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Understanding the Molecular Mechanisms of Antibiotic Resistance Through Horizontal Gene Transfer in *Pseudomonas aeruginosa*

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ABSTRACT

Pseudomonas aeruginosa is a gram-negative bacterium whose resistance mechanism has been linked to several different diseases, most notably lung infections in people with cystic fibrosis, which account for more than 60% of infections in adults and affect 300 million people worldwide at a prevalence rate of 12.2%. This study aims to do two things: provide a thorough explanation of the molecular mechanisms underlying its antibiotic resistance via horizontal gene transfer (HGT), and highlight approaches to counteracting this rising global menace. To highlight the importance of metallo-lactamases, ribosomal protection proteins, and modifying enzymes, we conducted a systematic literature review that zeroed in on intrinsic and acquired resistance mechanisms made possible by HGT. CRISPR-Cas technology and other snoRNAs have considerable potential for combating antibiotic resistance in P. aeruginosa, according to the study's main findings. Since spacer proteins, crRNA, and sgRNA all play a role in horizontal gene transfer (HGT), altering them with CRISPR-cas can reduce the prevalence of AMR. To create CRISPR-based antibiotics, phage therapy, and sensitize bacteria to antibiotics, current research is delving further into the processes of antimicrobial resistance.

Keywords: *Antibiotic resistance Genes, HGT, P. aeruginosa, CRISPR-cas Technology, and small non-coding RNAs*

INTRODUCTION

The multipurpose gram-negative bacteria Pseudomonas aeruginosa is infamous for its toxicity and drug resistance. Bacterial resistance to antibiotics is a complex defensive mechanism that has serious implications for human health. More than sixty percent of adult infections are associated with *P.aeruginosa* [13;40], and approximately thirty thousand people are living with CF in the United States and upwards of seven thousand people worldwide [33]. This resistance mechanism has also been linked to trauma-related infections and chronic obstructive pulmonary disease (COPD), which account for an additional three hundred million people being afflicted by these diseases. *P. aeruginosa* infections impact 32.4% to 42.8% of the world's population and account for 7.1% to 7.3% of all healthcare-associated infections. Antibiotic-resistant strains are linked to a significant number of these instances [51]. The treatment difficulties have been made worse by the evolution of antibiotic resistance in P. aeruginosa, which has led to an increase in morbidity and mortality among those affected.

Horizontal gene transfer (HGT) and genetic mutations have played crucial roles in the evolution of antibiotic resistance in *P. aeruginosa* [18]. HGT consists of The term "horizontal gene transfer" which is used to describe the process by which bacteria acquire additional DNA from their surroundings (transformation), from other bacteria in close proximit nearby (conjugation), and via viral infection (transduction). Detection of antibiotic-resistant *P. aeruginosa* in these bacterial groupings can be traced as far back as the 1940s, while the majority of discoveries began to surface in the 1970s (Figure 1). As early as 1979, *P. aeruginosa* and other antibiotic-resistant bacteria were identified in Finnish hospitals [6]. It has been shown through research that *P. aeruginosa* can survive and even thrive inside the CF lung, causing persistent infections. *P. aeruginosa* produces -lactamases, enzymes that can breakdown -lactam drugs, as a mechanism of antibiotic resistance. In 1994, it was found that P. aeruginosa's ability to recycle its cell wall results in the production of cytosolic muropeptides that act as effectors for the development of -lactamase. The spread of *P. aeruginosa* strains that are resistant to many antimicrobials due to a trait encoded on their chromosomes Since the turn of the millennium, efflux pumps have been a major problem all over the world [66]. The role of plasmids in the development of antibiotic resistance in *P. aeruginosa* is particularly noteworthy. They can help resistance mechanisms spread rapidly throughout bacterial populations since they carry the genes for doing so. P. aeruginosa's resistance can be boosted by plasmids, and plasmids can potentially spread resistance to other bacteria [14].

