

Review

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Review

Current Status and Future Perspectives of Antibiotic Therapy for MRSA Infections

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Abstract: *Staphylococcus aureus* (*S. aureus*), particularly Methicillin-resistant *S. aureus* (MRSA), is a life-threatening pathogen that causes a variety of infections in hospital and community settings. It poses a significant risk and challenge for global health that hinders our ability to control and treat bacterial infections. Recently, the emergence and spread of variant strains of bacteria, misuse of the limited available options of effective antibiotics, spread of fake drugs, and climate change that increased the contact between humans and animals' populations carrying different bacterial has significantly increased the incidence of Multi-Drug Resistance strains (MDR). This in turn, has created a severe problem in the infection control treatment of *S. aureus* infected patients. Treatment options for MRSA infection are increasingly limited and complicated. Unfortunately, different strains of MRSA are showing tolerance and resistance toward vancomycin, which is the standard of care for complicated MRSA. Therefore, it is crucial now to invest developing new effective antibiotics and synergies, develop and implement national action plans for the management of antibiotic resistance, as well as improving our understanding of the antimicrobial resistance mechanisms. Here, in this review we explain the main mechanisms of antibiotic resistance in MRSA and describes different approaches to manipulate it in MRSA, providing a basis for designing effective drugs, and shedding some light on the evolution of *S. aureus*.

Keywords: Methicillin-resistant *Staphylococcus aureus*; MRSA; antibiotic resistance mechanism; novel therapeutic approaches; national action plan for antibiotic resistance management

1. Introduction

Methicillin-Resistant *Staphylococcus aureus* (MRSA) is a major emerging threat to the global health that causes Skin and Soft Tissue Infections (SSTIs), lethal pneumonia, septic arthritis, osteomyelitis, sepsis, and endocarditis [1]. MRSA infections are associated with significant morbidity,

mortality, and severe socioeconomic impact due to the substantially increased treatment costs that burden patients and health systems [2]. MRSA was recently reported to be the leading drug-resistant cause of death pathogen in 27 European countries [3]. Recently, emergence of resistance to the currently available antibiotics has dramatically increased the incidence of Multi-Drug Resistance strains (MDR) and posed severe problems in the treatment and infection control of *S. aureus* [4]. Effective antibiotics for treating MRSA are exceedingly limited, costly, and complicated [4]. Besides resistance to β -lactam antibiotics, MRSA frequently exhibits resistance toward other antimicrobial agents such as macrolides, aminoglycosides, fluoroquinolones, and more recently vancomycin, which is the main current standard option of treatment for complicated infections caused by MRSA [5].

Moreover, MRSA strains exhibit resistance to topical drugs of choice, including mupirocin and fusidic acid, and many other new antibiotic compounds, including linezolid, daptomycin, and ceftaroline [5,6]. This has created a serious challenge for case management and infection control, particularly in limited resources settings. Therefore, there is an urgent need for novel effective antibiotics agents [7]. The primary strategies of pharmaceutical industries for developing new therapeutics is limited to pursue discovering new antibiotics or repurposing existing drugs, such as the groups of beta-lactams and aminoglycosides [8,9]. Other strategies that target intrinsic components in bacteria to mediate resistance to a particular antibiotic family, such as the use of Efflux Pump Inhibitors (EPI) and Methyltransferase Inhibitors to overcome the resistance mechanism of ciprofloxacin and Macrolide Lincosamide Streptogramin B (MLSB), respectively [10–14]. This review focuses on explaining the fundamental mechanisms of antibiotic resistance and novel therapeutic strategies to overcome the drug resistance mechanisms in MRSA.

2. Antimicrobial Resistance of MRSA

S. aureus has the ability to rapidly adapt, develop tolerance and resistance to new antibiotics [13]. Resistance to the most recently developed antibiotics such as linezolid, daptomycin, and fifth-generation cephalosporin (ceftaroline) have been reported among some strains of *S. aureus*. Several resistance mechanisms were described before among *S. aureus* including enzymatic modification of antibiotic targets, mutations that reduce antibiotic affinity for its target, and active efflux pumps that expel antibiotics outside the cell [15]. These mechanisms confer resistance to anti-MRSA drugs and are it is mediated by several factors (See Figure 1 and Table 1).

Table 1. Mechanism of resistance of MRSA to current antibiotics.

Drugs	Mechanism of resistance
Macrolide, Licosamid, and streptogramin B (MLSB)	MLSB resistance involves two possible mechanisms: (a) modification of ribosomal binding site by methylation in the A 2058 in 23S rRNA, mediated by methyltransferase encoded by erm genes, or (b) expression of active efflux mediated by msrA/B gene [16–18].
Vancomycin and Telavavin	Resistance to vancomycin is mainly by modification of peptidoglycan precursors to D-alanine-D-lactate instead of D-alanine-Alanine, mediated by vanA operon (vanH and vanA) and regulated by two genes (vanS and vanR) [15,19,20].
Linezolid	Linezolid resistance is mediated by mutation or changes in ribosomal proteins on the drug binding site and methylation of the drug target on 23S rRNA, encoded by the cfr gene [21,22].
Daptomycin	Daptomycin resistance occurs due to modification of the drug target by an increased positive charge of the bacterial cell surface that is mediated by Multiple Peptide Resistance Factor encoded by mrpf gene [23].
Tigecycline	Resistance to tigecycline is conferred by alterations of rpsJ protein or by expression of an active efflux pump called mepA [24].

Ceftaroline	Mutations within the PBP2a allosteric domain alter the triggering mechanism and possibly affect protein-protein interactions needed for peptidoglycan biosynthesis [25,26].
Fusidic acid	A mutation in the chromosomal fusA gene encoding Factor-G (EF-G) confers resistance to fusidic acid [27,28].
Mupirocin	A point mutation in Isoleucyl-tRNA synthetase (IleRS), which is the drug target [15].
Fluoroquinolone	Fluoroquinolone resistance is conferred by a mutation in the quinolone determining region on DNA gyrase and topoisomerases IV that reduce drug binding efficiency and/or by overexpression of endogenous efflux pumps such as NorA efflux pump [29,30].
Aminoglycoside	Aminoglycoside resistance is conferred by modification of antibiotics to prevent binding to the ribosomal target, which is mediated by bi-functional acetyltransferase-phosphotransferase enzymes [15].

