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Review article

Understanding multidrug resistance in *Staphylococcus aureus*: The role of efflux pumps and key protein structures

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Abstract

A rising health concern is the occurrence of illnesses brought on by multidrug-resistant bacteria in healthcare facilities or in public spaces. The growth of microbial biofilms and efflux pumps are the main causes of *S. aureus's* antibiotic resistance. Multidrug EPs cells utilizing proteins to detoxify from toxic substances, appear to be essential in the development of this drug-resistant bacteria. Through the extrusion of numerous unrelated chemicals, experimental data have demonstrated their role in reduced antibacterial resistance in bacteria as well as the possible significance in the emergence of resistant phenotypes. Efflux systems plays an important role in MDR resistance in addition to helping to transport molecules that are important for cell signaling, and because they are common, there is a real danger that we could soon return to a time before antibiotics. According to recent research, cells may use the efflux systems as the initial step of defence to prevent drugs from reaching deadly concentrations while waiting for a stable, more effective modification to occur. The primary facilitator superfamily-belonging *S. aureus* efflux pump *NorA* imparts resistance to various substrates. Even though many EPI i.e inhibitors of the efflux pump have been found, from them, no one has received clinical approval because of unfavourable toxicity. In this review article, we will discuss the current understanding of the MDR efflux pump and its clinical importance, concentrating on current discoveries on the efflux system of *S. aureus*, and the regulation of efflux pump based on protein/gene expression, and emphasize significant genes and proteins along with their PDB IDs.

Introduction

One of the defining moments in medicine in the 20th century was the development of antibiotics for the treatment of various infections. However, the first germs exhibiting antibiotic resistance were described not long after they were first used in clinical settings. But since, the development of new antibiotics has coincided with an ongoing increase in the number of microbial strains that are resistant to them as well as a diversity of strategies employed by bacteria to evade their fatal effects. Today, each class of widely used antibiotics has at least one documented mechanism of resistance [1]. Additionally, many bacterial species exhibit phenotypes that are multi-resistant. Numerous of these multidrug-resistant (MDR) bacteria can result in infections that are fatal, which makes them a serious issue for both the community and the hospital [2, 3].

One of the main bacterial pathogens, Gram-positive *Staphylococcus aureus*, can cause infections that are mild to fatal [4]. *S. aureus* exhibits a surprising range of resistance pathways along with its potential pathogenicity [5]. The MRSA i.e methicillin-resistant *S. aureus* strains, which have become increasingly isolated from the general population and are resistant to every type of β-lactam antibiotics, are a

serious issue. For many years, these strains were a major cause of outbreaks in nosocomial settings [6].

There are many ways that bacteria can resist antibiotics, including degradation or alteration of the antibiotic, switching the antibiotic's bacterial target, safeguarding the target, and lowering the intracellular concentration of the antibiotic through either reduced permeability to cell wall or antibiotic efflux from the cell. Resistance mediated by efflux, in contrast to the other recognised mechanisms, has received less attention [7].

S. aureus is a persistent pathogen that silently persists as our natural flora but occasionally poses a threat to our lives. Its multi-drug resistance phenotype makes it one of the most unbeatable pathogenic bacteria in the history of antibiotic treatment, in addition to its capacity to outsmart human immune system. Almost every antibiotic created since the 1940s was defeated by it. The first MRSA was discovered in clinical isolates of S. aureus in 1961 [98]. Methicillin and vancomycin resistance are the two most notable antibiotic resistances that *Staphylococcus aureus* has attained. A special staphylococcal mobile genetic element facilitated the interspecies transfer of the mecA gene from an ancestral Staphylococcus species to S. aureus, resulting in methicillin resistance. By horizontally transferring a plasmid-born vanA-gene transposon from *vancomycin-resistant Enteriococcus* to *S. aureus* across the genus barrier, vancomycin resistance was attained. The other kind of vancomycin resistance, known as VISA, is brought about by adaptive mutations that are integrated into the genes that control the physiology of bacterial cells [8,9]. Comparative genomics of paired *S. aureus* isolates has been used in several studies to identify mutations that arise in the resistant strain relative to the parent strain. One of the most notable of these studies was conducted by Mwangi et al., which found that accumulating mutations in the increasingly resistant strain were linked to increasing vancomycin resistance in successive clinical isolates of *S. aureus* [9].

The topic has, however, become more popular as we have come to understand that a large number of efflux pumps have the ability to remove from the cell a variety of different classes of antimicrobial compounds, which promotes the emergence of resistant strains phenotypes [8]. Drugs may be "accidental substrates" of these transporters due to natural involvement of efflux system in bacteria's ability to eliminate harmful endogenous metabolites, secrete virulence inhibitors, and respond to stress [9, 10].

Efflux pump

When tetracycline resistance in enterobacteria was found to be a mechanism in 1980, antibiotic efflux was first identified. Over the years, bacterial resistance to almost all antibiotics has been associated with efflux mechanisms. The zones of inhibition (MIC) of substrate antibiotics is typically two to eight times higher for bacteria overexpressing an efflux pump than for a susceptible strain of that species [11].

