microbial cell

Review

www.microbialcell.com

Efflux pumps: gatekeepers of antibiotic resistance in *Staphylococcus aureus* biofilms

Shweta Sinha[1], Shifu Aggarwal[2,3] and Durg Vijai Singh[1,*]

[1]Department of Biotechnology, School of Earth, Biological and Environmental Sciences, Central University of South Bihar, 824236, Gaya, India. [2]Department of Infectious Disease Biology, Institute of Life Sciences,, 751023, Bhubaneswar, India. [3]Current Address: Department of Surgery, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts USA.

*Corresponding Author:
Durg Vijai Singh, Tel: +91 7978911048; E-mail: dvsingh@cusb.ac.in

ABSTRACT *Staphylococcus aureus*, a versatile human pathogen, poses a significant challenge in healthcare settings due to its ability to develop antibiotic resistance and form robust biofilms. Understanding the intricate mechanisms underlying the antibiotic resistance is crucial for effective infection treatment and control. This comprehensive review delves into the multifaceted roles of efflux pumps in *S. aureus*, with a focus on their contribution to antibiotic resistance and biofilm formation. Efflux pumps, integral components of the bacterial cell membrane, are responsible for expelling a wide range of toxic substances, including antibiotics, from bacterial cells. By actively extruding antibiotics, these pumps reduce intracellular drug concentrations, rendering antibiotics less effective. Moreover, efflux pumps have emerged as significant contributors to both antibiotic resistance and biofilm formation in *S. aureus*. Biofilms, structured communities of bacterial cells embedded in a protective matrix, enable *S. aureus* to adhere to surfaces, evade host immune responses, and resist antibiotic therapy. Efflux pumps play a pivotal role in the development and maintenance of *S. aureus* biofilms. However, the interplay between efflux pumps, antibiotic resistance and biofilm formation remains unexplored in *S. aureus*. This review aims to elucidate the complex relationship between efflux pumps, antibiotic resistance and biofilm formation in *S. aureus* with the aim to aid in the development of potential therapeutic targets for combating *S. aureus* infections, especially those associated with biofilms. The insights provided herein may contribute to the advancement of novel strategies to overcome antibiotic resistance and disrupt biofilm formation in this clinically significant pathogen.

doi: 10.15698/mic2024.11.839
Received originally: 12. 06. 2024;
in revised form: 01. 10. 2024,
Accepted: 08. 10. 2024
Published: 11. 11. 2024

Keywords: S aureus, antibiotic resistance, biofilm, efflux pumps, efflux pump inhibitors

Abbreviations:
ABC – ATP binding cassette,
CCCP – carbonyl cyanide-3-chlorophenyl hydrazone,
EPIs – efflux pump inhibitors,
EPS – extracellular polymeric matrix substance,
MATE – multidrug and toxin extrusion,
MFS - major facilitator superfamily,
MRSA - methicillin-resistant *S. aureus*,
MSCRAMM – microbial surface component recognizing adhesive matrix molecules,
RND – resistance nodulation cell division,
SMR – small multidrug resistance.

INTRODUCTION

The emergence of antimicrobial-resistant bacteria has become a global health crisis, posing significant challenges to modern medicine [1]. Among these pathogens, *Staphylococcus aureus* is the most prevalent human pathogen, causing a wide range of infections from mild skin and soft tissue infections to life-threatening conditions such as pneumonia, endocarditis, and device-associated infections [2]. *S. aureus* is a commensal organism, colonizing the anterior nasal passages of 20% to 80% of the human population [3]. However, its ability to rapidly develop resistance to a broad spectrum of antimicrobial compounds has made it a major concern in clinical settings [4]. The U.S. Centre for Disease Control reports *S. aureus* as the second most prevalent pathogenic bacteria, highlighting its significance in public health.

Historically, *S. aureus* antimicrobial resistance is marked by the widespread use of methicillin and semi-synthetic anti-staphylococcal penicillin in the 1960s that led to the emergence of methicillin-resistant *S. aureus* (MRSA) [5]. Since then, MRSA has become a leading hospital-associated pathogen [6-9]. Vancomycin, long considered the drug of last resort for severe MRSA infections, has shown decreased efficacy with the emergence of vancomycin-intermediate *S. aureus* (VISA) and vancomycin-resistant *S. aureus* (VRSA) in some regions [10]. *S. aureus* has developed resistance to multiple antibiotic classes, including aminoglycosides, penicillin, macrolides, and tetracycline [11], leading to frequent outbreaks and treatment challenges [12].

Among the various known antibiotic resistance mechanisms, efflux pumps play a crucial role in *S. aureus*. These membrane-bound transporters act as critical defence mechanisms by expelling antibiotics and other toxic compounds from the bacterial cell, thereby reducing their

intracellular concentration and efficacy [13]. Recent studies have also suggested a link between efflux pumps, virulence and biofilm formation, highlighting their multifaceted role in *S. aureus* pathogenesis [14].

Biofilm formation represents a sophisticated survival strategy employed by *S. aureus* and other pathogens [15–17]. In natural and clinical environments, *S. aureus* forms complex microbial communities encased in a protective extracellular matrix [18]. This mode of growth significantly enhances *S. aureus* tolerance to various antibiotic classes, promotes persistence, and complicates treatment strategies. Studies have shown that efflux pumps contribute to biofilm formation by facilitating the export of quorum-sensing molecules necessary for biofilm development, enhancing tolerance to antimicrobial compounds within the biofilm structure and modulation of gene expression involved in extracellular matrix production [13, 19, 20].

This review presents an understanding of the role of efflux pumps in developing antibiotic resistance in *S. aureus* biofilm which will help in the development of effective therapeutic strategies against efflux pumps to overcome the increasing problem of biofilm associated infections.

ANTIBIOTIC RESISTANCE MECHANISMS

Antibiotics are designed to eliminate microorganisms that are harmful to human health. However, the increased use of antibiotics has led to the emergence of resistant bacteria, suggesting that such organisms are present in the environment and may have evolved due to antibiotic exposure [13, 19, 20]. *S. aureus* has developed resistance to various antibiotics. The mechanisms of antibiotic resistance in *S. aureus* are diverse and can be broadly categorized into intrinsic and acquired resistance. Acquired antibiotic resistance often results from plasmid-mediated resistance [21], mutations in chromosomal genes or by acquisition of external genetic elements of resistance [21, 22]. MRSA exemplifies acquired resistance, primarily encoded by the *mecA* gene carried on the Staphylococcal Cassette Chromosome *mec* (SCC*mec*). This genetic element can be transferred between staphylococcal species, thus contributing to the spread of resistance [13].

S. aureus utilizes various mechanisms to develop intrinsic antimicrobial resistance, including limiting drug uptake, modifying drug targets, enzymatically inactivating drugs and actively effluxing the drugs [23, 24]. Among these mechanisms, the efflux system is a major mode of intrinsic drug resistance in *S. aureus* and is described in detail in the following section.

KNOWN STRUCTURE AND FUNCTION OF EFFLUX PUMPS IN *S. AUREUS*

Efflux pumps are ubiquitous membrane proteins involved in the export of the harmful substances from bacterial cells to the external environment [25]. These proteins, either present on bacterial chromosomes or on plasmids [26] are employed by *S. aureus*, a key mechanism adopted to cope with the diverse range of antimicrobials used to treat infections [27].

Based on energy requirements and structure, efflux pumps in *S. aureus* are classified into five membrane protein families: Major Facilitator Superfamily (MFS), Small Multidrug Resistance (SMR), Multidrug and Toxin Extrusion (MATE) family, Resistance Nodulation Cell Division (RND) superfamily, and ATP-binding Cassette (ABC) superfamily (**Figure 1**) [28]. MFS and SMR transporters utilize proton motive force to drive substrate extrusion via an anti-port H^+ drug mechanism. The MATE family can use the sodium membrane gradient, while the ABC superfamily uses ATP hydrolysis to drive substrate extrusion [29].

Among the known efflux transporters in *S. aureus*, the MFS is the primary class, encoded by NorA, NorB, NorC, MdeA, SdrM, LmrS, QacA, and QacB efflux proteins (**Table 1**) [30]. NorA is the most studied and predominant efflux pump associated with the first line defence against antimicrobials in *S. aureus*. It is often overexpressed in MRSA strains [31, 32]. The NorA protein consists of twelve transmembrane segments with 388 amino acids, sharing 44% identity to *Escherichia coli*'s tetracycline efflux pump TetA and is 24% identical with *Bacillus subtilis* Bmr [33]. NorA pumps expel a variety of compounds, including hydrophilic fluoroquinolones such as norfloxacin and ciprofloxacin, dyes like ethidium bromide, and biocides like quaternary ammonium compounds [34].

