Letters in Applied NanoBioScience
Open-Access Journal (ISSN: 2284-6808)

Review
Volume 15, Issue 1, 2026, 40
https://doi.org/10.33263/LIANBS151.040

Specific Approaches for the Development of New Drugs Against *Staphylococcus aureus*, Through Intra- and Extracellular Targets

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Received: 4.08.2025; Accepted: 7.02.2026; Published: 30.03.2026

Abstract: The World Health Organization (WHO) estimates that by 2050, bacterial resistance will cause 10 million deaths, of which approximately one million are associated with multidrug-resistant (MDR) microorganisms. *Staphylococcus aureus* is one of the bacteria with high resistance to the most widely used antibiotics, which cause infections worldwide. This bacterium possesses highly developed mechanisms of resistance to drugs and the host's immune system. It is worth highlighting its capacity for cross-protective mechanisms, since plasmids can be transmitted between bacteria in an infectious event, promoting a component that increases bacterial resistance in that event. Reports show that approximately 80% of *S. aureus* strains are resistant to penicillin (methicillin resistance), and that this resistance has been developing since the 1960s (WHO). This demonstrates the importance of continuing to study this bacterium and of developing new drugs against it. This study reviews several proteins involved in key stages of the *S. aureus* infection process, where metabolism and intra- and extracellular resistance mechanisms require proteins such as 3-dehydroquinate dehydratase (SaDHQD) and Shikimate kinase (SaSK), as well as Protein A, Nuclease 1, Beta-lactamase, PBP2a and Triosephosphate isomerase (SaTIM), which have functions that can contribute to increased drug resistance or susceptibility. Therefore, it is necessary to consider the multiple mechanisms of resistance to the different groups of antimicrobial drugs used in clinical practice, and to promote and conduct studies to develop new antibiotics.

Keywords: antibiotic resistance; *Staphylococcus aureus*; bacterial targets.

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1. Introduction

The World Health Organization (WHO) estimates that bacterial resistance will cause 10 million deaths by 2050, and it has declared the “Combat against bacterial resistance: if we do not act today, there will be no cure tomorrow” [1], due to cases of bacterial resistance have multiplied, the WHO has positioned infectious diseases as the third leading cause of death worldwide, with nearly 6.5 million deaths, of which approximately 1 million are associated with multidrug-resistant (MDR) microorganisms. The most frequent pathogenic organisms that are relevant to drug resistance are reported, where the important bacteria that have been developing greater resistance to antibacterial treatments are: *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella spp*., *Pseudomonas aeruginosa*, *Acinetobacter spp*., *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Neisseria gonorrhoeae*, and *Shigella spp*. (WHO). Some

of them are in the ESKAPE group that require more effective treatments, which are summarized in the set of *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter spp*. (WHO) [2,3], among these bacteria, there is a greater increase in resistance to the most commonly used antibiotics, such as *Klebsiella pneumoniae* (MDR) and extended-spectrum beta-lactamase-producing bacteria (ESBL), Pseudomonas aeruginosa MDR, *Staphylococcus aureus* MDR, and *Enterococcus faecium* [1].

Therefore, this review focuses on approaches to developing new drugs against S. aureus, which, as we know, is a relevant pathogen for public health (WHO). This review provides a general overview of the important components of bacteria, focusing on their role in developing new drugs.

1.1. Bacterial morphology and structure.

Some differences between eukaryotic cells (animal and plant cells) and prokaryotic cells (bacteria) are mainly size, nucleus, organelles, and cell wall [4] where we can emphasize the characteristics of bacteria, such as some proteins or the cell wall, where we can find therapeutic targets; such as cell wall-binding proteins (PBPs) [5–8] or proteins that are important in metabolic processes, either for the synthesis of amino acids or proteins [9–11].

1.1.1. Macroscopic and microscopic distinction of bacteria.

The macro- and microscopic characteristics of bacteria may contribute to the study and analysis of potential therapeutic targets, starting with bacterial development in specialized culture media or in the environment, which can be observed macroscopically as colonies [12]. Remembering that bacteria cannot form other specialized structures, such as tissues or organs. Colonies take on different forms depending on the nutritional status of the bacteria; some characteristics can be distinguished by color, size, shape, and even odor [13,14]. Determining whether bacteria can degrade erythrocytes, secrete toxins, form biofilm, generate drug resistance, or adopt different forms will depend on each type of bacteria, where this could be considered to relate to the prognosis or infectious capacity of the bacteria, given the conditions and factors that favor or diminish it [15,16]. The microscopic appearance includes the size of the bacteria, the configuration (bacilli, cocci, and spirals), the grouping, and their ability to retain Gram stain dyes (Gram-positive or Gram-negative), which is determined by the chemical structure of the cell wall [4,17]. Bacteria that have a spherical structure are called "cocci," the most representative example being the bacterium of the genus *Staphylococcus spp*., and rod-shaped bacteria are called “bacilli”, of which the most representative example in science is *Escherichia coli*., some other bacteria that belong to the group of cocci or bacilli have different shapes, some of them closely resemble coffee beans (*Neisseria spp*.) or cells in the shape of isosceles triangles (*Streptococcus spp*.), and of the latter we have bacteria that tend to be branched like *Nocardia spp*. or curved bacilli as in the case of *Vibrio cholerae*, to mention an example. To observe bacteria under a microscope, they must be stained with Gram stain dyes [18].

