleberg, S. (2009). The biofilm life cycle and virulence of Pseudomonas aeruginosa are dependent on a filamentous prophage. *ISME Journal*, *3*(3), 271–282. https://doi.org/10.1038/ismej.2008.109

Riou, M., Avrain, L., Carbonnelle, S., el Garch, F., Pirnay, J. P., de Vos, D., Plésiat, P., Tulkens, P. M., & van Bambeke, F. (2016). Increase of efflux-mediated resistance in Pseudomonas aeruginosa during antibiotic treatment in patients suffering from nosocomial pneumonia. *International Journal of Antimicrobial Agents*, *47*(1), 77–83. https://doi.org/10.1016/j.ijantimicag.2015.11.004

Robinson, J. T., Thorvaldsdóttir, H., Winckler, W., Guttman, M., Lander, E. S., Getz, G., & Mesirov, J. P. (2011). Integrative Genome Viewer. *Nature Biotechnology*, *29*(1), 24–26. https://doi.org/10.1038/nbt.1754.Integrative

Rodríguez-Rojas, A., Mena, A., Martín, S., Borrell, N., Oliver, A., & Blázquez, J. (2009). Inactivation of the hmgA gene of Pseudomonas aeruginosa leads to pyomelanin hyperproduction, stress resistance and increased persistence in chronic lung infection. *Microbiology*, *155*(4), 1050–1057. https://doi.org/10.1099/mic.0.024745-0

Rombouts, S., Volckaert, A., Venneman, S., Declercq, B., Vandenheuvel, D., Allonsius, C. N., van Malderghem, C., Jang, H. B., Briers, Y., Noben, J. P., Klumpp, J., van Vaerenbergh, J., Maes, M., & Lavigne, R. (2016). Characterization of novel bacteriophages for biocontrol of bacterial blight in leek caused by Pseudomonas syringae pv. porri. *Frontiers in Microbiology*, *7*(MAR), 1–15. https://doi.org/10.3389/fmicb.2016.00279

Rosenblatt-Farrell, N. (2009). The landscape of antibiotic resistance. *Environ Health Perspect*, *117*(6), A244–A250.

Ruokoranta, T. M., Grahn, A. M., Ravantti, J. J., Poranen, M. M., & Bamford, D. H. (2006). Complete Genome Sequence of the Broad Host Range Single-Stranded RNA Phage PRR1 Places It in the Levivirus Genus with Characteristics Shared with Alloleviviruses . *Journal of Virology*, *80*(18), 9326–9330. https://doi.org/10.1128/jvi.01005-06

Safaei, H., Moghim, S., Isfahani, B., Fazeli, H., Poursina, F., Yadegari, S., Nasirmoghadas, P., & Nodoushan, S. H. (2017). Distribution of the Strains of Multidrug-resistant, Extensively Drug-resistant, and Pandrug-resistant Pseudomonas aeruginosa Isolates from Burn Patients. *Advanced Biomedical Research*, *6*(1), 74. https://doi.org/10.4103/abr.abr_239_16

Saleem, S., & Bokhari, H. (2020). Resistance profile of genetically distinct clinical Pseudomonas aeruginosa isolates from public hospitals in central Pakistan. *Journal of Infection and Public Health*, *13*(4), 598–605. https://doi.org/10.1016/j.jiph.2019.08.019

Samson, I. (2005). A new class of antimycobacterial drugs: the diarylquinolines. *Thorax*, *60*(6), 495. https://doi.org/10.1136/thx.2005.la0156

Samson, J. E., Magadán, A. H., Sabri, M., & Moineau, S. (2013). Revenge of the phages: Defeating bacterial defences. *Nature Reviews Microbiology*, *11*(10), 675–687. https://doi.org/10.1038/nrmicro3096

Sandhu, A., Gross, M., Wylie, J., van Caeseele, P., & Plourde, P. (2017). *Pseudomonas aeruginosa Necrotizing Chondritis Complicating High Helical EarPiercing Case Report Clinical and Public Health Perspectives*. https://doi.org/10.1007/BF03405390.

