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EFFLUX PUMPS IN THE INSURGENCE OF MDR CONCEIVABLY CURATIVE WITH EFFLUX PUMP INHIBITORS OF THERAPEUTICALLY ORIGIN FROM PLANT SOURCES

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ABSTRACT

With the appearance of antibiotics, bacterial infections have been speculated to be a factor of past. However, this rather brought about the selection and evolution of microorganism with mechanisms to counter the movement of antibiotics. Antibiotic efflux is one many of the principal mechanisms, wherein microorganism pump out the antibiotics from their cell indoors to the outside surroundings the use of unique transporter proteins referred to as efflux pumps. Inhibiting those pumps appears to be a stunning approach at a time whilst novel antibiotic materials are dwindling. Molecules able to be inhibiting those pumps, referred to as efflux pump inhibitors (EPIs), had been considered as capability healing marketers that may rejuvenate the interest of antibiotics which are not powerful towards bacterial EPIs comply with a few well-known mechanisms of efflux inhibition and are derived from numerous herbals additionally as artificial sources. This assessment makes a specialty of EPIs and identifies the demanding situations which have stored those futuristic therapeutics far far from the industrial realm so.

KEYWORDS: Antibiotic resistance, Efflux pumps, EPIs, Plant-derived EPIs.

INTRODUCTION

Antibiotic resistance is the ability of bacteria or other microbes to resist the consequences of an antibiotic. Antibiotic resistance occurs when bacteria change in how they reduce or eliminates the effectiveness of drugs, chemicals, or other agents designed to cure or prevent infections. The bacteria survive and still multiply causing more harm.[1] Bacteria can do this through several mechanisms. Some bacteria develop the facility to neutralize the antibiotic before it can do harm, others can rapidly pump the antibiotic out, and still, others can change the antibiotic attack site so it cannot affect the function of the bacteria. Antibiotics kill or inhibit the expansion of susceptible bacteria. Sometimes one among the bacteria survives because it can neutralize or escape the effect of the antibiotic; that one bacterium can then multiply and replace all the bacteria that were killed off. Exposure to antibiotics therefore provides selective pressure, which makes the surviving bacteria more likely to be resistant. In addition, bacteria that were at only one occasion susceptible to an antibiotic can acquire resistance through mutation of their genetic material or by acquiring pieces of DNA that code for the resistance properties from other bacteria.[1]

1.1 Antibiotic Resistant towards Bacteria

Disease-causing bacteria can cause illness. Viruses can also cause illness. Antibiotics are drugs used to treat bacterial infections. Viral infections do not respond to antibiotic treatment. Antibiotics are used for an extended time and are frequently prescribed. Because of this widespread use, the infectious bacteria the antibiotics were designed target have adapted and altered, making the drugs less effective. This is antibiotic resistance. Antibiotics aren't effective against viral infections like the cold, most sore throats, and therefore the flu. Using antibiotics once they aren't needed contributes to antibiotic resistance and unwanted side effects.

The more antibiotics are used, the more resistant the bacteria can become because sensitive bacteria are killed, but stronger germs resist the treatment and grow and multiply. Repeated and improper use of antibiotics contributes to the present process. The Food and Drug Administration (FDA) states antibiotic resistance may be

a common public ill health because nearly all kinds of bacteria became stronger and fewer aware of antibiotic treatment. Antibiotic resistance in children and older adults are of concern thanks to high rates of antibiotic use. Once a specific bacterium becomes immune to an antibiotic, treating that infection becomes harder and, in some cases, medically impossible. Untreated, bacterial infections can spread rapidly.[1]

1.2 Prevention of Drug resistant infections

Do not take antibiotics for viral infections. Finished your prescribed course of treatment exactly as directed by your healthcare provider. don't stop taking your medicine albeit you are feeling better, and don't save any antibiotics for future use. don't take someone else's antibiotics because different sorts of antibiotics treat different types of bacterial infections.[1]

