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Chemotherapy and Mechanisms of Action of Antimicrobial Agent

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Abstract

Pseudomonas aeruginosa is a widespread opportunistic pathogen that causes bloodstream, urinary tract, burn wounds infections and is one of the largest pathogens that infect cystic fibrosis patients' airways and can be life-threatening for *P. aeruginosa* infections. In addition, *P. aeruginosa* remains one of the most significant and difficult nosocomial pathogens to handle. Increasingly, multi-drug resistance (MDR) strains are identified and the option of therapy is often very limited in these cases, particularly when searching for antimicrobial combinations to treat serious infections. The fact that no new antimicrobial agents are active against the MDR strains of *P. aeruginosa* is an additional matter of concern. In recent decades, bacterial drug resistance has increased, but the rate of discovery of new antibiotics has decreased steadily. The fight for new, powerful antibacterial agents has therefore become a top priority. This chapter illustrates and explores the current state of several innovative therapeutic methods that can be further discussed in clinical practice in the treatment of *P. aeruginosa* infections.

Keywords: *P. aeruginosa*, drug resistance, alternative therapies, vaccine, phage therapy

1. Introduction

We are currently facing an international crisis with many troublesome aspects: new antibiotics are no longer being detected, resistance mechanisms are developing in almost all clinical isolates of bacteria, and the effective treatment of infections is hampered by recurrent infections caused by persistent bacteria. Antibiotic failure is one of the most worrying health issues worldwide [1]. Although resistance acquisition is a natural phenomenon, it is accelerated by antibiotic misuse, inadequate inspection and poorly regulated management of antibiotics have resulted in the appearance and spread of multidrug-resistant (MDR) bacteria abroad in clinical medicine and in the livestock industry [2, 3].

Empirical antibiotic treatment requires monotherapy and combination therapy for suspected cases of *P. aeruginosa* and reduces mortality in patients with serious *P. aeruginosa* infections [4, 5]. However, because of the ability of this bacterium to avoid many of the currently available antibiotics, treatment of *P. aeruginosa* infections has become a great challenge [6]. Recently, the World Health Organization (WHO) has identified carbapenem-resistant *P. aeruginosa* as one of three bacterial species with an urgent need for new antibiotics to be developed to treat infections [7]. In addition, inappropriate treatment use of antibiotics accelerates the production of multidrug-resistant strains of *P. aeruginosa*, resulting in the ineffectiveness

of empirical antibiotic therapy against this microorganism [8]. Resistance to a range of antibiotics, including aminoglycosides, quinolones and β -lactams, is demonstrated by *P. aeruginosa* [9]. Generally, the main mechanisms of *P. aeruginosa* used to fight antibiotic attack can be divided into intrinsic, acquired and adaptive resistance. Low external membrane permeability, the expression of efflux pumps that remove antibiotics from the cell, and the development of antibiotic inactivating enzymes are part of the intrinsic resistance of *P. aeruginosa*. Either horizontal transfer of resistance genes or mutational modifications will achieve the acquired resistance of *P. aeruginosa* [10]. *P. aeruginosa*'s adaptive resistance requires the development of biofilm in the lungs of infected patients, where the biofilm functions as a diffusion barrier to inhibit the access of antibiotics to bacterial cells [11].

The effectiveness and safety of murepavadin in the treatment of infections of the lower respiratory tract caused by *P. aeruginosa* (suspected or confirmed) in patients with ventilation-associated pneumonia or CF-unrelated bronchiectasis (Clinical Trials.gov identifiers NCT02096315 and NCT02096328) have been tested in two clinical trials. However, by July 17, 2019, the trials were stopped because in research participants who had obtained murepavadin, an unusually high level of renal failure had been found. This decision would not impact the production of an aerosolized formulation of murepavadin for topical use [12]. Murepavadin is a particular weapon against *P. aeruginosa*, which separates it from the broad pipeline of antimicrobial natural and synthetic peptides acting against multiple taxa, *P. aeruginosa* included. Recently, several novel peptides with broad antimicrobial activity have been identified, such as antimicrobial peptide DGL13K, Mel4 and melamine (Melimine and Mel4 are chimeric cationic peptides with broad-spectrum antimicrobial activity), Cecropin B, Lysine-based peptidomimetics (LBP-2), Truncated pseudin-2 analogs (Pse-T2), antimicrobial peptide, termed 6 K-F17 (sequence: KKKKKK-AAFAAWAAFAA-NH₂), Melittin-derived peptides (MDP1, MDP2) [13–20]. In addition, multidrug-tolerant persistent cells can form in the biofilm that are capable of surviving antibiotic attack; in cystic fibrosis (CF) patients, these cells are responsible for prolonged and recurrent infections [21]. For patients whose infections are resistant to traditional antibiotics, the development of new antibiotics or alternative therapeutic methods for treating *P. aeruginosa* infections is urgently needed. In recent years, new antibiotics with novel modes of action have been investigated, as have new routes of administration and resistance to bacterial enzyme alteration. Compared to traditional antibiotics, some of these newer antibiotics demonstrate excellent in vitro antibacterial activity against *P. aeruginosa* as well as lower minimum inhibitory concentration (MIC) [22, 23]. Moreover, several novel non-antibiotic therapeutic approaches that are highly successful in destroying antibiotic resistant *P. aeruginosa* strains have been documented in recent studies [24]. These approaches include: antimicrobial peptides, phage therapy, inhibition of quorum sensing, iron chelation, the use of nanoparticles, probiotic and vaccine strategy. In order to combat *P. aeruginosa* infections, these therapeutic approaches may be used either as an alternative to or in conjunction with traditional antibiotic therapies.

2. Mechanisms of action of antimicrobial agents

There are six basic mechanisms of antimicrobial agents presented below:

1. Inhibition of microbial cell wall synthesis.
2. Inhibition of microbial cell membrane function.

3. Inhibition of microbial protein synthesis.
4. Inhibition of microbial DNA synthesis.
5. Inhibition of microbial RNA synthesis.
6. Inhibition of microbial metabolic pathways.

