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Review

Staphylococcus aureus antimicrobial efflux pumps and their inhibitors: recent developments

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Abstract: The microorganism *Staphylococcus aureus* is a notorious causative agent of bacterial infection. The widespread presence of this pathogen has caused significant morbidity and mortality rates in clinical healthcare settings and communities. Due to its increasingly frequent recalcitrant nature towards clinically available antimicrobial agents, the bacterium poses a considerable public health crisis. A significant bacterial mechanism of antimicrobial agent resistance includes multidrug efflux pump systems. These antimicrobial efflux determinants translate into several large superfamilies of transporters that share related amino acid sequences, similarities in three-dimensional structures, modes of energization, and solute transport catalysis across the membrane. Because of their ubiquitous nature and functional role in virulence, these multidrug transporters make good targets for inhibition. This review briefly summarizes recent key findings regarding multidrug efflux activity and modulation in the MATE, SMR, and MFS transporters.

Keywords: antimicrobial efflux; bacteria; efflux pump inhibitors; modulation; multidrug resistance; pathogens; *Staphylococcus aureus*

1. Introduction

The morbidity and mortality rates of *Staphylococcus aureus* are alarming [1]. First characterized, isolated, and named by Ogston [2], *S. aureus* has been well documented as a causative agent in many

infections [3]. In particular, methicillin-resistant *S. aureus* (MRSA) and multidrug-resistant variants have been exceptionally troublesome, and clinical case estimations are predicted to worsen with time [3–7]. As a pathogen, *S. aureus* has developed a series of antimicrobial resistance mechanisms to ensure its survival [8], and multidrug-resistant strains are known to compromise the clinical efficacy of chemotherapy against infection, posing a considerable public health concern [9]. Bacteriological machinery that confers antimicrobial and multidrug resistance can serve as potential targets for modulation to restore the efficacy of antimicrobial agents compromised by such resistance determinants in *S. aureus* [10,11].

2. Mechanisms of antimicrobial resistance in *S. aureus*

Of the various virulence factors associated with a clinical infection, antimicrobial resistance mechanisms possessed by *S. aureus* represent critical factors in determining clinical outcomes regarding morbidity and mortality [12]. *S. aureus* has amassed an impressive arsenal of antimicrobial resistance systems [13].

One of the first such resistance mechanisms involves the enzymatic degradation of the penicillin-derivative methicillin, a physiological characteristic associated with poor clinical outcomes in hospitalized patients with infection [14]. The production of the enzyme β-lactamase was demonstrated to hydrolyze the β-lactam ring, the active site of the β-lactam class of antimicrobial agents [15]. More recently, extended-spectrum β-lactamases (ESBLs) have been reported [16]. These ESBLs enjoy broad spectra for structurally-distinct molecular substrates and at significantly high levels of enzymatic activities [17]. Furthermore, ESBL-encoding determinants are transferable between bacterial species, especially to and from *S. aureus* clinical isolates [18].

A related antimicrobial resistance mechanism involves the well-characterized alterations in the cell wall [19], such as that reported in vancomycin-resistant *S. aureus* (VRSA), and those mediated by modulation of cell wall peptidoglycan synthetic enzymes, such as the well-studied glycosyltransferases [20]. These pathogens have been problematic in food processing industries, necessitating the development of new molecular detection methods for monitoring resistance determinants as they move through these environments [21].

Another bacterial resistance mechanism of *S. aureus* involves modifying the antimicrobial target [22]. One well-studied example of this type of resistance mechanism encompasses alterations of DNA gyrase, a target of the fluoroquinolone antimicrobials [23]. Similarly, alterations in the A subunit of the RNA polymerase enzyme confer resistance to the nucleic acid synthesis inhibitor class of compounds called rifamycins, such as rifampicin [24,25].

The antimicrobial resistance system that involves target protection represents another class of resistance mechanisms utilized by *S. aureus* [26]. One noteworthy antimicrobial resistance apparatus includes protecting the ribosome, a target of protein synthesis inhibitors such as tetracycline [27]. The Tet(M) and Tet(O) proteins bind the 30S subunit of the prokaryotic ribosome, preventing the action of the antibiotic on translational inhibition and permitting protein synthesis and bacterial growth [28–30].

More recently, the development of biofilms has provided a novel means of antimicrobial tolerance and persistence in *S. aureus* and other pathogens [11,31]. In particular, biofilm formation has been effectively measured using bioluminescent markers for biomass and physiological analyses, while chemical dyes, such as crystal violet, safranin, and resazurin, have been utilized to assess biofilm

structure [32]. Such new methods for assessing biofilm activity and integrity undoubtedly continue to be of clinical relevance in directing avenues for chemotherapy of infectious diseases.

Antimicrobial efflux is a prominent resistance mechanism in clinical isolates of *S. aureus* [3,10,33], (Figure 1). These exporters reside in the membrane and frequently extrude multiple structurally-distinct antimicrobial agents [34]. Some of these efflux pumps are energized by ATP hydrolysis in a primary active transport process discussed extensively elsewhere [35,36]. Other antimicrobial efflux pump systems are driven by electrochemically-based ion motive forces, such as those held by proton- or sodium-gradients, processes termed secondary active transport [37], and they represent bacterial systems that constitute suitable targets for modulation [10]. Because of their extensive presence in *S. aureus*, this review primarily considers recent developments concerning superfamilies of bacterial secondary active efflux pump systems.

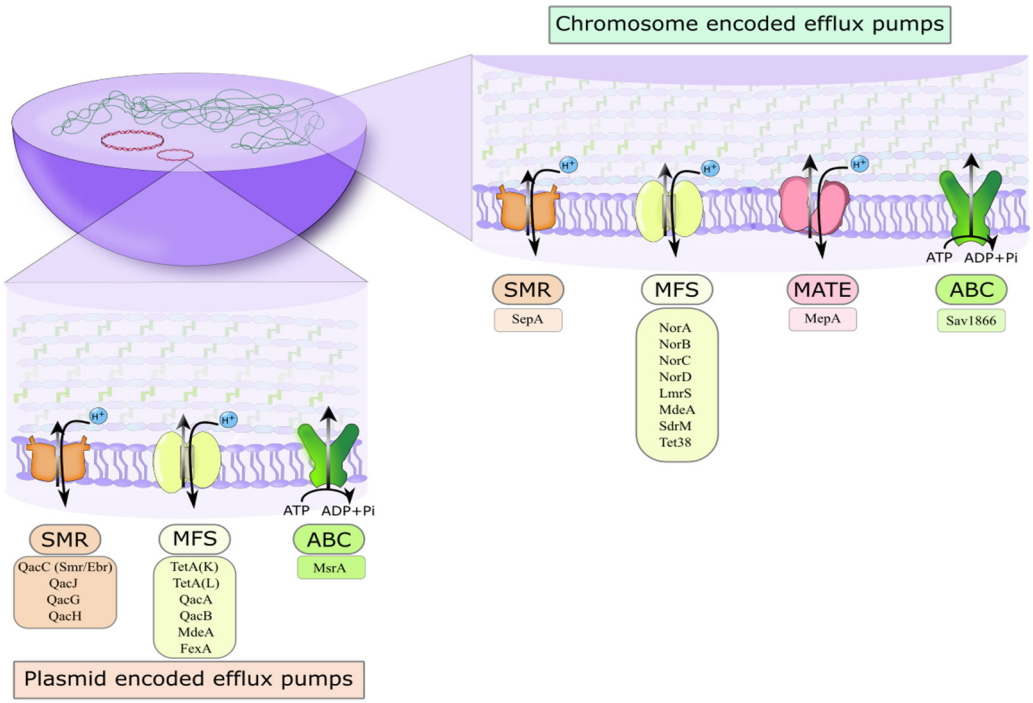

Figure 1. *Staphylococcus aureus* efflux pumps. Important drug efflux pumps of *S. aureus* belonging to MFS, MATE, SMR, and ABC families are encoded on the chromosome and plasmids.

3. MATE superfamily of multidrug efflux pumps in *S. aureus*

The MATE (Multidrug and Toxic Compound Extrusion) family of proteins possess 12 transmembrane helices comprising 400–500 amino acids similar to the MFS family of efflux pumps [38] and are widely distributed in bacteria, plants, and animals, although their functions are poorly understood. In plants and animals, MATE proteins are presumed to play essential roles in detoxifying cellular metabolites and excretion of xenobiotics [39]. In bacteria, the MATE proteins use the energy derived from H^+ or Na^+ electrochemical gradients to transport antimicrobial compounds outside the

cell, thereby lowering their intracellular concentrations [40]. Bacteria carrying MATE efflux proteins can resist diverse compounds, including antibiotics (aminoglycosides, fluoroquinolones), DNA-binding dyes such as ethidium bromide and acridine orange, and anticancer drugs. These efflux pumps were initially placed under the MFS family of proteins due to their structural and functional similarities. Based on the amino acid sequence similarity, the MATE family of proteins is broadly grouped into three groups represented by the prototype efflux pumps like Na^+-dependent NorM of *Vibrio parahaemolyticus*, H^+-dependent efflux pumps such as YdhE of *Escherichia coli*, and DinF of *Pyrococcus furiosus* (PfMATE), and the eukaryotic subfamilies [41]. The crystal structures of Na^+-dependent NorM from *V. cholerae* (NorM-VC) and *Neisseria gonorrhoeae* (NorM-NG) [42] and the H^+-dependent pumps DinF from *Pyrococcus furiosus* (PfMATE) and *Bacillus halodurans* (DinF-BH) [43,44] have been determined, forming the basis for the elucidation of molecular mechanisms underlying the efflux behavior of MATE proteins [40,45].

The staphylococcal efflux pump MepA belongs to the chromosomally-encoded MATE family of efflux proteins and is the only efflux pump under this group reported from *S. aureus* so far [46,47]. The gene encoding MepA is located on an operon *mepRAB* (multidrug export protein), which also has a gene coding for a transcriptional regulator protein MepR that binds to the promoter regions of both *mepA* and *mepR*, and the overexpression of *mepR* resulted in the reversal of MDR phenotype of *S. aureus* due to the transcriptional inhibition of *mepA* [46,48]. The predicted secondary structure of MepA has 12 transmembrane helices formed by 451 amino acids [48]. Due to the lack of homology with proteins of known function, the fundamental role of MepA in *Staphylococcus* physiology is largely obscure. At the amino acid level, MepA shows 26% identity with CdeA of *Clostridium difficile* and 21% identity with NorM of *Vibrio parahaemolyticus*, both belonging to the MATE family of proteins with fluoroquinolones as efflux substrates [48–50]. Multiple compounds act as substrates for MepA, including fluoroquinolones (norfloxacin, ciprofloxacin, moxifloxacin), tigecycline, benzalkonium, cetrimide, chlorhexidine, and ethidium bromide [46]. The low level of identity of MepA with NorM is also evident from its low affinity for fluoroquinolones compared to NorM. Efflux pump inhibitors (EPIs) such as reserpine, paroxetine, and certain phenothiazines and thioxanthenes inhibit the efflux activity of MepA [48,51]. Using site-directed mutagenesis and *in silico* modeling, Schindler et al. [52] predicted a substrate transport pathway involving helices 1, 2, 4, 7, 8, and 10 that form a large central cavity, with amino acid residues Ser-81, Ala-161, Met-291, and Ala-302 within the cavity assuming essential roles in substrate binding and the efflux activity [52]. More recently, cells harboring MepA exposed to the monoterpene estragole had lowered MICs for ethidium bromide and ciprofloxacin [53]. In the same study, estragole showed a similar reduction in the MIC for norfloxacin in *S. aureus* expressing NorA [53]. Another report showed that the synthetic compounds 1,8-naphthyridines sulfonamides synergized with ciprofloxacin or ethidium bromide in cells of *S. aureus* harboring MepA as measured by MIC assays [54]. Fluorescence emission analysis of ethidium bromide transport showed inhibition of drug efflux in MepA-containing cells with 2,3,4-trifluoro-*N*-(5-chloro-1,8-naphthyridin-2-yl)-benzenesulfonamide [54]. An evaluation of the molecular docking properties demonstrates that the efflux pump inhibition effect directly affects MepA [54]. A monocyclic monoterpene phytochemical, called limonene, was evaluated in *S. aureus* with MepA for efflux activity, demonstrating a direct inhibitory effect on drug transport [55]. In the same study, molecular docking analysis showed interactions of limonene with multiple amino acid residues of MepA [55]. The new work indicates that limonene is a suitable efflux pump inhibitor and suggests that it may be a suitable platform for developing new derivatives to enhance inhibitory modulation [55].