Efflux system in bacteria

Bacterial efflux systems can either be specialized, extruding only one antibiotic or class of antibiotics, or they can be multidrug-resistant efflux pumps, capable of extruding numerous classes of antimicrobial chemicals. The major facilitator superfamily (MFS), the small multidrug resistance (SMR) family, the multidrug and toxic compound extrusion (MATE) family, the resistance-nodulation-cell division (RND) superfamily, and the adenosine triphosphate (ATP)-binding cassette (ABC) superfamily are the five families that these MDR efflux systems are divided into based on their energy requirements and structural characteristics. The transporters of the first four families are secondary transporters that drive the extrusion of their substrates by an antiport H+: drug mechanism, with the exception of the family MATE, which may also take energy from the sodium membrane gradient. In contrast, the ABC superfamily of transporters are the main transporters that propel the ejection of their substrates using ATP as given in figure 1 [12].

Antimicrobial resistance mediated by efflux pump

Changes in drug targets, structural alterations or drug degradation, reduced outer membrane proteins permeability to stop medications to enter cells, and improved transporters are the basis for antimicrobial resistance mechanisms.to lower medication concentrations inside cells. It was previously thought that protein of outer membrane and efflux pumps did not work together to reduce intracellular drug concentrations. In *Burkholderia thailandensis*, a recent study discovered a connection of efflux pumps and the membrane permeability barrier [14]. In reality, efflux pump overexpression is an important factor in the development of MDR as well as antimicrobial resistance. The creation of efflux pump inhibitors depends critically on our knowledge of the chemical structures of pumps and the important drug-binding sites on those molecules. Following a thorough study of the architectures of various efflux pump families, it will be briefly described [15, 16].

Efflux pumps role in virulence and formation

It has been demonstrated that bile salts and other antimicrobials produced from the host facilitate colonisation and enhance bacterial adaptation to the host digestive system through efflux [17]. The RND Efflux pump *AcrAB-TolC*, which is mostly implicated in drug efflux in *E. coli*, can also pass on bile salt resistance [18]. Complex microbial communities known as biofilms adhere to many surfaces, including implanted gadgets like urine catheters. It is commonly known that bacteria that have formed biofilms are more susceptible to antibiotics than germs in the water. In various bacterial species, the connection between the antimicrobial resistance of biofilm and EPs has been documented [19].

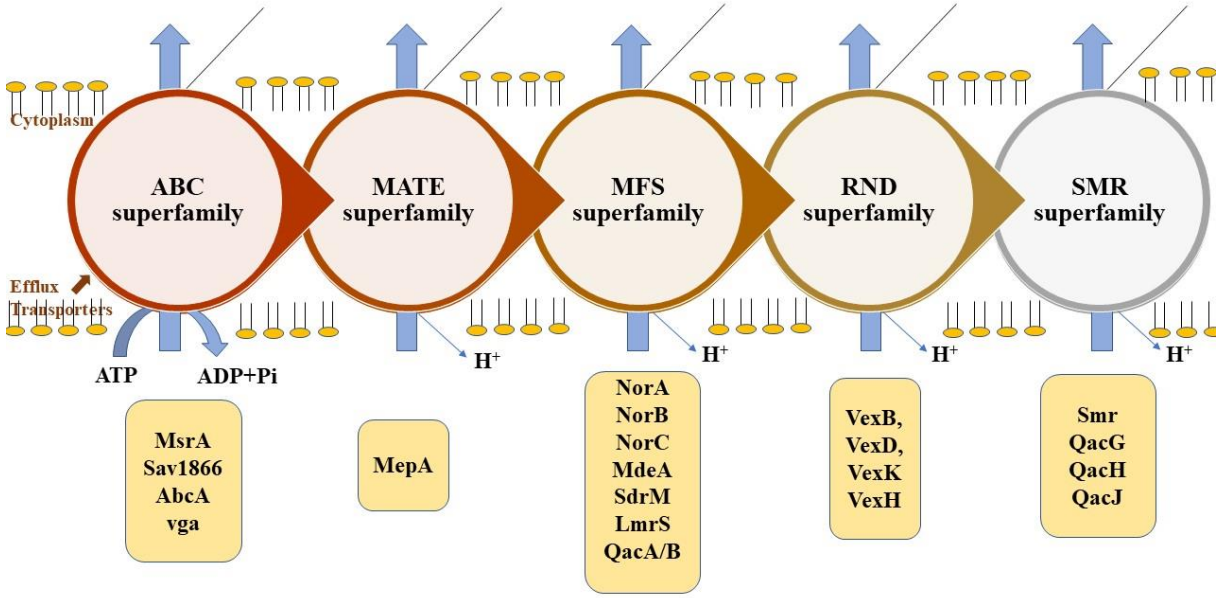


Figure 1. Five classes of multidrug efflux pumps with examples in *S. aureus* [13].