Mechanisms of resistance associated with antibiotic reported in bacteria are causing a worldwide unhealthiness. In MDR bacteria, the excessive expression of efflux pumps plays a big part in the reduced susceptibility by decreasing the intracellular concentration of antibiotics.[2] Efflux pumps are transport proteins involved within the extrusion of toxic compounds like antibiotics and present in both sorts of bacteria i.e., Gram-positive and Gram-negative, and even in some eukaryotic cells.[3] Functional efflux may be a long-spread process for bacterial resistance to antibiotics, which contributes to low intrinsic susceptibility and cross-resistance to structurally diverse classes of drugs.[4] Functional efflux is a wide-spread process for bacterial resistance to antibiotics, which contributes to poor intrinsic susceptibility and cross-resistance to structurally diverse classes of medicine. Efflux is the process in which bacteria transport compounds outside the cell wall which are potentially toxic in nature, such as drugs or chemicals.[5] Many of the efflux systems in bacteria are non-drug-specific proteins and can identify and extract out a wide range of chemically and physically unrelated compounds from bacteria in an energy-dependent mechanism.[6] Because of their overwhelming presence in pathogenic bacteria, these active multi-drug efflux mechanisms are a serious area of intense study, in order that measures could also be discovered to inhibit these active multi-drug efflux pumps.[7]

Efflux pumps show their strongest innate and acquired antibiotic resistance in Gram-negative bacteria, as this is attributed to the combined effect of the trans envelope efflux and reduced uptake across the outer membrane.[8] To fight drug resistance three methods are often employed First is the primary approach to satisfy this example is that the development of the latest antibiotics. Second is an alternate therapy to treat antibiotic-resistant microorganisms is the use of plant extracts. The third is high level acquired resistance to standard antibiotics is frequent, it's reasonable to use combination therapy to realize bactericidal synergism. Plants derived antimicrobials are found to be synergistic enhancers. Though they'll not have any antimicrobial properties individually, once they are taken concurrently with standard drugs, they enhance the effect of that drug.[9] Efflux pump inhibitors (EPIs) are particularly the substances that give many of promising approach shot in blocking the passage of efflux pumps. They are the molecules which interfere with the process of removing toxic substances and antibiotics from the bacterial cell. Efflux pump inhibitors act as adjuvant to potentiate the activities of conventional antibiotics by inhibiting them either competitively or non-competitively.[10] Therefore, an effort has been made during this review to enlist the synthetic and plant derived EPIs discovered till date against Gram negative and Gram-positive bacteria of human pathogenesis to the simplest of our knowledge.

In Gram-negative bacteria, many of the efflux pumps that contribute to resistance to most antibiotics are three component structures that traverse both inner membrane & outer membrane. This structural organization permits extrusion of substrates directly into the external medium bypassing the periplasmic space and makes efflux pumps more efficient.[11-12]

There are many modes of action of efflux pumps: I) The EPI may bind directly to the pump in a competitive or noncompetitive manner with the substrate, causing the blocking of the efflux pump; II) EPI can also cause a depletion of energy, through the inhibition of the binding of ATP or the disturbance of the proton gradient across the membrane; III) EPI may have affinity for substrates, and bind them, forming a posh that facilitates the entry of the drug within the cell and prevents its efflux.[13-14] The utilization of efflux pump inhibitors can easily facilitate the re-introduction of therapeutically ineffective antibiotics back into clinical use and might even suppress the emergence of MDR strains.[15]

1.3 Efflux Pumps Presents in Bacteria

There are five super families of efflux pumps found in microorganisms, these are, ATP –binding cassette superfamily (ABC), major facilitator super family (MFS), resistance – nodulation cell division superfamily (RND), small multidrug resistance family (SMR), multi-antimicrobial extrusion protein family (MATE).[16] Resistance nodulation division (RND) transporters are most frequently found in gram-negative bacteria and are known to export a variety of antimicrobial agents.[17-18] They transport a wide variety of substrates including antibiotics, detergents, dyes, and host-derived molecules from the periplasm to the extracellular space 18. The conventional methods for detection of efflux pumps are costly affairs and time-consuming, also the use of radioactive substances is dangerous and biohazardous. Hence there is a need to develop a fast and cost-effective method for detecting efflux pumps in efflux mediated multi-drug resistant (MDR) bacteria (19-20). The Ethidium bromide (Et Br) agar cartwheel assay is a freshly discovered easy, free from instruments, safe, and

effective in cost process utilized for the demonstration of efflux pump activity in bacteria.[21]

