Review

Metal nanoparticles as inhibitors of enzymes and toxins of multidrug-resistant *Staphylococcus aureus*

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A B S T R A C T

Staphylococcus aureus is an aerobic Gram-positive spherical bacterium known to cause a broad range of infections worldwide. It is a major cause of infective skin and soft infections and severe and life-threatening conditions, such as pneumonia, bloodstream infections, and endocarditis. The emergence of drug-resistant strains of *S aureus*, particularly methicillin-resistant *S aureus* (MRSA), has become a significant concern in the healthcare community. Antibiotic-resistant *S aureus* is commonly acquired in hospitals and long-term care facilities. It often affects patients with weakened immune systems, those undergoing invasive medical procedures, or those who have been hospitalized for extended periods. In the US, *S aureus* is known to cause potentially fatal illnesses, such as toxic shock syndrome (TSS) and acute-onset toxic shock syndrome (TSS), which are characterized by fever and hypotension. It develops resistance to antibiotics through several mechanisms, such as the production of enzymes that inactivate antibiotics, target site modification, efflux pumps, and plasmid-mediated resistance. Therefore, preventing the spread of drug-resistant *S aureus* is needed, and there is an urgent need to explore novel approaches in the development of anti-*staphylococcal* agents. This article reviews the principal infections caused by *S aureus,* major virulence factors, mechanisms of resistance development, and nanotechnology-based solutions for the control of drug-resistant *S aureus*.

1. Introduction

According to a World Health Organization (WHO) assessment, one of the biggest concerns to public health in the 21st century is the development of antibiotic resistance among pathogens included in the WHO priority list. Deaths caused directly by antibiotic-resistant pathogens are estimated to be highest in Sub-Saharan Africa and South Asia, at 24 deaths per 100,000 population and 22 deaths per 100,000 populations, respectively. Methicillin-resistant *Staphylococcus aureus (*MRSA) directly caused more than 100,000 deaths in 2019, while 6 more each caused between 50,000 and 100,000 deaths in the same year [1].

Injudicious use of antibiotics has caused a dramatic increase in bacterial resistance to antibiotics that have threatened the therapeutic value of several antibiotics. It is becoming increasingly difficult to treat many bac-

terial diseases since the existing medicines are becoming less effective or ineffective against specific bacterial species. Moreover, the emergence of antibiotic-resistant pathogens has made our battle against tuberculosis and HIV-AIDS extremely difficult. *Staphylococcus aureus,* a Gram-positive bacterium, is part of the normal human flora and is known to cause several life-threatening infections. Approximately 20% of healthy individuals are carriers of *S aureus*, and they are at a greater risk of infection and serve as an important source of *S aureus*. Multidrug resistance in *S aureus* has been achieved in a variety of ways, such as altered target sites/enzymes, target protection, decreased cell permeability, target overproduction, and enzyme inactivation [2].

Furthermore, its multidrug resistance phenotype makes *S aureus* one of the most difficult pathogenic bacteria to treat in the history of antibiotics. MRSA has become prevalent worldwide, and currently, more than half

of *S aureus* clinical strains are methicillin-resistant. MRSA was developed when methicillin-susceptible strains of *S aureus* acquired the methicillin-resistance gene mecA by horizontal gene transfer through a mobile genetic element staphylococcal cassette chromosome (SCC) [3]. Second, spontaneous mutations were found to play a major role in the development of multidrug resistance in *S aureus*. It appears that staphylococci never stop evolving. It may acquire a highly efficient plasmid carrying the vanA gene in the near future, leading to the development of resistance toward vancomycin. Therefore, the discovery and development of a new paradigm for future chemotherapy against the threat of multidrug-resistant *S aureus* infection is needed [2]. This review highlights staphylococcal infections, the main virulence factors of *S aureus*, multidrug resistance, and mechanisms of resistance development. The article also sheds light on commonly used antibiotics in the control of methicillin-resistant staphylococcal infections and their limitations. The last section of the article highlights the recent state of understanding on the potential of metal nanoparticles as inhibitors of staphylococcal enzymes and virulence factors.

2. Staphylococcal infections

Staphylococcus aureus can cause a wide range of infections ranging from mild skin and soft tissue infections to invasive infections, such as sepsis and pneumonia [4]. *Staphylococcus aureus* is notorious for causing boils, furuncles, styes, impetigo and other superficial skin infections in humans. It may also cause more serious infections, particularly in persons debilitated by chronic illness, traumatic injury, burns, or immunosuppression. These infections include pneumonia, deep abscesses, osteomyelitis, endocarditis, phlebitis, mastitis, and meningitis and are often associated with hospitalized patients rather than healthy individuals in the community. *Staphylococcus aureus* and *Staphylococcus epidermidis* are common causes of infections associated with indwelling devices such as joint prostheses, cardiovascular devices, and artificial heart valves. *Staphylococcus aureus* causes a range of infections in humans and animals.

Staphylococcus aureus can cause a range of benign to immediately life-threatening skin and soft tissue infections, including impetigo and simple cellulitis [5]. It is the most typical pathogen seen in purulent cellulitis, cutaneous abscesses, and surgical site infections (SSIs).

Bacteremia is a bloodstream infection. Bacteremia due to *S aureus* has been reported to be associated with mortality rates of 15% to 60%. MRSA is an important cause of bacterial endocarditis, which can cause mortality in approximately one-third of infected patients (30%–37%), triggering a generalized inflammatory response. One of the most harmful side effects of *S aureus* is that it can

spread throughout the body and impair the operation of internal organs [6].

