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Review article

Membrane protein-driven drug development in *Staphylococcus aureus*: Bridging the gap in antibiotic efficacy for resistant strains

Kokila Manickam[1], Suganya Kannan[1]*, Jeyakumar Balakrishnan[1], Guru Aadavan Kuppusamy [1], Gunasekaran Chinnathambi [2]

1- Central Research Laboratory for Biomedical Research, Vinayaka Mission's Medical College and Hospital, Vinayaka Mission's Research Foundation (Deemed to be University), Karaikal, Puducherry, India

2- Vinayaka Mission's Medical College and Hospital, Vinayaka Mission's Research Foundation (Deemed to be University), Karaikal, Puducherry, India

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ABSTRACT

Background: The rising prevalence of the antibiotic-resistant strains of *Staphylococcus aureus*, notably to the Methicillin-resistant *Staphylococcus aureus* (MRSA), presents a critical challenge in contemporary healthcare. By examining the unique challenges posed by antibiotic-resistant strains, we underscore the urgent need for innovative therapeutic interventions. The review navigates the current landscape of antibiotic development, focusing on the exploitation of membrane proteins as viable targets for drug design. Membrane proteins in *S. aureus*, including adhesins, transporters, and signal transduction proteins, are dissected for their contributions to bacterial pathogenicity and resilience. The article elucidates the interplay between membrane proteins and antibiotic resistance, offering insights into the potential vulnerabilities that can be harnessed for drug development. Drawing attention to the multifaceted nature of membrane proteins, we discuss their critical roles in biofilm formation, nutrient acquisition, and evasion of host defences, underscoring their significance as druggable targets. In conclusion, this review critically examines the prospects and challenges of membrane protein-driven drug development in *Staphylococcus aureus*, highlighting the promising avenues for bridging the gap in antibiotic efficacy against resistant strains. The comprehensive exploration of this paradigm aims to inform and inspire researchers in the pursuit of innovative strategies to mitigate the looming threat to the antibiotic resistance in one of the most clinically significant bacterial pathogens.

Introduction

Staphylococcus aureus (*S. aureus*) is a major pathogen associated with hospital-acquired and community-acquired infections. This bacterium can cause a wide range of infectious diseases, from minor skin and soft tissue infections to severe conditions such as bacteraemia, lethal pneumonia, osteomyelitis, and infectious endocarditis [1]. *Staphylococcus aureus* is notable for its pathogenicity in both humans and animals, making it a significant species within its family [2]. It is also present in environmental sources, including soil, water, and air. Typically, *S. aureus* resides in mixed microbial communities, where interactions among different species can influence its pathogenic behaviour, affecting factors like virulence, biofilm formation, and antibiotic resistance [3].

Antibiotics target specific bacterial structures and pathways crucial for survival, such as the cell wall, proteins, RNA, DNA synthesis

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* *Corresponding author:* Suganya Kannan
E-mail address: suganya.k34@gmail.com

machinery, and intermediate metabolism [4]. Notably, *S. aureus* has developed resistance to all known antibiotic mechanisms. It has a remarkable ability to rapidly acquire antibiotic resistance and adapt to diverse environments [5]. Resistance in *S. aureus* can emerge through genetic mutations and rearrangements or through the acquisition of resistance determinants. β-lactam antibiotics, such as penicillin G, historically marked the first successful treatment against *S. aureus*, targeting its penicillin-binding proteins (PBPs) and disrupting peptidoglycan cross-linking in the cell wall, leading to cell death [6]. The pathogenicity of *S. aureus* is complex, involving numerous factors and regulatory mechanisms. It produces a variety of toxins that compromise cell integrity and function, including haemolysis, exfoliative toxins A and B, enterotoxins, toxic shock syndrome toxin-1, and Panton-Valentine leucocidin. These factors significantly contribute to the virulence of clinical isolates [7].

