Review Article

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Pathogenicity and Antibiotic Resistance of *Pseudomonas aeruginosa*: A Comprehensive Review

Microbiology Section

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ABSTRACT

Pseudomonas aeruginosa (P. aeruginosa) presents a complex challenge in terms of pathogenicity and antibiotic resistance. This versatile pathogen adeptly colonises various host tissues and evades the immune system through its intricate virulence factors. The review delves into the antimicrobial resistance mechanisms at play, which encompass inherent resistance characteristics and those acquired through genetic mutations and horizontal gene transfer. Notably, efflux pump systems and limited membrane permeability underpin its inherent resistance, rendering many conventional antibiotics ineffective. Multidrug Resistant (MDR) strains are on the rise, posing a substantial threat to patient care and infection control. In response, innovative strategies are being explored, including combination therapies to enhance the effectiveness of existing antibiotics and drug repurposing, redirecting existing medications to target *P. aeruginosa*. Phage therapy, which leverages bacteriophages to combat *P. aeruginosa* infections, is gaining attention as a promising solution. Infection prevention and control are pivotal, particularly in healthcare settings, to curtail the spread of *P. aeruginosa*. Surveillance programs are crucial for monitoring the prevalence and dissemination of antibiotic-resistant strains and guiding response strategies during outbreaks. Comprehending *P. aeruginosa's* complex virulence and resistance mechanisms is paramount for developing efficient treatments and effective infection control measures. Ongoing research and collaborative efforts are instrumental in mitigating the substantial impact of *P. aeruginosa* infections on public health, underscoring the need for sustained vigilance and innovation in infectious disease management.

Keywords: Advanced therapies, Multidrug resistant, Virulence

INTRODUCTION

Pseudomonas aeruginosa, a versatile gamma-proteobacterium, utilises binding elements such as pili, flagella, and biofilms to thrive in water, diverse substrates, and healthcare settings. It is prevalent in natural and manmade environments, including bodies of water, hospitals, and drains [1]. The bacterium's capacity to cause infections varies in severity, ranging from regional to potentially fatal. This gram negative opportunistic pathogen has emerged as a leading cause of nosocomial illnesses, including Ventilator Associated Pneumonia (VAP), infections in Intensive Care Units (ICUs), circulatory system infections from central lines, surgical site infections, Urinary Tract Infections (UTI), burn wound infections, keratitis, and otitis media, resulting in significant morbidity and mortality [2,3]. The prognosis remains grim for ICU sepsis and pneumonia. Recurrent airway infections by *P. aeruginosa* are common in patients with Cystic Fibrosis (CF) and Chronic Obstructive Pulmonary Disease (COPD) [4]. This aerobic pathogen rapidly develops antibiotic resistance, adapts to external changes, and produces diverse virulence factors. Its ability to evade immune defenses through binding, colonisation, biofilm formation, and production of pathogenic agents poses a risk to weakened immune systems [5]. *P. aeruginosa* outbreaks, fueled by adaptive mechanisms that enhance resistance, have become global epidemics [6].

This review aims to update readers on *P. aeruginosa*'s pathogenicity, antibiotic resistance, diagnostic advancements, and therapeutic potential. Researchers extensively searched Medline/PubMed and Cochrane Library datasets for relevant studies on virulence and drug resistance in *P. aeruginosa*.

Phenotypic Characteristics and Ecology of *P. aeruginosa*

Gilardi categorised non fermenter Gram-Negative Bacteria (GNB) into seven groups based on visible traits [6,7]. Meanwhile, Palleroni NJ classified them into five identical rRNA categories (*Pseudomonas*, *Burkholderia, Comamonas, Brevimundas*, and *Stenotrophomonas*) using rRNA-DNA sequence similarities. *Pseudomonas aeruginosa*, isolated from green pus, was later proposed as the type species [7,8]. Members of the Pseudomonadaceae family are widely distributed in the environment. *P. aeruginosa* is a major pathogen for humans and warm-blooded animals [9]. Other *Pseudomonas* species affect fish, causing diseases such as septicaemia and inflammatory syndrome [10]. *Pseudomonas fluorescens* and *Pseudomonas putida* contribute to food spoilage and contamination of transfusions. Uncommon pathogens like *Pseudomonas stutzeri, Pseudomonas mendocina, Pseudomonas fulva*, and *Pseudomonas monteilii* affect severely ill individuals. Plant pathogens include *Pseudomonas baetica, Pseudomonas syringae, Pseudomonas plecoglossicida*, and *Pseudomonas viridiflava* [11]. *P. aeruginosa*, a GNB, possesses non fastidious traits and appears as straight or slightly curved rods (1.5±3 mm length, 0.5-0.7 mm width). It is aerobic and motile, bearing one or more polar flagella. It grows on various media such as nutrient agar, Luria-Bertani, and blood agar. Selective media include Cetrimide agar, King-A, and King-B. Optimal growth occurs at 37°C, with a tolerance range of 4-40°C [12]. Distinct odors ("grape juice," "fresh tortilla"), beta-haemolysis on blood agar, and colony colour aid in rapid identification [13].