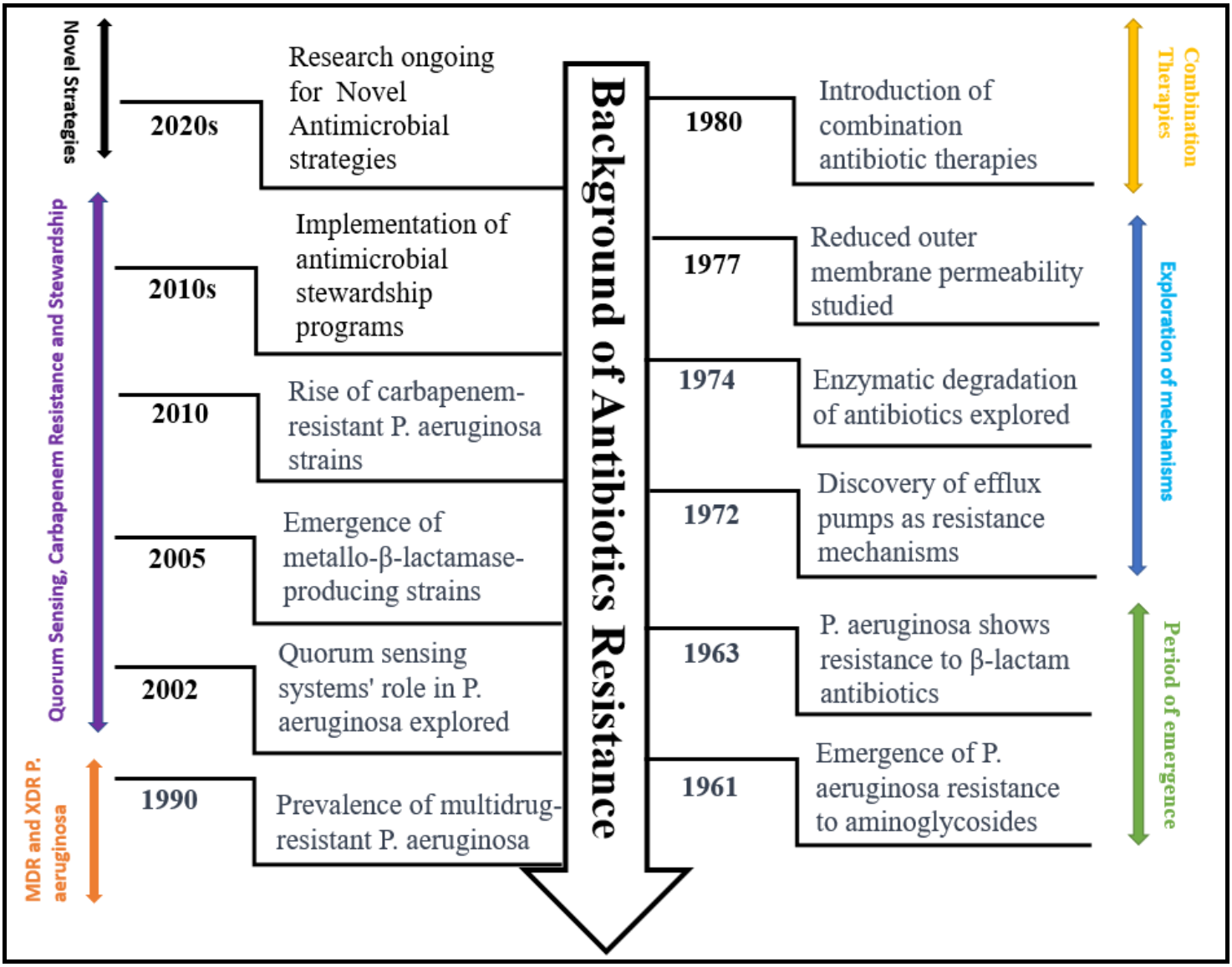

Figure 1. *A Diagram showing the timeline of Antibiotic Resistance. It typically starts with the introduction of antibiotics in the mid-20th century, followed by the emergence of resistance, the development of new antibiotics, and a continuous cycle of resistance escalation. This figure provides a visual representation of the historical progression of antibiotic resistance over time, highlighting key milestones and trends.*

In the 1940s and 1950s, *P. aeruginosa* was first isolated, identified, and commercialized. Since it can cause so many different illnesses and is so adaptable, it is considered a model microbe [15]. Especially in immunocompromised and chronically ill people, it causes a wide variety of infections, making it of clinical relevance. Its adaptability to genetic manipulation has made it a useful research tool in molecular biology and genetics. Scientists are actively looking for methods to overcome antibiotic resistance in P. aeruginosa, particularly its use of quorum-sensing to produce extra polymeric substances (EPS) that protect it from antibiotics. CRISPR and RNA-based treatments provide inspiration for the creation of new antibiotics, which are now being investigated. Scientific relevance can be shown in the improvement of phages, the primary engineers of CRISPR-Cas systems for precise gene editing in antibiotic-resistant bacteria. This review focuses on the potential of CRISPR-cas technology and RNA-based therapeutics used to combat AR, as well as the molecular mechanisms driving antibiotic resistance in Pseudomonas aeruginosa, the role of horizontal gene transfer in these processes, and how current strategies like membrane sensitizers, efflux pump inhibitors, phage therapy, and antimicrobial peptides, etc., use combination therapies.

Antibiotic Resistance in Pseudomonas

Because of its unique characteristics, *P. aeruginosa* can develop and maintain its resistance to antibiotics. They can develop this resistance in one of two ways: either naturally or by exposure to antibiotics [59]. (Figure 2)

Intrinsic resistance

Some features of the bacterial genome, such as a selectively permeable outer membrane that prevents antibiotics from entering the cell and efflux pumps that pump out antibiotics, biofilm formation, adaptive resistance mechanisms, mutation accumulation, and production of AmpC-Lactamase, contribute to the bacteria's innate resistance to antibiotics.

