Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v30i16.4846

EFFLUX PUMP INHIBITORS: PAVING THE WAY FROM RESEARCH BENCH TO BEDSIDE BATTLE AGAINST BACTERIAL PATHOGENS

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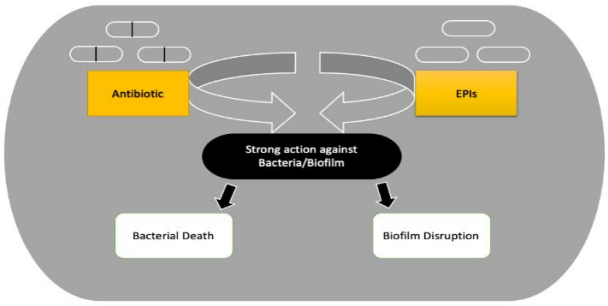
Abstract

It was thought that bacterial illnesses would go extinct with the development of antibiotics. Instead, it caused microorganisms with defence mechanisms against antibiotics to be selected for and evolve. Antibiotic efflux is a key process by which bacteria use unique transporter proteins known as efflux pumps to pump antibiotics out of their cellular interior and into the surrounding environment. Given the scarcity of new antibiotics, inhibiting these pumps appears to be a desirable tactic. Efflux pump inhibitors (EPIs) are molecules that have the potential to block these pumps. They are being considered as possible therapeutic agents that could reactivate antibiotics that have lost their effectiveness against bacterial diseases. EPIs are characterised by their general efflux inhibition methods.

Graphical abstract



Keywords: Antibiotics, efflux pumps, multiple drug resistance, pathogens, therapeutics

Introduction

The early 20th century saw the discovery of penicillin and streptomycin, ushering in the antibiotic age in which previously thought to be fatal bacterial illnesses could be readily treated. The "golden age" of antibiotic discovery occurred in the middle of the 20th century, as about half of the drugs currently in use were found during that time [1]. However, the widespread unnecessary, mishandling, and abuse of antibiotics hastened bacterial evolution and conduct to the selection of microorganisms resistant to antibiotics [2]. According to estimates from the USA's Centres for Disease Control and Prevention (CDC), over 30% of antibiotic prescriptions for outpatients are superfluous [3]. The issue has also been made worse by the careless application of broad-spectrum antibiotics as growth promoters in animal agriculture. The fact that about two million people in the United States suffer from hospital-acquired illnesses caused by drug-resistant germs, which result in approximately 100,000 deaths, illustrates the seriousness of the condition [4]. Calculation on medical expense per patient with antibiotic-resistant illnesses vary from $18,588 to $29,069 which ultimately equates to a healthcare loss as high as $20 billion and a productivity loss of $35 billion every year [5]. Poor hygienic, health, and medical conditions are common in economically disadvantaged countries, where the condition is significantly worse. The prevalence of microbes that are resistant to multiple drugs, including multidrug-resistant (MDR), extensively drug-resistant (XDR) and pan-drug-resistant (PDR) strains, has increased. This has led to the possibility of a "post-antibiotic era," in which antibiotics will no longer be effective and common infections could become fatal [6]. The UN General Assembly acknowledged the grave threat this situation poses and created a framework for international cooperation in the fight against antimicrobial resistance [7]. Four main mechanisms **(Fig. 1)** are used by bacteria to develop resistance to antibiotics: (i) changing cellular permeability to prevent antibiotics from entering the cells; (ii) changing the antibiotics' molecular targets so they can no longer act on them; (iii) enzymatically altering the antibiotics to render them inactive; and (iv) expressing efflux pumps to remove antibiotics from the cellular milieu [8]. These resistance-causing variables may be acquired through different processes or they may be innate. Drug resistance has spread to a broad range of bacterial genera and geographical places due to the availability of resistance determinants on mobile genetic components like plasmids and transposons as well as the unrestricted mobility of human carriers. The goal of the research project, which was originally limited to finding new antibiotics, has expanded to include studying how resistance develops and developing countermeasures. This review addresses the advancements made thus far in blocking these resistance determinants, with a particular emphasis on the efflux pumps, one of the sources of antibiotic resistance.

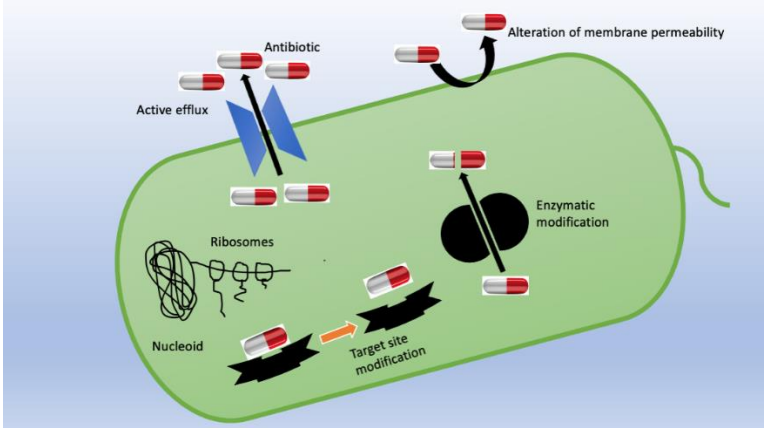


Fig.1 The four main processes by which bacterial cells acquire multiple drug resistance are as follows: (i) changing permeability to prevent antibiotics from entering the cells; (ii) changing the targets of the antibiotics so that they can no longer act on them; (iii) enzymatically changing the antibiotics to make them inactive; and (iv) expressing efflux pumps to remove antibiotics from the interior of the cell.

The role of bacterial efflux systems in predicting multidrug resistance

Bacterial transport proteins called efflux pumps are involved in the extrusion of substrates from inside cells into the surrounding environment. These substrates frequently contain antibiotics, which provide an antibiotic resistance phenotype on the bacteria that express the efflux pump [9]. Since the discovery of the first drug-resistant efflux pump in the 1990s, advances in molecular microbiology have allowed for the characterization of numerous efflux pumps in both Gram-positive (GPB) and Gram-negative (GNB) bacteria, including *Acinetobacter baumannii, Escherichia coli, Klebsiella pneumoniae, Stenotrophomonas maltophilia, Campylobacter jejuni, Pseudomonas aeruginosa, Neisseria gonorrhoeae, Vibrio cholerae,* and *Salmonella* spp. [10,11]. These efflux pumps rely on energy because they move substrates against a gradient in concentration. Generally speaking, there are two types of efflux pumps based on the process by which they obtain this energy. While the secondary efflux pumps obtain their energy from chemical gradients created by protons or ions like sodium, the primary efflux pumps derive their energy from the active hydrolysis of ATP. Prokaryotes have been found to have five major families of efflux pumps **(Fig. 2):** (i) primary active transporters, the ATP binding cassette (ABC) family; (ii) small multidrug resistance family; (iii) multidrug and toxin extrusion (MATE) family; (iv) major facilitator superfamily (MFS); and (v) resistance nodulation cell division (RND) family. All of these families are secondary active transporters [12]. These pump proteins' intricate organisation has shed light on their structure and the molecular process behind substrate transport. The efflux pumps in GNB are more complex because of their multi-layered cell envelop: the inner, or cytoplasmic, membrane and the outer membrane, which are separated by the periplasmic space and combine to form a tripartite protein channel through which the drug is effluxed. In contrast, the drug resistance in GPB is primarily mediated by efflux transporters located in the cytoplasmic membrane. Tripartite in structure, RND family efflux pumps are the primary cause of intrinsic antibiotic resistance in GNB, which eliminates a wide range of antibiotics and biocides such as fluoroquinolones, β-lactams, tetracycline, and linezolid. Nonetheless, MFS transporters, such as NorA of *S. aureus,* PmrA of *S. pneumoniae,* and EmeA of *E. faecalis,* are prevalent in GPB and extrude a wide variety of antibiotics from various classes [10,11].