Sanz-garcía, F., Hernando-amado, S., & Martínez, L. (2018). Mutation Driven Evolution of Pseudomonas aeruginosa in the Presence of either Ceftazidime or Ceftazidime-Avibactam. *Antimicrobial Agents and Chemotherapy*, *62*(10), 1–13. https://doi.org/10.1128/AAC.01379-18

Schneider, G., Szentes, N., Horváth, M., Dorn, Á., Cox, A., Nagy, G., Doffkay, Z., Maróti, G., Rákhely, G., & Kovács, T. (2018). Kinetics of Targeted Phage Rescue in a Mouse Model of Systemic Escherichia coli K1. *BioMed Research International*, *2018*. https://doi.org/10.1155/2018/7569645

Schooley, R. T., Biswas, B., Gill, J. J., Hernandez-Morales, A., Lancaster, J., Lessor, L., Barr, J. J., Reed, S. L., Rohwer, F., Benler, S., Segall, A. M., Taplitz, R., Smith, D. M., Kerr, K., Kumaraswamy, M., Nizet, V., Lin, L., McCauley, M. D., Strathdee, S. A., … Hamilton, T. (2017). Development and use of personalized bacteriophage-based therapeutic cocktails to treat a patient with a disseminated resistant Acinetobacter baumannii infection. *Antimicrobial Agents and Chemotherapy*, *61*(10). https://doi.org/10.1128/AAC.00954-17

Schwechheimer, C., & Kuehn, M. J. (2015). Outer-membrane vesicles from Gram-negative bacteria: Biogenesis and functions. In *Nature Reviews Microbiology* (Vol. 13, Issue 10, pp. 605–619). Nature Publishing Group. https://doi.org/10.1038/nrmicro3525

Seemann, T. (2014). Prokka: Rapid prokaryotic genome annotation. *Bioinformatics*, *30*(14), 2068–2069. https://doi.org/10.1093/bioinformatics/btu153

Serra, C., Bouharkat, B., Touil-Meddah, A. T., Guénin, S., & Mullié, C. (2019). MexXY multidrug efflux system is more frequently overexpressed in ciprofloxacin resistant French clinical isolates compared to hospital environment ones. *Frontiers in Microbiology*, *10*(FEB), 1–9. https://doi.org/10.3389/fmicb.2019.00366

Sharma, A., Sharma, R., Bhattacharyya, T., Bhando, T., & Pathania, R. (2017). Fosfomycin resistance in Acinetobacter baumannii is mediated by efflux through a major facilitator superfamily (MFS) transporter-AbaF. *Journal of Antimicrobial Chemotherapy*, *72*(1), 68–74. https://doi.org/10.1093/jac/dkw382

Shen, M., Le, S., Jin, X., Li, G., Tan, Y., Li, M., Zhao, X., Shen, W., Yang, Y., Wang, J., Zhu, H., Li, S., Rao, X., Hu, F., & Lu, S. (2016). Characterization and Comparative Genomic Analyses of Pseudomonas aeruginosa Phage PaoP5: New Members Assigned to PAK-P1-like Viruses. *Scientific Reports*, *6*(September), 1–6. https://doi.org/10.1038/srep34067

Shen, M., Zhang, H., Shen, W., Zou, Z., Lu, S., Li, G., He, X., Agnello, M., Shi, W., Hu, F., & Le, S. (2018). Pseudomonas aeruginosa MutL promotes large chromosomal deletions through non-homologous end joining to prevent bacteriophage predation. *Nucleic Acids Research*, *46*(9), 4505–4514. https://doi.org/10.1093/nar/gky160

Shi, Q., Huang, C., Xiao, T., Wu, Z., & Xiao, Y. (2019). A retrospective analysis of Pseudomonas aeruginosa bloodstream infections: Prevalence, risk factors, and outcome in carbapenem-susceptible and -non-susceptible infections. *Antimicrobial Resistance and Infection Control*, *8*(1), 1–9. https://doi.org/10.1186/s13756-019-0520-8

Sidhu, S. S. (2000). Phage display in pharmaceutical biotechnology. *Current Opinion in Biotechnology*, *11*(6), 610–616. https://doi.org/10.1016/S0958-1669(00)00152-X

