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RESEARCH ARTICLE
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REVIEW ON EFFLUX PUMP BLOCKER FOR DRUG RESISTANCE BACTERIA

Biswayani Chakraborty[1*]

[1*]Integrated B.tech/M.tech Biotechnology Gautam Buddha University Uttar Pradesh, India
Email- cbiswayani31@gmail.com

***Corresponding Author:** Biswayani Chakraborty
[*]Integrated B.tech/M.tech Biotechnology Gautam Buddha University Uttar Pradesh, India
Email- cbiswayani31@gmail.com

Overview of Antibiotic Resistance

Antimicrobial Resistance (AMR) occurs when bacteria, viruses, fungi and parasites no longer respond to antimicrobial medicines. As a result of drug resistance, antibiotics and other antimicrobial medicines become ineffective and infections become difficult or impossible to treat, increasing the risk of disease spread, severe illness, disability and death.[1] Antibiotic resistance is a critical global health issue where bacteria evolve mechanisms to withstand the effects of antibiotics, rendering these drugs less effective or even useless. This resistance arises through natural selection, driven by the overuse and misuse of antibiotics in both healthcare and agriculture. Resistance mechanisms include modifying the antibiotic target, inactivating the antibiotic, and actively effluxing the drug out of the bacterial cell. The growing prevalence of resistant bacteria poses significant challenges to treating infections and necessitates urgent actions to manage and mitigate its impact.[2] Antibiotic resistance is a pressing global health concern where bacteria evolve to withstand the effects of antibiotics. This resistance can emerge through various mechanisms such as the alteration of antibiotic targets, enzymatic degradation of antibiotics, and efflux pumps that expel antibiotics out of the bacterial cell. The misuse and overuse of antibiotics in medicine and agriculture accelerate the development of resistance. This issue complicates the treatment of bacterial infections, leading to increased morbidity, mortality, and healthcare costs.[3]

Antibiotics are the “magic bullets” for fighting against bacteria and are thoughtout as ultimate unusual healing discovery of the 20th century. The installation of medicines has changed the healing example and resumes preserving millions of lives from bacterial contaminations. Antibiotics have certainly existed a godsend to humanity; they do not just have curative uses, but they have further been used in various purposes containing farming and animal production as deterrent measures in many undeveloped and underdeveloped countries for decades.[4]

With their ever-growing use and misuse, microorganisms have grown
antimicrobial fighting (AMR). The wonder of antimicrobial resistance refers to the potential of microorganisms containing microorganisms, viruses, fungi, and bootlickers to thrive and touch evolve among drugs planned to kill bureaucracy. Infections provoked by antimicrobial-opposing organisms are not only troublesome to treat, skilled is too always a raised chance of harsh ailment and even demise due to these contaminations. There are various types of antimicrobial powers including medicines, antifungal, antiviral, disinfectant, and cuisine preservatives that either restrain the growth and duplication of bacteria or destroy them. Antibiotics are a class of antimicrobials expressly used to combat bacterial contaminations and medicine opposition that is much often secondhand than some

other class of antimicrobials. AMR is a necessary metamorphic wonder shown by all animals through happening of ancestral mutations in consideration of safeguard the lethal excerpt pressure. To bear the tangible selection pressure, microorganisms seek to expand opposition against antibacterial drugs, interpretation these drugs useless.[5] With the always-increasing use of medicines, particularly in underdeveloped countries, microorganisms have ample freedom to evolve AMR accompanying profound results containing much taller morbidity and death. The occurrence and predominance of antimicrobialopposing-bacterial infections has accomplished absurd levels all along 21st century and warns all-encompassing community health as an understood pandemic, making necessary interferences. Antibiotic resistance can occur in some country and can influence one irrespective adult and feminine. With allure current scenario, AMR is individual of the supreme warnings not only to allencompassing health but further to feed safety.[6] Evolution and dissemination of AMR is together troubled by a tremendous number of interdependent determinants had connection with healthcare and farming. In addition, it can too be affected by determinants donating from pharmaceuticals, unfit waste management, business, and finance, founding AMR all at once of ultimate intricate community health concerns general. With the fast global spread of "superbugs" (microorganisms that are opposing to most famous antimicrobials), the position of drug-opposing pathogens has attained a palpable and disquieting rank. AMR has been recognized expected individual among the top three main community health dangers apiece World Health Organization (WHO).