Bacteria whose wall structure is high in protein will be called Gram-positive, and those bacteria whose bacterial wall is thinner and has a greater amount of lipids will not be able to fix the dyes well, so it will be necessary to add a contrast dye called safranin, and they will be called Gram-negative [4,17,19].

1.1.2. The bacterial wall, one of the most frequent therapeutic targets.

The cell wall is a bacterial characteristic, a distinctive element of bacteria, and is widely used as a therapeutic target. Bacterial cell structure shows very specific differences in staining affinity in Gram stain preparations, which are highly correlated with the wall structure. PBPs bind to their components, primarily peptidoglycans, and exert their therapeutic effect [5–8]. Therefore, it is important to keep in mind the characteristics of the main components or elements of bacterial walls, which can be divided into thick and thin walls. The wall of Gram-positive bacteria is thick and has multiple layers of peptidoglycan (links of four peptides linked to two carbohydrates arranged in a 3D configuration that are joined by structural bridges in each of the layers), and that surrounds the cytoplasmic membrane. The peptidoglycan layers confer a wall thickness of approximately 150 to 500 angstroms (thick), essential for replication and survival in the hostile environment in which bacteria develop. Without the bacterial wall, both in Gram-positive and Gram-negative bacteria, cells succumb to a large difference in osmotic pressure: the interior of bacterial cells is much denser than the extracellular medium, so a large amount of water would passively but rapidly cross the membrane, causing cell lysis [20]. Lipoteichoic acid plays a very important role in the immune response, acting in a very similar way to lipopolysaccharide and its endotoxin in Gram-negative bacteria.

Compared to Gram-positive bacteria (thicker wall), the cell wall of Gram-negative bacteria is much more complex than that of Gram-positive bacteria. Unlike Gram-positive bacteria, Gram-negative bacteria have a double lipid bilayer: the inner bilayer surrounds the cytoplasm, and the outer bilayer forms the outer membrane. This layer is composed of phospholipids and lipopolysaccharides on its extracellular surface. Between the spaces of the outer membrane and the cytoplasmic membrane is the peptidoglycan cell wall, with a very small number of peptidoglycan layers. There are empty spaces known as the periplasmic space, which contain nutrients and ions important for bacterial metabolism, exotoxins, and other substances. One of the most important components of the outer membrane is lipopolysaccharide, often called an endotoxin, as it is a potent stimulator of the immune response. It is released into the extracellular medium and into the host [20].

Therefore, it is important to note that most penicillins or methicillins are directed at the walls of bacteria, and that this therapeutic target is widely used and that they have developed mutations [21], hence also the loss of efficacy and the requirement for other drugs or combinations to be able to use this target [22-25].

1.2. Main types of antibiotic resistance.

Antibiotic (antibacterial) resistance is the ability of microorganisms to survive and remain viable under the influence of antimicrobial agents, such as soaps, antibiotics, food preservatives, cleaning solutions, and disinfectants, which significantly reduce their growth or inhibit multiplication [26].

There are processes by which microorganisms become resistant to antimicrobial agents. The most important of these are: 1) the bacteria produce enzymes that destroy the antimicrobial agent before it reaches its target or modify the antimicrobial agent in such a way that it can no longer be recognized by its target; 2) the cell wall becomes impermeable to the antimicrobial agent; 3) the attack site is altered by mutation in such a way that it no longer allows the antimicrobial agent to bind; 4) the bacteria have an efflux pump that expels the antimicrobial agent from the cell before it reaches its target [27] (Figure 1).

