

# Methicillin-resistant *Staphylococcus aureus* (MRSA) efflux pump inhibitors from natural products



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## **Abstract**

Among different nosocomial infections, *Staphylococcus aureus*, a Gram-positive bacterium, is a highly adaptive human pathogen. Over the years it had acquired resistance to multiple classes of antibiotics including methicillin. The multidrug resistance towards multiple antibiotics and poor pipeline of safe and effective drugs has rendered bacterial infections a life-threatening problem. Multidrug efflux pumps play an essential role in antibiotic resistance by extrusion of drugs via different mechanisms. Natural products especially derived from plants have emerged as an important source of effective efflux pump inhibitors. In this article different classes of plant- and microbe-derived natural products have been described as efflux pump inhibitors of MRSA that act synergistically in combination with antibiotics to modulate efflux pump-mediated extrusion of antibiotics and thereby help in combating the multidrug resistance.

## **1. Introduction**

*Staphylococcus aureus* is a major cause of nosocomial- and community-acquired infections. The emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) has occurred over the years which contributes to the development of new strains resistant against multiple classes of antibiotics (1). Around 90–95% of *S. aureus* strains worldwide are found to be resistant to penicillin, and methicillin-resistant strains account for 70–80% of its total count in most Asian countries (2). At least 50,000 deaths are recorded due to *S. aureus* infection in Europe every year, and it is predicted that infections due to drug resistance would be the reason for the deaths of nearly ten million people worldwide by 2050 (3). MRSA is a leading cause of endocarditis, bacteremia, soft tissue skin infections, and hospital acquired infections. Since 1990, there has been a rapid spread of MRSA infections in the human community which poses a great challenge for their treatment. It is already known that staphylococci are able to act against each new antimicrobial agent by adopting one or more resistance mechanisms. Several mechanisms associated with the resistance to antibiotics include target protein mutation, antibiotic inactivation by enzymes, or antibiotic accumulation inhibition due to overexpression of the efflux system in bacterial cells. Among these, drug efflux is the most widespread reason for antimicrobial resistance (4). Gram-positive bacteria such as *S. aureus* lacks an outer membrane. Efflux pumps are helpful in limiting the accumulation of toxic compounds within the cell. So far, various efflux pumps have been discovered in microorganisms. These EPs are mainly classified in five different superfamilies, such as (1) adenosine triphosphate-binding cassette transporters (ABC), (2) multidrug and toxic compound extrusion (MATE), (3) major facilitator superfamily (MFS), (4) small multidrug resistance (SMR), and (5) resistance nodulation division (RND) family (Figure 1) (5-7).