Singh, M., Yau, Y. C. W., Wang, S., Waters, V., & Kumar, A. (2017). MexXY efflux pump overexpression and aminoglycoside resistance in cystic fibrosis isolates of pseudomonas aeruginosa from chronic infections. *Canadian Journal of Microbiology*, *63*(12), 929–938. https://doi.org/10.1139/cjm-2017-0380

Skurnik, M., Pajunen, M., & Kiljunen, S. (2007). Biotechnological challenges of phage therapy. In *Biotechnology Letters* (Vol. 29, Issue 7, pp. 995–1003). https://doi.org/10.1007/s10529-007-9346-1

Skurnik, M., & Strauch, E. (2006). Phage therapy: Facts and fiction. In *International Journal of Medical Microbiology* (Vol. 296, Issue 1, pp. 5–14). Elsevier GmbH. https://doi.org/10.1016/j.ijmm.2005.09.002

Sosin, M., Weissler, J. M., Pulcrano, M., & Rodriguez, E. D. (2015). Transcartilaginous ear piercing and infectious complications: A systematic review and critical analysis of outcomes. In *Laryngoscope* (Vol. 125, Issue 8, pp. 1827–1834). John Wiley and Sons Inc. https://doi.org/10.1002/lary.25238

Sousa, J. A. M. de, & Rocha, E. P. C. (2019). Environmental structure drives resistance to phages and antibiotics during phage therapy and to invading lysogens during colonisation. *Scientific Reports*, *9*(1), 1–13. https://doi.org/10.1038/s41598-019-39773-3

Stewart, P. S. (2002). Mechanisms of antibiotic resistance in bacterial biofilms. In *Int. J. Med. Microbiol* (Vol. 292). https://doi.org/10.1078/1438-4221-00196.

Stewart, P. S., & J William, C. (2001). Antibiotic resistance of bacteria in biofilms. *Lancet*, *358*(135), 38. https://doi.org/10.1016/s0140-6736(01)05321-1.

Stover, C. K., Pham, X. Q., Erwin, A. L., Mizoguchi, S. D., Warrener, P., Hickey, M. J., Brinkman, F. S. L., Hufnagle, W. O., Kowallk, D. J., Lagrou, M., Garber, R. L., Goltry, L., Tolentino, E., Westbrock-Wadman, S., Yuan, Y., Brody, L. L., Coulter, S. N., Folger, K. R., Kas, A., … Olson, M. v. (2000). Complete genome sequence of Pseudomonas aeruginosa PAO1, an opportunistic pathogen. *Nature*, *406*(6799), 959–964. https://doi.org/10.1038/35023079

Stratton, C. W. (2003). Dead Bugs Don't Mutate: Susceptibility Issues in the Emergence of Bacterial Resistance. In *Emerging Infectious Diseases* • (Vol. 9, Issue 1). https://doi.org/10.3201/eid0901.020172

Stover, C. K., Pham, X. Q., Erwin, A. L., Mizoguchi, S. D., Warrener, P., Hickey, M. J., Brinkman, F. S. L., Hufnagle, W. O., Kowallk, D. J., Lagrou, M., Garber, R. L., Goltry, L., Tolentino, E., Westbrock-Wadman, S., Yuan, Y., Brody, L. L., Coulter, S. N., Folger, K. R., Kas, A., … Olson, M. v. (2000). Complete genome sequence of Pseudomonas aeruginosa PAO1, an opportunistic pathogen. *Nature*, *406*(6799), 959–964. https://doi.org/10.1038/35023079

Swarts, D. C., Jore, M. M., Westra, E. R., Zhu, Y., Janssen, J. H., Snijders, A. P., Wang, Y., Patel, D. J., Berenguer, J., Brouns, S. J. J., & van der Oost, J. (2014). DNA-guided DNA interference by a prokaryotic Argonaute. *Nature*, *507*(7491), 258–261. https://doi.org/10.1038/nature12971

Tan, S. Y., & Tatsumura, Y. (2015). Alexander Fleming (1881–1955): Discoverer of penicillin. In *Singapore Medical Journal* (Vol. 56, Issue 7, pp. 366–367). Singapore Medical Association. https://doi.org/10.11622/smedj.2015105