P. aeruginosa Major Virulence Factor and Pathogenicity

P. aeruginosa, initially identified in wound infections, has emerged as a significant pathogen with complex pathogenic mechanisms.

Outer Membrane Proteins (OMPs): These proteins facilitate amino acid and peptide transport, antibiotic absorption, carbon source transport, bacterial adherence, virulence secretion, and host recognition [14].

Lipopolysaccharides (LPS): LPS, a structural component on the bacterial surface, protects the outer membrane and exhibits toxicity towards host cells. It contributes to tissue injury, adhesion, and host receptor recognition, influencing antimicrobial resistance and biofilm formation [15,16].

Biofilm formation: *P. aeruginosa*'s biofilm formation involves flagella, pili, adhesins, and other factors, contributing to antibiotic resistance and increased persistence [17].

Secretory systems: Six secretion systems, including T6SS, T4SS, and T3SS components, aid in colonisation, adhesion, swimming, swarming, and chemotactic signaling. Secreted toxins modify host cell signaling, disrupt the extracellular matrix, cause tissue damage, and alter the local microbiota.

Exopolysaccharides (EPS): Alginate, Psl, and Pel EPSs promote biofilm formation, bacterial aggregation, and microcolony development in pneumonia. The anionic matrix protects against phagocytes, antibodies, and complement [17].

Toxins: *Pseudomonas* produces toxins such as T3SS-delivered ExoU, ExoT, ExoS, and ExoY, which alter the intracellular environment. Exotoxin A interferes with host protein production, pyocyanin causes oxidative damage, and various toxins impact immune response and tissue damage [18].

Lytic enzymes: Elastases LasA and LasB, alkaline protease (AprA), lipases, and esterase A damage epithelial cells through lung surfactant degradation and disruption of junctions [19,20].

Siderophores: Pyoverdine and pyochelin siderophores aid in iron acquisition and the production of virulence factors, including biofilms [21].

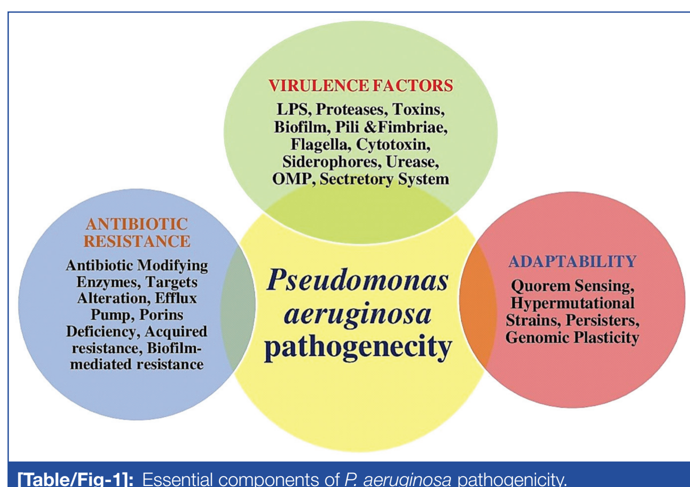
Antioxidant enzymes: Catalases, alkyl hydroperoxide reductases, and superoxide dismutase neutralise Reactive Oxygen Species (ROS), evading phagocyte elimination [22].

Quorum Sensing (QS): *Pseudomonas* utilises QS, involving Ls, Rhl, and Pqs pathways, to regulate gene expression and microbial activity during infection. This cooperation promotes survival and dampens the immune response [23,24].

P. aeruginosa's intricate pathogenicity hinges on these mechanisms, contributing to its adaptability and virulence across various contexts.

Mechanism of Pathogenesis [Table/Fig-1]

P. aeruginosa exhibits diverse pathogenic capabilities, infecting wounds, surgical sites, and the urinary system, and causing bloodstream infections, particularly prevalent in healthcare settings. Its primary focus is respiratory infections. The bacterium possesses a single polar flagellum and numerous type 4 pili crucial for mobility and initiating respiratory infections. The presence of flagella and pili sparks inflammation. Bacterial chemotaxis relies on the whip-like motion of the flagellum for corkscrew-like swimming in liquids. During infection, interaction with host epithelial cells occurs via the glycolipid asialoGM1, triggering NF-B signaling through TLR5 and caspase-1 responses via the Nod-like receptor, Ipaf, inducing significant inflammation [25].