Osteomyelitis (OM) is an infection of the bone that causes inflammatory destruction or bone necrosis. Bone can become infected via the hematogenous route of infection by bacteremic seeding of bone from a distant source of infection, contiguous spread from surrounding tissue and joints, or direct inoculation from trauma or surgery [7]. Hematogenous osteomyelitis occurs more frequently in children than in adults, and long bones are usually affected. The overall incidence of osteomyelitis in the United States is mostly unknown, but reports show it to be as high as 1 in 675 in the United States hospital admissions each year or approximately 50,000 cases annually [8]. Endocarditis is an infection of the inner lining of the heart chambers and valves. In wealthy nations, the annual incidence of endocarditis is between 2.6 and 7 cases per 100,000 people. Patients with endocarditis are 58 years old on average [9]. MRSA is an important cause of bacterial endocarditis, which can cause mortality in approximately one-third of infected patients (30%–37%). Food poisoning connected to *staph* is caused by eating foods contaminated by toxins that are produced by bacteria. Pneumonia is a frequent infection that results in swelling and fluid build-up in the lungs' air sacs, making it extremely difficult to breathe properly. Three percent of patients with community-acquired pneumonia (CAP) who were hospitalized for it developed it because of MRSA, according to a significant, global multicenter study. Depending on the patient's residence and geography, this had a different incidence. It was shown that 51% of the *S aureus* isolates were secondary to MRSA, and 49% were secondary to MSSA [5]. According to a different study, 1.7% of inpatients who were hospitalized in the United States for CAP had secondary *S aureus* infections, of which 0.7% had MRSA and 1% had MSSA [10,11]. Toxic shock syndrome (TSS) is a potentially fatal illness caused by toxins produced by specific bacteria, such as *S aureus*. Acute-onset toxic shock syndrome (TSS) is characterized by fever, hypotension, a rash resembling a sunburn, and end-organ destruction. In the United States, the incidence of TSS is thought to range from 0.8 to 3.4 per 100,000 people [12].

3. Main virulence factors of *S aureus*

It produces several virulence factors, such as exotoxins, cytotoxins, superantigens, and cytotoxic enzymes, which modulate the host's immune responses that help to spread the pathogen. In addition to the above virulence factors, *S aureus* also produces many virulence factors that have enzymatic properties. These enzymes are of 2 categories: cofactors that activate host zymogens and exoenzymes responsible for the degradation of tissue components. Cytotoxic exoenzymes damage host cells

and modulate the host immune system and therefore play an important role in *S aureus* infections. These exoenzymes breakdown host molecules for the acquisition of nutrients, bacterial survival, and spreading. They work through various substrates and methods. Staphylococcal enzymes that degrade host tissue components include nucleases, coagulase, proteases (metalloproteases, serine, and cysteine proteases), hyaluronidase, and lipases. Lipases are known to support the persistence of *S aureus* in fatty secretions in mammalian skin and therefore directly contribute to their pathogenic potential. Additionally, lipase prevents host granulocytes from phagocytosing infectious *S aureus* cells that produce lipase, demonstrating that lipase directly contributes to pathogenesis [13,14].

Another exoenzyme, hyaluronidase (also called hyaluronate lyase HysA), cleaves the hyaluronic acid polymer at the β-1, 4 glycosidic bonds, yielding disaccharide units of *N*-acetylglucosamine and d-glucuronic acid. Hyaluronic acid (HA) is synthesized and secreted from the plasma membrane of mammalian cells, and it serves as a cementing structure of connective tissues and is also involved in water homeostasis, assisting with cell proliferation, and acting as an immune regulator [15]. Many of these tissues with high HA concentrations are frequently infected with *S aureus* due to their ability to produce hyaluronidase [16].

4. Multidrug resistance in *S aureus*

Antimicrobial resistance is recognized as one of the extreme risks to human health and accounts for millions of deaths every year worldwide. In recent decades, there has been continuous effort taken by academics and pharmaceutical industries to discover new antimicrobial agents for treating infections caused by antibiotic-resistant pathogens. However, the overprescription and improper use of antibiotics lead to the emergence of multidrug and even pandrug-resistant bacteria. The production of extended-spectrum β-lactamases and carbapenemases is the major cause of resistance to β-lactam antibiotics. Moreover, other factors, such as target modification, overexpression of efflux pumps, and downregulation of outer membrane porin (OMP) channels, are also responsible for resistance to antibiotics [17,18].

In recent times, the increasing emergence of carbapenem/β-lactam resistance has been a major challenge for clinicians to treat infections caused by these resistant pathogens. Carbapenems are used as last-resort antibiotics in salvage therapy for various critical bacterial infections. In Enterobacterales, strains harboring carbapenem hydrolyzing enzymes (carbapenemase), along with down regulation of porin channels and expression of efflux pumps in Pseudomonas, are the principal reasons for resistance to carbapenems.

4.1. Methicillin-resistant S aureus

Another barrier to treating *S aureus* infections is raising resistance. Due to their multidrug resistance, they can avoid the pharmacologic effects of antibiotics. However, recent reports have shown that *S aureus* has already developed resistance to daptomycin [19] and glycopeptide antibiotics (teicoplanin and vancomycin), which have been used to treat MRSA, particularly in severe infections. Previously, we knew more about the resistant strains of *S aureus* when they were resistant to β-lactams [20].

Staphylococcus aureus has several fundamental resistance mechanisms. One important resistance mechanism is the emergence of resistance genes. The mecA gene and its related genes, mec B and mec C, provide methicillin or cephalosporin resistance [21]. PBP2a or PBP2′, a type of penicillin-binding protein, is produced by the mecA gene. These proteins are associated with the bacterial cell envelope and are target sites for β-lactam antibiotics. The β-lactam ring, which grants penicillin, cephalosporin, and methicillin their action, will be broken down by these proteins. Additionally, the *S aureus* chromosomal cassette mecA genetic element can facilitate the spread of mecA [22]. The efflux pumps, which can aggressively efflux antimicrobial drugs out of bacteria, is another resistance tactic used by *S aureus*. Resistance and *S aureus* biofilm formation are related [23]. It was reported that decreased drug permeability increased *S aureus* resistance in the biofilm state [24]. At this time, it is critical to take effective strategic actions incorporating alternate medicines that can lessen *S aureus* resistance. Several mechanisms are known for antibiotic resistance in *S aureus*.