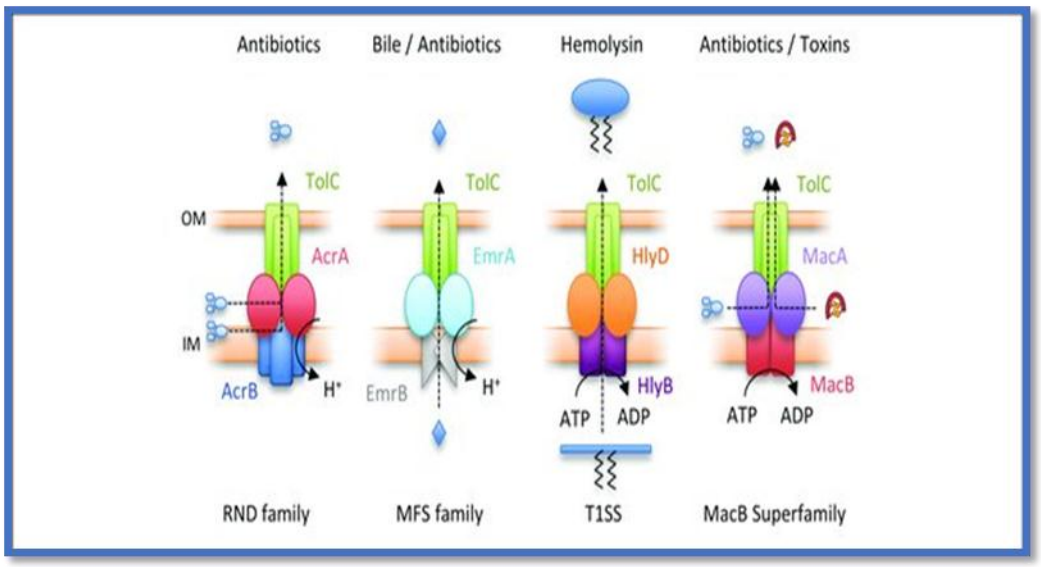

Figure 1: Different classes of efflux pumps in Bacteria.

Although many molecules have shown potential as EPIs, the mechanism of action is not known for many of them. Therefore, it becomes not easy to fit such molecules in a classification scheme based on their mode of action. To put up EPIs with no express mode of action, the EPIs can be categorized based on their source. This leads to three broad categories that include EPIs derived from plant products, synthetic chemistry, and microorganisms.

Table 1: Efflux Pump of Pathogens.[22,23]

Nature of substrate	Efflux pump family	Bactria containing efflux pump	Antibiotics used
Lipophilic, multicationic Substrates	SMR	Staphylococcus aureus & Acinetobacter baumannii	Tetracycline, erythromycin, sulfadiazine
Aphiphilic, charged substrates	RND	Escherichia coli & Pseudomonas aeruginosa	Tetracycline, fluoroquinolone, erythromycin, rifampicin, β- lactam, fusidic acid, chloramphenicol, aminoglycosides
Amphiphilic, mono or dicationic substrates	MFS	Staphylococcus aureus & Escherichia coli	Tetracycline, fluoroquinolone, erythromycin, lincosamides, rifampicin, pristinamycin, chloramphenicol, aminoglycosides
Amphiphilic neutral or cationic substrates	ABC	Staphylococcus aureus & Lactococcus lactis	Teracycline, fluoroquinolone, macrolids, lincosamides, rifanpicin, chloramphenicol, aminoglycosides
Low molecular weight Cationic substrates	MATE	Staphylococcus aureus, Escherichia coli & Vibrio parahaemolyticus	Norfloxacin, Fluoroquinolone, amioglycosides

1.4 Plant derived EPI's

Plant-derived phytochemicals comprise a wide variety of chemical adjuvants that synergistically stimulate the effectiveness of antibiotics up to several folds. Major subclasses of plant-derived EPIs are listed as follows.[24] Plant alkaloids: Reserpine, an antipsychotic drug extracted from the roots of Rauwolfia serpentina, is a promising EPI that targets efflux pumps of the MFS and RND superfamily.[24] Reserpine is reported to potentiate the antimicrobial activity of antibiotics by interacting directly with amino acid residues in the efflux transporter protein Bmr, which mediates tetracycline efflux in B. subtilis. In addition, reserpine has also been shown to reverse NorA-mediated resistance in S. aureus by enhancing the activity of norfloxacin up to four-fold.[25] The clinical application of reserpine with clinically used antibiotics, however, has not yet been achieved thanks to its nephrotoxic nature.[26]

a) Piperine (isolated from Piper nigram) is another alkaloid known to inhibit the human P-glycoprotein of ABC transporters via cytochrome P450-mediated pathways. The efflux pump inhibitory activity of both piperine and its derivative, piperidine, has also been

