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Review

# Critically Resistant *Pseudomonas aeruginosa* : a Short Review on the Mechanisms of Resistance and Therapeutic Strategies

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**Abstract**

*Pseudomonas aeruginosa* is a critically resistant pathogen that is responsible for the morbidity of immunodeficient patients throughout the world. The bacterium possesses unique resistance to several antibiotics. Numerous resistance mechanisms like reliable efflux pumps, reduced permeability of the cell membrane, inactivation of antimicrobial drugs by enzymatic mechanisms and modification of antibiotic target sites enable the survival of the bacteria in adverse conditions. The causes and extent of antibiotic resistance in this bacterial species have been elucidated clearly. The ability of the pathogen to express several mutations in crucial genes makes it lethal. The everlasting threat of intrinsic as well as acquired antimicrobial resistance in common bacterial species is studied extensively to devise newer and effective therapeutics for the general public. Several studies have worked on the methods to deal with *P. aeruginosa* infections. The highest level of the newest antibiotics along with combinatorial therapy is administered to severely affected patients. Modern therapeutic strategies of bacteriophage therapy have been tested preclinically. The problem statement of curing *P. aeruginosa* infections thoroughly is yet to be resolved constructively.

**Keywords**: *Pseudomonas aeruginosa*, resistance mechanisms, antibiotic resistance, newest antibiotics, bacteriophage therapy.

**Резюме**

*Pseudomonas aeruginosa* е критично резистентен патоген, който е отговорен за заболеваемостта на пациентите с имунен дефицит по целия свят. Бактерията притежава уникална резистентност към няколко антибиотици. Способността на *P. aeruginosa* да оцелее като биофилм в конюгация с други видове само повишава нейната жизненост. Нивото на антибиотична резистентност при този бактериален вид е изключително високо и много тревожно, особено в нозокомиални среди. Надеждните ефлуксни помпени системи и ензими като β-лактамаза позволяват оцеляването на бактериите при неблагоприятни условия. Механизмите на резистентност, дължащи се на постоянни мутации в ключови гени в *P. aeruginosa*, го правят смъртонсен. Постоянната заплаха от вътрешна и придобита антимикробна резистентност при често срещани бактериални видове е обект на нарастващ интерес. Целта е да се разработят нови и по-ефективни терапевтични средства за клинична употреба. Публикувани са проучвания, посветени на методите за справяне с инфекциите, причинени от *P. aeruginosa*. При тежко засегнатите пациенти се прилага комбинирана терапия от нови антибиотици. Съвременните терапевтични стратегии за терапия с бактериофаги са тествани предклинично. Проблемът за цялостното лечение на инфекциите с *P. aeruginosa* все още не е решен конструктивно.

## Introduction

*Pseudomonas aeruginosa* is a Gram-negative bacillus which is about 1-5µm long and 0.5-1.0µm wide. Taxonomically, the heterotrophic bacterium, with 273 strains (Girard *et al*., 2021), can be classified under Kingdom Monera, phylum Proteobacteria, class gamma sub-division, order Pseudomonadaceae, genus *Pseudomonas*, species *P. aeruginosa* (Diggle and Whitely, 2020). The lethal bacterium belongs to the *Pseudomonas* genus which possesses a whopping 144 species (Gomila *et al*., 2015). The microorganism is studied in laboratories using the two commonly isolated strains