[Table/Fig-1]: Essential components of *P. aeruginosa* pathogenicity.

Type 4 pili govern biofilm development and twitching motility, acting as pivotal adhesins in *P. aeruginosa*. Found at cell extremities, these pili extend and retract, enabling "twitching motility" on solid surfaces. They also contribute to flocking motility along with flagella, fostering bacterial aggregation to create small colonies. These microcolonies protect against host defenses and antibiotics. In CF patients, persistent lung infection leads to the formation of microcolonies resembling lab-developed mucoid colonies [17]. Pili also play a role in non opsonic phagocytosis. Mutants lacking pilin or with poor twitching movement exhibit reduced pathogenicity. Pili are targeted in anti-pseudomonal therapy, including vaccination, yet the antigenic diversity of pili across *P. aeruginosa* strains complicates these efforts [25]. *P. aeruginosa*'s adherence to host cells relies on lectins, predominantly LecB, a fucose-binding lectin. LecB's strong binding stems from specific charge delocalisation, low-barrier hydrogen bonds, collaborative hydrogen bond rings, and water molecule motion [26]. In CF patients, increased bacterial Vav3 enzyme in airway epithelial cells enhances *P. aeruginosa* adherence through 1 integrin and fibronectin synthesis, reinforcing early-stage adherence [27].

P. aeruginosa's pathogenesis involves more than adherence, with certain clinical isolates showing faulty LasR genes, leading to constitutive biofilm development without external stimuli. The primary QS regulator, LasR, encoded by the lasR gene, is implicated in the growth of these isolates [28]. Further insights into *P. aeruginosa* QS and its control of virulence have emerged. Quantum-Sensing Autoinducer (QSAI) molecules initiate QS, requiring about 2000 cells to deliver significant amounts of QSAI [29,30]. Key QS circuits like LasI and RhlI produce QSAIs, which are recognised by LasR and RhlR. Genes such as pyocyanin and rhamnolipids, important virulence factors, are controlled by QSAIs binding to LasR and RhlR [31]. The non coding RNA, RhlS, associated with the RNA-binding molecule Hfq, promotes Vfr synthesis, a universal virulence regulator [32].

P. aeruginosa releases EPS during biofilm formation, including Psl, Pel, and alginate. Psl is vital for microbial growth, aiding bacterial adhesion on surfaces. Pel, a highly charged EPS, promotes cell-to-cell communication within biofilms. Alginate's high molecular weight enhances biofilm stability and protects against dehydration. These EPSs foster microbial growth in airways, as exemplified by *S. salivarius* interacting with Psl to initiate and maintain biofilm development in CF lungs [33]. Biofilm production and antimicrobial tolerance in *P. aeruginosa* are not fully understood, emphasising the need for ongoing research. The conserved putative protein expressed by the PA2146 gene controls biofilm formation and antibiotic endurance. Although it does not affect planktonic cells, its deletion significantly impairs *P. aeruginosa* PAO1 biofilm structure and tolerance to tobramycin [34]. Additionally, *P. aeruginosa* isolates with a mucoid trait, crucial for CF pathogenicity [35], secrete QSAI molecules C4-HSL and PQS. C4-HSL engages with EPS through van der Waals contacts, while PQS forms thermodynamically resistant ionic complexes with EPS-bound Ca2+ [36]. Iron/siderophore acquisition systems play a role in *P. aeruginosa*'s virulence, contributing to biofilm formation and the development of hypervirulent variants in wound infections [37]. *P. aeruginosa* generates virulence factors like pyocyanin, phenazine, rhamnolipid, and pyoverdine, controlled by QS pathways for pathogenicity [38]. To successfully infect hosts, *P. aeruginosa* adheres, forms biofilms, and evades immunity. Research into type II, III, IV, and VI secretion systems reveals their role in delivering effectors to host cells. Type III secretion system effectors, like exotoxin T, disrupt NLRC4 inflammasome activation, hindering *P. aeruginosa* pathogenicity [39].

P. aeruginosa biofilms (e.g., Psl/Pel) interact with human immune cells via C-type lectin receptors: DC-SIGN, Mannose Receptor (MR), and Dectin-2. DC-SIGN recognises both planktonic and biofilm cells, while MR and Dectin-2 exhibit weaker biofilm recognition. Biofilm

carbohydrates, particularly mannose-rich ones, can interfere with immune receptor activities [38]. *P. aeruginosa* adjusts metabolic pathways to evade immune clearance and thrive in inflamed human airways, utilising host macrophage-produced itaconate. This shift encourages biofilm growth, boosting EPS production while sacrificing LPS. EPS shields against itaconate-induced membrane stress. Altered metabolism stimulates myeloid cell reprogramming, fostering a chronic infection-prone environment [40]. *P. aeruginosa's* virulence relies on factors such as QS, flagella, and biofilm formation. Key players include pyoverdine, the lasR gene, capsules, alginate D, elastase B, exotoxin A, and Transcription Factors (TFs). Master regulators of QS include RsaL, QscR, RhlR, CdpR, MvfR, PchR, PhoB, LasR, while ExsA governs T3SS and GacA T6SS [41]. The role of the AlgKX protein in alginate synthesis and biofilm adhesion is highlighted [42].