announced in opposition to pathogenic bacteria including S. aureus and Mycobacteria spp.[27] A study conducted in S. aureus showed that piperine enhances the buildup of ciprofloxacin by inhibiting NorA efflux pump. In M. tuberculosis H37Rv and several clinical isolates, piperine has been reported to potentiate the activity of rifampicin by inhibiting an uncharacterized efflux pump – Rv1258c. In Mycobacterium smegmatis, piperine has been shown to decrease the MIC of ethidium bromide indicating its application as an EPI across bacterial genera.[28]

b) Flavonoids: Baicalein, a 5,6,7-tri-hydro flavone, maybe a less strong antimicrobial flavone isolated from thyme leaves (Thymus vulgaris). It improves the susceptibility of clinical MRSA strain towards ciprofloxacin and β-lactam antibiotics including oxacillin, cefmetazole, and ampicillin.[29,30] Baicalein is additionally reported to extend the potency of tetracycline in TetK-overexpressing Staphylococci by inhibiting the uptake of [H] tetracycline.[30]

c) 5'-methoxy-hydnocarpin, a flavolignan isolated from Berberis fremontii, has been announced to reinforce the effectiveness of several NorA substrates, including norfloxacin and berberine by inhibiting this proton pump. However, thanks to its toxic nature, its clinical success is doubtful.[31] a number of the opposite plant-derived isoflavones (isolated from Lupinus argenteus) including genistein, orobol and biochanin A, are reported to scale back the MIC of berberine and norfloxacin in clinical S. aureus and M. smegmatis by blocking the MDR efflux pumps.[32]

d) Polyphenols: Catechin gallates, a gaggle of phenolic metabolites, are reported to reverse the MRSA resistance. Catechin gallates like epicatechin gallate and epigallocatechin gallate are weak inhibitors of NorA efflux pump, with epicatechin gallate being slightly stronger. Interestingly, both compounds are reported to reinforce the efflux at low concentrations.[33] it's been proposed that these molecules have two different binding sites on the NorA efflux transporter with different affinities. At low concentrations, catechins occupy high-affinity binding sites resulting in the increased efflux of NorA substrate. Their effect as EPI is observed only at a better concentration. Epigallocatechin gallate has also been reported to reinforce the potency of tetracycline, erythromycin and ciprofloxacin in TetK-overexpressing Gram-positive Staphylococci and in Gram-negative Campylobacter spp. However, thanks to toxicity concerns related to it, further in vivo and preclinical studies weren't undertaken.[34]

e) Phenolic diterpenes: Phenolic diterpenes, such as carnosic acid and carnosol, isolated from the herb Rosemary (Rosmarinus officinalis), have been reported as EPIs. These enhance the potency of antibiotics such as tetracycline and erythromycin against the macrolide-resistant strain of S. aureus expressing the ABC transporter MsrA and TetK efflux pumps.[35] Geraniol (monoterpenoid alcohol), isolated from Helichrysum italicum, has also been reported to modulate drug resistance in several GNB species by targeting MDR efflux mechanisms. It reduces the MIC of chloramphenicol in Enterobacter aerogenes CM-64 strain that overexpresses the tripartite efflux pump, AcrAB-TolC.[36]

1.5 EPIs of synthetic origin

Apart from natural plant-derived products, screening of novel semi-synthetic or synthetic diversified chemical libraries may be a useful thanks to identify potential EPIs. Many screening efforts have yielded results with varying amounts of success. Such synthetic small molecule EPIs can be further classified as follows:

a) Peptidomimetic compounds: The dipeptide amide compound PAβN was one among the primary EPIs discovered through a chemical genetics approach. It has been announced to potentiate the activity of the many antibiotics including fluoroquinolones, macrolides, and chloramphenicol in GNB by inhibiting RND efflux pumps.[37,38] However, it had limited clinical potential due to toxicity towards mammalian cells. Although some synthetic derivatives with different basic properties such as reduced toxicity, enhanced stability, and better solubility were evaluated, none of the active analogs could significantly reduce the drawback of the parent molecule. Thus, PAβN and its novel derivatives are limited to use in laboratories as standards to work out the extent of inhibitor-sensitive efflux for specific antibiotics in various bacterial pathogens.[39]

b) Quinoline derivatives: This new class of compounds was discovered by using many screening approaches against clinical MDR bacterial strains. Quinoline derivatives such as pyridoquinolones can restore the activity of norfloxacin in E. aerogenes overexpressing the AcrAB-TolC efflux pump, by acting as a competitive inhibitor of this RND pump.[40] Few other synthetic analogs like 4substituted thioalkyl, alkylamino and alkoxy quinolone have also been reported to reinforce the activity of tetracyclines, norfloxacin, and chloramphenicol in clinical isolates of K. pneumoniae and E. aerogenes.[41] A series of 2-phenyl-4(1H)-quinolone and 2-phenyl-4-hydroxyquinoline derivatives are synthesized by modifying the flavone scaffold and these are reported as potent inhibitors of NorA efflux pump in S. aureus.[42]

