

Application of chemically and physically synthesized metal nanoparticles to *Staphylococcus aureus*

Abstract

The infections caused by *S. aureus* have emerged as a grave challenge to human health worldwide. Further, conventional antibiotic therapies for *S. aureus*-mediated infections are gradually becoming ineffective due to the emergence of drug-resistant strains like methicillin-resistant *Staphylococcus aureus* (MRSA). In search of alternative novel therapeutic strategies against *S. aureus*, the use of metal nanoparticles is proliferating. Among different synthesis methods of metal nanoparticles, chemical and physical methods are the most common. Despite reports of metal nanoparticles' efficacy against drug-resistant *S. aureus* strains, contemporary reports that the bacteria can evolve resistance to nanoparticles are a significant source of concern. There is also the issue of metal nanoparticle toxicity, which affects a variety of organisms. The clinical translatability of published research conclusions is another major hurdle in nanotherapeutics research. More research is needed to make nanoparticle-based treatments a viable and long-term therapy for infections caused by *S. aureus*. The present review provides an overview of the therapeutic application of physicochemically synthesized nanoparticles (electron beam, mechanical grinding, milling, spray pyrolysis, vapour phase synthesis, electrolysis, photochemical, solutions and gels, wound healing, anticancer, antioxidant, biosensing, cosmetics, antimicrobial, human health care and water treatment) of various metals (transition metals, post-transition metals, alkaline earth metals, rare earth metals, etc.) against various *S. aureus* strains.

Keywords: Nanoparticles, chemical synthesis, physical synthesis, *Staphylococcus aureus*, MRSA, antibacterial

Abrevationed: MRSA: Methicillin-resistant *Staphylococcus aureus*, AgNPs: Silver nanoparticles, ZnO NPs: Zinc oxide nanoparticles, AuNPs: Gold nanoparticles, MBC:

Maximum bactericidal concentration, DMF: Dimethylformamide, PAA: Polyacrylic acid, TBO: Toluidine blue O, CTAB: Cetyl trimethyl ammonium bromide, NiO NPs: Nickel oxide nanoparticles, ROS: Reactive oxygen species, PdNPs: Palladium nanoparticles and MIC: Minimum inhibitory concentration

Introduction

Staphylococcus aureus (*S. aureus*) is a gram-positive bacterium that belongs to *Staphylococcus* and was first isolated by surgeon Alexander Ogston from human pus in 1880. It is a standard part of the body's microbiota, commonly found in the upper respiratory tract and skin. It is one of the most frequent causes of skin and soft tissue infections that generally start as a minor boil or abscesses but may lead to severe life-threatening blood, muscle, and bone infections. Moreover, it may spread to the other vital internal organs such as the brain, lungs, hearts, etc. (McCaig et al., 2006; David and Daum, 2017). Endocarditis, meningitis, osteomyelitis, pyomyositis, necrotizing fasciitis, impetigo, etc., are some significant diseases caused by *S. aureus* (Lowy, 1998; David and Daum, 2017). It is the most commonly isolated bacterial strain from hospitalized patients in the United States from 1998 to 2005 (Styers et al., 2006) and the second most cause for nosocomial infections related deaths in the United States (Magill et al., 2014). *S. aureus* can also form biofilm by colonizing both host tissue and artificial surfaces like medical implants.

The biofilm-forming ability of *S. aureus* is an important virulence factor that provides resistance against antibiotics, allows bacterial persistence in host tissues, and helps the bacteria overcome host defense (Oliveira et al., 2006). The severity of *S. aureus*-associated infections is further exacerbated by the emergence of antibiotic-resistant strains such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Staphylococcus aureus*, vancomycin-intermediate *Staphylococcus aureus*, and delafloxacin-resistant *Staphylococcus aureus* — a newly isolated strain from hospitals in Brooklyn, New York, USA (Iregui et al., 2020). Importantly, *S. aureus* has created a consid-

erable economic burden on society and healthcare institutions due to the need for treatment and hospitalization (Lee et al., 2013; Suaya et al., 2014). The current antimicrobial approaches against *S. aureus* are plagued with multiple problems, such as human toxicity, bacterial resistance, and inadequacy against bacterial biofilms (Church et al., 2006; Monroe, 2007).