Antibiotic resistance has become a major global public health concern, with resistance rates in organisms like MRSA (Methicillin-resistant *Staphylococcus aureus*) and *Escherichia coli* exceeding 50% in some regions. This issue is recognized by the World Health Organization in five of its six global regions [8]. Current research on anti-MRSA drugs often focuses on derivatives or classes of antibiotics already known to be effective against these pathogens [9]. The spread of antibiotic-resistant bacteria like MRSA presents a significant challenge to traditional treatments, necessitating novel approaches to manage these resilient pathogens [10].

Membrane proteins have recently gained attention as potential drug targets due to their critical roles in essential cellular processes and their accessibility on the bacterial cell surface. In *S. aureus*, membrane proteins are involved in key functions such as nutrient transport, signal transduction, and cell adhesion. This makes them attractive targets for developing new antibacterial agents [11]. This article explores the crucial role of membrane protein-targeted drug development in bridging the gap in antibiotic efficacy against resistant *S. aureus* strains [12]. We discuss the unique challenges posed by antibiotic-resistant strains and the importance of targeting membrane proteins, providing insights into promising avenues for next-generation antibiotics capable of combating *S. aureus's* evolving resistance mechanisms [13].

We examine the complex relationship between membrane proteins and antibiotic resistance, navigating the current landscape of antibiotic development and underscoring the need for innovative strategies to effectively counter *S. aureus's* adaptability. By enhancing our understanding of membrane protein dynamics and their impact on antibiotic efficacy, this article aims to contribute to the development of therapeutic interventions that can stay ahead of the evolving landscape of antibiotic resistance, offering renewed hope in the fight against drug-resistant *S. aureus* infections [14].

2. Membrane proteins in *Staphylococcus aureus*: key players in physiology and antibiotic resistance

The adaptable Gram-positive bacterium *S. aureus* is well-known for its capacity to cause a wide range of illnesses, relies heavily on a diverse array of membrane proteins to adapt, survive, and counteract antibiotic therapies [7]. The intricate interplay between these membrane proteins and the bacterium's physiological processes underscores their significance in both normal cellular functions and the development of antibiotic resistance **(Figure 1)** [10].

2.1 Cell wall-associated proteins

2.1.1 Adhesins and Invasins:

Cell wall-associated proteins facilitate the initial stages of infection by mediating the attachment of host cells and tissues of *S. aureus*. Examples includes protein A (SpA) and fibronectin-binding proteins (FnBPs), which contribute to the bacterium's ability to colonize diverse host niches [15].

2.1.2 Biofilm-associated proteins:

The proteins anchored in the cell wall, similar to the microbial surface components recognizing adhesive matrix molecules (MSCRAMMs), participates crucially in the development of biofilms. Biofilms contribute to chronic infections and serve as a protective niche for *S. aureus*, rendering it less susceptible to antibiotics [11].

2.2 Transport proteins

2.2.1 Efflux pumps:

Membrane transporters, particularly efflux pumps, contribute to multidrug resistance by expelling antibiotics from the cell of bacteria [4]. One well-known efflux pump in *S. aureus* is NorA,

is known for its role in decreasing the intracellular concentration of various antibiotics, thereby diminishing their efficacy [16].

2.2.2 Nutrient transporters:

Staphylococcus aureus relies on membrane proteins for nutrient acquisition. The oligopeptide permeases (Opp) system, for instance, facilitates the uptake of peptides, playing a crucial role in the bacterium's adaptability to different host environments [8].

2.3 Signal transduction proteins

2.3.1 Two-component systems:

Sensor kinases that are membrane-bound and response regulators form two-component systems that *S. aureus* allows to sense and respond to the environment changes. The WalKR and GraRS systems, for example, influence cell wall metabolism and antibiotic resistance, highlighting the interconnectedness of signal transduction and resistance mechanisms [3].