Antimicrobial-resistant contamination has happened ordered third as the chief cause of passing afterwards cardiovascular afflictions. An estimated 1.27 heap afterlife were unpaid to antimicrobial-resistant contaminations in 2019 unique, while almost 5 heap deaths were anyway guide drug-opposing infections, in accordance with a important study written in January 2022. This number is estimated expected raised to 10,000,000 occurring by 2050, considerably exceeding extermination from tumor. As a familiar example of first "superbug", methicillin-opposing Staphylococcus aureus (MRSA) is guide a extreme victim from antimicrobial-resistant contaminations across the earth.[7] Currently, 3.5% of alive TB and 18% of previously considered TB cases concern MDR-TB (multidrugopposing infection) worldwide and skilled is a increasing concern for XDR-TB (widely drug-resistant infection) between many MDR-TB cases.[8] Although medicines are essential in combating bacterial contaminations, their misuse and abuse accompanying unfit quantity and duration over the decades have happened in collection pressure accompanying the emergence of opposing microorganisms. Apart from human healthcare, the rise and spread of AMR from the unsystematic use of antimicrobials in livestock feed in many underdeveloped countries has existed a main contributing determinant. It makes necessary raised following on the impact of excessive and deregulated use of medicines in animal feeds to drop the incidence of drug-opposing microorganisms.[9] Antibiotic opposition can have an affect human health in agreements of two together healing and preventive results. The healing suggestion is direct and seen through situation collapse and complexities, while deterrent implications are visualized through compromise of situation alternatives for immunosuppressive situations to a degree malignancy a destructive agent, state-of-the-art surgical procedures in the way that transplantation, and obtrusive processes such as intubation or catheterization. The current money in the incident of new artificial narrow and natural-amount-derivative fragments stand in sharp contrast accompanying an always-increasing demand of novel antimicrobials to treat life-threatening antimicrobial-opposing contaminations. Pharmaceutical behemoths have deserted their interest in antibiotic finding, established their own interpretation and have ceased growing their meaningful medicines stock since 1980s. Fluoroquinolone was additional in the group of the last general medicines discovery in the 1980s and was influenced to display in 1987. Since before, skilled has been a lack in the growth and various new antibiotic groups are in the passage. Use of medicines is twisted with the growth of opposition, meaning that fighting can be heavily weakened by preventing unnecessary devouring of medicines. Given the experience that antimicrobials are necessary tools to treat and avert catching ailments, it is now critical to continue the productiveness of now available antimicrobials because skilled has happened no significant finding of new fragments all the while recent decades.[10]