Additional Elements Influencing the Survival and Infections of *Pseudomonas aeruginosa*

P. aeruginosa exhibits two functional paralogs of DksA, namely DksA1 with a zinc-finger motif and DksA2, facilitating resistance to oxidative stress. Both planktonic and biofilm cells rely on DksA1 for H_2O_2 tolerance by regulating katA and katE gene expression, evading macrophage destruction. DksA2, produced under zinc scarcity, substitutes for DksA1 in oxidative stress defense [43]. The Type VI Secretion System (T6SS) empowers *Pseudomonas aeruginosa's* anaerobic advantage through the release of an anion-binding protein and molybdate acquisition [44]. Its T6SS toxin (Tse8) binds to VgrG1a, entering target cells to hinder protein synthesis [45]. *Pseudomonas aeruginosa* mutations in CF patients' infections heighten only when other species are absent, suggesting benefits of polymicrobial infections in eradication efforts [46]. Antibiotic resistance develops swiftly in *P. aeruginosa* populations within days based on therapy type and duration. Unidentifiable culture-based rare mutations arise in 5-12 days, while non targeted resistance diminishes [47]. Strains with deactivated fgE genes exhibit enhanced biofilm cell resistance to diverse antibiotics like gentamicin and colistin due to altered cell aggregation, surface adherence, and biofilm formation [48].

Diagnosis

Diagnosing *P. aeruginosa* infections hinges on timely and accurate cultures from appropriate sites. Blood cultures should precede antibiotic therapy in suspected severe cases, obtained within an hour of identification [49]. Urine cultures are essential for suspected UTIs and Catheter-Associated UTIs (CAUTIs). Sputum cultures aid pneumonia diagnosis, especially in productive individuals, while tailored approaches are used for CF patients. Detection involves recognising colony appearance and growth on media; cetrimide-containing media can aid amidst diverse bacteria. Antimicrobial susceptibility testing guides effective antibiotic selection post-culture detection, often utilising automated systems for minimal inhibitory doses and resistance profiling [50]. Advanced and emerging diagnostics for early *P. aeruginosa* detection benefit from molecular techniques. Molecular methods improve regular recognition and epidemiological studies and reduce dependency on cultivation. Challenges of culture-based methods include specific media requirements, microbial growth compatibility, and prolonged incubation times. Molecular diagnostics directly identify bacteria from clinical samples, enhancing safety and nucleic acid preservation. Storage temperature ensures prolonged preservation of nucleic acids' quantity and quality [51].

Advance and Emerging Diagnostic Tools for Early Detection of *Pseudomonas aeruginosa*

Molecular diagnostic methods have gained significant importance in clinical laboratories due to their advantages in recognising pathogenic microorganisms, fingerprinting, and epidemiological studies. These techniques reduce the need for cultivation, thereby expediting phenotypic and biochemical diagnoses. Drawbacks of cultivation-based approaches include microbe-specific artificial media requirements, compatibility with chosen media, and lengthy incubation times. Molecular diagnostics directly identify bacteria from clinical specimens, minimising risks to lab workers and preserving nucleic acids' quantity and quality with proper storage conditions [51]. Polymerase Chain Reaction (PCR) is a well-established technique for detecting and categorising *P. aeruginosa*. It amplifies DNA with catalytic DNA replication and targeted primers. Genes like ecfX, oprL, and gyrB are used as targets in clinical samples due to their high specificity and sensitivity. False positives and negatives may arise due to *P. aeruginosa*'s genomic flexibility and horizontal gene transfer to other species. Multiplex PCR, examining multiple specific genes concurrently, can mitigate these issues. Multiplex PCR offers benefits such as internal controls, cost savings, material preservation, and template assessment. Primer concentration and primer-primer competition are critical considerations. Though evolving, a standardised procedure for *P. aeruginosa* detection through multiplex PCR is lacking [51,52]. Quantitative real-time PCR (qPCR) is increasingly used in clinical microbiology to detect pathogens. It offers a quick turnaround, simplicity, reproducibility, and enhanced quantitative capabilities compared to traditional PCR [53]. These newer techniques offer benefits such as simple equipment, minimal training, and rapid, accurate results within an hour. They are particularly valuable for point-of-care testing, especially in resource-limited areas. Both Loop-mediated Isothermal Amplification (LAMP) and PCR assays exhibit high sensitivity and specificity, making them effective tools [54,55]. LAMP stands out by directly detecting *P. aeruginosa* in clinical plasma within 20 minutes, bypassing DNA purification steps [55]. Polymerase Spiral Reaction (PSR) utilises DNA polymerase with strand-dispersion activity and isothermal DNA amplification targeting the tox A gene, demonstrating higher sensitivity (10 times that of PCR) and rapidity without preliminary denaturation [56].