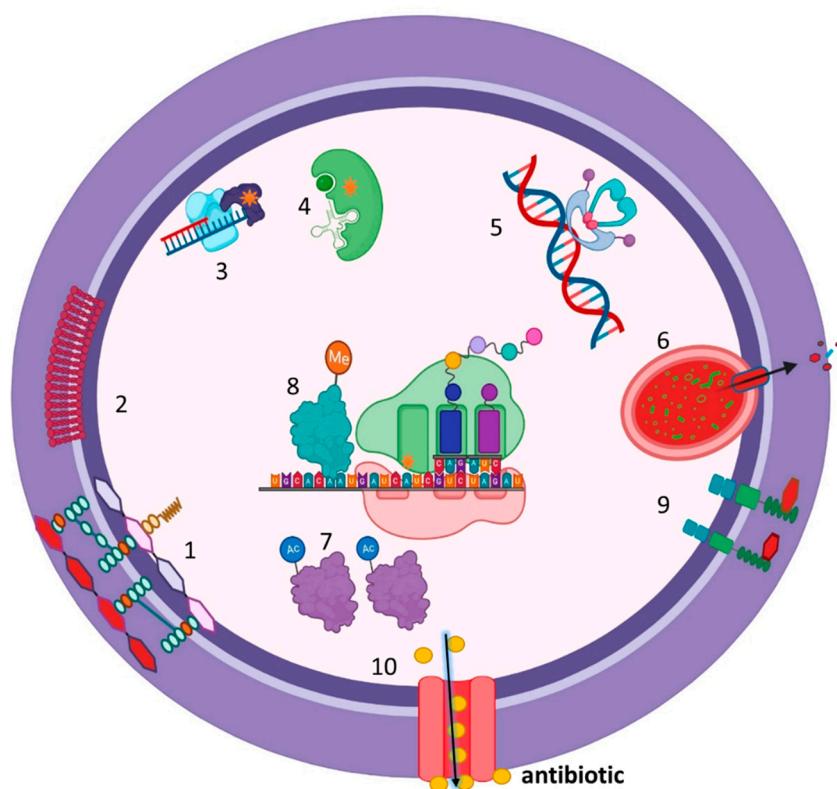


Figure 1. Mechanism of resistance of anti-MRSA. 1. Vancomycin: Alteration of the antibiotic target from D-ala D-ala to D-ala D-lac. 2. Daptomycin: Modification of phosphatidylglycerol (PG) by MrpF protein, which adds a positively charged lysine residue to PG, forming lysylphosphatidyl glycerol (L-PG). 3. Fusidic acid: Mutation in Elongation Factor-G (EF-G). 4. Mupirocin: Mutation in Isoleucyl-tRNA synthetase (IleRS). 5. Fluoroquinolones: Mutation in DNA gyrase/Topoisomerase IV. 6. Degradation of antibiotics and release by extracellular vesicles. 7. Aminoglycosides: Enzymatic modification of antibiotics, such as Acetyltransferase. 8. MLSB and Linezolid: Mutation/Methylation of 23s rRNA, MLSB (erm MTase), Linezolid (cfr MTase). 9. Ceftaroline and Ceftobiprole: Mutation/Alteration of PBP2a. 10. Ciprofloxacin and Tigecycline: Active efflux pump (EP): NorA for ciprofloxacin, TetM, and MepA EP for tigecycline. Created with BioRender.com.

2.1. Penicillin & Methicillin Resistance

S. aureus resistance to penicillin is primarily conferred by the blaZ gene, which encodes a β -lactamase enzyme that inactivates penicillin by hydrolyzing the beta-lactam ring of penicillin. Methicillin, a semi-synthetic beta-lactam antibiotic, it was introduced in the late 1950s as a therapy

for penicillin-resistant *S. aureus* (PRSA) infection; particularly for treating infections caused by beta-lactamase-producing staphylococci. However, interestingly within a year of its introduction, MRSA emerged [13]. MRSA strains were first reported in 1961 in the United Kingdom. The resistance was acquired via incorporating a *mecA* gene into the chromosome at a specific site [15]. *mecA* encodes an alternative penicillin-binding protein (PBP2a or PBP2') that has a low affinity for most semi-synthetic penicillins, including methicillin, nafcillin, and oxacillin [11]. Briefly, PBPs are transpeptidase enzymes responsible for the final step of bacterial cell wall synthesis and are primary targets for all beta-lactam antibiotics [15]. Modified PBPs are the major cause of beta-lactam antibiotic resistance, especially among gram-positive cocci. In MRSA, PBP2a (PBP2') is encoded by the *mecA* gene located on the mobile genetic element SCCmec, along with its regulators *mecR1* and *mecI* [15]. The majority of healthcare-associated (HA)-MRSA infections are linked to SCCmec types II or III, whereas community-associated (CA)-MRSA is associated with SCCmec types IV [15,31]. Recently, *mecC*, which encodes a homolog of PBP2a (designated PBP2c by the European Committee on Antimicrobial Susceptibility Testing; EUCAST) from the *S. aureus* strain LGA251, has been described, which differs in beta-lactam binding characteristics [4]. Unlike penicillinase-mediated resistance, the resistance conferred by *mecA* is broad-spectrum to the entire class of lactam-related drugs, except for ceftaroline and ceftobiprole [15]. Therefore, MRSA is currently a major risk of hospital admission, particularly in immunocompromised people and a significant challenge for the infection control teams globally [32].

2.2. Vancomycin-resistant *S. aureus* (VRSA)

The major component of the *S. aureus* cell wall is peptidoglycan, a heavily cross-linked polymer consisting of N-acetylmuramic acid (NAM) and N-acetylglucosamine (NAG) glycan chains, cross-linked by glycine bridges and pentapeptide stems (UDP-Mur-NAc-L-Ala-D-iso-Gln-L-Lys-D-Ala-D-Ala) [13]. Vancomycin disrupts downstream peptidoglycan assembled by binding non-covalently to the D-Ala-D-Ala residues of newly synthesized UDP-MurNAc-pentapeptides during the later stages of peptidoglycan synthesis [33]. The *vanA* operon confers resistance to vancomycin by hydrolyzing D-Ala-D-Ala peptidoglycan precursors and synthesizing D-Ala-D-lactate peptidoglycan precursors that are essential for vancomycin binding. The *vanA* operon consists of several genes including *vanA*, *vanH*, *vanX*, *vanS*, *vanR*, *vanY*, and *vanZ* [2]. The expression of the *vanA* operon is regulated by a two-component sensor-regulator system encoded by *vanS* and *vanR*, which activate the transcription of the operon [19]. *VanA* is a ligase that catalyzes the ester-bond formation of the D-Ala-D-Lac depsipeptide, and *VanH* is a dehydrogenase that forms D-Lac by reducing pyruvate [33]. *VanA*, *VanH*, and *VanX* are essential for producing vancomycin-resistant phenotypes. *VanA* and *VanH* synthesize the depsipeptide D-Ala-D-Lac, and *VanX* hydrolyzes the D-Ala-D-Ala ester bond, ensuring that the newly formed D-Ala-D-Lac depsipeptide has little competition to bind the UDP-linked tripeptide peptidoglycan precursor [15]. *VanY* is a D, D-carboxyl peptidase that facilitates the cleavage of D-Ala-D-Ala dipeptides already attached to the C-terminal end of stem pentapeptide structures [33]. On the other hand; the function of *VanZ* is not well understood, but it may be involved in the resistance mechanism to teicoplanin. Incorporating altered D-Ala-D-Lac into peptidoglycan results in a cell wall that is no longer susceptible to vancomycin [33].

2.3. Macrolide, Lincosamid and Streptogramine B (MLSB) Resistant *S. aureus*

MLSB antibiotics, including erythromycin, clindamycin, and streptogramin B, exert their antibacterial activity mainly by binding reversibly to the bacterial 50S ribosomal subunits, blocking the progression of nascent proteins through the exit tunnel in bacterial protein biosynthesis [34]. The resistance mechanisms to MLSB antibiotics primarily involve inhibiting protein synthesis via ribosomal binding site modification through methylation or creating mutations in the 23S rRNA gene. This process is encoded by the erythromycin ribosomal methylase (*erm*) genes; activating efflux mediated by *msrA/B* gene; or enzymatically modifying the antibiotics [16]. In *S. aureus*, the important mechanism of MLSB resistance is the N6 methylation of nucleotide A2058 in the macrolide binding site in 23S rRNA by the *erm* group of Methyltransferase (MTase) 5,6. This group of enzymes uses S-

adenosyl methionine (SAM) to mono- or dimethylate a 50S ribosomal precursor substrate specifically [17]. The erm genes were initially identified in microorganisms that have natural macrolides as a mechanism of self-protection against their antibiotics. Furthermore, the dimethylation of adenine A2058 at the N6 position in the peptidyl transferase loop region in domain V of the 50S ribosomal subunit leads to cross-resistance between lincosamides, macrolides, and the streptogramin group B (MLSB) [18]. As the erythromycin binding site on the 50S ribosome subunit overlaps the binding site of the newer MLSB. The modification by methylase(s) reduces the binding of all three classes of antibiotics, resulting in the MLSB resistant phenotype [17].

2.4. Linezolid

Linezolid is a type of synthetic oxazolidinone antibiotic that can effectively inhibit enterococci, staphylococci, and most strains of *Streptococcus* [35]. The drug works by binding to the 23S site of ribosomal RNA on the 50S subunit of bacteria, it inhibits the 50S and 30S ribosomal subunits and ultimately prevents the formation of the 70S initiation complex, thus interfering with protein synthesis [35]. One of the most common mechanisms of resistance to linezolid is through drug-binding site mutations in the genes encoding the 23S rRNA [36]. Enzymatic modification of the 23S rRNA through methylation of adenine at position 2503 has also been observed in enterococci [37]. The gene responsible for this modification, Cfr, encodes for methylase and is a plasmid-borne determinant of resistance found in clinical isolates of *E. faecalis* and other gram-positive bacteria such as staphylococci [21,37]. The Cfr gene has been linked to the mobile transposable element IS256, which has a common sequence in multidrug-resistant staphylococci and enterococci. This sequence has been shown to mediate the transfer of antibiotic resistance genes, as well as alter the promoter sequence of regulatory proteins or activate the expression of existing resistance determinants [38]. This phenomenon could explain the ability of cfr to spread across species and the possibility of widespread dissemination in clinical settings. The Cfr protein is a methyltransferase that methylates C-8 of A2503 in 23S rRNA [39]. This base is located near the overlapping binding sites for these drugs, and its methylation prevents the molecules from binding [35].