Efflux pump and membrane interplay

MexAB-OprM and MexXY-OprM are examples of efflux pumps from the Resistance-Nodulation-Division (RND) family that are located within the inner membrane of bacterial cells. Antibiotics are pumped out of the cell using energy from the proton motive force across the cell membrane, which is generated by the

molecular pumps that serve as molecular transporters [68]. MexA, MexB, and OprM are all part of the MexAB-OprM complex, which aids in the extrusion of several antibiotics. MexA and MexB combine to generate a multi-membrane complex. The intracellular concentration of antibiotics is reduced by efflux, which is extremely substrate-specific in *P. aeruginosa* and can make it difficult for medicines to reach their target locations and acquire the necessary therapeutic levels. In contrast, the exclusion of plasma membranes is largely dependent on their selectively permeable membrane. Loss or alteration of outer membrane porin proteins, such as OprD, lowers the uptake of particular antibiotics because they function as channels for specific antibiotic compounds to enter the periplasmic region. Overexpressed or properly functioning efflux pumps can "capture" antibiotics that have passed through porin channels in the outer membrane. By recapturing antibiotics, efflux pumps limit antibiotics' time inside the bacterial cell and hence the antibiotics' ability to reach their intracellular targets [27].

Adaptive resistance mechanisms and mutation

Quorum sensing (QS) and two-component systems (TCSs) are examples of global regulatory systems that keep it under control. Biofilm formation is controlled by the expression of virulence factors and antibiotic resistance genes, which is regulated by quorum sensing through the creation and sensing of signaling molecules such as acyl-homoserine lactones (AHLs). AHLs are created by bacteria and then released into the environment, where they spread quickly. LasR and RhlR, two examples of the LuxR family of transcriptional regulators, play critical roles in the QS system. As the biofilm grows larger due to cell division, the concentration of AHL rises, whereas, in a low-density cell, it remains below the threshold required for activation of the LuxR-type receptors [61].

When AHL molecules connect to LuxR-type receptors like LasR and RhlR, the receptors undergo structural alterations that enable them to function as transcription factors. Biofilm generation and LPS secretion are both boosted when the LasR-AHL and RhlR-AHL complexes bind to the promoter regions of the respective target genes.

Target-based resistance occurs when antibiotic-target genes (such as those encoding DNA gyrase and topoisomerase IV) accumulate mutations. During DNA replication, repair, and transcription, the enzymes DNA gyrase and topoisomerase IV play important roles in maintaining DNA's integrity and structure [1]. Mutations in the genes for DNA gyrase (gyrA and gyrB) and topoisomerase IV (parC and parE) are caused by several antibiotics, including fluoroquinolones (e.g., ciprofloxacin) and some quinolones (e.g., levofloxacin). Because of this, antibiotics may lose some of their binding potency. Genes like gyrA and gyrB code for the DNA gyrase component. Resistance to fluoroquinolone drugs like ciprofloxacin is typically linked to mutations in the gyrA gene, specifically in the quinolone resistance-determining region (QRDR). Resistance can also be caused by less common mutations in gyrB. Both parC and parE are genes that encode topoisomerase IV, therefore mutations in either can result in resistance to antibiotics that target the enzyme. This is especially true for mutations in parC's QRDR.

Acquired antibiotic resistance

Mutagenesis, horizontal gene transfer (HGT), metallo--lactamases (MBLs), small colony variants (SCVs), and horizontal gene transfer (HGT) are just a few of the processes by which antimicrobial drugs acquire resistance over time. These genes from the outside world are transported by mobile genetic elements like plasmids or transposons, as opposed to the intrinsic resistance that originates from within the cell itself [23].

Metallo-β-Lactamases

Antibiotics lose their efficacy against bacterial infection because these enzymes use the zinc ions in their active sites to catalyze the activation of a water molecule, which hydrolyzes and opens the -lactam ring. Due of their prevalence in Gram-negative infections, MBLs have garnered attention. Mobile genetic cassettes within integrons or bracketed by composite transposons facilitate the extensive distribution of MBLs, which are encoded on chromosomes or plasmids. Based on zinc coordination and amino acid sequence variations, MBLs are divided into subclasses B1, B2, and B3. In particular, subclass B1 is of clinical importance and is well-known for its propensity to transmit on plasmids, making it a serious concern in terms of producing infections resistant to antibiotics [55].

Horizontal Gene Transfer (HGT)

Antibiotic resistance and genetic variation among acquired features during GHT. Synergistic effects, adaption to novel niches, cooperation, and social activities like extracellular enzyme synthesis or the creation of biofilms can all help *P. aeruginosa* evolve quickly without relying on genetic mutation and natural selection. [34] describe the HGT process in terms of conjugation, transformation, and transduction.