Next-Generation Sequencing (NGS) has replaced Sanger DNA sequencing, enabling comprehensive analysis of bacterial genomes, including transcription, translation, and more. This approach is widely used in clinical microbiology for advanced pathogen characterisation, offering precise results with less DNA, and has gained adoption since its 2005 launch [57,58]. NGS benefits include accurate data with reduced noise, though expertise in lab procedures, data processing, and interpretation is vital [59]. Overcoming technical challenges and updated software are necessary as sequencing technology evolves [60]. NGS metagenomic studies based on the 16S rRNA gene have utilised this technology for quick identification of diverse bacteria in heterogeneous clinical samples, without prior cultivation. Compared to conventional culture methods, NGS sequencing of 16S rRNA genes proves reliable, quantitatively sensitive, and precise for determining the microbial nature and proportions in polymicrobial samples [61].

Antimicrobial Resistance in *Pseudomonas aeruginosa*

Clinical settings are experiencing a rise in Multi-Drug Resistant (MDR), Extensively Drug-Resistant (XDR), Pan-Drug Resistant (PDR), and Totally Drug-Resistant (TDR) bacterial isolates. The European Society of Clinical Microbiology and Infectious Diseases (ESCMID) has established guidelines for these classifications [62]. Global bodies like the World Health Organisation (WHO) and the US Centers for Disease Control and Prevention (CDC) are alarmed by the escalating cases of MDR bacteria due to their threat to public health [63,64]. Such infections lead to prolonged hospital stays, diminished quality of life, challenges, and increased mortality when adequate treatments are absent. Strong correlations between MDR bacteria and poor clinical

outcomes are evident in numerous cases [65]. Clinical settings attribute antibiotic resistance to inappropriate usage and the waning interest of pharmaceutical companies in antibacterial research [66]. *P. aeruginosa* exhibits resistance mechanisms encompassing innate drug resistance, biofilm formation, and rapid adaptation [67,68].

These mechanisms (summarised in [Table/Fig-2]) encompass intrinsic factors like membrane permeability, overexpression of efflux systems, and production of antibiotic-inactivating enzymes (refer to [Table/Fig-3]). Acquired resistance stems from mutations and horizontal gene transfers impacting efflux pumps, porins, Penicillin-Binding Proteins (PBP), and enzymes. Adaptive resistance arises from continued antibiotic use, biofilm development, latent forms, and exposure to environmental stress [69]. Often, multiple mechanisms co-exist, collectively conferring resistance to various antimicrobials in *Pseudomonas aeruginosa*, posing significant treatment challenges [70,71]. *P. aeruginosa* emerges due to ICU admission, prior hospital stays, and previous diverse antibiotic use, including quinolones, cephalosporins, and carbapenems [74]. ICU isolates often exhibit higher protease and elastase levels, key virulence factors linked to severe and invasive diseases.

Resistance Acquisition in *Pseudomonas aeruginosa* toward Antimicrobials other than β-lactams

The intricate cell wall of Gram-negative microbes acts as a selective barrier, impacting antibiotic binding and pharmacological efficacy. *P. aeruginosa's* less permeable outer membrane renders it less sensitive to certain medications, influenced by porins that act as β-barrel proteins on the membrane's surface. Porins include non specific (OprF), specific (OprB, OprD), gated (OprC, OprH), and efflux (OprM, OprN, OprJ) types [75,76]. The most common non-lipoprotein porin, OprF, maintains membrane integrity, influences QS, fosters biofilms, encourages adherence, and causes infections. Fluoroquinolones and beta-lactams use porin channels aminoglycoside uptake requires oxygen/nitrogen-dependent transport, while colistin interacts with LPS [77]. Kidney toxicity is a concern for transplant recipients due to potential drug effects. DTR categorises *Pseudomonas* isolates as DTR if resistant to cephalosporins, carbapenems, and quinolones [73]. MDR *Pseudomonas* results from ICU admissions, prior hospital stays, and antibiotic use [74]. Proteases and elastases are higher in ICU isolates and linked to severe infections.