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PAO1 (Stover *et al*., 2000) and PA14 (Mikkelsen *et al*., 2011). *P. aeruginosa* is a highly resistant bacterium that has been classified as a pathogen of paramount concern by the WHO due to multidrug resistance and widespread carbapenem resistance. The contents of the layers of the plasma membrane, specifically its lipopolysaccharide (Huszczynski *et al*., 2019), are responsible for the symptoms of its infection. Studies prove that the bacterium is predominantly present in hospitals, and it tends to infect patients who are immunocompromised due to pneumonia (Ding *et al*., 2016), cystic fibrosis (Silva Filho *et al*., 2013), extensive burn wounds (Brandenburg *et al*., 2019), rhinosinusitis (Cho *et al*., 2020) and AIDS (Dropulic *et al*., 1995). The mortality rate due to *P. aeruginosa* infections is alarming with nosocomial statistics showing that around 8% of 32,600 succumbent patients lost their lives in 2017 (CDC, 2019). Lu *et al*. (2014) have postulated that the most life-threatening serotypes of *P. aeruginosa* are the O6 and O11 serotypes in nosocomial environments. It must also be noted that pediatric patients are affected more adversely (Grisaru-Soen *et al*., 2000) with almost 50% of the bacteremia cases occurring in cancer patients. Although the bacterium is opportunistic, it rarely affects healthy individuals (Pang *et al*., 2019). *P. aeruginosa* has a fascinating ability to form thick layers called biofilms (Thi *et al*., 2020) in conjugation with other pathogens like *Candida albicans* (Phuengmaung *et al*., 2020) on ventilators, catheters, and several hospital appliances. Several unique techniques for eradicating such biofilms formed by various bacteria have been proposed by Chakraborty *et al*. (2020). Like most other bacteria, *P. aeruginosa* also displays the potential to take possession of extensive resistance genes to antibiotics via horizontal gene transfer or mutations (Poole, 2011). It also displays high levels of intrinsic resistance by reducing membrane permeability, increasing the number of efflux pumps for the extrusion of antibiotics, and producing antibiotic-inactivating enzymes like lactamase. It adapts to antibiotic exposure and environmental stresses, even in critical care centers (Pachori *et al*., 2019), thereby increasing its vitality.

## Mechanisms of antibiotic resistance in MDR *P. aeruginosa*

Antibiotic resistance is delineated as the ability of the bacteria to survive in the presence of antibiotics which have been developed to decimate their effect. Thus, many bacteria have developed specific mechanisms that protect them from the effects of antimicrobial drugs. According to Nikaido (2009), the everlasting issue of pathogenic bacteria acquiring or developing resistance to multiple antimicrobial drugs due to the accumulation and effect of resistance genes is termed multidrug resistance (MDR). The complex genome of the bacterial species is one of the chief reasons for drug resistance. The genes for resistance may be part of extra-chromosomal DNA. Upon PCR Amplification and the use of the clonality analysis technique, Sawa *et al*. (2020) found that the accessory genome content of the pathogenic strains is very variable. The high degree of variability marks the capability of the pathogen to become highly virulent. The efforts to limit the cellular uptake of antibiotics, active extrusion of a drug molecule, inactivation of the drug by chemical alterations, and modification of the drug target are the four major mechanisms of resistance responsible for the increased pathogenicity of bacteria (Reygaert, 2018). While bacteria can selectively alter their cell membrane to prevent the entry of drug molecules, they also have efflux pumps present on the same membrane to remove the active drug molecule from the bacterial cell before it can elicit its mechanism of action. Bacterial enzymes often chemically alter and degrade drug molecules before they act on the target. Certain drug target proteins may also undergo conformational changes to prevent the binding and subsequent action of antibiotics. All the aforementioned modes of resistance are shown by *P. aeruginosa* (Mares *et al*., 2021), and these are discussed in detail to understand and tackle infections more accurately.

### Efflux pumps

Efflux pumps are energy pumps that are present in bacterial membranes and selectively extrude harmful chemicals and compounds that have entered the cell. There are 5 major superfamilies of efflux pumps namely the SMR, MFS, ABC, RND, and MATE superfamilies (Fernández and Hancock, 2012). The 4 significant pumps in *P. aeruginosa* described by Poole (2011) namely MexAB-OprM, MexEF-OprN, MexCD-OprJ, and MexXY are all part of the Resistance-nodulation-division (RND) family. According to Masuda *et al*. (2000), MexAB-OprM and MexXY-OprM are the two pumps that are capable of actively extruding fluoroquinolones, tetracycline, and chloramphenicol antibiotics. MexAB-OprM is involved in β-Lactam exclusion. MexXY-OprM is upregulated due to mutations in the fusA1 gene, which codes for the elongation factor G, conferring as high as 8-fold resistance to aminoglycosides (del Barrio-Tofiño *et al.,* 2017).

Phenylalanine arginyl β-naphthylamide (PAβN) is a chemical that decreases bacterial efflux by competitive inhibition of efflux pumps and increasing the permeability of bacterial membranes (Lamers *et al*., 2013; Fernández and Hancock, 2012). OprF protein is a general membrane protein in the pathogen that enables the survival of the cell in very low osmolarity conditions and enables the acquisition of antibiotic resistance genes from several species. The efflux proteins OmpH, OprM, OprN, OprJ, OmpL, OmpM, and TolC are highly specific pumps involved in antibiotic extrusion. The alkaline protease secretions of AprF pumps can minimize the effect of several antibiotics (Hancock and Brinkman, 2002).