2.4 Toxin and virulence factor transport

2.4.1 Sec System:

The Sec translocon system in the bacterial membrane is responsible for the secretion of toxins and virulence factors. This system is crucial for the delivery of proteins like alpha-toxin, a potent virulence factor that contributes to tissue damage and immune evasion. Understanding the functional and structural aspects to these membrane proteins provides valuable insights into the vulnerabilities that can be exploited for therapeutic purposes [4]. As researchers delve deeper into the intricate world of membrane proteins in *Staphylococcus aureus*, novel drug development strategies targeting these key players offer promising avenues for overcoming antibiotic resistance and ensuring effective treatment against this formidable pathogen **(Table 1)** [17].

3. Membrane transporters and their involvement in antibiotic efflux

Bacterial efflux transporters, with significant sequence similarity in their amino acids, have been systematically categorized into five primary families: the ATP-binding cassette (ABC) family, the resistance-nodulation-division (RND) family, the small multidrug resistance (SMR) family, the multiple antimicrobial and toxin extrusion (MATE) family, and the major facilitator (MF) superfamily [15]. These pumps, with the exception of those in the ABC family, are considered secondary transporters because they utilize proton motive force (H+ antiport) to expel specific molecules from the interior of the cell. MATE family pumps are driven by the exchange of either sodium ions or protons, while the RND, SMR, and MF families use a proton-drug antiport system for energy. The ABC family, in contrast, couples ATP hydrolysis with drug extrusion [18].

In Gram-positive bacteria like *S. aureus*, drug efflux is mediated by transporters from the SMR, MF, or ABC families located in the cytoplasmic membrane. Many identified Efflux Pump Inhibitors (EPIs) are potential blockers of these efflux pumps in *S. aureus*, a significant pathogen in human disease [19]. Drug ejection is a key defence mechanism for S. aureus against antimicrobial agents. To date, about five such efflux pumps have been identified in *S. aureus*, including members of the MFS family and plasmid-encoded QacA/B, as well as chromosome-encoded efflux pumps such as NorA, NorB, NorC, MdeA, and SdrM **(Figure 2)** [20].

Strategies to mitigate the impact of efflux pumps include: (i) modifying existing antibiotics to decrease their affinity for binding proteins; (ii) enhancing membrane permeability to increase antibiotic influx and intracellular drug concentration; (iii) inhibiting bacterial membrane activity or mutating genes encoding efflux pumps; (iv) interfering with the energy supply required for drug transport [18]; (v) permeabilizing membranes; (vi) creating antagonistic interactions between antibiotics and substrates during transport by pumps. Clinical specimens overexpressing multidrug efflux pumps exhibit reduced susceptibility to drugs [13].

Resistance processes may be influenced by chemical agents that modify resistance [21]. Coupling antibiotic substrates of efflux pumps with inhibitors can increase intracellular antibiotic concentrations, helping restore drug efficacy and impact the development of antibiotic-resistant strains [22].

4. **Receptors and signalling proteins affecting antibiotic susceptibility**

Within the *S. aureus* genome, fifteen two-component systems (TCSs) are involved in regulating bacterial physiological processes. These TCSs inevitably impact bacterial antimicrobial resistance [19]. Each TCS comprises response regulators (RRs) and histidine protein kinases (HPKs), which are membrane-bound and respond to external stimuli. After environmental stimulation,

HPKs become phosphorylated and transfer the phosphate group to RRs. This phosphorylation enhances bacterial adaptability by activating the promoter regions of downstream target genes [16].

S. aureus, a significant opportunistic human pathogen, can either exist as a harmless microbiome member or become an invasive pathogen overcoming immune defenses [5]. The YycFG TCS, for instance, is closely linked to the host's inflammatory response during infection, influencing various virulence genes related to host-matrix interactions (efb, emp, fnbA, and fnbB), cytolysis (hlgACB, hla, and hlb), and evasion of innate immunity (scn, chp, and sbi) [20].