2.5. Tigecycline

Tigecycline is a type of glycylcycline antibiotic that prevents amino acid incorporation into the bacterial peptide chains on the 30S ribosomal subunit. Structurally, tigecycline is similar to minocycline and tetracycline; however, it is still effective against most tetracycline-resistant isolates of *S. aureus* [24]. There have been reports of tigecycline resistance in *S. aureus* due to the serial passage of bacterial cultures in increasing drug concentrations. Exposed MRSA strains N315 and Mu3 resulted in mutants with 16- to 32-fold increased MICs. In both strains, mutations in a repressor gene resulted in increased gene transcription of a previously uncharacterized multidrug and toxin extrusion family transporter called MepA [24].

2.6. Ceftaroline and Ceftobiprole

Ceftaroline and Ceftobiprole are last-generation parenteral cephalosporins that have an extended spectrum of activity against multi-drug resistant bacteria including MRSA. They inhibit integral peptidoglycan transpeptidases, interfering with cell wall synthesis, and binding to abnormal PBPs like PBP2a in MRSA and PBP2b and PBP2x in β -lactam-resistant pneumococci, ultimately leading to rapid bacterial cell death [25]. X-ray crystallography has revealed that Ceftaroline can bind to two sites in PBP2a of MRSA, one inactivating the serine-active site, while the other attaches to an allosteric binding site, leading to a conformational change that increases the exposure to lethal dose of the drug [26]. However, the persistent usage of ceftaroline has placed a selection pressure that accelerated the development of resistance by mutations. These mutations are characterized by amino acid substitutions close to the active site and the allosteric site of PBP2a [40]. Low-level resistance to Ceftaroline is associated with PBP2a mutations in the allosteric and transpeptidase domains, while high-level resistance is caused by mutations that modify the geometry of the active site [41]. While

high-level Ceftaroline resistance was rendered by the transpeptidase domain mutations, which modifies the geometry of the active site. The low-level resistance was associated with modifications within the allosteric site and defined by a novel allosteric modulation of the active site [42].

At therapeutic concentrations, Ceftaroline activates the opening and acylation process of the active site by binding to the PBP2a allosteric site [43]. Mutations within the PBP2a allosteric domain can modulate the triggering mechanism, affecting protein-protein interactions needed for peptidoglycan biosynthesis [43]. However, additional factors contributing to Ceftaroline resistance remain to be analyzed. Reduced susceptibility to Ceftaroline has been described in *S. aureus* isolates predating its commercial introduction and in countries where it is not commercially available [22].

2.7. Daptomycin

Daptomycin is a lipopeptide antibiotic produced by *Streptomyces roseosporus*. It exhibits activity against a broad spectrum of gram-positive bacteria and is approved for the treatment of *S. aureus* bacteremia and endocarditis, making it a crucial antibiotic for MRSA treatment [44]. The antimicrobial activity of daptomycin is dependent on the presence of calcium, which forms a complex called Calcium-Daptomycin (Ca-Dap) [44]. The Ca-Dap complex binds to the negatively charged head groups of phosphatidylglycerol (PG) and penetrates the cell wall into the cytoplasmic membrane, leading to depolarization, permeabilization, and leakage of ions that ultimately results in cell death. Resistance to daptomycin arises due to mutations in genes that activate the bacterium's defenses towards cell envelope damage, including host cationic antimicrobial peptides [45]. The resistance usually occurs during prolonged therapy in infections involving high bacterial densities. Prior treatment of the patient with vancomycin is associated with a more rapid development of daptomycin resistance during therapy due to resistance mechanisms enhancement [23].

To resist daptomycin, *S. aureus* must prevent the drug from reaching the cytoplasmic membrane and interfere with its entry into the membrane. However, mutations in different genes can occur, resulting in the minimum inhibitory concentration (MIC) surpassing the susceptibility breakpoint [44]. The most common mutational changes conferring resistance to daptomycin are found in the multiple peptide resistance factors (mrpF) gene [39]. The mrpF protein plays a role in daptomycin resistance by adding a positively charged lysine residue to PG, forming lysylphosphatidyl glycerol (L-PG) [15]. L-PG is synthesized in the cytosol and is then translocated to the outer face of the membrane, resulting in increased levels of L-PG in the cell membrane, which increases the charge of the outer face of the membrane. This, in turn, repels the Ca-Dap complex, reducing the negatively charged PG needed for drug binding to initiate membrane damage [23].

2.8. Telavancin

Telavancin is a vancomycin derivative that contains a hydrophobic side chain on the vincamine sugar [46]. Like vancomycin, Telavancin inhibits bacterial cell wall synthesis by interacting with the polymerization and peptidoglycan biosynthesis [46]. In addition, Telavancin disrupts membrane barrier function by binding to the bacterial membrane, facilitated by targeted interaction with lipid II precursor in the cell wall [47]. Telavancin exerts concentration-dependent bactericidal activity against Gram-positive organisms in vitro, including a wide range of multi-drug resistant strains [46,47]. The FDA has approved Telavancin for treating cSSSI in adult patients caused by susceptible isolates of the following Gram-positive microorganisms: *S. aureus* (including methicillin-susceptible and -resistant isolates) [20].

2.9. Fusidic acid

Fusidic acid is commonly used topically to treat *S. aureus* skin infections and to reduce skin colonization in eczema patients. However, when the drug is used alone, resistance rapidly emerges by mutation due to the high selection pressure. A combination of rifampicin and fusidic acid effectively combats difficult-to-treat MRSA infections and is a useful alternative to linezolid. It is concerning that widespread topical usage could compromise the drug effectiveness [27]. Fusidic acid

targets elongation factor G, a cytoplasmic protein that binds to the ribosome after peptide bond formation and transformation of the growing peptide chain to the aa-tRNA at the A site [28]. Normally, the EF-G GTPase promotes translocation of the ribosome, such that the peptidyl-tRNA returns to the P site, and the deacylated tRNA is expelled from the E site. EF-G then detaches, allowing the next round of translation to begin with an aa-tRNA molecule entering the vacant A site. The presence of fusidic acid prevents the release of EF-G after the translocation event and blocks the next round of aa-tRNA binding [48]. There has been a recent increase in the reported resistance to fusidic acid, mainly involving plasmids expressing the FusB and FusC mechanisms. While the fusB gene is plasmid-encoded, fusC is located within SCC elements (SCCFus and SCCmec) and linked to the TirS gene, which encodes the TirS protein that inhibits Toll-like receptor signaling and likely contributes to virulence [27,48].

Furthermore, fusC and tirS seem are co-expressed because TirS expression is elevated in subinhibitory concentrations of fusidic acid. FusA resistance is due to spontaneous chromosomal mutants affecting EF-G that prevents the drug from binding to its target [48]. Flubs is a small ~25 kDa protein that binds EF-G. It binds to the EF-G fusidic acid complex on the stalled ribosome in the post-translocation state. This triggers a conformational change in EF-G, promoting its release and allowing translation to proceed [48].

2.10. Mupirocin

Mupirocin is another topical antibiotic commonly used to reduce nasal carriage of MRSA among hospital patients and staff, as well as to treat skin infections. It targets the isoleucyl-tRNA synthetase, an enzyme involved in protein synthesis. Resistance to mupirocin can occur at both low and high levels [49]. Low-level resistance is usually caused by mutations (amino acid substitutions) in the target-site, thus reducing the drug's binding efficiency. On the other hand, high-level resistance is typically associated with plasmid-borne MupA determinants that specify an intrinsically insensitive IleRS enzyme, which allows the sensitive target to bypass the drug's inhibitory action [15].