**Reduced membrane permeability**

Gram-negative bacteria commonly have extremely complex LPS, phospholipid bilayer, and porins which restrict several components from entering the cell. The majority of antibiotics that are administered to tackle *P. aeruginosa* infections are broad-spectrum antibiotics having intracellular targets. According to Yang *et al*. (2015), the administration of Fluoroquinolones like Ciprofloxacin to inhibit DNA Gyrase and Topoisomerase □ and consequently stop the process of DNA replication in bacterial cells is extremely challenging for impermeable membranes. Aminoglycosides like Gentamicin must enter cells to attack 30S ribosomal units of bacteria and cause the production of deformed proteins (Kapoor *et al*., 2017). Porins are fluid-filled membrane channels that allow the penetration of antibiotics into bacterial cells. There are several porin superfamilies and families that modulate permeability levels. According to the experiment conducted by Choi and Lee (2019), all the porins like OmpA and OmpC, which are non-specific, play a key role in response to stressful conditions encountered by the cell. A predominantly occurring porin named OprF mediates closed conformal channels in the membrane. It is responsible for ion channels and antibiotic penetration, and the closed conformation is responsible for low antibiotic penetration (Pang *et al*., 2019). Additionally, the bacterium has many specific proteins (Choi and Lee, 2019) like OprB (carbohydrate specific), OprD (amino-acid specific), and OprP (phosphate specific), which tend to contribute to reduced antibiotic entry. Li *et al*. (2012) delineated that OprD contains the binding site of β-lactam antibiotics of the carbapenem group and a reduced expression of this porin can result in Carbapenem resistance. The specificity of *P. aeruginosa* porins is extremely high and although the penetration of most antibiotics in mutants is not majorly affected by the presence of porins, some antibiotics like Azithromycin and Rifampicin display very poor performance (Ude *et al*., 2021) in bacteria with altered porins. Since the aforementioned antibiotics have intracellular targets, their efficacy is highly diminished, and they are often refrained from being administered as first choice drugs.

**Antibiotic inactivation**

Over the years bacteria have developed specialized enzymatic mechanisms to alter the chemical structure of antimicrobials and decimate their action. Most of these enzymes ensure the catalysis of the hydrolysis reactions of β-lactam antibiotics and aminoglycosides. β-Lactamase enzyme is one of the most crucial enzymes produced by Gram-negative bacteria and there are four main molecular classes of the enzyme, namely A, B, C, and D. The A, C, and D class enzymes make use of serine residues to hydrolyze the β-lactam rings whereas B class metalloenzymes require divalent cations like zinc for their action (Bush and Jacoby, 2010). Intrinsically, the chromosomal material of *P. aeruginosa* contains the ampC gene which acts as cephalosporinase, and the poxB gene coding for the class D oxacillinase (Poole, 2011). The ampC gene along with several mutations hydrolyzes the amide linkages of the β-lactam ring portion of β-lactam antibiotics and completely breaks the ring (Berrazeg *et al.*, 2015), thus rendering the antibiotic ineffective. ampC gene is induced with the help of the accessory ampR (codes for positive transcriptional regulator for β-lactamase), ampG (encodes transmembrane permease protein required for signaling pathway), and ampD (suppresses the repressor for ampC) genes (Pachori *et al*., 2019). A recent study by Shalmashi *et al.* (2022) found the presence of the class B TEM and SHV enzyme genes, and class A CTX enzyme gene in *P. aeruginosa*-infected patients who failed to respond to newer β-lactams like ceftazidime and imipenem. Additionally, the Oxacillin-hydrolyzing (OXA), Penicillin sensitive enzyme (PSE-4) (Livermore *et al*., 1985), and Carbenicillin-hydrolyzing (CARB) β-lactamase groups are present in this species (Nordmann *et al*., 1993). The set of β-lactamases that contribute to carbapenem resistance are called carbapenemases and they are the most significant clinically since pathogens expressing these proteins are resistant to all β-lactam antibiotics (Meletis, 2016). According to Poole (2011), AIM, GIM, IMP, NDM, SIM, SPM, and VIM, are the various metallo β-lactama-