c) Arylpiperidines and aryl piperazine derivatives: Arylpiperidine and its derivatives like 3arylpiperidine are reported to revive susceptibility to linezolid and enhance its accumulation in E. coli.[43] Another series of analogs, phenylpiperidines, which are selective serotonin re-uptake inhibitors, are known to inhibit the function of S. aureus MDR efflux pumps. These compounds also affect the activity of the AcrAB-TolC pump in E. coli partially but have no effect on the efflux activity of the P. aeruginosa RND efflux pumps such as MexAB-OprM or MexCD-OprJ.[44] One of the leading arylpiperazine

compounds, NMP, has been shown to revive the activity of RND pump substrates including levofloxacin and EtBr in E. coli overexpressing AcrAB and AcrEF. However, due to serotonin re-uptake inhibitor property of arylpiperazines, these compounds are likely to be toxic to mammalian cells.[45]

d) Pyridopyrimidine and pyranopyridine derivatives: Pyridopyrimidine analogues D2 and D13-9001 have been reported as MexAB-OprM-specific pump inhibitor in MexABoverexpressing P. aeruginosa under both in vitro and in vivo conditions.[46] It has been proposed that D13-9001 is in a position to inhibit the efflux of antibiotics by binding to specific site in efflux pumps (AcrB in E. coli and MexB in P. aeruginosa). Further, the crystallographic data suggested that the hydrophobic tert-butyl thiazolyl aminocarboxyl moiety of D13-9001 binds tightly to the hydrophobic trap in deep substrate binding pocket of the pump and prevents the conformational changes that are needed for the right activity of the pump. In addition, the hydrophilic component of D13-9001 is additionally reported to interact with the substrate binding channel of pump, thereby preventing the substrate binding to the pumps.[39] MBX2319, a synthetic pyrazolopyridine, was screened as a potentiator of fluoroquinolones antibiotics from a library of small molecules. It enhances the efficacy of ciprofloxacin, levofloxacin and piperacillin up to eight-fold against *E. coli.*[47] Further, MBX2319 also led to increased intracellular accumulation of Hoechst dye in wild type and AcrAB-TolC-overexpressing *E. coli*. In addition, many synthetic/semisynthetic derivatives are synthesized artificially that mainly target MDR efflux pump of both GPB and GNB.[39]

1.6 EPIs derived from microbes

Although most of the EPIs have their origin in natural products or semi-synthetic/synthetic chemical libraries, a small fraction of EPIs has been reported to originate from microbes. EA-371α and EA-371d, first extracted from fermentation extract of Streptomyces spp., have been recognized as specific inhibitors of the MexAB-OprM pump in P. Aeruginosa.[48] The novel structure of these compounds offers an opportunity to the researchers to synthesize novel derivatives with increased potency, bioavailability and reduced toxicity. With the three-dimensional crystal structure of efflux pumps available, further computational studies could also be useful to identify the molecular interaction of these compounds with such MDR pumps.

CONCLUSION

Massive usage of antibiotics in clinical practice reported in resistance of bacteria to antimicrobial agents. Bacteria use innate and acquired resistance procedures to protect themselves. Acquired resistance raised from mutations, gene transfer by conjugation or transformation, transposons, integrons, and bacteriophages. The following biochemical types of resistance procedures are used by bacteria: antibiotic inactivation, target modifi cation, altered permeability, and “bypass” metabolic pathway.

Until this point, no efflux pump inhibitors has been authorized for use in the treatment of bacterial diseases in human or veterinary settings, although examination proceeds. In the treatment of bacterial malady cystic fibrosis, one medication improvement program including co-organization of an EPI with an anti-microbial operator has arrived at human clinical preliminaries. In this preliminary, an aerosolized detailing of the EPI compound MC-601, 205 is being joined with ciprofloxacin for the treatment of aspiratory intensifications in cystic fibrosis patients in a stage II preliminary being directed by Mpex Pharmaceuticals.[49] In this infection, the most genuine side effects are seen in lungs, expanding the danger of bacterial contamination of the microscopic organisms like B. Cepacia, P. aeruginosa and S. aureus.[50] Regardless of whether a look is given on the writing of optional metabolites of plants they additionally show action against Gram positive microscopic organisms and not against Gram negative microbes since Gram negative microorganisms have advanced successful hindrances for all amphipathic mixes (cationic, nonpartisan and anionic). In Gram negative microscopic organisms an extra external film is available which restrains the section of amphipathic mixes. While in Gram positive microbes just a solitary layer is available. So, the passage of amphipathic mixes is simple in Gram positive bacteria.[50] Therefore, there is an incredible need to investigate novel plant hotspots for EPIs against Gram-negative microscopic organisms.