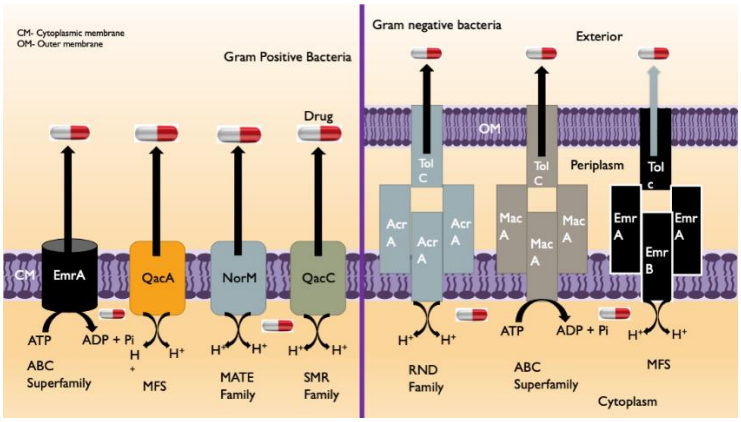


Fig.2 The five categories of bacterial efflux pumps, (i) ATP-binding cassette superfamily, (ii) major facilitator superfamily, (iii) multidrug and toxic compound extrusion family, (iv) small multidrug resistance family, and (v) resistance nodulation division family. The arrangement of these efflux pumps varies between bacteria that are Gram-positive and those that are Gram-negative.

Efflux pumps are more frequently intrinsic than most other resistance determinants. Both sensitive and resistant bacteria include the genes encoding for these transporters [13]. These genes are frequently components of an operon whose transcriptional regulation controls its expression. Drug resistance is the consequence of the hyperexpression of these efflux pumps caused by mutations in the regulatory proteins or in the promoters [13]. Bacterial efflux systems are classified as MDR efflux pumps if they are able to pump out multiple classes of antibiotics, such as MexAB-OprM, NorA, and BmrA, which extrude distinct classes of antibiotics, disinfectants, dyes, and detergents. Alternatively, they can be specific, extruding only one or a single class of antibiotics (e.g., TetA and AbaF, which

selectively exclude specific antibiotics, such as tetracycline and fosfomycin, respectively) [14]. While some MDR efflux pumps are encoded on plasmids (QacA/B of *S. aureus*) or transposons (MefA and MefB of *Streptococcus* spp.) that provide the transferable mode of resistance, the majority of MDR efflux pumps are chromosomally encoded, including NorA, NorB, MepA, and MdeA of *S. aureus* that are responsible for intrinsic resistance in bacteria to several antibiotics [15,16]. The physiological function of efflux pumps in bacteria goes beyond drug resistance and includes bile tolerance in enteric bacteria, which promotes colonisation, pathogenicity, biofilm production, and bacterial survival in the host [17].

Efflux pump inhibitors: novel drugs for treatment

It is reasonable to assume that avoiding these resistance factors may enhance the effectiveness of substrate antibiotics, given the role efflux plays in mediating antibiotic resistance. Redesigning antibiotics that are no longer recognised as substrates, (ii) downregulating the expression of efflux pump genes by interfering with genetic regulation, (iii) inhibiting the assembly of functional efflux pumps, (iv) blocking the pump to prevent substrate binding to the active site, and (v) collapsing the energy mechanism responsible for energising these pumps are some methods to achieve the elimination of efflux [18]. This review primarily concentrates on the final two groups, which make use of substances known as efflux pump inhibitors (EPIs) in an effort to block the efflux pumps. EPIs are the compounds that, through one or more methods, block efflux pumps, resulting in inactive drug transport. The effective accumulation of an antibiotic within the cell may result from this, hence these EPIs can be employed in conjunction with antibiotics as adjuncts to increase the antibiotics' efficacy against bacteria that produce efflux pumps. Since the turn of the century, there has been research being done on the potential use of EPIs to revive the activity of antibiotics. The first peptidomimetic EPI to be found was MC-207,110 [phenylalanyl arginyl β-naphthylamide (PAβN)], which was identified in 2001. It increases the antibacterial efficacy of erythromycin and levofloxacin against clinical isolates of *P. aeruginosa* that overexpress MexAB-OprM [19]. But there hasn't been much of a commercial success, with no EPI reaching that stage yet. A chemical entity seeking to become an approved EPI would need to pass a rigorous checklist. The chemical must not be inherently antibacterial first. The selection of mutants resistant to an antibacterial molecule's effect would ultimately have a negative impact on the molecule's usefulness as an EPI. Secondly, the chemical ought to be able to selectively block any eukaryotic efflux pump. Because efflux pumps are found in many different types of organisms and share many fundamental functions, it can be challenging to selectively inhibit bacterial efflux pumps. Thirdly, it should have the best possible pharmacological characteristics, including serum stability, good ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) profile, high therapeutic and safety indices, and non-toxicity. Lastly, the manufacture of the EPI needs to be economically viable in order to succeed commercially [18].

Types of EPIs according to mechanism of action

As therapeutic adjuvants, EPIs have demonstrated good potential in laboratory settings. Despite the fact that numerous EPIs with various mechanisms of action have been documented, these can be generally divided into two categories:

Energy dissipation

Since cellular energy is required for efflux pumps to function, separating energy from efflux activity offers an intriguing method of inhibiting efflux. Many EPIs have been explored targeting the proton gradient or the ATPase that powers these pumps. The efflux pump itself does not need to directly interact with the inhibitor in such an inhibition scheme. Given that numerous efflux pumps rely on the proton gradient, this strategy seems to be beneficial as it provides a universal means of blocking them. Most people are probably familiar with carbonyl cyanide-m-chlorophenylhydrazone (CCCP), a type of laboratory EPI. It is an ionophore that modifies both of the proton motive force's (PMF) components, $\Delta\psi$ and ΔpH [20], to cause disruption. This renders the bacterial cells metabolically inactive as well, sparking a discussion about whether the synergistic impact of CCCP with other

antibiotics is due to the cells' metabolic inactivity or the inactivity of the efflux pump. It has been documented that the CCCP restores tetracycline action in *Helicobacter pylori* and *Klebsiella* species [21,22]. It has also been shown that carbapenems and CCCP work in concert, independently of CCCP's efflux inhibition function. This finding lends credence to the earlier theory that CCCP causes cells to become metabolically inactive, which in turn produces antibiotics that work in concert with it [23]. This, together with its inherent toxicity to mammalian cells, has prevented CCCP from being used outside of laboratories.

Inhibition by direct binding

The binding of EPIs to functional efflux pumps, which reduces the pumps' capacity to interact with their substrates, is another way of efflux pump inhibition. This binding may be non-competitive, in which the binding of EPI to the pump results in a decrease in the affinity of the pump towards its substrates, or competitive, in which the EPI and the substrates compete for the same binding site. But bacteria can always change the target sites of these inhibitors by mutating their efflux pumps, which makes them ineffective. Screened from a synthetic library as a potentiator of levofloxacin against *P. aeruginosa* cells expressing MexAB, MexCD, and MexEF pumps, PAβN (or MC-207,110) is a paradigm in synthetic EPIs as it was the first inhibitor of the RND family pumps. Additionally, this molecule potentiates erythromycin and chloramphenicol [19]. It functions as a competitive inhibitor of substrate binding and efflux since it is also a substrate for the RND pumps. Combining PAβN with tetracycline and carbenicillin does not increase its effectiveness, which suggests that the binding sites of these antibiotics are not the same as PAβN's. The exact mechanism of action of PAβN is not well understood by science, although computational simulations using AcrB have suggested that it interacts with residues F135, F178, F615, F628, Q176, and E673 [24]. Further research is necessary to make a firm claim, even if there is some indication that it also influences the permeability of the outer membrane [19]. Verapamil is a medication used to treat hypertension that functions as an ion channel blocker. Verapamil has been demonstrated in studies involving *Mycobacterium tuberculosis* to enhance the effects of ofloxacin and bedaquiline [25, 26]. Verapamil has been found in additional research to impair MATE pump activity. Its low level of toxicity to bacterial cells that do not contain MATE efflux pumps suggests that it selectively inhibits bacteria that express these pumps through a competitive mechanism. Verapamil binds to the active site of MATE efflux pumps similarly to the pump's substrates, as verified by crystallisation experiments. Verapamil had distinct interactions with the two prototype MATE pumps, NorM and DinF, but overall, the inhibition of pump activity was the same [27]. 1-(1-napthylmethyl)-piperazine (NMP), another molecule that falls under this category, was created by deriving from a parent molecule that was filtered out of a synthetic compound library [28]. Potentiators of levofloxacin in *E. coli* cells overexpressing the efflux pumps AcrAB and AcrEF were evaluated in the library. Levofloxacin accumulated more in the cells as a result of NMP, which boosted its action. Additionally, it was discovered that NMP diminished the effects of fluoroquinolones, azithromycin, clindamycin, nitrofurantoin, and doxycycline while amplifying oxacillin, rifampin, chloramphenicol, and clarithromycin [28]. Error-prone PCR mutagenesis produced AcrB mutants that were resistant to NMP's potentiating action. As a consequence, the core residues G141, N282, and F610 were discovered, which are essential for NMP binding. NMP binds with the F610 residue in AcrB, changing its conformation and causing non-competitive inhibition [29]. The chemical may also have a secondary target because it also exhibits antibacterial activity at a concentration four times greater than that of an EPI.