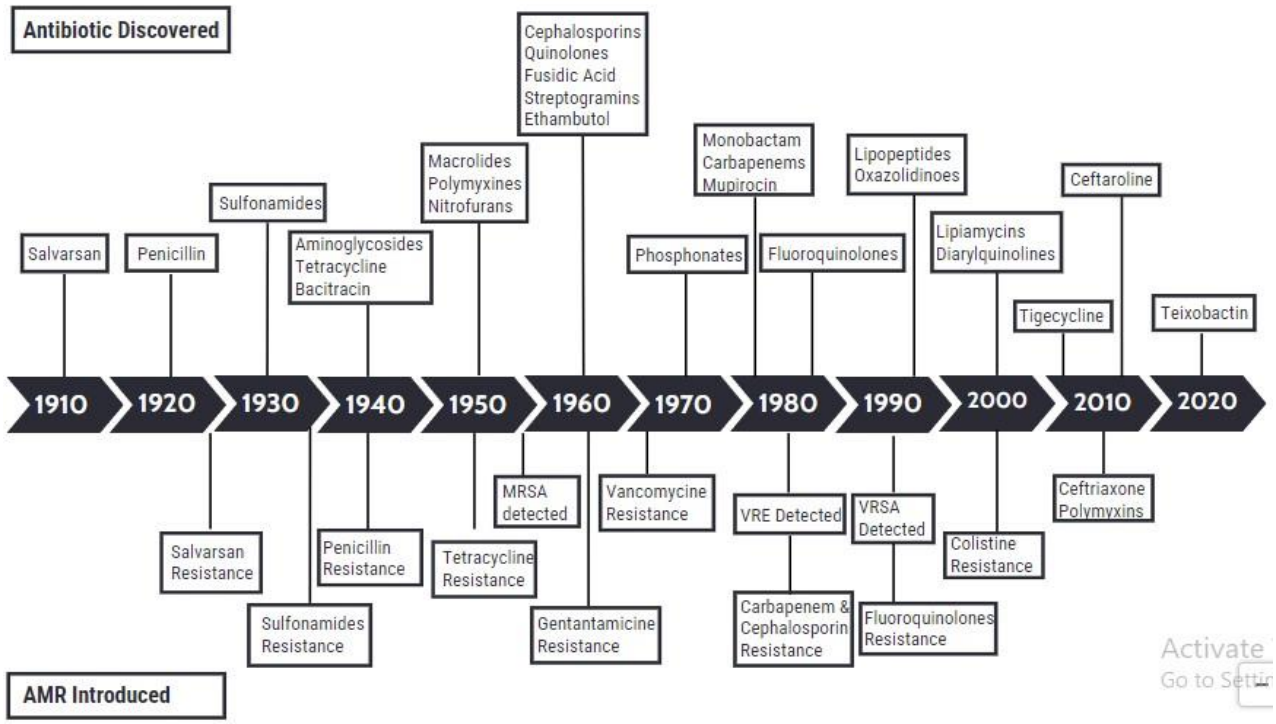

Figure 1: Timeline depicting Antibiotic discovered and AMR introduced from 1910-2020

Role of Efflux Pumps in Antibiotic Resistance

Efflux pumps are one of the primary mechanisms bacteria use to resist antibiotics.
These pumps actively transport antibiotics out of the bacterial cell, reducing the intracellular concentration of the drug to sub-lethal levels. Efflux pumps belong to several families, including the ATP-binding cassette (ABC) transporters and the major facilitator superfamily (MFS). They can be specific to a particular antibiotic or can expel a broad range of substances, contributing to multidrug resistance. Understanding the function and regulation of these pumps is crucial for developing strategies to combat antibiotic resistance.[11] Efflux pumps are crucial in bacterial antibiotic resistance. These membrane proteins actively transport antibiotics out of the bacterial cell, reducing the drug's intracellular concentration to sub-lethal levels. This mechanism is particularly concerning because it can provide resistance to multiple antibiotics simultaneously, contributing to multidrug resistance (MDR). For instance, the MexAB-OprM efflux pump in *Pseudomonas aeruginosa* is a well-documented example of such a resistance mechanism.[12]

Importance of Efflux Pump Inhibitors

Efflux pump inhibitors (EPIs) are compounds that inhibit the action of efflux pumps, thereby increasing the intracellular concentration of antibiotics and restoring their efficacy against resistant bacterial strains. EPIs are essential in combating antibiotic resistance because they can enhance the effectiveness of existing antibiotics and help in the treatment of infections caused by MDR bacteria. Developing EPIs is a significant strategy to manage and mitigate the impact of antibiotic resistance.[13]

Biology of Efflux pump

Structure and Classification of Efflux Pumps

Resistance-Nodulation-Cell Division (RND): These are primarily found in Gramnegative bacteria and form tripartite structures spanning the inner and outer membranes. A notable example is the AcrAB-TolC pump in *E. coli.*

Major Facilitator Superfamily (MFS): These pumps, found in both Gram-positive and Gram-negative bacteria, use a proton motive force to transport substrates. EmrD in *E. coli* is a representative member.

ATP-Binding Cassette (ABC): These pumps utilize ATP hydrolysis to transport substrates across membranes, with P-glycoprotein in humans being a well-known example.
Multidrug and Toxic Compound Extrusion (MATE): Typically utilizing a sodium ion gradient, these pumps expel drugs and other toxic compounds. NorM in *Vibrio parahaemolyticus* is an example.
Small Multidrug Resistance (SMR): The smallest efflux pumps, which use a proton gradient to export drugs. EmrE in *E. coli* is an example.[14]