se proteins, which are also carbapenemases (Tenover *et al.,* 2022), in *P. aeruginosa* (Gupta, 2008). *P. aeruginosa* strains which produce clones of the class A KPC and GES enzymes hydrolyze all carbapenems. Although the bacterium rarely produces class D enzymes, strains have been shown to produce OXA-40, OXA-48, OXA-181, and OXA-198 throughout the world (Yoon *et al*., 2021). Zhao and Hu (2010) estimated that *P. aeruginosa* has the ability to produce over 120 non-identical β-lactamases across different classes that enable effective resistance towards β-lactams. Several aminoglycoside antibiotics like tobramycin, gentamicin, and amikacin, which are administered to resolve *P. aeruginosa* infections worldwide, have encountered resistance due to Aminoglycoside Modifying Enzymes (AMEs), which hydrolyze these antibiotics (Ahmadian *et al*., 2021). The three types of AMEs are acetyltransferases (ACT), nucleotidyl transferases (ANT), and phosphotransferases (APH) that chemically alter the antimicrobials by acetylation, adenylation and phosphorylation respectively (Poole, 2005). Thus, aminoglycoside antibiotics get altered and become an entirely different compound before arriving at their ribosomal target site.

There are 4 main classes of β-lactam antibiotics namely Penicillins, Cephalosporins, Carbapenems, and Monobactams. Although they may be structurally similar, they have different efficacies and potencies. The 3-D conformers were procured from the PubChem database (Kim *et al*., 2021). Penicillin G is the antibiotic taken as the reference for the superpositioning of various other β-lactams since it is naturally occurring, conventional, and a narrow-spectrum antibiotic that is largely ineffective today (Temime *et al*., 2003; Sauvage *et al*., 2008). PyMOL software was used to systematically superpose several 3-D conformers of β-lactam antibiotics with penicillin G, to check whether the atoms, bond lengths, bond angles, conformations, and pharmacophoric groups coincide. The Root Mean Square Deviation (RMSD) value, which is the output of the superpositioning, is inversely proportional to the degree of similarity between the molecular structures. Inactivation of one antibiotic from the β-lactam group by β-lactamases may also inactivate the β-lactam rings of its close analogs. Due to structure similarity, resistance towards penicillin G may result in resistance towards other β-lactam antibiotics. The 11 antibiotics are selected from all 4 classes, and they are administered regularly for treating mild and serious *P. aeruginosa* infections. Carbapenems are relatively closer to Penicillin G as compared to the other classes of β-lactam antibiotics. The structures of most Cephalosporins along with the novel monobactam named Aztreonam, deviate the most from Penicillin G. Table 1 depicts the comparison of the structures of the β-lactams.

**Table 1.** Comparison of the class and structures of β-lactam antibiotics

| Name of Antibiotic | Class of Antibiotic | RMSD Value (in Å) |
|---|---|---|
| Penicillin G | Penicillin | 0.000 |
| Cloxacillin | Penicillin | 3.293 |
| Amoxicillin | Penicillin | 1.990 |
| Carbenicillin | Penicillin | 1.749 |
| Cefuroxime | Cephalosporin | 5.487 |
| Cefixime | Cephalosporin | 3.847 |
| Ceftriaxone | Cephalosporin | 5.505 |
| Cefepime | Cephalosporin | 3.880 |
| Imipenem | Carbapenem | 3.196 |
| Ertapenem | Carbapenem | 3.492 |
| Aztreonam | Monobactam | 5.134 |