4.2. Mechanism of methicillin resistance of S aureus

By obtaining the mecA gene, bacteria have become resistant to semisynthetic β-lactamase-insensitive β-lactams such as methicillin, oxacillin, and nafcillin. Both methicillin and all β lactams are ineffective against MRSA [25,26]. MecI and MecRI, which are independent regulators, control expression of the mecA gene. MecI, which is linked to the promoter-operator region of mecA, and the mecI-mecRI operon inhibit mecA in the absence of β-lactam antibiotics, preventing mecA transcription. When -lactam antibiotics are used or added to growth media, -lactam binds to MecRI, a -lactam-sensing signal transducer. Then, MecRI's metalloprotease domain, which is in the cytoplasm, is separated and cleaves MecI, which is already attached to the operator. MecA is thus translated to PBP2a, whose affinity for -lactams is modest [27]. Due to the formation of peptidoglycan in the presence of β-lactam concentrations that can inactivate the transpeptidase activity of PBPs, MRSA can spread since PBP2a has a low affinity for β-lactams. The transpeptidase

domain and nonpenicillin-binding protein are both found in PBP2a, a member of the PBP family.

The following biochemical mechanisms can be used to mediate AMR: (i) enzymatic modification of the antimicrobial binding site to reduce the affinity for the antimicrobial (for example, resistance to methicillin by PBP 2a); (ii) enzymatic inactivation/modification of the antimicrobial (for example, resistance to *β*-lactam antibiotics by production of *β*-lactamases); (iii) bypassing the metabolic pathway to avoid antimicrobial (e.g. resistance to fluoroquinolones by the NorA efflux pump); (iv) sequestering the antibiotic to protect the target (e.g. staphylokinase-mediated resistance to host defence antimicrobial peptides such -defensins); and (v) enhanced production of efflux pumps to expel antibiotic molecules (e.g. resistance to fluoroquinolones by the NorA efflux pump) [28–32].

4.2.1. Plasmids

Any plasmid that carries one or more genes for antibiotic resistance is referred to as a resistance plasmid. It can also be a metabolic plasmid if it encodes a metabolic function or a virulence plasmid if it has one or more virulence genes. The presence of one type of gene does not prohibit the presence of additional types that do not aid in the upkeep and spread of the plasmid [33]. According to their size, *S aureus* plasmids can be divided into 3 categories. The smallest plasmids, type I plasmids, have only one antibiotic-resistant determinant. The -lactamase gene is present in type II plasmids of intermediate sizes. The largest type is type III plasmids, which can resist a variety of drugs, including gentamycin, trimethoprim, and ethidium bromide [34]. Conjugative type III plasmids are horizontally transfected into other cells by their own *tra* genes.

4.2.2. Efflux-mediated antimicrobial resistance

The production of active efflux pumps is one of the important mechanisms of defense against antimicrobials [35]. In the cytoplasmic membrane of bacteria, archaea, and eukaryotes, a wide variety of transport proteins known as efflux pumps are dispersed [36]. Chemotherapy for bacterial infections and human tumors has become increasingly difficult to administer due to efflux-mediated drug resistance [37,38]. Numerous studies have emphasized the role that multidrug efflux pumps play in the development of bacterial persistence [39]. Because efflux pumps help bacteria endure for a while the likelihood of spontaneous mutations that result in the development of high-level resistance to antimicrobials is increased. As a result, the development of additional resistance mechanisms may be aided by efflux pump activity [40,41].

To address the wide variety of antimicrobials used in clinical settings to treat infections or as antiseptics and disinfectants to reduce bacterial load, bacterial pathogens, including *S aureus,* have evolved drug efflux pumps as an efficient resistance mechanism [42].

Tetracycline-specific pumps are an example of a bacterial efflux pump that is substrate-specific because it recognizes and expels only that substance or its closely related derivatives, as opposed to MDR efflux pumps that can recognize and export a wide range of structurally unrelated substrates [43]. In other words, the distinguishing trait of MDR efflux proteins is substrate polyspecificity (or promiscuity toward substrates) [44]. The extrusion of host-derived antimicrobials, endogenous toxic metabolites, and virulence factors by bacterial efflux pumps, in addition to their function in antimicrobial resistance, raises the possibility that synthetic antimicrobials may be "accidental substrates" of these membrane transport proteins [44]. Growing evidence from numerous studies points to the role of efflux pumps in the development of bacterial biofilms [45], particularly in a number of significant pathogenic bacterial species, such *S aureus, Acinetobacter baumannii, Escherichia coli, and Pseudomonas aeruginosa*. Clinical problems can result from biofilm infections caused by these microorganisms on medical devices [46].

Due to stringent regulation by several regulators, efflux pump gene expression levels are typically modest in typical environmental settings [47]. Notably, hospital-associated bacteria, including *S aureus that* use efflux pumps for antimicrobial resistance frequently express such pumps constitutively at higher levels as a result of regulatory alterations in the efflux pump promoter region or in its regulator gene [48]. This offers support for the idea that efflux pumps originally served physiological purposes unrelated to antimicrobial resistance but were accidentally used for those purposes by bacterial pathogens under strong antimicrobial selective pressures in hospital contexts [48]. To identify variations in antibiotic efflux activity, antimicrobial susceptibility measures, such as the minimum inhibitory concentration (MIC), are frequently used. When compared to bacteria with lower efflux pump expression, those with higher efflux pump expression are less vulnerable to some antimicrobials. However, various techniques have been developed to specifically identify resistance mediated by an efflux pump in bacterial cells, reflecting the method's poor sensitivity.

4.2.3. Modification of the target site

One of the most typical mechanisms of antibiotic resistance in bacterial pathogens, impacting practically all families of antimicrobial drugs, is the introduction of changes to the target site. Point mutations in the genes encoding the target site, enzymatic changes to the binding site (such as the addition of methyl groups) and/or replacement or bypass of the original target are some examples of these target changes. As previously mentioned, the end result is always the same, a reduction in the antibiotic's affinity for the target site. Here are some traditional instances of each of these tactics.