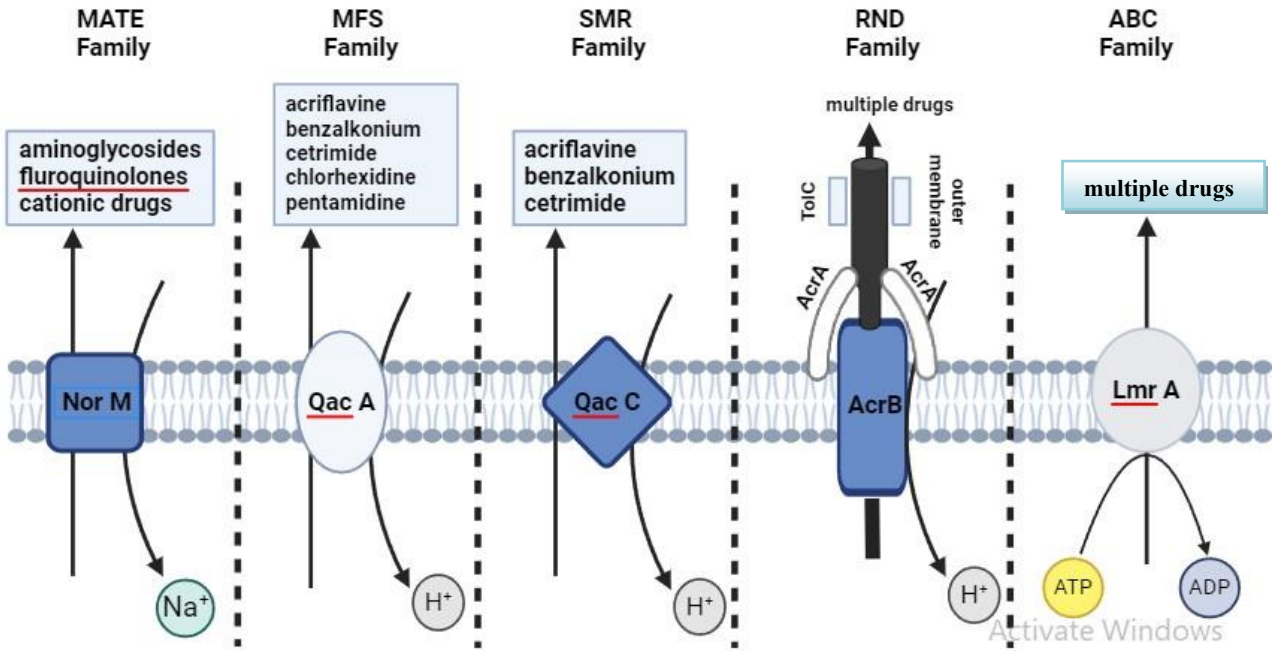

Figure 2: Schematic representation of the main types of bacterial efflux systems.

Regulation of Efflux Pump

1. Transcriptional Regulation

Efflux pumps in bacteria are regulated at the transcriptional level by various mechanisms, including local repressors, global regulators, and twocomponent systems. For instance, the AcrAB-TolC efflux pump in
Enterobacteriaceae is controlled by multiple transcriptional regulators, such as MarA, SoxS, and Rob, which respond to environmental stressors like antibiotics and oxidative stress.[7] Additionally, the MexAB-OprM efflux pump in Pseudomonas aeruginosa is regulated by the MexR repressor, which is sensitive to mutations that can lead to overexpression and increased antibiotic resistance.[15]

2. Post-Transcriptional Regulation

Post-transcriptional regulation of efflux pumps involves mechanisms that act on mRNA stability and translation efficiency. Small regulatory RNAs (sRNAs) play a crucial role in this process. They can bind to mRNA and either stabilize it or mark it for degradation. For example, the Hfq protein in Escherichia coli facilitates the interaction of sRNAs with target mRNAs, affecting the expression of efflux pumps like AcrAB-TolC.[16]

3. Environmental Factors Influencing Efflux Pump Expression

Environmental factors such as the presence of antibiotics, heavy metals, and biocides can induce the expression of efflux pumps. Bacteria often use two-component regulatory systems (TCS) to sense and respond to these environmental cues. For example, the CpxAR and BaeSR TCS in Salmonella spp. and Escherichia coli respond to envelope stress and activate the expression of efflux pumps, providing resistance to harmful compounds.[17]