**Target site related resistance**

All antibiotics target certain structures of the bacterial cell (Luthra *et al*., 2018) and inhibit bacterial infections. Commonly used antibiotics include Polymyxins which act on cell membranes, quinolones acting on DNA gyrase, macrolides acting on 50S ribosome subunits, β-lactams that act on cell walls, aminoglycosides and tetracyclines acting on 30S ribosome subunits and Rifampicin acting on RNA Polymerase. Bacterial lipopolysaccharides (LPS) are the major target of polymyxin antibiotics. Olaitan *et al*. (2014) observed the intrinsic modifications of the LPS by phosphoethanolamine and 4-amino 4-deoxy L-arabinose and ascertained that they reduce the negative charge of the LPS, thus reducing the extent of the interaction of polymyxins to the cell membrane target receptors. OprH is an exterior membrane protein that is basic and binds to divalent cations and the overexpression of this protein results in polymyxin resistance (Basetti *et al*., 2018). Ribosomal units of 16S rRNA are methylated by Rmt methylases thereby guarding the ribosomal target site to which aminoglycosides bind (Nafplioti *et al.*, 2021). Point mutations in the amino acid position of 83 succor the formation of leucine in place of serine, which directly impacts the active site of the DNA gyrase enzyme and significantly affects the action of fluoroquinolones (Bhatnagar and Wong, 2019). Additionally, muta-

tion at the 87th position of gyrA where tyrosine is replaced by asparagine or glycine, makes it very difficult for ciprofloxacin to bind to the DNA gyrase target (Langendonk *et al*., 2021). The MsrE gene in *P. aeruginosa* encodes the protein which binds to the E site as well as the peptidyl transfer region of ribosomes which constitutes the antibiotic resistance domain, thus protecting it from the action of macrolides like azithromycin and group B streptogramins (Wilson *et al*., 2020). Quinolone resistance due to ParC genes which can alter serine residues of Topoisomerase  and QNR genes from plasmids corresponds to resistance as high as 250 times that of any other antibiotic (Aldred *et al*., 2014).

**Common mutations in *Pseudomona aeruginosa* strains**

While *P. aeruginosa* possesses several intrinsic resistance mechanisms, the pathogen is capable of acquiring mobile genetic elements and developing resistance mutations to antibiotics (Cloeckaert *et al.*, 2017). The common *P. aeruginosa* strains are first isolated and cultured carefully to obtain homogenous colonies. This is followed by the genome sequencing of the species (Poulsen *et al*., 2019) to identify its core genome and essential genes for survival. Statistical genome outputs are also obtained thereafter. According to Dettman *et al*. (2016), the rate of mutations due to resistance can be 230 times higher than that of wild-type *P. aeruginosa*. Environmental DNA (eDNA) production and release by multiple pathways is known for contributing to widespread resistance in bacterial strains. The eDNA release mechanisms (Sarkar, 2020) and redox reactions of Pyocyanin, the highly active compound that is essential for the formation and fitness of biofilms, are responsible for mutational resistance. Through careful experimentation by Maxim *et al*. (2019), it was found that mutations in the quorum-sensing signal receptor LasR, responsible for activating many more resistance genes, are very common in *P. aeruginosa* CF patients. The traits affected by mutated genes are called Patho-adaptive traits which enhance the survival of bacterial colonies. The in-vitro analysis by Bolard *et al*. (2018) revealed 4 to 8-fold greater resistance of the bacterium to aminoglycosides due to acquired mutations on the fusA1 gene resulting in single specific amino acid substitution in Elongation factor G domains, which is responsible for translation. Braz *et al*. (2016) observed at least one mutated amino acid in the NalC transcription regulator protein which resulted in aztreonam resistance due to overexpression of Mex-AB-OprM efflux pumps in environmental isolates of *P. aeruginosa*. Mutations in genes like gyrA, coding for DNA gyrase, enable *P. aeruginosa* to enhance the negative supercoiling of DNA even in the presence of quinolones. Furthermore, mutations associated with genes coding for membrane transporters (oprD), transcription regulators of efflux pumps (nfxB, parE), and virulence factors (retS, gacS, exsD, and vgrG) were gained by the bacterium to enhance survival. The detailed description of all these mutations was portrayed by Winstanley *et al*. (2016). Increased genetic mutations in genes responsible for regular cellular activities like quorum sensing, metabolism, iron absorption and assimilation, mucosal secretion, DNA repair, and transport of metabolites have propelled the rapid spread of *P. aeruginosa* during infections.