4.2.4. Mutations of the target site

The emergence of rifampin (RIF) resistance is one of the most well-known instances of mutational resistance. RIF is a rifamycin that suppresses DNA-dependent RNA polymerase, a complex enzyme with a two-subunit structure, to prevent bacterial transcription. The RNA polymerase component, which is encoded by rpoB, contains a highly conserved structure known as the RIF binding pocket. After binding, the antibiotic molecule prevents transcription by directly obstructing the nascent RNA route [49]. Numerous other genetic alterations have been described, including single-step point mutations that result in amino acid substitutions in the rpoB gene, which have been demonstrated to cause high levels of RIF resistance. Notably, although these changes reduce the drug's affinity for its target, they typically preserve the polymerase's catalytic function, allowing transcription to continue [50].

4.2.5. Enzymatic alteration of the target site

One of the well-studied cases of resistance by enzymatic modification of the target site is the methylation of the ribosome, which is carried out by an enzyme-encoding generated by the erm genes (erythromycin ribosomal methylation) and leads to macrolide resistance. These enzymes are capable of mono- or dimethylating the adenine residue at position A2058 of domain V of the 23S rRNA of the 50S ribosomal subunit. The antimicrobial molecule's ability to bind to its target is compromised as a result of this metabolic alteration. Importantly, expression of the erm genes imparts cross-resistance to all individuals in the MLSB group because macrolides, lincosamides, and streptogramin B antibiotics have overlapping binding sites in 23S rRNA [51,52]. It has been reported that there are over 30 distinct erm genes, many of which are found in MGEs. This may explain why they are so widely distributed among various genera, including aerobic and anaerobic Gram-positive and Gram-negative bacteria.

4.2.6. Complete replacement or bypass of the target site

By employing this technique, bacteria can evolve new targets that perform comparable metabolic tasks to the original target but are not inhibited by the antimicrobial molecule. The two most pertinent clinical examples are vancomycin resistance in enterococci brought on by changes to the peptidoglycan structure mediated by van gene clusters and methicillin resistance in *S aureus* caused by the acquisition of an exogenous PBP (PBP2a). Finally, overproducing the antibiotic target is another technique to "bypass" the metabolic process that antibiotics inhibit. Trimethoprim-sulfamethoxazole (TMP-SMX) resistance is an appropriate illustration of this mechanism. It has been reported that there are over 30 distinct erm genes, many of which are found in mobile genetic elements (MGEs).

This may explain why they are so widely distributed among various genera, including aerobic and anaerobic Gram-positive and Gram-negative bacteria.

PBPs are crucial enzymes involved in the transpeptidation and transglycosylation of peptidoglycan units that emerge from the cytoplasm, and their inhibition is what gives lactams their antibacterial activity. A foreign gene called mecA, likely from *Staphylococcus sciuri*, is acquired by *S aureus* and causes resistance to methicillin, a semisynthetic penicillin stable against staphylococcal penicillinase. MecA is frequently found in a large DNA fragment known as the staphylococcal chromosomal cassette mec (SCCmec). PBP2a, a PBP that is encoded by the mecA gene, has a low affinity for all lactams, including penicillins, cephalosporins (with the exception of compounds from the most recent generation), and carbapenems. Most lactams are rendered worthless against MRSA by mecA acquisition, necessitating the adoption of other treatments for significant infections. Although PBP2a has a transpeptidase domain, this class B PBP is not a transglycosylase; hence, PBP2a requires the activity of other native PBPs to perform the latter function and entirely crosslink peptidoglycan. The penicillin-insensitive transglycosylase domain of PBP2 (a class A PBP) is particularly important for achieving transglycosylation of peptidoglycan in the presence of lactams, especially in mecA-carrying MRSA strains.

5. Commonly used antibiotics in the control of methicillin-resistant staphylococcal infections and their drawbacks

Staphylococcus aureus is a well-known and significant bacterial pathogen. It has a history of hospital epidemics, community-acquired infections, and a wide range of pyogenic lesions affecting several organs. Hospital-acquired β-lactam antibiotic resistance is linked to *S aureus* infections, which frequently have catastrophic outcomes [53]. This type of strain is referred to as MRSA (methicillin-resistant *S aureus*) [54]. Due to its connection to various nosocomial outbreaks and cross infections in the past, it has received particular attention since 1970 [55].

Over time, this organism's epidemiology has been altered. Previously exclusive to hospitals, life-threatening infections are increasingly pervasive in society [56]. Multiple drug resistance (MDR) in hospital-acquired MRSA (HA-MRSA) strains has been linked to high antibiotic usage in hospitals and antibiotic selection pressure. Similar to how more antibiotics are being used in animal feed, a novel MRSA strain called LA-MRSA with resistance to several non-β-lactam drugs has emerged. Strict infection control procedures and prudent antibiotic usage can significantly lower the spread of staphylococcal infection [57]. Currently used in clinical settings are common anti-MRSA drugs such as daptomycin, teicoplanin, vancomycin, and

Table 1

Mechanisms of action and limitations of currently available anti-MRSA antibiotics.

Antibiotic	Mechanism of action	Limitations
Vancomycin	Inhibition of cell wall synthesis	Nephrotoxicity risk at higher dosages and when combined with other nephrotoxic substances
Daptomycin	Rapid depolarization may disrupt potential of cell membrane.	Inactivation by pulmonary surfactant. Ineffective for treatment of MRSA pneumonia.
Linezolid	Inhibition of protein synthesis through binding of 50S ribosomal subunit. Bacteriostatic activity	Prolonged use may lead to multiple potentially serious side effects like marrow suppression, lactic acidosis, peripheral and optic neuropathy, serotonin syndrome etc.
Trimethoprim/sulfamethoxazole	Hampers multiple stages in bacterial folate and thymidine synthesis and it has bactericidal activity	May be ineffective in infections involving undrained pus due to thymidine scavenging. Limited data supporting use in bacteremia and endocarditis
Clindamycin	50S ribosomal subunit-mediated inhibition of protein synthesis. Shows bacteriostatic activity.	Ineffective for treatment of invasive infections in adults.
Tetracyclines	30S ribosomal subunit-mediated inhibition of protein synthesis. Shows bacteriostatic activity	Ineffective in treating invasive infections
Tigecycline	30S ribosomal subunit-mediated inhibition of protein synthesis. Shows bacteriostatic activity	Low serum levels. Ineffective against treatment of hospital-acquired MRSA pneumonia
Quinupristin/dalfopristin	Combination of two streptogramins that prevent protein production in a beneficial way. Bactericidal efficacy in absence of MLSB resistance	Symptoms of arthralgias, myalgias, venous intolerance) that occur frequently. Little evidence exists to support usage in invasive disease.
Rifampicin	Bactericidal action by inhibiting bacterial transcription	Multiple drug–drug interactions; fast development of resistance; cannot be administered as monotherapy. Possibility of liver damage