The World Health Organization (WHO, 2017) emphasized that methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Staphylococcus aureus*, vancomycin-intermediate *Staphylococcus aureus* are among the high-priority multi-drug resistant organisms that demand coordinated efforts in the research and development of new antibiotics and novel therapeutic approaches.

Nanomedicine is one such novel approach that has been exploited by numerous studies to combat *S. aureus*. Nanomedicine is a fast-growing multidisciplinary field combining material science, chemical science, pharmacological science, and biological science. It is the application of nanotechnology in medicine, serving clinical roles in screening, diagnosis, management, and therapy of diseases (Freitas, 2005; Fülöp et al., 2012; Moghimi et al., 2005; Riehemann et al., 2009). Over conventional medicine, nanomedicines offer better solubility and bioavailability, fewer side effects, and a lower likelihood of development of resistance by the biological system against them (Hock et al., 2011; Stankic et al., 2016). Further, nanomedicines, particularly nanoparticles (NPs), have been explored and, in some cases, approved. By the concerned agencies after successful clinical trials for treating various medical conditions. Such as cancer, infectious diseases, renal diseases, immune disorders, endocrine and exocrine disorders, neurodegenerative disorders, diabetes, and cardiovascular diseases (Kabanov and Gendelman, 2007; Etheridge et al., 2013; Lee Chung et al., 2015; Noorlander et al., 2015).

Nanoparticles are structures with a size range from 1 to 100 nm (although some studies include molecules up to 1000 nm with specific properties under the definition of nanoparticles) and play a leading role in nanomedicine (Kim et al.,

2010; Hawthorne et al., 2017). In this review paper, we have discussed the use of metal nanoparticles synthesized by chemical and physical methods for inhibiting the growth of *S. aureus*. Further, the review mainly focuses on the therapeutic use of monometallic nanoparticles against *S. aureus* infection.

Chemical and physical synthesis of nanoparticles

Depending on the starting material used in the reaction, nanoparticles can be synthesized by either a top-down or bottom-up approach. The physical method represents top-down strategy, whereas the chemical method can be applied in both top-down and bottom-up strategies of nanoparticles synthesis (Yu et al., 2013; Dhand et al., 2015; Reverberi et al., 2016; Jamkhande et al., 2019). Physical methods include the preparation of nano-sized structures by breaking-down bulk materials. Mechanical ball milling, electrospraying, physical vapor deposition (sputtering, electron beam evaporation, pulsed laser deposition), inert gas condensation, melt mixing, laser pyrolysis, and flash spray pyrolysis are some of the most frequently used physical methods for the fabrication of nanoparticles (Dhand et al., 2015).

The chemical synthesis of nanoparticles generally involves the reduction of metal ions in aqueous or non-aqueous solutions into their metallic form in the presence of a reducing agent and stabilizing agent. Such as polyvinyl alcohol (PVA), polyvinyl pyrrolidone (PVP), sodium dodecyl sulfate (SDS), dodecanoic acid (DDA), surfactin, etc., which prevent aggregation of nanoparticles (Iravani et al., 2014; Reverberi et al., 2016). The common reducing agents used in the chemical synthesis are ascorbic acid, dimethylformamide (DMF), ethylene glycol, hydrazine hydrate, sodium borohydride (NaBH_4), sodium citrate, polyols, etc. (Guzman et al., 2012; Iravani et al., 2014). Sol-gel method, microwave-assisted synthesis, sonochemical synthesis, microemulsion method, hydrothermal and solvothermal methods, polyol synthesis. The chemical reduction method, chemical vapor deposition technique, and supercritical fluid precipitation method are the most routinely used chemical methods for synthesizing nanoparticles (Dhand et al., 2015; Ealias and Saravanakumar, 2017; Jamkhande

et al., 2019). The metallic nanoparticles synthesized via physical and chemical methods have shown promising results in controlling *S. aureus* growth (Figure-1).