2.11. Aminoglycosides

Neomycin, an aminoglycoside, is frequently used topically to prevent or treat skin infections and as a nasal ointment for decolonizing carriers. Also, Gentamicin was introduced in the 1970s to address severe nosocomial infections caused by *S. aureus*, but its efficacy was hindered by the emergence of high-level resistance caused by mobile genetic elements [50]. Aminoglycosides are the only ribosome-targeting antibiotics that have bactericidal properties due to their unique mode of action that causes misreading during translation. Clinical strains of *S. aureus* develop resistance to aminoglycosides through the acquisition of cytoplasmic aminoglycoside-modifying enzymes (AMEs), the most common mechanism of resistance encoded by mobile genetic elements [50]. AMEs are classified into three groups, which render aminoglycosides inactive via acetylation (acetyltransferases; AAC), adenylation (nucleotidyltransferases; ANT), or phosphorylation (phosphotransferases; APH). Genes encoding these enzymes often exist on mobile elements such as plasmids or transposons [15]. Drug modification prevents ribosome binding. Tn4001 encodes a bi-functional acetyltransferase-phosphotransferase (aacA-aphD), which confers gentamicin and neomycin resistance. Neomycin resistance is caused by Tn5405-encoded phosphotransferase (aphA) or plasmid pUB110-encoded adenyltransferase (aadD). In some MRSA strains, the plasmid is incorporated within the SCCmecII cassette [15].

2.12. Fluoroquinolone

Fluoroquinolones are a highly successful class of entirely synthetic antimicrobial drugs. The breakthrough in their development came with the discovery that fluorination at position C6 could enhance the spectrum and potency of the prototype molecule nalidixic acid [51]. Since then, several generations of fluoroquinolones have been introduced with additional substitutions, leading to improved potency and pharmacokinetics of the drug.

In *S. aureus*, the molecular targets for fluoroquinolones include DNA gyrase and topoisomerase IV. DNA gyrase introduces negative supercoils into chromosomal DNA, while topoisomerase IV promotes chromosome decatenation following replication. Both enzymes are heterotetramers of A and B subunits, with GyrA and GyrB in DNA gyrase and ParC and ParE in topoisomerase IV. These enzymes catalyze staggered double-stranded breaks, with the 5' end of each cleaved DNA strand forming a phosphotyrosine bond with the active site tyrosine in the two A subunits. A DNA strand is then passed through the break, the gap is resealed, and the phosphodiester bond between the backbone deoxyriboses is regenerated [29].

The fluoroquinolones form ternary complexes with Mg²⁺ and the A subunit of the topoisomerases at the intermediate covalent protein-DNA complex stage. This prevents resealing of the cleaved DNA strand, leading to double-stranded DNA breaks that cannot be repaired and result in a rapid bactericidal effect [30]. *S. aureus* resistance to fluoroquinolones involves mutational changes to the topoisomerases that reduce drug binding efficiency and elevate the expression of endogenous efflux pumps [30]. The most common type of mutational change that leads to resistance to fluoroquinolones is due to topoisomerase mutants. This results in amino acid substitutions in residues that constitute the drug-binding site, also known as the quinolone resistance-determining region. Additionally, resistance to fluoroquinolones is mediated by overexpressing chromosomally encoded efflux pumps [98]. Three pumps can efflux fluoroquinolones: NorA handles the hydrophilic molecules norfloxacin and ciprofloxacin, while NorB and NorC target hydrophobic drugs such as sparfloxacin and moxifloxacin [52].

Nor proteins belong to the major facilitator superfamily and they are related to the Tet efflux pump. However, the increase in minimum inhibitory concentration is usually determined by the transporter, which depends on the pump's affinity for each particular fluoroquinolone. The expression of NorA, NorB, and NorC is governed by complex interactions between regulatory proteins, with the small transcriptional regulator MgrA being centrally involved [30]. Nor proteins can bind directly to the promoters of the nor genes depending on the degree of phosphorylation catalyzed by kinase PknB. For example, phosphorylated MgrA reduced binding to the NorA promoter and increased binding to norB [52].

2.13. Extracellular vesicles and *S. aureus* antibiotics resistance

Extracellular vesicles (EVs) are naturally secreted from bacteria, carrying specific cargo molecules from the cell of origin. They play significant roles in protecting bacterial cells and contribute to their survival in the presence of antibiotics [53]. EV secretion from MRSA increases significantly under antibiotic-stressed conditions. EVs released under these conditions were found to contain abundant proteins related to the degradation of β -lactam antibiotics. Therefore, EVs from MRSA can function as a first-line defense against β -lactam antibiotics, playing a crucial role in the survival of β -lactam susceptible bacteria. As a result, antibiotic stress leads to the release of EVs with high defense activity [53].

3. The current situation of MRSA treatment

Infections caused by MRSA strains have become a major global health concern due to their high morbidity and mortality rates [6]. Therefore, there is an urgent need for developing novel effective drugs to treat multi-drug resistant MRSA infections [14]. This has led to global investment that in turn accelerated scientific research, resulting in the discovery of several potentially effective anti-MRSA drugs [37]. Vancomycin, a glycopeptide that inhibits peptidoglycan biosynthesis in gram-positive bacteria by forming a strong hydrogen bond interaction with D-alanyl-D-alanine precursors of peptidoglycan, became the primary antibiotic for treating MRSA infections in hospital settings in the late 1980s [54]. Another effective anti-MRSA drug is daptomycin, a cyclic lipopeptide that was approved in 2003 for use in skin and soft tissue infections. It works by binding to the cell membranes of gram-positive bacteria [55]. Additionally, Mupirocin is an effective topical antibiotic used to treat bacterial skin infections that inhibits bacterial protein synthesis by interfering with isoleucyl transfer RNA synthase in gram-positive bacteria [32]. Furthermore, Linezolid is a recent drug used to treat

MRSA infections and other drug-resistant bacterial infections by inhibiting protein biosynthesis after binding to 50S ribosomal RNA [31].

Moreover, several semi-synthetic drugs, such as Tigecycline, Oritavancin, Dalbavancin, Iclaprim, Cethromycin, and Delafloxacin, have shown better activity against MRSA by inhibiting peptidoglycan biosynthesis or protein synthesis. Ceftaroline, a novel cephalosporin with a high affinity for the modified penicillin-binding protein (PBP 2A) and thus has in vitro activity against MRSA, is a recent anti-MRSA antibiotic [15]. Recently, several studies are investigating the safety and antibacterial effectiveness of using Snake Venom Phospholipases A2 as an innovative approach for treating drug resistant bacteria including *S. aureus* [56]. Nonetheless, the availability of a broad portfolio of antibiotics against MRSA is illustrated in Figure 2.