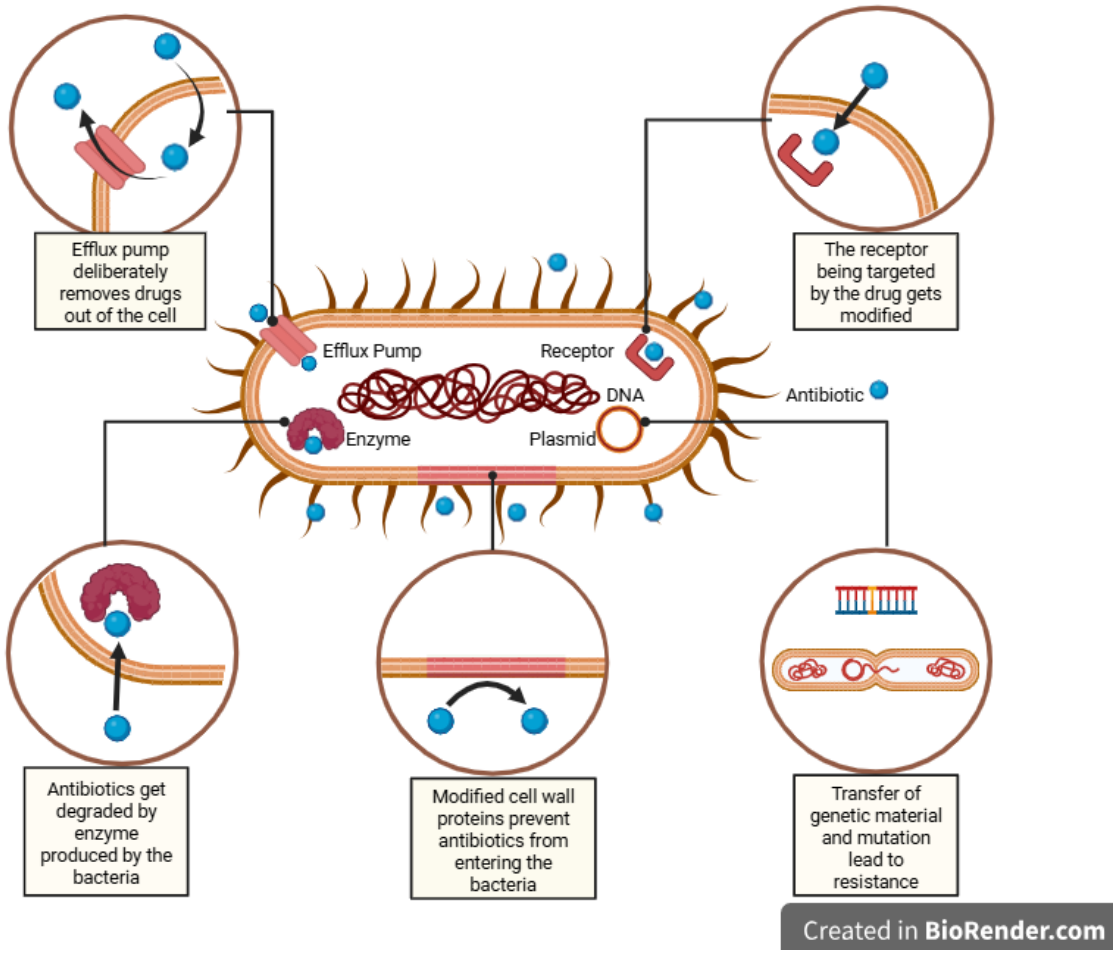

Figure 1. Main types of bacterial resistance to antibiotics. Image created in BioRender.com.

1.2.1. Enzyme inactivation.

Beta-lactamases are enzymes that hydrolyze beta-lactam antimicrobial agents, resulting in cell resistance to the action of beta-lactam drugs. In Gram-negative bacteria, beta-lactam drugs enter the cell through porins and encounter beta-lactamases in the periplasmic space. Beta-lactamases destroy beta-lactam drugs before they have a chance to reach penicillin-binding proteins (PBPs) [28] in the cell wall. In Gram-positive bacteria, beta-lactamases are secreted into the extracellular medium and destroy beta-lactam molecules before they can enter the bacteria [29]. In addition, Gram-negative bacteria can produce adenylating, phosphorylating, or acetylating enzymes that modify an aminoglycoside to inactivate it, and chloramphenicol acetyltransferase. Gram-negative bacteria can produce an acetyltransferase that modifies chloramphenicol, inactivating it [30].

1.2.2. Impermeability of bacterial membranes.

Gram-negative bacteria can become resistant to beta-lactam antibiotics by developing permeability barriers. This is usually caused by altered porins in the outer membrane that no longer allow the entry and transit of antibiotic molecules into the cell. When beta-lactams cannot reach PBPs, the cell is resistant [28,31].

1.2.3. Modification of the target site.

PBPs in both Gram-positive and Gram-negative bacteria can be altered by mutation so that beta-lactam drugs cannot bind to them [6]; therefore, the bacteria are resistant to

antimicrobial agents. In addition, there are other sites of mutation; for ribosomes, ribosomal RNA methylation confers resistance to macrolides, and mutations in DNA gyrase and topoisomerase IV in chromosomal genes confer resistance to quinolones [32].