4. SMR superfamily of multidrug efflux pumps of *S. aureus*

The SMR (small multidrug resistance proteins) superfamily of efflux proteins in *S. aureus*, such as the QacC (Smr/Ebr), QacJ, QacG, and QacH, are plasmid-encoded, while SepA is encoded on the chromosome [3]. Small membrane proteins represent the SMR family of efflux proteins with 100–150 amino acid residues forming four transmembrane helices, and these are distinctly different from their MFS counterparts, QacA and QacB, with little or no sequence homology [56]. The plasmid-encoded Smr/Ebr (Staphylococcal multidrug resistance/Ethidium bromide resistance) protein was the first efflux pump discovered in *S. aureus* responsible for ethidium bromide resistance, which was subsequently renamed QacC [57,58]. QacC extrudes diverse biocides such as quaternary ammonium compounds, DNA-intercalating dyes, and phosphonium ions but differs from QacA/B in their inability to efflux acriflavine [57,58]. QacC has 107 amino acid residues in its 4 TMS, which form dimers across bacterial membranes, creating a pore-like structure that allows the substrate to pass through [59]. Using site-directed mutagenesis, Grinius and Goldberg [60] showed that a Glu-13 residue located on a hydrophobic domain of QacC is crucial for the drug/H^+ antiport activity, while Glu-24 is predicted to be responsible for drug specificity. Among the *qac* family of efflux protein conferring genes, *qacC* is highly conserved and is located on conjugative, rolling-circle replicating (RCR) plasmids with a novel gene transfer mechanism responsible for spreading the *qacC* gene [61,62]. The gene gets transferred between rolling-circle plasmids of variable backgrounds without the assistance of insertion sequences or other similar gene mobility mechanisms [62]. QacG was discovered as a 107 amino acid long efflux pump with 69.2% identity to QacC and encoded on a 2.3 kb pST94 resistance plasmid [63].

The QacH protein was first reported by Heir and colleagues in *Staphylococcus saprophyticus* as a 107 amino acid protein encoded on a 2.4-kb plasmid (p2H6) with 78% and 70% identity with Smr and the QacG proteins, respectively [64]. Homologous proteins of QacH in Gram-negative bacteria include QacE and EmrE, with about 40% similarity [65]. Bjorland and colleagues reported a 2.65 kb rolling circle plasmid pNVH01 in equine *Staphylococcus* species harboring the gene encoding a 107 amino acid efflux protein QacJ [66]. The QacJ protein is homologous with Smr/QacC (72.5% identity), QacG (82.6%), and QacH (73.4%). The pNVH01 plasmid carrying the *qacJ* gene is widely distributed in coagulase-positive and -negative *Staphylococcus* species that exhibit resistance to a wide range of biocides.

A comparison of amino acid sequences of over sixty SMR efflux proteins revealed the highly hydrophobic nature of these sequences with a highly conserved glutamate at position 14 across all sequences and TMS-specific motifs [67]. Paulsen et al. [68] performed *qacC-phoA* and *qacC-lacZ* fusions and mutational analysis to understand the functional roles of conserved amino acids in the QacC protein. This study attributed an essential role for Cys-42 in substrate recognition. Two other amino acid residues, Tyr-59 and Trp-62, were also proposed to have important functional roles in the efflux activity of QacC.

5. MFS multidrug efflux pumps of *S. aureus*

The major facilitator superfamily of solute transporters is one of the leading known constellations of related integral membrane proteins [69,70]. The transporters of this superfamily are known to share related primary sequences, highly conserved sequence motifs, and protein structures [71]. In terms of secondary structure, transport proteins of the MFS typically possess 12 or 14 membrane-spanning α-helices (Figure 2) [72]. The N- and C-termini of these transport systems reside on the cytoplasmic side of the membrane [73].

The members of the MFS differ in terms of the energetics that drive solute transport (i.e., passive or secondary active transport), the structurally diverse nature of their substrates (e.g., sugars, amino acids, antimicrobial agents, and ions), and directions of solute transport across the membrane (i.e., symport, uniport or antiport) [6,74]

Nevertheless, the similarities in sequences and primary and secondary structures predict that the transporters of the MFS undergo transport across the membrane by a shared catalytic mechanism [74,75]. A unifying principle that ties these seemingly disparate properties, i.e., similarities in sequence but differences in substrate profiles, modes of energetics, and transport direction, lies in discovering highly conserved amino acid sequence motifs [71,73–77].

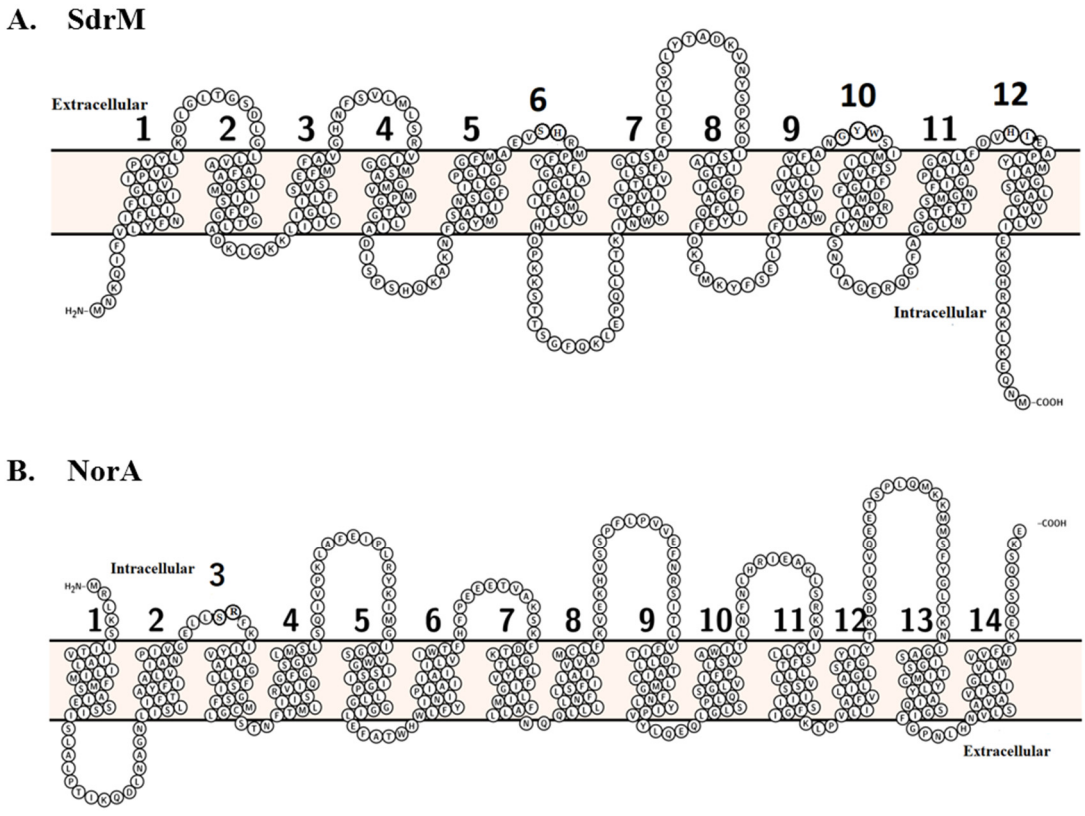

Figure 2. Major facilitator superfamily transporter predicted secondary structures. (A) The predicted two-dimensional structures in the membrane of (A) SdrM [59] and (B) NorA [78] are shown as generated by Protter [79].

Based on three-dimensional structural studies, transporters of the MFS are known to harbor two asymmetric domains consisting of a C-terminal bundle composed of helices 7 through 14 and N-terminal bundles characterized by helices 1 through 6, respectively [72]. Members of less well-characterized MFS transporters are also thought to harbor 12 or 14 membrane-spanning segments (Figure 3). Thus, studies of newly discovered transporters of the MFS benefit from the structural insights. The MFS transporters contain exposed cavities that alternately orient their substrate binding sites to either side of the membrane during solute transport [80]. These transport proteins are thought to operate by forming so-called inverted topological repeats composed of three-helix units repeated in tandem along the length of the transporter and functionally connected to a multi-helical hinge system to carry out conformational changes during transport [81,82]. Interestingly, these systems, i.e., alternating access, the inverted topology units, and the molecular hinge, appear to be unified by conserved amino acid sequence motifs that have been demonstrated to be essential for bacterial multidrug resistance [83].

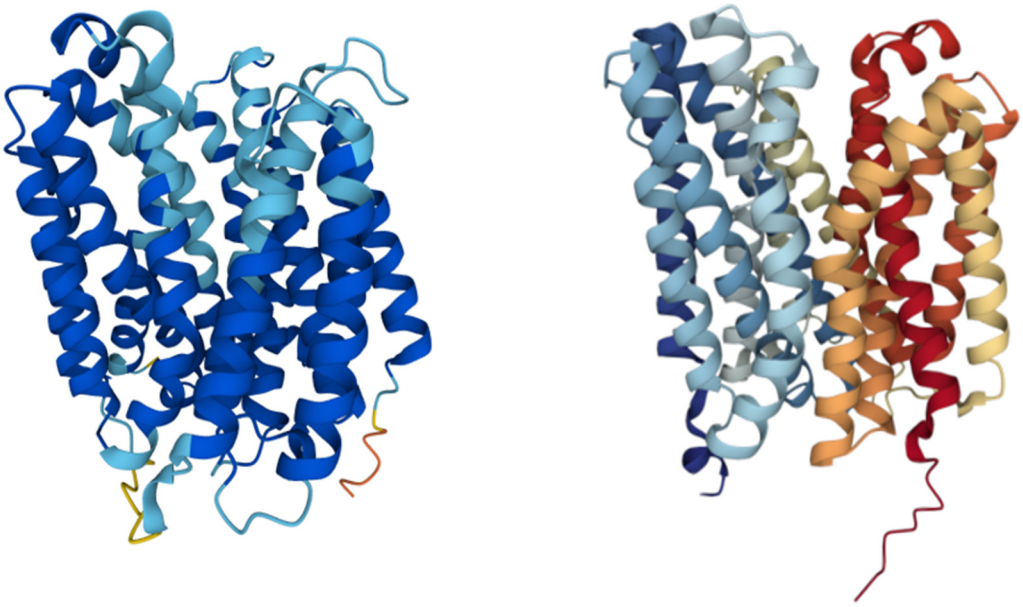

Figure 3. Predicted three-dimensional structures of major facilitator superfamily transporters. On the left is the predicted structure for SdrM (Q99S97, SDRM_STAAN) from *S. aureus*, a multidrug efflux pump with 14 predicted transmembrane domains [59]. The predicted structure for NorB from *S. aureus* is on the right with 12 putative membrane-spanning segments (A0A6B5H8J6_STAAU) [84].