Multidrug-resistant efflux pumps encoded by chromosomes

NorA

On *S. aureus NorA* is the extensively researched efflux systems drug efflux pump. A resistant isolate of fluoroquinolone found in a Japan hospital in 1986 was the first to carry the gene *NorA* that encodes it [20]. With three *norA* alleles that have been reported to far and nucleotide sequence differences of up to 10%, the *norA* gene exhibits some genetic variation [21, 22, 23]. The 388 amino acid protein *NorA*, which is part of the MFS and has 12 transmembrane segments, is identical to the multidrug *Bmr* efflux pump from *B. subtilis* in 44% of the ways and to the tetracycline *Tet(A)* efflux pump from *E. coli* in 24% of the ways [24, 25].

According to several investigations, *NorA* can extrude a wide variety of chemically different molecules, including fluoroquinolones (hydrophilic) like ciprofloxacin and norfloxacin, ethidium bromide like dye, and quaternary ammonium (biocide) compounds [26]. Since *norA* is known to express at a basal level, this lower sensitivity to certain antimicrobial agents can be partially explained [27]. Through the enhanced expression of the *norA*, efflux mediated by *NorA* has been linked to greater resistance to fluoroquinolones, biocides, and dyes [26, 29, 30]. Depending on the mutations that have been acquired in the promoter region of gene *NorA*, this enhanced expression may be either constitutive or inducible [28]. The details of efflux pumps are listed in Table 1.

NorB

The proof for existence of additional mechanisms of efflux in the *S. aureus* chromosome has been accumulated since the initial experiments in *NorA* [31, 32]. The *NorA* and *QacA S. aureus* efflux pumps, as well as the *Blt* efflux pumps (41%) and *Bmr* of *B. subtilis* (30%), share structural similarities with the efflux pump *NorB*. It is a 463 amino acid MFS proton-driven efflux pump with 12 segments of transmembrane. *NorB* imparts resistance to some substrates of NorA, including the hydrophobic fluoroquinolones moxifloxacin and sparfloxacin, the tetraphenylphosphonium biocide and cetrimide, and tetracycline. It also imparts resistance to substrates of non-*NorA*, including the hydrophilic norfloxacin and ciprofloxacin [33].

NorB may have a role in the pathogenesis of staphylococcal bacteria, according to research using subcutaneous abscess model of mouse that demonstrated its importance for *S. aureus* competitiveness [34]. According to subsequent investigations from the same team, *NorB* may have a role in *S. aureus* reaction to acidic shock and decreased airflow, conditions that led to the overexpression of the *norB* gene [35, 36].

NorC

The *norC* chromosomal gene encodes efflux pump *norC*. Its an MFS protein with 462 amino acids and 12 transmembrane segments that is 61% identical to *NorB* [37]. *NorC* is linked to low-level resistance to ciprofloxacin, moxifloxacin, garenoxacin, and the dye rhodamine, as well as hydrophilic and hydrophobic fluoroquinolones [37, 38]. Studies have shown that *norC* overexpression is necessary to establish low-level resistance because *norC* wild-type expression does not appear to be adequate to influence the sensitivity to these compounds [37].

Table 1. MDR efflux pump of chromosomes and plasmid along with family, substrate and regulators.

Sr. No.	Efflux pump	Substrate for efflux pump	Efflux pump family	Regulator of efflux pump	References
	Multidrug-resistant efflux pumps encoded by chromosomes				
1	*NorA*	Ciprofloxacin, norfloxacin, tetraphenylphosphonium, benzalkonium chloride, ethidium bromide, rhodamine	MFS	*MgrA, NorR, NorG**	2, 21
2	*NorB*	Ciprofloxacin, norfloxacin, moxifloxacin, sparfloxacin, Tetracycline, tetraphenyl phosphonium, cetrimide ethidium bromide	MFS	*MgrA, NorG*	31, 32, 33
3	*NorC*	Ciprofloxacin and moxifloxacin rhodamine	MFS	*MgrA, NorG*	37, 38
4	*MepA*	Fluoroquinolones (e.g. hydrophilic: ciprofloxacin, sparfloxacin, norfloxacin moxifloxacin, Tigecycline, tetraphenylphosphonium, benzalkonium chloride, cetrimide, ethidium bromide)	MATE	*MepR*	39
5	*MdeA*	Ciprofloxacin, mupirocin, norfloxacin, fusidic acid, Virginiamycin, novobiocin, tetraphenylphosphonium, benzalkonium chloride, dequalinium, ethidium bromide	MFS	Not identified	41, 42
6	*SepA*	benzalk-onium chloride, chlorhexidine gluconate and the dye acriflavine	N/I	Not identified	43
7	*SdrM*	Norfloxacin, ethidium bromide, acriflavine	MFS	Not identified	44
8	*LmrS*	Erythromycin, linezolid, choramphenicol, fusidic acid kanamycin, florfenicol Trimethoprim, tetraphenylphosphonium, sodium docecyl sulphate, ethidium bromide	MFS	Not identified	45
	Multidrug-resistant efflux pumps encoded by plasmid				
9	*QacA/B*	Benzalkonium chloride, dequalinium Diamidines, Biguanidines, tetraphenylphosphonium, ethidium bromide, rhodamine	MFS	*QacR*	46, 47, 48
10	*Smr*	Cetrimide, benzalkonium chloride, ethidium bromide	SMR	Not identified	53, 54, 57
11	*QacG*	Benzalkonium chloride, cetyltrimethylammonium, ethidium bromide	SMR	Not identified	60
12	*QacH*	Benzalkonium chloride, cetyltrimethyl ammonium, Proflavin	SMR	Not identified	61
13	*QacJ*	Benzalkonium chloride, cetyltrimethyl ammonium, ethidium bromide	SMR	Not identified	62