Another TCS, SaeSR (*S. aureus* exoprotein expression), regulates pathogenic genes and is positively regulated by YycF. SaeSR controls virulence factors crucial for evading innate defence by lysing polymorphonuclear leukocytes (PMNs) during phagocytosis [11]. Both innate and adaptive immune systems respond to *S. aureus* during host-pathogen interactions. Chronic infections are linked to adaptive immunity, which enhances innate immune cell activation and alters host sensitivity to *S. aureus*. *S. aureus* has also developed mechanisms to evade adaptive immune responses. For example, the immunoglobulin-binding protein Sbi induces cell wall turnover and disintegration [20]. Sbi is a surface protein that interacts with the soluble complement component C3, modulating adaptive immune responses to *S. aureus* [23].

A mutation in the yycF gene, specifically the A96T mutation, which changes base G to base A at position 24673 in the *S. aureus* genome, affects the yycFG gene regions in vancomycin-intermediate *Staphylococcus aureus* (VISA) strains. This mutation in a conserved region of the yycF gene is associated with a conformational change in the phosphorylated control protein. These mutations reduce the bacteria's autolytic activity by decreasing YycFG activities and downregulating autolysin production. VISA strains exhibit higher mutation rates in the yycHI gene than vancomycin-sensitive *S. aureus* strains. Further research is needed to determine if yycHI mutations contribute to increased cell wall production and antibiotic resistance [24]."

5. Membrane protein-driven drug development

5.1 Membrane transporters and antibiotic efflux in *Staphylococcus aureus*: Unravelling resistance mechanisms

Methicillin-resistant *S. aureus* (MRSA) in particular has emerged as an antibiotic-resistant strain that has shown to be a formidable challenge to conventional antibiotic therapy. Membrane transporters, mainly efflux pumps, plays a major role in *S. aureus* antibiotic resistance by actively expelling antibiotics from the bacterial cell, thereby reducing their intracellular concentration [12]. Understanding the intricacies of these transporters is crucial for devising strategies to overcome antibiotic resistance in this clinically significant pathogen [25].

5.2 Efflux pump systems

5.2.1 NorA:

The pump for multidrug efflux NorA, which is a member of the major facilitator superfamily (MFS), is one of the most extensively researched efflux pumps of *S. aureus* [18]. Resistance to many antibiotics, including tetracyclines, β-lactams, and fluoroquinolones, has been associated with the gene NorA. By actively pumping these antibiotics out of the cell, NorA reduces their effectiveness and it contributes for the development of multidrug resistance [25].

5.2.2 MepA and MepR:

The MepA efflux pump, a member of the multidrug and toxic compound extrusion (MATE) family, is associated and resistance to various quinolones. The transcriptional regulator MepR controls the expression of MepA, which also affects total efflux activity in response to external stimuli [26].

5.2.3 QacA/B:

These efflux pumps, belonging to the Small Multidrug Resistance (SMR) family, is responsible to a resistance and to a variety of cationic compounds, in addition to disinfectants and antiseptics. Their overexpression can confer resistance to antibiotics like fluoroquinolones and chloramphenicol [24].

6. Targeting lipoproteins: Case study on drug vancomycin

Antibiotic resistance is a continually evolving issue, with the emergence of MRSA strains resistant to vancomycin or macrolides identified in the community setting [22]. The intracellular nature of *S. aureus* infections notably diminishes antibiotic efficacy and may even facilitate the development of drug tolerance. Antibiotics must penetrate the cell,

and depending on the bacterial location, they must traverse the cell membrane either once (in cytosol) or twice (in phagolysosome) [13]. The inability of antibiotics to eliminate intracellular bacteria, a phenomenon first described in vitro decades ago, highlights a significant challenge in treating *S. aureus* infections [25]. Up to 45% of patients experience treatment failure, indicating that antibiotic therapy is far from optimal. Vancomycin's efficacy is substantially reduced when targeting intracellular bacteria compared to extracellular targets, irrespective of the concentration used [27].

To effectively eradicate bacteria within the phagolysosome, the antibiotic must withstand acidic pH conditions. Merely penetrating membranes and targeting internal bacteria is insufficient [27]. Gentamicin, quinolones, and macrolides exhibit reduced intracellular concentrations in acidic environments [14]. Over a decade ago, a study demonstrated the effectiveness of gold nanoparticle-stabilized liposomes containing vancomycin against MRSA, showing superior bacterial growth inhibition compared to free vancomycin **(Table 2)** [28].