One of the first drug efflux pump systems to be characterized in *S. aureus* was reported to export tetracycline actively and was demonstrated to be extra-chromosomally encoded on plasmid-based mobile genetic elements [85]. These plasmid-encoded determinants are TetA(K) and TetA(L) [86,87]. Shortly afterward, the genome-encoded NorA efflux pump was discovered [78]. Initially shown to export norfloxacin, a fluoroquinolone, the NorA transporter was demonstrated to transport multiple structurally different antimicrobial agents, becoming a well-known multidrug efflux pump of central

importance [88,89]. Related determinants encoded NorB, NorC, and NorD [90–92]. Another notable multidrug efflux pump from *S. aureus* is the plasmid-encoded QacA, known for its export of a variety of seemingly unrelated variety of antimicrobial substrates [93]. Related transporters from *S. aureus* were denoted QacB, QacC, QacG, and QacJ, all plasmid-based [56]. The MdeA transporter was reported to be encoded as a genomic element and harbor multiple substrates for transport [94,95]. More recently, the chromosomally based LmrS from *S. aureus* was discovered by our laboratory and shown to actively export a large variety of structurally-distinct antimicrobials [96]. Other MFS transporters from *S. aureus* include SdrM, Sav1866, Tet(38), and MepA [10,69,97].

Recently, the functional roles of acidic residues were evaluated in QacA, where Asp-34 and Asp-411 were shown to recognize substrate, whereas Glu-407 could bind substrate and participate in protonation during transport catalysis [98]. These residues, conserved amongst other MFS drug transporters, may serve as suitable targets for novel efflux pump inhibition. Highly conserved amino acid sequence motifs are crucial for structural stability, transport, and modulation of MFS symporters and antiport-based efflux pumps [83,99]. In addition to previously known motifs A, B, and C, Shang et al. recently discovered conserved motifs, called Motif-1 and Motif-2, with influences on transporter stability and binding of ethidium bromide [100]. Molecular physiological studies involving conserved amino acid sequence motifs and multidrug efflux pump modulation are needed and show strong promise toward reestablishing the efficacy of antimicrobial action against pathogenic strains of *S. aureus* [10,99,101].

Studies from our laboratory showed that an extract of cumin spice from *Cuminum cyminum*, cumin seed oil, and a principal bioactive agent called cuminaldehyde inhibited the growth of *E. coli* host cells harboring the LmrS multidrug efflux pump from *S. aureus* [96,102], (Table 1). We also demonstrated that cumin extract inhibited the ethidium bromide transport activities of LmrS, such as efflux and accumulation [102]. Interestingly, TetR21, a member of the TetR family of repressors, suppressed the gene expression levels of *lmrS* and the gene encoding Tet(38) [103]. A recent study by Nava et al. showed that calcium ion (Ca^{2+}) enhanced the ethidium bromide efflux activity of LmrS [104]. Furthermore, these investigators demonstrated that when in the presence of antibiotics, a Ca^{2+} mediated transient was generated in cells of *S. aureus*, which then positively modulated LmrS after inducing a physiological process that could aid bacterial survival in harsh pH conditions [104]. This new work points to Ca^{2+} as a potential regulator of antimicrobial transport activity and controlling gene expression programs [104].

Thiazol and a well-known group of thiazolidinedione derivatives had been shown to interact with NorA of *S. aureus* elements using molecular docking systems [105]. These compounds demonstrated a synergistic relationship with norfloxacin in host cells harboring NorA [105]. Capsaicin, a known efflux pump inhibitor [106], was conjugated to newly developed molecules of 1,3,4-oxadiazole and shown to increase the antimicrobial activity of ciprofloxacin and reduce the transport of ethidium bromide by NorA, thus, pointing to these conjugates as potentially novel efflux pump inhibitors with promising potency [107], (Table 1). Molecular docking simulations were performed on a predicted NorA structure in which substrate ciprofloxacin and putative NorA-inhibitors derived from capsaicin were shown to make contacts with amino acid residues lining the hydrophobic cavities of the substrate-binding core and the inward and outward-facing drug binding sites [108]. In this study, specific residues formed distinct interactions between NorA and ciprofloxacin, capsaicin, and the novel putative efflux pump inhibitor CID-44330438 [108]. Interestingly, residues common to all three molecules included Phe-47 and Trp-293 [108], indicating shared elements in the solute transport and

modulatory systems of antimicrobial efflux at transmembrane helix one, a known drug-binding site region of MFS transporters [109–111], and helix ten where Trp-293 resides [78].

Another naturally occurring plant compound, a terpene-based agent called eugenol, and several compounds derived from it showed reductions in the MICs for efflux substrates ethidium bromide and norfloxacin by NorA [112]. Though efflux by NorA was not directly measured in this study, the investigators demonstrated synergy with these transport substrates and eugenol or isoeugenol [112]. Further, molecular docking simulations showed a close association between many amino acid residues in a predicted model of NorA and 4-allyl-2,6-dimetoxyphenol or allylbenzene [112]. These eugenol derivatives show promise as efflux pump inhibitors and synergistic modulators involving NorA. Other terpene-based compounds called carvacrol and thymol were effective antibacterial agents for *S. aureus* cells that contained NorA [113]. Molecular simulation docking studies showed that these compounds made close contact with many aliphatic or aromatic residues in the interior pocket of NorA, suggesting they dictate a suitable target for modulation of transport activity [113].

Another study of the terpene-based agents combined α-terpinene with essential oil from *Chenopodium ambrosioides* was conducted on Tet(K), showing a reduced MIC of tetracycline and ethidium bromide [114]. Quercetin, a flavonoid-based compound known to modulate Tet(K) and NorA [76,115,116], was recently demonstrated to enhance the antimicrobial actions of the antimicrobials erythromycin, tetracycline, and norfloxacin in host cells of *S. aureus* through its stabilizing interaction with Ser-138 of NorA [117]. Similarly, new chalcone derivatives, known efflux pump inhibitors of NorA of the MFS and MepA proteins from the MATE family [118–121], were recently shown to synergize with norfloxacin and ethidium bromide in cells harboring NorA or MepA [122]. Along these same lines, synergy was observed between ethidium bromide, norfloxacin, and the so-called 1,8-naphthyridine sulfonamides, suggesting the latter could be an efflux pump inhibitor of new interest [123]. More recently, a compound from various citrus fruits and vegetables, a phenolic-based compound called ferulic acid and esterified derivatives, showed reductions in the MICs for NorA substrates and demonstrated synergy between them [124]. In another study, propyl ferulate conferred a reduction in the Tet(K)-mediated MICs of ethidium bromide [125]. Lapachol and norlachol agents were used as a platform to synthesize various hydroxylamine derivatives, which showed lowered MIC values for ethidium bromide and norfloxacin [126]. Although direct measurement of drug efflux activities via the NorA or Tet(K) pumps was not demonstrated experimentally, the ferulic acids and hydroxylamines from lapachol and norlachol represent new chemical classes of interest for inhibiting *S. aureus* growth clinically [125,126].

Another group of modulators involving NorA and Tet(K) efflux pumps is represented by the sesquiterpene α-bisabolol from plants like *Matricaria chamomilla* L [127]. Recently, α-bisabolol was used to form a so-called inclusion complex with a β-cyclodextrin to enhance water solubility and provide greater bioavailability [127]. Several inclusion complexes reduced the MICs for norfloxacin in cells with NorA and tetracycline in host bacteria with Tet(K) [127].

An antibiotic called elaiophylin produced by species of the *Streptomyces* genus, such as *S. hygroscopicus* was recently shown to inhibit ethidium bromide transport by NorA, and molecular docking analysis showed strong binding affinities with residues Tyr-57, Ile-258, Ser-262, Pro-384 of NorA [128]. Analogs of the putative NorA modulator dihydroquinazoline showed reduced gene expression of the *norA* determinant in an over-expressing strain of *S. aureus* [129]. Furthermore, the novel analogs demonstrated synergistic action with the transport substrate ethidium bromide and,

importantly, a significantly reduced amount of intracellularly located *S. aureus* cells within host-monocytes in culture [129].

Recently, a group of purified silymarin flavonolignans that had previously been observed to reverse multidrug resistance in *S. aureus* showed non-competitive inhibition of efflux pump activities in NorA and MepA multidrug transporters [130]. One of these flavonolignan-based compound derivatives, 2,3-dehydrosilybin B, repressed the gene expression programs for various antimicrobial transporters from distinct superfamilies [130]. The flavonolignans show quorum sensing attenuation properties, further pointing to these compounds as potential multidrug resistance modulators [130]. Further, modulating agents that can affect antimicrobial transport across the membrane and regulate gene expression of drug resistance or virulence factors will continue to be of particular interest. A recent investigation reported on the genomic nature of genetic elements for antimicrobial resistance and virulence factors for over 100 *S. aureus* isolates from lower animals (dogs, cats, and cows) [131]. In addition to sharing genes for superantigens, the new study showed that all such isolates shared genes encoding antimicrobial efflux pumps LmrS [96], Tet(38) [97,132], NorA [78], and MepA [48], and regulators of gene expression, such as MrgA (previously designated NorR) [133], and the ArlRS system [131].

Thus, studies of gene sharing amongst bacterial pathogens will shed light on developing novel strategies for reducing the conditions that foster the emergence of pathogens as they move through animal and human populations. Because the multidrug efflux pumps of the MFS are widespread and relatively well understood, they can serve as effective targets for transport inhibitors or modulation of gene expression programs in cells of *S. aureus* [134]. Future strategies for restoring the efficacy of antimicrobial agents against clinical pathogens of *S. aureus* entail a deeper understanding of the physiological mechanism of antimicrobial efflux systems and their relationship to the biochemistry of efflux pump inhibitors [135,136].

Table 1. MFS antimicrobial efflux pump substrates and inhibitors.

Efflux pump	MW (kDa)	TMS	Substrates	Inhibitors	References
LmrS	47.7	14	Linezolid (oxazolidinone), Phenicols (chloramphenicol, florfenicol), erythromycin, trimethoprim, lincomycin, kanamycin, fusidic acid, QACs (tetraphenylphosphonium), Dyes (ethidium bromide), Detergents (sodium dodecyl sulfate)	Cumin seed oil, Cumin aldehyde, Reserpine	[96,102]
NorA	42.3	12	Hydrophilic fluoroquinolones (norfloxacin, ciprofloxacin), QACs (benzalkonium), Dyes (ethidium bromide, Hoechst 33342), Biocides (acriflavine, cetrimide, benzalkonium chloride)	Verapamil, Capsaicin, Capsaicin 1,3,4-oxadiazole conjugates, Piperine and piperine analogs (SK-20, SK-56, SK-29), Chalcone, Baicalein, Caffeic acid, Coumarin, Boeravinone B, Benzophenanthridin, 15-copaenol, Caffeoylquininc acids, Genistein, Dimethyl octaol, Nerol, Estragole, Indirubin, Kaempferol Rhamnoside, Tannic acid, Phyllanthin, Curcumin, Osthol, Orizabins, Murucoidins, Biricodar (VX-710), Timcodar (VX-853), Ginsenoside, Dithiazole thione derivative (DTT10), Pieric acid amides derivatives, Phyllanthin, α-Bisabolol, 1,8-naphthyridines sulfonamides Brachydins (BR-A, BR-B), Berberine, Berberine INF55 (5-nitro-2-phenyl-1H-indole) and analogs, Diterpenes (ferruginol), 2-phenyl-4(1*H*)-quinolone and 2-phenyl-4-hydroxyquinoline derivatives, Menadione, Crysoplenol, Crysoplenetin, Sarothrin (5,7,4'-trihydroxy-3,6,8-trimethoxyflavone), Olympicin A, Reserpine, Aldonitrones (Z)-N-benzylidene2-(tert-butoxy carbonyl amino)-1-(5-iodo-1H-indol-3-yl) ethan), Indole analogue (compound 13 and 14), Sophoraflavanone G, Diosmetin, Tiliroside (kaempferol-3-O-β-D-(6"-E-p-coumaroyl), Chrysoeriol, Penduletin, Galangin, Carvacrol, Thymol	[78,88,89,106,107, 117,118,123,127, 137–165]
NorB	49	12	Hydrophilic and hydrophobic fluoroquinolones (norfloxacin and ciprofloxacin, moxifloxacin, sparfloxacin), Biocides (tetraphenylphosphonium, cetrimide), Dye (ethidium bromide), tetracycline, QACs (tetraphenylphosphonium, cetrimide)	$CuFe_2O_4$@Ag, Clerodane diterene 16α-hydroxycleroda-3,13 (14)-Z-dien-15,16-olid 6	[150,166,167]
NorC	48.9	12–14	Hydrophilic and hydrophobic fluoroquinolones (ciprofloxacin, norfloxacin, moxifloxacin, garenoxacin, sparfloxacin), Dye (rhodamine)	Nanobody (single-domain camelid antibody), Clerodane diterpene 16α-hydroxycleroda-3,13 (14)-Z-dien-15,16-olid	[167–169]