2.1 Inhibition of microbial cell wall synthesis

An integral microbial structure responsible for the shape of the cell is the cell wall. In addition, because of the high cytoplasmic osmotic pressure, the cell wall prevents cell lysis and facilitates the anchoring of membrane components and extracellular proteins, such as adhesins [25]. Bacterial cell wall synthesis has perhaps become the target field most commonly exploited for antimicrobial production on the basis of the number of antimicrobial drugs in clinical usage. Due to the absence of equivalents in human biology, the components of the cell wall synthesis machinery are attractive antimicrobial targets, thus providing intrinsic objective selectivity. The cytoplasmic synthesis of building blocks composed of N-acetyl muramic acid (M) linked to N-acetyl glucosamine (G) with an attached pentapeptide (P) side chain (referred to as MGP subunits) comprises the sequential late steps in cell wall synthesis. The linkage of the MGP subunit to the lipid II molecule enables subsequent translocation to the outside or periplasmic space of the cell through the cytoplasmic membrane. By catalyzing glycosidic linkages between the M and G components of the MGP subunits, transglycosylase enzymes then assemble the MGP subunits into a linear backbone. An immature peptidoglycan structure is constituted by linearly connected MGP subunits. Transpeptidase enzymes then work to cross-link pentaglycine bridges to the peptide side chains, in the process, the terminal 2 D-alanines of the peptide side chain are cleaved, creating the mature, lattice-like peptidoglycan that provides the form and osmotic stability of the bacterium [26]. β -lactam antibiotics, such as penicillins and cephalosporins, are the most widely used antimicrobials that prevent cell wall biosynthesis [27]. These β -lactam antibiotics interact directly with bacterial transpeptidases and inhibit them effectively. As transpeptidase inhibitors, β -lactams thus obstruct the transition from immature to mature peptidoglycan, so these enzymes are also referred to as penicillin-binding proteins (PBPs). Due to the stereochemical similarity of the β -lactam moiety with the D-alanine-D-alanine substrate, they are capable of doing this. Transpeptidases form a lethal covalent penicilloyl enzyme complex in the presence of the drug, which helps to inhibit the usual transpeptidation reaction. This results in peptidoglycan that is weakly cross-linked, which makes the developing bacteria extremely susceptible to cell lysis and death [28].

2.2 Inhibition of microbial cell membrane function

Lipids, proteins and lipoproteins are essentially made of biological membranes. The cytoplasmic membrane for water, ions, nutrients and transport systems serves as a diffusion barrier. Most health workers now assume that membranes are a lipid matrix with uniformly distributed globular proteins to penetrate through the bilayer of the lipid. A number of antimicrobial agents may cause disorganization of the membrane. These agents can be categorized into cationic, anionic, and neutral agents. Polymyxin B and colisthemethate (polymyxin E) are the best-known compounds [29]. For several antimicrobial agents, the cytoplasmic membrane forms an important barrier.

The mode of action of certain antimicrobial agents may be due to the ability of such medicines to increase membrane permeability, making it easier for them and other compounds to penetrate. Antibacterial cationic agents, increased permeability of the outer membrane to the lysozyme and hydrophobic compounds has been identified, such as polymyxin B. The initial function of these antimicrobial agents is to interrupt the structure of the outer membrane, allowing the cell to join itself and other compounds and inhibit unique metabolic processes [30]. There are several cell-damaging properties of Polymyxin B: (i) the surface charge, lipid composition and membrane structure are disturbed; (ii) the K⁺ gradient on the cytoplasmic membrane is dissipated; and (iii) the cytoplasmic membrane is depolarized. One of the key factors regulating bacterial exposure to polymyxin B is the permeability of the external membrane to lipophilic compounds. Since polymyxin B is bulkier than its displacement of inorganic divalent cations, in the presence of polymyxin B, the packing order of lipopolysaccharides (LPS) is changed. This results in increased permeability of a variety of molecules to the outer membrane and also promotes polymyxin B uptake ("self-promoted" uptake) [31].

2.3 Inhibition of microbial protein synthesis

Microbial protein synthesis inhibition a range of groups of antimicrobial agents work by inhibiting the synthesis of bacterial proteins (ribosome function). That include aminoglycosides, macrolides, tetracyclines, ketolides, lincosamides, streptogramins, chloramphenicol and oxazolidinones [26, 32]. The synthesis of microbial proteins is led by ribosomes in conjunction with cytoplasmic factors which, during the initiation phase, elongation phase and termination phases, bind transiently to particles. Microbial ribosomes contain 70S particles consisting of two 50S and 30S subunits, which join at the initiation stage of the synthesis of proteins and split at the termination stage. In bacterial protein synthesis, antimicrobial agents block various steps by interfering with the work of either the cytoplasmic factors or the ribosomes. Inhibitors which bind to the ribosomal subunit of 30S primarily interfere with initiation, although some interfere with the pairing of the AA- tRNA anticodon with the mRNA codon, elongation is thus impaired. The steps involved in the elongation process interact with inhibitors that bind to the 50S ribosomal subunit or to elongation factors that are transiently connected to ribosomes at certain stages of the cycle.

Through binding to particular ribosomal subunits [33], aminoglycosides function. By inducing the development of aberrant, non-functional complexes as well as causing misreading, aminoglycoside-type drugs may combine with other binding sites on 30S ribosomes and destroy bacteria. Spectinomycin is an antimicrobial agent that is closely linked to the aminoglycosides of aminocyclitol. It binds and is bacteriostatic but not bactericidal to a particular protein in the ribosome. Tetracyclines are other agents which bind to 30S ribosomes. These agents tend to inhibit aminoacyl tRNA binding to the A site of the bacterial ribosome. Tetracycline binding is temporary, so it's bacteriostatic for these agents. Nevertheless, a wide range of bacteria, *chlamydias* and *mycoplasmas* are inhibited and highly helpful agents [29]. There are three major groups of medicines that inhibit the ribosomal subunit of 50S. A bacteriostatic agent that inhibits both gram-positive and gram-negative bacteria is chloramphenicol. By binding to a peptidyltransferase enzyme on the 50S ribosome, it prevents peptide bond formation. Macrolides are large compounds of the lactone ring that bind to 50S ribosomes and tend to impair the reaction or translocation of peptidyltransferase, or both. Erythromycin, which inhibits gram-positive species and a few gram-negative species, such as *haemophilus*, *mycoplasma*, *chlamydia* and

legionella, is the most significant macrolide. Against many of these pathogens, new molecules including azithromycin and clarithromycin have greater activity than erythromycin. There is a similar activity site for lincinoids, the most important of which is clindamycin. Generally, macrolides and lincinoids are bacteriostatic and only inhibit the development of new peptide chains [29].

2.4 Inhibition of microbial DNA synthesis

The modulation of chromosomal supercoiling by topoisomerase-catalyzed strand breakage and rejoining reactions is needed for DNA synthesis, mRNA transcription and cell division [34]. Depending on whether they catalyze reactions involving transient breakage of one (type I) or both (type II) strands of DNA, DNA topoisomerase enzymes are classified into two groups, I and II [35]. The topological state of DNA inside cells is regulated by topoisomerases and is important for the vital processes of protein translation and cell replication. The enzyme that negatively super-coils DNA in the presence of ATP is DNA gyrase, a type II DNA topoisomerase [36]. Moreover, in the absence of ATP, this enzyme plays a role in the catenation and decatenation reaction of a double-stranded DNA circle, resolves knots in DNA, and also relaxes supercoiled DNA negatively. As a result, for almost all cellular procedures involving duplex DNA, including replication, recombination and transcription, the enzyme is vital. It is unique to the prokaryotic kingdom and is essential to the organism's survival. Thus, for antibacterial drugs, DNA gyrase remains an ideal and attractive target. The most effective DNA gyrase-targeted antimicrobial agents are quinolones. Nalidixic acid, a naphthyridone inadvertently discovered as a by-product during chloroquine synthesis, was the source of the compounds [37].