7. Exploiting transporters: In-depth analysis of drug daptomycin

Daptomycin, a cyclic lipopeptide, was approved in 2003 for treating soft tissue infections [21]. It employs a unique mechanism involving attachment to Gram-positive bacterial membranes, with its bactericidal activity being concentration-dependent [23]. In vitro studies suggest that daptomycin's antibacterial activity is at least as potent as that of linezolid and vancomycin. Daptomycin has shown promising results in removing MRSA biofilms and in animal model studies for treating MRSA infections. High-dose daptomycin has been used as a life-saving treatment in patients with severe Gram-positive infections, with a 94% success rate (63 out of 67 patients) [29]. The advent of target-based antibiotic development led to the inclusion of compounds targeting areas like fatty acid biosynthesis (FAB) or inspired by daptomycin's efficacy in the bacterial membrane. Proteome analysis has furthered understanding of daptomycin's mode of action [27]. A recent study by Meeker et al. employed gold nanocages coated with polydopamine and noncovalently bound daptomycin. To achieve selectivity, the carrier was attached to an antibody targeting staphylococcal protein A, commonly present on *S. aureus* surfaces [30]. This approach effectively eradicated MRSA biofilms, as evidenced by colony counting methods showing no viable bacteria [30]. Exploring the application of daptomycin (DPD), surface modification via thin-film hydration enhanced the ability of liposomes containing daptomycin DPD-L[D] to combat MRSA. Additionally, combining clarithromycin with daptomycin encapsulation significantly reduced the required daptomycin dosage while maintaining therapeutic efficacy, potentially lowering toxicity risk, although further research is needed for safety confirmation **(Table 3)** [31].

8. Comparative membrane protein expression profiles in resistant vs. susceptible strains

Daptomycin exhibits rapid bactericidal action by inserting into the bacterial membrane's outer leaflet, leading to cytosolic leakage. Its effectiveness is limited to Gram-positive bacteria, as it cannot penetrate the outer membrane of Gram-negative organisms [21]. Daptomycin's preferential interaction with phosphatidylglycerol distinguishes it from eukaryotic cells [24]. Recent research indicates that daptomycin interacts with eukaryotic membranes without compromising their integrity, as it is a substrate for P-glycoprotein. However, daptomycin resistance has been observed [32]. A subset of mutants shows a gradual increase in minimum inhibitory concentration (MIC), primarily linked to decreased bacterial binding or mutations in specific genes [16]. One such gene, mprF, encodes lysylphosphatidyl glycerol synthetase, which is implicated in phosphatidylglycerol production and associated with daptomycin-resistant clinical isolates [4].

VISA strains exhibit reduced daptomycin susceptibility, likely due to the drug's decreased ability to penetrate the thicker cell wall and reach the membrane [21]. Additionally, daptomycin and other non-ribosomal lipopeptides synthesized from amino acids could potentially be enhanced by inhibiting intrinsic processes competing for precursor molecules [33].

9. Interfering with signalling proteins: Assessing the efficacy of linezolid

Linezolid, the first synthetic oxazolidinone antibiotic, exhibits potent antibacterial effects against Gram-positive bacteria. It has been prescribed for a variety of conditions including complicated skin and soft tissue infections, pneumonia, endocarditis, and bacteraemia [12]. Linezolid has also been recently considered as a treatment for tuberculosis [32]. Its mechanism

involves binding to the 50S large subunit of the 23S rRNA, thereby inhibiting the formation of the initiation complex at the beginning of the protein synthesis process. Being fully synthetic reduces the likelihood of inherent resistance mechanisms [33]. Linezolid shows superior efficacy against a broad spectrum of significant Gram-positive pathogens, including vancomycin-resistant enterococci, methicillin-resistant staphylococci, penicillin-resistant pneumococci, and macrolide-resistant streptococci. To date, resistance to this compound has been relatively infrequent [34].