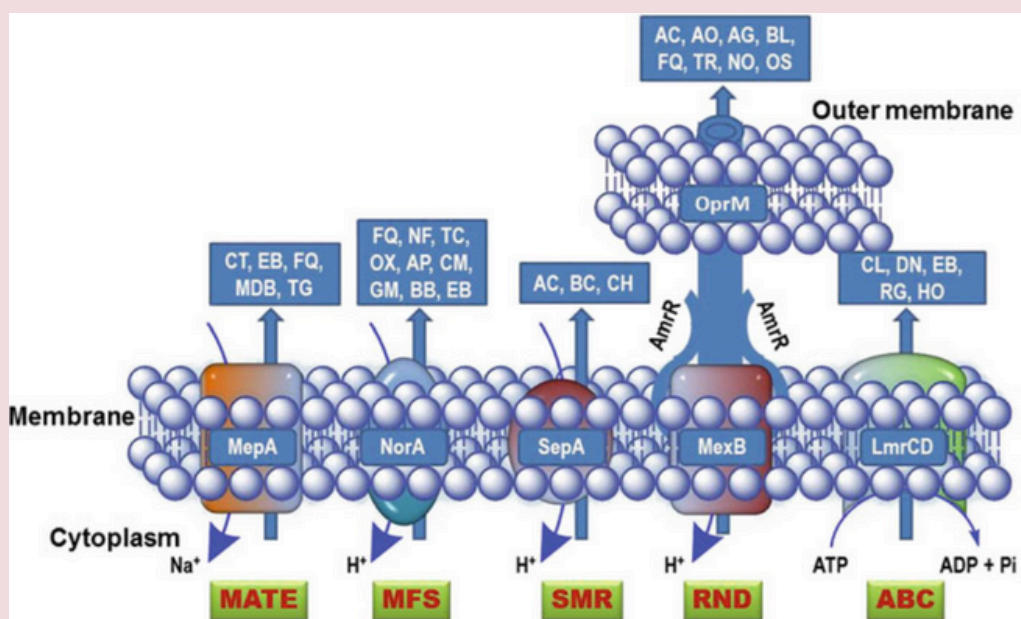


Figure 1. EPs and the substrates that are effluxed out of the bacterial cell (Jachak et al. 2012). ABC ATP-binding cassettes, RND resistance nodulation division, SMR small multidrug resistance, MFS major facilitator superfamily, MATE multidrug and toxic compound extrusion, AC acriflavine, AG aminoglycoside, AO acridine orange, AP ampicillin, BB berberine, BL  $\beta$ -lactam, BC benzalkonium chloride, CH chlorhexidine, CL cholate, CM chloramphenicol, CT ceftazidime, DN daunomycin, EB ethidium bromide, FQ fluoroquinolones, GM gentamicin, HO Hoechst 33342, MDB monovalent and divalent biocides, NF norfloxacin, NO novobiocin, OS organic solvents, OX oxacillin, RG rhodamine, TC tetracyclines, TG tigecycline, TR triclosan (adopted from: Pallavi Ahirrao et.al. In: Kumar, V., Shriram, V., Paul, A., Thakur, M. (eds) Antimicrobial Resistance. Springer, Singapore. (2022), [https://doi.org/10.1007/978-981-16-3120-7\\_19](https://doi.org/10.1007/978-981-16-3120-7_19))

The above efflux pump families have been classified on the basis of amino acid sequence similarity, single or multiple components, specificity, energy source, and the number of transmembrane spanning regions. The ABC, MATE, SMR, and MFS superfamilies have been widely distributed in Gram (+)ve and Gram (-)ve bacteria whereas the RND family is distributed in Gram (-)ve bacteria only.

Among all MDR microorganisms, MRSA strain is of main concern, liable for both hospital- and community-acquired infections (8). Among MRSA, NorA overexpressed strains are the most common ones (9). MsrA efflux pump of the ABC transporter family causes resistance to streptogramins and macrolides. MsrA is a transmembrane protein made up of 488 amino acids with two ATP-binding motifs.

Efflux pumps are an important and major antibacterial drug target. Hence, it is necessary to identify and develop potent efflux pump inhibitors (10-11). Efflux pumps can be inhibited by the following strategies: a) interference in genetic regulation by deregulating the EP expression, b) antibiotic redesigning that are considered as substrates earlier, c) suppressing the functional EPs assembly, d) avoiding the substrate binding by blocking the active site, and e) disintegrating the energy mechanism liable for reinvigorating the Eps (12). To tackle antibiotic resistance, drug resistance reversal agents especially efflux pump modulators/inhibitors would be promising leads. These compounds may possess either

antimicrobial activity of their own or possess the ability to enhance the activity of ineffective antibiotics by inhibiting/modulating efflux pumps. Thus, the susceptibility or sensitivity of resistant strains to antibacterial agents can be reinstated with the aid of EPIs (13-14). The actions of EPI mechanisms are not precisely known yet. But, it has been suggested that the inhibitor binds directly to the pump and thus blocks it competitively or noncompetitively with the substrates. By inhibiting ATP binding or by disrupting the proton gradient, EPIs can cause depletion of energy. A complex of EPI with an antibiotic enhances the entry of an antibiotic into the bacterial cell and further inhibits the efflux of an antibiotic due to the larger complex size (15-16). To combat antibiotic resistance, presently the researchers are working on the development of synergistic antibiotic combinations to reduce the dose of antibiotics many times. In this regard, drug resistance reversal agents especially efflux pump inhibitors (EPIs)/modulators would be the promising agents.

## **2. Natural product inhibitors of efflux pumps**

Historically NPs have been a major source of biologically active molecules exhibiting numerous scaffolds and displaying various activities against both noninfectious and infectious diseases. The NPs are formed biogenetically by the processes catalyzed using enzymes that are highly regio-, enantio-, and diastereospecific. A few efflux pumps selectively efflux out a particular class of antibiotics, while other EPs extrude a diverse class of antibiotics; these are termed as MDR. EPIs may be useful in restoring the clinical efficacy of some earlier antibiotics, by enhancing the potency of antibiotics or by decreasing their resistance development. Numerous natural products are known to act as EPIs on different EPs located on the bacterial cell membrane (17).