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Efflux pump	MW (kDa)	TMS	Substrates	Inhibitors	References
MdeA	52	14	QACs (benzalkonium chloride, dequalinium, tetraphenylphosphonium), Dye (ethidium bromide, Hoechst 33342, acriflavine, and rhodamine 6G), Hydrophilic fluoroquinolones (virginiamycin, novobiocin, mupirocin, fusidic acid, norfloxacin, ciprofloxacin), Anthracyclines (doxorubicin, daunorubicin), Macrolides	Piperine, Clerodane diterpene 16α-hydroxycleroda-3,13 (14)-Z-dien-15,16-olid, Osthol, Imperatorin, Tangeretin	[95,167,170,171]
SdrM	56.4	14	Dyes (acriflavine, ethidium bromide), Fluoroquinolone (norfloxacin)	carbonyl cyanide *m*-chlorophenylhydrazone	[59]
QacA	55	14	QACs (tetraphenylphosphonium, benzalkonium chloride, dequalinium), Biguanidines (chlorhexidine), Diamides (pentamidine), Dyes (ethidium bromide, rhodamine, acriflavine), Cetyltrimethylammonium bromide (Ct), Tetraphenylarsonium chloride (Guanylhydrazones), Propamidine isethionate, Diamidinodiphenylamine dihydrochloride	Silybin, Volkensiflavone, Morelloflavone, Verapamil, Reserpine, Hydantoin PI8a	[93,161,172–177]
QacB	55	14	QACs (tetraphenylphosphonium, benzalkonium chloride, diamidinodiphenylamine dihydrochloride, cetrimide), Dyes (ethidium bromide, rhodamine, acriflavine), Biguanidines (chlorhexidine),	Silybin, Volkensiflavone, Morelloflavone, Hydantoin PI8a	[161,175,177]
Tet38	48	14	Tetracycline, Unsaturated fatty acids (palmitoleic and undecanoic acid), Fosfomycin, Tunicamycin, Congo red	Minocycline, glycerol-3-phosphate (G3P)	[97,132,168,178]
TetA(K)	50.7	14	Tetracyclines	Nocardamines, Essentials oil, Isopimarane diterpenes Osthol, 5,7-Diacetoxy-8-(3-methyl-2-butenyl)-coumari, 3-(2-Methyl but-3-en-2-yl), Xanthyletin	[165,179–181]
Tet63	-	14	Tetracycline and Doxycycline	Tigecycline	[182]
FexA	49.3	14	Florfenicol, Chloramphenicol		[183]

6. Conclusions and future directions

Morbidity and mortality rates reported by clinical studies of multidrug-resistant *S. aureus* isolates are of tremendous concern from a public health standpoint [10,11,184,185]. Addressing the concern will involve continued studies of the molecular mechanisms that confer multiple antimicrobial resistance in these pathogens [3,186]. Antimicrobial transporters are known to dictate multidrug resistance and represent promising targets for inhibition to restore clinical efficacy against infection [135]. Towards this, investigators have garnered a great deal of mechanistic and structural features of the drug transporters that can permit the development of suitable new modulators of resistance and infection [187]. Modulators that directly affect antimicrobial transport and show gene expression regulation are promising [131]. Comparative analyses of bacterial genomes can discover new targets for modulation [188–190].

While these efforts are promising, the field still lacks a detailed molecular understanding of antimicrobial translocation across the membrane [191]. Once the molecular pathways are delineated through dedicated transport systems and are definitively elucidated, efflux pump inhibitors can be designed with improved accuracy and, thus, improved antimicrobial efficacy for clinical treatment of infection [192,193].

New developments regarding the nature of transport for multiple structurally disparate antimicrobial agents via specific efflux pumps are constantly being reported [194,195]. However, we do not yet understand how multidrug efflux pumps dictate the specific translation of certain substrates while keeping others out and preventing unwanted leakages through the pumps in the cells of pathogens, especially for *S. aureus*. We anticipate that molecular analyses of the transport systems that confer single- versus multiple-drug transport yield new advances for improved chemotherapies. The molecular mechanisms that dictate passive versus active transport are not yet clearly understood. For example, we do not yet understand how these various antimicrobial transporters mediate ion-selectively in antiporters while not requiring such specificities in passive transporters. Along these lines, it remains poorly understood how multidrug pumps prevent unwanted translocation of ions which could collapse ion-motive forces that drive secondary active transporters of clinically relevant chemotherapeutics. Thus, we foresee that studies of the energetic mechanisms that drive the activities of the multidrug efflux pumps and how these systems related to the accumulation of antimicrobial agents to one side of the bacterial membrane will shed light on the nature of molecular configurations that will make effective targets for new modulators [10,134,196].

Lastly, the highly conserved nature of the members within each of the known transporter superfamilies predicts that such related members operate by mechanisms shared between them. Thus, elucidating molecular mechanisms that are believed to be commonly shared by members within each of the transporter superfamilies represents the Holy Grail of multidrug resistance in pathogenic microorganisms. We anticipate that once the detailed molecular mechanisms of antimicrobial transport are understood, treating severe infection by *S. aureus* will provide efficacious clinical outcomes.

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Conflict of interest

The authors declare no conflicts of interest in this review article.

References

1. Tong SY, Davis JS, Eichenberger E, et al. (2015) *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. *Clin Microbiol Rev* 28: 603–661. https://doi.org/10.1128/CMR.00134-14
2. Newsom SW (2008) Ogston's coccus. *J Hosp Infect* 70: 369–372. https://doi.org/10.1016/j.jhin.2008.10.001
3. Andersen JL, He GX, Kakarla P, et al. (2015) Multidrug efflux pumps from Enterobacteriaceae, *Vibrio cholerae* and *Staphylococcus aureus* bacterial food pathogens. *Int J Environ Res Public Health* 12: 1487–1547. https://doi.org/10.3390/ijerph120201487
4. Turner NA, Sharma-Kuinkel BK, Maskarinec SA, et al. (2019) Methicillin-resistant *Staphylococcus aureus*: an overview of basic and clinical research. *Nat Rev Microbiol* 17: 203–218. https://doi.org/10.1038/s41579-018-0147-4
5. Murray CJL, Ikuta KS, Sharara F, et al. (2022) Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *Lancet* 399: 629–655. https://doi.org/10.1016/S0140-6736(21)02724-0
6. Varela MF, Wilson TH (1996) Molecular biology of the lactose carrier of *Escherichia coli*. *Biochim Biophys Acta* 1276: 21–34. https://doi.org/10.1016/0005-2728(96)00030-8
7. Kumar S, Lekshmi M, Parvathi A, et al. (2017) Antibiotic resistance in seafood borne pathogens, In: Singh, O.V. Editor(s), *Foodborne Pathogens and Antibiotic Resistance*, Wiley-Blackwell, 397–415. https://doi.org/10.1002/9781119139188.ch17
8. Blair J, Webber MA, Baylay AJ, et al. (2015) Molecular mechanisms of antibiotic resistance. *Nat Rev Microbiol* 13: 42–51. https://doi.org/10.1038/nrmicro3380
9. Aslam B, Wang W, Arshad MI, et al. (2018) Antibiotic resistance: a rundown of a global crisis. *Infect Drug Resist* 11: 1645–1658. https://doi.org/10.2147/IDR.S173867
10. Lekshmi M, Ammini P, Adjei J, et al. (2018) Modulation of antimicrobial efflux pumps of the major facilitator superfamily in *Staphylococcus aureus*. *AIMS Microbiol* 4: 1–18. https://doi.org/10.3934/microbiol.2018.1.1
11. Varela MF, Stephen J, Lekshmi M, et al. (2021) Bacterial resistance to antimicrobial agents. *Antibiotics (Basel)* 10: 593. https://doi.org/10.3390/antibiotics10050593
12. Vestergaard M, Frees D, Ingmer H (2019) Antibiotic resistance and the MRSA problem. *Microbiol Spec*tr 7. https://doi.org/10.1128/microbiolspec.GPP3-0057-2018
13. Pantosti A, Sanchini A, Monaco M (2007) Mechanisms of antibiotic resistance in *Staphylococcus aureus*. *Future Microbiol* 2: 323–334. https://doi.org/10.2217/17460913.2.3.323
14. Sabath LD, Finland M (1962) Inactivation of methicillin, oxacillin and ancillin by *Staphylococcus aureus*. *Proc Soc Exp Biol Med* 111: 547–550. https://doi.org/10.3181/00379727-111-27850

15. Frère JM, Sauvage E, Kerff F (2016) From "An Enzyme Able to Destroy Penicillin" to Carbapenemases: 70 years of β-lactamase misbehaviour. *Curr Drug Targets* 17: 974–982. https://doi.org/10.2174/1389450116666151001112859
16. Rodriguez-Bano J, Pascual A (2008) Clinical significance of extended-spectrum β-lactamases. *Expert Rev Anti Infect Ther* 6: 671–683. https://doi.org/10.1586/14787210.6.5.671
17. Sawa T, Kooguchi K, Moriyama K (2020) Molecular diversity of extended-spectrum β-lactamases and carbapenemases, and antimicrobial resistance. *J Intensive Care* 8: 13. https://doi.org/10.1186/s40560-020-0429-6
18. Lee S, Mir RA, Park SH, et al. (2020) Prevalence of extended-spectrum β-lactamases in the local farm environment and livestock: challenges to mitigate antimicrobial resistance. *Crit Rev Microbiol* 46: 1–14. https://doi.org/10.1080/1040841X.2020.1715339
19. Bayer AS, Schneider T, Sahl HG (2013) Mechanisms of daptomycin resistance in *Staphylococcus aureus*: role of the cell membrane and cell wall. *Ann N Y Acad Sci* 1277: 139–158. https://doi.org/10.1111/j.1749-6632.2012.06819.x
20. Guo Y, Pfahler NM, Volpel SL, et al. (2021) Cell wall glycosylation in *Staphylococcus aureus*: targeting the *tar* glycosyltransferases. *Curr Opin Struct Biol* 68: 166–174. https://doi.org/10.1016/j.sbi.2021.01.003
21. Shahid AH, Nazir K, El Zowalaty ME, et al. (2021) Molecular detection of vancomycin and methicillin resistance in *Staphylococcus aureus* isolated from food processing environments. *One Health* 13: 100276. https://doi.org/10.1016/j.onehlt.2021.100276
22. Lambert PA (2005) Bacterial resistance to antibiotics: modified target sites. *Adv Drug Deliv Rev* 57: 1471–1485. https://doi.org/10.1016/j.addr.2005.04.003
23. Badshah SL, Ullah A (2018) New developments in non-quinolone-based antibiotics for the inhibition of bacterial gyrase and topoisomerase IV. *Eur J Med Chem* 152: 393–400. https://doi.org/10.1016/j.ejmech.2018.04.059
24. Tremblay S, Lau TT, Ensom MH (2013) Addition of rifampin to vancomycin for methicillin-resistant *Staphylococcus aureus* infections: what is the evidence? *Ann Pharmacother* 47: 1045–1054. https://doi.org/10.1345/aph.1R726
25. Shanson DC (1981) Antibiotic-resistant *Staphylococcus aureus*. *J Hosp Infect* 2: 11–36. https://doi.org/10.1016/0195-6701(81)90003-7
26. Nguyen F, Starosta AL, Arenz S, et al. (2014) Tetracycline antibiotics and resistance mechanisms. *Biol Chem* 395: 559–575. https://doi.org/10.1515/hsz-2013-0292
27. Liu WT, Chen EZ, Yang L, et al. (2021) Emerging resistance mechanisms for 4 types of common anti-MRSA antibiotics in *Staphylococcus aureus*: A comprehensive review. *Microb Pathog* 156: 104915. https://doi.org/10.1016/j.micpath.2021.104915
28. Dantley KA, Dannelly HK, Burdett V (1998) Binding interaction between Tet(M) and the ribosome: requirements for binding. *J Bacteriol* 180: 4089–4092. https://doi.org/10.1128/JB.180.16.4089-4092.1998
29. Burdett V (1996) Tet(M)-promoted release of tetracycline from ribosomes is GTP dependent. *J Bacteriol* 178: 3246–3251. https://doi.org/10.1128/jb.178.11.3246-3251.1996
30. Burdett V (1991) Purification and characterization of Tet(M), a protein that renders ribosomes resistant to tetracycline. *J Biol Chem* 266: 2872–2877. https://doi.org/10.1016/S0021-9258(18)49928-0