Conjugation

Conjugants are the result of cellular interactions in this case. At the outset, the formation of the pilus is controlled by a set of genes called tra genes that are located in the transfer area. DNA processing and transfer necessitates the cleavage of plasmid DNA at the origin of transfer (oriT) site, which is accomplished by the relaxase enzyme encoded by tra genes. This pilus, comprised of pilin proteins encoded by the tra genes, initiates the contact and extends from the donor cell, attaching itself to the recipient cell. The enzyme relaxase forms a relaxosome complex by covalently binding to the 5' end of the DNA strand that has been nicked. Additional proteins, such as coupling proteins and those of the type IV secretion system (T4SS), work with this complex to deliver the DNA strand to the recipient cell. The tra genes code for a system called the type IV secretion system (T4SS), which creates channels for DNA strand translocation from the donor to the receiving cell [48].

Transformation

Competence is first activated in response to environmental cues such as nutrition deprivation, stress, or DNA fragmentation. Specific regulatory genes, known as com genes, involved in DNA processing are turned on by this activation. ComA, ComB, and ComC, all encoded by the com genes, are part of a complex that spans the outer membrane of the bacterial cell. This complex aids in the cell's ability to capture, digest, and import extracellular DNA. Enzymes called nucleases and recombinases are essential to this process because they degrade and recombine the foreign DNA with the cell's genetic material. This mingling of species has the potential to spread novel genes, such as those for resistance to antibiotics [41].

Transduction

The DNA of a bacteriophage is housed in a protein coat called the capsid, which is part of the bacteriophage's complex structure. Infecting Pseudomonas bacteria, these viruses can integrate their genetic material into the bacterial genome. During the lytic cycle of infection, any bacterial DNA can be packaged into the bacteriophage by a process called generalized transduction. The process of specialized transduction differs slightly. Temperate bacteriophages are a type of virus that integrates into bacterial DNA and, when it leaves, may unwittingly carry with it some of the host bacteria's genes.

The prophage may inadvertently eliminate neighboring bacterial genes in the process of excision from the bacterial genome. Therefore, when the removed phage infects a recipient bacterium, it transfers not only the phage DNA but also the surrounding bacterial genes. For the transferred DNA to be passed down reliably during transduction, an integration process involving recombination between the phage DNA and the recipient genome is necessary. The transferred genes are expressed and passed on to future generations after they are inserted into the recipient genome via recombination. CRISPR-Cas technology came to light through research on this process [34].