Linezolid (PNU-100766) was selected for further clinical development due to its enhanced absorption and serum levels, allowing for twice-daily dosing [31]. Compared to DuP-721, it exhibits considerably lower toxicity and remarkable in vitro activity. Studies have evaluated linezolid's efficacy against various microorganisms including *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococci*, and *Pneumococci*, alongside antimicrobials such as imipenem, clavulanic acid, clindamycin, erythromycin, gentamicin, ampicillin, vancomycin, rifampin, aztreonam, cefotaxime, cefpodoxime, and chloramphenicol [29]. For instance, a recent study demonstrated that linezolid alone had a limited effect on the mortality of susceptible *M. tuberculosis* isolates [33]. Epidemiological data indicates a 40% mortality rate for patients with MRSA pneumonia, linked to rapid lung function decline, increased morbidity, and reduced survival rates [28]. When combined with other antibiotics, linezolid, approved for MRSA meningitis and bacteraemia treatment, has shown enhanced activity against this formidable pathogen [26].

Currently, there are no human clinical trials on linezolid's effectiveness in treating meningitis. A recent study using a rabbit meningitis model investigated linezolid's effectiveness against penicillin-susceptible and -resistant pneumococci. The study found significant penetration of linezolid into rabbit cerebrospinal fluid, with reduced efficacy against penicillin-susceptible *Staphylococcus pneumoniae* compared to ceftriaxone. However, the efficacy of linezolid in eradicating colonization bacteria has been variable [35].

Initially, linezolid proved effective in eradicating *S. aureus* nasal carriage. However, it showed no efficacy in eliminating vancomycin-resistant enterococci from stool samples.

Myelosuppression, though relatively rare and reversible upon discontinuation of linezolid, should be closely monitored in patients undergoing prolonged treatment or those with predisposing conditions for bone marrow suppression. In vitro studies suggest linezolid's potential in treating various mycobacterial infections [32]. Its interaction with the P450 enzyme system is minimal, neither inducing nor inhibiting these enzymes, nor is it metabolized by them in the liver [36].

Pending further trials, current evidence strongly supports linezolid's effectiveness against a variety of bacteria resistant to vancomycin, β-lactams, and other agents [8]. Although early observations indicate that linezolid is not immune to resistance development, particularly in enterococci, its development represents a significant advancement in treating resistant Gram-positive infections. The potential of oxazolidinones as a novel and promising class of antibacterial agents may be further enhanced with additional research on other compounds [37].

10. Case studies: Membrane protein-targeted drugs

The treatment of MRSA infections continues to be a major focus of discussion among experts in antimicrobial resistance [17]. Significant efforts are being made in this area by infectious disease specialists and microbiology laboratories. This section examines the potential treatments for various MRSA infections [13]. The notion that "The mecA gene encodes methicillin resistance" in MRSA is overly simplistic; the pathogen's extensive resistance to antibiotic therapy actually fosters a multidrug resistance mechanism, with methicillin resistance being just the initial stage [4]. Moreover, there are numerous *S. aureus* "resistant phenotypes" under development. These include reduced susceptibility to linezolid and daptomycin, hetero-resistance to vancomycin, and constitutive or induced resistance to macrolides, lincosamides, and type B streptogramins [38].

MRSA is prevalent in both healthcare and community settings, with its epidemiology constantly evolving. Currently, complicated skin and soft-tissue infections (cSSTIs) can be treated with vancomycin and daptomycin; severe MRSA infections can be treated with vancomycin and daptomycin; hospital-associated pneumonia (HAP) can be treated with vancomycin or linezolid.