Quinolones are unique DNA-gyrase inhibitors. DNA gyrase reactions such as supercoiling and relaxation involving DNA breakage and reunion are inhibited by quinolones, specifically interfering with the DNA gyrase breakage-reunion reaction by interacting with subunit A (GyrA) [38]. Relatively poor antimicrobial activity is found in first-generation quinolones, nalidixic acid and oxolinic acid. However, the synthesis and improvement over many generations of fluoroquinolones, such as norfloxacin and ciprofloxacin (second generation), levofloxacin (third generation), and moxifloxacin and gemifloxacin (fourth generation), has resulted in a variety of potent antimicrobial agents [38]. Most bacterial pathogens possess an additional essential topoisomerase, topoisomerase I (Topo I), in addition to the type II topoisomerases. Topo I is architecturally and mechanistically distinct from gyrase and topoisomerase IV, and is an attractive candidate for new antibacterial chemotypes to be discovered as such [36].

2.5 Inhibition of microbial RNA synthesis

Rifamycins inhibit DNA-dependent transcription by binding the DNA-bound and effectively transcribing RNA polymerase with a high affinity to the β -subunit (coded by *rpoB*). In the channel formed by the RNA polymerase-DNA complex, from which the newly synthesized RNA strand emerges, the β -subunit is located. Rifamycins clearly require that RNA synthesis has not progressed beyond two ribonucleotides being added; This is due to the drug molecule's capacity to sterically inhibit the initialization of nascent RNA strands. It should be noted that rifamycins are not believed to work by blocking the RNA synthesis elongation stage, although a recently discovered class of RNA polymerase inhibitors (based on the CBR703 compound) could inhibit elongation by modifying the enzyme allosterically [34].

2.6 Inhibition of microbial metabolic pathways

By competitively blocking the biosynthesis of tetrahydrofolate, which acts as a carrier of one-carbon fragments and is required for the ultimate synthesis of DNA, RNA and bacterial cell wall proteins, trimethoprim and sulfonamides interfere with folic acid metabolism in the microbial cell. Bacteria and protozoan parasites typically lack a transport mechanism in order to extract preformed folic acid from their host, unlike mammals [29]. Most of these species, while some are capable of using exogenous thymidine, must synthesize folic acid, circumventing the need for metabolism of folic acid. The conversion of pteridine and p-aminobenzoic acid (PABA) to dihydrofolic acid by the pteridine synthetase enzyme is competitively inhibited by sulfonamides. Sulfonamides have a greater affinity for pteridine synthetase than for PABA. Trimethoprim has a huge affinity (10,000 to 100,000 times greater than that of the mammalian enzyme) for bacterial dihydrofolate reductase; it inhibits tetrahydrofolate synthesis when bound to this enzyme [29].

3. Mechanisms of resistance to antimicrobial agents

The ability of an organism to overcome the action of an antimicrobial agent to which it was previously susceptible is a general definition of antimicrobial resistance [39]. With the growing production of MDR strains (i.e. resistance to at least three antibiotics), nosocomial infection caused by antibiotic resistant *P. aeruginosa* has emerged as a major concern in clinical care settings [40]. Because of its outer membrane with low permeability (1/100 of the permeability of the outer membrane of *E. coli*), *P. aeruginosa* exhibits intrinsic resistance to various antimicrobial agents (β -lactam and penem group of antibiotics) [41, 42]. While several other processes are also responsible for their intrinsic resistance, including the efflux system that expels antibiotics from the cell's bacteria and the production of inactivating enzyme antibiotics. This bacterium, however is a highly diverse pathogen capable of adapting to the conditions around it. When subjected to selective pressure from antibiotics, the mediated reaction encourages bacterial survival and improves resistance to antibiotics [43–45].

The development of antibiotic resistance during host colonization of patients with CF has been confirmed, with *P. aeruginosa* strains developing and gaining resistance during antimicrobial therapy [46]. Studies have shown a clear link between increased applications of ciprofloxacin, with a growing incidence of strains resistant to ciprofloxacin [47]. Therefore the excessive use of antimicrobial agents is another factor associated with the rise in MDR-*Pseudomonas aeruginosa*. This acquired resistance may be attributable to the effects of the mutational event or the acquisition by horizontal gene transfer of the resistance gene and may occur during the mutational event of antibiotic therapy, leading to over-expression of endogenous β -lactamases or efflux pump, specific porin expression [48].

3.1 Resistance to β -lactam

Inhibition of the synthesis of the bacterial peptidoglycan cell wall requires β -lactam antibiotics [39]. Penicillin, cephalosporin, carbapenem and monobactam are included in this class. These classes include piperacillin and ticarcillin (penicillin), ceftazidime (cephalosporin 3rd generation), cefepime (cephalosporin 4th generation), aztreonam (monobactam), imipenem, meropenem and doripenem (carbapenems) are most powerful β -lactam widely used to treat *P. aeruginosa* is β -lactam [49]. These enzymes break the amide bond of the β -lactam ring through

resistance to the β -lactam mediated by the action of β -lactamases, rendering the antimicrobial ineffective. The expression of endogenous β -lactamases or the expression of acquired β -lactamases may be due to this inactivation of the drug. To date, hundreds of β -lactamases have been recognized and are distinguished by their substrate specificity. There are four major groups of beta-lactamases known in *P. aeruginosa* on the basis of Ambler's molecular classification system: A-D [50]. Through the serine-residue catalytic activity, classes A, C and D inactivate the β -lactams, while class B or metallo- β -lactamases (MBLs) require zinc for their action [51].

3.2 AmpC β -lactamase (Cephalosporinase)

In particular, the development of endogenous β -lactamase, such as chromosomal cephalosporinase (AmpC β -lactamase). A variety of β -lactams, such as benzyl penicillin, narrow spectrum cephalosporin and imipenem, can be induced in *P. aeruginosa*. Naturally, *P. aeruginosa* is susceptible to carboxypenicillins, ceftazidime and aztreonam, but it can develop resistance through a mutation in the gene that contributes to AmpC β -lactamase hyper-production [52, 53]. The enzyme is usually produced in small amounts ('low-level' expression), resistance to aminopenicillins and to most early cephalosporins is determined. *P. aeruginosa* produces an inducible chromosome-coded AmpC β -lactamase (cephalosporinase) belonging to the Ambler-based molecular class C and the first functional group according to Bush et al. [54, 55].