Types of EPIs based on their origin

While a large number of compounds have demonstrated potential as EPIs, most of them have unknown mechanisms of action. As a result, it becomes challenging to include these compounds in a classification system that takes into account their method of action. EPIs without a clear mode of action can be accommodated by classifying them according to their source. This gives rise to three general groups of EPIs: those originating from microbes, synthetic chemicals, and plant products.

Plant-derived EPIs

A vast range of chemical adjuvants found in plants called phytochemicals can work in concert to increase an antibiotic's potency by multiple orders of magnitude [30]. The following is a list of significant subclasses of EPIs generated from plants:

Plant alkaloids: Rauwolfia serpentina roots are the source of the antipsychotic medication resperine, a potential EPI that inhibits the efflux pumps of the MFS and RND superfamily [30]. By directly interacting with amino acid residues in the efflux transporter protein Bmr, which causes tetracycline efflux in *B. subtilis*, serpine is believed to enhance the antibacterial action of antibiotics. Furthermore, it has been demonstrated that reserpine reverses NorA-mediated resistance in *S. aureus* by up to four times the activity of norfloxacin [31]. However, because reserpine is nephrotoxic, its practical application with commonly used antibiotics has not yet been accomplished [32]. Another alkaloid that inhibits the human P-glycoprotein of ABC transporters through cytochrome P450-mediated pathways is piperine, which is extracted from Piper nigram. Both piperine and its derivative piperidine have been shown to have efflux pump inhibitory action against pathogenic bacteria, such as *S. aureus* and *Mycobacteria* spp.[33]. According to a study done on *S. aureus*, piperine increases ciprofloxacin accumulation by blocking the NorA efflux pump. Piperine has been found to increase the action of rifampicin in *M. tuberculosis* H37Rv and numerous clinical isolates by blocking an uncharacterized efflux pump, Rv1258c. It has been demonstrated that piperine reduces the MIC of ethidium bromide in *Mycobacterium smegmatis*, suggesting that it can be used as an EPI in a variety of bacterial genera [34].

Flavonoids: Isolated from thyme leaves (Thymus vulgaris), baicalein, a 5,6,7-trihydroflavone, exhibits mild antibacterial properties. It increases the clinical MRSA strain's resistance to oxacillin, cefmetazole, ampicillin, and ciprofloxacin, as well as other β-lactam antibiotics [35, 36]. By preventing the uptake of [3H] tetracycline, baicalein is also reported to boost the efficacy of tetracycline in TetK-overexpressing *Staphylococci* [36]. By blocking this proton pump, 5'-methoxy-hydnocarpin, a flavolignan that was isolated from *Berberis fremontii*, has been shown to increase the effectiveness of a number of NorA substrates, such as norfloxacin and berberine. However, there is question about its clinical success due to its poisonous nature [37]. By inhibiting the MDR efflux pumps, genistein, orobol, and biochanin A—some of the other plant-derived isoflavones that were separated from *Lupinus argenteus*—have been shown to lower the MIC of berberine and norfloxacin in clinical *S. aureus* and *M. smegmatis* [38].

Polyphenols: It has been reported that a class of phenolic metabolites called catechin gallates can reverse the MRSA resistance. The NorA efflux pump is weakly inhibited by catechin gallates, with epicatechin gallate being somewhat more effective than epigallocatechin gallate. It's interesting to note that both substances have been shown to increase efflux at low concentrations [39]. It has been suggested that these molecules interact to the NorA efflux transporter through two distinct sites with varying affinities. Catechins occupy high-affinity binding sites at low concentrations, which increases NorA substrate outflow. They only show an effect as an EPI at larger concentrations. Additionally, it has been observed that epigallocatechin gallate increases the effectiveness of ciprofloxacin, erythromycin, and tetracycline in TetK-overexpressing Gram-positive *Staphylococci* and Gram-negative *Campylobacter* spp. However, more in vivo and pre-clinical research were not conducted because of associated toxicity concerns [40].

Phenolic diterpenes: It has been reported that phenolic diterpenes, including carnosic acid and carnosol, which were isolated from the plant rosemary (*Rosmarinus officinalis*), are EPIs. These increase the effectiveness of antibiotics like erythromycin and tetracycline against macrolide-resistant strains of *S. aureus* that exhibit TetK efflux pumps [41] and the ABC transporter MsrA. It has also been reported that geraniol, a monoterpenoid alcohol derived from *Helichrysum italicum*, modifies drug resistance in a number of GNB species by focusing on MDR efflux pathways. When the *Enterobacter aerogenes* CM-64 strain overexpresses the tripartite efflux pump AcrAB-TolC [42], it lowers the minimum inhibitory concentration (MIC) of chloramphenicol.

Synthetically derived EPIs

In addition to naturally occurring plant-derived compounds, one helpful method for identifying possible EPIs is the screening of innovative semi-synthetic or synthetic diverse chemical libraries. Numerous screening attempts have produced results, but with differing degrees of success. These artificial small molecule EPIs fall into the following further categories:

Peptidomimetic compounds: One of the first EPIs identified using a chemical genetics approach was the dipeptide amide molecule PAβN. By blocking RND efflux pumps, it has been shown to enhance the effects of several antibiotics, such as macrolides, fluoroquinolones, and chloramphenicol, in GNB [19,24]. However, because it was poisonous to mammalian cells, its clinical potential was limited. Despite the evaluation of some synthetic derivatives with various basic qualities like improved solubility, increased stability, and decreased toxicity, none of the active analogues were able to considerably lessen the disadvantage of the parent molecule. Therefore, PAβN and its new derivatives can only be used as standards in the lab to measure the amount of antibiotic-specific inhibitor-sensitive efflux in different bacterial pathogens [43].

Quinoline derivatives: Using a variety of screening techniques against clinical MDR bacterial strains, this new family of chemicals was found. By functioning as a competitive inhibitor of the AcrAB-TolC efflux pump in *E. aerogenes* overexpressing it, quinoline derivatives such pyridoquinolones can reinstate the activity of norfloxacin [44]. In clinical isolates of *K. pneumoniae* and *E. aerogenes* [45], it has also been shown that certain additional synthetic analogues, such as 4-substituted thioalkyl, alkylamino, and alkoxy quinolone, increase the action of tetracyclines, norfloxacin, and chloramphenicol. By altering the flavone scaffold, a number of 2-phenyl-4 (1H)-quinolone and 2-phenyl-4-hydroxyquinoline derivatives have been produced, and these have been shown to be effective NorA efflux pump inhibitors in *S. aureus* [46].

Arylpiperidines and derivatives of aryl piperazine: It has been observed that arylpiperidine and its derivatives, such as 3-arylpiperidine, improve linezolid accumulation in *E. coli* [47] and restore susceptibility to the drug. Phenylpiperidines, a different class of analogues, are known to selectively block serotonin reuptake, which in turn prevents *S. aureus* MDR efflux pumps from functioning. These substances also have a little impact on the AcrAB-TolC pump activity in *E. Coli*, but they have no effect on the efflux activity of the RND efflux pumps in *P. aeruginosa*, such as MexAB-OprM or MexCD-OprJ [48]. It has been demonstrated that NMP, one of the most well-known arylpiperazine compounds, can reinstate the activity of RND pump substrates, such as levofloxacin and EtBr, in *E. coli* strains that overexpress AcrAB and AcrEF. However, arylpiperazines' ability to impede serotonin reuptake means that these substances are probably hazardous to mammalian cells [28].