Tandogdu, Z., Cek, M., Wagenlehner, F., Naber, K., Tenke, P., van Ostrum, E., & Bjerklund Johansen, T. (2014). Resistance patterns of nosocomial urinary tract infections in urology departments: 8-year results of the global prevalence of infections in urology study. *World Journal of Urology*, *32*(3), 791–801. https://doi.org/10.1007/s00345-013-1154-8

Tanji, Y., Hattori, K., Suzuki, K., & Miyanaga, K. (2008). Spontaneous deletion of a 209-kilobase-pair fragment from the Escherichia coli genome occurs with acquisition of resistance to an assortment of infectious phages. *Applied and Environmental Microbiology*, *74*(14), 4256–4263. https://doi.org/10.1128/AEM.00243-08

Thaden, J. T., Park, L. P., Maskarinec, S. A., Ruffin, F., Fowler, V. G., & van Duin, D. (2017). Results from a 13-year prospective cohort study show increased mortality associated with bloodstream infections caused by pseudomonas aeruginosa compared to other bacteria. *Antimicrobial Agents and Chemotherapy*, *61*(6), 1–11. https://doi.org/10.1128/AAC.02671-16

Thi, M. T. T., Wibowo, D., & Rehm, B. H. A. (2020). Pseudomonas aeruginosa biofilms. In *International Journal of Molecular Sciences* (Vol. 21, Issue 22, pp. 1–25). MDPI AG. https://doi.org/10.3390/ijms21228671

Thomassen, E., Gielen, G., Schütz, M., Schoehn, G., Abrahams, J. P., Miller, S., & van Raaij, M. J. (2003). The structure of the receptor-binding domain of the bacteriophage T4 short tail fibre reveals a knitted trimeric metal-binding fold. *Journal of Molecular Biology*, *331*(2), 361–373. https://doi.org/10.1016/S0022-2836(03)00755-1

Turner, D., Kropinski, A. M., & Adriaenssens, E. M. (2021). A roadmap for genome-based phage taxonomy. *Viruses*, *13*(3). https://doi.org/10.3390/v13030506

Turner, K. H., Wessel, A. K., Palmer, G. C., Murray, J. L., & Whiteley, M. (2015). Essential genome of Pseudomonas aeruginosa in cystic fibrosis sputum. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(13), 4110–4115. https://doi.org/10.1073/pnas.1419677112

Twort, F. W. (1915). an Investigation on the Nature of Ultra-Microscopic Viruses. *The Lancet*, *186*(4814), 1241–1243. https://doi.org/10.1016/S0140-6736(01)20383-3

Ul Haq, I., Chaudhry, W. N., Akhtar, M. N., Andleeb, S., & Qadri, I. (2012). Bacteriophages and their implications on future biotechnology: A review. *Virology Journal*, *9*, 1–8. https://doi.org/10.1186/1743-422X-9-9

Vicente, L., Ferreira Da, R., Filho, S., De, F., Ferreira, A., José, F., Reis, C., Amorim De Britto, C., Levy, C. E., Clark, O., Ribeiro, J. D., & Ferreira, R. (2013). Pseudomonas aeruginosa infection in patients with cystic fibrosis: scientific evidence regarding clinical impact, diagnosis, and treatment. *J Bras Pneumol*, *39*(4), 495–512. https://doi.org/10.1590/S1806-37132013000400015

Vogne, C., Aires, J. R., Bailly, C., Hocquet, D., & Plésiat, P. (2004). Role of the Multidrug Efflux System MexXY in the Emergence of Moderate Resistance to Aminoglycosides among Pseudomonas aeruginosa Isolates from Patients with Cystic Fibrosis. *Antimicrobial Agents and Chemotherapy*, *48*(5), 1676–1680. https://doi.org/10.1128/AAC.48.5.1676-1680.2004

Wagenlehner, F. M. E., Bjerklund Johansen, T. E., Cai, T., Koves, B., Kranz, J., Pilatz, A., & Tandogdu, Z. (2020). Epidemiology, definition and treatment of complicated urinary tract infections. In *Nature Reviews Urology* (Vol. 17, Issue 10, pp. 586–600). Nature Research. https://doi.org/10.1038/s41585-020-0362-4