Efflux Pumps and Multidrug Resistance (MDR)

Contribution of Efflux Pumps to Multidrug Resistance (MDR)

Efflux pumps are crucial elements in bacterial cells that contribute significantly to multidrug resistance (MDR). These pumps, which can extrude a wide range of substrates, including antibiotics, from bacterial cells, thereby lowering intracellular drug concentrations and enabling bacteria to survive otherwise lethal doses of antimicrobials. There are several families of efflux pumps, such as the resistance-nodulation-division (RND), small multidrug resistance (SMR), major facilitator superfamily (MFS), multidrug and toxic compound extrusion (MATE), and ATP-binding cassette (ABC) superfamilies.

Efflux pumps can contribute to intrinsic, acquired, and transient resistance. Intrinsic resistance arises from the basal expression of efflux pumps, while acquired resistance involves mutations leading to their overexpression. Transient resistance occurs in the presence of inducers, such as specific environmental conditions or substrates. The overexpression of these pumps can significantly impact bacterial pathogenicity by promoting biofilm formation, quorum sensing, and overall bacterial survival in hostile environments.[18]

Clinical Relevance of Efflux Pumps in Antibiotic Resistance

The clinical relevance of efflux pumps is profound, especially in the context of treating infections caused by multidrug-resistant (MDR) and extensively drugresistant (XDR) bacteria. In pathogens such as Pseudomonas aeruginosa, efflux pumps like MexAB-OprM, MexXY, MexCD-OprJ, and MexEF-OprN are well-studied for their role in resistance to multiple antibiotics. These pumps can export various classes of antibiotics, contributing to both MDR and XDR phenotypes.

Efflux pumps are not only involved in resistance to antibiotics but also in the regulation of virulence factors, such as adhesins and toxins, which are critical for bacterial colonization and infection. This dual role underscores the importance of efflux pumps in the persistence and pathogenicity of bacterial infections.

Targeting these pumps with specific inhibitors could enhance the efficacy of existing antibiotics and help in managing resistant infections more effectively.[19]

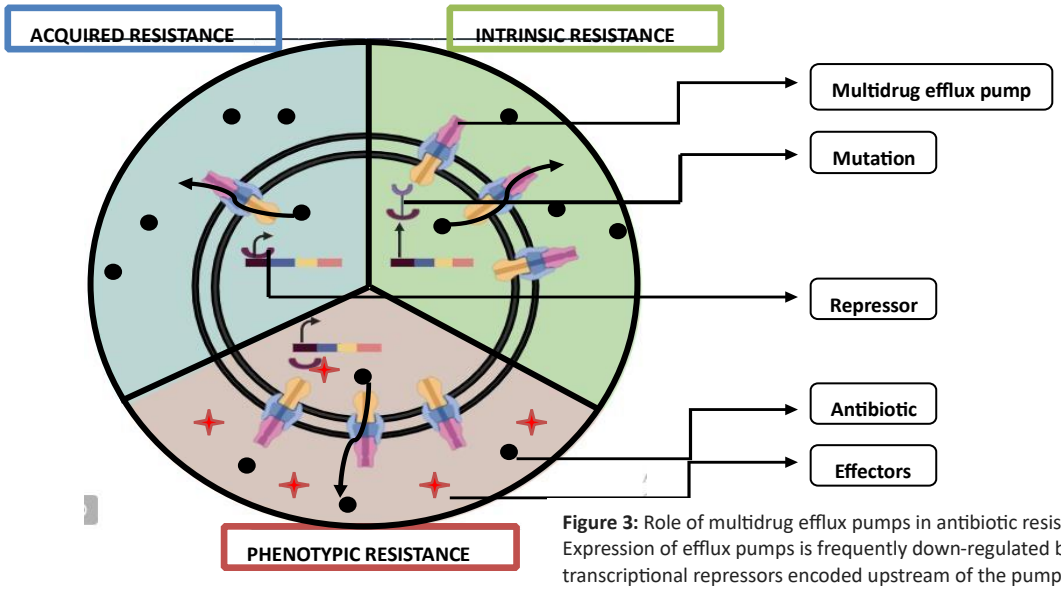

Figure 3: Role of multidrug efflux pumps in antibiotic resistance. Expression of efflux pumps is frequently down-regulated by transcriptional repressors encoded upstream of the pump operon.