Pyridopyrimidine and pyranopyridine derivatives: In *P. aeruginosa* that overexpresses MexAB, pyridopyrimidine analogues D2 and D13-9001 have been shown to be MexAB-specific pump inhibitors in both in vitro and in vivo settings [49]. It has been suggested that D13-9001 binds to a particular location on efflux pumps (AcrB in *E. coli* and MexB in *P. aeruginosa*) to block the efflux of antibiotics. The hydrophobic tert-butyl thiazolyl aminocarboxyl moiety of D13-9001 reportedly binds securely to the hydrophobic trap in the deep substrate binding pocket of the pump, preventing conformational changes that are necessary for the pump's appropriate operation, according to additional crystallographic studies. Furthermore, it has been shown that the hydrophilic component of D13-9001 interacts with the pump's substrate binding channel, blocking substrate binding to the pumps [43]. From a library of small compounds, the synthetic pyrazolopyridine MBX2319 was tested as a fluoroquinolone antibiotic potentiator. It upregulates the effectiveness of piperacillin, levofloxacin, and ciprofloxacin against *E. Coli* AB1157 [29]. Moreover, MBX2319 caused AcrAB-TolC-overexpressing *E. coli* [29] and wild type *E. coli* to accumulate more Hoechst dye intracellularly. According to a thorough X-ray crystallographic analysis, MBX2319 interacts with the AcrB pump's hydrophobic trap through a ring stacking contact with the amino acid residues [43,52] that is anticipated to occur on the pyridine ring. Furthermore, a large number of synthetic and semisynthetic compounds that primarily target the MDR efflux pump of both GPB and GNB have been intentionally synthesised (**Table**).

List of efflux pump inhibitors from various sources

Bacterial Strain	EPIs	Substrate	Target efflux pump	References
Streptococcus aureus, Pseudomonas aeruginosa	Pheophorbide A	Berberine, ciprofloxacin	NorA, MexAB-OprM	50
S. aureus	5′-MHC	Berberine	NorA	37,38
S. aureus	Carnosic acid	Erythromycin	MsrA	41
S. aureus	Carnosol	Tetracycline	MsrA, TetK	41
Salmonella Typhimurium	Cathinone	Ciprofloxacin	acrAB-TolC	51,52
S. Typhimurium, *Klebsiella pneumoniae*	Theobromine	Ciprofloxacin, tetracycline	acrAB-TolC	51
Mycobacterium spp.	Reserpine	Ciprofloxacin, ofloxacin	ABC: Rv2936-Rv2937-Rv2938 (DrrABC) Rv0933 (PstB) Rv2686c-Rv2687c-Rv2688c RND: Rv0678, Rv1145, Rv1146, Rv2942 (mmpL7) MFS: Rv1410c (P55), Rv1877 Rv2846c SMR: Rv3065 (mmr)	53
S. aureus	4’,5’- O-dicaffeoylquinic acid	Berberine, norfloxacin	NorA	54
S. aureus	Curcumin	Norfloxacin, ciprofloxacin	NorA	55
S. aureus	Kaempferol	Norfloxacin, ciprofloxacin	NorA	56
S. aureus	N-trans-feruloyl 4’- O-methyldopamine	Norfloxacin, ciprofloxacin	NorA	57
S. aureus	Silibinin	Norfloxacin	NorA	49
S. aureus	Genistein, Isoflavone	Berberine	NorA	38
Escherichia coli	Artesunate	Penicillin G; ampicillin, cefazolin, cefuroxime, cefoperazone	AcrAB-TolC	58
S. aureus	Orizabins	Norfloxacin, berberine	NorA	49
S. aureus	Resin glycosides (Orizabins IX, Murucoidins, Stoloniferin)	Norfloxacin, ciprofloxacin	NorA	59
S. aureus	Citropten and furocoumarins	Norfloxacin, ciprofloxacin	NorA, ErmA, ErmB	60
S. aureus	Coumarins	Norfloxacin, ciprofloxacin	NorA	61
S. aureus	Crysoplenol and Crysoplenetin	Berberine, norfloxacin	NorA	62
S. aureus	Diosmetin	Erythromycin, norfloxacin	MsrA, NorA	63
S. aureus	Murucoidins	Norfloxacin	NorA	59
S. aureus	Chrysosplenol-D	Berberine	NorA	62
Mycobacterium spp.	Phenylpropanoid	Et-Br	Rv1145, Rv1146 Rv1877, Rv2846c Rv3065(mmr)	64
S. aureus	Compound 1	Norfloxacin	NorA	65
Staphylococcus epidermidis	Essential oils (Salvia species)	Tetracycline	Tet (K)	66
Mycobacterium spp.	Spectinamides	Clarithromycin, Doxycycline and Clindamycin	Rv1258c	67
S. aureus, Mycobacterium spp.	Diterpenes (ferruginol)	Tetracycline, erythromycin, norfloxacin isoniazid	MsrA, TetK, NorA	68
S. aureus, Mycobacterium spp.	Totarol	Erythromycin, isoniazid	MsrA, TetK	69
S. aureus	Boeravinone B	Norfloxacin, ciprofloxacin	NorA	70
S. aureus	α-Terpinene	Tetracycline	TetK	71
S. aureus	Biochanin A	Berberine, norfloxacin	NorA	38
S. aureus	Cumin seed oil, cuminaldehyde	Et-Br	LmrS	72
S. aureus	Epigallocatechin gallate, Epicatechin gallate	Tetracycline	TetK	39,40
S. aureus	Galbanic acid	Norfloxacin, ciprofloxacin	NorA	73
S. aureus	Orobol	Berberine	NorA	38

S. aureus, E. coli	Baicalein	Ciprofloxacin, tetracycline	NorA, TetK	35,36
S. aureus	Tannic acid	Tetracycline, norfloxacin	TetK, NorA	74
Pseudomonas aeruginosa, Acinetobacter baumannii	Conessine	Cefotaxime, levofloxacin, tetracycline, novobiocin and rifampicin	MexAB-OprM, AdeIJK	75,76
S. aureus	Linoleic and oleic acids	Erythromycin	MsrA	77
S. aureus	Tiliroside, kaempferol-3-O-b-d- (6-E-p-coumaroyl)Glucopyranoside	Norfloxacin, ciprofloxacin	NorA	78
S. aureus	Capsaicin (8-methyl-N-vanillyl-6 nonenamide)	Norfloxacin, ciprofloxacin	NorA	79
Enterococcus faecalis, S. aureus	Caeffeoylquinic acid	Berberine	NorA	54
S. aureus, Mycobacterium spp.	Piperine	Norfloxacin, ciprofloxacin	NorA, MdeA, Rv1258c	33,34
S. aureus	Clerodane diterpene 16α-hydroxycleroda-3,13 (14)-Z-dien-15,16-olide	Norfloxacin, ciprofloxacin	norA, norB, norC, mepA, mdeA	80
S. aureus	Chalcone	Berberine, norfloxacin	NorA	30
S. aureus	Olaanolic acid, Ulvaol	Norfloxacin, oxacillin	NorA	81
Mycobacterium spp.	Quercetin	-	Rv3065(mmr)	53
Mycobacterium spp.	Tetrandrine	Isoniazid and ethambutol	Rv2459 (jefA), Rv3728 Rv3065(mmr)	53
Mycobacterium spp.	Farnesol	Et-Br	-	53
S. aureus	4-acetyl-3-(4-fluorophenyl) - 1-(p-tolyl)-5-methylpyrrole	Norfloxacin, ciprofloxacin	NorA	82
S. aureus	N-trans-3,4-O dimethylcaffeoyl Tryptamine	Norfloxacin, ciprofloxacin	NorA	83
S. aureus	5,7 deoxyhydnocarpin-D (5,7-DHC-D)	Berberine	NorA	31
S. aureus	Chalcone and derivatives	Norfloxacin, ciprofloxacin	NorA	84
S. aureus	4-phenoxy-4'- dimethylaminoethoxy chalcone, (4-DAEC)	Norfloxacin, ciprofloxacin	NorA	57
S. aureus	SK-20 and SK-56 (Piperine analogs)	Norfloxacin, ciprofloxacin	NorA	33
E. coli	SLUPP-225, SLUPP-417	Novobiocin and erythromycin	AcrAB-TolC	85
A. baumannii	PAβN	Trimethoprim, chloramphenicol and clindamycin	AdeFGH	86
A. baumannii, E. coli, Enterobacter aerogenes, K. pneumonia	NMP (1-(1naphthylmethyl)-piperazine)	Levofloxacin	AdeABC, AcrAB, AcrEF	28
S. aureus	5-MPC	Norfloxacin, ciprofloxacin	NorA	83
M. tuberculosis	Verapamil	Isoniazid	(efpA [Rv2846c], Rv1258c, jefA [Rv2459], and P55 [Rv1410c]) and (Rv1819c and pstB [Rv0933]	25,26, 53
E. coli	Piperazine Arylideneimidazolones	Fluoroquinolones	AcrAB Tol-C and AcrEF	87
S. aureus	Ethyl 6-amino-1 cyclopropyl- 7-[4-(hydroxyimino)-3-methyl-3,4,7,8-tetrahydro-2H-thiopyrano[3,2-c] pyridin-6 (5H)-yl]-8-methyl-4-oxo-1,4-dihydroquinoline-3-carboxylate (EDCQ)	Norfloxacin, ciprofloxacin	NorA	83
S. aureus	10-(4-(-3-phenylureido)- benzylamino)-9-fluoro-3,7- dihydro-3-methyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij] quinoline-6-carboxilic acid (Q6CA)	Norfloxacin, ciprofloxacin	NorA, MepA	68
E. aerogenes	Pyridoquinolines	Norfloxacin	AcrAB-TolC	44
S. aureus	2-phenyl-4-hydroxyquinoline derivativesN, N-diethyl-2- {[2-(4-propoxyphenyl) quinolin-4-yl] oxy}-ethanamine hydrochloride (PPQE)	Norfloxacin, ciprofloxacin	NorA	46
S. aureus	4-(2-piperidin-1-ylethoxy)- 2-(4 propoxyphenyl) quinoline (PPQ)	Norfloxacin, ciprofloxacin	NorA	46
E. coli	4-(2-(piperazin-1-yl) ethoxy)-2-(4-propoxyphenyl) quinolone - PQQ4R	Ofloxacin, tetracycline	AcrAB-TolC	88
E. aerogenes	(Z)-5-(2,4-dimethoxybenzylidene)-3- (2-hydroxy-3-(isopropylamino) propyl) imidazolidine-2,4-dione	Chloramphenicol, nalidixic acid and sparfloxacin	AcrAB-TolC	89