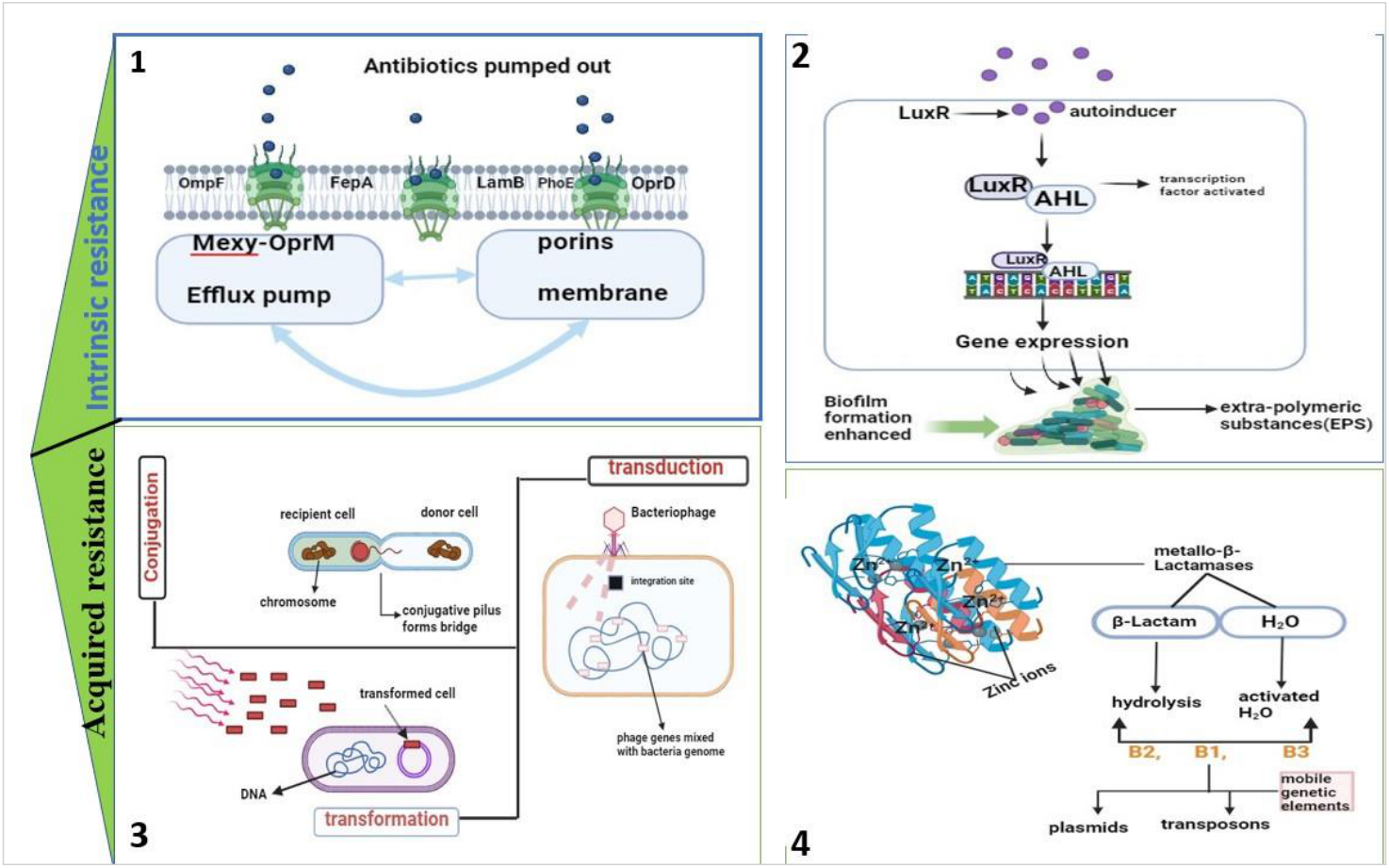

Figure 2. *Diagrams of Various Means Antibiotic Resistance in P. aeruginosa: 1. Efflux Pump-Mediated Resistance: Demonstrates how efflux pumps in the bacterial membrane, like porins and MexY-OprM, actively expel antibiotics, reducing their effectiveness. 2. Depicts the process of quorum sensing using LuxR and only AHL pathway is used in this process because P. aeruginosa is a gram-negative bacterium. This allows bacteria to coordinate resistance gene expression based on cell density. 3. Shows the mechanisms of horizontal gene transfer through conjugation, transformation, and transduction, which enable the exchange of antibiotic resistance genes between bacteria. 4. Highlights how Metallo beta-lactamases interact with genetic mobile elements, transposons, and plasmids, using essential ions in their structure, to facilitate the spread of antibiotic resistance genes within bacterial populations.*

The impact of biofilms and the host environment on horizontal gene transfer (Michaelis & Grohmann, 2023).

Bacterial cells in a biofilm release extracellular vehicles (EVs), which are tiny membrane-bound vesicles containing genetic material like ARGs. The host environment is critical for HGT to develop during an infection. By boosting the expression of genes involved in HGT, such as those encoding conjugative plasmids or integrative and conjugative elements (ICEs), the bacterial genome responds to the stress induced by the host immunological response and the presence of drugs. Another way in which antibiotics contribute to the transmission of antibiotic resistance is by creating selection pressure inside the host, thereby increasing the survival and proliferation of bacteria harboring ARGs, which in turn facilitates the transfer of ARGs to susceptible bacteria.

Factors influencing the rate and efficiency of HGT

Several factors, such as bacterial competence, environmental factors, genetic compatibility, selective pressure, the host's immune system, and Mobile genetic elements (MGEs), influence the rate and efficiency with which genetic material is transferred between bacteria.

Gene exchange between bacteria is often facilitated by mobile genetic elements (MGEs) like plasmids and transposable elements (TEs) and the genes they carry. A plasmid's ability to replicate autonomously and transfer across bacteria via conjugation is an example of MGE activity that affects the rate of HGT [23]. It's possible that MGEs' fitness goals and those of their hosts won't always coincide. Plasmids are a good example of a

cellular component that is under-selected to expand its copy quantity, even though doing so can have negative consequences for the host [27]. DNA restriction-modification systems and CRISPR-Cas loci, which detect and selectively destroy foreign DNA, including potentially useful MGEs are examples of mechanisms that impede HGT and are involved in the control of MGEs [38]. There are times when the host is susceptible to gene transfer and times when it is not, and this is due to fluctuations in the regulation of MGEs and how they interact with the host's immune system. The amount of DNA taken in by a competent cell is also affected by factors such as nutrition availability and stress.

Table 1. Mechanisms of Antibiotic Resistance Genes and Their Functional Roles with How and which antibiotics they resist in Pseudomonas aeruginosa