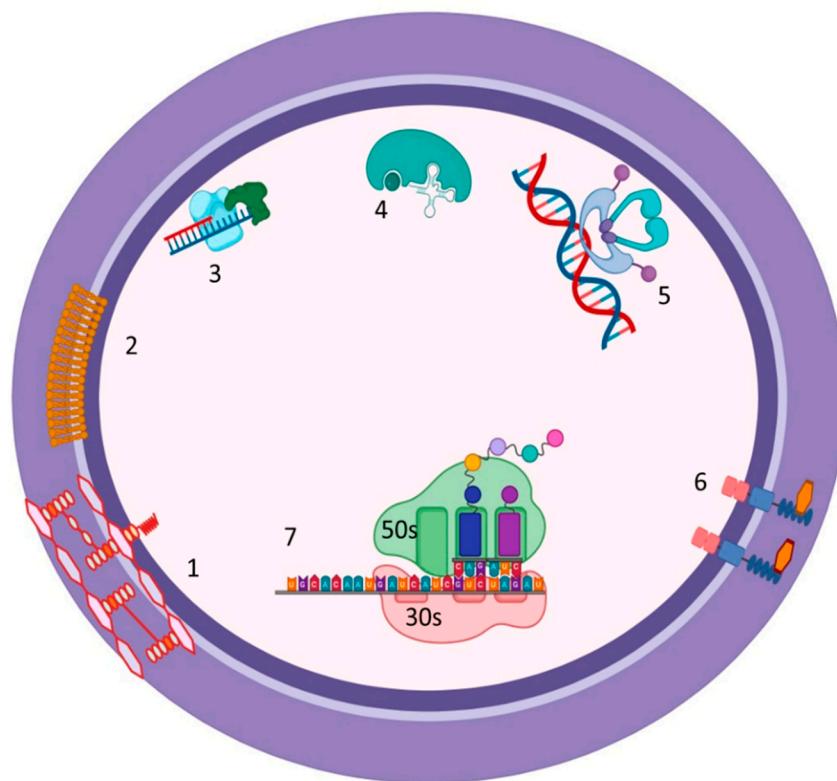


Figure 2. Mechanisms of action of anti-MRSA. 1. Vancomycin and Telavancin: interfere with late-stage peptidoglycan synthesis by forming non-covalent hydrogen bonds with the D-Ala-D-Ala residues of newly synthesized UDP-MurNAc-pentapeptides, thereby disrupting downstream peptidoglycan assembly. 2. Daptomycin: inserts into the cytoplasmic membrane by binding to negatively charged phosphatidylglycerol (PG) head groups, inducing strain into the lipid bilayer, which results in depolarization, permeabilization, and leakage of ions, notably K⁺, and ultimately cell death. 3. Fusidic acid: prevents the release of EF-G after the translocation event and blocks the next round of aa-tRNA binding. 4. Mupirocin: binds to isoleucyl-tRNA synthetase, which recognizes amino acids, ATP, and cognate tRNA. 5. Fluoroquinolones: inhibit DNA gyrase and topoisomerase IV. 6. Ceftaroline: inhibits the action of peptidoglycan transpeptidases, which interfere with cell wall synthesis through binding to abnormal PBPs like PBP2a. 7. Macrolides, lincosamides, and linezolid: bind to the bacterial 23S rRNA on 50S ribosomal subunits and block nascent protein synthesis. Created with BioRender.com.

4. Novel Therapeutic Strategies for MRSA Treatment:

The emergence of MRSA strains with intrinsic resistance to the current used antimicrobials, particularly MDR strains, has reduced the effectiveness of these drugs and made infection control and case management more challenging. Thus, it is crucial to develop efficient strategies to combat

antimicrobials drug resistance and to develop innovative drugs for control of MRSA-associated infections (See Figure 3 and Table 2) [13].

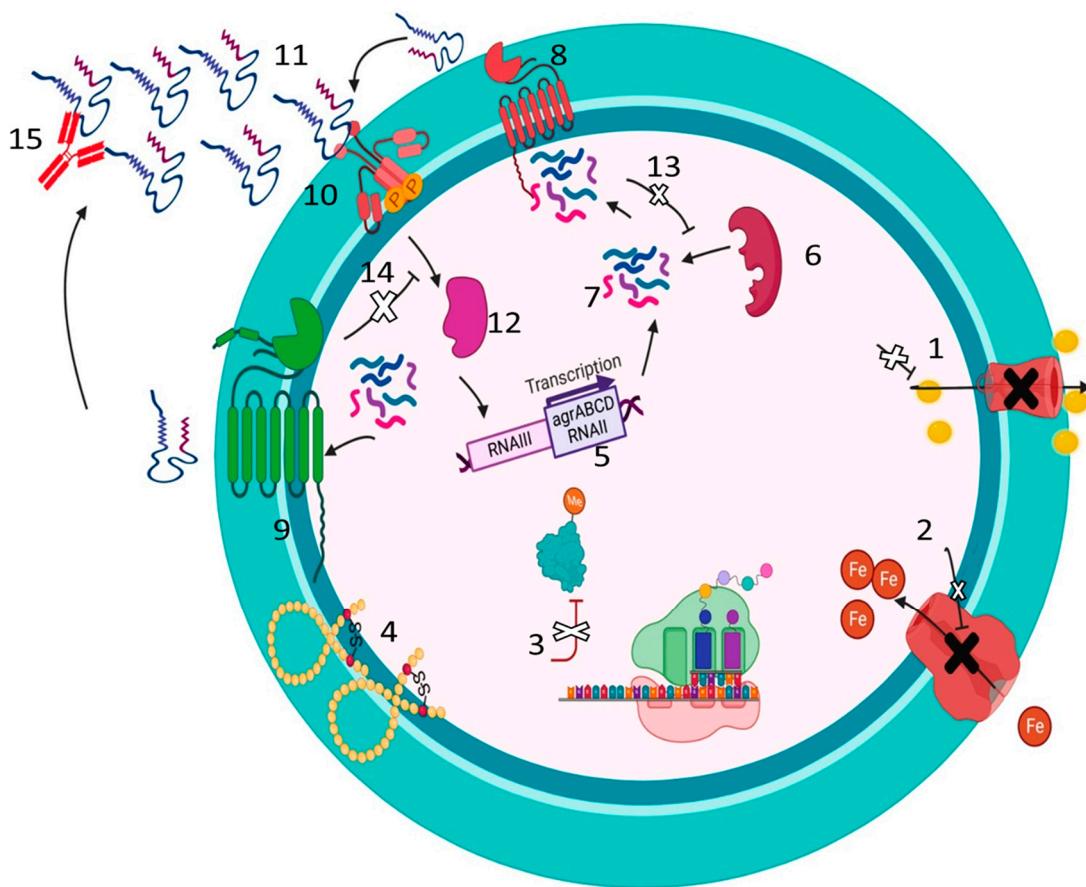


Figure 3. Novel approaches for MRSA treatment. 1. Inhibition of NorA efflux pump. 2. Blocking of Ironsiderophore by Iron chelators. 3. Inhibition of ermC methyltransferases. 4. Synthetic Anti-microbial Peptides to disrupt the cell wall. 5. Inhibition of the accessory gene regulator (agr) locus. 6. Autoinducing peptides (AIPs) synthase. 7. Pro-AIP. 8. Inhibition of the type I signal peptidase (SpsB). 9. AgrB: a transmembrane endopeptidase. 10. AgrC: Histidine Kinase, the sensor kinase of the TCS. 11. AIPs. 12. AgrA: which binds to RNAlII. 13. Inhibition of quorum sensing by inhibiting AIP synthase, which inhibits the synthesis of pro-AIP. 14. Inhibition of quorum sensing by inhibiting the regulatory factor agrA. 15. Inhibition of quorum sensing by using monoclonal antibodies against AIP to inhibit AIP binding to AgrC Histidine Kinase. Created with BioRender.com.

Table 2. Novel Therapeutic Strategies for treating MRSA infection.

New approaches	Mechanism of action / Uses	Examples
Quorum Sensing Inhibitor	Inhibits the switch of agr quorum sensing, preventing expression of virulence factors and subsequent inhibition of biofilm formation [57,58].	Alanine-5 (D5A), tri-AIP-1, triAIP2, solonamide A and B, Savirin, Hamamelitannin, anti-autoinducer monoclonal antibody AP4-24H11 [59–61].
Iron Chelators	Inhibit <i>S. aureus</i> iron acquisition mechanisms, suppressing MRSA wound infection and nares MRSA carriage, and potentially enhancing antibiotic activities [62].	Deferiprone, Deferasirox, Hydroxypyridinone (DIBI) [63].

NorA efflux pump inhibitors	Inhibit the NorA efflux pump [64].	Reserpine, Thioridazine, Orizabin XV, Verapamil, Omeprazole, Paroxetine, Chlorpromazine [30,52].
ErmC' methyl transferase inhibitor	Inhibit bacterial methyl transferase [65].	Sinefungin, UK-10573, ZINC database (ZINC code 327479060 compound) [65].
Antimicrobial peptide (AMP)	Display a direct activity by disrupting the plasma membrane and/or acting on specific intracellular targets to inhibit DNA, RNA, or protein synthesis processes, or inactivate essential intracellular enzymes [66].	CAMA, LL-37, Magainin-II, Nisin, WR12, D-IK8 [66,67].
Teichoic acid and lipoteichoic acid Inhibition	Inhibition Inhibit teichoic acid and lipoteichoic acid biosynthesis, resulting in the inhibition of biofilm formation and disruption of bacterial growth, cell wall physiology, and membrane homeostasis [68].	Tunicamycin, Targocil, HSGN-189, and HGSN-94 [68].