Efflux Pump Inhibitors (EPIs)

Mechanisms of EPI action

Efflux pump inhibitors (EPIs) work by blocking the action of efflux pumps, which are proteins that bacteria use to expel antibiotics and other toxic substances. This blockage increases the intracellular

concentration of antibiotics, making them more effective. EPIs can inhibit efflux pumps through several mechanisms:
Competitive Inhibition: EPIs bind directly to the substrate-binding sites of the efflux pumps, preventing antibiotics from being expelled.

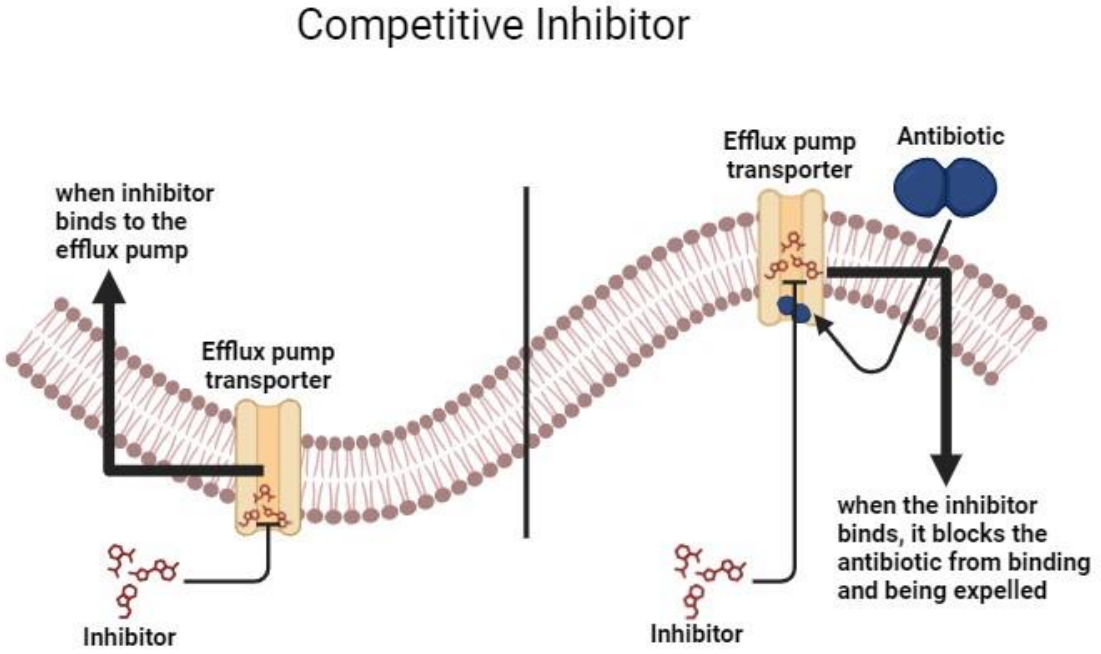

Figure 4: Competitive Inhibitor

Non-competitive Inhibition: EPIs bind to different parts of the efflux pumps, causing conformational changes that impair the pump's function.