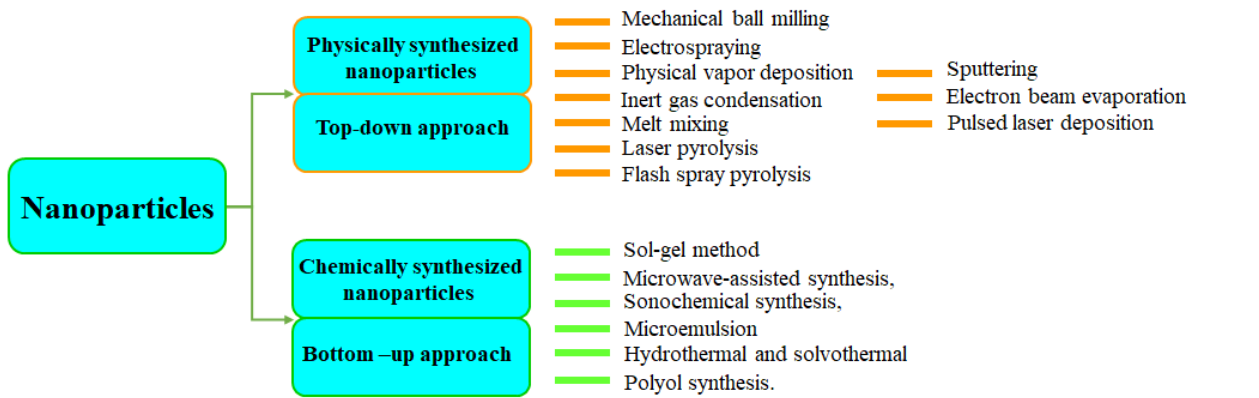


Figure 1: Schematic illustration of the various mechanisms of nanoparticles against *Staphylococcus aureus*. This review catalogs the antibacterial application of metal (gold, silver, copper, zinc, iron, and other transition metals; post-transition metals; rare-earth metals; alkaline earth metals) nanoparticles fabricated through either chemical or physical methods against *S. aureus* infection (Figure-2).

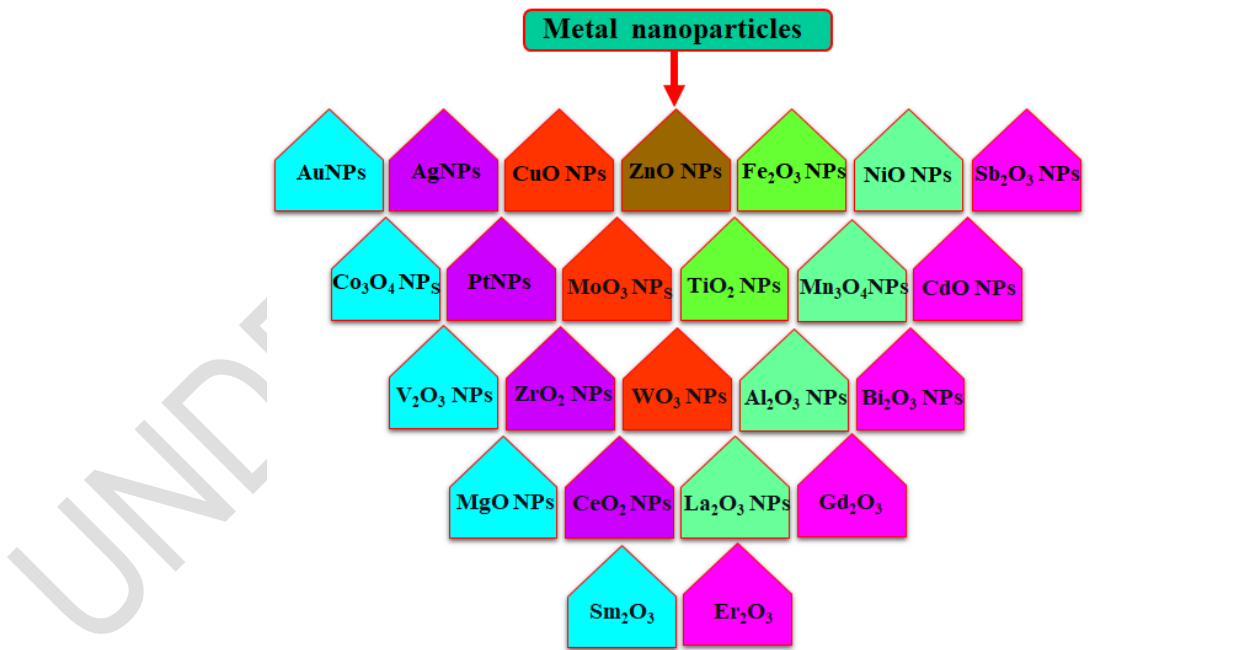


Figure 2: Graphic representation of the various antibacterial metal nanoparticles against *Staphylococcus aureus*.