31. Lewis K (2008) Multidrug tolerance of biofilms and persister cells. *Curr Top Microbiol Immunol* 322: 107–131. https://doi.org/10.1007/978-3-540-75418-3_6
32. Elkhatib WF, Khairalla AS, Ashour HM (2014) Evaluation of different microtiter plate-based methods for the quantitative assessment of *Staphylococcus aureus* biofilms. *Future Microbiol* 9: 725–735. https://doi.org/10.2217/fmb.14.33
33. Smith KP, Kumar S, Varela MF (2009) Identification, cloning, and functional characterization of EmrD-3, a putative multidrug efflux pump of the major facilitator superfamily from *Vibrio cholerae* O395. *Arch Microbiol* 191: 903–911. https://doi.org/10.1007/s00203-009-0521-8
34. Kumar S, Varela MF (2013) Molecular mechanisms of bacterial resistance to antimicrobial agents, In: Méndez-Vilas A. Editor, *Microbial pathogens and strategies for combating them: science, technology and education*, Formatex Research Center, 522–534.
35. Velamakanni S, Yao Y, Gutmann DA, et al. (2008) Multidrug transport by the ABC transporter Sav1866 from *Staphylococcus aureus*. *Biochemistry* 47: 9300–9308. https://doi.org/10.1021/bi8006737
36. Orelle C, Mathieu K, Jault JM (2019) Multidrug ABC transporters in bacteria. *Res Microbiol* 170: 381–391. https://doi.org/10.1016/j.resmic.2019.06.001
37. Forrest LR, Kramer R, Ziegler C (2011) The structural basis of secondary active transport mechanisms. *Biochim Biophys Acta* 1807: 167–188. https://doi.org/10.1016/j.bbabio.2010.10.014
38. Kuroda T, Tsuchiya T (2009) Multidrug efflux transporters in the MATE family. *Biochim Biophys Acta* 1794: 763–768. https://doi.org/10.1016/j.bbapap.2008.11.012
39. Moriyama Y, Hiasa M, Matsumoto T, et al. (2008) Multidrug and toxic compound extrusion (MATE)-type proteins as anchor transporters for the excretion of metabolic waste products and xenobiotics. *Xenobiotica* 38: 1107–1118. https://doi.org/10.1080/00498250701883753
40. Kusakizako T, Claxton DP, Tanaka Y, et al. (2019) Structural basis of H^+-dependent conformational change in a bacterial MATE transporter. *Structure* 27: 293–301.e3. https://doi.org/10.1016/j.str.2018.10.004
41. Brown MH, Paulsen IT, Skurray RA (1999) The multidrug efflux protein NorM is a prototype of a new family of transporters. *Mol Microbiol* 31: 394–395. https://doi.org/10.1046/j.1365-2958.1999.01162.x
42. He X, Szewczyk P, Karyakin A, et al. (2010) Structure of a cation-bound multidrug and toxic compound extrusion transporter. *Nature* 467: 991–994. https://doi.org/10.1038/nature09408
43. Lu M, Symersky J, Radchenko M, et al. (2013) Structures of a Na^+-coupled, substrate-bound MATE multidrug transporter. *Proc Natl Acad Sci U S A* 110: 2099–2104. https://doi.org/10.1073/pnas.1219901110
44. Lu M, Radchenko M, Symersky J, et al. (2013) Structural insights into H^+-coupled multidrug extrusion by a MATE transporter. *Nat Struct Mol Biol* 20: 1310–1317. https://doi.org/10.1038/nsmb.2687
45. Kusakizako T, Miyauchi H, Ishitani R, et al. (2020) Structural biology of the multidrug and toxic compound extrusion superfamily transporters. *Biochim Biophys Acta Biomembr* 1862: 183154. https://doi.org/10.1016/j.bbamem.2019.183154
46. McAleese F, Petersen P, Ruzin A, et al. (2005) A novel MATE family efflux pump contributes to the reduced susceptibility of laboratory-derived *Staphylococcus aureus* mutants to tigecycline. *Antimicrob Agents Chemother* 49: 1865–1871. https://doi.org/10.1128/AAC.49.5.1865-1871.2005

47. Costa SS, Viveiros M, Amaral L, et al. (2013) Multidrug efflux pumps in *Staphylococcus aureus*: an update. *Open Microbiol J* 7: 59–71. https://doi.org/10.2174/1874285801307010059
48. Kaatz GW, McAleese F, Seo SM (2005) Multidrug resistance in *Staphylococcus aureus* due to overexpression of a novel multidrug and toxin extrusion (MATE) transport protein. *Antimicrob Agents Chemother* 49: 1857–1864. https://doi.org/10.1128/AAC.49.5.1857-1864.2005
49. Morita Y, Kataoka A, Shiota S, et al. (2000) NorM of *Vibrio parahaemolyticus* is an Na^+-driven multidrug efflux pump. *J Bacteriol* 182: 6694–6697. https://doi.org/10.1128/JB.182.23.6694-6697.2000
50. Dridi L, Tankovic J, Petit JC (2004) CdeA of *Clostridium difficile*, a new multidrug efflux transporter of the MATE family. *Microb Drug Resist* 10: 191–196. https://doi.org/10.1089/mdr.2004.10.191
51. Kaatz GW, Moudgal VV, Seo SM, et al. (2003) Phenylpiperidine selective serotonin reuptake inhibitors interfere with multidrug efflux pump activity in *Staphylococcus aureus*. *Int J Antimicrob Agents* 22: 254–261. https://doi.org/10.1016/S0924-8579(03)00220-6
52. Schindler BD, Patel D, Seo SM, et al. (2013) Mutagenesis and modeling to predict structural and functional characteristics of the *Staphylococcus aureus* MepA multidrug efflux pump. *J Bacteriol* 195: 523–533. https://doi.org/10.1128/JB.01679-12
53. da Costa RHS, Rocha JE, de Freitas TS, et al. (2021) Evaluation of antibacterial activity and reversal of the NorA and MepA efflux pump of estragole against *Staphylococcus aureus* bacteria. *Arch Microbiol* 203: 3551–3555. https://doi.org/10.1007/s00203-021-02347-x
54. Oliveira-Tintino CDM, Tintino SR, Muniz DF, et al. (2021) Chemical synthesis, molecular docking and MepA efflux pump inhibitory effect by 1,8-naphthyridines sulfonamides. *Eur J Pharm Sci* 160: 105753. https://doi.org/10.1016/j.ejps.2021.105753
55. Freitas PR, de Araujo ACJ, Dos Santos Barbosa CR, et al. (2022) Inhibition of the MepA efflux pump by limonene demonstrated by *in vitro* and *in silico* methods. *Folia Microbiol (Praha)* 67: 15–20. https://doi.org/10.1007/s12223-021-00909-6
56. Wassenaar TM, Ussery D, Nielsen LN, et al. (2015) Review and phylogenetic analysis of *qac* genes that reduce susceptibility to quaternary ammonium compounds in *Staphylococcus species*. *Eur J Microbiol Immunol (Bp)* 5: 44–61. https://doi.org/10.1556/EuJMI-D-14-00038
57. Sasatsu M, Shima K, Shibata Y, et al. (1989) Nucleotide sequence of a gene that encodes resistance to ethidium bromide from a transferable plasmid in *Staphylococcus aureus*. *Nucleic Acids Res* 17: 10103. https://doi.org/10.1093/nar/17.23.10103
58. Grinius L, Dreguniene G, Goldberg EB, et al. (1992) A staphylococcal multidrug resistance gene product is a member of a new protein family. *Plasmid* 27: 119–129. https://doi.org/10.1016/0147-619X(92)90012-Y
59. Yamada Y, Hideka K, Shiota S, et al. (2006) Gene cloning and characterization of SdrM, a chromosomally-encoded multidrug efflux pump, from *Staphylococcus aureus*. *Biol Pharm Bull* 29: 554–556. https://doi.org/10.1248/bpb.29.554
60. Grinius LL, Goldberg EB (1994) Bacterial multidrug resistance is due to a single membrane protein which functions as a drug pump. *J Biol Chem* 269: 29998–30004. https://doi.org/10.1016/S0021-9258(18)43980-4
61. Littlejohn TG, DiBerardino D, Messerotti LJ, et al. (1991) Structure and evolution of a family of genes encoding antiseptic and disinfectant resistance in *Staphylococcus aureus*. *Gene* 101: 59–66. https://doi.org/10.1016/0378-1119(91)90224-Y

62. Wassenaar TM, Ussery DW, Ingmer H (2016) The *qacC* gene has recently spread between rolling circle plasmids of *Staphylococcus*, indicative of a novel gene transfer mechanism. *Front Microbiol* 7: 1528. https://doi.org/10.3389/fmicb.2016.01528
63. Heir E, Sundheim G, Holck AL (1999) The *qacG* gene on plasmid pST94 confers resistance to quaternary ammonium compounds in staphylococci isolated from the food industry. *J Appl Microbiol* 86: 378–388. https://doi.org/10.1046/j.1365-2672.1999.00672.x
64. Heir E, Sundheim G, Holck AL (1998) The *Staphylococcus qacH* gene product: a new member of the SMR family encoding multidrug resistance. *FEMS Microbiol Lett* 163: 49–56. https://doi.org/10.1111/j.1574-6968.1998.tb13025.x
65. Jiang X, Xu Y, Li Y, et al. (2017) Characterization and horizontal transfer of *qacH*-associated class 1 integrons in *Escherichia coli* isolated from retail meats. *Int J Food Microbiol* 258: 12–17. https://doi.org/10.1016/j.ijfoodmicro.2017.07.009
66. Bjorland J, Steinum T, Sunde M, et al. (2003) Novel plasmid-borne gene *qacJ* mediates resistance to quaternary ammonium compounds in equine *Staphylococcus aureus*, *Staphylococcus simulans*, and *Staphylococcus intermedius*. *Antimicrob Agents Chemother* 47: 3046–3052. https://doi.org/10.1128/AAC.47.10.3046-3052.2003
67. Ninio S, Rotem D, Schuldiner S (2001) Functional analysis of novel multidrug transporters from human pathogens. *J Biol Chem* 276: 48250–48256. https://doi.org/10.1074/jbc.M108231200
68. Paulsen IT, Brown MH, Dunstan SJ, et al. (1995) Molecular characterization of the staphylococcal multidrug resistance export protein QacC. *J Bacteriol* 177: 2827–2833. https://doi.org/10.1128/jb.177.10.2827-2833.1995
69. Kumar S, Lekshmi M, Parvathi A, et al. (2020) Functional and structural roles of the major facilitator superfamily bacterial multidrug efflux pumps. *Microorganisms* 8: 266. https://doi.org/10.3390/microorganisms8020266
70. Ranjana K, Shrestha U, Kumar S, et al. (2017) Molecular biology of multidrug resistance efflux pumps of the major facilitator superfamily from bacterial food pathogens. In: Singh, O.V. Editor(s), *Foodborne Pathogens and Antibiotic Resistance*, Wiley-Blackwell. https://doi.org/10.1002/9781119139188.ch13
71. Maiden MC, Davis EO, Baldwin SA, et al. (1987) Mammalian and bacterial sugar transport proteins are homologous. *Nature* 325: 641–643. https://doi.org/10.1038/325641a0
72. Ranaweera I, Shrestha U, Ranjana KC, et al. (2015) Structural comparison of bacterial multidrug efflux pumps of the major facilitator superfamily. *Trends Cell Mol Biol* 10: 131–140.
73. Henderson PJ (1990) The homologous glucose transport proteins of prokaryotes and eukaryotes. *Res Microbiol* 141: 316–328. https://doi.org/10.1016/0923-2508(90)90005-B
74. Griffith JK, Baker ME, Rouch DA, et al. (1992) Membrane transport proteins: implications of sequence comparisons. *Curr Opin Cell Biol* 4: 684–695. https://doi.org/10.1016/0955-0674(92)90090-Y
75. Henderson PJ (1991) Studies of translocation catalysis. *Biosci Rep* 11: 477–453. https://doi.org/10.1007/BF01130216
76. Henderson PJ (1990) Proton-linked sugar transport systems in bacteria. *J Bioenerg Biomembr* 22: 525–569. https://doi.org/10.1007/BF00762961
77. Varela MF, Griffith JK (1993) Nucleotide and deduced protein sequences of the class D tetracycline resistance determinant: Relationship to other antimicrobial transport proteins. *Antimicrob Agents Chemother* 37: 1253–1258. https://doi.org/10.1128/AAC.37.6.1253