teicomycin. Worldwide, it has been noted that several of these medications are developing drug resistance. There have been infrequent reports of vancomycin-resistant and intermediate MRSA strains (VRSA and VISA). Further evidence of decreased susceptibility includes an increase in the minimum inhibitory concentration (MIC) to glycopeptides over time. Future therapy choices are projected to become even more limited due to the growth of resistance to existing medications and the lack of novel anti-MRSA medicines in development (Table 1).

5.1. Methicillin

Methicillin (originally called Celbenin) was the first β lactamase-resistant semisynthetic penicillin developed in 1960 to treat infections with penicillin-resistant *S aureus*. However, methicillin-resistant strains of *S aureus* emerged within 1 year of its clinical use [58]. The early reports of MRSA among European countries were from the UK and Denmark [59]. MRSA was also reported in India as early as 1964 [60].

5.2. Vancomycin

Vancomycin is currently the antibiotic of choice for treating MRSA infections. It is a branched glycosylated tricyclic peptide belonging to the glycopeptide antibiotic class. It binds to the growing ends of peptide chains and prevents their interaction with transpeptidase enzymes. Although reports of MRSA strains with diminished susceptibility to this antibiotic are not infrequent [61], only a few reports of vancomycin-resistant *S aureus* (VRSA) showing MIC $\geq$ 32 μg/mL have been documented.

5.3. Linezolid

Linezolid is a new drug class, *oxazolidinones*. It binds to domain V of 23S RNA and prevents correct protein synthesis. Linezolid resistance occurs when at least 2 copies of 23S RNA genes are mutated, especially with increased clinical use, and the control measure is aggressive antibiotic stewardship (reducing its clinical use) [62]. The first case of linezolid resistance in MRSA was reported in 2001 [63], and subsequently, 8 cases were reported in the United States, 2 in Germany and 1 each in Brazil, Colombia and the United Kingdom [64].

5.4. Daptomycin

Daptomycin is a calcium-dependent cyclic lipopeptide anti-MRSA drug that depolarizes the bacterial cell membrane. However, due to its lipophilic nature, it is incorporated into alveolar surfactant and deposited in alveoli instead of the bacterial cell membrane, resulting in eosinophilic pneumonia and limiting its therapeutic use [65]. There are no defined resistance breakpoints for *S aureus,* and isolates are either categorized as susceptible or nonsusceptible [66,67].

5.5. Clindamycin

Clindamycin is a lincosamide antibiotic classically used for infections by aerobic Gram-positive cocci and anaerobes. Clindamycin resistance in *S aureus* may be classified into one of three phenotypes, designated MLSBi, MLSBc and MS [68]. Inducible resistance to *streptogramin* B, macrolide and lincosamide in *S aureus* is attributed to

the *erm* gene encoding an enzyme that methylates the adenine residue of 23S rRNA. This inducible clindamycin resistance has been found more frequently among MRSA strains and often leads to treatment failure, as it is not detected in routine antibiotic susceptibility tests [69].

6. Nanoparticles as inhibitors of staphylococcal enzymes and their significance in the control of staphylococcal infections/metallic nanoparticles as inhibitors of staphylococcal toxins and enzymes

Enzyme inhibitors are widely distributed in living systems. The clinical and technological applications of enzyme inhibitors as antibacterial drugs include the treatment of diabetes, Alzheimer's disease and some cancers. Unfortunately, due to low solubility, poor absorption, and rapid metabolism, the use of several natural and synthetic products as inhibitors of key enzymes involved in bacterial pathogenesis at the clinical level is not satisfactory. In this regard, modern nanotechnology can certainly provide important leads.

Nanotechnology has emerged as one of the most crucial technologies in all fields of research. It involves the creation and manipulation of nanoparticles, which necessitates major changes in metal characteristics. Nanotechnology may offer a promising alternative to emerging multidrug resistance in *S aureus* [70]. A variety of materials, including liposomal and polymer-based nanodrug carriers, have been investigated, and metallic vectors, such as gold NPs, are appealing as core materials due to their essentially inert and harmless nature [71]. NPs with a size less than 20 nm can penetrate the bacterial cell wall and, in turn, hamper biochemical pathways through the destruction of cell organelles, inhibit enzyme activity by generating reactive oxygen species (ROS) and cause mechanical damage to cell membranes.

Metal NPs exert their bactericidal effect by cell wall damage, cytoskeleton damage, ROS generation, disruption of various signaling pathways and inhibition of membrane synthesis enzymes; therefore, NPs can be used as effective antibacterial agents against widespread antibiotic resistance. Earlier studies have reported the mechanism of the antibacterial activity of NPs on pathogenic bacteria. Urease inhibition was found to be greater when ciprofloxacin capped AgNPs/AuNPs were used [71]. It was demonstrated the β-galactosidase inhibitory potential of small zinc oxide nanoparticles in a biomimetic fashion and showed strong antibacterial activity against MRSA [72,73]. According to these findings, the cellular enzyme is either directly or indirectly inhibited by the NPs, leading to an antibacterial effect. However, no systematic study using nanotechnology-based principles has yet been undertaken to target the virulent exoenzymes of *S aureus*.

Lipase and hyaluronidase are the major virulence factors during staphylococcal infections. The inhibition of these virulent enzymes can be a simple and effective approach to controlling drug-resistant staphylococci. More-

Table 2
Antibiotic-NP combinations used to treat *S aureus*.