Antimicrobial class	Resistance mechanism			
	Mechanism 1	Mechanism 2	Mechanism 3	Mechanism 4
Beta-lactam (e.g.,: penicillin, ampicillin etc.,)	AmpC hyper expression (chromosomal)	Opr M porin mutation or loss	OXA-1 and OXA-2 enzyme production	MexXY efflux pump over-expression
Flouroquinolones (e.g.,: levofloxacin, ofloxacin, norfloxacin etc.,)	Gyrase (gyrase A)-topoisomerse expression; (par C) mutations	Altered permeability	Efflux systems	-
Aminoglycosides (e.g.,: gentamicin, amikacin, tobramycin etc.,)	Altered permeability	Aminoglycoside modifying enzymes, such as aminoglycoside-2”-O- nucleotidyltransfer ANT (ANT 2”Ia) and aminoglycoside 4’-o-adenyltransferase (ANT 4’-IIb	Overexpression of MexXY efflux pumps	-
Carbapenems (e.g.,: imipenem, meropenem, ertapenem etc.,)	Opr D porin loss	MexXY efflux pump expression	Beta-lactamase production	-

[Table/Fig-2]: Major resistance mechanisms of *Pseudomonas aeruginosa*.

Antimicrobials to which Pseudomonas species are intrinsically resistant	Therapeutic alternatives
Amino-penicillins	Aminoglycosides, neoglycosides: gentamicin, tobramycin, amikacin, Plazmomycin
Amino-penicillin/Beta-lactamase inhibitor combination	
Streptogramins	β-lactam antibiotics: 3rd and 4th generation parenterally administered cephalosporins (cefepime, ceftazidime, piperacillin/tazobactam), monobactams (aztreonam), carbapenems (meropenem, imipenem, and doripenem), novel β lactam/Beta-lactamase inhibitor combinations (ceftolozane/ tazobactam, ceftazidime/avibactam, imipenem/cilastatin/relebactam, meropenem/vaborbactam
Lincosamides	
Glycopeptides	
Daptomycin	
Macrolides	
Oxazolidinones	
Rifampin	
Trimethoprim-sulfamethoxazole	Fluoroquinolones: levofloxacin, ciprofloxacin, delafloxacin, moxifloxacin
Tetracycline	
1st and 2nd generation cephalosporins	Polymyxins: colistin
Oral 3rd generation cephalosporins	

[Table/Fig-3]: Alternative antimicrobial therapy for *pseudomonas* infection with intrinsic resistance.

Beta-lactam antibiotics are commonly used to treat *Pseudomonas* infections in clinical settings, especially in vulnerable patient groups like newborns, children, pregnant women, and the elderly. Alternative medications like aminoglycosides, colistin, and fluoroquinolones should be avoided due to potential side-effects such as tendon issues, light sensitivity, and liver toxicity. Last-resort regimens might be necessary if resistance increases or hypersensitivity to beta-lactams occurs, impacting patients' quality of life [12,72]. Kidney toxicity is a major concern for transplant recipients given their multiple medications affecting kidney function. The “difficult-to-treat resistance” classification evaluates bacterial resistance based on clinical efficacy and the risk-benefit ratio, categorising *Pseudomonas* isolates as “Difficult-To-Treat Resistant” (DTR) if they are robust against cephalosporins, carbapenems, and quinolones [73]. MDR

Efflux pumps, notably the RND subfamily, cause MDR by impacting drug effects. Twelve RND pumps exist, with four overexpressed due to gene mutations. MexAB-OprM (β-lactams, quinolones), MexCD-OprJ (β-lactams), MexEF-OprN (quinolones), and MexXY-OprM (aminoglycosides) show various substrate profiles. Efflux resistance is clinically relevant when combined with other mechanisms [78]. Resistance mechanisms differ in impact; isolates might resist amikacin but not tobramycin. Fluoroquinolone resistance involves efflux, mutations in DNA gyrase (gyrA, gyrB), and topoisomerase IV (parC, parE) [12]. Aminoglycoside resistance arises from ribosome target modification or Aminoglycoside-Modifying Enzymes (AMEs) altering drug structure [79]. AMEs spread via horizontal gene transfer. Antimicrobial resistance amplifies colistin use, a last-resort therapy. Initially, resistance was linked to chromosomal mutations, but the mobile colistin resistance gene mcr-1 introduced plasmid-mediated resistance in 2015. The arn BCADTEF operon modifies lipopolysaccharide, hindering colistin-LPS binding. Regulatory mechanisms (PhoPQ, PmrAB) activate under inadequate cation levels, furthering resistance [80]. In conclusion, *Pseudomonas* outer membrane and porins, efflux pumps, and specific resistance mechanisms challenge effective antibiotic treatment.