1.2.4. Efflux pumps.

There is a wide variety of efflux pumps that contribute to antimicrobial resistance in both Gram-positive and Gram-negative bacteria. Active antibiotic efflux is mediated by transmembrane proteins embedded in the cytoplasmic membrane and, in Gram-negative organisms, also involves components of the outer membrane and the periplasm. These proteins form channels that actively export an antimicrobial agent from the cell as quickly as it enters [33].

1.2.5. Alteration of metabolic pathways.

Some microorganisms develop altered metabolic pathways that evade the action of antimicrobial agents. Mutations that inactivate thymidylate synthase block the conversion of deoxyuridylate to thymidylate. These mutants require exogenous thymine or thymidine for DNA synthesis and are therefore resistant to folate pathway antagonists such as sulfonamides and trimethoprim [34].

Now, with an overview of bacteria and their mechanisms of resistance to conventional drugs, let's review key points about *Staphylococcus aureus*.

2. Staphylococcus aureus

Humans are a natural reservoir of *Staphylococcus aureus* (*S. aureus*) [35], and this bacterium can cause infections in several animals [36-39]. This bacterium can be present in between 30% and 50% of healthy adults. After an infection, it could remain persistently colonized in between 10 and 20% people [40], which is also related to complications in other diseases (diabetes, addictions, hemodialysis, surgeries, HIV, among others).

S. aureus is a bacterium that can be present in intra- and extra-hospital infections [41,42]. The main risk group is hospitalized or immunocompromised patients, making this bacterium of global importance. For example, in 2017, it was estimated that 52% of multidrug-resistant infections in hospitalized patients in the US were caused by methicillin-resistant *S. aureus* (MRSA), with the main complications being bacteremia, pneumonia, endocarditis, and vascular infections [41,43]. The above is even more relevant due to the resistance to the most common treatments currently available, and there are reports that bacteremia has a fatality rate between 15 and 30% and causes about 300,000 deaths annually (90-day mortality in patients is 27.0%) [43].

Furthermore, *S. aureus* is the leading bacterial cause of death in 135 countries [43,44]. This shows that it is necessary to develop new drugs against this bacterium, since the levels and types of resistance to the different antibiotics on the market have increased (WHO), including methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant Staphylococcus aureus (VRSA) [40,41,45]. Common strains of *S. aureus* are resistant to penicillin (approximately 80%), making it necessary to use other antibiotics and their combination (aminoglycosides, oxacillin or nafcillin) (WHO) [40], which has emphasized the development of new drugs against this bacterium [46], it is considered in the objective 3 of the UN Sustainable Development Goals (SDG) for 2030.

2.1. Infection process of S. aureus.

This review highlights targets (proteins) in the infectious process of this bacterium, so these proteins could be therapeutic targets [16,43,47-49] (Figure 2), and these proteins could be located in the intra- or extracellular space or in the wall/membrane of *S. aureus*; which have specific functions, with regions of amino acid sequences little conserved with other organisms, which could give greater selectivity to the treatments that could be developed in this bacterium. It is important to note that, for a better study of these targets, the availability of the crystallographic structure (PDB or predicted protein structure) and tests/assays to determine interactions between the recombinant protein complex and the ligand (molecule) should be considered. With all this, a development integrating several theoretical-experimental tests could be proposed. In this way, selective drugs targeting specific therapeutic targets important in the infectious process of this bacterium could be developed (Figure 2), by performing a theoretical design and an in vitro validation of the protein-ligand interaction, and relating this to an antibacterial effect in in vitro cultures.

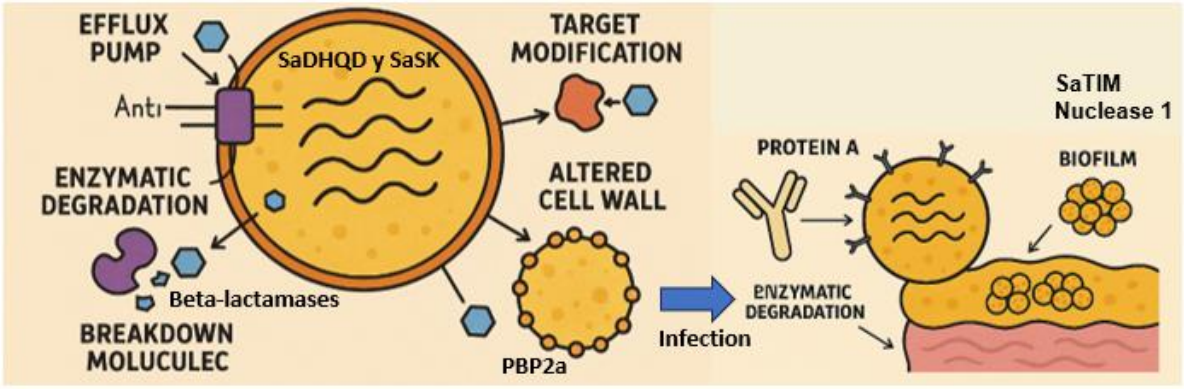

Figure 2. Main components for the infection process of *S. aureus*. Image created in copilot.microsoft.com

2.2. Identified molecular targets in S. aureus.

2.2.1. 3-dehydroquinate dehydratase (SaDHQD).