Silver-based nanoparticles

Silver has been known to humanity for its antibacterial property for hundreds of years. Many silver-based nanoparticles have been prepared by chemical or physical methods to kill *S. aureus*. Hwang and group (2012) reported that chemically synthesized spherical-shaped silver nanoparticles (AgNPs) have a mean size of 3 nm. In combination with antibiotics (ampicillin, chloramphenicol, and kanamycin), inhibit the growth of and biofilm formation by *S. aureus*. Guzman et al. (2012) synthesized AgNPs with a size between 10 and 20 nm from the chemical reduction of aqueous silver nitrate solution in the presence of reducing agents hydrazine hydrate and sodium citrate, and stabilizer SDS. The authors reported excellent antibacterial activity for the AgNPs against both *S. aureus* and drug-resistant strain MRSA.

Further, according to the authors, the silver ions released by AgNPs and the affinity of silver with sulfur and phosphorus groups present in the bacterial cell membrane were responsible for the bactericidal effect of AgNPs against *S. aureus* and MRSA (Guzman et al., 2012). Chudobova and group (2014) synthesized silver phosphate nanoparticles having a size between 200 and 300 nm via a chemical method. The silver phosphate nanoparticles inhibited the growth of *S. aureus*, with the minimum inhibitory concentration (MIC) and total inhibitory concentration of 10 μ M and 300 μ M, respectively (Chudobova et al., 2014). AgNPs fabricated in a microwave-assisted method in the presence of ascorbic acid as reductant and starch as stabilizers exhibited antibacterial properties against *S. aureus* in disk diffusion assay (Valodkar et al., 2011). Wady and group (2014) synthesized AgNPs in a chemical reduction method that utilized sodium borohydride as a reductant and PVA as a stabilizing agent. The authors reported the bacteriostatic and bactericidal effect of AgNPs on *S. aureus* and MRSA planktonic cells. Ayala-Núñez and group (2009) found that the size of AgNPs affects its antibacterial property against *S. aureus*. The authors used AgNPs in three different sizes: 10 nm, 30-40 nm, and 100 nm. The smallest-sized AgNPs (10 nm) had the highest antibacterial activity against MRSA. They were non-toxic to the HeLa cells, whereas

AgNPs with large sizes (30-40 nm and 100 nm) had a moderate effect on MRSA and had a toxic effect on HeLa cells (Ayala-Núñez et al., 2009).

Similar to the size, the shape of AgNPs also affects their antibacterial activity against *S. aureus*. For example, Gao et al. (2013) synthesized spherical-shaped AgNPs in a chemical reduction method with L-ascorbic acid as the reductant and PVP as the surface modifier. The authors demonstrated superior antibacterial activity for spherical-shaped AgNPs than triangle-shaped nanoplates versus *S. aureus*.

Bankier and group (2019) showed the antibacterial effect of rod-shaped AgNPs prepared from thermal plasma techniques against *S. aureus*. Actis et al. (2015) prepared, through two different chemical methods (a novel thermal method and polyol process), AgNPs in three different geometries: spherical with size 34.10 ± 3.18 nm, triangular with size 28.80 ± 4.89 nm, and cuboid with size 75.29 ± 7.15 nm. The authors did not find any significant effect of shape on the antibacterial activity of AgNPs against *S. aureus*; however, MRSA showed higher susceptibility to cuboid-shaped AgNPs than either to spherical- or triangular-shaped AgNPs (Actis et al., 2015). AgNPs can also be used to prepare surfaces to prevent the growth of *S. aureus* strains. In one such study, AgNPs coated on glass surfaces inhibited biofilm formation by clinically isolated strains MRSA and methicillin-sensitive *S. aureus* (MSSA) (Ansari et al., 2015).