MepA

MepA was discovered in research using mutants of *S. aureus* with *norA* disrupted [39]. It was the first multidrug transporter from the MATE family to be described in *S. aureus*. It is encoded by the chromosomal *mepA* gene. 12 transmembrane segments make up this 451amino acid protein, which shares 26% and 21% of its amino acid sequence with the *CdeA* from *Clostridium difficile* and NorM of *Vibrio parahaemolyticus* (MATE) transporter respectively. MepA was discovered to be linked to an Resistant phenotype, which confers Reduced resistance to quaternary ammonium substances like cetrimide, benzalkonium chloride, tetraphenyl-phosphonium, dequalinium, and ethidium bromide dye, as well as to pentamidine, chlorhexidine, and tigecycline, a glycylcycline antibiotic. Norfloxacin and ciprofloxacin were found to be ineffective substrate of *MepA gene* [39, 40]. The *mepRAB*

operon contains the *mepA* gene. The encoded *MepR* protein shares similarities with *MarR* family regulatory proteins, according to sequence analysis. *MepB* and other proteins with recognized functions were not found to have any significant similarities, and there was no correlation between the *MepB* gene and Resistant Multidrug phenotypes [39].

mdeA

An open expression library of the genome of *S. aureus* revealed the chromosomal *mdeA* gene, which encodes the efflux pump *MdeA*. *MdeA* is a member of the MFS, has 479 amino acids, and 14 transmembrane segments, and uses the proton motive force to energise the transport of its substrates. *MdeA* and the efflux pump *LmrB* from *B. subtilis*, *EmrB* from *E. coli*, and *QacA* from S. aureus share 37% of their genetic makeup. The overexpression of *mdeA* has been connected to increased resistance to the biocides (e.g. benzalkonium chloride, dequalinium, and tetraphenylphosphonium), the dye (e.g. ethidium bromide), and the antibiotics (e.g. virginiamycin, novobiocin, mupirocin, and fusidic acid). Mutations that occur in the *mdeA* promoter area can cause the overexpression of *mdeA*, but they only slightly increase the phenotype of MDR resistance [41]. The norfloxacin and ciprofloxacin (fluoroquinolones) are less active substrates of this pump, according to a subsequent study [42].

SepA

As an efflux pump, the *SepA* protein, which is coded by the chromosomal *sepA* gene, confers Reduced antiseptic resistance (e.g. benzalkonium chloride, chlorhexidine gluconate, and the acriflavine dye. This transporter has four putative transmembrane segments and 157 amino acids, which are shared by SMR family transporters. *SepA* lacks the conserved motifs of this family even though several residues essential for the transport specificity and the antiport H+: drug are present in a different place., which raises the possibility that it may be a member of an unidentified family of transporters [43].

SdrM

The chromosomal gene *sdrM* codes for the efflux pump *SdrM.* The pumps *NorB* and *QacA S. aureus* Resistant efflux share 23% and 21% of their identity with SdrM, respectively. Sequence research suggests that SdrM, which has 14 transmembrane segments, maybe a member of the MFS. By encouraging an energy-dependent outflow of these substances, it was demonstrated that this efflux pump is linked to Reduced resistance to ethidium bromide, acriflavine, and norfloxacin [44].

LmrS

lincomycin resistance protein *S. aureus* i.e *LmrS* was recently characterized by Floyd and colleagues. t is 39% identical to the *B. subtilis* lincomycin resistance protein *LmrB* and 25% identical to the efflux pumps *FarB* (*Neisseria gonorrhoeae*) and *EmrB* (*E. coli*) The improved linezolid resistance, sodium dodecyl sulphate, tetraphenyl phosphonium chloride, chloramphenicol, and trimethoprim, was attributed to *LmrS,* a 480 amino acid MFS protein with 14 putative membrane-spanning domains [45].

Multidrug-resistant efflux pumps encoded by plasmid

QacA/B

Clinical isolates of *S. aureus* carries pSK1 plasmid, a resistance-conferring gene to a variety of disinfectants and antiseptics was discovered in the early 1980s [46]. The *QacA gene* member of the MFS, which has 514 amino acids and 14 transmembrane segments, is encoded by this gene, which was later given the name *qacA* [47]. Large conjugative plasmids from coagulase-negative staphylococci as well as strains of S. aureus include the *qacA* gene [48].