However, production of chromosomal cephalosporin in *P. aeruginosa*, in the presence of inducing β -lactams (especially imipenem), can increase from 100 to 1000 times [56]. β -lactamase inhibitors used in clinical practice, such as clavulanic acid, sulbactam and tazobactam, do not inhibit AmpC cephalosporinase function. β -lactamase of AmpC is encoded by the gene *ampC* [57, 58]. Several genes, including *ampR*, *ampG*, and *ampD*, are involved in *ampC* gene induction. *AmpR* encodes a positive transcriptional regulator and this regulator is required for the induction of β -lactamase. *AmpG*, a transmembrane protein that functions as a permease for 1,6-anhydromuropeptides, which are known to be the signal molecules involved in the induction of *ampC*, is the second gene involved. The third gene, *ampD*, encodes a cytosolic amidase of N-acetyl-anhydromuramyl-L-alanine that hydrolyses 1,6-anhydromuropeptides, which functions as an *ampC* expression repressor. The 4th chromosome, *ampE*, encodes the protein of the cytoplasmic membrane that serves as the molecule of the sensory transducer necessary for induction. Except for avibactam, the activity of this AmpC β -lactamase is not inhibited by commercially available β -lactam.

3.3 Class A carbenicillin hydrolysing β -lactamases

Four β -lactamases (PSE- of *Pseudomonas* specific enzyme) carbenicillin hydrolysing enzymes were identified in *P. aeruginosa*: PSE-1 (CARB-2), PSE-4 (CARB-1), CARB-3 and CARB-4 [59]. These enzymes belong to the group of β -lactamases of molecular class A and include carboxypenicillins, ureidopenicillins and cefsulodine in their substrate profile. These enzymes belong to functional group 2c and molecular class A [60]. Commercially available β -lactam inhibitors, such as clavulanic acid, tazobactam, and sulbactam, can inhibit the activity of this β -lactamase [61].

3.4 Resistance to aminoglycoside

Aminoglycosides are a microbial protein synthesis inhibitor which act by binding to the ribosomal subunit of the bacterial 30S and interfering with the initiation of protein synthesis. Resistance to aminoglycosides in *Pseudomonas* is mediated by

transferable aminoglycoside modifying enzymes (AMEs), low permeability of the outer membrane, active efflux and, in rare cases, target modification [62–64].

3.5 Aminoglycoside-modifying enzymes

AMEs inactivate the aminoglycoside by adding the antibiotic molecule to a phosphate, adenylyl or acetyl radical, and thus modified antibiotics minimize the binding affinity of the bacterial cell (30S ribosomal subunit) to its target [65, 66]. Aminoglycoside phosphoryl transferases (APHs), aminoglycoside adenylyl transferases (also known as nucleotidyltransferases) (AADs or ANTs) and aminoglycoside acetyltransferases (AACs) are three types of AMEs involved in aminoglycoside alteration. The following AMEs are most commonly expressed by *P. aeruginosa*: AAC(69)-II (resistant to gentamicin, tobramycin and netilmicin), AAC(3)-I (resistant to gentamicin), AAC(3)-II (resistant to gentamicin, tobramycin and netilmicin), (69)-I (resistant to tobramycin, netilmicin and amikacin) and ANT(29)-I (resistant to tobramycin and gentamicin) [67].

3.6 Low outer membrane permeability

Membrane impermeability or reduced permeability is a mechanism known to provide resistance to many antibiotic forms, including aminoglycosides, β -lactams and quinolones [68]. For instance, this resistance mechanism is often encountered in cystic fibrosis isolates that are continually under antibiotic attack. Several mechanisms, such as lipopolysaccharide (LPS) modifications, alteration of membranous proteins involved in substratum absorption, and inactivation of enzymatic complexes involved in the energetic membrane necessary for transport system activity, may cause membrane impermeability [69].

3.7 Active efflux pumps

The combination of low membrane permeability and active efflux pumps is partially due to the natural resistance of *P. aeruginosa* to many groups of antibiotics. *P. aeruginosa*'s efflux systems involved in antibiotic resistance belong to the family of resistance-nodulation-division (RND) [70]. In order to confer resistance to several antibiotics, four major efflux systems have been described: MexAB-OprM, MexCD-OprJ, MexEF-OprN and MexXY-OprM. These systems consist of three proteins: (1) the efflux pump protein found in the cytoplasmic membrane (MexB, MexD, MexF and MexY), (2) the pore-acting outer membrane protein (OprM, OprJ and OprN) and (3) A protein in the periplasmic space that bridges the cytoplasmic and outer membrane proteins (MexA, MexC, MexE and MexX). In both natural and acquired resistance, MexAB-OprM and MexXY-OprM are active, whereas only the other two mechanisms are observed in cumulative resistance.

Acquired resistance is observed following mutations in the regulatory systems that can be caused by antibiotic pressure and that can confer resistance to all groups of antibiotics upon over-expression of these efflux systems. Polymyxins, except [69]. Resistance to multiple groups of antibiotics that are substrates of these efflux systems can be caused by exposure to a single antibiotic. Quinolones are substrates of all efflux systems and are an important trigger factor that can generate cross-resistance to efflux systems of several major classes of antibiotics, including β -lactams and aminoglycosides, for pseudomonal therapy [71]. It is understood that efflux systems confer a moderate degree of resistance, but they typically act simultaneously with other mechanisms of resistance, thus taking part in the high-level resistance that can be observed in *P. aeruginosa*.

3.8 Target modification

Due to the low affinity of the drug to the bacterial ribosome, bacteria may be resistant to aminoglycosides. This can be achieved by 16S rRNA methylation by target modification. Various 16S rRNA methylases have been identified for *P. aeruginosa*: RmtA, first reported in clinical isolates of *P. aeruginosa* resistant to aminoglycosides and conferred resistance to all parenterally administered aminoglycosides, including amikacin, tobramycin, isepamicin, kanamycin, arbecacin and gentamicin, secondary 16S rRNA methylases including RmtB, ArmA and RmtD [72].

3.9 Resistance to fluoroquinolones

Resistance to fluoroquinolones arises by mutation in the DNA gyrase or topoisomerase 1 V coding bacterial chromosome gene or by successful drug transport out of the cell [73]. Topoisomerase 1 V mutations can occur in *gyrA* / *gyrB* genes within the motif of the quinolone-resistant determinative region (QRDR), which is considered to be the active site of the enzyme. This contributes to the altered amino acid sequences of the subunits A and B, and hence to the altered topoisomerase II with a low affinity for quinolone molecules. As a result of point mutations in *parC* and *parE* genes encoding the ParC and ParE enzyme subunits, modifications of a secondary target (topoisomerase IV) occur. The over-expression of efflux includes other types of fluoroquinolone tolerance in *Pseudomonas*. Mutations in the *nalB*, *nfxB* and *nfxC* genes, resulting in overexpression of MexA-MexB-OprM, MexC-MexD- OprJ and MexE- MexF- OprN following efflux [74].