Beta-lactamase Production in *Pseudomonas aeruginosa*

Scientific concerns including Multidrug Resistance (MDR) and the production of extended-spectrum beta-lactamases (ESBLs) by enteric gram-negative rods in hospitals. Because MDR bacteria are

resistant to antibiotic therapy, they are a constant source of concern [81]. *Pseudomonas* infections rely on β-lactam antibiotics, with carbapenems as the last resort against MDR strains [82]. However, widespread carbapenem use has led to concerning carbapenem resistance [83]. Piperacillin-tazobactam and vancomycin use have been linked to acquiring carbapenem-resistant *Pseudomonas aeruginosa*, supported by a recent meta-analysis. Mechanisms of beta-lactam resistance include porin mutations, efflux pump overexpression, and PBP changes. Diverse β-lactamases contribute, with resistance often resulting from several factors [84]. *Pseudomonas* PBPs also undergo alterations affecting Beta-lactam susceptibility [12]. AmpC β-lactamase is chromosomally encoded and inducible, derepressed by antibiotics like ceftazidime, carbapenems, and clavulanic acid [85]. AmpD gene mutations can lead to AmpC hyperproduction [12].

Pseudomonas, classified as "SPACE" (*Serratia, Pseudomonas, Acinetobacter, Citrobacter, Enterobacter*), displays inducible AmpC-based resistance, not inhibited by first-generation β-lactamase inhibitors. Plasmid-mediated carbapenemases render many antibiotics ineffective, causing significant treatment challenges [86]. Carbapenem-resistant *Pseudomonas* is a major concern, particularly in low to middle-income nations. Infections from carbapenem-resistant GNB incur higher costs, prolonged hospital stays, and poorer outcomes compared to carbapenem-susceptible strains [87]. Carbapenem-resistant *Pseudomonas* bacteremia is associated with a tripled mortality risk [88]. Initially, OprD inactivation and efflux pumps were highlighted in carbapenem resistance, but recent findings emphasise carbapenemases' increasing role [89]. The ST235 clone is a widespread carbapenem-resistant *Pseudomonas* strain, carrying metallo-β-lactamases like IMP, NDM, and VIM enzymes [90].

New and Emerging Therapies for Resistant *Pseudomonas aeruginosa* [Table/Fig-4]

Antimicrobial research has shifted its focus to the investigation of novel strategies beyond conventional antibiotics due to the worrisome growth in antimicrobial resistance among bacterial strains. Bacteriophages, antimicrobial peptides with various structural and functional characteristics, virulence inhibitors, siderophores, naturally occurring substances like essential oils, and other adjuvants, including efflux pump blockers and monoclonal antibodies, are a few of these cutting-edge approaches [91,92]. These medications have the potential to play a vital role in the treatment of serious bacterial infections brought on by *P. aeruginosa* and other dangerous pathogens.

New and emerging therapies	Its role
Antibiotic combination therapies	
Synergy-based approaches	Synergistic antibiotic combinations target multiple processes and enhance efficacy against *Pseudomonas aeruginosa* by overcoming resistance mechanisms.
Targeting virulence factors	Inhibiting virulence factors weakens *Pseudomonas aeruginosa* pathogenicity, targeting QS, secretion, and biofilm for improved treatment.
Repurposing existing drugs	Repurposing approved drugs against *Pseudomonas aeruginosa*, like statins and antifungals, offers efficient and cost-effective treatment development.
New antimicrobial agents	
Quorum sensing (QS) inhibitors	QSIs disrupt *Pseudomonas aeruginosa* signaling, reducing virulence. Furanones, halogenated furanones, and natural compounds are potential therapeutic agents.
Efflux pump inhibitor	EPIs hinder *Pseudomonas aeruginosa* efflux pumps, enhancing antibiotic potency. PaβN derivatives and natural compounds are promising against resistance.
Novel antibiotics	Cefiderocol targets *Pseudomonas aeruginosa* by binding iron receptors, overcoming resistance, and potent against MDR strains. Polymyxin derivatives disrupt cell membranes, and combat carbapenem-resistant *Pseudomonas*. Lefamulin inhibits protein synthesis, active against *Pseudomonas*. Imipenem/relebactam combo was effective against MDR *Pseudomonas* infections. Murepavadin targets outer membrane protein, potential for Multidrug-Resistant (MDR) infections. Eravacycline broad-spectrum activity against Drug-Resistant (MDR) *Pseudomonas*.
Antimicrobial peptides	AMPs like colistin-derived and synthetic peptide WLBU2 disrupt *Pseudomonas aeruginosa* membrane, and combat MDR with low resistance potential.
Bacteriophage therapy	Bacteriophage therapy is promising for *Pseudomonas aeruginosa* infections, proven in trials and models like AB-SA01 in Cystic Fibrosis (CF).
Immune-based approach	
Monoclonal antibodies	Monoclonal antibody (mAb) therapy targets *Pseudomonas aeruginosa*'s virulence factors, such as MEDI3902 targeting OprD, showing promise in clinical trials.
Vaccine	Efforts to create a *Pseudomonas aeruginosa* vaccine target antigens like PcrV-Retapamulin, showing potential in preclinical studies but requiring human efficacy evaluation.
Immunomodulatory therapies	Immunomodulatory therapies for *Pseudomonas aeruginosa* aim to enhance clearance, including anti-PD-1 antibodies and IL-17 inhibitors, requiring efficacy and safety evaluation.
Alternative treatment strategies	
Photodynamic	Photodynamic Therapy (PDT) employs photosensitisers and light to generate Reactive Oxygen Species (ROS), showing promise against *Pseudomonas aeruginosa* infections.
Antisense Peptide Nucleic Acid (PNAs)	Antisense Peptide Nucleic Acids (PNAs) inhibit *Pseudomonas aeruginosa* genes like lasR and pqsA, reducing virulence and biofilm formation.
Nanoparticle based therapies	Nanoparticle therapy for *Pseudomonas aeruginosa* uses nanoparticles to deliver antimicrobials, modulate immunity, and inhibit virulence, requiring further clinical optimisation.