Walker, T. S., Bais, H. P., Déziel, E., Schweizer, H. P., Rahme, L. G., Fall, R., & Vivanco, J. M. (2004). Pseudomonas aeruginosa-Plant Root Interactions. Pathogenicity, Biofilm Formation, and Root Exudation. *Plant Physiology*, *134*(1), 320–331. https://doi.org/10.1104/pp.103.027888

Walters, M. C., Roe, F., Bugnicourt, A., Franklin, M. J., & Stewart, P. S. (2003). Contributions of antibiotic penetration, oxygen limitation, and low metabolic activity to tolerance of

Pseudomonas aeruginosa biofilms to ciprofloxacin and tobramycin. *Antimicrobial Agents and Chemotherapy*, *47*(1), 317–323. https://doi.org/10.1128/AAC.47.1.317-323.2003

Weppelman, R. M., & Brinton, C. C. J. (1971). The infection of Pseudomonas aeruginosa by RNA pilus phage PP7: the adsorption organelle and the relationship between phage sensitivity and the division cycle. *Virology*, *44*(1), 1–17. https://doi.org/10.1016/0042-6822(71)90147-4

WHO. (2018). *World health organization releases global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics*. WHO GLOBAL PRIORITY LIST. https://www.who.int/medicines/publications/WHO-PPL-Short_Summary_25Feb-ET_NM_WHO.pdf

WHO. (2020). *Lack of new antibiotics threatens global efforts to contain drug-resistant infections*. https://www.who.int/news/item/17-01-2020-lack-of-new-antibiotics-threatens-global-efforts-to-contain-drug-resistant-infections

WHO. (2021). *2020 Antibacterial Agents in Clinical and Preclinical Development*. World Health Organization 2021. https://www.who.int/publications/i/item/9789240021303

Wick, R. R., Judd, L. M., Gorrie, C. L., & Holt, K. E. (2017). Unicycler: Resolving bacterial genome assemblies from short and long sequencing reads. *PLoS Computational Biology*, *13*(6), 1–22. https://doi.org/10.1371/journal.pcbi.1005595

Wilton, M., Charron-Mazenod, L., Moore, R., & Lewenza, S. (2016). Extracellular DNA acidifies biofilms and induces aminoglycoside resistance in Pseudomonas aeruginosa. *Antimicrobial Agents and Chemotherapy*, *60*(1), 544–553. https://doi.org/10.1128/AAC.01650-15

Wingett, S. W., & Andrews, S. (2018). Fastq screen: A tool for multi-genome mapping and quality control [version 1; referees: 3 approved, 1 approved with reservations]. *F1000Research*, *7*(0), 1–13. https://doi.org/10.12688/f1000research.15931.1

Wolska, K. I., Grudniak, A. M., Rudnicka, Z., & Markowska, K. (2016). Genetic control of bacterial biofilms. *Journal of Applied Genetics*, *57*(2), 225–238. https://doi.org/10.1007/s13353-015-0309-2

Wood, T. K., Knabel, S. J., & Kwan, B. W. (2013). Bacterial persister cell formation and dormancy. In *Applied and Environmental Microbiology* (Vol. 79, Issue 23, pp. 7116–7121). https://doi.org/10.1128/AEM.02636-13

Wright, G. D. (2005). Bacterial resistance to antibiotics: Enzymatic degradation and modification. In *Advanced Drug Delivery Reviews* (Vol. 57, Issue 10, pp. 1451–1470). https://doi.org/10.1016/j.addr.2005.04.002

Wu, X., Siehnel, R. J., Garudathri, J., Staudinger, B. J., Hisert, K. B., Ozer, E. A., Hauser, A. R., Eng, J. K., Manoil, C., Singh, P. K., & Bruce, J. E. (2019). In Vivo Proteome of Pseudomonas aeruginosa in Airways of Cystic Fibrosis Patients. *Journal of Proteome Research*, *18*(6), 2601–2612. https://doi.org/10.1021/acs.jproteome.9b00122

Xian-Zhi, L., & Hiroshi, N. (2009). Efflux-Mediated Drug Resistance in Bacteria: an Update. In *Drugs* (Vol. 69, Issue 12). https://doi.org/10.2165/11317030-000000000-00000.