3.10 Biofilm-mediated resistance

A biofilm is an aggregate of microorganisms that bind to each other on a living or non-living surface and are embedded in an extracellular polymeric (EPS) matrix of self-produced substances, including exopolysaccharides, proteins, metabolites, and eDNA [75, 76]. The microbial cells grown in biofilms are less sensitive than the cells grown in free aqueous suspension to the antimicrobial agents and the host immune response [77]. Even bacteria that are deficient or lack protective mutations in their intrinsic resistance, when they grow in a biofilm, they can become less susceptible to antibiotics [78]. The general mechanisms of biofilm-mediated resistance that protect bacteria from antibiotic attack include antibiotic penetration prevention, altered microenvironment that induces slow biofilm cell growth, adaptive stress response induction, and differentiation of persistent cells [78–80].

P. aeruginosa causes chronic lung infections in CF patients and, through the production of DNA, proteins and exopolysaccharides, forms a biofilm on lung epithelial cell surfaces. The regulation of the formation of *P. aeruginosa* biofilm is multifactorial and mainly depends on quorum sensing systems, GacS / GacA and RetS / LadS two-component regulatory systems, exopolysaccharides and cdi- GMP [81]. Quorum sensing is a form of communication between bacterial cells and cells that regulates gene expression in response to changes in cell population density. *P. aeruginosa* has three major systems of quorum sensing, LasI/LasR, RhII-RhlR, and PQS-MvfR, all of which contribute to mature and differentiated biofilm formation. During biofilm formation, *P. aeruginosa* undergoes numerous physiological and phenotypic changes [82]. For example, *P. aeruginosa* strains convert to a mucoid phenotype in CF chronic infection that displays upregulated production of alginate driven by the CF microenvironment, enabling the formation of colonies of biofilms. Due to its ability to show swarming and twitching motility, *P. aeruginosa* flagellum is important for the initiation of biofilm formation. However, *P. aeruginosa* significantly

decreases flagellum expression after surface attachment and may also permanently lose the flagellum due to genetic mutations, reducing host immune response activation, allowing *P. aeruginosa* to evade immune detection and phagocytosis [83].

4. The global economic scenario of antibiotic resistance

It is still an immense global challenge to quantify the exact economic effect of resistant bacterial infections. Measuring the distribution of the disease associated with antibiotic resistance is a crucial prerequisite in this situation. A major economic burden for the entire world is antibiotic resistance. In the USA alone, 99,000 deaths are caused annually by antibiotic-resistant pathogen-associated hospital-acquired infections (HAIs). Approximately 50,000 Americans died in 2006 because of two popular HAIs, namely pneumonia and sepsis, costing the US economy around \$8 billion [84]. Patients with antibiotic-resistant bacterial infections need to remain in the hospital for at least 13 days, creating an extra 8 million hospital days each year. There have been estimates of costs of up to \$29,000 per patient infected for an antibiotic-resistant bacterial infection. In total, economic losses of approximately \$ 20 billion were recorded in the US, while losses of approximately \$35 billion per year were also recorded in terms of loss of productivity due to antibiotic resistance in health care systems [85].

A worst-case scenario could emerge in the coming future, according to the analysts of the Research and Development Corporation, a US non-profit global organization, where the planet could be left without any effective antimicrobial agent to treat bacterial infections. In this case, the global economic burden will be nearly \$120 trillion (\$3 trillion per annum), roughly equal to the entire actual annual health care budget of the United States. In general, the world population will be significantly affected: about 444 million people will succumb to infections as of 2050, and birth rates will decrease rapidly in this scenario [86, 87]. These losses are calamitous, but these estimates reflect imperfect images of the economic costs of antibiotic resistance due to data limitations such as the inclusion of total conditions and resistance-susceptible diseases. The use of antibiotics in the livestock and food industries is another very critical trait of antimicrobial resistance (AMR), that was missing from the investigation. It is an important player in the rising AMR, likely causing its own projected economic losses. There is also a misappropriation of the use of antimicrobials as growth promoters in many developing countries. This activity has been outlawed in the European Union since 2006 [88, 89].

5. Novel alternative antimicrobial therapy for *P. aeruginosa* treatment

The overuse and misuse of antibiotics, which can lead to unwanted side effects and the production of drug-resistant bacterial strains, is a growing public health issue. The production of new antibiotics, in addition, is very limited and timely. The development of innovative therapeutic approaches to the treatment of infections with *P. aeruginosa* is therefore highly desirable and has received further interest over the past decade. These innovative therapeutic techniques, which involve antimicrobial peptides, phage therapy, inhibition of quorum sensing as well as the use of iron chelation, nanoparticles, probiotics and vaccine strategies.

5.1 Antimicrobial peptides

A number of species, from bacteria to animals, develop antimicrobial peptides (AMPs), also called host defense peptides, and they are active against a wide range

of microorganisms [90]. There is no complete understanding of the mode(s) of operation of AMPs. It is widely agreed that the cytoplasmic membrane is attacked by AMPs, leading to cell death [91]. AMPs have also been shown to possess anti-biofilm and immunomodulatory properties, in addition to antimicrobial activity, AMPs have been proposed as an alternative to traditional antibiotics to battle bacterial infections as a result of their broad-spectrum activity; AMPs exhibit rapid killing kinetics, low mediated resistance levels, and low host toxicity [92]. Many antimicrobial peptides, including GL13 K, LL-37, T9W, NLF20, cecropin P1, indolicidin, magainin II, nisin, ranalexin, melittin, and defensin, have demonstrated powerful antimicrobial effects of either direct bactericidal effects or biofilm disruption against *P. aeruginosa* [93]. In addition, by facilitating antibiotic absorption, disrupting biofilm formation or inhibiting bacterial quorum, some AMPs have demonstrated synergy with traditional antibiotics against several bacteria, including *P. aeruginosa* [94]. For instance, it has been shown that the clearance of *P. aeruginosa* biofilm was increased by a combination of GL13 K with tobramycin [95]. In 2017, Zheng et al. [96] observed that when combined with tetracycline in vitro, the minimum inhibitory concentration of cecropin A2 against clinical isolates of *P. aeruginosa* was reduced 8-fold.