Sr. No.	Antibiotic/metal	Nanomaterial carrier	Application	Reference
1.	Gentamicin	PLGA	Elevated intracellular drug	[74,75]
2.	Gentamicin	Liposomes	Increased level of intracellular gentamicin	[76]
3.	Ceftazidime	Liposomes	Suppressed biofilm formation	[77]
4.	Tetracyclin	Chitosan NPs	Suppressed *S aureus* infections.	[78]
5.	Ciprofloxacin	PLGA	Suppressed biofilm formation	[79]
6.	Bacillus natto	Chitosan nano	Suppressed biofilm formation	[80]
7.	Gold	Nanoparticles	Improved removal of MRSA biofilm	[81]
8.	Penicillin G	Self-assembled	improved cell penetration effects	[82]
9.	Enrofloxacin	SLNs	increased capacity for cell accumulation	[83]
10.	Silver	Nanoparticles	Increased impact of MRSA inside cells	[84]
11.	Tilmicosin	SLNs	better mastitis treatment effectiveness	[85]
12.	Gold	Gold nanoclusters	Efficient at preventing MRSA infection	[86]
13.	Daptomycin	Liposomes	Increased anti-MRSA activity	[87]
14.	Azithromycin	DP7-C liposomes	Improved MRSA protection	[88]
15.	Ampicillin	AuNps	A significant antibacterial effect Against MRSA and compatibility with human dermal fibroblasts	[89]
16.	Amoxicillin	AgNps	Antibiotic effectiveness against MRSA	[90]

over, NPs under physiological conditions have better adsorption and less cytotoxicity. Upon proving their safety, biosynthesized NPs can be of great medical importance in our fight against staphylococcal infections (Table 2).

7. Use of nanoparticles in combination with antibiotics

NPs can be customized and packed with various antimicrobial agents to combat antibiotic resistance. Antimicrobial resistance is unlikely to arise if NPs are used with antibiotics since numerous simultaneous mutations in the same microorganism are needed. NPs operate on bacteria through multiple targets and/or a distinct mechanism; hence, the probability of resistance development towards NPs would be less as compare to antibiotics [91,92]. A promising strategy to fight bacterial resistance is the functionalization of NPs with antibiotics. Additionally, NPs can target or transport antimicrobial drugs to diseased locations while lowering the dosage and toxicity of medicines (Table 2) [93].

Ag NPs, for instance, have been shown to have synergistic antibacterial effectiveness against *S aureus*, *β*-lactamase- or carbapenemase-producing *E coli*, *P aeruginosa*, and *A baumannii* strains at extremely low concentrations [94–96], while Ag, Au, and ZnO NPs and antibiotics have been shown to have synergistic antibacterial effects against *S aureus, E faecium, E coli, A baumannii, and P aeruginosa* through penetration of the bacterial cell membrane and disruption of crucial molecular pathways; thus, developing original antimicrobial mechanisms is necessary [93]. Antibiotics infused with NPs were equally effective in killing Gram-positive and Gram-negative bacteria, in contrast to how difficult it is to destroy MDROs with antibiotics alone [93]. Functionalized Ag, Au, or ZnO NPs may be used in combination with antibiotics to reverse antimicrobial resistance and boost the antibacterial properties of multiple drugs, such as polymyxin B, ciprofloxacin, ceftazidime, ampicillin, clindamycin, vancomycin, or erythromycin, against MDROs, such as antibiotic-resistant *A baumannii and P aeruginosa* [93].

In terms of antibiotic resistance, the particular physical structure of NPs offers clear advantages over traditional antibiotics [97]. The current state of NPs indicates a significant future potential for topically treating skin infections [97]. The application of NPs to the contact surfaces of medical equipment, fibers, and textiles has been attempted (Table 3).

Antimicrobial nanoparticles show targeted drug delivery via specific accumulation, have fewer side effects than chemical antimicrobials, are less prone to bacterial resistance, and can cross tissue barriers (e.g. the blood–brain barrier). Antimicrobial nanoparticles also have an extended therapeutic lifetime due to slow elimination, con-

trolled drug release, a broad therapeutic index, improved solubility, and low immunosuppression. These characteristics make this drug delivery system highly desirable for the treatment of diseases that require targeted delivery to specific tissues or organs. Additionally, it has the potential to improve patient compliance and reduce the frequency of dosing needed. Despite the potential benefits of using nanomaterials for drug delivery, concerns have been raised regarding their accumulation in tissues and organs. This accumulation can lead to unintended side effects and toxicity. However, when administered locally at proper doses, nanomaterials have shown high therapeutic efficacy without causing harm to vital organs such as the lungs, kidneys, liver, brain, or germ cells. To fully understand the behavior of these nanoparticles in the body, reliable characterization techniques are needed that are not affected by their unique properties.

8. Potential of metal nanoparticles as inhibitors of staphylococcal enzymes and virulence factors

Metal nanoparticles (MNPs) have gained considerable attention as potential inhibitors of enzymes and virulence factors in various pathogenic microorganisms, including bacteria, viruses, and fungi. Their unique properties, such as size, shape, surface charge, and resistance to degradation in environmental conditions, make them promising candidates for biomedical applications, including antimicrobial therapies. Noble MNPs such as AuNPs, PtNPs and AgNPs have been used in a variety of biomedical applications, such as the treatment of cancer, the diagnosis of diseases, the improvement of radiation efficacy, the eradication of pathogens and fungi, thermal ablation, medication delivery, and gene transport. Metal nanoparticles can be functionalized with a wide range of functional groups, including antibodies, peptides, DNA, and RNA, as well as biocompatible polymers, such as polyethylene glycol, to target a wide variety of cell types [157]. MNPs interfere with the activity of enzymes essential for the survival and replication of pathogens. MNPs can physically block the active site of the enzyme, preventing its interaction with the substrate and inhibiting its catalytic activity [158,159]. Nanoparticles are expected to act as broad-spectrum enzyme inhibitors with potential applications in the control of infectious diseases [160]. MNPs can induce conformational changes in the enzyme's structure, rendering it inactive. Some metal nanoparticles, such as silver and copper nanoparticles, can produce ROS upon contact with the pathogen. ROS can damage enzymes and inhibit their activity. MNPs, which are inorganic nanoparticles, do not contain carbon. Inorganic nanoparticles have the advantages of being hydrophilic, nontoxic, and biocompatible with living systems. The stability of inorganic nanoparticles is superior to that of organic nanoparticles [161].