NorB efflux pumps have structural similarities with Blt (41%), and Bmr (30%) of *B. subtilis* and NorA (30%), QacA (39%) of *S. aureus*. NorB confers resistance to a diverse range of antimicrobial compounds, including biocides like cetrimide, tetraphenylphosphonium, and dyes such as ethidium bromide, and hydrophobic and hydrophilic fluoroquinolones like norfloxacin and ciprofloxacin [43]. The NorC efflux pump is comprised of 462 amino acids having twelve transmembrane domains and shares 61% similarity with the *norB* efflux gene of *S. aureus* [43]. NorC is associated with low-level resistance to ciprofloxacin, moxifloxacin, garenoxacin, and the dye rhodamine. The Nor efflux pumps (NorA, NorB, NorC) are regulated by the global regulator MgrA. MgrA acts as a positive regulator for *norA* gene expression but a negative regulator of *norB* and *norC* gene expression (**Table 1**) [62, 63]. This differential regulation of Nor efflux pumps by MgrA enables the bacterium to modulate its efflux pump expression in response to diverse environmental stressors and antimicrobial agents, thereby optimizing its survival and resistance strategies.

The MdeA efflux pump is a 479 amino-acid protein with 14 transmembrane segments shared similarities with LmrB of *B. subtilis* (24%), EmrB of *E. coli* (24%), and QacA of *S. aureus* (23%) [46]. It confers resistance to biocides like benzalkonium chloride, dequalinium, and tetraphenylphosphonium, and dyes like ethidium bromide, and antibiotics such as virginiamycin, novobiocin, mupirocin, and fusidic acid [46, 61].

SdrM is a 447-amino-acid protein with 14 transmembrane segments, shows 23% similarity with NorB and 21% similarity with the QacA protein. The SdrM transporter confers resistance to antimicrobials like norfloxacin and dyes such as acriflavine and ethidium bromide.

The lincomycin resistance protein LmrS from *S. aureus* is 480 amino acids long with 14 putative membrane-spanning domains. It shows similarity with ImrB from *B. subtilis* (39%), *farB* from *Neisseria gonorrhoeae* (25%), and *emrS* from *E. coli* (3%). Antibiotics such as linezolid, tetraphenylphosphonium chloride, sodium dodecyl sulphate, trimethoprim, and chloramphenicol are less likely to be removed by LmrS efflux pump (**Table 1**) [48].

QacA and QacB are 514-amino-acid proteins with 14 transmembrane segments [64]. QacA mediates resistance to antimicrobials such as ethidium bromide and rhodamine,

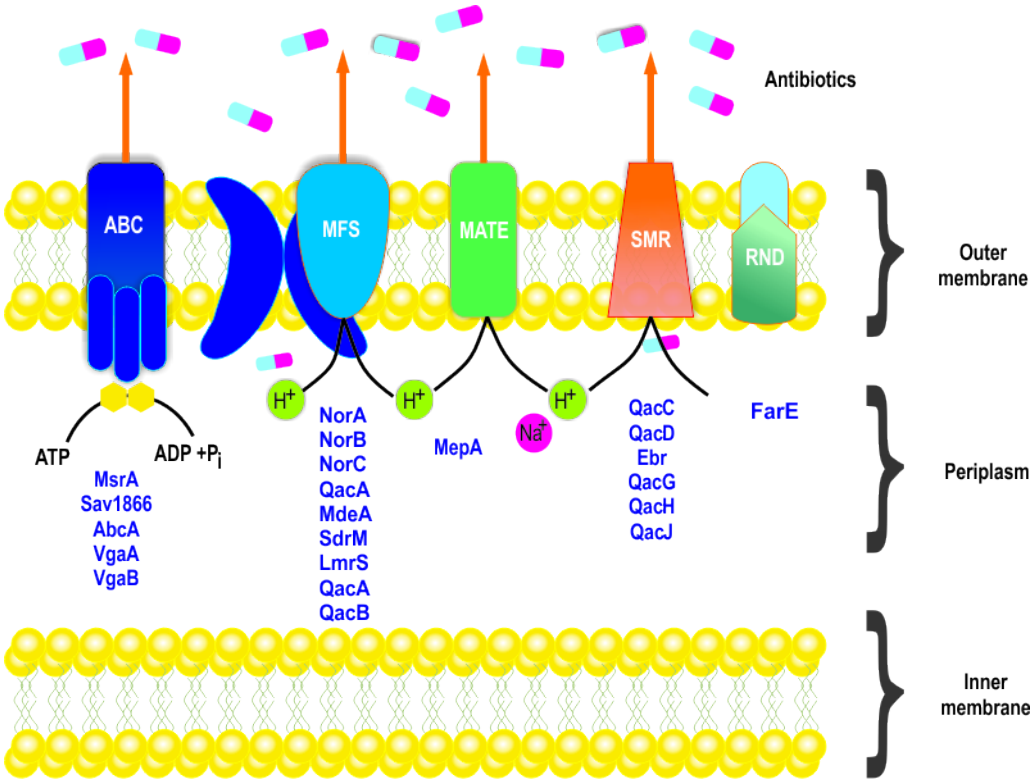

FIGURE 1 ● **Known classes of multidrug efflux pumps in *S. aureus*.** Multidrug efflux pumps identified in *S. aureus* are categorized into five families of membrane proteins: ATP binding cassette (ABC) superfamily, major facilitator superfamily (MFS), multidrug and toxin extrusion (MATE) family, small multidrug resistance (SMR) family and FarE, a resistance -nodulation division (RND) type efflux pumps.

quaternary ammonium compounds like benzalkonium chloride, tetraphenylphosphonium, and dequalinium, diamidines such as pentamine and DAPI, biguanides like chlorhexidine, and guanyl hydrazones [49, 52]. On the other hand, plasmid-encoded QacB pump provides protection against monovalent lipophilic cations [64].

S. aureus TetA(K) and Tet38 efflux pumps mediate high levels of tetracycline resistance [65]. The TetA(K) efflux gene is plasmid-encoded, functioning as a $Na^+(K^+)/H^+$antiporter comprising 459 amino acids and 14 transmembrane regions [66]. Antibiotics such as tetracycline, oxytetracycline, and chlortetracycline are rendered ineffective by TetK, but to a lesser extent to minocycline, doxycycline, and 6-methyl-6-deoxytetracycline. Bacteria equipped with the TetK efflux pump are also resistant to extrinsic stimuli such as sodium stress, alkali stress, and potassium deficiency stress [67]. Like the NorB efflux pump, TetK contributes to *S. aureus* colonisation on mouse skin and survival during abscess development [68]. Chromosomally encoded Tet38 with 450 amino acids and 14 transmembrane domains [68, 69] shares 46% similarity with *S. aureus tetK* and 45% similarity with *B. subtilis tetA* [70]. Tet38 is negatively regulated by MgrA, which confers resistance to quinolones and tetracyclines [71]. Studies have shown that Tet38 also plays a role in *S. aureus* internalization into host cells through interaction with the CD36 receptor [72], suggesting its potential role in biofilm formation and host-pathogen interactions.

Another MFS efflux pump, SepA, comprising 157 amino acids is also encoded by the *S. aureus* genome. It confers low-scale resistance to antiseptics such as benzalkonium chloride, chlorhexidine gluconate, and chromosomal-encoded dye acriflavine (**Table 1**) [54].

The SMR transporters are episome encoded [36] comprised of 110 amino acids and possess four transmembrane helices [73]. The SMR transporters include QacC (QacD, Ebr, or Smr), QacG, QacH, and QacJ. Despite differences in amino acid sequences, Smr and QacG/H/J have similar substrate specificities [36]. This efflux pump confers resistance to quaternary ammonium compounds, such as benzalkonium chloride, and monovalent cationic dyes, such as ethidium bromide [74].

The MATE family member MepA efflux pump consists of 452-amino-acid protein having twelve transmembrane regions and is located on chromosome 2. It shares 26% identity with MATE transporters belonging to other organisms: CdeA from *Clostridium difficile* and NorM from *Vibrio parahaemolyticus* [75].