5.2 Phage therapy

By inducing lysis, bacteriophages (phages) are viruses that infect and destroy bacteria [97]. In 1915, the British bacteriologist Frederick Twort first discovered phages. Two years later, Félix d'Herelle made a similar discovery independently in Paris and presented the phage therapy notion. With the advent of antibiotic therapy, phage therapy was abandoned in several countries, but has been continuously developed in Eastern European countries with facilities in Warsaw, Poland, and Tbilisi, Georgia [98]. Shotgun metagenome sequencing showed that there were antipseudomonal phages in the phage cocktails sold in pharmacies in Georgia and Russia [99]. The successful treatment of infections with MDR *P. aeruginosa* has been reported in a few case reports from Belgium and the US, but has not gained broad acceptance in the Western world [100]. Phage therapy has many benefits, including replication at the infection site, high precision for attacking bacteria without effects on commensal flora, less side effects than other therapies, antibiotic-resistant bacteria bactericidal activity and simple administration [101]. The use of phages as an alternative to antibiotics has been extensively studied for the treatment of *P. aeruginosa* infections. There are 137 different phages that have been characterized to date that target the *Pseudomonas* genus [102]. Many in vitro and in vivo studies have been performed to test the efficacy of phages against chronic infections of *P. aeruginosa*. For instance, co-incubation of phage PA709 with the clinical strain *P. aeruginosa* 709 has been shown to significantly reduce the viability of *P. aeruginosa*. Another research found that intranasal administration of P3-CHA bacteriophage to mice receiving a lethal dose of *P. aeruginosa* strain CHA substantially improved the rate of survival and reduced the bacterial load in the lungs [103].

Another benefit of phage therapy is that phages can be genetically modified as vehicles to transport bacteria with antimicrobial agents, thus increasing treatment efficacy [104]. While phages have been shown to be successful in vitro and in animal models against bacterial infection, only a small number of phage therapy clinical trials have been performed to date. The reasons for this include: safety issues about post-treatment phage clearance and impurity of phage preparations; poor stability of phage preparations; and lack of knowledge of the comprehensive phage mode of action and bacterial resistance to phage growth [105]. In clinical trials, the use of phages against *P. aeruginosa* infections has been studied in patients with venous leg ulcers, burn wounds and otitis, and no adverse reactions have been identified during these clinical trials [106].

5.3 Quorum sensing inhibition

Quorum sensing is a mechanism that enables bacteria to regulate the expression of genes in a manner based on cell density. To control virulence and biofilm formation, *P. aeruginosa* utilizes quorum sensing [107]. Las and Rhl are two major *P. aeruginosa* quorum-sensing systems responsible for the synthesis of the signal molecules of N-acyl homoserine lactone (AHL), N-(3-oxododecanoyl)-L-homoserine lactone (3O-C12-HSL) and N-butanoyl-L-homoserine lactone (C4-HSL). 3O-C12-HSL and C4-HSL bind to and activate their LasR and RhlR cognate transcription factors, respectively, inducing the formation of biofilms and the expression of various virulence factors, including elastase, proteases, pyocyanin, lectins, rhamnolipids, and toxins [108]. The third *P. aeruginosa* quorum-sensing system, PQSMvfR, has been reported to facilitate the formation of biofilms in addition to the LasI-LasR and RhlI-RhlR systems. This mechanism regulates the development of the *Pseudomonas* quinolone signal (PQS), 2-heptyl-3-hydroxy-4-quinolone, by the transcriptional regulator MvfR, also known as PqsR, by controlling the pqsABCDE operon. In addition, PqsA and PqsD proteins have been implicated in the development of biofilms [82].

A promising technique for treating *P. aeruginosa* infections is known to be the inhibition of quorum sensing. This approach is capable of preventing or decreasing the formation of biofilms, reducing bacterial virulence and has a low risk of bacterial resistance growth. In addition, this strategy has a small scope, such that any unwanted inhibitory effects on beneficial bacteria are impossible. For the Las and Rhl systems, quorum sensing inhibitors may be either natural or synthetic and are capable of reducing the activity of AHL synthase, inhibiting the development of AHL, degrading AHLs or competing for AHL receptor binding [109]. In recent years, the use of quorum sensing inhibitors for the treatment of infections with *P. aeruginosa* has been intensively studied. The carotenoid zeaxanthin, typically found in plants, algae and lichens, for example, reduced the formation of biofilms in *P. aeruginosa* by binding to the signal receptors for quorum sensing, lasR and RhlR, and blocking the expression of virulence genes, lasB and rhlA [110]. Flavonoids are a class of naturally developed plant metabolites that have acted as LasR and RhlR antagonists and substantially decreased their ability to bind to the *P. aeruginosa* promoters of quorum sensing-regulated genes [111].

5.4 Iron chelation

Iron is important for bacterial growth and is involved in a number of cellular processes, such as the production of electricity, the replication of DNA and the transport of electrons [112]. Compared to healthy people, the iron content of human sputum was found to be substantially elevated in CF patients, indicating that an increased amount of iron promotes chronic CF lung infection [113]. *P. aeruginosa* utilizes pyoverdine and pyochelin siderophores to obtain iron from the extracellular environment [114]. Therefore, a technique to fight *P. aeruginosa* infections is to limit the concentration of extracellular iron or disrupt iron uptake by *P. aeruginosa*. Several studies have related iron metabolism to the pathogenesis of chronic infections, indicating that iron analogues and chelators may work against *P. aeruginosa* as potential therapeutic agents. For example, iron chelators, 2,2'-dipyridyl (2DP), diethylenetriaminepentaacetic acid (DTPA) and EDTA, have been reported to impair growth and biofilm formation of *P. aeruginosa* and have been more effective under anaerobic conditions [115].

Gallium is a nonredox iron III analog that disrupts the metabolism of bacterial iron by acting in several biological processes as an iron replacement, so it is a US

FDA-approved medication for cancer-associated hypercalcemia treatment [116]. In 2007, Kaneko et al., reported that gallium was able to inhibit the growth of *P. aeruginosa*, prevent the development of biofilms, and manifest excellent bactericidal activity in vitro by reducing the uptake of bacterial iron and repressing the synthesis of pyoverdine mediated by the transcriptional regulator PvdS. In addition, in mouse infection models, gallium has also been found to remove *P. aeruginosa* effectively.

5.5 Nanoparticles

Currently, a variety of diseases, including cancer and bacterial infectious diseases, have received significant attention from nanoparticles to treat them. Nanoparticles are small materials that have been used in a number of chemical, biological and biomedical applications, having a size of less than 100 nm and a large surface area to mass ratio [117]. The nanoparticles used for their antimicrobial activity are highly penetrable in the bacterial membranes, may interfere with the formation of biofilms, have several antimicrobial mechanisms, and are strong antibiotic carriers [118]. For the prevention of *P. aeruginosa* infections, metallic and antimicrobial agent-loaded nanoparticles have been extensively examined. Silver nanoparticles, for example, are powerful antimicrobial agents that generate silver ions responsible for the inhibition, like DNA synthesis, of bacterial enzymatic systems. Silver nanoparticles have shown important antimicrobial effects on the clinical strains of *P. aeruginosa*, killing *P. aeruginosa* effectively and inhibiting its in vitro growth. In addition, silver nanoparticles have demonstrated low mammalian cell cytotoxicity, although this requires more in vivo research [119].