Intracellularly, SaDHQD can be considered to inhibit the shikimate pathway (absent in mammals), which is key to the survival of methicillin-resistant *S. aureus* (MRSA). This pathway is involved in the biosynthesis of chorismate, an intermediate for the synthesis of aromatic amino acids, folates, and ubiquinone. The third step of the pathway is the function of SaDHQD [11,50], and it is reported that the amino acids Glu35, Arg37, Arg70, Lys160, His133, Arg202, and Gln225 are important for its catalytic activity [50], Therefore, molecular docking (PDB:6SFH) could be performed in this region, and subsequently evaluate its interaction with the recombinant protein, for which in vitro tests have already been reported [45,50] (Figure 2).

2.2.2. Shikimate kinase (SaSK).

SaSK, another enzyme within the shikimate pathway which is an important metabolic pathway for the survival of methicillin-resistant *S. aureus* (MRSA), in the fifth step of this metabolic pathway is carried out by the enzyme SaSK [11]. It is reported an important region for interaction between Gly17, Lys18, Arg113 amino acid, related to the activity of this protein [11] which could be used to perform molecular docking, and subsequently evaluate the interaction and effect in in vitro assays with recombinant SaSK protein [9,10,51] (Figure 2).

The predicted protein structure is only available from the AlphaFold database (AF_AFA5IT66F1) [52].

2.2.3. PBP2a.

Resistance to methicillin, nafcillin, and oxacillin is independent of beta-lactamase production. This resistance is encoded and regulated by a series of genes located in the region of the *S. aureus* chromosome called *Staphylococcal cassette chromosome mec (SCCmec)*. This gene encodes a penicillin-binding protein, PBP2a, with low affinity for beta-lactam antimicrobials (penicillin, methicillin, etc.), which is involved in resistance, thereby conferring resistance to these drugs [21]. Therefore, PBP2a is a therapeutic target in the bacterial cell wall (Figure 2). Studies have been developed to improve the effects of antimicrobial agents [22,24], and PDBs (6Q9N or 6H5O) reported could be used for molecular docking [22,25,53].

2.2.4. Protein A.

Another protein that could be used as a therapeutic target is the Protein A (42 kDa/508 aa), which is covalently tethered to the peptidoglycan layer of *S. aureus* (Figure 2) [19,54,55]. It has affinity for the Fc region of the IgG immunoglobulins in the affected organism's immune system (IgG1, IgG2, IgG4 subtypes), which allows it to bind to the Fc region, thereby preventing S. aureus from being opsonized and phagocytosed. Amino acids have been identified in this protein that are relevant to this protein (YSIRK-G/S) [56,57]. Therefore, could be performed molecular docking using the PDB:1DEE, to determine potential ligans with the sequence between Gln26, Asn28, Gly29, Phe30, Ile31, Gln32, Ser33, Lys34, Asp35, Asp36, Gln40 aminoacids in Protein A [58], with this probably the ligands avoid the the interactions with IgGs so that this target could be promising, where there are promising studies [59,60].

2.2.5. Beta-lactamases.

A widely used target currently is beta-lactamases, and there are several beta-lactamase inhibitors used against *S. aureus*. These drugs are co-administered with beta-lactam antimicrobials (PBPs; penicillins, cephalosporins, monobactams, and carbapenems) and thus exert their effect on the bacterial cell wall. Therefore, beta-lactamases increase antimicrobial resistance and decrease the effect of PBPs. Currently, there are several beta-lactamase inhibitors (avibactam, clavulanic acid, sulbactam, vaborbactam, among others), and their main function is through the inhibition of serine beta-lactamase, the enzyme that inactivates the beta-lactam ring, a chemical structure common to all beta-lactam antimicrobials [61] (Figure 2).

S. aureus has a constitutive production of a large amount of beta-lactamases, which gives it the ability to resist beta-lactam antimicrobial drugs. Currently, mutations in beta-lactamases have been identified (amino acid positions 104, 164, 238, and 240) [62,63], which increase resistance to drugs that inhibit these enzymes. This shows that it would be important to target molecular docking (PDB: 3Q81 or 3Q82) to regions encompassing these positions and/or other interaction regions.