Zeng, J., Wang, Y., Zhang, J., Yang, S., & Zhang, W. (2021). Multiple novel filamentous phages detected in the cloacal swab samples of birds using viral metagenomics approach. *Virology Journal*, *18*(1), 1–6. https://doi.org/10.1186/s12985-021-01710-0

Zhang, L., Hinz, A. J., Nadeau, J. P., & Mah, T. F. (2011). Pseudomonas aeruginosa tssC1 links type VI secretion and biofilm-specific antibiotic resistance. In *Journal of Bacteriology* (Vol. 193, Issue 19, pp. 5510–5513). https://doi.org/10.1128/JB.00268-11

Zhang, L., & Mah, T. F. (2008). Involvement of a novel efflux system in biofilm-specific resistance to antibiotics. *Journal of Bacteriology*, *190*(13), 4447–4452. https://doi.org/10.1128/JB.01655-07

Zhang, Y., & LeJeune, J. T. (2008). Transduction of blaCMY-2, tet(A), and tet(B) from Salmonella enterica subspecies enterica serovar Heidelberg to S. Typhimurium. *Veterinary Microbiology*, *129*(3), 418–425. https://doi.org/https://doi.org/10.1016/j.vetmic.2007.11.032

13.0. SUPPLEMENTARY

Table S1. List of PAO1 transposon mutants used in the study

Mutant	Defective structure(s)
ΔoprM	Resistance-Nodulation-Cell Division (RND)-OprM precursor
ΔmexY	RND-Multidrug efflux transporter
ΔgalU	Lipopolysaccharide core region biosynthesis
ΔopdO	Pyroglutamate porin OpdO
ΔsppR	Cell surface receptor
ΔlepA	GTP-binding protein LepA
ΔopdE	membrane protein OpdE
ΔgalE	UDP-glucose 4-epimerase
Δsppr	Membrane Receptor-SppR
ΔgalE	UDP-glucose 4-epimerase
ΔopdO	pyroglutamate porin OpdO
ΔcupA3	usher CupA3

Table S2: GO enrichments of the deleted region in the mutants.

GO.ID	Term	Annotated	topgoFisher
GO:0071453	Cellular response to oxygen levels	11	0,000000047
GO:0008300	Isoprenoid catabolic process	14	0,000063
GO:0031408	Oxylipin biosynthetic process	2	0,0017
GO:0006572	Tyrosine catabolic process	2	0,0017
GO:0098743	Cell aggregation	2	0,0017
GO:0006552	Leucine catabolic process	8	0,0032
GO:0009405	Pathogenesis	63	0,0037
GO:0019441	Tryptophan catabolic process to kynurenine	3	0,0049
GO:0046247	Terpene catabolic process	11	0,0087
GO:0007155	Cell adhesion	21	0,0093
GO:0007166	Cell surface receptor signaling pathway	13	0,0142
GO:0042413	Carnitine catabolic process	6	0,0224
GO:0006559	L-phenylalanine catabolic process	6	0,0224
GO:0043711	Pilus organization	28	0,0305
GO:0061077	Chaperone-mediated protein folding	7	0,0305
GO:0044042	Glucan metabolic process	5	0,0404
GO:0006011	Udp-glucose metabolic process	1	0,0409
GO:0032329	Serine transport	1	0,0409
GO:0009439	Cyanate metabolic process	1	0,0409
GO:0090503	Rna phosphodiester bond hydrolysis	1	0,0409
GO:0005992	Trehalose biosynthetic process	1	0,0409
GO:0071407	Cellular response to organic cyclic compound	1	0,0409
GO:0015826	Threonine transport	1	0,0409
GO:0005980	Glycogen catabolic process	1	0,0409
GO:0005978	Glycogen biosynthetic process	1	0,0409
GO:0072707	Cellular response to sodium dodecyl sulfate	1	0,0409
GO:0005975	Carbohydrate metabolic process	194	0,0412
GO:0055114	Oxidation-reduction process	493	0,0475
GO:0071555	Cell wall organization	9	0,0496