Nanoparticles are capable of delivering antimicrobial agents such as antibiotics to bacteria, as described earlier. Kwon et al., developed porous silicon nanoparticles with a novel antimicrobial peptide fused with a synthetic bacterial toxin, containing membrane-interacting peptides. This engineered nanoparticle was discovered in a mouse model of *P. aeruginosa* lung infection to increase the survival rate and bacterial clearance. Moreover, it has been found that the binding of antibiotics to nanoparticle surfaces greatly improves the effectiveness of both antibiotics and nanoparticles. In this respect, silver ampicillin-attached nanoparticles have a higher rate of in vitro killing of ampicillin-resistant *P. aeruginosa* isolates compared to silver ampicillin-attached nanoparticles [120].

5.6 Probiotic as an alternative antimicrobial therapy

Probiotics are living microorganisms which, when ingested in appropriate quantities, provide health benefits [121]. The majority of probiotic bacteria are gram-positive, and their primary functions are related to intestinal tract health regulation and maintenance (e.g., *Lactobacillus* and *Bifidobacterium*) [122]. The probiotics in the intestines that colonize the human host are the most numerous. The commensal intestinal microbiome leads to enhanced infection tolerance, differentiation of the host immune system, and nutrient synthesis [123]. The probiotic *Pediococcus acidilactici* HW01 was studied against *P. aeruginosa* and observed decreased *P. aeruginosa* motility as well as decreased pyocyanin development, decreased protease and rhamnolipid production, and decreased stainless steel surface biofilm formation. Another research conducted by Moraes et al., showed that *Lactobacillus brevis* and *Bifidobacterium bifidum* were effective against *S. aureus* biofilms grown on titanium discs. The findings showed a decrease in *S. aureus* growth on titanium discs when both probiotics were used, but *L. brevis* strains was shown to have the greatest inhibitory effect on biofilm formation.

Recent studies by Xu et al. [124], have indicated that probiotics can be used by patients infected by COVID 19 to prevent secondary infections. There was intestinal microbial dysbiosis in some patients with COVID-19. In all patients, nutritional and gastrointestinal functions must be measured. To control the composition of the intestinal microbiota and reduce the risk of secondary infection due to bacterial translocation, nutritional support and application of probiotics was suggested.

5.7 Vaccine strategy

The concept of a vaccine strategy is to avoid infection until it can be produced. The production of vaccines aims to prevent and decrease infections of *P. aeruginosa* [125]. However, no approved vaccine against this pathogen is yet available. *P. aeruginosa* antigens, which are responsible for pathogenesis, induce potent immune responses. LPS O-antigen, polysaccharide protein conjugates, outer membrane proteins OprF and OprI, type III secretion system portion PcrV, flagella, pili, DNA, live-attenuated *P. aeruginosa* and whole killed cells are possible candidates for *P. aeruginosa* vaccines [126]. Among the potential *P. aeruginosa* vaccines, phase III clinical trials in CF patients were performed only with the flagella vaccine and the recombinant vaccine IC43, containing OprF and OprI.

Related to the ability of this pathogen to undergo phenotypic changes in variable environmental conditions, the existing vaccines for *P. aeruginosa* demonstrate poor efficacy in clinical trials. For example, *P. aeruginosa* strains downregulate the expression of highly immunogenic virulence factors in CF patients' lungs, such as LPS O-antigen, type III secretion systems, flagella and pili [127]. In addition, impaired mechanisms of host protection often reduce vaccination effectiveness. Due to the CF lungs having an altered mucus layer, impaired phagocytosis, and dysregulated inflammatory responses, including aberrant cytokine and chemokine production, and reduced phagocyte recruitment, the lung microenvironment in CF patients has become a great challenge for effective vaccination [128].

6. Role of combination therapy *versus* monotherapy

Early administration of adequate antibiotic therapy was associated with a favorable clinical outcome, especially among critically ill patients with serious *P. aeruginosa* infections [129]; on the other hand, delays in administering adequate antibiotic therapy were associated with a substantial increase in mortality. The progressive rise in antibiotic resistance in *P. aeruginosa* has been established in recent years as the key explanation for the inadequacy of antibiotics, with a negative effect on patient survival [130].

The evidence available indicates that the greatest advantage of combination therapy derives from an increased probability of selecting an appropriate agent during empirical therapy, rather than avoiding resistance during definitive therapy or benefiting from synergistic action in vitro. Therefore, researchers recommend early administration of a combination regimen when *P. aeruginosa* is suspected, followed by a prompt de-escalation when the antimicrobial susceptibility test becomes available, to balance between early antibiotic administration and the risk of resistance selection. An approach consisting of the prescription of an anti-pseudomonal beta-lactam (piperacillin / tazobactam, ceftolozane / tazobactam, ceftazidime, cefepime, or carbapenem) plus a second (aminoglycoside or fluoroquinolone) anti-pseudomonal agent is encouraged.

7. New antipseudomonal antibiotics

Related to the emergence of multidrug-resistant strains, traditional antibiotic therapies against *P. aeruginosa* infections have become increasingly ineffective. The use of various antibiotic combinations and the development of new antibiotics are existing therapeutic options for *P. aeruginosa* treatment. New antibiotics have been shown to be more effective in destroying *P. aeruginosa* and have a lower frequency of production of resistance compared to current antibiotics due to their novel modes of action, efficient delivery of drugs (e.g. inhaled antibiotics) and resistance to bacterial enzyme alteration. Novel antibiotics with action against *P. aeruginosa* have been available in Europe in recent years and others are in advanced stages of clinical development. In certain instances, indirect evidence indicates their possible superiority over standard anti-pseudomonal regimes.

7.1 Doripenem

Doripenem is a new carbapenem antibiotic with wide spectrum activity against gram-negative and gram-positive bacteria by binding to penicillin-binding proteins by inhibiting bacterial cell wall synthesis; it has been approved for the treatment of complicated intra-abdominal infection and urinary tract infection by the US Food and Drug Administration (FDA) [131]. Except for the metallo- β -lactamases of class B, doripenem is resistant to hydrolysis by several β -lactamases. Importantly, compared to other carbapenem antibiotics such as meropenem and imipenem, the in vitro antibacterial activity of doripenem against the *P. aeruginosa* isolates from CF patients was found to be more active [132]. In addition, the effectiveness of doripenem was tested in patients with *P. aeruginosa* ventilator-associated pneumonia, a phase III clinical trial of patients with *P. aeruginosa* ventilator-associated pneumonia found that patients treated with doripenem had higher rates of cure compared to patients treated with imipenem. Of note, headache, nausea, diarrhea, rash, and phlebitis are among the side effects of doripenem [133].