From 12 different chemical classes more than 30 mono and divalent cations (lipophilic), including dyes (e.g. ethidium bromide and rhodamine), quaternary ammonium compounds (e.g. tetraphenyl phosphonium, benzalkonium chloride, and dequalinium, diamidines (e.g. DPAI and pentaamine), biguanidines (guanylhydrazones, and chlorhexidine) are used. The proton motive force drives the transport of these substrates through an antiport H+: drug mechanism [49-52].

Smr

The smr efflux pump gene, which confers resistance of ethidium bromide and antiseptic, was discovered in numerous plasmids by several groups in the late 1980s. The determinants identified by these authors as belonging to this gene were given the names *ebr, qacC/D,* or *smr* [53-55] but sequencing examination revealed that these all determinants identical. *Smr* gene, which encodes the efflux pump Smr, can be seen in *S. aureus* as well as *staphylococci* (coagulase-negative) in the form of tiny non-conjugative plasmids (pSK89) or big conjugative plasmids (pSK41) [56, 57].

Smr, which comprises 107 amino acids and four transmembrane segments, is a member of the SMR family and uses the proton motive force to energise the transport of toxic chemicals, according to hydropathy research. Comparing this efflux pump to *QacA/B*, a smaller subset of chemicals cationic dyes (ethidium bromide) and quaternary ammonium compounds (benzalkonium chloride) convey reduced resistance. *Smr* may undertake efflux, as demonstrated by in vitro transport tests using pure *Smr* gene and a site-directed mutagenesis, albeit it is unclear whether this is done as an oligomer or monomer as is the case for other efflux pumps in the family of SMR [58, 59].

QacG

S. aureus isolates gathered from the food sector included the efflux pump gene *qacG.* The plasmid pST94 (2.3 kb) contained the gene in question. It codes the efflux pump

QacG, a member of the SMR of transporters with one hundred and seven amino acids and four segments of transmembrane [60].

QacH

QacH is 69.2% identical to *smr* efflux pump. The *S. saprophyticus* food sector strain contained a 2.4 kb isolated plasmid that contained the determinant *qacH* for the first time. The efflux pump *QacH*, which has one hundred and seven amino acids and four segments of transmembrane. and is a SMR member, is encoded by the *qacH*, which shares 76% of its nucleotides with the *smr* gene and 70% of its nucleotides with the *qacG* gene [61].

QacJ

S. aureus, *S. simulans*, and *S. intermedius* staphylococcal species all obtained from horses, were discovered to share the qacJ efflux pump gene on a plasmid (2.65 kb). The encoded efflux pump *QacJ* is 72.5% identical to Smr, and 73.4% and 82.6% identical to *QacH* and *QacG* efflux pumps respectively. It is a member of the SMR and has 107 amino acids and four segments of transmembrane. All of these pumps have equal specificities for substrate, imparting same levels of resistance to ethidium bromide, benzalkonium chloride, and cetyltrimethylammonium bromide. This is true even though the *QacG/H/J* amino acid having different sequences than *Smr* [62].

Clinical values and significance of efflux pumps

Efflux pump inhibitors are prospective antibacterial medicines because of the extensive distribution of efflux pump gene in bacteria *S. aureus* and the strong link with resistance to antibiotics in the clinic. Inhibiting efflux pumps is anticipated to have the following effects:

i) Reactivating antibiotics that were previously made inactive by the extrusion method;
ii) Lowering the frequency of occurrence of mutants(resistant); and
iii) Lowering the sensitivity of the bacteria, as shown in figure number 2.

Over the last ten years lot of research have been undertaken to find efflux pump inhibitors that are effective against *S. aureus*, with a particular focus on *NorA* [63,64]. Reserpine and phenothiazines are two antipsychotic/antihypertensive medications that have been shown to inhibit *S. aureus* efflux pumps, although their use has been restricted due to their cytotoxicity [65].

Bacteria's natural defensive mechanism against dangerous compounds in their ecological environment is the efflux of antibacterial agents. In order to compete with other bacterial pathogens, eukaryotic hosts, and other bacteria, bacterial pathogens have evolved to withstand a variety of antibiotic compounds. Since efflux pumps are the initial step of defence against antibacterial agents, their inherent function may be a defence against such naturally occurring compounds. While the intrinsic activity function of efflux pumps has primarily been studied in species of Gram-negative and Gram-positive (*S. aureus)* [66,67]. Drug efflux-mediated clinically meaningful resistance in bacteria can include biocides, antibiotics, or both. The extrusion of multiple classes of antibiotics by MDR efflux pumps, like the ones discussed in this review, or efflux systems that can extrude a single class of antibiotics, like the Tet determinants that transmit resistance to tetracycline, are examples of efflux-mediated antibiotic resistance. A multidrug resistance phenotype might arise from the overexpression of MDR efflux pumps because of their promiscuous substrate selectivity, which can increase resistance to many antibiotic classes while simultaneously decreasing susceptibility to biocides [112,113].