MepA is associated with a multidrug-resistant phenotype in clinical *S. aureus* strains, providing minimal resistance against ethidium bromide, chlorhexidine, pentamidine, tetraphenylphosphonium, quaternary ammonium compounds, fluoroquinolones (ciprofloxacin, norfloxacin), benzalkonium chloride, cetrimide, dequalinium, tetraphenylphosphonium, chloroquine, and tigecycline [40, 76–78]. The *mepA* gene is controlled by MepR, which belongs to the MarR family of transcriptional repressors [39, 79].

S. aureus possess two chromosomally encoded ABC transporter, Sav1866 and abc. These transporters are single polypeptides possessing transmembrane and nucleotide-

TABLE 1 ● Multidrug resistantefflux pumps reported in *S. aureus*.

Efflux pump family	Gene	Gene location	Regulator	Substrate	Reference
SMR	Smr (QacC, QacD, Ebr) QacG, QacH QacJ	Plasmid	Not known	Benzalkonium Chloride, Cetrimide, Chlorhexidine Diacetate, Ethidium Bromide, Proflavine, Cetyltrymethylammonium	[35–38]
MATE	MepA	Chromosome	MepR	Ciprofloxacin, Norfloxacin, Moxifloxacin Sparfloxacin Tigecycline, Pentamidine, Cetrimide, Benzalkonium Chloride, Dequalinium Tetraphenylphosphonium, Chlorhexidine, Ethidium Bromide, Acriflavine, Crystal Violet, Hoechst 33342, 4-6-Diamidino-2-Phenylindole	[39–42]
MFS	NorA, NorB, NorC	Chromosome	MgrA, NorG	Hydrophilic Fluoroquinolones (Ciprofloxacin, Norfloxacin, Dparofloxacin, Gemifloxacin, Premafloxacin), Qacs (Tetraphenyl Phosphonium, Benzalkonium Chloride), Cetrimide, Tetraphenyl Ammonium, Dyes (E.G. Ethidium Bromide, Rhodamine)	[43–54]
	MdeA	Chromosome	Not known	Virginiamycin, Novobiocin, Mupirocin, Fusidic Acid, Doxorubicin, Daunorubicin, Benzalkonium Chloride, Tetraphenylphosphonium, Ethidium Bromide, Hoechst 33342	
	SdrM	Chromosome	Not known	Norfloxacin, Acriflavine, Ethidium Bromide	
	LmrS	Chromosome	Not known	Linezolid, Chloramphenicol, Florfenicol, Thrimethoprim Erythromycin, Kanamycin, Fusidic Acid, Lincomycin, Streptomycin Tetraphenylphosphonium, Ethidum Bromide	
	QacA/B	Plasmid	QacR	Pentamidine, Benzalkonium Chloride, Cetrimide, Chlorhexidine, Ethidium Bromide (Over 30 Mono And Divalent Cations). Benzalkonium Chloride, Tetraphenylphosphonium, Ethidium Bromide, Acriflavine, Rhodamine	
	TetA (K) Tet38	Plasmid	TetR MgrA	Tetracycline, Omadacycline, Amino Methylcycline, Tunicamycin, Fosfomycin, Fatty Acids	
	SepA	Chromosome	Not known	Benzalkonium Chloride, Chlorhexidine Gluconate, Dye Acriflavine	
ABC	Sav1866 AbcA	Chromosome	Not known SarA	Doxorubicin, Vinblastine, Ethidium Bromide, Hoechst 33342, Oxacillin, Imipenem, Nafcillin, Penicillin G, Methicillin, Cefotaxime, Moenomycin Tetraphenylphosphonium, Rhodamine, Ethidium Bromide	[44, 55–61]
	MsrA, VgaA Vga(A) LC VgaB	Plasmid	SarZ MgrA NorG Not known	Erythromycin, Macrolides, Type B Streptogramins; Type A Streptogramin. Lincomycin, Clindamycin; Type A Streptogramin	

binding domains that, upon dimerization, produce a functional transporter. The crystal structure of Sav1866 has been identified [55]. Functional studies have shown that Sav1866 can transport diverse substrates such as ethidium bromide, Hoechst 33342, tetraphenylphosphonium, verapamil and vinblastine [56]. The other plasmid encoded ABC transporter belonging to the MsrA efflux pumps and possessing a single-nucleotide-binding-domain may interact with other transmembrane proteins [59] . In addition, the Vga proteins are also expressed by genes present on plasmids with a ATP-binding-domain (ABD) transporters (Table 1, Figure 2) [80, 81].

The role of efflux pumps is widely known in antibiotic resistance, however, they regulate the internal environment by extruding toxic substances, biofilm formation molecules, quorum sensing molecules and virulence factors (Figure 2) [13]. This multifaceted role of efflux pumps underscores their importance not only in antibiotic resistance but also in the overall pathogenicity and survival strategies of *S. aureus*.

BIOFILM FORMATION IN *S. AUREUS*

Biofilms are complex, sessile microbial communities encased in a self-produced extracellular polymeric substance (EPS) that

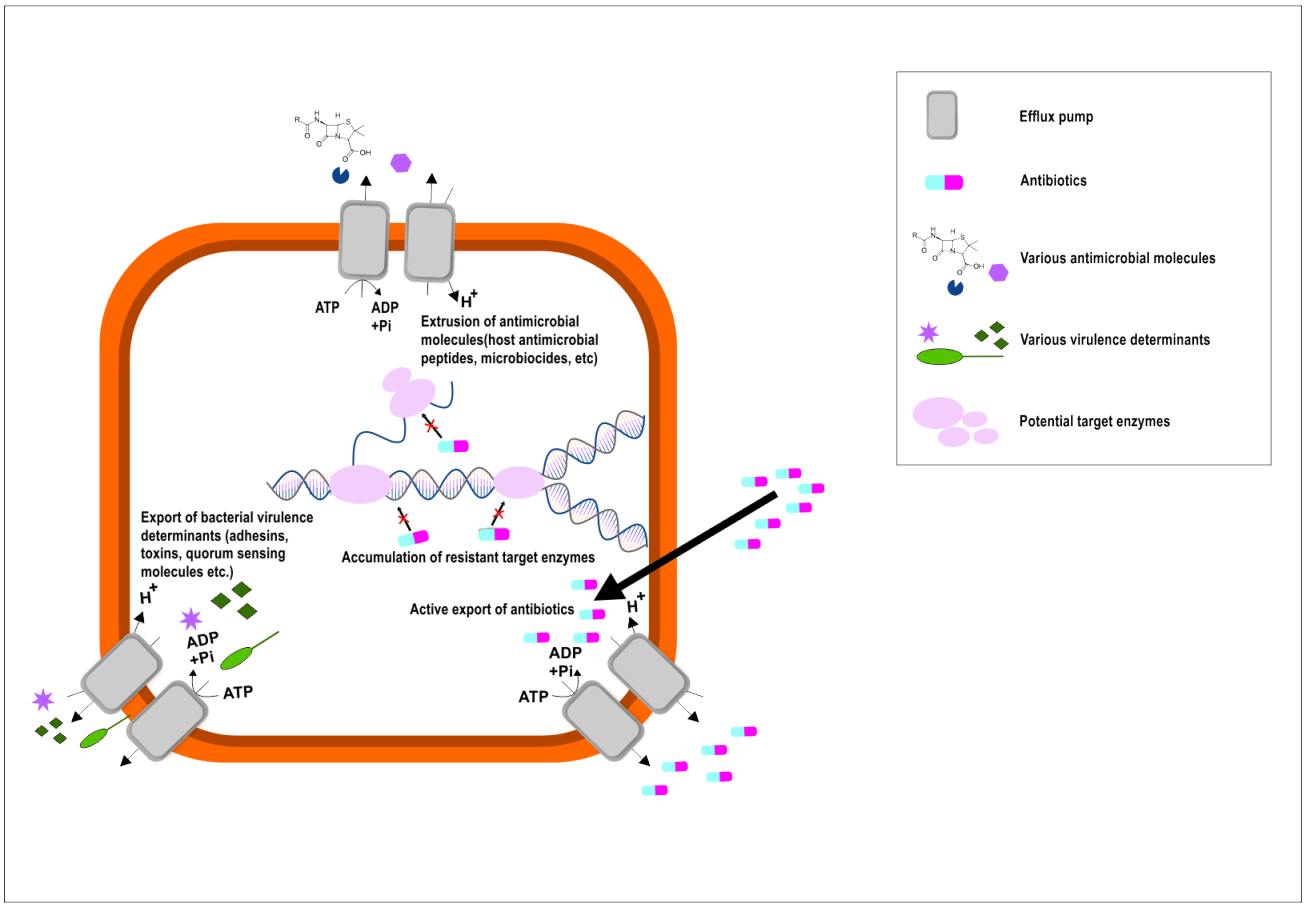

FIGURE 2 **Intrinsic and antibiotic induced implications of bacterial efflux pumps.** Bacterial efflux pumps function by (1) increasing bacterial pathogenicity by extruding antibacterial molecules produced from the host and secreting bacterial virulence factor, (2) reducing antibiotic efficacy by pumping them out of the bacteria, thus lowering intracellular concentration of antibiotics, which can increase development of further resistance.