7.2 Plazomicin

Plazomicin is a semisynthetic aminoglycoside antibiotic of the next generation that is synthetically derived from the natural product sisomicin. A wide range of aminoglycoside modifying enzymes, but not 16S rRNA ribosomal methyltransferases, are able to resist plazomicin [134]. Plazomicin exhibits potent in vitro activity against both gram-negative and gram-positive bacterial pathogens and has an activity close to that of amikacin against strains of multidrug-resistant *P. aeruginosa*. In addition, Pankuch et al., reported in vitro synergistic activity of plazomicin against clinical isolates of *P. aeruginosa* in combination with cefepime, doripenem, imipenem or piperacillin-tazobactam and no antagonism was observed in this study, indicating that plazomicin is a possible candidate for combination therapy in the treatment of infections with multidrug-resistant *P. aeruginosa*. Plazomicin can cause nephrotoxic and ototoxic effects that are mild to moderate [135].

7.3 POL7001

As a novel class of antibiotics against *P. aeruginosa*, protein epitope mimetic (PEM) molecules have emerged; some PEM molecules inhibit the transfer of LPS to the outer bacterial membrane [136]. A macrocycle molecule belonging to the PEM antibiotic family is POL7001. The efficacy of POL7001 was tested by Cigana et al.,

both in vitro and in murine *P. aeruginosa* acute and chronic pneumonia models. They observed that *P. aeruginosa* multidrug-resistant isolates were susceptible to POL7001 in CF patients, and that POL7001-treated mice had substantially decreased bacterial burden and decreased levels of lung inflammation during acute and chronic *P. aeruginosa* infection. POL7001 as a novel therapeutic agent for potential clinical trials is indicated by the new mode of action, effective pulmonary delivery and potent in vitro and in vivo activity. The side effects of POL7001 have not yet been identified [137].

7.4 Arikayce™

Arikayce™ has been approved by the FDA for the treatment of *Mycobacterium avium* complex (MAC) lung disease, and is a liposomal amikacin treatment. Clinical trials for this drug and its efficacy in the treatment of *P. aeruginosa* in patients with cystic fibrosis have been performed. While these are early phases and some experiments would have to resolve the drawbacks of this compound in order to improve safety, some experimental clinical trials have been performed [138].

7.5 Ceftolozane-tazobactam

To resolve *P. aeruginosa* antimicrobial resistance mechanisms, such as changes in porine permeability and upregulation of efflux pumps, ceftolozane-tazobactam is being created. Due to a higher affinity for all essential PBPs, including PBP1b, PBP1c and PBP3, this drug has an intrinsically potent anti-pseudomonal effect [139]. Ceftolozane/ Tazobactam has been shown to have a strong in vitro activity against most strains of MDR *P. aeruginosa* [including strains developing extended-spectrum β -lactamase (ESBL) but not carbapenemase]. The therapeutic use of ceftolozane-tazobactam in complicated intra-abdominal and urinary tract infections has been suggested by the FDA [140]. In addition, a study is currently underway for the treatment of HAP, including ventilator-associated pneumonia (VAP). In 71 percent of patients with MDR *P. aeruginosa* infections, evidence from real-world trials using ceftolozane-tazobactam for the treatment of MDR *P. aeruginosa* infections showed promising results.

7.6 Ceftazidime-avibactam

Ceftazidime-avibactam is a novel combination of β -lactam / BLI approved for the treatment of complicated urinary tract infections (UTIs) and complicated intra-abdominal infections by the Food and Drug Administration (FDA) and European Medicines Agency (EMA). In vitro studies have shown that the combination of ceftazidime-avibactam is highly effective against KPC- producing *Klebsiella pneumoniae* carbapenemase (KPCs), oxacillinase (OXA), extended- spectrum β -lactamases (ESBLs) and AmpC enzymes producing Enterobacteriaceae. The drug does not, however, have any action against metallo-beta β - lactamases (MBL, VIM and NDM) and avibactam does not have any improved activity against *P. aeruginosa* [141]. In phase III research compared ceftazidime-avibactam to meropenem (NTC01808092), the efficacy of ceftazidime-avibactam against VAP was analyzed [142]. The predominant isolated baseline gram-negative pathogens were *K. pneumoniae* and *P. aeruginosa*, with 28% of patients possessing a non-susceptible isolate of 1% ceftazidime. 356 patients in the clinically evaluable population were treated with ceftazidime-avibactam and 370 with meropenem. The research met the non-inferiority criterion for ceftazidime-avibactam as there was no disparity in the outcome between the groups. In addition, the efficacy of ceftazidime-avibactam

was close to that of ceftazidime-susceptible pathogens against ceftazidime-non-susceptible strains and was also comparable to meropenem.

7.7 Imipenem-cilastatin-relebactam

Relebactam is a β -lactamase inhibitor (BLI) diazabicyclooctane that inhibits β -lactamase class A and C activity, but has no activity against metallo- β -lactamase. It has been shown that the combination of imipenem-cilastatin with relebactam has synergistic activity against a broad range of MDR gram negative pathogens including *P. aeruginosa*, KPC-producing *K. pneumoniae* and *Enterobacter* spp. [143]. This medication has been tested predominantly in patients with IAI, complicated UTI, and pyelonephritis, although a trial is currently underway in patients with HAP/VAP. Some new medications have a small effect on *P. aeruginosa*, such as plazomycin, meropenem-vaborbactam and aztreonam-avibactam [144].

8. Conclusions

Treatment of infections with *P. aeruginosa* continues to be significant challenging. Improving the early diagnosis and empirical treatment of serious *P. aeruginosa* infections is an urgent need. First, to quickly announce the detection and susceptibility results for *Pseudomonas* in blood cultures and other clinically important cultures, matrix-assisted-laser-desorption-ionization time-of-flight mass spectrometry (MALDI-TOF) and modern molecular techniques should be routinely introduced. However, in order to decide if such diagnostic methods have a real effect on hospitalization time and patient mortality, controlled trials would be required. Secondly, more studies are urgently required to classify patients at risk of infection with MDR *P. aeruginosa* (bloodstream infections, urinary tract infections) based on clinical risk factors. Ultimately, clinical response depends on factors such as underlying diseases, seriousness of infection, form of infection, adequate control of the source, and response to prior antibiotics. There is an immediate need to determine the true impact of the latest anti-*Pseudomonas* drugs recently approved for the treatment of these infections on patient outcomes. To date, however, due to high cost, side effects and safety issues, few of these newer methods have continued to clinical practice.

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