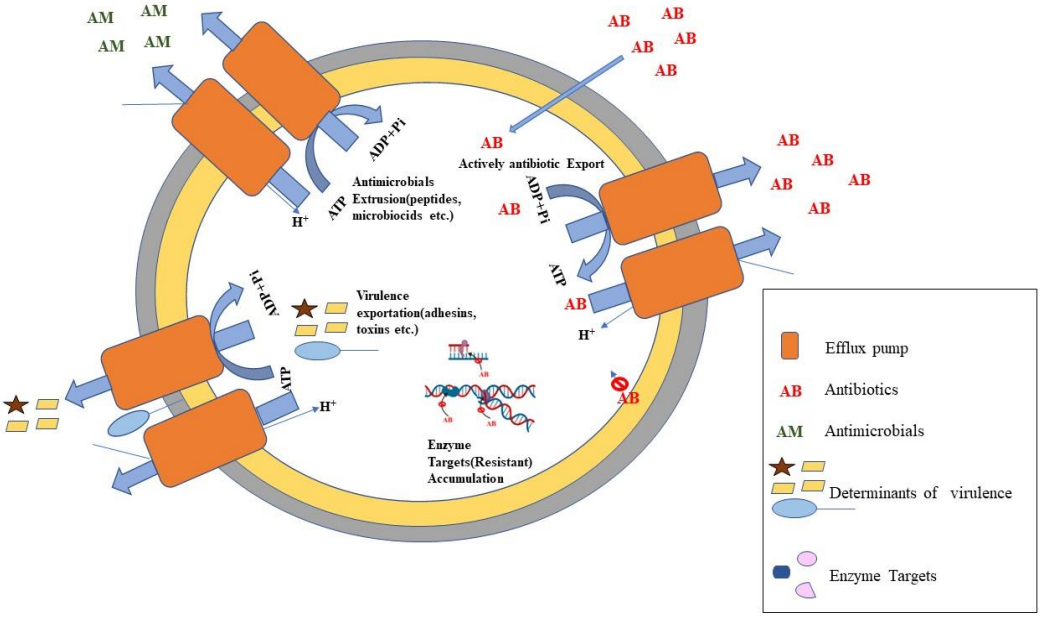


Figure 2. Clinical significance of bacterial EPs function [66, 67].

Assessment of efflux activity

One must ensure that the methodology used is the most suitable to reveal efflux activity in order to completely determine the function that a particular efflux system plays in antibiotic and/or biocide resistance. The majority of research on efflux-mediated resistance in clinical isolates of *S. aureus* uses the so-called efflux inhibitors, which are substances that have been shown to impede efflux activity, to lower the antibiotic minimum inhibitory concentration (MIC) [101, 102]. This method is time-consuming and depends on how susceptible the efflux system or systems are to that specific inhibitor, which can vary greatly and for which the cellular mechanism of action is typically still unclear [103-105].

Ethidium bromide, an extensive variety of efflux pump substrate, has been investigated more recently as a means of measuring efflux activity in *S. aureus* cells. The Ethidium Bromide-Agar Cartwheel Method, which assesses the cells' ability to retain/extrude ethidium bromide after an overnight stay [106], the measurement of ethidium bromide MICs to identify *S. aureus* strains that exhibit increased efflux activity [107, 108] in the the presence or absence of efflux inhibitors [109, 110], or assays that directly assess the efflux activity using real-time fluorometry [111] are examples of how this substance can be used as a marker for the indirect evaluation of efflux activity.

Regulation and application of efflux pumps related to protein/*gene* expression

Mechanism of efflux pump regulation

Various regulatory systems and proteins also have a role in controlling efflux pump expression, in addition to exposure to disinfectants or antibacterials. Currently, single MDR pump regulatory proteins are mostly categorised into four groups: *MarR, MerR, AraC*, and *TetR* [68]. Both domains ligand-binding and DNA-binding are present in these regulatory proteins. *tet* genes related to efflux, which provide tetracyclines resistance, are controlled in expression by the *TetR*, a transcriptional repressor that depends on substrate [69]. Overexpression of the new MATE efflux pump *FepA* leads to fluoroquinolone resistance. The norfloxacin and ciprofloxacin MIC values in *L. monocytogenes* are raised as a result of a *TetR*-type repressor *FepR*, which controls it [70]. MgrA controls the ABC efflux pump AbcA, the non-MDR tetracycline efflux pump Tet38, and the three S. aureus MDR efflux pumps NorA, NorB, and NorC [113].

For instance, it is known that the regulation systems*, AcrR, Rob, EnvR, MprA, MarA, PhoP,* and *RpoE* all have a role in the regulation of *AcrAB* (*E. coli).* These regulatory mechanisms are triggered by signals from the environment, such as pH, antimicrobial concentrations, organic solvents, metal ions, growth phase, and oxidative stress [71].

Certain regulators can also alter the transcription of the genes that code for certain MDR efflux pumps in *S. aureus*. For *MepA* and *QacA/B*, which are regulated by *MepR* and *QacR,* respectively, this is true. Both of these regulators are sensors that bind to the MDR efflux pumps' substrates and trigger their expression, making them substrate-responsive regulators [113]. *MepR* is a self-repressive protein that attaches to the motif GTTAG, which is found in the promoter areas of *mepR* and *mepA*, as well as to sequences that contain pseudo palindromes. The *mepB* promoter region is not bound by *MepR* [114]. *MepR* binds to the *mepA* promoter more strongly than it does to the *mepR* promoter, and the ratio of this binding is most likely different [115].