adheres to surfaces and forms aggregates [82]. The EPS, comprised of polysaccharides, proteins and nucleic acids, constitutes up to 90% of the biofilm's dry weight and provides the immediate environment for the microorganisms to form a biofilm [83, 84]. The interaction between EPS and bacterial aggregates confers cohesion and viscoelasticity to the biofilm structure [85].

Like most of the bacterial species, *S. aureus* possesses similar stages of biofilm development, namely, attachment, accumulation, and detachment [86]. During the attachment stage, *S. aureus* initiates biofilm formation through the adhesion of planktonic cells to natural or biomaterial surfaces. This event is mediated through the organization of microbial surface components recognizing adhesive matrix molecules (MSCRAMMs). The major components of MSCRAMM includes fibronectin-binding proteins (FnbA and FnbB) [87], clumping factors such as ClfA, ClfB [88], and serine-aspartate repeat family proteins such as SdrC, SdrD and SdrE [89].

Following initial attachment, bacterial cells proliferate and begin to produce EPS in response to environmental cues [90]. During the aggregation stage, bacteria form biofilms by recognizing environmental signals that stimulate intracellular signal molecules and regulatory networks which leads to the proliferation and thickening of the biofilm [91]. Thus, a compact three-dimensional mushroom like structure of the formed biofilm is encased in a extracellular matrix which provides resistance against human immune system and antibiotics [92].

As the biofilm matures, dispersal of the biofilm is triggered leading to the release of cells from the biofilm [93]. This stage is important for colonization of new surfaces, dissemination of infection and continuation of the biofilm life cycle (Figure 3).

BIOFILM FORMATION AND EFFLUX MEDIATED ANTIBIOTIC RESISTANCE

Despite being susceptible to antibiotics, bacteria have an inherent capacity to survive, which occurs by forming a sessile community called biofilms [94]. The role of efflux pumps in biofilms is known to be functioning through excretion of extracellular matrix molecules and quorum sensing molecules that mediate biofilm formation, in addition to effluxing the harmful molecules and influencing the surface adhesion [13]. The expression of efflux genes in *S. aureus* biofilms has been the determining factor for efflux pump mediated resistance. A study reported that the expression of several efflux and transporter genes was altered during biofilm growth compared to exponential and stationary phase cells [95]. A comparative transcriptomic study on *S. aureus* cells under planktonic and biofilm conditions showed that the expression of transporter genes was higher in biofilms than in planktonic cells [96]. Of the

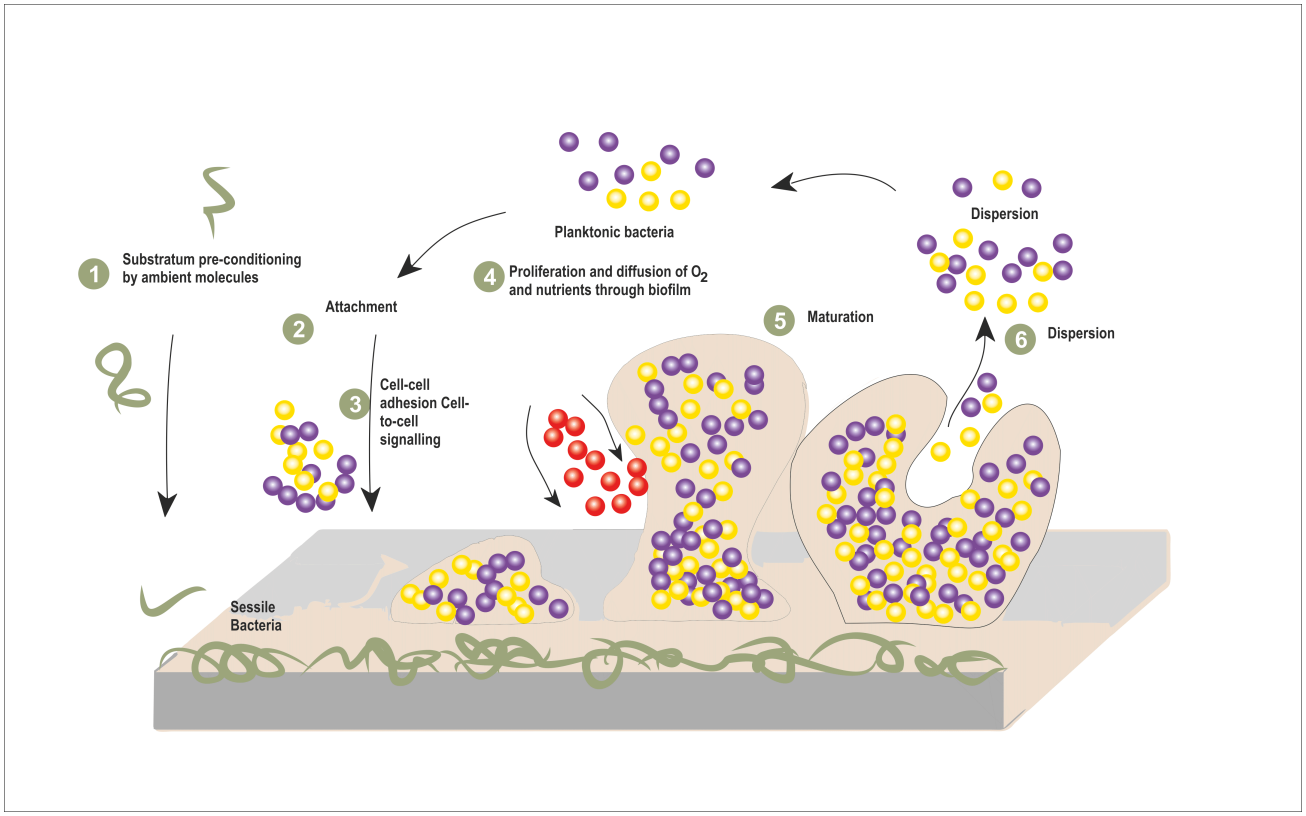

FiGURE 3 ● Steps involved in biofilm formation of *S. aureus*.

MFS transporters *mdeA*, *norB*, and *norC*, which are upregulated in S. aureus biofilms, norB and norC pumps extrude cetrimide, ethidium bromide, quinolones, and tetraphenylphosphonium and the *mdeA* efflux pump exports a range of quaternary ammonium compounds and antibiotics [46, 97]. These observations thus suggest that *norB* gene expression is upregulated in response to acid shock but reduced under other conditions, thus suggesting that it may be involved in maintaining low pH in the biofilm [75]. Also, the *norB* gene functions to ensure that biofilm cells are protected from the toxic effects of organic acids produced during anaerobic respiration [98]. Studies demonstrated that MgrA, a pleiotropic regulator of *S. aureus*, acts as a negative regulator for the *norB* and *norC* efflux gene, repressing the biofilm formation and thus establishing a link between efflux pump and biofilm formation in *S. aureus* [71]. Moreover, a hypothetical gene showing characteristics of the MFS family was identified in an insertional mutant library in a high biofilm-forming clinical isolate of *S. aureus*, which, when disrupted, led to a defective biofilm [99]. Although several efflux pumps have been described, most of the studies explain the role of MFS type efflux pumps in biofilm formation of *S. aureus*. However, the exact mechanism of efflux pumps in mediating antibiotic resistance in biofilms, the role of non-MFS efflux pump families in biofilm formation and maintenances and the potential of targeting efflux pumps as a strategy to combat biofilm-associated infections remains elusive in *S. aureus*.