Gene Name & Functional Role	Resisted Antibiotics	Origin (Intrinsic/Acquired) & specific factor	Mode of Action & Sequences	References
mexA (Part of MexAB-OprM efflux pump)	Multiple	Acquired (mutation in MexR genes)	Efflux of antibiotics	[46]
mexB (Part of MexAB-OprM efflux pump)	multiple	Acquired (mutation)	Efflux of antibiotics via MexAB-OprM pump	[68]
mexR (Repressor of MexAB-OprM pump)	Multiple	Acquired (Mutation)	overexpression of efflux pump	[43]
oprD (Outer membrane porin)	Carbapenems	Intrinsic (Loss or reduced expression)	Reduced antibiotic permeability	[39]
fosA (Fosfomycin resistance)	Fosfomycin	Intrinsic (n/a)	FosA enzymatic activity inactivates fosfomycin	[58]
catB7 (Chloramphenicol resistance)	Chloramphenicol	Intrinsic (N/A)	CatB7 enzyme inactivates chloramphenicol	[5]
blaOXA-50 (Beta-lactam resistance)	Beta-lactam	Intrinsic	Production of β-lactamase, contributing to beta-lactam resistance	[26]
aac(3)-Ic (Aminoglycoside resistance)	Aminoglycosides (e.g., amikacin	Acquired (HGT)	Enzyme inactivates aminoglycosides	[16]
tet(G) (Tetracycline resistance)	Tetracycline	Acquired (HGT)	Ribosomal protection protein confers tetracycline resistance	[21]
cmlA1 (Phenicol resistance)	Phenicol	Acquired (HGT)	Enzyme inactivates phenicol antibiotics	[63]
rmtF (Aminoglycoside resistance)	Aminoglycosides	Acquired (HGT)	Encodes a 16S rRNA methylase, conferring aminoglycoside resistance	[12]
aadA6 (Aminoglycoside resistance)	Aminoglycosides	Acquired (HGT)	Enzyme inactivates aminoglycosides	[16]
rpsL-F (Ribosomal Protein)	Streptomycin	Acquired (Mutations)	Reduced binding of streptomycin	[59]
mexE/mexF (efflux pump)	Aminoglycosides	Acquired (over-expression of efflux genes	Active efflux of antibiotics	[32]
nfxB (Regulator Protein)	Various antibiotics	Acquired (mutation)	Upregulation of efflux pumps	[64]
oprM (outer membrane Porin)	Carbapenems	Intrinsic (reduced porin expression)	impaired antibiotic entry	[20]

Mechanisms of some genes that are involved in the resistance of more/most antibiotics

In this article, I define weight genes as those that serve several purposes and provide resistance to multiple drugs. The following are some of the unique qualities that contribute to their success:

I. mexA/mexB (Part of MexAB-OprM efflux pump): The proteins MexA and MexB are essential to this process. Pseudomonas aeruginosa's efflux pump, MexB, is part of a tripartite efflux system that also includes the periplasmic membrane MexA and the outer membrane OprM. Through this mechanism, bacteria are able to actively excrete a wide variety of drugs. MexB is an ATPase-encoding inner membrane protein, and MexA connects it to OprM in the periplasm. The outer membrane channel is formed by OprM. Antibiotic resistance is conferred by the MexAB-OprM pumping system, which expels antibiotics from the periplasm into the extracellular environment.

II. blaOXA-50 (Beta-lactam resistance): A beta-lactamase enzyme is encoded by this gene. Beta-lactamases are enzymes that break down the beta-lactam ring structure of penicillin and other cephalosporin medicines. cut the beta-lactam ring, neutralizing the medicines. In particular, BlaOXA-50 is effective against a wide variety of beta-lactam antibiotics.

III. tet(G) (Tetracycline resistance): A ribosomal protection protein is encoded by the tet(G) gene. Antibiotics like tetracycline can't damage the bacterial ribosome because of ribosomal protective proteins. tet(G) prevents tetracycline antibiotics from halting protein synthesis by attaching to the ribosome. In the presence of tetracycline, it works as a "bodyguard" for the ribosome, allowing the bacterium to keep making proteins.

IV. aac(3)-Ic (Aminoglycoside resistance): The aminoglycoside modifying enzyme aac(3)-Ic is encoded by the aac(3)-Ic gene and is capable of covalently altering aminoglycoside antibiotics. Aminoglycoside antibiotics are modified chemically by enzymes like acetyltransferase AAC(3)-Ic. The antibiotics are rendered ineffective because their capacity to bind to the target location in the bacterial ribosome has been diminished by these alterations.

V. Strategies for Combating Antibiotic Resistance

Combating antibiotic resistance requires a multipronged strategy. Antibiotic innovation and the use of metallo--lactamase (MBL) inhibitors are also important tactics. Two of these are increasing membrane permeability and employing efflux pump inhibitors (EPIs). An all-encompassing strategy is needed to effectively combat resistance, and this includes maximizing the efficacy of combination therapies, investigating novel approaches like phage therapy and CRISPR-Cas systems, performing constant genomic surveillance, creating targeted vaccines, and advocating for antibiotic stewardship. Here we describe strategies for combating efflux pumps and membranes, HGT, quorum sensing (QS), and metallo-lactamases [54].