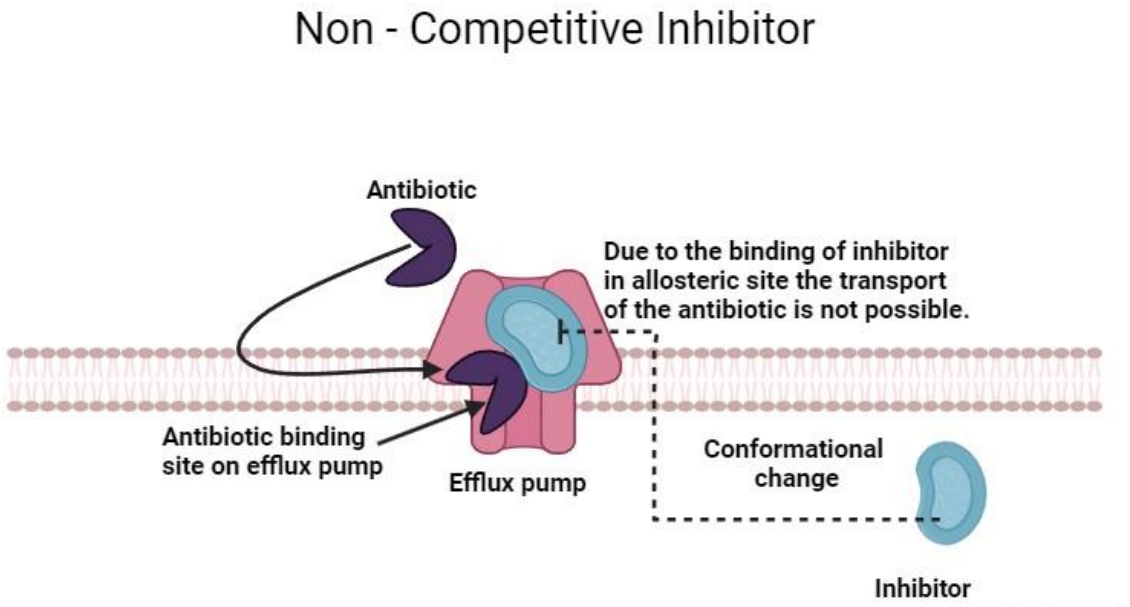

Figure 5: Non-Competitive Inhibitor

Blocking Energy Sources: Some EPIs disrupt the energy sources (such as ATP or proton motive force) required for the efflux pump's operation

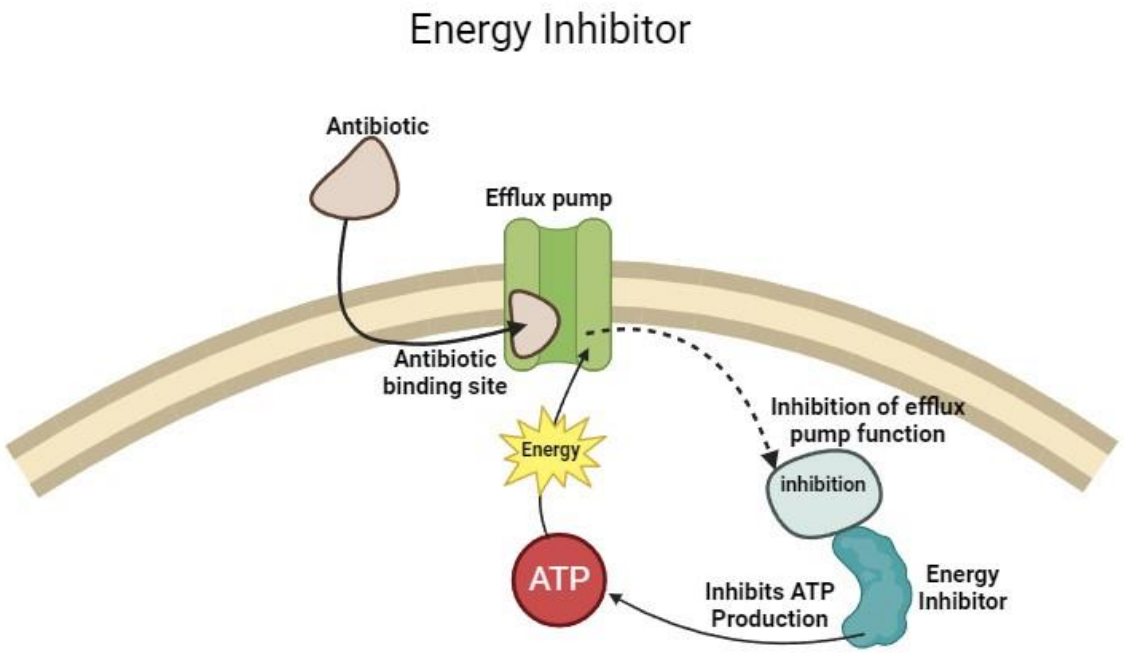

Figure 6: Blocking energy Inhibitor [20]

Classification and Types of EPIs

Efflux pump inhibitors are classified based on their chemical structure and mechanism of action. Some common types include:

Peptide-based Inhibitors: These mimic natural substrates of efflux pumps but do not get expelled, thereby blocking the pump.

Acridine Derivatives: These compounds interact with efflux pump proteins, altering their function.