EFFLUX PUMPS INHIBITORS

The emergence of antimicrobial resistance among clinical strains of *S. aureus* has necessitated the development of novel therapeutic strategies. Efflux pump inhibitors (EPIs) have emerged as a promising approach to combat antibiotic resistance. EPIs function by blocking the extrusion of antibiotics, thereby restoring antimicrobial susceptibility and enhancing the clinical efficacy of the existing antibiotics [100].

Traditional EPIs, such as thioridazine and PAβN, have been found to reduce the biofilm formation in biofilm-forming strains of *S. aureus*. CCCP, a broad-spectrum efflux inhibitor, is effective against efflux pumps requiring proton motive force for their function [101]. CCCP has a permeabilizing effect on membranes affecting various cellular processes within bacterial cells, including cell division and metabolism. CCCP has been found to reduce biofilm formation under both static and flow conditions in *S. aureus* [19]. Interestingly, NMP (1-(1-Napthylmethyl)-piperazine) was found to be ineffective against *S. aureus*, highlighting the specificity of certain inhibitors. The role of phenothiazines, e.g. thioridazine, in biofilm reduction remains unclear. Studies have shown no effect on the expression levels of the *norA* and *abcA* efflux genes in *S. aureus*, suggesting that their anti-biofilm activity may occur through alternative mechanisms.

Apart from traditional EPIs, several plant products have been shown to act as efflux pump inhibitors [102]. A compound namely 4', 5'-O-dicaffeoylquinic acid isolated from the plant *Artemisia absinthium* has been shown to be effective against MFS efflux pumps in *S. aureus*, exhibiting its potential as EPI and an anti-biofilm agent [103]. Additionally, thymoquinone showed impairment of the NorA efflux pump activity in multidrug

resistant *S. aureus* [104].

Indeed, it is evident that EPIs can be utilized as anti-biofilm agents and can be used in conjunction with antibiotics to overcome antibiotic resistance. Till now, no EPI has been approved for clinical use due to their toxicity and their effectiveness at high concentrations.

CONCLUSION AND PERSPECTIVES

S. aureus remains as the leading human pathogen causing infections in hospital and community settings. Efflux pump-mediated antibiotic resistance is the clinical problem rendering current antibiotics ineffective in eradicating biofilm-associated infections. The prevalence of efflux pumps poses a significant challenge in the treatment of *S. aureus* biofilm-related infections, necessitating the development of alternative therapeutic strategies that target both the biofilm matrix and efflux pump activity.

Understanding the interplay between efflux pumps and biofilm formation sheds light on the complexity of *S. aureus* resistance mechanisms. Combating biofilm-related infections requires a multifaceted approach that considers not only the inhibition of efflux pumps but also the strategies targeting key components of the biofilm matrix, such as polysaccharide intercellular adhesion (PIA).

The accessory gen regulator (agr) is a key of element of quorum sensing system that controls the cell density and expression of genes in *S. aureus*. agr regulates many traits like virulence factor, biofilm formation and protects them from oxidative stress. Inhibiting the agr quorum sensing system may prevent biofilm dispersal and the spread of infection. Other potential strategies include use of quorum sensing inhibitors, enzymes that degrade extracellular matrix components such as DNase or dispersin B, phage therapies using bacteriophages that can penetrate biofilm and combinatorial approaches using anti-biofilm agents with antibiotics.

Additionally, continuous research into the genetic and molecular mechanisms underlying efflux pump-mediated resistance will provide valuable insights for the development of novel antimicrobial agents and therapeutic interventions.

ACKNOWLEDGEMENTS

The Research Fellowship provided by Central University of South Bihar, Gaya to Shweta Sinha is gratefully acknowledged. SA is grateful to the Institute of Life Sciences, Bhubaneswar, for providing Senior Research Fellowships. The funder had no role in study design, data collection, analysis, or decision to publish or prepare the manuscript. We thank our laboratory colleagues for their helpful discussions and critical comments. We apologize that not all of the work related to this field could be discussed or cited due to space limitations.

CONFLICT OF INTEREST

Authors declare that they have read the contents of the paper and do not have any competing interests.

Please cite this article as: Shweta Sinha, Shifu Aggarwal, Durg Vijai Singh (**2024**). Efflux pumps: gatekeepers of antibiotic resistance in *Staphylococcus aureus* biofilms. **Microbial Cell** 11: 368-377. doi: 10.15698/mic2024.11.839

REFERENCES

1. Blair JMA, Webber MA, Baylay AJ, Ogbolu DO, Piddock LJV (**2015**). Molecular mechanisms of antibiotic resistance. **Nat Rev Microbiol** 13 (1): 42–51. doi:10.1038/nrmicro3380

2. Lekshmi M, Stephen J, Ojha M, Kumar S, Varela M (**2022**). Staphylococcus aureus antimicrobial efflux pumps and their inhibitors: recent developments. **AIMSMEDS** 9 (3): 367–393. doi:10.3934/medsci.2022018

3. Sakr A, Brégeon F, Mège JL, Rolain JM, Blin O (**2018**). Staphylococcus aureus nasal colonization: an update on mechanisms, epidemiology, risk factors, and subsequent infections. **Front Microbiol** 9: 2419–2419. doi:10.3389/fmicb.2018.02419

4. Chambers HF, Deleo FR (**2009**). Waves of resistance: Staphylococcus aureus in the antibiotic era. **Nat Rev Microbiol** 7 (9): 629–641. doi:10.1038/nrmicro2200

5. Turner NA, Sharma-Kuinkel BK, Maskarinec SA, Eichenberger EM, Shah PP, Carugati M, Holland TL, Fowler VG (**2019**). Methicillin-resistant Staphylococcus aureus: an overview of basic and clinical research. **Nat Rev Microbiol** 17 (4): 203–218. doi:10.1038/s41579-018-0147-4

6. Xu Z, Li L, Shirtliff ME, Peters BM, Li B, Peng Y, Alam MJ, Yamasaki S, Shi L (**2001**). Resistance class 1 integron in clinical methicillin-resistant Staphylococcus aureus strains in southern China. **Clin Microbiol Infect** 17 (5): 714–718. doi:10.1111/j.1469-0691.2010.03379.x

7. Deng Y, Liu J, Peters BM, Chen L, Miao J, Li B, Li L, Chen D, Yu G, Xu Z, Shirtliff ME (**2001**). Antimicrobial resistance investigation on Staphylococcus strains in a local hospital in Guangzhou, China, 2001-2010. **Microb Drug Resist** 21: 102–104. doi:10.1089/mdr.2014.0117

8. Shrestha LB, Syangtan G, Basnet A, Acharya KP, Chand AB (**2021**). Methicillin-resistant Staphylococcus aureus in Nepal. **J Nepal Med Assoc** 59 (237): 518–522. doi:10.31729/jnma.6251

9. Shrestha NK, Fraser TG, Gordon SM (**2019**). Methicillin resistance in Staphylococcus aureus infections among patients colonized with methicillin-susceptible Staphylococcus aureus. **Clin Microbiol Infect** 25 (1): 71–75. doi:10.1016/j.cmi.2018.03.045

10. Gherardi G (**2023**). Staphylococcus aureus infection: pathogenesis and antimicrobial resistance. **IJMS** 24 (9): 8182–8182. doi:10.3390/ijms24098182

11. Tigabu A, Getaneh A (**2021**). Staphylococcus aureus, ESKAPE bacteria challenging current health care and community settings: a literature review. **Clin Lab** 67 (7). doi:10.7754/Clin.Lab.2020.200930

12. Van Loo I, Huijsdens X, Tiemersma E, De Neeling A, Van De Sande-Bruinsma N, Beaujean D, Voss A, Kluytmans J (**2007**). Emergence of methicillin-resistant Staphylococcus aureus of animal origin in humans. **Emerg Infect Dis** 13 (12): 1834–1839. doi:10.3201/eid1312.070384

13. Alav I, Sutton JM, Rahman KM (**2018**). Role of bacterial efflux pumps in biofilm formation. **J Antimicrob Chemother** 73 (8): 2003–2020. doi:10.1093/jac/dky042

14. Kvist M, Hancock V, Klemm P (**2008**). Inactivation of efflux pumps abolishes bacterial biofilm formation. **Appl Environ Microbiol** 74 (23): 7376–7382. doi:10.1128/AEM.01310-08