CRISPR-Cas technology against HGT

This innovation equips bacteria with a sophisticated immune system that can recognize and eliminate a wide variety of foreign DNA including phages, plasmids, and integrative conjugative elements (ICE). Smaller genome size and higher GC content, both indicative of an active CRISPR-Cas system, have been linked to a decreased propensity for the acquisition of rogue DNA (Kunz, A. J. 2022). CRISPR-Cas spacers mostly target phages, although more than 80% of CRISPR-Cas-active isolates include spacers that target ICE or the conserved conjugative transfer machinery employed by plasmids and ICE. Conjugation is facilitated by CRISPR-Cas, which focuses on the Type VI Secretion System (T6SS). T6SS is a complicated machine that prevents the spread of plasmids and ICE by delivering toxic effectors into recipient cells. By eliminating prospective donor cells and stopping the transmission of mobile genetic material, the T6SS serves as a defensive mechanism against HGT. In addition, specific regions of T4SS genes can be targeted using improved gRNAs. The presence of a protospacer adjacent motif (PAM) sequence is essential for Cas proteins to identify and bind to the target DNA [52]. This is just one of several criteria that must be met when designing gRNAs. If we take the pilV gene as an example, we may build the gRNA sequence to target a specific area inside the gene. The gRNA sequence should contain the appropriate PAM sequence and be complementary to the target area. The sequence 5'-GTTGCTGCTGCTGCTGCTGG-3' (target sequence) + PAM (NGG) could be used as a template for a gRNA directed against the pilV gene. Together, this and the Cas9 nuclease can be utilized to target and sever the pilV gene.

The CRISPR-Cas systems can be activated during transformation to degrade the incoming foreign DNA by interfering with the ComA, ComB, and ComC complex, which is involved in the digestion of extracellular DNA. Transduction can be blocked by CRISPR-cas boosted spacers, which are anti-CRISPR protein spacers made by certain phages [62].

Genome reduction in bacteria, particularly Pseudomonas aeruginosa, has been linked to the presence of functional CRISPR-Cas systems. Since the acquisition of resistance genes through HGT contributes to the spread of antibiotic resistance in bacterial populations, the correlation between active CRISPR-Cas systems and smaller genome sizes, especially in the context of antibiotic resistance, suggests that CRISPR-Cas systems play a role in constraining HGT and limiting the acquisition of foreign DNA [65].

Small non-coding RNA (ribozyme) against antibiotic resistance

Pseudomonas aeruginosa RNAse P is an endogenous ribozyme that shows promise as a therapeutic agent [2]. This ribozyme may selectively target and cleave predetermined mRNA sequences. Antibiotic resistance pathways can be suppressed by RNAse P's ability to selectively target and degrade particular mRNA. Researchers have produced RNAse P nuclease-resistant analogs such as locked nucleic acids/DNA co-oligomers and phosphorodiamidate morpholino oligonucleotide EGSs coupled to permeabilizer peptides, expanding its utility even further. These analogs improve RNAse P's stability and make it easier to transport into Pseudomonas aeruginosa cells. Antibiotic resistance can be effectively combated by combining the sequence-specific mRNA degradation performed by RNAse P with the usage of nuclease-resistant analogs [44].