2.2.6. Triosephosphate isomerase from *S. aureus* (SaTIM).

Triosephosphate isomerase from *S. aureus* (SaTIM) is an enzyme that participates in the glycolytic pathway. This enzyme has been reported as a therapeutic target [64-67]. Still, SaTIM has also been reported to have extracellular functions (Figure 2), this could contribute

to using the enzyme as a therapeutic target as well, since this protein has been identified with functions that help this bacterium to adhere to other cells and invade host cells, and that SaTIM also participates in the formation of biofilms (Figure 2), which is very important in the infectious process of this bacterium [68,69]. Therefore, molecular docking could be performed using PDB: 3UWW, and the potential binding site involving Lys11, His97, and Glu169 in SaTIM could be identified. [70]. Thus, to determine whether the effect would be extracellular or intracellular, it is worth noting that TIMs from other organisms have already been reported to promote cell adhesion [71].

2.2.7. Nuclease 1.

Finally, emphasizing the reported extracellular functions of this protein, micrococcal *S. aureus* nuclease 1 (*Nuc1*) plays important roles in biofilm formation and the persistence of infections. This enzyme degrades extracellular DNA, helping bacteria avoid detection and elimination by the immune system. This contributes to the formation of the biofilm matrix (Figure 2). Studies have demonstrated its role in the infectious process of this bacterium [72-74], so, this protein could be a therapeutic target in the future and a molecular docking could be performed using the PDB: 6XSF [75], and the potential site between amino acids positions: 103, 117, 122, 123, 125, and 169 (Uniprot: P00644) [76].

3. Discussion

Developing more effective drugs and reducing bacterial resistance to current treatments are global problems. The WHO proposes an action plan to raise awareness of the problem of bacterial resistance to current treatments, emphasizing the need to develop new antibiotics against resistant bacteria [46]. As already mentioned, it is necessary to develop new drugs, even more selective antibiotics that can attack antibiotic resistance mechanisms, and a clear example is the resistance to conventional treatments that *S. aureus* can present, since important mechanisms of evasion, resistance, and infection have been identified; through proteins (sometimes with mutations in amino acids) that can contribute to evading/inactivating drugs [62,63,77,78]. Therefore, the development of new antibiotics against *S. aureus* will require consideration of protein-ligand interaction regions at the amino acid level; this could be achieved through molecular docking. As mentioned above, *S. aureus* has developed several mechanisms to survive different types of antibiotics, with resistance mechanisms that could be located both intracellularly and extracellularly (Figure 2), and these mechanisms have been studied in recent decades [40,43,79] (OMS), and addressing these mechanisms is of utmost importance, since there are reports showing that approximately 80% of *S. aureus* strains are resistant to penicillin (methicillin resistance) [79] (OMS). This review highlights seven therapeutic targets that could serve as points of attack against this bacterium, each with characteristics that could be considered for developing a comprehensive treatment for the infectious process of *S. aureus.*

There are studies aimed at developing new, specific antibacterial drugs. There are advances in SaDHQD and SaSK inhibitors, where derivatives of known antibacterial drugs are being tested [11,45,51], those that possibly interact with the bacterial wall as PBPs [21,23,80], and those that are performing molecular docking using specific chemical libraries [25]. It is worth mentioning beta-lactamase as a target [81]; it is the target most studied across different bacteria [84-86], as well as Protein A [85], since their immune evasion functions are

undoubtedly important in addressing the problem of bacterial resistance. On the other hand, extracellular targets, which could be considered in adhesion or biofilm components, mainly SaTIM and nuclease 1, are amenable to molecular docking. There are other studies that use biofilm components as targets; advances in new drugs with an effect on the membrane or on the biofilm D-3263 molecule exhibited potent antibacterial and antibiofilm activities against *S. aureus* by targeting the cell membrane [86], but the specific target or sortase A (SrtA) with functions related to biofilm and Protein A remains to be determined [59,60], there are also other targets being studied and reported, others targeting proteins relevant to bacterial metabolism; in *E. coli* the Gyrase B or the Lanosterol 14-α-demethylase (CYP51) [87], and which describe the interaction and probable selectivity of the drug. As we move forward, future research should focus on identifying the specific antibacterial targets that disrupt the infectious process [59].

4. Future Directions, Therapeutic Approaches

The current therapies are demonstrating that there are not enough efforts to address the problem of bacterial resistance reported (WHO). Researchers have demonstrated the possible mechanisms of this bacterium's evasion and have begun to understand them to develop new approaches [2,26,27,33,50,88]. The efforts to develop new antibiotics are insufficient, as is the ongoing research on several targets, including those mentioned in this review. However, it may be necessary to be more selective at the amino acid level, where specific designs can target regions that are unique or less conserved across bacteria. This allows the new drug to achieve selectivity and generate interactions that result in a therapeutic effect. This selectivity could be achieved through current techniques, such as molecular docking, recombinant proteins, and directed mutations, to corroborate interactions, describe protein-ligand interactions, and correlate the antimicrobial effect of new drugs.