### **2.1. *S. aureus* NorA multidrug efflux pump inhibitors**

In MF superfamily, the most studied example is NorA multidrug transporter that contributes to the resistance of *S. aureus*. Berberine and fluoroquinolones like norfloxacin and ciprofloxacin are effluxed out of the cell by NorA EP (18).

#### **2.1.1 Polyphenols**

##### **2.1.1.1 - Arylbenzofuran**

SpinosanA from *Dalea spinosa*, at 48  $\mu\text{M}$ , Reduced Berberine MIC by Eightfold against Wild-Type *S. aureus*, whereas, (+)-Medicarpin at 56  $\mu\text{M}$  decreased Berberine MIC by Fourfold (19).

##### **2.1.1.2- N-Caffeoylphenylkylamides**

N-Trans-Feruloyl-4'-O-Methyldopamine isolated from *Mirabilis jalapa* exhibited moderate EPI activity against NorA-Overexpressed *S. aureus* 1199B strain. It showed an eightfold reduction in the Minimum Inhibitory Concentration of Norfloxacin at 100  $\mu\text{g}/\text{mL}$  (20).

##### **2.1.1.3 Caffeoylquinic Acids**

40,5'-O-caffeoylquinic acid isolated from chloroform extract of *Artemisia absinthium* potentiated activity of Berberine by eight-fold, EtBr by 4-fold, and Fluoroquinolones, Ciprofloxacin, and Norfloxacin by 4–Eight-Fold in NorA overexpressed *S. aureus* Strain (21). Curcumin was reported to exhibit significant inhibition of NorA EP in *S. aureus*. There is an Eightfold Reduction of Ciprofloxacin MIC at 25  $\mu\text{M}$  by curcumin. The molecular modeling study of curcumin with the human Pgp and NorA efflux protein revealed favorable binding interactions (22).

#### 2.1.1.4 Chalcones

A chalcone compound characterized from *Dalea versicolor* showed a four-fold increase in berberine activity against MDR *S. aureus* at 10 µg/mL (23). 3, 4'-dihydroxy-3,4,5-trimethoxy-chalcone isolated from the flowers of *Arrabidaea brachypoda* showed a significant decrease in MIC of norfloxacin by fourfold (24).

#### 2.1.1.5 Coumarins

A 20-fold reduction of norfloxacin MIC in *S. aureus*-resistant strains (MRSA16565, 9543, 5, and 7) was observed due to coumarins, viz., 4-[[[E]-5-(3,3-dimethyl-2-oxiranyl)-3-methyl-2-pentenyl]oxy]-7H-furo(3,2-g)chromen-7-one and 7-[[[E]-5-(3,3-dimethyl-2-oxiranyl)-3-methyl-2-pentenyl]oxy]-2H-2-chromenone isolated from grapefruit oil at 35.7 µg/mL and 30 µg/mL concentrations, respectively (25). The ciprofloxacin MIC reduced from 10-80 µg/mL to 2.5-5 µg/mL and of EtBr from 4-16 µg/mL to 0.5-2 µg/mL by galbanic acid (A10), a sesquiterpene coumarin isolated from the roots of *Ferulazowitsiana*, against several *S. aureus*-resistant clinical isolate strains, at 300 µg/mL (26).

#### 2.1.1.6 Flavones and flavonols

Chrysosplenol-D and chrysosplenetin, methoxy flavonols reported from the herbaceous plant *Artemisia annua*, showed inhibition of *S. aureus* growth at a sub inhibitory concentration (30 µg/mL) with MIC of 25 µg/mL and 6.25 µg/mL, respectively (27).

#### 2.1.1.7 Flavonolignans

50-Methoxyhydrocarpin-D (5'-MHC-D), a flavonolignan reported from *Berberis aetnensis* leaves, exhibited EPI activity by reducing norfloxacin MIC to 0.25 µg/mL at 10 µg/mL for wild-type *S. aureus* (27).