4.1. Quorum Sensing Inhibition

In *S. aureus*, the accessory gene regulator (agr) locus is a crucial part of the global virulence response. The operon is composed of two transcripts, RNAII and RNAIII. The RNAII encodes the constituents of the agr system itself, namely agrB, agrD, agrC, and agrA [59]. The agr system is a two-component signal transduction system (TCS) that uses cyclic thiolactone peptides as signaling molecules. agrD produces auto-inducing peptides (AIPs) as pro-peptides, which are then processed into active AIPs by agrB, a transmembrane endopeptidase. The AIPs are secreted and accumulate in the extracellular environment. Upon reaching a certain concentration, they bind to agrC, the sensor kinase of the TCS. agrC, which is phosphorylated in an AIP-dependent manner, activates agrA, the response regulator. Activated agrA binds to RNAIII, a regulatory mRNA that acts as the effector of the agr QS system [57]. The agr system in *S. aureus* controls the expression of 70 to 150 genes and is mainly involved in the invasiveness of *S. aureus*. It upregulates toxins and enzymes (proteases, nucleases, lipases, etc.), and almost all known virulence factors are upregulated by agr [57].

Hence, inhibiting the agr quorum sensing can significantly attenuate bacterial virulence factors and result in the inhibition of biofilm formation, which plays a major role in MRSA drug resistance [58].

4.2. Iron Chelator

Iron (Fe) ions are essential nutrients for most organisms. Research has revealed that Fe ions play a significant role in essential biological enzymes such as oxidoreductase, and are involved in various life processes including electron transport, antioxidant reactions, and the nucleic acid synthesis [58]. Additionally, Fe carriers are essential for controlling pathogenic microorganisms. Chelation of Fe can prevent pathogenic bacteria from using Fe, thereby inhibiting their growth and metabolic activity. Since human cells do not possess a pathway for iron carrier synthesis, their biosynthesis and absorption pathways can be leveraged for antimicrobial treatment [62].

Targeting iron acquisition represents a potential strategy to overcome MRSA drug resistance by introducing new anti-MRSA agents that may act independently or synergistically support other antibiotics to enhance their efficacy. Thus, compounds that affect iron acquisition have been recognized as potential candidates [62]. *S. aureus* is dependent on iron and must acquire this essential nutrient from its host during infection via molecules such as transferrin, lactoferrin, and heme. Therefore, the dependence of *S. aureus* on Fe makes it an excellent target for Fe-chelating agents that could interfere with the bacterium's Fe acquisition mechanisms [49,63].

4.3. *NorA* efflux pumpsinhibitors:

To overcome drug resistance, another promising strategy is the inhibition of efflux pumps; compounds that prevent antibiotics from being pumped out. NorA, the most important efflux pump in *S. aureus*, is implicated in drug extrusion and subsequently decreased intracellular drug concentration [64]. Inhibiting the action of NorA efflux pumps with compounds represents a good opportunity to manipulate the antimicrobial resistance [69]. These compounds alone are not growth inhibitors but act synergistically with antibiotics, this makes the use of efflux pump inhibitors (EPIs) an effective approach to decreasing the MIC of drug agents. There are many anti-EPIs compounds, most of which are non-antibiotic drugs [70]. An early example of efflux pump inhibition in *S. aureus* that demonstrates the potential of the plant alkaloid, a known inhibitor of mammalian MDR efflux pumps, which reduced the ciprofloxacin MIC. Besides reserpine, various other natural products have been shown to inhibit bacterial efflux pumps. It appears that a variety of Berberis medicinal plants that produce berberine, a cationic antimicrobial, also synthesize the flavone 5 methoxyhydnocarpin (5'-MHC, 24), an inhibitor of the NorA efflux pump of *S. aureus*. Without 5-MHC, *S. aureus* readily extrudes the plant-derived antimicrobial alkaloid.

Efflux pump inhibitors are not restricted to natural products [71]. For instance, the nonsteroidal anti-inflammatory drug (NSAID) celecoxib has recently been demonstrated to re-sensitize *S. aureus* to multiple antibiotics. Sabatini and co-workers confirmed the efflux pump inhibitor activity of the NSAID and identified a new class of analogs acting as inhibitors of the *S. aureus* NorA efflux pump. The most active inhibitor showed only modest anti-staphylococcal activity and was able to restore the antibacterial activity of ciprofloxacin in norA-overexpressing *S. aureus* strains [69,71].

4.4. *ErmC'* methyltransferase (*ermCMTases*) Inhibitors:

S. aureus develops cross-resistance to MLSB antibiotics by modifying the drug target enzymatically [17]. This modification occurs through methylation of the 23S rRNA at A2058 that creates a macrolide binding site that is no longer accessible to the antibiotics. The erythromycin ribosomal methyltransferase encoded by erm genes (such as ermC, ermE, ermA) is responsible for this conversion, using S-adenosyl-L-Methionine (SAM) as a methyl donor for the process [72]. The crystal structure of the rRNA methyltransferase of ErmC and ErmE has provided a model for drug design aimed at overcoming and preventing MLSB cross-resistance [73]. Therefore, identifying novel compound combinations with antibiotics that act as potential inhibitors of resistance mechanisms could be useful in inhibiting the methylation of SAM substrates and thus restore the activity of antibiotics against bacteria [74].

Several strategies for developing inhibitors have been proposed and developed. Enzymatic activity was initially used to screen and identify combinations with potential inhibitory activity [74]. Followed by nuclear magnetic resonance-based screening and chemical synthesis based on the enzymatic activities. This approach led to the discovery of novel compounds that bound to the binding sites of ErmC and ErmB, such as SAM, and demonstrated the potential to overcome resistance [18].

Virtual screening of structural compounds and in vitro potency characterization of the Erm proteins helped design inhibitors based on the minimal substrate features [74]. Although, the SAM binding site is highly conserved across different methyltransferase (MTases) families, different conformational aspects of ErmC could be exploited to design new inhibitors. Additionally, the conserved water structures observed in the ErmC-SAM binding site could be substituted, and structural mimics of the transition state could be used to enhance the potency [65].

Similarly, the recent explication of the functions of the tentative carbon-oxygen (CH-O) hydrogen bond that was found between the methyl group of SAM and oxygen from particular active site residue(s) contributes to high-affinity SAM binding, limiting the motion of the SAM methyl group to maintain the alignment of the methyl group for optimal transfer geometry, and transition state stabilization. It could provide two distinct strategies for the specific inhibitor development of MTases, like designing ligands to act as CH- O hydrogen bond acceptors instead of SAM or mimicking SAM CH -O hydrogen-bond donors using cofactor or transition state analogs [65].

5. Synthetic Antimicrobial Peptides against multidrug resistant MRSA

Antimicrobial peptides (AMPs) and their synthetic analogs are from an extensive group of compounds of great structural diversity and multifunctionality, different modes of antimicrobial action, and considerable market potential. That proven antibacterial, antifungal, and antiviral activities [66]. Recently, the functions of the carbon-oxygen (CH-O) hydrogen bond that was found between the methyl group of SAM and oxygen from particular active site residue(s) have been elucidated. This bond contributes to high-affinity SAM binding, limiting the motion of the SAM methyl group to maintain the alignment of the methyl group for optimal transfer geometry, and stabilizing the transition state. This knowledge opened the door for two distinct strategies for the specific inhibitor development of MTases. One strategy is to design ligands that act as CH-O hydrogen bond acceptors instead of SAM, while the other strategy involves mimicking SAM CH-O hydrogen-bond donors using cofactor or transition state analogs.

Antimicrobial peptides (AMPs) and their synthetic analogs are a diverse group of compounds with multifunctionality, different modes of action, and significant market potential, with proven antibacterial, antifungal, and antiviral activity [67]. AMPs have emerged as promising therapeutic agents for treating infections caused by multidrug-resistant bacteria including MRSA. One of the unique characteristics of AMPs is their broad-spectrum activity against all pathogens, with minimal chance for developing resistance [67,75]. Therefore, this review explores the potential of synthetic AMPs, WR12 and D-IK8, for use as topical antimicrobials in the treatment of SSTIs caused by *S. aureus* [76].