S. aureus	5-nitro-2-phenylindole, (INF 55, INF 240, INF 240, INF 271, INF 277)	Ciprofloxacin	NorA	83
S. aureus	[4-benzyloxy-2-(5-nitro-1H-2-yl)-phenyl]-methanol (BNPM)	Berberine, norfloxacin	NorA	83
S. aureus	2-phenylbenzo[b] thiophene-3 carboxaldehyde (2-PTC)	Ciprofloxacin	NorA	83
S. aureus	3-(3,4-dihydronapth-2-yl)-propenoic acid isobutyl amide (3-PIA)	Ciprofloxacin	NorA	83
S. aureus	2-((2-(4-propoxyphenyl) quinolin-4-yl) oxy) alkylamines 1-4	Ciprofloxacin	NorA	46
E. coli	13-cyclopentylthio-5-OH-TC (13-CPTC), semisynthetic tetracycline (TC) analogs	Tetracycline	TetA or TetB	90
S. aureus	Cholecalciferol and alpha-tocopherol	Erythromycin, tetracycline	TetK, MsrA	91
P. aeruginosa	Phe-Arg-β-naphthylamide (MC-207, 110)	Levofloxacin	MexAB-OprM	19
S. aureus, E. faecalis	Biricodar, G-918	FQs, Norfloxacin	NorA	49
S. aureus, Mycobacterium spp.	Timcodar	Norfloxacin, isoniazid, rifampicin	-	49
Mycobacterium spp.	SILA 421	-	mdr-1	92
S. aureus, E. coli	Phenothiazine and its derivatives (methylene blue, promethazine, chlorpromazine and thioridazine)	Norfloxacin, FQs	NorA, AcrB	49
Burkholderia pseudomallei	Phenothiazine and its derivatives (methylene blue, promethazine, chlorpromazine and thioridazine)	Erythromycin, levofloxacin and azithromycin	-	49
S. enterica	Chlorpromazine	Et-Br	AcrB	49
S. aureus	phenyl-1,4-benzothiazine derivatives	Ciprofloxacin	NorA	93
K. pneumonia, E. aerogenes	Pyridoquinolines	Tetracycline, norfloxacin, chloramphenicol	AcrAB-ToIC	44
S. aureus	2-(4-Propoxy-phenyl) quinolone derivatives	Ciprofloxacin	NorA	46
Mycobacterium spp.	Valinomycin	Isoniazid	Rv1410c (P55)	57
E. coli, P. aeruginosa	Pyridopyrimidine analogues (D13-9001, D2)	FQs	AcrB and MexB	49
E. coli	Pyranopyridine derivatives (MBX2319)	Ciprofloxacin	AcrAB	29
A. baumannii	(E)-N-(3,4-difluorophenyl)- 2-(2-(3-(methylthio) phenylimino)-4-oxothiazolidin-5-yl	Norfloxacin, ciprofloxacin	AbeM	20
E. coli	DHA7, DHA 27	FQs	AcrB	94
S. aureus	Riparin-B	Ciprofloxacin, norfloxacin	NorA	95
S. aureus	Nerol, Dimethyl octanol and Estragole (monoterpenes)	Norfloxacin	NorA	96
S. aureus	PA EPA amides	Norfloxacin	NorA	97
S. aureus	6-(aryl) alkoxypyridine-3-boronic acids, 6-(3-Phenylpropoxy) pyridine-3-boronic acid 3i and 6-(4-phenylbutoxy) pyridine-3-boronic acid 3j	Ciprofloxacin	NorA	98
S. aureus	Ginsenoside 20(S)-Rh2 (Rh2)	Ciprofloxacin	NorA	99
E. coli	Pimozide (neuroleptic drug)	Et-Br	AcrAB-TolC	100
E. coli	Sertraline	Levofloxacin, tetracycline	AcrAB, AcrEF, MdtEF and MexAB	45
P. aeruginosa	EA-371α and EA-371δ (EPI from microbial source)	Levofloxacin	MexAB-OprM	101

PA, piperic acid; EPA, 4-ethylpiperic acid; DHA7, dihydroartemisinine 7; PaβN, Phenylalanine-arginine β-naphthylamide; 5′-MHC, 5’- methoxyhydnocarpin

EPIs derived from microbes

A tiny percentage of EPIs have been documented to come from microorganisms, despite the fact that the majority of EPIs come from natural products or semi-synthetic/synthetic chemical libraries. It has been established that EA-371α and EA-371d, which were initially isolated from the fermentation extract of *Streptomyces* species, are particular inhibitors of the MexAB-OprM pump in *P. aeruginosa* [101]. Because of these molecules' unique structures, researchers can create novel derivatives that are more potent, bioavailable, and less poisonous. The availability of the efflux pumps' three-dimensional crystal structure means that additional computational research may be helpful in determining how these chemicals interact molecularly with such MDR pumps.

Current challenges for EPIs as therapeutic agents

Even though EPIs have been used in lab settings for research purposes since the 1990s, they represent one of the most promising developments in the fight against bacteria that are resistant to antibiotics. There are many obstacles in the way of a profitable commercial EPI, though. These difficulties come in a variety of forms, from intellectual and scientific to administrative and financial. The economic value of an EPI is a significant barrier to its development and sale. Since EPI is essentially a new chemical entity, major firms in the pharmaceutical industry typically avoid this field (NCE). The concept of changing the existing approved antibiotics, which have a well-established pharmacological profile and clinical data from multiple patient records, is more appealing to drug professionals who are well-versed in the issues surrounding NCE [102]. Researchers have searched for EPIs derived from both natural and synthetic compounds, but their commercial production has not been considered at the laboratory level. The synthesis of naturally derived EPIs is a laborious process due to their complex and bulky structure; synthesis of synthetic molecules is simpler, but the resulting molecules frequently have poor solubility, toxicity, and cell permeability issues. A great deal of work is also expended on meeting the very strict regulatory requirements. The majority of pharmaceutical corporations avoid the discovery of EPIs and NCEs altogether due to the financial impossibility of such endeavours when paired with average economic returns. In essence, an EPI therapy would be a combination therapy. This presents an additional obstacle to the EPI and antibiotic partner's compatibility. For a medicinal combination to be successful, the pharmacokinetics of both partners must be complementary [102]. Although these factors are frequently disregarded in lab trials, they are crucial from a clinical perspective. For instance, the US FDA has issued a stern warning [103] on the potentially lethal combination of clarithromycin, a macrolide antibiotic, with verapamil, a Ca++ channel blocker. The cytochrome that is in charge of verapamil metabolism is the target of clarithromycin. When these medications are taken together, verapamil can build up to exceedingly toxic levels, which can cause kidney failure, hypotension, and even death [103]. Targets are a significant obstacle for EPIs as therapeutic agents in and of themselves. Although they are not necessarily the only mechanism, efflux pumps are one type of antibiotic resistance mechanism. Fluoroquinolone resistance in bacteria like *A. baumannii* and *P. aeruginosa* is frequently caused by point mutations in the gyrase-coding genes as well as efflux pumps [104]. Redundant substrates and the co-expression of several pumps exacerbate the issue. This creates a case-specificity in the EPI-antibiotic combinatorial therapy and raises questions about its effectiveness at the community level. Even though EPIs typically work well as an antibiotic against the efflux pump, it is frequently observed that an EPI does not increase the activity of other efflux pump substrates. Only a specific group of antibiotics can be effectively potentiated by PAβN; other substrate antibiotics of the pump MexAB are not significantly potentiated by PaβN [19]. Similar to PAβN, a large number of EPIs act at a specific substrate-binding site and are substrates of the pumps. This discovery implies, indirectly, that a high concentration of EPI would be necessary to guarantee that they competitively prevent substrate antibiotics from interacting with the pump. Sadly, the do well with antibiotics that have a distinct substrate-binding site but are nonetheless the pump's substrate. As a result, an EPI's spectrum is significantly reduced, making it extremely selective for a small number of substrates. While it is challenging to identify an NCE that blocks an antibiotic's efflux from a pump, it is very challenging to identify an EPI that would block several pumps in several bacterial species. Despite sharing a common mechanism of inhibition, several compounds have also been observed to inhibit animal efflux pumps, leading to toxicity and an undesirable pharmacological profile [102]. A further obstacle to the effectiveness of EPIs is the dearth of pre-clinical and clinical data. To support the work of EPIs, there is a limited amount of data on model organisms and patient information. To advance the field of EPI research, more pre-clinical and clinical work is needed [102]. One benefit of employing EPIs is the low to nonexistent frequency of mutant production. Nevertheless, PCR-based random mutagenesis has produced efflux pump variants that are resistant to EPI32's effect while still maintaining their activity. It is undeniable that, in the face of intense selection pressure, bacteria may undergo such alterations that, in the end, protect them from the EPI-antibiotic combination therapy, even though it may seem like a remote possibility.