15. Van Houdt R, Michiels CW (**2010**). Biofilm formation and the food industry, a focus on the bacterial outer surface: Biofilm formation and the bacterial outer surface. **J Appl Microbiol** 109 (4): 1117–1131. doi:10.1111/j.1365-2672.2010.04756.x

16. Chmielewski RAN, Frank JF (**2003**). Biofilm formation and control in food processing facilities. **Comp Rev Food Sci Food Safe** 2 (1): 22–32. doi:10.1111/j.1541-4337.2003.tb00012.x

17. Karatan E, Watnick P (**2009**). Signals, regulatory networks, and materials that build and break bacterial biofilms. **Microbiol Mol Biol Rev** 73 (2): 310–347. doi:10.1128/MMBR.00041-08

18. Flemming HC, Wingender J (**2010**). The biofilm matrix. **Nat Rev Microbiol** 8 (9): 623–633. doi:10.1038/nrmicro2415

19. Baugh S, Phillips CR, Ekanayaka AS, Piddock LJV, Webber MA (**2014**). Inhibition of multidrug efflux as a strategy to prevent biofilm formation. **J Antimicrob Chemother** 69 (3): 673–681. doi:10.1093/jac/dkt420

20. Knight R, Vrbanac A, Taylor BC, Aksenov A, Callewaert C, Debelius J, Gonzalez A, Kosciolek T, Mccall LI, Mcdonald D, Melnik AV, Morton JT, Navas J, Quinn RA, Sanders JG, Swafford AD, Thompson LR, Tripathi A, Xu ZZ, Zaneveld JR, Zhu Q, Caporaso JG, Dorrestein PC (**2018**). Best practices for analysing microbiomes. **Nat Rev Microbiol** 16 (7): 410–422. doi:10.1038/s41579-018-0029-9

21. Foster TJ (**2017**). Antibiotic resistance in Staphylococcus aureus. Current status and future prospects. **FEMS Microbiol Rev** 41 (3): 430–449. doi:10.1093/femsre/fux007

22. Haaber J, Penadés JR, Ingmer H (**2017**). Transfer of antibiotic resistance in Staphylococcus aureus. **Trends Microbiol** 25 (11): 893–905. doi:10.1016/j.tim.2017.05.011

23. Yılmaz EŞ, Aslantaş Ö (**2017**). Antimicrobial resistance and underlying mechanisms in Staphylococcus aureus isolates. **Asian Pac J Trop Med** 10 (11): 1059–1064. doi:10.1016/j.apjtm.2017.10.003

24. Piddock LV (**2006**). Clinically relevant chromosomally encoded multidrug resistance efflux pumps in bacteria. **Clin Microbiol Rev** 19 (2): 382–402. doi:10.1128/CMR.19.2.382-402.2006

25. Tanaka Y, Hipolito CJ, Maturana AD, Ito K, Kuroda T, Higuchi T, Katoh T, Kato HE, Hattori M, Kumazaki K, Tsukazaki T, Ishitani R, Suga H, Nureki O (**2013**). Structural basis for the drug extrusion mechanism by a MATE multidrug transporter. **Nature** 496 (7444): 247–251. doi:10.1038/nature12014

26. Webber MA, Piddock LJV (**2003**). The importance of efflux pumps in bacterial antibiotic resistance. **J Antimicrob Chemother** 51 (1): 9–11. doi:10.1093/jac/dkg050

27. Blanco P, Hernando-Amado S, Reales-Calderon J, Corona F, Lira F, Alcalde-Rico M, Bernardini A, Sanchez M, Martinez J (**2016**). Bacterial multidrug efflux pumps: much more than antibiotic resistance determinants. **Microorganisms** 4: 14–14. doi:10.3390/microorganisms4010014

28. Mahapatra SR, Dey J, Kushwaha GS, Puhan P, Mohakud NK, Panda SK, Lata S, Misra N, Suar M (**2022**). Immunoinformatic approach employing modeling and simulation to design a novel vaccine construct targeting MDR efflux pumps to confer wide protection against typhoidal Salmonella serovars. **J Biomol Struct Dyn** 40 (22): 11,809–11,821. doi:10.1080/07391102.2021.1964600

29. Putman M, Van Veen HW, Konings WN (**2000**). Molecular properties of bacterial multidrug transporters. **Microbiol Mol Biol Rev** 64 (4): 672–693. doi:10.1128/MMBR.64.4.672-693.2000

30. Papkou A, Hedge J, Kapel N, Young B, Maclean RC (**2020**). Efflux pump activity potentiates the evolution of antibiotic resistance across S. aureus isolates. **Nat Commun** 11 (1): 3970. doi:10.1038/s41467-020-17735-y

31. Handzlik J, Matys A, Kieć-Kononowicz K (**2013**). Recent advances in multi-drug resistance (mdr) efflux pump inhibitors of gram-positive bacteria. **S aureus Antibiotics** 2 (1): 28–45. doi:10.3390/antibiotics2010028

32. Bouter AL, Leclercq R, Cattoir V (**2011**). Molecular basis of resistance to macrolides, lincosamides and streptogramins in Staphylococcus saprophyticus clinical isolates. **Int J Antimicrob Agents** 37 (2): 118–123. doi:10.1016/j.ijantimicag.2010.10.008

33. Yoshida H, Bogaki M, Nakamura S, Ubukata K, Konno M (**1990**). Nucleotide sequence and characterization of the Staphylococcus aureus norA gene, which confers resistance to quinolones. **J Bacteriol** 172 (12): 6942–6949. doi:10.1128/jb.172.12.6942-6949.1990

34. Neyfakh AA, Borsch CM, Kaatz GW (**1993**). Fluoroquinolone resistance protein NorA of Staphylococcus aureus is a multidrug efflux transporter. **Antimicrob Agents Chemother** 37 (1): 128–129. doi:10.1128/AAC.37.1.128

35. Leelaporn A, Paulsen IT, Tennent JM, Littlejohn TG, Skurray RA (**1994**). Multidrug resistance to antiseptics and disinfectants in coagulase-negative staphylococci. **J Med Microbiol** 40 (3): 214–220

36. Bjorland J, Steinum T, Sunde M, Waage S, Heir E (**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 (10): 3046–3052. doi:10.1128/AAC.47.10.3046-3052.2003

37. 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 (3): 378–388

38. 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 (1): 49–56

39. Kaatz GW, Demarco CE, Seo SM (**2006**). MepR, a repressor of the Staphylococcus aureus MATE family multidrug efflux pump MepA, is a substrate-responsive regulatory protein. **Antimicrob Agents Chemother** 50 (4): 1276–1281. doi:10.1128/AAC.50.4.1276-1281.2006

40. Kaatz GW, Thyagarajan RV, Seo SM (**2005**). Effect of promoter region mutations and mgrA overexpression on transcription of norA, which encodes a Staphylococcus aureus multidrug efflux transporter. **Antimicrob Agents Chemother** 49 (1): 161–169. doi:10.1128/AAC.49.1.161-169.2005

41. Kumaraswami M, Schuman JT, Seo SM, Kaatz GW, Brennan RG (**2009**). Structural and biochemical characterization of MepR, a multidrug binding transcription regulator of the Staphylococcus aureus multidrug efflux pump MepA. **Nucleic Acids Res** 37 (4): 1211–1224

42. Schindler BD, Jacinto PL, Buensalido J, Seo SM, Gw K (**2015**). Clonal relatedness is a predictor of spontaneous multidrug efflux pump gene overexpression in Staphylococcus aureus. **Int J Antimicrob Agents** 45 (5): 464–470

43. 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 (10): 2525–2534. doi:10.1128/JB.00018-10

44. Truong-Bolduc QC, Hooper DC (**2007**). The transcriptional regulators NorG and MgrA modulate resistance to both quinolones and β-lactams in Staphylococcus aureus. **J Bacteriol** 189 (8): 2996–3005

45. Truong-Bolduc QC, Bolduc GR, Okumura R, Celino B, Bevis J, Liao CH, Hooper DC (**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 (7): 3214–3219

46. Huang J, O'toole PW, Shen W, Amrine-Madsen H, Jiang X, Lobo N, Palmer LM, Voelker L, Fan F, Gwynn MN, Mcdevitt D (**2004**). Novel chromosomally encoded multidrug efflux transporter MdeA in Staphylococcus aureus. **Antimicrob Agents Chemother** 48 (3): 909–917. doi:10.1128/AAC.48.3.909-917.2004