5.1. Nanoparticles and Nanomedicine

Metal nanoparticles possess multiple antimicrobial properties that may be useful for combating intracellular infections as these properties reduce the selection pressure for developing antibiotic resistance. For instance, silver nanoparticles (AgNPs) release Ag⁺ ions that can disrupt electron transport and trigger ROS production, causing harm to bacteria [77]. Growing evidence has demonstrated that these AgNPs can kill 100% of extracellular bacteria and 76% of intracellular *S. aureus* within osteoblasts, with intracellular killing rates similar to those of rifampicin treatment, whereas gentamicin treatment alone did not show any intracellular killing effect [77]. In a recent study, *S. aureus* bacteria were isolated and tested on culture plates with AgNP-discs, which showed effective growth inhibition for both MRSA and VRSA variants. If AgNPs' efficacy is demonstrated *in vivo*, they could become valuable tools for combating drug-resistant *S. aureus* infections [78].

The physicochemical characteristics of nanocarriers signify their bactericidal effect, which differs for every material. The nanosize and higher surface area of NPs enable better interactions with bacterial cells compared to typical antibiotics [79,80]. Furthermore, these nanocarriers can effectively overcome the bacterial cell envelope, which limits the internalization of antibacterial drugs. For example, NPs such as metal NPs, dendrimers, carbon nanotubes, chitosan (CS), antimicrobial peptides (AMPs), and cyclodextrin (CD) can directly penetrate the bacterial envelope, causing envelope disruption. However, some delivery systems such as the fusogenic liposomes system do not penetrate bacteria but deliver the drug intracellularly to the bacteria [79].

When designing carriers for the specific and tolerable release of antibiotics in treating bacteria, several aspects must be considered. These aspects include minimizing antibiotic interactions with healthy tissue, measuring the accumulation of antibiotics at the infection site, improving drugs' efficacy, and lowering the toxicity risks and vulnerability of commensal microflora to the antibiotic. One approach is to develop a stimuli-responsive system that can either react in a self-motivated way and recognize the bacterial microenvironment or is responsive to certain physical factors [81]. This approach is a promising strategy for improving antibiotic delivery systems, enhancing their efficiency, targeting properties, and effectiveness of antibiotics while minimizing the side effects [82]. Additionally, NPs can enter cells via endocytosis, a mechanism that can treat intracellular microbial infections. Several studies have shown that different types of NPs can overcome bacterial resistance to antibiotics by escaping from drug degradation enzymes such as β -lactamase, inhibiting efflux pumps, or penetrating thick cell walls. Several NP-based drug delivery systems designed for MRSA

treatment are either candidates for clinical trials or have recently been approved [80]. In Figure 4, we demonstrate the potential usefulness of Synthetic Antimicrobial Peptides against multidrug resistant MRSA.

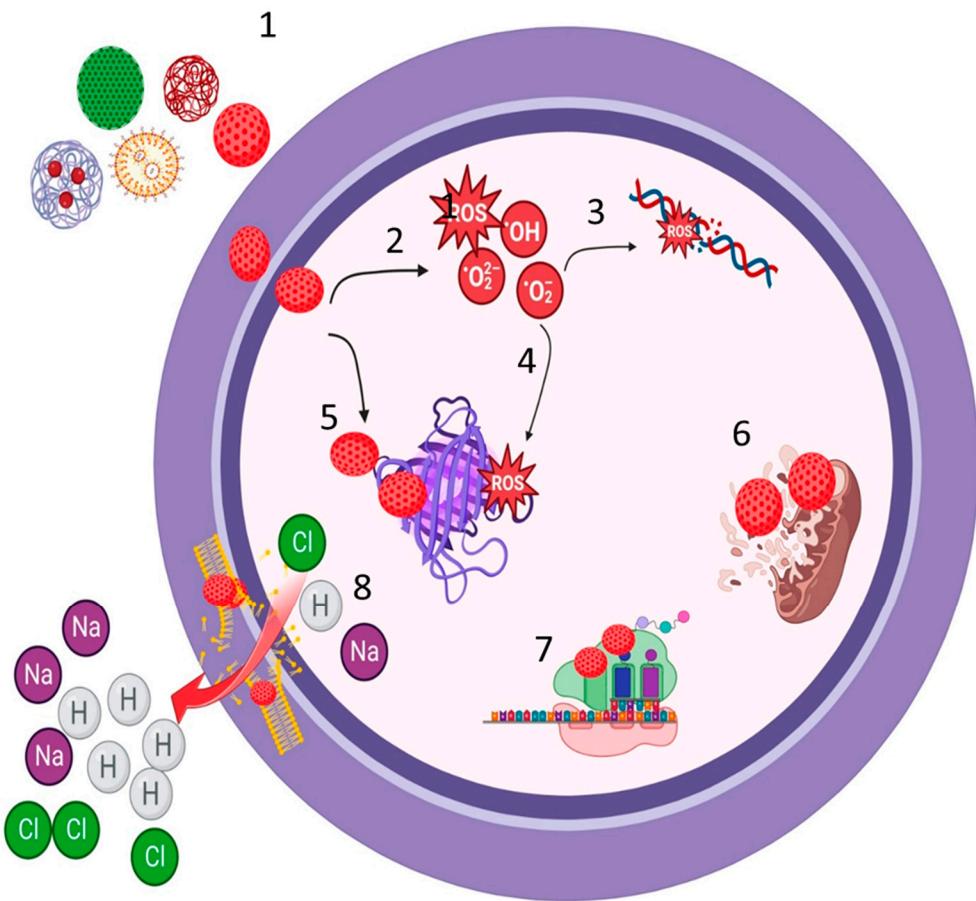


Figure 4. Synthetic Antimicrobial Peptides against multidrug resistant MRSA 1. Nanoparticles ex: silver nanoparticles (AgNPs). 2. cellular toxicity by the generation of ROS. 3. ROS oxidize and damage the DNA. 4. ROS oxidize the proteins. 5. Nanoparticles can directly be destabilized and denature the proteins. 6. Nanoparticles interact and act with mitochondria leading to mitochondrial dysfunction. 7. Nanoparticles interact and destabilize ribosomes. 8. Nanoparticle adhesion to the cell membrane alters membrane structure and permeability, resulting in leakage of electrolyte and cellular content. Created with BioRender.com.

5.2. Inhibition of Teichoic acid biosynthesis

Most chronic MRSA infections are characterized by the formation of biofilms, in which group of bacterial pathogens including MRSA are attached to biological or non-biological surfaces such as medical devices. Biofilm bacteria are much more resistant to antibiotics than planktonic bacteria and more challenging to treat. Current treatments involve long-term antibiotic therapy, which can lead to increased tissue damage and persistence of the infection. Therefore, new agents that can eradicate or inhibit MRSA biofilm formation via novel mechanisms are needed [81].

Teichoic acid (TA) is a glycopolymer that represents a significant component of *S. aureus* cell walls. TAs consist of polyribitol-phosphate polymers cross-linked to MurNAc residues of peptidoglycan and covered with D-alanine and GlcNAc residues [77]. There are two types of TA: wall teichoic acid (WTA) and lipoteichoic acid (LTA) [77]. LTA is mainly anchored in the plasma membrane, while WTA is covalently linked to peptidoglycan. Although, WTA and LTA have similar structures, they are synthesized by different pathways, with WTA being a ribitol phosphate polymer and LTA being composed of glycerol phosphate [68]. Therefore, enzymes involved in biosynthesis

and export could be effective drug targets. Several WTA biosynthesis inhibitors have been reported. For example, Targocil inhibits the membrane-bound ABC transporter, which exports WTA across the membrane. When used in combination with a β -lactam, a synergistic effect was observed, restoring the efficacy of the β -lactam towards MRSA [68,77].

Assessing potential antimicrobial strategies involves considering several factors, such as the ability to target specific bacterial infections, minimize harm to healthy tissue, improve drug efficacy, and reduce toxicity. One promising approach involves developing a stimuli-responsive drug delivery system that can react to the bacterial microenvironment or certain physical factors to enhance targeting properties and effectiveness while minimizing side effects [81].