Table 3

Nanoparticles as inhibitors of staphylococcal enzymes and their applications in the control of *S aureus* infections.

Sr. no	Nanoparticles	Method of synthesis	Target enzyme(s)	Application(s)	Reference
1.	2D-MoS$_2$(−)	Chemical synthesis	ChT	To fight against multidrug-resistant bacterial infections.	[98]
2.	2D-MoS$_2$(+)	Chemical synthesis	β-galactosidase.	To fight against multidrug-resistant bacterial infections.	[98]
3.	3-D copper-β-cyclodextrin-graphene oxide (Cu-β-CD-GO) porous nanocomposite	Chemical synthesis	Antibacterial activity against MRSA	Vancomycin and copper nanoparticles' synergistic effect on pathogenic bacteria (MRSA) was investigated.	[99]
4.	AgNPs	Green synthesis	Urease	Biomedical field	[100]
5.	AgNPs	Green synthesis	Xanthine Oxidase	Biomedical field	[100]
6.	AgNPs	Green synthesis	Urease	Antimicrobial agent	[101]
7.	AgNPs	Green synthesis	Urease	Homeopathic and pharmaceutical fields. Also opens a new Nano approach of antiulcer therapies	[102]
8.	AgNPs	Green synthesis	Xanthine oxidase and Urease enzymes	Against MRSA	[103]
9.	AgNPs	Chemical synthesis	Enzymes responsible for biofilm formation	Inhibition of Biofilm formation	[104]
10.	AgNPs	Physicochemical	Replicase	as a novel tool to study chromosomal DNA replication	[105]
11.	AgNPs	Chemical synthesis		Antibacterial activity against *S aureus*.	[106]
12.	Ag vanadate nanowires (β-AgVO$_3$) with AgNPs.	Chemical synthesis		Antibacterial activity against *S aureus*.	[107]
13.	Ag/MMT/Cts bionanocomposites (BNCs)	Green synthesis		Useful for a variety of biological applications, including surgical instruments and medication administration systems.	[108]
14.	AgNPs	Green synthesis		Antibacterial activity against MRSA in HIV infections.	[109]
15.	AgNPs	Biogenic synthesis		Inhibit the Biofilm Formation and Virulence Activities of MRSA Strain	[110]
16.	AgNPs	Green synthesis		Antibacterial activity against *S aureus*.	[111]
17.	AgNPs	Biogenic synthesis		Antibacterial activity against *S aureus*.	[112]
18.	AgNPs	Chemical synthesis		Antibacterial activity against *S aureus* (MRSA) and *E coli.*	[113]
19.	AgNPs (with chitosan)	Chemical synthesis		MRSA, a Gram-positive bacteria, and three Gram-negative bacteria (*P aeruginosa, P mirabilis*, and *A baumannii*) prevented from growing in vitro),	[114]
20.	AgNPs-Amp	Chemical synthesis		Antibacterial activity against MRSA	[115]
21.	AuNPs	Green synthesis	Urease	Homeopathic and pharmaceutical fields. Also opens a new Nano approach of antiulcer therapies	[102]
22.	AuNPs	Chemical synthesis	α-chymotrypsin		[116]
23.	AUNC-L-Amp	Chemical synthesis		Antibacterial activity against MRSA	[117]
24.	AuNPs (&laser)	Chemical synthesis		Antibacterial activity against MRSA	[118]
25.	Carbon nanotubes	Chemical synthesis	VIM-2 Metallo-β-lactamases (MBLs)	Useful for rational design inhibitors for MBLs	[119]
26.	CeO$_2$ NPs	Chemical synthesis	Enzymes responsible for biofilm formation	Inhibition of Biofilm formation	[104]
27.	CeO$_2$ NPs	Green synthesis	α-amylase and Urease	Not reported	[103]
28.	chitosan-coated silver nanoparticles	Chemical synthesis	Antibacterial activity against MRSA	Effective for the treatment of MRSA-infected wounds	[120]
29.	Cr$_2$O$_3$ NPs	Green synthesis	AChE and BChE	Not reported	[121]
30.	CuO NPs (R.d-CuO NPs)	Green synthesis	Urease	Not reported	[122,123]
31.	CuO NPs (R. f-Cu NPs)	Green synthesis	α-amylase	Not reported	[122]
32.	CuO NPs (R.k-Cu NPs)	Green synthesis	lipase	Not reported	[122]
33.	Cur-Au@ZnO	laser ablation	α-hemolysin toxin	Biomedical and pharmaceutical applications.	[124]

Table 3 (*continued*)