Similarly, Piçarra and groups (2019) coated glass, polystyrene, and steel surfaces with AgNPs and noted good antibacterial activity for the coated surfaces against *S. aureus*. Combining AgNPs with antibiotics, drugs, blue light, or other chemical groups can increase AgNPs' antibacterial efficacy versus *S. aureus*. For example, Akram et al. (2016) found that the triple combination of AgNPs (15-20 nm) blue light (460 nm and 250 mW for one hour). Moreover, different antibiotics (amoxicillin, azithromycin, clarithromycin, linezolid, and vancomycin) more effectively killed clinical MRSA isolates in comparison with the double combination of AgNPs and antibiotics or AgNPs and blue light. Cavassin et al. (2015) syn-

thesized citrate-, chitosan-, and PVA-functionalized spherical AgNPs, respectively, and reported antibacterial activity for the functionalized AgNPs against oxacillin-resistant *Staphylococcus aureus*. Similarly, in another study, PEG-functionalized AgNPs of 14 nm exhibited the most miniature MIC (3.31 ± 0.03 $\mu\text{g/mL}$) and thus the highest bactericidal activity compared with the T80-functionalized AgNPs of 45 nm size and SDS-functionalized AgNPs of 54 nm size against *S. aureus* (Bhattacharya et al., 2012).

AgNPs functionalized with antibiotics such as ampicillin (Brown et al., 2012), vancomycin (Hur and Park, 2016; Esmacillou et al., 2017; Kaur et al., 2019), cephradine (Masri et al., 2018), rifampicin (Farooq et al., 2019) have been effectively utilized as antibacterial agents against *S. aureus* and MRSA. Li and group (2011) studied the mechanism of AgNPs against *S. aureus* and reported that AgNPs damage the cell membrane integrity, interfere with healthy cell metabolism, and condense the bacterial DNA.

Gold-based nanoparticles

Like silver, gold is another metal whose nanoparticles are extensively explored for its antibacterial properties against *S. aureus*. Shamaila et al. (2016), in a chemical method using NaBH_4 as a reducing agent, synthesized gold nanoparticles (AuNPs) in two size ranges: 7-34 nm and 30-40 nm. Both types of AuNPs exhibited antibacterial activities against *S. aureus*, with MIC values of 3.92 $\mu\text{g/mL}$ and 3.98 $\mu\text{g/mL}$ for AuNPs with sizes 7-34 nm and size 30-40 nm, respectively.

On the other hand, some studies found no or deficient antibacterial activity against *S. aureus* for naked AuNPs that is AuNPs without any surface modification (Rai et al., 2010; Brown et al., 2012; Yang et al., 2018). Conjugation of AuNPs with antibiotics such as gentamicin (Ahangari et al., 2013); amoxicillin (Kalita et al., 2016); ampicillin (Fan et al., 2019); vancomycin (Gu et al., 2003; Hur and Park, 2016); streptomycin, and kanamycin (Saha et al., 2007), etc. have been shown to improve the antibacterial efficacy of both AuNPs and antibiotics against *S. aureus*. Darabpour's group (2017) attached methylene blue

dye to the surface of AuNPs and used the conjugated AuNPs in photodynamic antimicrobial chemotherapy to deactivate the MRSA biofilm. Kuo et al. (2009) prepared gold nanorods coated with polyacrylic acid (PAA) and conjugated with toluidine blue O (TBO).

The modified gold nanorods in the presence of 633 nm HeNe laser caused photothermal deactivation of MRSA (Kuo et al., 2009). In two separate studies, antibiotic-loaded, antibody-conjugated, polymer-coated gold nano-constructs were combined with photothermal heating to kill *S. aureus* (Meeker et al., 2016; Meeker et al., 2018). AuNPs conjugated with vascular endothelial growth factor A165 (VEGF-A165) and (11-mercaptopoundecyl)-N, N, N-trimethylammonium (11-MTA) cation showed wound healing property on MRSA-induced wounds in diabetic mice (Wei et al., 2019). AuNPs have also been used in therapeutic systems devised to inhibit MRSA biofilm formation (Hu et al., 2017; Ramasamy et al., 2017; Mizdal et al., 2018; Xie et al., 2018). The shape of AuNPs affects its antibacterial properties against *S. aureus*. In one such exciting study, authors found flower-shaped AuNPs (40.6 ± 2.2 nm) to have more potent antibacterial activity than sphere- and star-shaped AuNPs towards *S. aureus* (Penders et al., 2017).