78. Yoshida H, Bogaki M, Nakamura S, et al. (1990) Nucleotide sequence and characterization of the *Staphylococcus aureus norA* gene, which confers resistance to quinolones. *J Bacteriol* 172: 6942–6949. https://doi.org/10.1128/jb.172.12.6942-6949.1990
79. Omasits U, Ahrens CH, Muller S, et al. (2014) Protter: interactive protein feature visualization and integration with experimental proteomic data. *Bioinformatics* 30: 884–886. https://doi.org/10.1093/bioinformatics/btt607
80. Law CJ, Maloney PC, Wang DN (2008) Ins and outs of major facilitator superfamily antiporters. *Annu Rev Microbiol* 62: 289–305. https://doi.org/10.1146/annurev.micro.61.080706.093329
81. Yaffe D, Radestock S, Shuster Y, et al. (2013) Identification of molecular hinge points mediating alternating access in the vesicular monoamine transporter VMAT2. *Proc Natl Acad Sci U S A* 110: E1332–E1341. https://doi.org/10.1073/pnas.1220497110
82. Radestock S, Forrest LR (2011) The alternating-access mechanism of MFS transporters arises from inverted-topology repeats. *J Mol Biol* 407: 698–715. https://doi.org/10.1016/j.jmb.2011.02.008
83. Kumar S, Ranjana KC, Sanford LM, et al. (2016) Structural and functional roles of two evolutionarily conserved amino acid sequence motifs within solute transporters of the major facilitator superfamily. *Trends in Cell & Molecular Biology* 11: 41–53.
84. Truong-Bolduc QC, Hooper DC (2010) Phosphorylation of MgrA and its effect on expression of the NorA and NorB efflux pumps of *Staphylococcus aureus*. *J Bacteriol* 192: 2525–2534. https://doi.org/10.1128/JB.00018-10
85. Yamaguchi A, Shiina Y, Fujihira E, et al. (1995) The tetracycline efflux protein encoded by the *tet*(K) gene from *Staphylococcus aureus* is a metal-tetracycline/H^+ antiporter. *FEBS Lett* 365: 193–197. https://doi.org/10.1016/0014-5793(95)00455-I
86. Jin J, Guffanti AA, Bechhofer DH, et al. (2002) Tet(L) and Tet(K) tetracycline-divalent metal/H^+ antiporters: characterization of multiple catalytic modes and a mutagenesis approach to differences in their efflux substrate and coupling ion preferences. *J Bacteriol* 184: 4722–4732. https://doi.org/10.1128/JB.184.17.4722-4732.2002
87. Sheridan RP, Chopra I (1991) Origin of tetracycline efflux proteins: conclusions from nucleotide sequence analysis. *Mol Microbiol* 5: 895–900. https://doi.org/10.1111/j.1365-2958.1991.tb00763.x
88. Neyfakh AA, Borsch CM, Kaatz GW (1993) Fluoroquinolone resistance protein NorA of *Staphylococcus aureus* is a multidrug efflux transporter. *Antimicrob Agents Chemother* 37: 128–129. https://doi.org/10.1128/AAC.37.1.128
89. Neyfakh AA (1992) The multidrug efflux transporter of *Bacillus subtilis* is a structural and functional homolog of the *Staphylococcus* NorA protein. *Antimicrob Agents Chemother* 36: 484–485. https://doi.org/10.1128/AAC.36.2.484
90. Truong-Bolduc QC, Strahilevitz J, Hooper DC (2006) NorC, a new efflux pump regulated by MgrA of *Staphylococcus aureus*. *Antimicrob Agents Chemother* 50: 1104–1107. https://doi.org/10.1128/AAC.50.3.1104-1107.2006
91. Truong-Bolduc QC, Bolduc GR, Okumura R, et al. (2011) Implication of the NorB efflux pump in the adaptation of *Staphylococcus aureus* to growth at acid pH and in resistance to moxifloxacin. *Antimicrob Agents Chemother* 55: 3214–3219. https://doi.org/10.1128/AAC.00289-11

92. Ding Y, Fu Y, Lee JC, et al. (2012) *Staphylococcus aureus* NorD, a putative efflux pump coregulated with the Opp1 oligopeptide permease, contributes selectively to fitness *in vivo*. *J Bacteriol* 194: 6586–6593. https://doi.org/10.1128/JB.01414-12
93. Brown MH, Skurray RA (2001) Staphylococcal multidrug efflux protein QacA. *J Mol Microbiol Biotechnol* 3: 163–170.
94. Yamada Y, Shiota S, Mizushima T, et al. (2006) Functional gene cloning and characterization of MdeA, a multidrug efflux pump from *Staphylococcus aureus*. *Biol Pharm Bull* 29: 801–804. https://doi.org/10.1248/bpb.29.801
95. Huang J, O'Toole PW, Shen W, et al. (2004) Novel chromosomally encoded multidrug efflux transporter MdeA in *Staphylococcus aureus*. *Antimicrob Agents Chemother* 48: 909–917. https://doi.org/10.1128/AAC.48.3.909-917.2004
96. Floyd JL, Smith KP, Kumar SH, et al. (2010) LmrS is a multidrug efflux pump of the major facilitator superfamily from *Staphylococcus aureus*. *Antimicrob Agents Chemother* 54: 5406–5412. https://doi.org/10.1128/AAC.00580-10
97. Truong-Bolduc QC, Wang Y, Hooper DC (2018) Tet38 efflux pump contributes to fosfomycin resistance in *Staphylococcus aureus*. *Antimicrob Agents Chemother* 62: e00927–18. https://doi.org/10.1128/AAC.00927-18
98. Majumder P, Khare S, Athreya A, et al. (2019) Dissection of protonation sites for antibacterial recognition and transport in QacA, a multi-drug efflux transporter. *J Mol Biol* 431: 2163–2179. https://doi.org/10.1016/j.jmb.2019.03.015
99. Kakarla P, Ranjana K, Shrestha U, et al. (2017) Functional roles of highly conserved amino acid sequence motifs A and C in solute transporters of the major facilitator superfamily. In: Arora, G., Sajid, A., Kalia, V. Editor(s), *Drug resistance in bacteria, fungi, malaria, and cancer*, Springer, 111–140. https://doi.org/10.1007/978-3-319-48683-3_4
100. Shang Y, Lv P, Su D, et al. (2022) Evolutionary conservative analysis revealed novel functional sites in the efflux pump NorA of *Staphylococcus aureus*. *J Antimicrob Chemother* 77: 675–681. https://doi.org/10.1093/jac/dkab453
101. Varela MF, Sansom CE, Griffith JK (1995) Mutational analysis and molecular modelling of an amino acid sequence motif conserved in antiporters but not symporters in a transporter superfamily. *Mol Membr Biol* 12: 313–319. https://doi.org/10.3109/09687689509072433
102. Kakarla P, Floyd J, Mukherjee M, et al. (2017) Inhibition of the multidrug efflux pump LmrS from *Staphylococcus aureus* by cumin spice *Cuminum cyminum*. *Arch Microbiol* 199: 465–474. https://doi.org/10.1007/s00203-016-1314-5
103. Truong-Bolduc QC, Wang Y, Chen C, et al. (2017) Transcriptional regulator TetR21 controls the expression of the *Staphylococcus aureus* LmrS efflux pump. *Antimicrob Agents Chemother* 61: e00649–17. https://doi.org/10.1128/AAC.00649-17
104. Nava AR, Mauricio N, Sanca AJ, et al. (2020) Evidence of calcium signaling and modulation of the LmrS multidrug resistant efflux pump activity by Ca^{2+} ions in *S. aureus*. *Front Microbiol* 11: 573388. https://doi.org/10.3389/fmicb.2020.573388
105. Pereira PS, Lima M, Neto PPM, et al. (2019) Thiazolidinedione and thiazole derivatives potentiate norfloxacin activity against NorA efflux pump over expression in *Staphylococcus aureus* 1199B strains. *Bioorg Med Chem* 27: 3797–3804. https://doi.org/10.1016/j.bmc.2019.07.006

106. Kalia NP, Mahajan P, Mehra R, et al. (2012) Capsaicin, a novel inhibitor of the NorA efflux pump, reduces the intracellular invasion of *Staphylococcus aureus*. *J Antimicrob Chemother* 67: 2401–2408. https://doi.org/10.1093/jac/dks232
107. Naaz F, Khan A, Kumari A, et al. (2021) 1,3,4-oxadiazole conjugates of capsaicin as potent NorA efflux pump inhibitors of *Staphylococcus aureus*. *Bioorg Chem* 113: 105031. https://doi.org/10.1016/j.bioorg.2021.105031
108. Zarate SG, Morales P, Swiderek K, et al. (2019) A molecular modeling approach to identify novel inhibitors of the major facilitator superfamily of efflux pump transporters. *Antibiotics (Basel)* 8: 25. https://doi.org/10.3390/antibiotics8010025
109. Shinnick SG, Varela MF (2002) Altered sugar selection and transport conferred by spontaneous point and deletion mutations in the lactose carrier of *Escherichia coli*. *J Membr Biol* 189: 191–199. https://doi.org/10.1007/s00232-002-1013-9
110. Varela MF, Wilson TH, Rodon-Rivera V, et al. (2000) Mutants of the lactose carrier of *Escherichia coli* which show altered sugar recognition plus a severe defect in sugar accumulation. *J Membr Biol* 174: 199–205. https://doi.org/10.1007/s002320001044
111. Varela MF, Brooker RJ, Wilson TH (1997) Lactose carrier mutants of *Escherichia coli* with changes in sugar recognition (lactose versus melibiose). *J Bacteriol* 179: 5570–5573. https://doi.org/10.1128/jb.179.17.5570-5573.1997
112. Muniz DF, Dos Santos Barbosa CR, de Menezes IRA, et al. (2021*) In vitro* and *in silico* inhibitory effects of synthetic and natural eugenol derivatives against the NorA efflux pump in *Staphylococcus aureus*. *Food Chem* 337: 127776. https://doi.org/10.1016/j.foodchem.2020.127776
113. Dos Santos Barbosa CR, Scherf JR, de Freitas TS, et al. (2021) Effect of Carvacrol and Thymol on NorA efflux pump inhibition in multidrug-resistant (MDR) *Staphylococcus aureus* strains. *J Bioenerg Biomembr* 53: 489–498. https://doi.org/10.1007/s10863-021-09906-3
114. Limaverde PW, Campina FF, da Cunha FAB, et al. (2017) 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* 109: 957–961. https://doi.org/10.1016/j.fct.2017.02.031
115. Butaye P, Cloeckaert A, Schwarz S (2003) Mobile genes coding for efflux-mediated antimicrobial resistance in Gram-positive and Gram-negative bacteria. *Int J Antimicrob Agents* 22: 205–210. https://doi.org/10.1016/S0924-8579(03)00202-4
116. Piddock LJ (2006) Clinically relevant chromosomally encoded multidrug resistance efflux pumps in bacteria. *Clin Microbiol Rev* 19: 382–402. https://doi.org/10.1128/CMR.19.2.382-402.2006
117. Dos Santos JFS, Tintino SR, da Silva ARP, et al. (2021) Enhancement of the antibiotic activity by quercetin against *Staphylococcus aureus* efflux pumps. *J Bioenerg Biomembr* 53: 157–167. https://doi.org/10.1007/s10863-021-09886-4
118. Holler JG, Slotved HC, Molgaard P, et al. (2012) Chalcone inhibitors of the NorA efflux pump in *Staphylococcus aureus* whole cells and enriched everted membrane vesicles. *Bioorg Med Chem* 20: 4514–4521. https://doi.org/10.1016/j.bmc.2012.05.025
119. Rezende-Junior LM, Andrade LMS, Leal A, et al. (2020) Chalcones isolated from *Arrabidaea brachypoda* flowers as inhibitors of NorA and MepA multidrug efflux pumps of *Staphylococcus aureus*. *Antibiotics (Basel)* 9: 351. https://doi.org/10.3390/antibiotics9060351