47. Yamada Y, Hideka K, Shiota S, Kuroda T, Tsuchiya T (**2006**). Gene cloning and characterization of SdrM, a chromosomally-encoded multidrug efflux pump, from Staphylococcus aureus. **Biol Pharml Bull** 29 (3): 554–556

48. Floyd JL, Smith KP, Kumar SH, Floyd JT, Varela MF (**2010**). LmrS is a multidrug efflux pump of the major facilitator superfamily from Staphylococcus aureus. **Antimicrob Agents Chemother** 54 (12): 5406–5412. doi:10.1128/AAC.00580-10

49. Tennent JM, Lyon BR, Midgley M, Jones G, Purewal AS, Skurray RA (**1989**). Physical and biochemical characterization of the qacA gene encoding antiseptic and disinfectant resistance in Staphylococcus aureus. **Microbiology** 135 (1): 1–10. doi:10.1099/00221287-135-1-1

50. Grkovic S, Brown MH, Roberts NJ, Paulsen IT, Skurray RA (**1998**). QacR is a repressor protein that regulates expression of the Staphylococcus aureus multidrug efflux pump QacA. **J Biol Chem** 273 (29): 18,665–18,673

51. Mitchell BA, Paulsen IT, Brown MH, Skurray RA (**1999**). Bioenergetics of the Staphylococcal multidrug export protein QacA. **J Biol Chem** 274 (6): 3541–3548

52. Brown D, Demeter M, Turner RJ (**2019**). Prevalence of multidrug resistance efflux pumps (MDREPs) in environmental communities. In: Microbial diversity in the genomic era. Elsevier. vol 135. pp 545–557

53. Nakaminami H, Noguchi N, Nishijima S, Kurokawa I, Sasatsu M (**2008**). Characterization of the pTZ2162 encoding multidrug efflux gene qacB from Staphylococcus aureus. **Plasmid** 60 (2): 108–117

54. Narui K, Noguchi N, Wakasugi K, Sasatsu M (**2002**). Cloning and characterization of a novel chromosomal drug efflux gene in Staphylococcus aureus. **Biol Pharm Bull** 25 (12): 1533–1536. doi:10.1248/bpb.25.1533

55. Dawson RJP, Locher KP (**2006**). Structure of a bacterial multidrug ABC transporter. **Nature** 443 (7108): 180–185. doi:10.1038/nature05155

56. Velamakanni S, Yao Y, Gutmann D, Van Veen HW (**2008**). Multidrug transport by the ABC transporter Sav1866 from Staphylococcus aureus. **Biochemistry** 47 (35): 9300–9308. doi:10.1021/bi8006737

57. Villet RA, Truong-Bolduc QC, Wang Y, Estabrooks Z, Medeiros H, Hooper DC (**2014**). Regulation of expression of abcA and its response to environmental conditions. **J Bacteriol** 196 (8): 1532–1539

58. Ross JI, Eady EA, Cove JH, Baumberg S (**1995**). Identification of a chromosomally encoded ABC-transport system with which the staphylococcal erythromycin exporter MsrA may interact. **Gene** 153 (1): 93–98

59. Reynolds E, Ross JI, Cove JH (**2003**). Msr(A) and related macrolide/streptogramin resistance determinants: incomplete transporters? **Int J Antimicrob Agents** 22 (3): 228–236. doi:10.1016/s0924-8579(03)00218-8

60. Allignet J, Solh E, N (**1997**). Characterization of a new staphylococcal gene, vgaB, encoding a putative ABC transporter conferring resistance to streptogramin A and related compounds. **Gene** 202 (1-2): 133–138

61. De Marco CE, Cushing LA, Frempong-Manso E, Seo SM, Jaravaza TAA, Kaatz GW (**2007**). Efflux-related resistance to norfloxacin, dyes, and biocides in bloodstream isolates of Staphylococcus aureus. **Antimicrob Agents Chemother** 51 (9): 3235–3239. doi:10.1128/AAC.00430-07

62. Truong-Bolduc QC, Hsing LC, Villet R, Bolduc GR, Estabrooks Z, Taguezem GF, Hooper DC (**2012**). Reduced aeration affects the expression of the NorB efflux pump of Staphylococcus aureus by posttranslational modification of MgrA. **J Bacteriol** 194 (7): 1823–1834. doi:10.1128/JB.06503-11

63. Briaud P, Camus L, Bastien S, Doléans-Jordheim A, Vandenesch F, Moreau K (**2019**). Coexistence with Pseudomonas aeruginosa alters Staphylococcus aureus transcriptome, antibiotic resistance and internalization into epithelial cells. **Sci Rep** 9 (1): 16,564. doi:10.1038/s41598-019-52975-z

64. Paulsen IT, Brown MH, Littlejohn TG, Mitchell BA, Skurray RA (**1996**). Multidrug resistance proteins QacA and QacB from Staphylococcus aureus: membrane topology and identification of residues involved in substrate specificity. **Proc Natl Acad Sci** 93 (8): 3630–3635. doi:10.1073/pnas.93.8.3630

65. Guay GG, Rothstein DM (**1993**). Expression of the tetK gene from Staphylococcus aureus in Escherichia coli: comparison of substrate specificities of TetA(B), TetA(C), and TetK efflux proteins. **Antimicrob Agents Chemother** 37 (2): 191–198. doi:10.1128/AAC.37.2.191

66. 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 (6): 1492–1498. doi:10.1128/JB.182.6.1492-1498.2000

67. Krulwich TA, Guffanti JJ, Bechhofer AA, H (**2001**). Functions of tetracycline efflux proteins that do not involve tetracycline. **J Mol Microbiol Biotechnol** 3 (2): 237–246

68. Truong-Bolduc QC, Bolduc GR, Medeiros H, Vyas JM, Wang Y, Hooper DC (**2015**). Role of the Tet38 efflux pump in Staphylococcus aureus internalization and survival in epithelial cells. **Infect Immun** 83 (11): 4362–4372. doi:10.1128/IAI.00723-15

69. Ding Y, Fu Y, Lee JC, Hooper DC (**2012**). Staphylococcus aureus NorD, a putative efflux pump coregulated with the Opp1 oligopeptide permease, contributes selectively to fitness in vivo. **J Bacteriol** 194 (23): 6586–6593. doi:10.1128/JB.01414-12

70. Truong-Bolduc QC, Dunman PM, Strahilevitz J, Projan SJ, Hooper DC (**2005**). MgrA Is a multiple regulator of two new efflux pumps in Staphylococcus aureus. **J Bacteriol** 187 (7): 2395–2405. doi:10.1128/JB.187.7.2395-2405.2005

71. Trotonda MP, Tamber S, Memmi G, Cheung AL (**2008**). MgrA represses biofilm formation in Staphylococcus aureus. **Infect Immun** 76 (12): 5645–5654. doi:10.1128/IAI.00735-08

72. 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 (7). doi:10.1128/iai.00194-19

73. Wassenaar TM, Ussery D, Nielsen LN, Ingmer H (**2015**). Review and phylogenetic analysis of qac genes that reduce susceptibility to quaternary ammonium compounds in Staphylococcus species. **Eur J Microbiol Immunol** 5 (1): 44–61. doi:10.1556/EUJMI-D-14-00038

74. 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 (47). doi:10.1016/s0021-9258(18)43980-4

75. Costa SS, Junqueira E, Palma C, Viveiros M, Melo-Cristino J, Amaral L, Couto I (**2013**). Resistance to antimicrobials mediated by efflux pumps in Staphylococcus aureus. **Antibiotics** 2 (1): 83–99. doi:10.3390/antibiotics2010083

76. Huet AA, Raygada JL, Mendiratta K, Seo SM, Gw K (**2008**). Multidrug efflux pump overexpression in Staphylococcus aureus after single and multiple in vitro exposures to biocides and dyes. **Microbiology** 154: 3144–3153. doi:10.1099/mic.0.2008/021188-0

77. Fang R, Sun Y, Dai W, Zheng X, Tian X, Zhang X, Wang C, Cao J, Zhou T (**2020**). Mutations in the MepRAB efflux system contribute to the in vitro development of tigecycline resistance in Staphylococcus aureus. **J Glob Antimicrob Resist** 22: 631–636. doi:10.1016/j.jgar.2020.06.005