In a similar study, the maximum antibacterial activity toward *S. aureus* was shown by gold nanocubes (zone of inhibition of 16.5 mm) followed by gold nanospheres (zone of inhibition of 13.5 mm) and gold nanostars (zone of inhibition of 12.5 mm) (Hameed et al., 2020). Similarly, other studies reported the therapeutic potential of gold nanorods with various surface modifications against *S. aureus* (Kuo et al., 2009; Pissuwan et al., 2010; Bermúdez-Jiménez et al., 2019).

Iron- based nanoparticles

Iron nanoparticles have emerged as a promising antibacterial agent because of their superpara magnetic properties and biocompatibility (Berry and Curtis, 2003; Mahmoudi et al., 2011). Another advantage of magnetic nanoparticles is that they can be retrieved after being used in the treatment (Manna et al., 2018). The commonly used iron oxide nanoparticles for

antibacterial properties are α -Fe₂O₃ (hematite), γ -Fe₂O₃ (maghemite), and Fe₃O₄ (magnetite). Tran et al. (2010) synthesized PVA-coated iron oxide nanoparticles (mixture of γ -Fe₂O₃ and Fe₃O₄) using a matrix-mediated method. The PVA-coated iron nanoparticles had the size of 9±4 nm and arrested the growth of *S. aureus* in the live/dead assay (Tran et al., 2010). Rhombohedral-shaped Fe₂O₃ NPs with an average size of 35.16±1.47 nm were bactericidal against *S. aureus* at a maximum bactericidal concentration (MBC) value of 80±1.5 µg/mL (Azam et al., 2012). Ravikumar and group (2012) found a zone of inhibition in agar well diffusion assay for Fe₂O₃ NPs (size, 9-11 nm) against *S. aureus*. Hematite NPs (50-110 nm) synthesized by pulsed laser ablation method in dimethylformamide (DMF) and SDS solutions showed excellent antibacterial activity toward *S. aureus* (Ismail et al., 2015). Similarly, Fe₃O₄ NPs (9.7 nm) synthesized by flame spray pyrolysis method displayed antibacterial activity against *S. aureus*, with MBC value between 10 and 100 µg/mL (Aruoja et al., 2015). In another study, spherical magnetite NPs (50–100 nm) coated with oleic acid showed excellent anti-biofilm activity against *S. aureus* (Velusamy et al., 2016). Similarly, EDTA-Na₃-functionalized magnetite NPs were found to have biofilm removing ability against MRSA (Manna et al., 2019). Kim's group (2013) conjugated magnetite NPs with anti-*S. aureus* protein-A antibody. Under the influence of heat generated by the high-amplitude, high-frequency, alternating magnetic field, the conjugated magnetite NPs effectively killed *S. aureus*. They promoted wound healing in *S. aureus*-infected mouse model (Kim et al., 2013). Iron oxide nanoparticles can also be used as a drug delivery vehicle to treat *S. aureus*-associated infections (Manna et al., 2018; Nickel et al., 2020). For example, Manna and group (2018) used amine-functionalized, biocide-coated, non-spherical Fe₃O₄ NPs of varied shapes (cubic, disk-like, hexagonal, rectangular, and rod-like) to deactivate MRSA bacteria entirely in only two hours. Very recently, Nickel and group (2020) synthesized magnetic nanoparticles (a mixture of Fe₃O₄ and γ -Fe₂O₃) of distinct shapes (spherical, cubic, and tetrapod) via thermal decomposition of iron oleate. The researchers used magnetic

nanoparticles to transport biocidal agent cetyl trimethyl ammonium bromide (CTAB) within the extracellular matrix of the bacterial cells to eradicate MRSA biofilms (Nickel et al., 2020).