11. Challenges, future directions, and synergistic approaches

Integral membrane proteins (IMPs) are embedded within the lipid membranes of cells and organelles and play pivotal roles in various cellular functions such as nutrient transport, molecular recognition, and maintenance of cell integrity. Their significance is underscored by the fact that they constitute 20–30% of the proteome in most organisms and are targets for 60% of pharmacological interventions. Recent discoveries regarding the influence of specific mecA alleles on β-lactam susceptibility in MRSA have opened new avenues for understanding β-lactam resistance in this pathogen. Methicillin-resistant *S. aureus* is notably challenging, accounting for 20% of deaths in hospital-acquired bloodstream infections. MRSA infections are more difficult to treat than those caused by Methicillin-susceptible *S. aureus* (MSSA) due to resistance to nearly all β-lactams, which are the preferred treatment for MSSA. Therefore, a crucial goal in developing new antibacterial therapies is to achieve efficient delivery of antibiotics into host cells harbouring these infections.

Bacterial resistance to antibiotics arises through spontaneous mutations and the acquisition of resistance traits. A well-known example is the acquisition of β-lactamases, enzymes that hydrolyse β-lactam antibiotics, rendering them ineffective. However, resistance trait acquisition is not the only cause of treatment failure. For an antibiotic to be effective in clinical settings, it must reach its molecular targets and the bacteria in therapeutic concentrations without harming the host. The plasma membrane of mammalian cells comprises a lipid bilayer interspersed with integral and peripheral proteins.

Table 1. Membrane proteins implicated by antibiotic resistance mechanisms of *Staphylococcus aureus.*

Membrane protein	Class/Type	Function in antibiotic resistance	Examples
Adhesins	Cell wall-associated	Mediate attachment to host cells and tissues	Protein A (SpA), Fibronectin-binding proteins
Efflux pumps	Transporters	Remove antibiotics from the bacterial cell actively.	NorA, MepA, QacA/B, and others
Two-component systems	Signal transduction	Regulate gene expression in response to environmental cues	NorG/NorR, GraXRS, WalKR, and others
Biofilm-Associated	Cell wall-associated	Contribute to biofilm formation and chronic illness.	Microbial surface components recognizing adhesive matrix molecules (MSCRAMMs)
Toxin transporters	Sec system	Facilitate secretion and transport of toxins and virulence factors	SecA, SecY, SecE, SecG, and others

Table 2. Membrane protein-driven drug targets in *Staphylococcus aureus.*

Membrane protein	Potential drug target	Relevant antibiotic classes	Targeted mechanisms
NorA (Efflux Pump)	Efflux pump inhibitors	Fluoroquinolones, Tetracyclines, β-Lactams	Inhibition of active antibiotic expulsion
Adhesins (e.g., SpA)	Anti-adhesive compounds	Various antibiotics, host immune proteins	Disruption of host-cell binding and biofilm formation
Two-component systems	Modulators of signal transduction	Multiple antibiotic classes, antiseptics	Modulation of gene expression and regulatory pathways

Table 3. Exploiting transporters: In-depth analysis of drug Daptomycin.

Membrane protein	Antibiotic- resistant strains	Antibiotic - susceptible strains	Variations in expression/mutations
NorA (Efflux Pump)	Upregulated	Downregulated	Increased expression levels contributing to resistance
Adhesins (e.g., FnBPs)	Variable	Consistent	Strain- dependent variations in adhesin expression
Two- component systems	Altered activation states	Normal Activation	Mutations affecting regulatory elements

Figure 1. Pathogenic factors and cell structure of *Staphylococcus aureus*.