Fastly emerging resistant bacteria warning the extraordinary health benefits that have been attained with antibiotics. This disaster is global, reflecting the worldwide overuse of these drugs and the absence of the development of noble antibiotic agents by pharmaceutical companies to address the challenging situation. Antibiotic-resistant infections place substantial health issues and economic crises on the Indian health care system and population. Coordinated efforts to implement new plans & policies, renew research efforts favoring, and pursue steps to manage and reduce the crisis are greatly needed Although most physician view antibiotics resistance as a serious problem, perceptions about its local importance, its causes and possible solutions may vary widely which helps in controlled prescriptions of these antibiotics

REFERENCES

1. Aviello G, AbenavoliL, BorrelliF, CapassoR, IzzoAA, LemboF, RomanoB, CapassoF. Garlic: empiricismorscience?.NatProdCommun, 2009; 4: 1785–1796.
2. Nikaido H, Pagès JM. Broad-specificity efflux pumps and their role in multidrug resistance of Gram-negative bacteria, FEMS Microbiology Reviews, 2012; 36: 340–63.

3. Bambeke FV, Balzi E, Tulkens PM. Antibiotic efflux pumps, Biochemical Pharmacology, 2000; 60: 457-70.
4. Bambeke FV, Pages JM, Lee VJ, Inhibitors of bacterial efflux pumps as adjuvants in antibacterial therapy & Diagnostic tools for detection of resistance by efflux, Frontiers in anti-infective drug discovery, 2010; 1: 138-75.
5. Ryan BM, Dougherty TJ, Beaulieu D, Chuang J, Dougherty BA, Barrett JF, Efflux in bacteria: what do we really know about it? Expert Opinion on Investigational Drugs, 2001; 10(8): 1409-22.
6. Kumar A, Schweizer HP, Bacterial resistance to antibiotics: Active efflux and reduced uptake, Adv Drug Deliv Rev, 2005; 57: 14861513.
7. Kumar S, Varela MF, Biochemistry of Bacterial Multidrug Efflux Pumps, Int J Mol Sci., 2012; 13: 4484-95.
8. Rouveix B, Clinical implications of multiple drug resistance efflux pumps of pathogenic bacteria, J Antimicrob Chemother, 2007; 59: 1208-09.
9. Kamatou GPP,Zyl RL, Vuuren SF, Viljoen AM, Figueiredo AC, Barroso JG, Pedro LG, Tilney PM, Chemical Composition, Leaf Trichome Types and Biological Activities of the Essential Oils of Four Related Salvia Species Indigenous to Southern Africa, J Ess Oil Res, 2006; 18: 72-79.
10. Lamers RP, Cavallari JF, Burrows LL, The Efflux Inhibitor Phenylalanine-Arginine Beta-Naphthylamide (PAβN) Permeabilizes the Outer Membrane of Gram-Negative Bacteria, PLoS ONE, 2013; 8(3): e60666.
11. Zgurskaya HI, Nikaido H, AcrA is a highly asymmetric protein capable of spanning the periplasm, J Mol Bio, 1999; 285: 409-420.
12. Zgurskaya HI, Nikaido H, Bypassing the periplasm: reconstitution of the AcrAB multidrug efflux pump of Escherichia coli, Proc Natl Acad Sci USA, 1999; 96: 7190-7195.
13. Zloh MG, Simon W, Glenn, Kaatz, Inhibitors of Multidrug Resistance (MDR) have Affinity for MDR Substrates, Bioorganic & Medicinal Chemistry Letters, 2004; 14: 881–885.
14. Marquez, Béatrice, Bacterial Efflux Systems and Efflux Pumps Inhibitors, Biochimie, 2005; 87: 1137–1147.