14.0. LIST OF PUBLICATIONS

LIST OF PUBLICATION RELATED TO THIS THESIS

MTMT Author ID: 10053210

Cumulative impact factor: 17.167

Koderi Valappil, S., Shetty, P., Deim, Z., Terhes, G., Urbán, E., Váczi, S., Patai, R., Polgár, T., Pertics, B. Z., Schneider, G., Kovács, T., & Rákhely, G (2021). Survival Comes at a Cost: A Coevolution of Phage and Its Host Leads to Phage Resistance and Antibiotic Sensitivity of Pseudomonas aeruginosa Multidrug Resistant Strains. *Frontiers in Microbiology* 12, 1–17. DOI:10.3389/fmicb.2021.783722. (IF_{2021}: 5.640)

Horváth, M., Kovács, T., **Koderi Valappil, S**., Ábrahám, H., Rákhely, G., and Schneider, G. (2020). Identification of a newly isolated lytic bacteriophage against K24 capsular type, carbapenem resistant Klebsiella pneumoniae isolates. *Scientific Reports* 10. doi:10.1038/s41598-020-62691-8. (IF_{2020}: 4.130)

OTHER PUBLICATIONS

Molnár, J., Magyar, B., Schneider, G., Laczi, **Koderi Valappil, S**. K., Kovács, Á. L., Nagy, I. K., Rákhely, G., & Kovács, T. (2020). Identification of a novel archaea virus, detected in hydrocarbon polluted Hungarian and Canadian samples. *PLoS ONE* 15. DOI:10.1371/journal.pone.0231864. (IF_{2020}:3.240)

Deim, Z., Dencső, L., Erdélyi, I., **Koderi Valappil, S**. K., Varga, C., Pósa, A., Makrai, L., & Rákhely (2019). Porcine circovirus type 3 detection in a Hungarian pig farm experiencing reproductive failures. *Veterinary Record* 185, 84. DOI:10.1136/vr.104784. (IF_{2019}:3.357)

Kovács, T., Molnár, J., Varga, I., Nagy, I. **Koderi Valappil, S**. K., Papp, S., Vera Cruz, C. M., Oliva, R., Vizi, T., Schneider, G., & Rákhely, G. (2019). Complete Genome Sequences of 10 Xanthomonas oryzae pv. oryzae Bacteriophages. *Microbiology Resource Announcements* 8. DOI:10.1128/mra.00334-19. (IF_{2019}:0.800)

CONFERENCE ABSTRACTS

Koderi Valappil, S., Shetty, P., Deim, Z., Terhes, G., Urbán, E., Váczi, S., Patai, R., Polgár, T., Pertics, B. Z., Schneider, G., Kovács, T., & Rákhely, G. (2018). Characterization of Novel Lytic Bacteriophages Against Pseudomonas aeruginosa Biofilm. The 5th World Congress on Targeting Infectious Diseases: Targeting Phage & Antibiotic Resistance 2018**,** Florence, Italy in May 17-18, 2018. Suppl. 1 Pp. 123. (2018)

Rákhely, G., Varga, I., Molnár,J., Gazdag, A., Szűcs, D., Doffkay, Z., **Koderi Valappil, S.,**, Papp, S., Pintér, R., Cruz, C., Vizi, T., Schneider, G., Kovács, T. Comparative Genomics Of Xop2-Like Xanthomonas Oryzae Pv. Oryzae Bacteriophages Acta Microbiologica Et Immunologica Hungarica 64 : Suppl. 1 Pp. 160-161. , 2 P. (2017)

Koderi Valappil, S., Shetty, P., Deim, Z., Terhes, G., Urbán, E., Váczi, S., Patai, R., Polgár, T., Pertics, B. Z., Schneider, G., Kovács, T., & Rákhely, G. Characterization Of Novel Lytic Bacteriophages Against Pseudomonas Aeruginosa Clinical Isolates Acta Microbiologica Et Immunologica Hungarica 64 : Suppl. 1 Pp. 186-186. , 1 P. (2017)