120. Alves Borges Leal AL, Teixeira da Silva P, Nunes da Rocha M, et al. (2021) Potentiating activity of Norfloxacin by synthetic chalcones against NorA overproducing *Staphylococcus aureus*. *Microb Pathog* 155: 104894. https://doi.org/10.1016/j.micpath.2021.104894
121. Freitas TS, Xavier JC, Pereira RLS, et al. (2021) *In vitro* and *in silico* studies of chalcones derived from natural acetophenone inhibitors of NorA and MepA multidrug efflux pumps in *Staphylococcus aureus*. *Microb Pathog* 161: 105286. https://doi.org/10.1016/j.micpath.2021.105286
122. Rocha JE, de Freitas TS, da Cunha Xavier J, et al. (2021) Antibacterial and antibiotic modifying activity, ADMET study and molecular docking of synthetic chalcone (E)-1-(2-hydroxyphenyl)-3-(2,4-dimethoxy-3-methylphenyl)prop-2-en-1-one in strains of *Staphylococcus aureus* carrying NorA and MepA efflux pumps. *Biomed Pharmacother* 140: 111768. https://doi.org/10.1016/j.biopha.2021.111768
123. Oliveira-Tintino CDM, Muniz DF, Barbosa C, et al. (2021) The 1,8-naphthyridines sulfonamides are NorA efflux pump inhibitors. *J Glob Antimicrob Resist* 24: 233–240. https://doi.org/10.1016/j.jgar.2020.11.027
124. Pinheiro P, Santiago G, da Silva F, et al. (2021) Antibacterial activity and inhibition against *Staphylococcus aureus* NorA efflux pump by ferulic acid and its esterified derivatives. *Asian Pac J Trop Biomed* 11: 405–413. https://doi.org/10.4103/2221-1691.321130
125. Pinheiro PG, Santiago GMP, da Silva FEF, et al. (2022) Ferulic acid derivatives inhibiting *Staphylococcus aureus* TetK and MsrA efflux pumps. *Biotechnol Rep* 34: e00717. https://doi.org/10.1016/j.btre.2022.e00717
126. Figueredo FG, Ramos ITL, Paz JA, et al. (2020) Effect of hydroxyamines derived from lapachol and norlachol against *Staphylococcus aureus* strains carrying the NorA efflux pump. *Infect Genet Evol* 84: 104370. https://doi.org/10.1016/j.meegid.2020.104370
127. Pereira da Cruz R, Sampaio de Freitas T, Socorro Costa MD, et al. (2020) Effect of α-Bisabolol and its β-Cyclodextrin complex as TetK and NorA efflux pump inhibitors in *Staphylococcus aureus* strains. *Antibiotics (Basel)* 9: 28. https://doi.org/10.3390/antibiotics9010028
128. Rodrigues Dos Santos Barbosa C, Feitosa Muniz D, Silvino Pereira P, et al. (2021) Evaluation of Elaiophylin extracted from *Streptomyces hygroscopicus* as a potential inhibitor of the NorA efflux protein in *Staphylococcus aureus*: An *in vitro* and *in silico* approach. *Bioorg Med Chem Lett* 50: 128334. https://doi.org/10.1016/j.bmcl.2021.128334
129. Deka B, Suri M, Sarma S, et al. (2022) Potentiating the intracellular killing of *Staphylococcus aureus* by dihydroquinazoline analogues as NorA efflux pump inhibitor. *Bioorg Med Chem* 54: 116580. https://doi.org/10.1016/j.bmc.2021.116580
130. Holasová K, Křížkovská B, Hoang L, et al. (2022) Flavonolignans from silymarin modulate antibiotic resistance and virulence in *Staphylococcus aureus*. *Biomed Pharmacother* 149: 112806. https://doi.org/10.1016/j.biopha.2022.112806
131. Bruce SA, Smith JT, Mydosh JL, et al. (2022) Shared antibiotic resistance and virulence genes in *Staphylococcus aureus* from diverse animal hosts. *Sci Rep* 12: 4413. https://doi.org/10.1038/s41598-022-08230-z
132. Truong-Bolduc QC, Wang Y, Hooper DC (2019) Tet38 of *Staphylococcus aureus* binds to host cell receptor complex CD36-Toll-Like receptor 2 and protects from teichoic acid synthesis inhibitors tunicamycin and congo red. *Infect Immun* 87: e00194–19. https://doi.org/10.1128/IAI.00194-19

133. Truong-Bolduc QC, Zhang X, Hooper DC (2003) Characterization of NorR protein, a multifunctional regulator of *norA* expression in *Staphylococcus aureus*. *J Bacteriol* 185: 3127–3138. https://doi.org/10.1128/JB.185.10.3127-3138.2003
134. Kumar S, He G, Kakarla P, et al. (2016) Bacterial multidrug efflux pumps of the major facilitator superfamily as targets for modulation. *Infect Disord Drug Targets* 16: 28–43. https://doi.org/10.2174/1871526516666160407113848
135. Kumar S, Varela MF (2012) Biochemistry of bacterial multidrug efflux pumps. *Int J Mol Sci* 13: 4484–4495. https://doi.org/10.3390/ijms13044484
136. Varela MF, Kumar S (2019) Strategies for discovery of new molecular targets for anti-infective drugs. *Curr Opin Pharmacol* 48: 57–68. https://doi.org/10.1016/j.coph.2019.04.015
137. Bame JR, Graf TN, Junio HA, et al. (2013) Sarothrin from *Alkanna orientalis* is an antimicrobial agent and efflux pump inhibitor. *Planta Med* 79: 327–329. https://doi.org/10.1055/s-0032-1328259
138. Buonerba F, Lepri S, Goracci L, et al. (2017) Improved potency of indole-based NorA efflux pump inhibitors: from serendipity toward rational design and development. *J Med Chem* 60: 517–523. https://doi.org/10.1021/acs.jmedchem.6b01281
139. Chan BC, Ip M, Lau CB, et al. (2011) Synergistic effects of baicalein with ciprofloxacin against NorA over-expressed methicillin-resistant *Staphylococcus aureus* (MRSA) and inhibition of MRSA pyruvate kinase. *J Ethnopharmacol* 137: 767–773. https://doi.org/10.1016/j.jep.2011.06.039
140. Coelho ML, Ferreira JH, de Siqueira Junior JP, et al. (2016) Inhibition of the NorA multi-drug transporter by oxygenated monoterpenes. *Microb Pathog* 99: 173–177. https://doi.org/10.1016/j.micpath.2016.08.026
141. Falcao-Silva VS, Silva DA, Souza Mde F, et al. (2009) Modulation of drug resistance in *Staphylococcus aureus* by a kaempferol glycoside from *Herissantia tiubae* (Malvaceae). *Phytother Res* 23: 1367–1370. https://doi.org/10.1002/ptr.2695
142. Hequet A, Burchak ON, Jeanty M, et al. (2014) 1-(1H-indol-3-yl)ethanamine derivatives as potent *Staphylococcus aureus* NorA efflux pump inhibitors. *ChemMedChem* 9: 1534–1545. https://doi.org/10.1002/cmdc.201400042
143. Holler JG, Christensen SB, Slotved HC, et al. (2012) Novel inhibitory activity of the *Staphylococcus aureus* NorA efflux pump by a kaempferol rhamnoside isolated from *Persea lingue* Nees. *J Antimicrob Chemother* 67: 1138–1144. https://doi.org/10.1093/jac/dks005
144. Kaatz GW, Seo SM, Ruble CA (1993) Efflux-mediated fluoroquinolone resistance in *Staphylococcus aureus*. *Antimicrob Agents Chemother* 37: 1086–1094. https://doi.org/10.1128/AAC.37.5.1086
145. Kumar A, Khan IA, Koul S, et al. (2008) Novel structural analogues of piperine as inhibitors of the NorA efflux pump of *Staphylococcus aureus*. *J Antimicrob Chemother* 61: 1270–1276. https://doi.org/10.1093/jac/dkn088
146. Lan JE, Li XJ, Zhu XF, et al. (2021) Flavonoids from *Artemisia rupestris* and their synergistic antibacterial effects on drug-resistant *Staphylococcus aureus*. *Nat Prod Res* 35: 1881–1886. https://doi.org/10.1080/14786419.2019.1639182
147. Lowrence RC, Raman T, Makala HV, et al. (2016) Dithiazole thione derivative as competitive NorA efflux pump inhibitor to curtail multi drug resistant clinical isolate of MRSA in a zebrafish infection model. *Appl Microbiol Biotechnol* 100: 9265–9281. https://doi.org/10.1007/s00253-016-7759-2