Sr. no	Nanoparticles	Method of synthesis	Target enzyme(s)	Application(s)	Reference
34.	DNA-Au NPs	Chemical synthesis	Nucleases	Recognizing polyvalent DNA-Au NPs as gene regulation tools	[125]
35.	Doxycycline conjugated Ag-Au NPs	Chemical synthesis		Antimicrobial activity. Promising for significant application in burn healing therapy.	[126]
36.	Gold nanoclusters	Chemical synthesis		Antibacterial activity against MRSA	[127]
37.	Graphene oxide	Chemical synthesis	α-chymotrypsin		[128]
38.	Graphene oxide	Chemical synthesis	VIM-2 Metallo-β-lactamases (MBLs)	Useful for rational design inhibitors for MBLs and more specific inhibition might be achieved by further surface modifications on these nanocarbons.	[119]
39.	MoS_2	Chemical synthesis	β-galactosidase	Future development of 2D material-based enzyme inhibitors and for their other biological applications.	[129]
40.	Nanocrystalline silver dressing	Chemical synthesis		Antibacterial activity against MRSA	[130]
41.	Ni O NPs	Green synthesis	reactive oxygen species found responsible for bacterial cell death	Enhanced Bactericidal activity against multiple drug-resistant *S aureus*	[131]
42.	Rose Bengal (RB)-decorated silica (SiO_2–NH_2–RB) nanoparticles	Chemical synthesis		Suppress the growth of Gram-positive bacteria, including Methicillin-resistant *S aureus* (MRSA).	[132]
43.	SiO_2 NPs	Sol-gel method	Urease	Antimicrobials	[133]
44.	SnO_2 NPs	Chemical synthesis	Enzymes responsible for biofilm formation	Inhibition of Biofilm formation	[104]
45.	$sPLA_2$i-loaded micellar NPs ($sPLA_2$i-NPs)	Chemical synthesis	Phospholipase A_2	Promising therapeutic agents for OA treatment	[134]
46.	TiO_2	Chemical synthesis		Antibacterial activity against MRSA	[135]
47.	TiO_2 NPs	Hydrothermal	Urease	Antimicrobials	[102]
48.	TiO_2 NPs	Chemical synthesis	Enzymes responsible for biofilm formation	Inhibition of Biofilm formation	[136]
49.	TiO_2 NPs	Chemical synthesis		Antibacterial activity against MRSA	[137]
50.	ZnO NPs	Green synthesis	urease	Not reported	[138]
51.	ZnO NPs	Biogenic synthesis	A toxin	For use as nanomedicine to treat albino rats with *S aureus* infections.	[139,140]
52.	ZnO NP	Chemical synthesis	B galactosidase	Shape-specific antibacterial activity against methicillin-resistant *S aureus* (MRSA).	[141]
53.	ZnO NPs	Sol-gel method	Urease	Antimicrobials	[142]
54.	ZnO NPs	Coprecipitation method	(amylase, urease, and lipase)	In biomedical sciences, environmental sciences, and bioanalytical chemistry.	[143]
55.	ZnO NPs	Obtained from the US Research Nanomaterial Co.	α-haemolysin	The use of ZnO nanoparticle in sub-MIC concentration as cover of artificial instruments such as catheter, intravascular catheters or shunts to control bacterial infection is suggested for further study.	[144,145]
56.	ZnO NPs	Chemical synthesis	Biofilm formation (enzyme not given)	Support ZnO NPs' effectiveness in treating *S aureus* infections by using them as an antibiofilm agent.	[146]
57.	ZnO NPs	Chemical synthesis	Enzymes responsible for biofilm formation	Inhibition of Biofilm formation	[147]
58.	ZnO NPs	Chemical synthesis	Enzymes responsible for biofilm formation	Inhibition of Biofilm formation	[148]
59.	ZnO NPs	Chemical synthesis	Enzymes responsible for biofilm formation	Inhibition of Biofilm formation	[104]
60.	ZnO NPs	Chemical synthesis	Enzymes responsible for biofilm formation and hemolysin toxin	Inhibited biofilm formation, hemolysis by hemolysin toxin producing *S aureus*.	[149]
61.	ZnO NPs	Chemical synthesis		Inhibition of Biofilm formation	[151,150]
62.	ZnO NPs	Chemical synthesis	production of reactive oxygen species (ROS)	Not reported	[152]
63.	ZnO NPs /PVA	Chemical synthesis	-cell membrane damage - Cell adhesion inhibition.	Agricultural and food safety could be maintained by using ZnO NPs/PVA nanofibrous membranes as an efficient antibacterial agent.	[153]
64.	ZnO_2 NPs and ZnO NPs	Chemical synthesis	Enzymes responsible for biofilm formation	Inhibition of Biofilm formation	[136,154]
65.	ZnO NPs	Chemical synthesis		Inhibit biofilm formation	[155,156]

Staphylococcus aureus possesses over fifty proteins and virulence factors that evade the host's immune system and are involved in resistance mechanisms. MNPs can interfere with the production or function of these virulence factors, thereby reducing the pathogen's ability to cause harm. For example, disruption of quorum sensing. Quorum sensing is a communication mechanism used by bacteria to coordinate the expression of virulence factors. MNPs can prevent the formation of biofilms, which are protective structures that allow bacteria to adhere to surfaces and resist antimicrobial treatments. Moreover, MNPs such as silver and copper nanoparticles have shown broad-spectrum antimicrobial activity. They can inhibit a wide range of Staphylococcus serotypes, making them potentially useful in combating different types of infections. On the other hand, the synthetic drugs available to treat staphylococcal infections have severe side effects. Ligands from natural products, secondary metabolites from plants and nanomaterials show less drug resistance and therefore are widely explored as a source of new drugs. A number of metals and their nanoforms have been identified with potent antistaphylococcal activities. Silver and mercury, which form insoluble sulfides, act as potent urease inhibitors by reacting with sulfhydryl groups. Although no commercial use of nanomaterials as anti-staphylococcal molecules has been reported, various reports of these metal nanomaterials as urease inhibitors make them suitable anti-staphylococcal agents. Table 3 summarizes various nanoparticles, their synthesis methods and their applications as inhibitors of staphylococcal enzymes in the control of *S aureus* infections.

9. Conclusion

Most of the commercially available synthetic inhibitors act on pathogens by targeting important enzymes. This leads to widespread issue of resistance emergence. Metal nanoparticles offer a promising alternative to the traditional arsenal of enzyme inhibitors due to their size tenability, binding of multiple ligands on the surface, and diverse enzyme inhibitory strategies. Use of nanoparticles as enzyme inhibitors is novel and promising idea due to high surface area to mass ratio of nanomaterial, their diverse size, shape, chemical functionalization and their stability in diverse environmental conditions, etc. In near future, it nanoparticles are expected to be used as broad-spectrum enzyme inhibitors for wide range of biomedical applications.

Despite the several advantages of metal nanoparticles as a new class of enzyme inhibitor, more research is needed to study their specific and nonspecific interactions with other proteins normally present in living system. Toxicity studies, accumulation of nanoparticles in nontarget host as well as the environmental consequences are also needs to be addressed.

Declaration of competing interest

The authors declare that there are no known conflicts or personal financial interests in this work.

Data available statement

Data sharing is not applicable to this article.

Ethics statement

None.

Informed consent

None.

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