The gene *qacR*, which is located just upstream of the genes qacA and qacB, which are transcribed differently from these genes, encodes *QacR*. *QacR* is a member of the TetR family of transcriptional repressors and has a helix-turn-helix DNA binding motif that is typical of regulatory proteins [116]. By attaching itself to the *qacA* promoter, *QacR* has been demonstrated to be a direct suppressor of the *qacA* gene's expression [117]. *QacR* attaches itself to a big inverted repeat that crosses over the *qacA/B* transcription start sites and is situated directly downstream of the *qacA/B* promoters [118].

Antibiotic residue detection using efflux pump proteins

Only two regulatory proteins, *TetR* and *TtgR*, are utilized to screen for antibiotic residues at this time; proteins related to efflux pump are still being studied for this purpose (belong to *TetR* family). Utilizing proteins involved in gene transcription, a concentration of low antibiotics might be detected efficiently [72]. Hyerim *et al.* proposed a bioreporter technique focused on *TetR* and the *TetR* promoter for detecting doxycycline using a green fluorescent protein (GFP) gene as a reporter gene. GFP could be expressed in significant numbers in response to 5nM doxycycline [73]. Tetracycline antibiotics may be precisely and promptly determined using an in-vitro indirect ELISA developed by Weber et al. utilizing *TetR-tetO*, with limit of detection 0.1 and 1.9 ng/mL of doxycycline and tetracycline, respectively [74]. Furthermore, Espinosa-Urgel *et al.* proposed a unique microbial biosensor centered on TtgR that estimates drug concentrations as low as 22 M with high fluorescence. According to research, drugs such as ceftazidime, ciprofloxacin, and tetracyclines which are *P. putida's* GFP-fused *TtgR*, a *TtgABC* efflux pump transcription regulator, react the most [75].

Antibiotic residue detection using *gene* expression

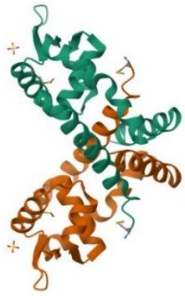
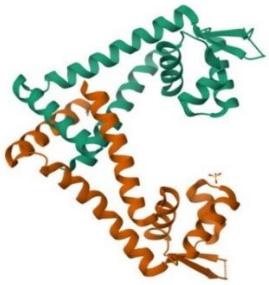
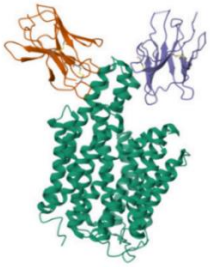
Resistant efflux pumps in microorganisms cause them to be naturally or unavoidably resistant to antimicrobial drugs. The efflux pumps' constituent or regulatory proteins, which serve as the first line of defence against medications and

ensure the bacteria's survival, are encoded by the resistance-related genes [76]. Antimicrobial resistance can be quickly detected using a variety of efflux pump genes, which can then be successfully confirmed using identification by PCR and determination of MIC. The RND pumps are capable of recognizing various substrates, causing the majority of medicines to be extruded and boosting antimicrobial resistance. Examples include *E. coli's Acr* pump, *A. baumannii's* Ade pump, and *P. aeruginosa's* Mex pump [77, 78].

Additionally, MICs and high levels of resistance are linked to several single-substrate efflux pumps. These single-substrate efflux proteins include the tetracycline-mediated efflux pump *TetA/TetO* in *E. coli* [79], the fluoroquinolones (hydrophilic) efflux pump *OqxAB* from *S. enterica* and *E. coli*, and *MacAB* the macrolide efflux pumps in *E. coli* and *Mef* in *S. pneumoniae* [80, 81].

Including PDB IDs for various efflux pumps in the review, article is highly significant as it provides researchers with precise structural information required for computational drug design and target-specific studies. These PDB IDs serve as a critical resource for understanding the three-dimensional structure and molecular dynamics of efflux pumps, enabling the identification of potential binding sites and the development of inhibitors to overcome multidrug resistance. By consolidating this information in a single table, the article facilitates streamlined access to essential data, promoting further research and innovation in targeting efflux pump proteins for therapeutic intervention. Table 2 and 3.

Table 2. Structure and PDB IDs of efflux gene regulators.