So, what could happen with broad-spectrum antibacterial drugs? By searching for more selective drugs, would they be developing drugs exclusively targeted to a specific bacterium? This could be the case if the drug interacts with unique regions of the target protein, or if these regions are conserved in other bacteria, a potentially similar effect would have to be evaluated. In this way, we could develop even more specific treatments that target the amino acid levels of each protein, thereby improving the treatment of bacteria already highly resistant to broad-spectrum antibiotics. For example, there is the triosephosphate isomerase protein, this is a protein used as a target for the development of selective drugs in different organisms, in which there is between 32 and 60 percent identity between the ESKAPE bacteria (which we have mentioned here), notably among *S. aureus* with *M. tuberculosis* and *E. faecium* there are an identity of 44 and 60% respectively [76]. Despite these, the probable ligands are not the same, this shows that despite being a similar protein in different organisms, there are regions that could be exclusive in one or more organisms, this has already been reported and shows the importance of the interaction regions between the TIMs of different organisms [64,65,67], so, these in vitro results and their descriptions differ between them, and demonstrate that selectivity varies for each organism. Therefore, they would likely need to be drugs exclusive to the proteins proposed here as targets against *S. aureus*.

Therefore, the development of new drugs could incorporate selectivity criteria and be specific at the amino acid level (Table 1) to achieve a better antimicrobial effect. As already mentioned, *S. aureus* has several therapeutic targets that can be studied at the molecular, structural, and amino acid sequence levels, enabling the development of potential drugs and addressing the problem of therapeutic inefficacy. For this development at the molecular and

structural levels of targets (proteins), molecular docking is a very important computational tool for determining compounds that interact with proposed potential targets (S. aureus proteins), analyzing biophysical and structural characteristics, and evaluating protein-ligand interactions.

Table 1. Proposed targets, cellular localization, reported PDB IDs, and potential sites for inhibition.

Target	Localization	PDB ID	Potential site	References
3-dehydroquinate dehydratase SaDHQD	Intracellular	6SFH	Glu35, Arg37, Arg70, Lys160, His133, Arg202, and Gln225 are important for its catalytic activity	[45,50]
Shikimate kinase SaSK	Intracellular	AF_AFA5IT66F1	Gly17, Lys18, Arg113	[52]
Penicillin-Binding Protein 2a PBP2a	Extracellular	6Q9N or 6H5O	amino acid positions 403, 406, and 464 Allosteric site: amino acid positions 237, 242, 446	[22,53]
Protein A	Extracellular	1DEE	Gln26, Asn28, Gly29, Phe30, Ile31, Gln32, Ser33, Lys34, Asp35, Asp36, Gln40	[58]
Beta-lactamases	Extracellular	3Q81 or 3Q82	amino acid positions 104, 164, 238, and 240	[62,63]
SaTIM	Intracellular/ extracellular	3UWW	Lys11, his97, Glu169	[70]
Nuclease 1	Extracellular	6XSF	amino acid positions 103, 117, 122, 123, 125, and 169	[76]

Future developments will have to integrate analysis and development at the molecular level, where studying and choosing therapeutic targets (proteins) sometimes requires a whole team of researchers, and in this study, at least seven proteins for *S. aureus* are mentioned, each of with characteristics to be used to get selectivity; where with each one of these a theoretical-experimental study can be performed. Drug selectivity will be of utmost importance, for which carrying out a validation of the interaction at different stages of development, initially theoretical, related to molecular docking, determining potentially selective molecules, subsequently an evaluation of the interaction between the potential drugs with the recombinant protein, and the interaction evaluation; these through assays of enzymatic activity, fluorescence, stoichiometry, or other that can determine a protein-molecule interaction by in vitro assays [64,67,89-91] (Figure 3).