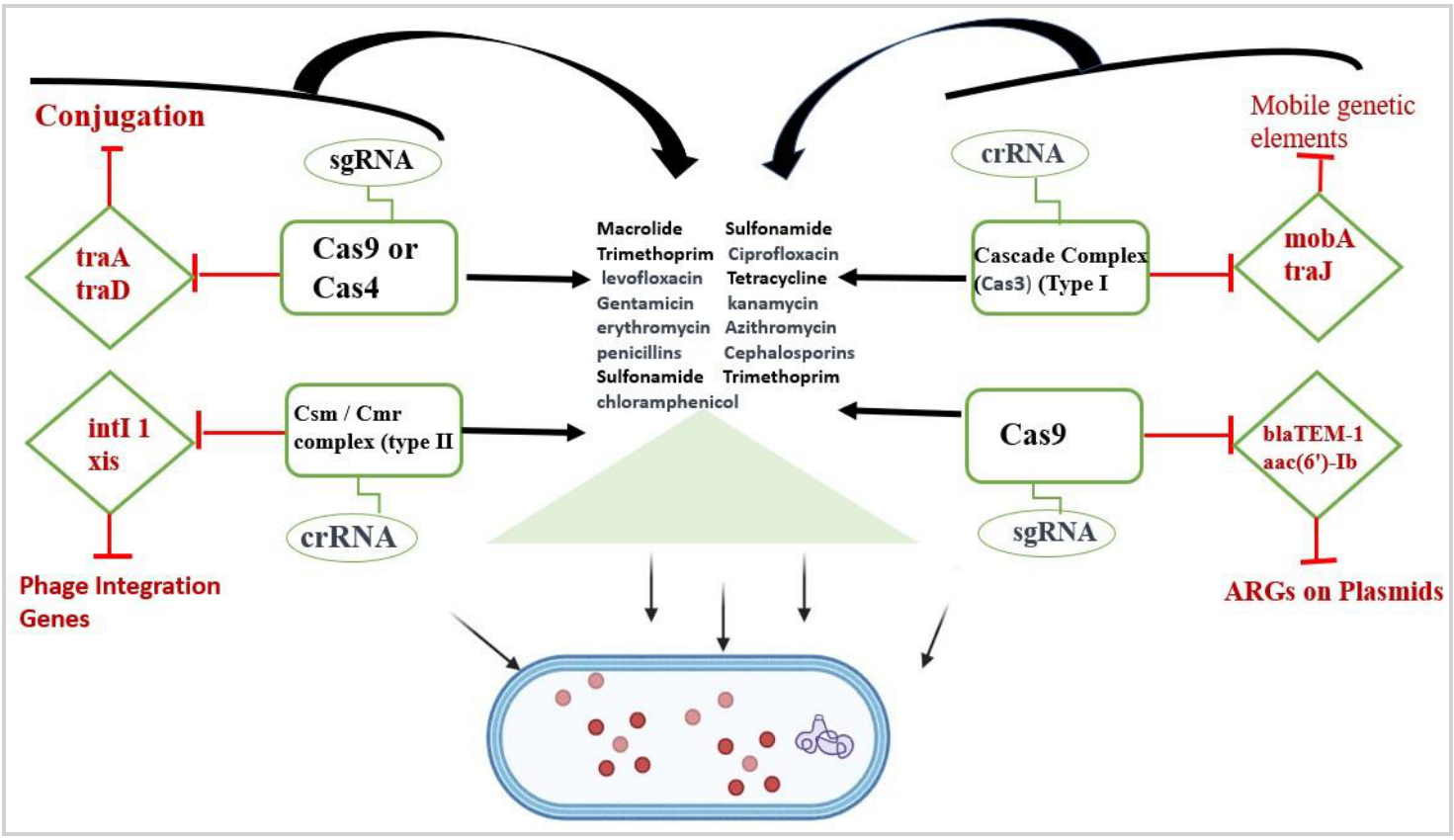

Figure 3*. CRISPR-cas technology-mediated augmentation of antibiotics through small guide RNA (sgRNA) and CRISPR RNA (crRNA) cleavage. sgRNA uses enhanced Cas9 or 4 to inhibit tra genes that are known to be promoters of conjugative genes, while CRISPR complex type II inhibits the intI xis genes that promote phage integration during transduction. On the other hand, crRNA uses enhanced type I, specifically cas 3 endonucleases to inhibit mobile genetic elements genes while sgRNA targets the blaTEM-1 acc (6')-Ib, inhibiting antibiotics resistance genes on plasmids.*

Future Prospects

Mobile genetic components like plasmids and phages tend to have a lower GC content than their bacterial hosts. The genome of *P. aeruginosa* has a GC content of between 65% and 67% [60]. The recent incorporation of these mobile elements into the host genome is indicated by the existence of genomic areas defined by reduced GC content [35]. An increased genomic GC content has been linked to CRISPR-Cas systems, leading to the hypothesis that this system can suppress HGT [66]. The role of sRNAs as post-transcriptional regulators in regulating the spread of mobile genetic elements is the subject of active research. In addition, scientists are looking into ways to tailor such proteins to interfere with the function of certain genes known to have a role in HGT. For instance, Cas9 can be modified to hone in on and cleave mobile element-carried genes that code for antibiotic resistance or virulence factors.

HGT frequently occurs by a process called conjugation, which involves a set of genes called tra genes. Targeting tra genes in plasmids or bacterial chromosomes is possible with CRISPR-Cas systems, notably Type I and Type II. Specific regions inside tra genes can be targeted for cleavage by Cas proteins. Cas9 is a member of the Type II CRISPR-Cas system that uses a guide RNA (sgRNA) to target a specific DNA sequence and create a double-strand break there. The targeted gene may become inactive as a result of this cleavage.

Conclusion

In this ongoing drama of antibiotic resistance, HGT's facilitation of antibiotic resistance adds a new component. Plasmids, transposons, and bacteriophages all play a role in the fast spread of genes encoding resistance mechanisms including metallo--lactamases (MBLs), ribosomal protective proteins, and modifying enzymes. The fight against antibiotic resistance is a top priority for the field of microbiology. Researchers are exploring novel methods, such as efflux pump inhibitors (EPIs), increasing membrane permeability, and taking advantage of phage therapy's potential. Several snoRNAs and the CRISPR-Cas9 system show potential for targeting and altering HGT-related genes. This strategy provides a glimpse of hope in the fight against resistance by interfering with the transfer of antibiotic-resistance genes in CRISPR-Cas techniques, which involve the construction of specialized guide RNAs. RNAse P and other ribozymes have been harnessed because of their capacity to selectively degrade mRNA involved with antibiotic resistance pathways, making them promising candidates for future therapeutic use. Their accuracy is very astounding. Antibiotic stewardship, targeted vaccine development, and genomic surveillance are also crucial parts of the defense. The potential of CRISPR-Cas systems to restrict HGT and the acquisition of foreign DNA is shown by the correlation between active CRISPR-Cas systems and smaller bacterial genomes.

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