Conclusion and Future Perspectives

While there are several obstacles in the way of using EPIs as therapeutic agents, this does not lessen their significance or benefits. By reviving the activity of currently available antibiotics, EPIs offer a glimmer of hope during periods when the antibacterial pipeline has nearly dried up. The usage of EPIs prevents the development of new antibiotics, which is an tactic that saves a significant amount of time, money, and effort when developing a novel antibiotic. It enables medical professionals to take advantage of the well-established pharmacological characteristics of well-known antibiotics. The potential of EPIs as therapeutic agents to reverse antibiotic resistance is a significant significance. It takes on significant significance when we take into account that the present economic climate is likewise favourable to the large-scale production of antibiotics that are already optimised and in stock. One notable benefit of employing EPIs is the incredibly low incidence of resistant mutant creation. As a result, the combination of antibiotic and EPI works well to both combat germs that are already resistant and to temporarily halt the spread of resistance in the future. After assessing the potential of EPIs, it seems that while using them is a desirable approach, its implementation is still far from complete. There are numerous holes that must be filled and a great deal of ground to cover. There is an urgent need to address the technical drawbacks and limitations of the EPIs. To emphasise the EPIs' benefits from a scientific and financial standpoint, more study is needed. In the end, this would aid in drawing in additional funding and the attention of the pharmaceutical businesses. In summary, a great deal of work is being done at the bench level right now, but more thought and work will need to be done before the EPIs can ideally reach the bedside.

Footnotes

This work is supported by an extramural research grant, SERB, DST grant number (CRG/2018/001317)

Conflict of Interest

The authors declare no conflict of interest.

References

1. Lewis K. Antibiotics: Recover the lost art of drug discovery. Nature. 2012;485:439–40.
2. Ory EM, Yow EM. The use and abuse of the broad spectrum antibiotics. JAMA. 1963;185:273–9.
3. Fleming-Dutra KE, Hersh AL, Shapiro DJ, Bartoces M, Enns EA, File TM., Jr Prevalence of inappropriate antibiotic prescriptions among US ambulatory care visits, 2010-2011. JAMA. 2016;315:1864–73.
4. Haque M, Sartelli M, McKimm J, Abu Bakar M. Health care-associated infections-an overview. Infect Drug Resist. 2018;11:2321–33.
5. Ventola CL. The antibiotic resistance crisis: Part 1: Causes and threats. P T. 2015;40:277–83.
6. Falagas ME, Karageorgopoulos DE. Pandrug resistance (PDR), extensive drug resistance (XDR), and multidrug resistance (MDR) among Gram-negative bacilli: Need for international harmonization in terminology. Clin Infect Dis. 2008;46:1121–2.
7. Hoffman SJ, Outterson K. Introduction: What will it take to address the global threat of antibiotic resistance? J Law Med Ethics. 2015;43(Suppl 3):6–11.
8. Blair JM, Webber MA, Baylay AJ, Ogbolu DO, Piddock LJ. Molecular mechanisms of antibiotic resistance. Nat Rev Microbiol. 2015;13:42–51.
9. Piddock LJ. Clinically relevant chromosomally encoded multidrug resistance efflux pumps in bacteria. Clin Microbiol Rev. 2006;19:382–402.
10. Schindler BD, Kaatz GW. Multidrug efflux pumps of Gram-positive bacteria. Drug Resist Updat. 2016;27:1–3.

11. Li XZ, Plésiat P, Nikaido H. The challenge of efflux-mediated antibiotic resistance in Gram-negative bacteria. Clin Microbiol Rev. 2015;28:337–418.
12. Blair JM, Richmond GE, Piddock LJ. Multidrug efflux pumps in Gram-negative bacteria and their role in antibiotic resistance. Future Microbiol. 2014;9:1165–77.
13. Webber MA, Piddock LJ. The importance of efflux pumps in bacterial antibiotic resistance. J Antimicrob Chemother. 2003;51:9–11.
14. Sharma A, Sharma R, Bhattacharyya T, Bhando T, Pathania R. Fosfomycin resistance in Acinetobacter baumannii is mediated by efflux through a major facilitator superfamily (MFS) transporter-AbaF. J Antimicrob Chemother. 2017;72:68–74.
15. Costa SS, Ntokou E, Martins A, Viveiros M, Pournaras S, Couto I, et al. Identification of the plasmid-encoded QacA efflux pump gene in meticillin-resistant Staphylococcus aureus (MRSA) strain HPV107, a representative of the MRSA iberian clone. Int J Antimicrob Agents. 2010;36:557–61.
16. Santagati M, Iannelli F, Cascone C, Campanile F, Oggioni MR, Stefani S, et al. The novel conjugative transposon TN1207.3 carries the macrolide efflux gene mef(A) in Streptococcus pyogenes. Microb Drug Resist. 2003;9:243–7.
17. Piddock LJ. Multidrug-resistance efflux pumps – Not just for resistance. Nat Rev Microbiol. 2006;4:629–36.
18. Bhardwaj AK, Mohanty P. Bacterial efflux pumps involved in multidrug resistance and their inhibitors: Rejuvinating the antimicrobial chemotherapy. Recent Pat Antiinfect Drug Discov. 2012;7:73–89.
19. 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.
20. Kothari A, Kherdekar R, Mago V, Uniyal M, Mamgain G, Kalia RB, Kumar S, Jain N, Pandey A, Omar BJ. Age of Antibiotic Resistance in MDR/XDR Clinical Pathogen of Pseudomonas aeruginosa. Pharmaceuticals. 2023 Aug 30;16(9):1230.
21. Anoushiravani M, Falsafi T, Niknam V. Proton motive force-dependent efflux of tetracycline in clinical isolates of Helicobacter pylori. J Med Microbiol. 2009;58:1309–13.
22. Fenosa A, Fusté E, Ruiz L, Veiga-Crespo P, Vinuesa T, Guallar V, et al. Role of tolC in Klebsiella oxytoca resistance to antibiotics. J Antimicrob Chemother. 2009;63:668–74.
23. Osei Sekyere J, Amoako DG. Carbonyl cyanide m-chlorophenylhydrazine (CCCP) reverses resistance to colistin, but not to carbapenems and tigecycline in multidrug-resistant Enterobacteriaceae. Front Microbiol. 2017;8:228.
24. 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.
25. Gupta S, Cohen KA, Winglee K, Maiga M, Diarra B, Bishai WR. Efflux inhibition with verapamil potentiates bedaquiline in Mycobacterium tuberculosis. Antimicrob Agents Chemother. 2014;58:574–6.
26. Singh M, Jadaun GP, Ramdas, Srivastava K, Chauhan V, Mishra R, et al. Effect of efflux pump inhibitors on drug susceptibility of ofloxacin resistant Mycobacterium tuberculosis isolates. Indian J Med Res. 2011;133:535–40.
27. Radchenko M, Symersky J, Nie R, Lu M. Structural basis for the blockade of MATE multidrug efflux pumps. Nat Commun. 2015;6:7995.
28. 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.
29. 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.
30. Stavri M, Piddock LJ, Gibbons S. Bacterial efflux pump inhibitors from natural sources. J Antimicrob Chemother. 2007;59:1247–60.