148. Mullin S, Mani N, Grossman TH (2004) Inhibition of antibiotic efflux in bacteria by the novel multidrug resistance inhibitors biricodar (VX-710) and timcodar (VX-853). *Antimicrob Agents Chemother* 48: 4171–4176. https://doi.org/10.1128/AAC.48.11.4171-4176.2004
149. Ponnusamy K, Ramasamy M, Savarimuthu I, et al. (2010) Indirubin potentiates ciprofloxacin activity in the NorA efflux pump of *Staphylococcus aureus*. *Scand J Infect Dis* 42: 500–505. https://doi.org/10.3109/00365541003713630
150. Randhawa HK, Hundal KK, Ahirrao PN, et al. (2016) Efflux pump inhibitory activity of flavonoids isolated from *Alpinia calcarata* against methicillin-resistant *Staphylococcus aureus*. *Biologia* 71: 484–493. https://doi.org/10.1515/biolog-2016-0073
151. Braga Ribeiro AM, Sousa JN, Costa LM, et al. (2019) Antimicrobial activity of *Phyllanthus amarus* Schumach. & Thonn and inhibition of the NorA efflux pump of *Staphylococcus aureus* by Phyllanthin. *Microb Pathog* 130: 242–246. https://doi.org/10.1016/j.micpath.2019.03.012
152. Roy SK, Kumari N, Pahwa S, et al. (2013) NorA efflux pump inhibitory activity of coumarins from *Mesua ferrea*. *Fitoterapia* 90: 140–150. https://doi.org/10.1016/j.fitote.2013.07.015
153. Sabatini S, Gosetto F, Manfroni G, et al. (2011) 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* 54: 5722–5736. https://doi.org/10.1021/jm200370y
154. Samosorn S, Bremner JB, Ball A, et al. (2006) Synthesis of functionalized 2-aryl-5-nitro-1H-indoles and their activity as bacterial NorA efflux pump inhibitors. *Bioorg Med Chem* 14: 857–865. https://doi.org/10.1016/j.bmc.2005.09.019
155. Shiu WK, Malkinson JP, Rahman MM, et al. (2013) A new plant-derived antibacterial is an inhibitor of efflux pumps in *Staphylococcus aureus*. *Int J Antimicrob Agents* 42: 513–518. https://doi.org/10.1016/j.ijantimicag.2013.08.007
156. Smith EC, Kaatz GW, Seo SM, et al. (2007) The phenolic diterpene totarol inhibits multidrug efflux pump activity in *Staphylococcus aureus*. *Antimicrob Agents Chemother* 51: 4480–4483. https://doi.org/10.1128/AAC.00216-07
157. Stermitz FR, Scriven LN, Tegos G, et al. (2002) Two flavonols from *Artemisa annua* which potentiate the activity of berberine and norfloxacin against a resistant strain of *Staphylococcus aureus*. *Planta Med* 68: 1140–1141. https://doi.org/10.1055/s-2002-36347
158. Tintino SR, Oliveira-Tintino CD, Campina FF, et al. (2016) Evaluation of the tannic acid inhibitory effect against the NorA efflux pump of *Staphylococcus aureus*. *Microb Pathog* 97: 9–13. https://doi.org/10.1016/j.micpath.2016.04.003
159. Tintino SR, Souza VCA, Silva J, et al. (2020) Effect of Vitamin K3 inhibiting the function of NorA efflux pump and its gene expression on *Staphylococcus aureus*. *Membranes (Basel)* 10: 130. https://doi.org/10.3390/membranes10060130
160. Waditzer M, Bucar F (2021) Flavonoids as inhibitors of bacterial efflux pumps. *Molecules* 26: 6904. https://doi.org/10.3390/molecules26226904
161. Wang D, Xie K, Zou D, et al. (2018) Inhibitory effects of silybin on the efflux pump of methicillin resistant *Staphylococcus aureus*. *Mol Med Rep* 18: 827–833. https://doi.org/10.3892/mmr.2018.9021
162. Wani NA, Singh S, Farooq S, et al. (2016) Amino acid amides of piperic acid (PA) and 4-ethylpiperic acid (EPA) as NorA efflux pump inhibitors of *Staphylococcus aureus*. *Bioorg Med Chem Lett* 26: 4174–4178. https://doi.org/10.1016/j.bmcl.2016.07.062

163. Yu JL, Grinius L, Hooper DC (2002) NorA functions as a multidrug efflux protein in both cytoplasmic membrane vesicles and reconstituted proteoliposomes. *J Bacteriol* 184: 1370–1377. https://doi.org/10.1128/JB.184.5.1370-1377.2002
164. Zhang J, Sun Y, Wang Y, et al. (2014) 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* 740: 277–284. https://doi.org/10.1016/j.ejphar.2014.07.020
165. Joshi P, Singh S, Wani A, et al. (2014) Osthol and curcumin as inhibitors of human Pgp and multidrug efflux pumps of *Staphylococcus aureus*: reversing the resistance against frontline antibacterial drugs. *MedChemComm* 5: 1540–1547. https://doi.org/10.1039/C4MD00196F
166. Shokoofeh N, Moradi-Shoeili Z, Naeemi AS, et al. (2019) Biosynthesis of Fe_3O_4@Ag nanocomposite and evaluation of its performance on expression of *norA* and *norB* efflux pump genes in ciprofloxacin-resistant *Staphylococcus aureus*. *Biol Trace Elem Res* 191: 522–530. https://doi.org/10.1007/s12011-019-1632-y
167. Gupta VK, Tiwari N, Gupta P, et al. (2016) A clerodane diterpene from *Polyalthia longifolia* as a modifying agent of the resistance of methicillin resistant *Staphylococcus aureus*. *Phytomedicine* 23: 654–661. https://doi.org/10.1016/j.phymed.2016.03.001
168. Truong-Bolduc QC, Bolduc GR, Medeiros H, et al. (2015) Role of the Tet38 efflux pump in *Staphylococcus aureus* internalization and survival in epithelial cells. *Infect Immun* 83: 4362–4372. https://doi.org/10.1128/IAI.00723-15
169. Kumar S, Athreya A, Gulati A, et al. (2021) Structural basis of inhibition of a transporter from *Staphylococcus aureus*, NorC, through a single-domain camelid antibody. *Commun Biol* 4: 836. https://doi.org/10.1038/s42003-021-02357-x
170. Jang S (2016) Multidrug efflux pumps in *Staphylococcus aureus* and their clinical implications. *J Microbiol* 54: 1–8. https://doi.org/10.1007/s12275-016-5159-z
171. Mirza ZM, Kumar A, Kalia NP, et al. (2011) Piperine as an inhibitor of the MdeA efflux pump of *Staphylococcus aureus*. *J Med Microbiol* 60: 1472–1478. https://doi.org/10.1099/jmm.0.033167-0
172. Rouch DA, Cram DS, DiBerardino D, et al. (1990) Efflux-mediated antiseptic resistance gene *qacA* from *Staphylococcus aureus*: common ancestry with tetracycline- and sugar-transport proteins. *Mol Microbiol* 4: 2051–2062. https://doi.org/10.1111/j.1365-2958.1990.tb00565.x
173. Littlejohn TG, Paulsen IT, Gillespie MT, et al. (1992) Substrate specificity and energetics of antiseptic and disinfectant resistance in *Staphylococcus aureus*. *FEMS Microbiol Lett* 74: 259–265. https://doi.org/10.1111/j.1574-6968.1992.tb05376.x
174. Tennent JM, Lyon BR, Midgley M, et al. (1989) Physical and biochemical characterization of the *qacA* gene encoding antiseptic and disinfectant resistance in *Staphylococcus aureus*. *J Gen Microbiol* 135: 1–10. https://doi.org/10.1099/00221287-135-1-1
175. AKF ES, Dos Reis AC, Pinheiro EEA, et al. (2021) Modulation of the drug resistance by *Platonia insignis* Mart. Extract, ethyl acetate fraction and morelloflavone/volkensiflavone (biflavonoids) in *Staphylococcus aureus* strains overexpressing efflux pump genes. *Curr Drug Metab* 22: 114–122. https://doi.org/10.2174/1389200221666200523155617
176. Mitchell BA, Paulsen IT, Brown MH, et al. (1999) Bioenergetics of the staphylococcal multidrug export protein QacA. Identification of distinct binding sites for monovalent and divalent cations. *J Biol Chem* 274: 3541–3548. https://doi.org/10.1074/jbc.274.6.3541

177. Dymek A, Armada A, Handzlik J, et al. (2012) The activity of 16 new hydantoin compounds on the intrinsic and overexpressed efflux pump system of *Staphylococcus aureus*. *In Vivo* 26: 223–229.
178. Truong-Bolduc QC, Dunman PM, Strahilevitz J, et al. (2005) MgrA is a multiple regulator of two new efflux pumps in *Staphylococcus aureus*. *J Bacteriol* 187: 2395–2405. https://doi.org/10.1128/JB.187.7.2395-2405.2005
179. Guay GG, Tuckman M, McNicholas P, et al. (1993) The *tet*(K) gene from *Staphylococcus aureus* mediates the transport of potassium in *Escherichia coli*. *J Bacteriol* 175: 4927–4929. https://doi.org/10.1128/jb.175.15.4927-4929.1993
180. Ginn SL, Brown MH, Skurray RA (2000) The TetA(K) tetracycline/H^+ antiporter from *Staphylococcus aureus*: mutagenesis and functional analysis of motif C. *J Bacteriol* 182: 1492–1498. https://doi.org/10.1128/JB.182.6.1492-1498.2000
181. Gibbons S, Oluwatuyi M, Veitch NC, et al. (2003) Bacterial resistance modifying agents from *Lycopus europaeus*. *Phytochemistry* 62: 83–87. https://doi.org/10.1016/S0031-9422(02)00446-6
182. Zhu Y, Wang C, Schwarz S, et al. (2021) Identification of a novel tetracycline resistance gene, *tet*(63), located on a multiresistance plasmid from *Staphylococcus aureus*. *J Antimicrob Chemother* 76: 576–581. https://doi.org/10.1093/jac/dkaa485
183. Kehrenberg C, Schwarz S (2004) *fexA*, a novel *Staphylococcus lentus* gene encoding resistance to florfenicol and chloramphenicol. *Antimicrob Agents Chemother* 48: 615–618. https://doi.org/10.1128/AAC.48.2.615-618.2004
184. Mancuso G, Midiri A, Gerace E, et al. (2021) Bacterial antibiotic resistance: the most critical pathogens. *Pathogens* 10: 1310. https://doi.org/10.3390/pathogens10101310
185. Lekshmi M, Ammini P, Kumar S, et al. (2017) The food production environment and the development of antimicrobial resistance in human pathogens of animal origin. *Microorganisms* 5: 11. https://doi.org/10.3390/microorganisms5010011
186. Varela MF, Andersen JL, Ranjana K, et al. (2017) Bacterial resistance mechanisms and inhibitors of multidrug efflux pumps belonging to the major facilitator superfamily of solute transport systems. In: *Frontiers in Anti-Infective Drug Discovery*, Bentham Science Publishers, 109–131. https://doi.org/10.2174/9781681082912117050006
187. Floyd JT, Kumar S, Mukherjee MM, et al. (2013) A review of the molecular mechanisms of drug efflux in pathogenic bacteria: A structure-function perspective. In: Shankar P. Editors, *Recent Research Developments in Membrane Biology*, Research Signpost, Inc., 15–66.
188. Mukherjee M, Kakarla P, Kumar S, et al. (2014) Comparative genome analysis of non-toxigenic non-O1 versus toxigenic O1 *Vibrio cholerae*. *Genom Discov* 2: 1–15. https://doi.org/10.7243/2052-7993-2-1
189. Kumar S, Lindquist IE, Sundararajan A, et al. (2013) Genome Sequence of Non-O1 *Vibrio cholerae* PS15. *Genome Announc* 1: e00227–12. https://doi.org/10.1128/genomeA.00227-12
190. Kumar S, Smith KP, Floyd JL, et al. (2011) Cloning and molecular analysis of a mannitol operon of phosphoenolpyruvate-dependent phosphotransferase (PTS) type from *Vibrio cholerae* O395. *Arch Microbiol* 193: 201–208. https://doi.org/10.1007/s00203-010-0663-8
191. Varela MF (2019) Antimicrobial efflux pumps, In: Capelo-Martínez J.L., Igrejas G. Editor(s), *Antibiotic Drug Resistance*, John Wiley & Sons, Inc., 167–179. https://doi.org/10.1002/9781119282549.ch8

192. Rao M, Padyana S, Dipin K, et al. (2018) Antimicrobial compounds of plant origin as efflux pump inhibitors: new avenues for controlling multidrug resistant pathogens. *J Antimicrob Agents* 4: 1000159. https://doi.org/10.4172/2472-1212.1000159
193. Shrestha U, Lekshmi M, Kumar S, et al. (2018) Bioactive agents as modulators of multidrug efflux pumps of the major facilitator superfamily in key bacterial pathogens. *Curr Trends Microbiol* 12: 15–37.
194. Stephen J, Lekshmi M, Ammini P, et al. (2022) Membrane efflux pumps of pathogenic *Vibrio* species: role in antimicrobial resistance and virulence. *Microorganisms* 10: 382. https://doi.org/10.3390/microorganisms10020382
195. Stephen J, Mukherjee S, Lekshmi M, et al. (2020) Antibiotic resistance in fish-borne pathogens of public health significance: An emerging food safety issue. *Trends Microbiol* 14: 11–20.
196. Kumar S, Mukherjee MM, Varela MF (2013) Modulation of bacterial multidrug resistance efflux pumps of the major facilitator superfamily. *Int J Bacteriol* 2013: 204141. https://doi.org/10.1155/2013/204141

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