15. Stavri M, Piddock LJV, Gibbons S, Bacterial efflux pump inhibitors from natural sources, J Antimicrob Chemother, 2007; 59: 1247– 1260.
16. Ren Q, Paulsen IT, Comparative analyses of fundamental differences in membrane transport capabilities in prokaryotes and eukaryotes. PLoS Comput Biol, 2005; 3: 27.
17. Elkins CA, Nikado H, Substrate specificity of the RND type multidrug efflux pumps AcrB and AcrD of Escherichia coli is determined predominantly by two large periplasmic loops, Bacteriol, 2002; 184: 64906498.
18. Mao W, Warren MS, Black DS, Satou T, Murata T, Nishino T, Geton N, Lomovskaya O, On the mechanism of substrate specificity by resistance pumps: the large periplasmic loops of MexD from P. aeruginosa are involved in substrate recognition. Mol Microbiol, 2002; 46: 889-901.
19. DeMarco CE, Cushing LA, Frempong-Manso E, Seo SM, Jaravaza TAA, Diixa Patel, Christos Kosmidis, Susan MS, Glenn WK, Ethidium Bromide MIC Screening for Enhanced Efflux Pump Gene Expression Or Efflux Activity in Staphylococcus aureus, Antimicrob. Agents Chemother, 2010; 54(12): 5070-5073.
20. Martins M, Viveiros M, Couto I, Costa SS, Pacheco T, Fanning S, Pagès JM, Amaral L, Identification of efflux pump-mediated multidrug-resistant bacteria by the Ethidium Bromide Agar Cartwheel Method, In Vivo, 2011; 25: 171–178.
21. Martins M, McCuskin MP, Viveiros M, Couto I, Fannings S, Pages JM et al., Simple Method for Assessment of MDR Bacteria for Over –Expressed Efflux Pumps, Open Microbiol J, 2013; 7(1-M6): 72-82.
22. Omote H, Hiasa M, Matsumoto T, Otsuka M, Moriyama Y, The MATE proteins as fundamental transporters of metabolic and xenobiotic organic cations, Trends Pharmacol Sci, 2006; 27: 587– 593.
23. Ana CFV, Identification of Characterisation of Efflux Pumps in Rhodococcus erythropolis, Instituto Superior Tecnico, 2012; 1-71.
24. Stavri M, Piddock LJ, Gibbons S. Bacterial efflux pump inhibitors from natural sources. J Antimicrob Chemother, 2007; 59: 1247–60.
25. Gibbons S, Oluwatuyi M, Kaatz GW. A novel inhibitor of multidrug efflux pumps in Staphylococcus aureus. J Antimicrob Chemother, 2003; 51: 13–7.
26. Pfeifer HJ, Greenblatt DK, Koch-Wester J. Clinical toxicity of reserpine in hospitalized patients: A report from the Boston collaborative drug surveillance program. Am J Med Sci., 1976; 271: 269–76.
27. Kumar A, Khan IA, Koul S, Koul JL, Taneja SC, Ali I, et al. Novel structural analogues of piperine as inhibitors of the NorA efflux pump of Staphylococcus aureus. J Antimicrob Chemother, 2008; 61: 1270–6.
28. Sharma S, Kumar M, Sharma S, Nargotra A, Koul S, Khan IA. Piperine as an inhibitor of Rv1258c, a putative multidrug efflux pump of Mycobacterium tuberculosis. J Antimicrob Chemother, 2010; 65: 1694–701.
29. Chan BC, Ip M, Lau CB, Lui SL, Jolivalt C, Ganem-Elbaz C, et al. Synergistic effects of baicalein with ciprofloxacin against NorA over-expressed methicillin-resistant Staphylococcus aureus (MRSA) and inhibition of MRSA pyruvate kinase. J Ethnopharmacol, 2011; 137: 767–73.
30. Fujita M, Shiota S, Kuroda T, Hatano T, Yoshida T, Mizushima T, et al. Remarkable synergies between baicalein and tetracycline, and baicalein and beta-