31. Gibbons S, Oluwatuyi M, Kaatz GW. A novel inhibitor of multidrug efflux pumps in Staphylococcus aureus. J Antimicrob Chemother. 2003;51:13–7.
32. 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.
33. 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.
34. 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.
35. Kothari A, Jain N, Kishor Kumar S, Kumar A, Kaushal K, Kaur S, Pandey A, Gaurav A, Omar BJ. Potential synergistic antibiotic combinations against fluoroquinolone-resistant Pseudomonas aeruginosa. Pharmaceuticals. 2022 Feb 18;15(2):243.
36. 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.
37. 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.
38. Morel C, Stermitz FR, Tegos G, Lewis K. Isoflavones as potentiators of antibacterial activity. J Agric Food Chem. 2003;51:5677–9.
39. Gibbons S, Moser E, Kaatz GW. Catechin gallates inhibit multidrug resistance (MDR) in Staphylococcus aureus. Planta Med. 2004;70:1240–2.
40. 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.
41. Oluwatuyi M, Kaatz GW, Gibbons S. Antibacterial and resistance modifying activity of Rosmarinus officinalis. Phytochemistry. 2004;65:3249–54.
42. 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.
43. Opperman TJ, Nguyen ST. Recent advances toward a molecular mechanism of efflux pump inhibition. Front Microbiol. 2015;6:421.
44. 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.
45. Pradel E, Pagès JM. The AcrAB-tolC efflux pump contributes to multidrug resistance in the nosocomial pathogen Enterobacter aerogenes. Antimicrob Agents Chemother. 2002;46:2640–3.
46. 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.
47. Thorarensen A, Presley-Bodnar AL, Marotti KR, Boyle TP, Heckaman CL, Bohanon MJ, et al. 3-arylpiperidines as potentiators of existing antibacterial agents. Bioorg Med Chem Lett. 2001;11:1903–6.
48. 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.
49. 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.
50. Zechini B, Versace I. Inhibitors of multidrug resistant efflux systems in bacteria. Recent Pat Antiinfect Drug Discov. 2009;4:37–50.

51. Piddock LJ, Garvey MI, Rahman MM, Gibbons S. Natural and synthetic compounds such as trimethoprim behave as inhibitors of efflux in Gram-negative bacteria. J Antimicrob Chemother. 2010;65:1215–23.
52. Neyfakh AA, Bidnenko VE, Chen LB. Efflux-mediated multidrug resistance in Bacillus subtilis: Similarities and dissimilarities with the mammalian system. Proc Natl Acad Sci U S A. 1991;88:4781–5.
53. Song L, Wu X. Development of efflux pump inhibitors in antituberculosis therapy. Int J Antimicrob Agents. 2016;47:421–9.
54. Fiamegos YC, Kastritis PL, Exarchou V, Han H, Bonvin AM, Vervoort J, et al. Antimicrobial and efflux pump inhibitory activity of caffeoylquinic acids from Artemisia absinthium against Gram-positive pathogenic bacteria. PLoS One. 2011;6:e18127.
55. Joshi P, Singh S, Wani A, Sharma S, Jain SK, Singh B, et al. Osthol and curcumin as inhibitors of human Pgp and multidrug efflux pumps of Staphylococcus aureus: Reversing the resistance against frontline antibacterial drugs. Med Chem Commun. 2014;5:1540–7.
56. Holler JG, Christensen SB, Slotved HC, Rasmussen HB, Gúzman A, Olsen CE, et al. Novel inhibitory activity of the Staphylococcus aureus NorA efflux pump by a kaempferol rhamnoside isolated from Persea lingue nees. J Antimicrob Chemother. 2012;67:1138–44.
57. Michalet S, Cartier G, David B, Mariotte AM, Dijoux-franca MG, Kaatz GW, et al. N-caffeoylphenalkylamide derivatives as bacterial efflux pump inhibitors. Bioorg Med Chem Lett. 2007;17:1755–8.
58. Li B, Yao Q, Pan XC, Wang N, Zhang R, Li J, et al. Artesunate enhances the antibacterial effect of {beta|-lactam antibiotics against Escherichia coli by increasing antibiotic accumulation via inhibition of the multidrug efflux pump system AcrAB-TolC. J Antimicrob Chemother. 2011;66:769–77.
59. Chérigo L, Pereda-Miranda R, Fragoso-Serrano M, Jacobo-Herrera N, Kaatz GW, Gibbons S. Inhibitors of bacterial multidrug efflux pumps from the resin glycosides of Ipomoea murucoides. J Nat Prod. 2008;71:1037–45.
60. Rana T, Singh S, Kaur N, Pathania K, Farooq U. A review on efflux pump inhibitors of medically important bacteria from plant sources. Int J Pharm Sci Rev Res. 2014;26:101–11.
61. Roy SK, Kumari N, Pahwa S, Agrahari UC, Bhutani KK, Jachak SM, et al. NorA efflux pump inhibitory activity of coumarins from Mesua ferrea. Fitoterapia. 2013;90:140–50.
62. Stermitz FR, Scriven LN, Tegos G, Lewis K. Two flavonols from Artemisa annua which potentiate the activity of berberine and norfloxacin against a resistant strain of Staphylococcus aureus. Planta Med. 2002;68:1140–1.
63. Chan BC, Ip M, Gong H, Lui SL, See RH, Jolivalt C, et al. Synergistic effects of diosmetin with erythromycin against ABC transporter over-expressed methicillin-resistant Staphylococcus aureus (MRSA) RN4220/pUL5054 and inhibition of MRSA pyruvate kinase. Phytomedicine. 2013;20:611–4.
64. Roy SK, Pahwa S, Nandanwar H, Jachak SM. Phenylpropanoids of Alpinia galangal as efflux pump inhibitors in Mycobacterium smegmatis mc2 155. Fitoterapia. 2012;83:1248–55.
65. Shiu WK, Malkinson JP, Rahman MM, Curry J, Stapleton P, Gunaratnam M, et al. A new plant-derived antibacterial is an inhibitor of efflux pumps in Staphylococcus aureus. Int J Antimicrob Agents. 2013;42:513–8.
66. Chovanová R, Mezovská J, Vaverková Š, Mikulášová M. The inhibition the Tet(K) efflux pump of tetracycline resistant Staphylococcus epidermidis by essential oils from three Salvia species. Lett Appl Microbiol. 2015;61:58–62.
67. Bruhn DF, Scherman MS, Liu J, Scherbakov D, Meibohm B, Böttger EC, et al. In vitro and in vivo evaluation of synergism between anti-tubercular spectinamides and non-classical tuberculosis antibiotics. Sci Rep. 2015;5:13985.
68. Smith EC, Williamson EM, Wareham N, Kaatz GW, Gibbons S. Antibacterials and modulators of bacterial resistance from the immature cones of Chamaecyparis lawsoniana. Phytochemistry. 2007;68:210–7.