Pyrrole Derivatives: These compounds interfere with efflux pumps, particularly those of the RND family, by inhibiting their transport capabilities.[21]

Examples of Successful EPIs and Their Efficacy

Several EPIs have shown promising results in reversing antibiotic resistance:

PAβN (Phenylalanine-Arginine Beta-Naphthylamide): Known for its potent efflux inhibitory ability, particularly against RND pumps, although its clinical use is limited due to nephrotoxicity.

NMP (1-(1-Naphthylmethyl)-Piperazine): Effective against various bacterial strains but has limitations due to side effects.

Pyrrole Derivatives: Recent studies have shown that certain pyrrole compounds significantly enhance the efficacy of antibiotics like tetracycline against E. coli and Pseudomonas aeruginosa. These compounds also exhibit anti-virulence properties, reducing the invasiveness and pathogenicity of these bacteria.[22]

Challenges and Limitations

Specificity and Selectivity of EPIs

EPIs need to be highly specific and selective to effectively inhibit bacterial efflux pumps without affecting similar human proteins. Achieving this specificity is challenging due to the structural similarities between bacterial and human transporters. Pyrrole-based EPIs, for instance, have been studied for their potential to selectively target bacterial efflux pumps such as AcrB in Escherichia coli without significant off-target effects on human cells. [23]

Toxicity Concerns

Many EPIs that show promising in vitro activity also exhibit significant toxicity in vivo, limiting their clinical application. Notable examples include phenylalanine arginine-β-naphthylamide (PAβN) and 1-(1-naphthylmethyl)-piperazine (NMP), which, despite their potent efflux inhibitory properties, are

associated with nephrotoxicity and other side effects. ocus on developing EPIs with reduced toxicity, such as certain diphenylmethane derivatives which have shown limited human-cell toxicity while effectively inhibiting efflux pumps in drug-resistant bacteria.[24]

Resistance Development Against EPIs

Bacteria can develop resistance to EPIs through various mechanisms, such as mutations in efflux pump genes or regulatory pathways. This resistance can diminish the efficacy of EPIs over time, similar to how bacteria develop resistance to antibiotics. Moreover, the broad substrate range of efflux pumps, particularly those of the resistance-nodulation-cell division (RND) family, complicates the development of EPIs that remain effective against diverse bacterial species and resistance mechanisms.[25]

Strategies for optimizing EPIs

Optimizing outflow pump inhibitors (EPIs) includes various strategies to reinforce their influence. Key approaches include guaranteeing mark specificity to bacterial outflow pumps, utilizing EPIs in combination accompanying medicines, and modifying their synthetic buildings for improved binding and balance. Highthroughput hide techniques can recognize new potential EPIs, while mechanistic studies and computational procedures like molecular tying up help enhance their interactions accompanying outflow pumps. Overcoming bacterial resistance devices, optimizing pharmacokinetic and pharmacodynamic characteristics, and employing artificial plant structure to engineer bacteria for EPI hide are again crucial. Additionally, biochemical assays to measure outflow tap activity in the ghost of EPIs supply valuable insights into their influence.

Conclusion

Efflux pumps are important for antibiotic resistance, actively transporting antibiotics out of cells and reducing their intracellular concentrations. This process not only inactivates antibiotics but also causes multidrug resistance (MDR). To address this problem, efflux pump inhibitors (EPIs) have been developed. These inhibitors increase the effectiveness of existing antibiotics by blocking the efflux pump, thus increasing intracellular antibiotic production. a variety of efflux pump series, such as RND, MFS, ABC, MATE and SMR, facilitate MDR by evacuating various products. EPI targets these pumps with a variety of strategies, including competitive and noncompetitive inhibition and blocking the energy required for pump operation. A However, the development and clinical use of EPIs are faced with challenges such as specificity and selectivity issues, toxicity issues, and potential resistance to EPIs. Despite these challenges, EPI is still a good strategy for controlling and reducing antibiotics with the potential to change the treatment of diseases caused by multidrug resistance.
Although efflux pumps pose serious problems in the fight against antibiotics, the development and implementation of strategies targeting EPIs offer solutions for the effective treatment of antibiotics and to cope with the threat of antibioticresistant bacteria.

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