#### 2.1.1.8 Isoflavones

Isoflavones, viz., genistein, orobol, and biochanin A reported from *Lupinus argenteus*, decreased norfloxacin MIC by 2-4-folds against *S. aureus* mutant strain, at 10 µg/mL. Spinosa A, an isoflavone compound characterized from *Dalea spinosa*, at 48 µM concentration showed an eightfold reduction of berberine MIC (89 µM) in wild-type *S. aureus* (28).

#### 2.1.1.9 Tannins

Catechin compounds, viz., epicatechin gallate and epigallocatechin gallate, increased the norfloxacin MIC by fourfold in wild-type (SA 1199) and NorA-overexpressed *S. aureus* (SA1199B) strain, at 20 µg/mL (29).

### **2.1.2 Terpenoids**

Ferruginol characterized from *Chamaecyparis lawsoniana* exhibited NorA pump inhibitory activity in *S. aureus*-resistant strain. Ferruginol at a subinhibitory concentration (2 µg/mL) showed a twofold potentiation of norfloxacin against SA1199B strain (30).

### **2.1.3 Oligosaccharides**

Five murucoidins (XII-XVI) were isolated and characterized from *Ipomoea murucoides*. Murucoidin XIV at 5 µg/mL exhibited a fourfold increase in norfloxacin activity against *S. aureus* strains (31).

#### 2.1.4 Alkaloids

Piperine, a piperidine alkaloid characterized from *Piper nigrum* fruits, displayed no growth of *S. aureus* mutant at 1 µg/mL concentration of ciprofloxacin when it was co administered at 50 µg/mL (32).

## 2.2. Miscellaneous *S. aureus* and MRSA efflux pump inhibitors of natural product origin

The essential oil extracted from *Origanum vulgare* L. as well as its constituents, viz., carvacrol and thymol, showed EPI activity. The essential oil showed a four fold reduction of tetracycline MIC (64 µg/mL to 16 µg/mL) whereas carvacrol and thymol exhibited a twofold reduction of tetracycline MIC (64 µg/mL to 32 µg/mL) against *S. aureus* IS-58 strain over expressing TetK efflux pump (33). Essential oil and its major constituent,  $\alpha$ -pinene(C03), extracted from *Croton grewoides* leaves showed EPI activity in SA-1199B (NorA-overexpressed strain) and IS-58 (TetK-overexpressed strain) by 64-fold and four-fold modulation in MIC of tetracycline and norfloxacin respectively (34). *Nigella sativa* essential oil and its constituents carvacrol, thymoquinone, and p-cymene were studied for their antibacterial effect and modulation of antibiotic resistance in methicillin-sensitive ATCC25923 and methicillin-resistant MRSA 272123 clinical isolate of *S. aureus*. All these constituents and essential oil displayed MIC values in mM range, indicating a weak antibacterial effect.

A microbial natural product, 2-(2-aminophenyl) indole isolated from *Streptomyces* sp. IMTB 2501 was found as the most potent NorA inhibitor, decreasing the MIC of ciprofloxacin, moxifloxacin, norfloxacin, and chloramphenicol in SA-1199B (NorA-overexpressed strain) by 64-, 16-, 4-, and 4-folds (FICI<sub>0.5</sub>), respectively (35).

## 3. Conclusion

Antibiotic resistance is emerging at an alarming rate. Thus, there is an urgent unmet need to develop alternative therapies that either reduce the bacterial resistance to antibiotics or potentiate the activity of existing antibiotics. Efflux pumps are one of the major targets that confer resistance to clinically used antibiotics. Presently no efflux pump inhibitor combination with existing antibiotics is clinically approved to tackle antimicrobial resistance. Over the decades natural products have demonstrated multiple biological activities in biomedical research and served as an important source of lead molecules in drug discovery and development.

In this article, natural compounds with potential efflux pump inhibition activity are described. This article describes the natural products that showed activity against NorA, efflux pump responsible for extrusion of drugs in *S. aureus* and MRSA. However, no plant/fungus/marine-derived antibiotic is used clinically yet. Since isolation and identification of plant-derived drugs are tedious and time consuming, in silico methodology and high-throughput screening can be utilized. A majority of bioactives discussed above have shown promising results as potent EPIs, determined using in vitro studies. The in vivo animal model studies and human clinical trials would be required to determine antibacterial action, efficacy, and toxicity studies to optimize a high therapeutic efficacy dosage of EPIs at an acceptable toxicity level.

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