78. McAleese F, Petersen P, Ruzin A, Dunman PM, Murphy E, Projan SJ, Bradford PA (**2005**). A novel MATE family efflux pump contributes to the reduced susceptibility of laboratory-derived Staphylococcus aureus mutants to tigecycline. **Antimicrob Agents Chemother** 49 (5): 1865–1871. doi:10.1128/AAC.49.5.1865-1871.2005

79. Opperman TJ, Williams JD, Houseweart C, Panchal RG, Bavari S, Peet NP, Moir DT, Bowlin TL (**2010**). Efflux-mediated bis-indole resistance in Staphylococcus aureus reveals differential substrate specificities for MepA and MepR. **Bioorg Med Chem** 18 (6): 2123–2130. doi:10.1016/j.bmc.2010.02.005

80. Allignet J, Loncle V, Solh E, N (**1992**). Sequence of a staphylococcal plasmid gene, vga, encoding a putative ATP-binding protein involved in resistance to virginiamycin A-like antibiotics. **Gene** 117 (1): 90,488. doi:10.1016/0378-1119(92)90488-B

81. Jacquet E, Girard JM, Ramaen O, Pamlard O, Lévaique H, Betton JM, Dassa E, Chesneau O (**2008**). ATP hydrolysis and pristinamycin IIA inhibition of the Staphylococcus aureus Vga(A), a dual ABC protein involved in streptogramin A resistance. **J Biol Chem** 283 (37): 25,332–25,339. doi:10.1074/jbc.M800418200

82. Costerton JW, Lewandowski Z, Caldwell DE, Korber DR, Lappin-Scott HM (**1995**). Microbial biofilms. **Annu Rev Microbiol** 49 (1): 711–745. doi:10.1146/annurev.mi.49.100195.003431

83. Kaplan JB, Izano EA, Gopal P, Karwacki MT, Kim S, Bose JL, Bayles KW (**2012**). Low levels of β-lactam antibiotics induce extracellular DNA release and biofilm formation in Staphylococcus aureus. **mBio** 3 (4): e00,198–00,112. doi:10.1128/mBio.00198-12

84. Idrees M, Sawant S, Karodia N, Rahman A (**2021**). Staphylococcus aureus biofilm: morphology, genetics, pathogenesis and treatment strategies. **Int J Environ Res Public Health** 18 (14): 7602. doi:10.3390/ijerph18147602

85. Martino PD (**2018**). Extracellular polymeric substances, a key element in understanding biofilm phenotype. **AIMS Microbiol** 4 (2): 274–288. doi:10.3934/microbiol.2018.2.274

86. Otto M (**2013**). Staphylococcal infections: mechanisms of biofilm maturation and detachment as critical determinants of pathogenicity. **Annu Rev Med** 64: 175–188. doi:10.1146/annurev-med-042711-140023

87. O'Neill E, Pozzi C, Houston P, Humphreys H, Robinson DA, Loughman A, Foster TJ, Gara JPO (**2008**). A novel Staphylococcus aureus biofilm phenotype mediated by the fibronectin-binding proteins, FnBPA and FnBPB. **J Bacteriol** 190 (11): 3835–3850. doi:0.1128/JB.00167-08

88. Wolz C, Goerke C, Landmann R, Zimmerli W, Fluckiger U (**2002**). Transcription of clumping factor A in attached and unattached Staphylococcus aureus in vitro and during device-related infection. **Infect Immun** 70 (6): 2758–2762

89. Sabat A, Melles DC, Martirosian G, Grundmann H, Van Belkum A, Hryniewicz W (**2006**). Distribution of the serine-aspartate repeat protein-encoding sdr genes among nasal-carriage and invasive Staphylococcus aureus strains. **J Clin Microbiol** 44 (3): 1135–1138. doi:10.1128/JCM.44.3.1135-1138.2006

90. Moormeier DE, Bayles KW (**2017**). Staphylococcus aureus biofilm: a complex developmental organism. **Mol Microbiol** 104 (3): 365–376. doi:10.1111/mmi.13634

91. Mangwani N, Kumari S, Das S (**2016**). Bacterial biofilms and quorum sensing: fidelity in bioremediation technology. **Biotechnol Genet Eng Rev** 32 (1-2): 43–73. doi:10.1080/02648725.2016.1196554

92. Rasamiravaka T, Vandeputte OM, Pottier L, Huet J, Rabemanantsoa C, Kiendrebeogo M, Andriantsimahavandy A, Rasamindrakotroka A, Stévigny C, Duez P, Jaziri ME (**2015**). Pseudomonas aeruginosa biofilm formation and persistence, along with the production of quorum sensing-dependent virulence factors, are disrupted by a triterpenoid coumarate ester isolated from Dalbergia trichocarpa, a tropical legume. **PLoS One** 10 (7): 132,791. doi:10.1371/journal.pone.0132791

93. Periasamy S, Joo HS, Duong AC, Bach TH, Tan VY, Chatterjee SS, Cheung G, Otto M (**2012**). How Staphylococcus aureus biofilms develop their characteristic structure. **Proc Natl Acad Sci U S A** 109 (4): 1281–1286. doi:10.1073/pnas.1115006109

94. Hall-Stoodley L, Stoodley P (**2002**). Developmental regulation of microbial biofilms. **Curr Opin Biotechnol** 13 (3): 228–233. doi:10.1016/S0958-1669(02)00318-X

95. Beenken KE, Dunman PM, Mcaleese F, Macapagal D, Murphy E, Projan SJ, Blevins JS, Smeltzer MS (**2004**). Global gene expression in Staphylococcus aureus biofilms. **J Bacteriol** 186 (14): 4665–4684

96. Resch A, Rosenstein R, Nerz C, Götz F (**2005**). Differential gene expression profiling of Staphylococcus aureus cultivated under biofilm and planktonic conditions. **Appl Environ Microbiol** 71 (5): 2663–2676. doi:10.1128/AEM.71.5.2663-2676.2005

97. He X, Ahn J (**2011**). Differential gene expression in planktonic and biofilm cells of multiple antibiotic-resistant Salmonella typhimurium and Staphylococcus aureus. **FEMS Microbiol Lett** 325 (2): 180–188. doi:10.1111/j.1574-6968.2011.02429.x

98. Rode TM, Møretrø T, Langsrud S, Langsrud O, Vogt G, Holck A (**2010**). Responses of Staphylococcus aureus exposed to HCl and organic acid stress. **Can J Microbiol** 56 (9): 777–792. doi:10.1139/w10-057

99. Quoc PHT, Genevaux P, Pajunen M, Savilahti H, Georgopoulos C, Schrenzel J, Kelley WI (**2007**). Isolation and characterization of biofilm formation-defective mutants of Staphylococcus aureus. **Infect Immun** 75 (3): 1079–1088. doi:10.1128/IAI.01143-06

100. Trifan A, Luca SV, Greige-Gerges H, Miron A, Gille E, Aprotosoaie AC (**2020**). Recent advances in tackling microbial multidrug resistance with essential oils: combinatorial and nano-based strategies. **Crit Rev Microbiol** 46 (3): 338–357. doi:10.1080/1040841X.2020.1782339

101. Mahamoud A, Chevalier J, Alibert-Franco S, Kern WV, Pagès JM (**2007**). Antibiotic efflux pumps in Gram-negative bacteria: the inhibitor response strategy. **J Antimicrob Chemother** 59 (6): 1223–1229. doi:10.1093/jac/dkl493

102. Stavri M, Piddock L, Gibbons S (**2007**). Bacterial efflux pump inhibitors from natural sources. **J Antimicrob Chemother** 59 (6): 1247–1260

103. Fiamegos YC, Kastritis PL, Exarchou V, Han H, Bonvin AMJJ, Vervoort J, Lewis K, Hamblin MR, Tegos GP (**2011**). Antimicrobial and efflux pump inhibitory activity of caffeoylquinic acids from Artemisia absinthium against gram-positive pathogenic bacteria. **PLoS One** 6 (4): 18,127. doi:10.1371/journal.pone.0018127

104. Ahmad A, Abdelgalil SY, Khamis T, Abdelwahab A, Atwa DN, Ga E (**2024**). Thymoquinone' potent impairment of multidrug-resistant Staphylococcus aureus NorA efflux pump activity. **Sci Rep** 14 (1): 16,483–16,483