**Antibiotic treatment for MDR *P. aeruginosa* infections**

*P. aeruginosa* is a highly invasive pathogen capable of causing serious lung, bloodstream, urinary tract, abdominal, and soft tissue infections. Many patients having comorbidities require a combination of therapeutic strategies to treat *P. aeruginosa* infections. Synergy of antibiotics like β-lactams and aminoglycosides and monotherapy of ciprofloxacin (quinolone) have been administered in patients, showing similar success rates (Driscoll *et al*., 2007). Aminoglycosides are selectively used to treat patients for infections caused by *P. aeruginosa* (Mensa *et al*., 2018). Although genetic polymorphisms and mutations of penicillin-binding protein are observed widely, extended-spectrum penicillins, cephalosporins, and carbapenems are widely used as antibiotics for the general treatment of *P. aeruginosa* infections (Clark *et al*., 2019). While ciprofloxacin is the main fluoroquinolone for first-line treatment, delafloxacin is the newest fluoroquinolone used for the treatment of ciprofloxacin-resistant *P. aeruginosa* in cystic fibrosis patients (Millar *et al.*, 2021). In recent findings, the novel drug named Cefiderocol, belonging to the cephalosporin class, has shown promising results against *P. aeruginosa* (Dima *et al.*, 2020). For almost all pan-resistant isolated infections of the bacterial species, a combination of monotherapy with β-lactamase inhibitors like ceftolozane-tazobactam, ceftazidime-avibactam, or imipenem-cilastatin-relebactam is administered (Ozma *et al.*, 2022). Polymyxin antibiotics are used as the last resort for hospitalized patients with severe multidrug-resistant infections. Lin *et al*. (2019) successfully noted the failure in the metabolic pathway of

the bacterium upon administering a combination of Polymyxin B and Enrofloxacin. Colistin, also known as polymyxin E, has been instrumental in obtaining high recovery rates among urinary tract infection patients infected with the highly resistant *P. aeruginosa* (Sorli *et al.*, 2019). The isolation of Pyoverdine inhibitory compounds can help to prevent the bacterial species from utilizing iron effectively, thus mitigating its pathogenicity (Kirienko *et al.*, 2019). Itaconimide compounds have been shown to block the quorum-sensing pathway of *P. aeruginosa* and thus it can function as a potential treatment for infections (Fong *et al.,* 2019). The detailed in-vitro susceptibility test on five strains of *P. aeruginosa* conducted by Rajivgandhi *et al*. (2019) yielded metal-conjugated cefoperazone-sulbactam, piperacillin-tazobactam, ticarcillin-clavulanic acid, and ciprofloxacin as the most suitable antibiotic combinations for most strains. For immunocompromised individuals, a combination of drugs are administered, with no other choice, and the number of antibiotics can be decreased based on the susceptibility studies of the microbe (Chamot *et al*., 2003).