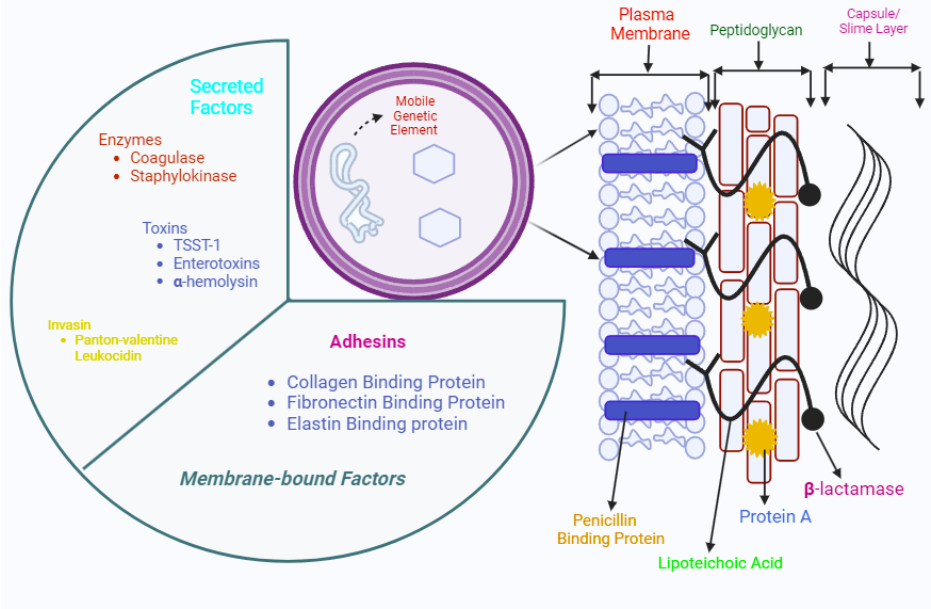
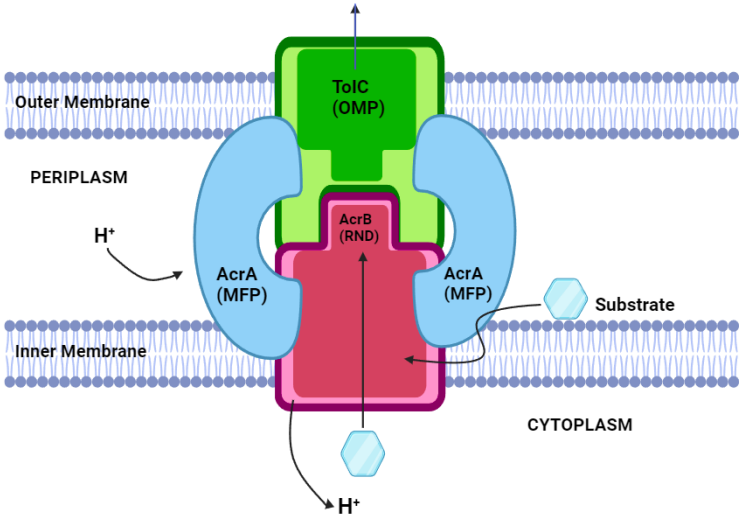


Figure 2. Bacterial multidrug efflux pumps.



An RND efflux pump's structure. The *E. coli* AcrAB-TolC system's structural layout is depicted in the image. The system looks like as a three-part complex made up of the membrane protein fusion AcrA, the outer layer of the membrane protein TolC, and the interior membrane protein AcrB. The proton gradient is linked to the AcrB RND protein's function. It has been demonstrated that these efflux pumps are capable of extruding various substances from the periplasm and cytoplasm of bacteria. Adapted from Piddock and Blair (2009).

Conclusion

In conclusion, our analysis underscores the critical role of membrane proteins in developing effective drugs against resistant *S.aureus* strains. By elucidating the intricate interactions between these proteins and antibiotics, we have identified promising avenues for targeted drug development. The insights gained from this analysis lay the foundation for creating innovative treatments capable of combating *Staphylococcus aureus's* antibiotic resistance. Furthermore, establishing the link between drug development driven by membrane proteins and antibiotic efficacy not only enhances our understanding of the mechanisms underlying bacterial resistance but also offers viable solutions to the global challenge of antibiotic-resistant diseases. It is imperative to leverage this knowledge in guiding the development of next-generation antibiotics with improved effectiveness and reduced propensity for resistance. Continuing to investigate membrane protein targets and developing novel drug delivery methods will be crucial in combating bacterial infections worldwide and addressing the evolving pattern of antibiotic resistance in *S. aureus*.

Conflict of interest

The authors do not have any conflicts of interest.

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