lactams against methicillin-resistant Staphylococcus aureus. Microbiol Immunol, 2005; 49: 391–6.
31. Stermitz FR, Lorenz P, Tawara JN, Zenewicz LA, Lewis K. Synergy in a medicinal plant: Antimicrobial action of berberine potentiated by 5'-methoxyhydnocarpin, a multidrug pump inhibitor. Proc Natl Acad Sci., 2000; 97: 1433–7.
32. Morel C, Stermitz FR, Tegos G, Lewis K. Isoflavones as potentiators of antibacterial activity. J Agric Food Chem, 2003; 51: 5677–9.
33. Gibbons S, Moser E, Kaatz GW. Catechin gallates inhibit multidrug resistance (MDR) in Staphylococcus aureus. Planta Med, 2004; 70: 1240–2.
34. Sudano Roccaro A, Blanco AR, Giuliano F, Rusciano D, Enea V. Epigallocatechin-gallate enhances the activity of tetracycline in staphylococci by inhibiting its efflux from bacterial cells. Antimicrob Agents Chemother, 2004; 48: 1968–73.
35. Oluwatuyi M, Kaatz GW, Gibbons S. Antibacterial and resistance modifying activity of Rosmarinus officinalis. Phytochemistry, 2004; 65: 3249–54
36. Lorenzi V, Muselli A, Bernardini AF, Berti L, Pagès JM, Amaral L, et al. Geraniol restores antibiotic activities against multidrug-resistant isolates from Gram-negative species. Antimicrob Agents Chemother, 2009; 53: 2209–11.
37. Lomovskaya O, Warren MS, Lee A, Galazzo J, Fronko R, Lee M, et al. Identification and characterization of inhibitors of multidrug resistance efflux pumps in Pseudomonas aeruginosa: Novel agents for combination therapy. Antimicrob Agents Chemother, 2001; 45: 105–16.
38. Vargiu AV, Nikaido H. Multidrug binding properties of the AcrB efflux pump characterized by molecular dynamics simulations. Proc Natl Acad Sci., 2012; 109: 20637–42.
39. Opperman TJ, Nguyen ST. Recent advances toward a molecular mechanism of efflux pump inhibition. Front Microbiol, 2015; 6: 421.
40. Chevalier J, Atifi S, Eyraud A, Mahamoud A, Barbe J, Pagès JM. New pyridoquinoline derivatives as potential inhibitors of the fluoroquinolone efflux pump in resistant Enterobacter aerogenes strains. J Med Chem, 2001; 44: 4023–6.
41. Pradel E, Pagès JM. The AcrAB-tolC efflux pump contributes to multidrug resistance in the nosocomial pathogen Enterobacter aerogenes. Antimicrob Agents Chemother.
42. Sabatini S, Gosetto F, Manfroni G, Tabarrini O, Kaatz GW, Patel D, et al. Evolution from a natural flavones nucleus to obtain 2-(4-propoxyphenyl) quinoline derivatives as potent inhibitors of the S. aureus NorA efflux pump. J Med Chem, 2011; 54: 5722–36.
43. Thorarensen A, Presley-Bodnar AL, Marotti KR, Boyle TP, Heckaman CL, Bohanon MJ, et al. 3arylpiperidines as potentiators of existing antibacterial agents. Bioorg Med Chem Lett, 2001; 11: 1903– 6.
44. Kaatz GW, Moudgal VV, Seo SM, Hansen JB, Kristiansen JE. Phenylpiperidine selective serotonin reuptake inhibitors interfere with multidrug efflux pump activity in Staphylococcus aureus. Int J Antimicrob Agents, 2003; 22: 254–61.
45. Bohnert JA, Kern WV. Selected arylpiperazines are capable of reversing multidrug resistance in Escherichia coli overexpressing RND efflux pumps. Antimicrob Agents Chemother, 2005; 49: 849–52.
46. Mahmood HY, Jamshidi S, Sutton JM, Rahman KM. Current advances in developing inhibitors of bacterial multidrug efflux pumps. Curr Med Chem, 2016; 23: 1062–81.
47. Vargiu AV, Ruggerone P, Opperman TJ, Nguyen ST, Nikaido H. Molecular mechanism of MBX2319 inhibition of Escherichia coli AcrB multidrug efflux pump and comparison with other inhibitors. Antimicrob Agents Chemother, 2014; 58: 6224–34.
48. Lee MD, Galazzo JL, Staley AL, Lee JC, Warren MS, Fuernkranz H, et al. Microbial fermentation-derived inhibitors of efflux-pump-mediated drug resistance. Farmaco, 2001; 56: 81–5.
49. Barbara Z, Versace I. Inhibitors of multidrug resistant efflux systems in bacteria. Recent Pat Antiinfect Drug Discov, 2009; 4: 3750.
50. Coutinho HDM, Silva VSF, Goncalves GF, Pulmonary bacterial pathogens in cystic fibrosis patients and antibiotic therapy: a tool for the health workers, Int. Archives of Medicine, 2008; 1: 1-24.
51. Tegos G, Stermitz FR, Lomovskaya O, Lewis K, Multidrug Pump Inhibitors Uncover Remarkable Activity of Plant Antimicrobial, Antimicrob Agents Chemother, 2002; 46(10): 3133-3141.