**Bacteriophage therapy for MDR *P. aeruginosa***

Bacteriophages are the groups of viruses that are known to infect and kill bacteria. They specifically attach to bacterial surfaces and inject their DNA into bacterial cells. Thereafter, the DNA material of the phage takes over the bacterial cell machinery to replicate and produce numerous phages within the cell itself. Once the cellular volume is filled with phages, it bursts (lysis) open, completely annihilating the bacteria by deploying its lytic mechanism. A clear increase in bacteriophage therapy is essential in this era of ineffective antibiotics (Gordillo and Barr, 2019). They have been in use for a long time but their application in eliminating superbugs and MDR bacteria has surfaced and gained importance in recent times (Mertz, 2019). In such therapies, the bacteriophages are first isolated and replicated in large numbers, and then commonly effective strains are used to treat *P. aeruginosa* biofilms and infections. For example, LUZ24-like phages are the strongest phages for biofilm treatment (Chegini *et al.,* 2020). In a recent study by Adnan *et al.* (2020), bacteriophage MA-1 of the Myoviridae family was isolated from wastewater and they significantly inhibited the exponential growth of *P. aeruginosa*. It draws the conclusion of the effectiveness of phage MA-1 in decimating *P. aeruginosa* planktonic cells and biofilms. Another study by Pei and Lamas-Samanamud (2014) involved the engineering of T7 bacteriophages that expressed AiiA lactonase to degrade acyl homoserine lactones, which are vital cell signaling molecules, in *P. aeruginosa* biofilms (with *E. coli*). These quorum-quenching phages express enzymes to lyse diverse host bacteria and eliminate colonies as well as biofilms altogether. The experiment by Latz *et al.* (2017) employed hospital sewage that yielded three phage isolates namely SL1 (PB1-like virus), SL2 (phiKZ-like virus), and SL4 (LUZ24-like virus) phages. SL2 phages were most potent in suppressing planktonic bacterial cells, while SL4 phages degenerated biofilms efficiently. SL1 phages were not very effective and experimental bacteria had high survival rates upon exposure to them. Bacteriophage treatment is also used therapeutically in conjugation with antibiotics to resolve *P. aeruginosa* infections in serious patients according to Kutateladze and Adamia (2010). Fong *et al.* (2017) used stock of 4 lytic phages in equal quantities; Pa 193 and Pa 204 of the Myoviridae family, and Pa 222 and Pa 223 of the Podoviridae family to treat *P. aeruginosa* biofilms in chronic Rhinosinusitis patients with efficiency as high 76% in just two days. Phage PELP20 was used to kill the bacterial colonies in biofilms mimicking the biofilms in CF lungs with a 99.9% reduction in colonies (Waters *et al.,* 2017). Wright *et al.* (2009) used therapeutic Biophage-PA to treat chronic otitis caused due to *P. aeruginosa* in 24 patients in the UK with significantly low counts of bacteria after 42 days of treatment. A novel endolysin, LysPA26, contains a domain highly similar to lysozymes and it can eliminate up to 99.99% of *P. aeruginosa* colonies in half an hour (Guo *et al.,* 2017). Endolysins, as the name suggests, are lytic enzymes that disrupt bacterial cell walls by enabling peptidoglycan hydrolysis. There are several phages containing useful lysins that bind to specific bacterial cell wall receptors like peptidoglycan binding domain and Lysin motif to promote bacterial cell lysis towards the end of their lytic cycle (Jarábková *et al.,* 2015). Bacteriophage therapy is a relatively newer approach for the treatment of serious combinatorial infections caused by critically resistant bacteria like *P. aeruginosa*. Bacteriophages have been used for treating bacterial infections since 1966 (known to infect bacteria as well as archaebacteria) and they have their own advantages and disadvantages. A common advantage is the lower number of side effects caused due to this therapeutic practice. A major disadvantage is the fact that one particular action of the phage is the equivalent of a narrow-spectrum antibiotic as

opposed to a broad or an extended-spectrum antibiotic. Phages have been studied historically for several decades, but their therapeutic action has been extensively studied in recent years due to the gigantic menace of antibiotic resistance (Wittebole *et al*., 2014). The futuristic aim of bacteriophage therapies is to resolve the issue of multidrug and widespread resistance due to bacteria. The technique proves to be effective as bacteriophages inherently infect bacterial species and contribute most significantly (compared to any other species) to their deaths in colonies.

## Conclusion

In the wake of widespread antibiotic resistance, bacteria belonging to the *Pseudomonas* genus require extensive studies. *P. aeruginosa* has a highly sophisticated system of cellular communication of quorum sensing and the species has the propensity to form thick biofilms which are extremely cumbersome to eradicate. Microbial biofilm aggregation normally displays multidrug resistance and the newer techniques to obliterate them (in laboratory conditions) include the usage of direct currents of low voltage and anti-biofilm compounds like silver oxynitrate (Wolfmeier *et al.,* 2018). The best-known treatment regimens, including antibiotics, are not very effective against the exponential bacterial load. It is tough to detect the disease early as *P. aeruginosa* may be in latent form and effective serological tests for infection diagnosis are yet to be established (Silva Filho *et al.,* 2013). There are numerous different species of *Pseudomonas* in nature and 43 species were discovered recently (Girard *et al.,* 2021) by isolating over 300 strains from different geographical locations. Although the *Pseudomonas* genus is vast, the species responsible for infections, antibiotic resistance, and death in humans is none other than *P. aeruginosa*. Statistically, the number of *Pseudomonas* infections has not increased rapidly over the last few years, but the bacterium threatens the lives of thousands of immunocompromised patients. The therapeutic strategies to enable speedy recovery must focus on a combinational model, using susceptible antibiotics and bacteriophage therapy together. The future of antibiotics and treatment targets must prove to be more effective than current treatment standards against *P. aeruginosa* infections. Promising studies to eradicate biofilms and treat infections caused by critically resistant bacteria like *Acinetobacter baumannii*, *Enterobacteriaceae*, *Staphylococcus aureus*, *P. aeruginosa*, *Helicobacter pylori*, etc. pave the way for biological success.


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