Efflux Pump regulators	PDB IDs	Description	Structure	References
Mgr A	2BV6	Crystal structure of MgrA, a global regulator and major virulence determinant in Staphylococcus aureus		[119]
Mep R	3ECO	Crystal structure of MepR, a transcription regulator of the Staphylococcus aureus multidrug efflux pump MepA		[120]
QacA	7Y58	CryoEM structure of QacA (D411N), an antibacterial efflux transporter from Staphylococcus aureus		[121]

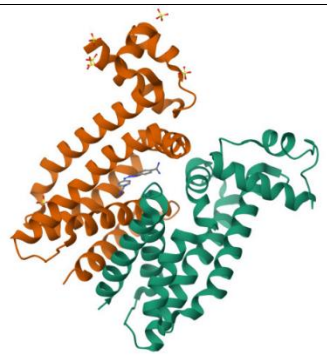
QacR	2GBY	Structure of QacR Multidrug Transcriptional Regulator Bound to Bivalent Diamidine Berenil		[122]

Table 3. PDB IDs for the various efflux pumps.

Sr. No	PDB ID	Description	Efflux pump	References
1	7LO7	*NorA* in complex with Fab25	*NorA*	84
2	7LO8	*NorA* in complex with Fab36	*NorA*	84
3	8TTE	Protonated state of *NorA* at pH 5.0	*NorA*	85
4	8TTF	*NorA* single mutant - E222Q at pH 7.5	*NorA*	85
5	8TTG	*NorA* double mutant - E222QD307N at pH 7.5	*NorA*	85
6	8TTH	*NorA* single mutant - D307N at pH 7.5	*NorA*	85
7	7D5Q	Structure of *NorC* transporter (K398A mutant) in an outward-open conformation in complex with a single-chain Indian camelid antibody	*NorC*	86
8	7D5P	Structure of *NorC* transporter in an outward-open conformation in complex with a single-chain Indian camelid antibody	*NorC*	86
9	4XYD	Nitric oxide reductase from *Roseobacter denitrificans* (*RdNOR*)	*NorC*	87
10	4LQE	Crystal Structure of *MepB*	*MepB*	88
11	3ECO	Crystal structure of *MepR*, a transcription regulator of the *Staphylococcus aureus* multidrug efflux pump *MepA*	*MepR*	89
12	5FFZ	*S. aureus MepR* bound to ethidium bromide	*MepR*	90
13	5FFX	*S. aureus MepR* G34K Mutant	*MepR*	91
14	4L9N	Crystal structure of *MepR* A103V mutant from multidrug resistant *S. aureus* clinical isolate 4L9N	*MepR*	92
15	5J44	Crystal structure of the Secreted Extracellular protein A (*SepA*) from *Shigella flexneri*	*SepA*	93
16	7Y58	CryoEM structure of *QacA* (D411N), an antibacterial efflux transporter from *Staphylococcus aureus*	*QacA*	94
17	2VKC	Solution structure of the B3BP Smr domain	*Smr*	95
18	3NPI	Crystal structure of a *TetR* family regulatory protein (DIP1788) from CORYNEBACTERIUM DIPHTHERIAE at 2.96 A resolution	*TetR*	96
19	2UXH	*TtgR* in complex with Quercetin	*TtgR*	97

Conclusions and future perspectives

Resistance to traditional antibiotics has grown significantly since the last few decades, posing a serious danger to antibacterial chemotherapy regimens used to treat a variety of persistent, life-threatening infections. There may someday be no viable antibiotics available for therapy due to the alarming rise in multidrug-resistant bacteria. This situation is referred to as "the post antibiotic age." Antibiotic active efflux, particularly through multidrug efflux pumps, is regarded as a significant mechanism of multidrug resistance for the emergence of resistance to antibiotic, and survival of bacteria in the environment in bacteria. Therefore, it makes sense to suppress bacterial efflux pumps in order to counteract multidrug resistance. By restoring the lost potency that active extrusion via pumps caused in existing antibiotics, efflux pumps inhibition is anticipated for improving their efficacy. Blocking efflux pumps would also lessen bacterial pathogenicity, the emergence of resistance to antibiotic, and bacterial survival in the surrounding [99].

Drug efflux pumps, an important factor in the resistance mechanism, has drawn a lot of interest from researchers working to tackle drug resistance. To improve the clinical efficiency of antibiotic, pathogenic *S. aureus*, MRSA can be treated with efflux pump inhibitor strategies. Due to these potential consequences, research on efflux pumps has been greatly accelerated, and *S. aureus* and other bacterial pathogens have benefited from the development of efflux pump inhibitors [100]. To create efficient efflux pump inhibitors, it is required to investigate the inhibitory mechanisms and conduct potential inhibitors testing by in-vivo studies. Additionally, a deeper comprehension of the design, mechanisms, control and relationship of the efflux pumps with clinical antibiotics resistance would make it possible to create better medications that are less vulnerable to bacterial resistance.

Researchers looking to investigate the structural Aspects and molecular of multidrug resistance will find PDB IDs helpful for important proteins and genes linked to the *S. aureus* efflux pumps. These structural understanding may help overcome current challenges in the war against antibiotic-resistant bacteria by enhancing the development of novel treatment approaches and targeted inhibitors. Future researcher can integrate the better understand the molecular and structural mechanisms of resistance by utilizing these PDB IDs, opening the door to more potential and practically applicable approaches.

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Competing interests

The authors declare no competing interests.

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Ethics approval and consent to participate

Not applicable

Data availability

All data generated or analyzed during this study are included in this published article.

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