5.3. Inhibition of *S. aureus* Aminoacyl-tRNA Synthetases (aaRSs)

Aminoacyl-tRNA synthetase (AaRS) enzymes have shown potential antimicrobial benefits because they play crucial roles in protein biosynthesis. Once these enzymes are inhibited, protein biosynthesis is halted, leading to inhibition of bacterial growth [77]. Recent studies suggest that AaRS could be used as therapeutic targets, and the loss of fitness associated with resistance could be compatible with antimicrobial restriction policies aimed at eliminating resistance [83].

AaRS enzymes meet several criteria defined by the pharmaceutical industry, making them ideal targets for antibacterial drug discovery. Although these enzymes are universally distributed in cell organisms, there has been evolutionary divergence between prokaryotic and eukaryotic enzymes, and these differences can be exploited to develop antibiotics that selectively inhibit bacterial enzymes. Moreover, AaRS enzymes are highly conserved across different bacterial pathogens, increasing the chances of making some discoveries of broad-spectrum antibiotics [83]. Their solubility, stability, and ease of purification in significant quantities by recombinant expression systems can be tested using one or more conventional methods amenable to high-throughput screening [84] (Figure 5).

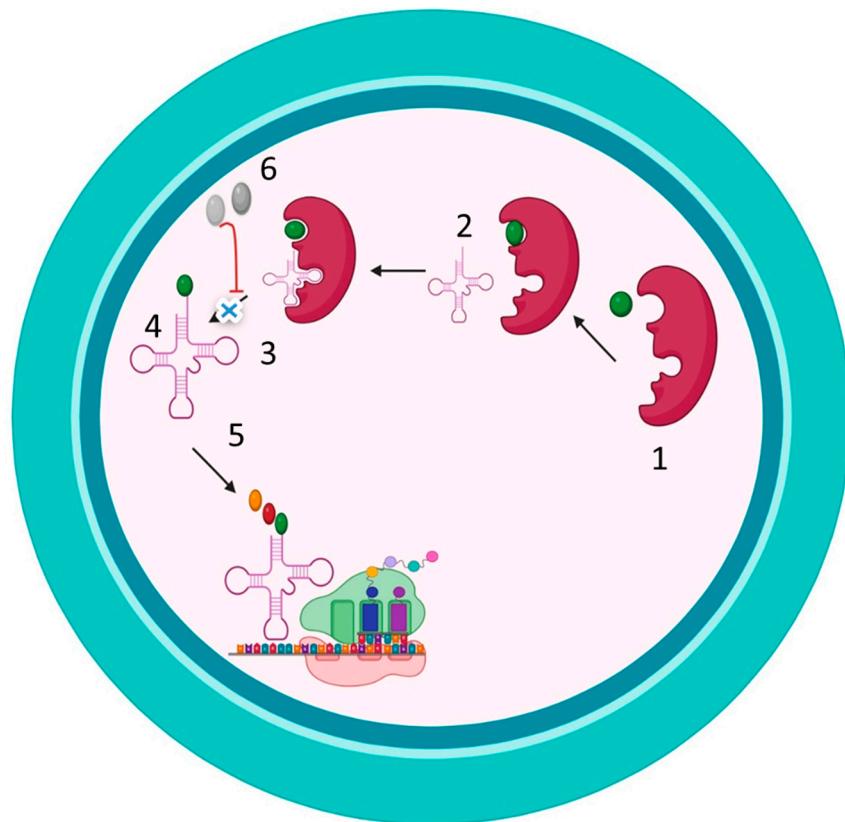


Figure 5. Inhibition of *S. aureus* Aminoacyl-tRNA Synthetases (aaRSs) 1. Aa-tRNA synthetases recognize specific amino acids. 2. Aa-tRNA synthetases bind to specific amino acids and recognize specific cognate uncharged tRNA. 3. Aa-tRNA synthetases bind to uncharged tRNA. 4. Aa-tRNA synthetases transfer amino acids to uncharged tRNA and become charged tRNA with specific amino

acid. 5. Charged or acylated tRNA goes to the ribosome, and the peptide grows. 6. Inhibition of aa-tRNA synthetases: Inhibition of these stages leads to the accumulation of uncharged tRNA, which attaches to ribosomes, resulting in an interruption in the elongation of the peptide chain and inhibition of protein synthesis. Created with BioRender.com.

6. Antibacterial Properties of Snake and Scorpion Venom: Potential for New Antimicrobial Agents

Snake and scorpion venoms are intricate mixtures of proteins, enzymes, and peptides that are primarily utilized for defense and capturing prey. Nonetheless, studies have indicated that some of the venom components possess antimicrobial activity. For instance, crotalicidin, a peptide extracted from the venom of South American rattlesnakes, has been demonstrated to be effective against a broad range of bacteria, including MRSA [85]. Similarly, cathelicidin-BF, a peptide present in the venom of Chinese cobras, has been found to have potent antibacterial activity against several bacteria, including multidrug-resistant strains [86].

Additionally, Pandinin 2, a peptide derived from the venom of the African scorpion Pandinus imperator, has demonstrated activity against both Gram-positive and Gram-negative bacteria [87]. Another peptide, IsCT, obtained from the venom of the North African scorpion Scorpio maurus, has also shown to have strong activity against various bacteria, including MRSA [88].

Although the antibacterial properties of snake and scorpion venom provide a promising avenue for the development of new antimicrobial agents, their toxicity should not be overlooked. Consequently, ongoing research is focused on identifying and isolating the most effective peptides for use in medical applications [56].

7. Antibacterial Properties of Snake and Scorpion Venom: Potential for New Antimicrobial Agents

In recent years, there has been a growing interest in developing vaccines for the prevention and control of MRSA infections [89]. The strategy of vaccination is to stimulate the body's immune system to recognize and destroy the pathogen, thus preventing the development of an infection [90]. Currently, there are no licensed vaccines available for MRSA, but many potential candidates are under development. One promising approach is to target the capsule polysaccharide of MRSA, which is a major virulence factor and plays a crucial role in the pathogenesis of the infection [89]. Several vaccine candidates have been developed to target this polysaccharide, such as conjugate vaccines and glycoconjugate vaccines, which link the polysaccharide to a carrier protein or a lipid molecule to enhance the immune response, respectively [89].

Another approach is to target specific surface proteins of MRSA, such as the clumping factor A (ClfA) and iron-regulated surface determinant B (IsdB), which are vital in adhesion and invasion of host cells. These proteins have shown promising results in preclinical studies [91,92].

The feasibility and potential use of an MRSA vaccine for the prevention and control of MRSA infections are significant. An effective vaccine would provide an essential tool in combating this highly resistant pathogen, potentially reducing the incidence of infections and the need for antibiotic treatment. Additionally, vaccination could assist in preventing the spread of MRSA in healthcare settings, where the risk of transmission is high. However, there are several challenges that need to be addressed in developing an MRSA vaccine, such as the high genetic variability of the organism and the requirement for effective adjuvants to enhance the immune response. Despite these challenges, the development of an effective MRSA vaccine remains a crucial goal in the fight against antibiotic-resistant bacteria.

8. Concluding Remarks

The emergence of multi-drug resistant *S. aureus* infections, particularly MRSA is a serious threat to the global health that poses a significant challenge to the control of infections, particularly during the COVID-19 pandemic. It is crucial to make significant efforts to keep up with the development of antibiotic resistance and devise new strategies to manipulate the drug resistance mechanism and

develop novel drugs to combat MRSA infections. Advances in microbial genomics, X-ray crystallography, and cryo-electron microscopy have provided promising avenues for the discovery of new antibacterial targets and the design and repurposing of antimicrobial compounds from existing antibiotics.

However, to effectively combat MRSA, it is essential to gain a deeper understanding of the molecular mechanisms of drug resistance. This understanding can be obtained by exploring the genetic and metabolic pathways involved in the development of drug resistance in MRSA. Moreover, investigating the bacterial cell's structural and functional features can provide crucial insights into the development of novel drugs and therapeutic strategies. In summary, the emergence of MRSA infections is a significant public health concern that demands urgent attention. It is essential to develop innovative strategies to combat this pathogen's drug resistance and discover new antibiotics to control MRSA infections including vaccination. Comprehensive molecular and structural investigations are critical to achieving these goals.

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