69. Smith EC, Kaatz GW, Seo SM, Wareham N, Williamson EM, Gibbons S. The phenolic diterpene totarol inhibits multidrug efflux pump activity in Staphylococcus aureus. Antimicrob Agents Chemother. 2007;51:4480–3.
70. Singh S, Kalia NP, Joshi P, Kumar A, Sharma PR, Kumar A, et al. Boeravinone B, A novel dual inhibitor of NorA bacterial efflux pump of Staphylococcus aureus and human P-glycoprotein, reduces the biofilm formation and intracellular invasion of bacteria. Front Microbiol. 2017;8:1868.
71. Limaverde PW, Campina FF, da Cunha FAB, Crispim FD, Figueredo FG, Lima LF, et al. Inhibition of the TetK efflux-pump by the essential oil of Chenopodium ambrosioides L. and α-terpinene against Staphylococcus aureus IS-58. Food Chem Toxicol. 2017;109:957–61.
72. Kakarla P, Floyd J, Mukherjee M, Devireddy AR, Inupakutika MA, Ranweera I, et al. Inhibition of the multidrug efflux pump LmrS from Staphylococcus aureus by cumin spice Cuminum cyminum. Arch Microbiol. 2017;199:465–74.
73. Fazly Bazzaz BS, Iranshahi M, Naderinasab M, Hajian S, Sabeti Z, Masumi E. Evaluation of the effects of galbanic acid from Ferula szowitsiana and conferol from F. badrakema, as modulators of multi-drug resistance in clinical isolates of Escherichia coli and Staphylococcus aureus. Res Pharm Sci. 2010;5:21–8.
74. Tintino SR, Morais-Tintino CD, Campina FF, Costa MDS, Menezes IRA, de Matos YMLS, et al. Tannic acid affects the phenotype of Staphylococcus aureus resistant to tetracycline and erythromycin by inhibition of efflux pumps. Bioorg Chem. 2017;74:197–200.
75. Siriyong T, Srimanote P, Chusri S, Yingyongnarongkul BE, Suaisom C, Tipmanee V, et al. Conessine as a novel inhibitor of multidrug efflux pump systems in Pseudomonas aeruginosa. BMC Complement Altern Med. 2017;17:405.
76. Siriyong T, Chusri S, Srimanote P, Tipmanee V, Voravuthikunchai SP. Holarrhena antidysenterica extract and its steroidal alkaloid, conessine, as resistance-modifying agents against extensively drug-resistant Acinetobacter baumannii. Microb Drug Resist. 2016;22:273–82.
77. Chan BC, Han XQ, Lui SL, Wong CW, Wang TB, Cheung DW, et al. Combating against methicillin-resistant Staphylococcus aureus – Two fatty acids from purslane (Portulaca oleracea L.) exhibit synergistic effects with erythromycin. J Pharm Pharmacol. 2015;67:107–16.
78. Falcão-Silva VS, Silva DA, Souza Mde F, Siqueira-Junior JP. Modulation of drug resistance in Staphylococcus aureus by a kaempferol glycoside from Herissantia tiubae (Malvaceae) Phytother Res. 2009;23:1367–70.
79. Kalia NP, Mahajan P, Mehra R, Nargotra A, Sharma JP, Koul S, et al. Capsaicin, a novel inhibitor of the NorA efflux pump, reduces the intracellular invasion of Staphylococcus aureus. J Antimicrob Chemother. 2012;67:2401–8.
80. Gupta VK, Tiwari N, Gupta P, Verma S, Pal A, Srivastava SK, et al. A clerodane diterpene from Polyalthia longifolia as a modifying agent of the resistance of methicillin resistant Staphylococcus aureus. Phytomedicine. 2016;23:654–61.
81. Martins A, Vasas A, Viveiros M, Molnár J, Hohmann J, Amaral L, et al. Antibacterial properties of compounds isolated from Carpobrotus edulis. Int J Antimicrob Agents. 2011;37:438–44.
82. Bharate JB, Singh S, Wani A, Sharma S, Joshi P, Khan IA, et al. Discovery of 4-acetyl-3-(4-fluorophenyl)-1-(p-tolyl)-5-methylpyrrole as a dual inhibitor of human P-glycoprotein and Staphylococcus aureus nor A efflux pump. Org Biomol Chem. 2015;13:5424–31.
83. Schindler BD, Jacinto P, Kaatz GW. Inhibition of drug efflux pumps in Staphylococcus aureus: Current status of potentiating existing antibiotics. Future Microbiol. 2013;8:491–507.
84. Holler JG, Slotved HC, Mølgaard P, Olsen CE, Christensen SB. Chalcone inhibitors of the NorA efflux pump in Staphylococcus aureus whole cells and enriched everted membrane vesicles. Bioorg Med Chem. 2012;20:4514–21.
85. Haynes KM, Abdali N, Jhawar V, Zgurskaya HI, Parks JM, Green AT, et al. Identification and structure-activity relationships of novel compounds that potentiate the activities of antibiotics in Escherichia coli. J Med Chem. 2017;60:6205–19.

86. Cortez-Cordova J, Kumar A. Activity of the efflux pump inhibitor phenylalanine-arginine β-naphthylamide against the AdeFGH pump of Acinetobacter baumannii. Int J Antimicrob Agents. 2011;37:420–4.
87. Bohnert JA, Schuster S, Kern WV, Karcz T, Olejarz A, Kaczor A, et al. Novel piperazine arylideneimidazolones inhibit the AcrAB-tolC pump in Escherichia coli and simultaneously act as fluorescent membrane probes in a combined real-time influx and efflux assay. Antimicrob Agents Chemother. 2016;60:1974–83.
88. Machado D, Fernandes L, Costa SS, Cannalire R, Manfroni G, Tabarrini O, et al. Mode of action of the 2-phenylquinoline efflux inhibitor PQQ4R against Escherichia coli. Peer J. 2017;5:e3168.
89. Handzlik J, Szymańska E, Alibert S, Chevalier J, Otrębska E, Pękala E, et al. Search for new tools to combat Gram-negative resistant bacteria among amine derivatives of 5-arylidenehydantoin. Bioorg Med Chem. 2013;21:135–45.
90. Nelson ML, Levy SB. Reversal of tetracycline resistance mediated by different bacterial tetracycline resistance determinants by an inhibitor of the Tet(B) antiport protein. Antimicrob Agents Chemother. 1999;43:1719–24.
91. Tintino SR, Morais-Tintino CD, Campina FF, Pereira RL, Costa Mdo S, Braga MF, et al. Action of cholecalciferol and alpha-tocopherol on Staphylococcus aureus efflux pumps. EXCLI J. 2016;15:315–22.
92. Simons SO, Kristiansen JE, Hajos G, van der Laan T, Molnár J, Boeree MJ, et al. Activity of the efflux pump inhibitor SILA 421 against drug-resistant tuberculosis. Int J Antimicrob Agents. 2013;41:488–9.
93. Sabatini S, Kaatz GW, Rossolini GM, Brandini D, Fravolini A. From phenothiazine to 3-phenyl-1,4-benzothiazine derivatives as inhibitors of the Staphylococcus aureus NorA multidrug efflux pump. J Med Chem. 2008;51:4321–30.
94. Song Y, Qin R, Pan X, Ouyang Q, Liu T, Zhai Z, et al. Design of new antibacterial enhancers based on AcrB's structure and the evaluation of their antibacterial enhancement activity. Int J Mol Sci. 2016;17 pii: E1934.
95. Costa LM, de Macedo EV, Oliveira FA, Ferreira JH, Gutierrez SJ, Peláez WJ, et al. Inhibition of the NorA efflux pump of Staphylococcus aureus by synthetic riparins. J Appl Microbiol. 2016;121:1312–22.
96. Coêlho ML, Ferreira JH, de Siqueira Júnior JP, Kaatz GW, Barreto HM, de Carvalho Melo Cavalcante AA, et al. Inhibition of the NorA multi-drug transporter by oxygenated monoterpenes. Microb Pathog. 2016;99:173–7.
97. Wani NA, Singh S, Farooq S, Shankar S, Koul S, Khan IA, et al. Amino acid amides of piperic acid (PA) and 4-ethylpiperic acid (EPA) as NorA efflux pump inhibitors of Staphylococcus aureus. Bioorg Med Chem Lett. 2016;26:4174–8.
98. Fontaine F, Héquet A, Voisin-Chiret AS, Bouillon A, Lesnard A, Cresteil T, et al. Boronic species as promising inhibitors of the Staphylococcus aureus NorA efflux pump: Study of 6-substituted pyridine-3-boronic acid derivatives. Eur J Med Chem. 2015;95:185–98.
99. Zhang J, Sun Y, Wang Y, Lu M, He J, Liu J, et al. Non-antibiotic agent ginsenoside 20(S)-rh2 enhanced the antibacterial effects of ciprofloxacin in vitro and in vivo as a potential NorA inhibitor. Eur J Pharmacol. 2014;740:277–84.
100. Bohnert JA, Schuster S, Kern WV. Pimozide inhibits the AcrAB-tolC efflux pump in Escherichia coli. Open Microbiol J. 2013;7:83–6.
101. 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.
102. Lomovskaya O, Bostian KA. Practical applications and feasibility of efflux pump inhibitors in the clinic – A vision for applied use. Biochem Pharmacol. 2006;71:910–8.
103. Gandhi S, Fleet JL, Bailey DG, McArthur E, Wald R, Rehman F, et al. Calcium-channel blocker-clarithromycin drug interactions and acute kidney injury. JAMA. 2013;310:2544–53.

104. Nakajima A, Sugimoto Y, Yoneyama H, Nakae T. High-level fluoroquinolone resistance in Pseudomonas aeruginosa due to interplay of the MexAB-oprM efflux pump and the DNA gyrase mutation. Microbiol Immunol. 2002;46:391–5.