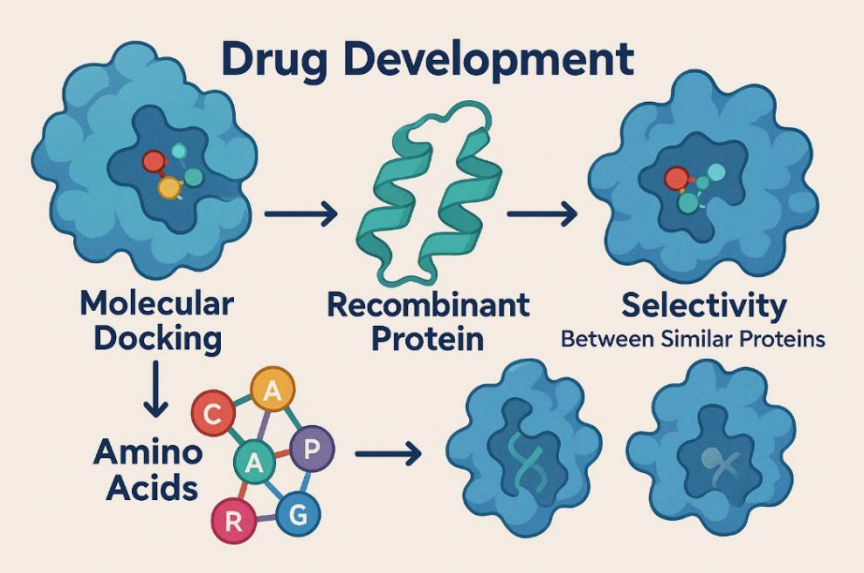

Figure 3. A graphical way for determining a new drug using a theoretical-experimental study is to assess the selectivity of new drugs between similar proteins in different organisms. Image created in copilot.microsoft.com.

Subsequently, through in vitro cultures, the antibacterial effect of the selected molecules/compounds could be evaluated by molecular docking (potential new drugs), these effects due to the interactions between the molecules and the potential targets selected, in this way to generate an antimicrobial effect and determine a minimum inhibitory concentration [39,92]. Therefore, regarding the development of a new drug against S. aureus, we propose a theoretical-experimental approach and validate it across many S. aureus strains. If a potential drug is identified using this methodology, we could propose a mechanism of action by integrating theoretical and experimental results, and we will perform a preclinical phase for this molecule (a new drug against *S. aureus*). This development will complement the assessment of the compound's toxicity: theoretical toxicity, cytotoxicity [89,90,93], and lethal dose 50 (LD50) [94]. Then, complement the development of the new drug with a proposal of the molecular interaction between the new drug and the target protein, using molecular dynamics [90,95], and thus relate the experimental results through theoretical-experimental analysis. This will apply to molecules/compounds that show favorable results in the assays mentioned. A preclinical phase will be conducted, and development will continue into a clinical phase. All is a wide way, more specifically, even per protein for each bacterium, but it will be justified for the new necessities to improve the treatments against drug resistance.

5. Conclusions

The World Health Organization emphasizes the urgency of addressing bacterial resistance, and Staphylococcus aureus is among the bacteria with high resistance to the most widely used antibiotics that cause infections worldwide [1,16]. In this review, we highlight some proteins that provide clues into the mechanisms of drug resistance that bacteria can develop.

The WHO proposes an action plan to raise awareness of the problem of bacterial resistance to current treatments. Therefore, this review proposes therapeutic targets located intracellularly and extracellularly, within the membrane, and even outside this bacterium. Therefore, the development of new drugs against *S. aureus* could be focused on targeting the therapeutic targets of this bacterium (the amino acids listed in Table 1). Furthermore, the impact of these therapeutic targets should be determined, using ligands/drugs specific to *S. aureus* amino acid sequences. While some of these targets are already used as antibacterial treatments, additive or synergistic effects could be determined when combining drugs for different therapeutic targets; for example, the combination of a phosphate-binding protein (PBP) inhibitor with an anti-beta-lactamase drug (amoxicillin + clavulanic acid). Therefore, evaluating or proposing these therapeutic targets is fundamental to understanding the mechanisms of action and impact of new drugs against multidrug-resistant strains of *S. aureus*.

Without a doubt, it is necessary to change the paradigm of broad-spectrum antibiotics. It must be a specific drug targeting a specific bacterium, based on specific targets. This will require greater effort and challenges to respond to the needs represented by resistance to conventional treatments. Finally, the development of new antibiotics is underway; all this will help design and develop specific drugs to reduce the resistance of bacteria.

Author Contributions

Conceptualization, J.L.V.S.; methodology, J.L.V.S.; investigation, J.L.V.S.; resources, J.L.V.S.; data curation, J.L.V.S.; writing—original draft preparation, J.L.V.S.; writing—

review and editing, J.L.V.S. The author has read and agreed to the published version of the manuscript.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

The data presented in this study are openly available at PubMed. Data supporting the findings of this study are available upon reasonable request from the corresponding author.

Funding

This project does not have support.

Acknowledgments

The author is very grateful for the financial support from C.I.P.I. 2024-2025 UABC, PREDEPA-UABC, SNII-SECIHTI, and Dr. Jose Manuel Avendaño Reyes.

Conflicts of Interest

The author declares no conflicts of interest.

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