[Table/Fig-4]: New and emerging therapies for resistant *Pseudomonas aeruginosa*.

Challenges in Developing Therapies for *Pseudomonas aeruginosa*

Developing effective therapies for *P. aeruginosa* infections is hindered by its intrinsic resistance mechanisms, biofilm-forming capability, and propensity for antibiotic resistance. The bacterium's innate resistance, attributed to efflux pumps, impermeable outer membranes, and β-lactamase production, hinders drug penetration and activity. Biofilm formation provides resilience against antimicrobials and immune responses. Disrupting biofilms is a priority, with research focusing on antimicrobial peptides, enzymes, and nanoparticles. Antibiotic resistance, achieved via mutations, gene transfer, and adaptive mechanisms, threatens the efficacy of last-resort antibiotics. Limited treatment options aggravate the situation, prompting increased use of drugs like colistin, further promoting resistance. *Pseudomonas* can manipulate host immunity, leading to chronic infections. Immunomodulatory therapies that enhance immunity or target virulence factors are being explored. Overcoming these challenges requires innovative strategies and collaborative efforts to effectively combat *P. aeruginosa* infections [90-92].

Future Perspectives

Addressing *P. aeruginosa* infections presents challenges due to intrinsic resistance, biofilm formation, and antibiotic resistance. Promising perspectives for the future include personalised medicine and tailoring treatment based on genetic and immune profiles. This strategy optimises therapeutic outcomes and minimises resistance risks by considering strain resistance and patient factors.

Combination therapies and treatment regimens offer potential by combining antimicrobial agents with diverse mechanisms and adjunctive therapies. Developing novel therapeutic targets is another focus, aiming to disrupt key components like virulence factors and metabolic pathways. Targets such as the type III secretion system and QS have been explored, but further research is needed to validate their efficacy and safety. Enhanced diagnostic techniques are crucial, as accurate and timely diagnosis guides effective treatment and prevents the spread of drug-resistant strains. Molecular methods and point-of-care tools offer sensitivity and rapidity. Prompt diagnosis allows targeted treatment, reduces antibiotic misuse, and enhances outcomes in *P. aeruginosa* infections. In conclusion, future perspectives encompass personalised medicine, combination therapies, the development of novel targets, and improved diagnostics. These strategies hold the potential to effectively combat this challenging pathogen.

CONCLUSION(S)

The recent update on *P. aeruginosa* highlights its multifaceted challenges. This adaptable pathogen possesses various virulence factors, contributing to its ability to cause infections. Furthermore, its intrinsic and acquired antibiotic resistance mechanisms complicate treatment options. Understanding the molecular mechanisms of pathogenicity and antibiotic resistance offers valuable insights for targeted therapies. Efforts focus on identifying therapeutic targets, exploring combination therapies, and utilising innovative approaches such as antimicrobial peptides, bacteriophage therapy, and nanotechnology. Improved diagnostic techniques are crucial for accurate detection, treatment decisions, and preventing the spread of drug-resistant strains. However, MDR strains pose a significant challenge, requiring ongoing research and collaboration. A comprehensive interdisciplinary approach involving researchers, clinicians, and policymakers is necessary to address pathogenicity and antibiotic resistance in *Pseudomonas aeruginosa*. Continued research, innovation, and collaboration are essential to combat this formidable pathogen, enhancing patient outcomes and mitigating its impact.

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