Nickel-based nanoparticles

Nickel-based nanoparticles have also been studied for their antibacterial activities toward *S. aureus*. Pang et al. (2009) synthesized nickel oxide (NiO) nanotubes and nanoflowers from the precursor bis (dimethylglyoximate) nickel (II) and NiCl₂, respectively. The NiO nanotubes exhibited more potent antibacterial activity with a MIC value of 6.25 µg/mL than NiO nanoflowers having a MIC value of 50 µg/mL against *S. aureus*. Baek and An (2011) compared the antibacterial activities of nickel oxide nanoparticles (NiO NPs), copper oxide nanoparticles (CuO NPs), zinc oxide nanoparticles (ZnO NPs), and antimony trioxide nanoparticles (Sb₂O₃ NPs) against *S. aureus*. All tested metal oxide nanoparticles showed antibacterial activities against *S. aureus*, and the order of their antibacterial activities was as follows: CuO NPs > NiO NPs > ZnO NPs > Sb₂O₃ NPs (Baek and An, 2011). Similarly, in another comparative study, Argueta-Figueroa and group (2014) reported that. In contrast, copper NPs exerted a bactericidal effect on *S. aureus*; nickel NPs only showed a bacteriostatic effect on the bacteria. Mirhosseini and group (2018) synthesized nickel nanoparticles (NiNPs) and nickel hydroxide nanoparticles (Ni(OH)₂ NPs) of 5 nm and 75 nm size, respectively, by chemical reduction method. NiNPs exhibited higher antibacterial activity with the MIC and MBC values of 0.81 mg/mL and 1.62 mg/mL than Ni(OH)₂ NPs for which the MIC and MBC values were 6.5 mg/mL and 13 mg/mL (Mirhosseini et al., 2018).

Copper--based nanoparticles

Nanoparticles of copper metal have also emerged as potent nanotherapeutics against infectious diseases caused by microbes, especially *S. aureus*. Copper-based nanoparticles synthesized through various chemical and physical methods, such as flame spray pyrolysis method (Aruoja et al., 2015), sol-gel method (Dadi et al., 2019), mechanical milling (Moniri Javad-

hesari et al., 2019), pulsed laser ablation method (Swarnkar et al., 2016), chemical reducing method (Argueta-Figueroa et al., 2014), hydrothermal technique (Giannousi et al., 2014) have been found to possess antibacterial activity against *S. aureus* strains. Kruk and coworkers (2015) presented the effectiveness of monodispersed copper NPs (50 nm) synthesized by reducing copper salt with hydrazine in the aqueous SDS solution against MRSA. Spherical-shaped copper oxide nanoparticles (CuO NPs) with a size range from 5 to 10 nm were synthesized through the electrochemical reduction method. The nanoparticles showed good antibacterial activity against *S. aureus* (Jadhav et al., 2011). Azam et al. (2012) revealed that CuO NPs restrict *S. aureus* growth in size- and concentration-dependent manner. CuO NPs exhibited higher antibacterial activity for Gram-positive bacteria than for Gram-negative bacteria. Ren et al. (2009) used thermal plasma technology to prepare CuO NPs. The CuO NPs were active against MRSA and other *S. aureus* strains, with MBC values ranging from 100 µg/mL to 2500 µg/mL (Ren et al., 2009). Chatterjee and coworkers (2012) prepared spherical-shaped copper NPs with 56.2 nm size by reducing CuCl₂ in the presence of gelatin as a stabilizer. The copper NPs inhibited the growth of *S. aureus*, with the MIC and MBC values of 4.5 and 9 µg/mL, respectively. Usman et al. (2013) synthesized copper NPs (2–350 nm) via chemical method using chitosan polymer as a stabilizer and reported the antibacterial activity of copper NPs toward MRSA. Nanoparticles of various oxide phases of copper (Cu, CuO, and Cu₂O) were synthesized at different pH (3, 5, 7, 9, and 11) in a low-temperature chemical reduction method by Moshalagae Motlatle et al. (2016). The researchers reported a difference in the bactericidal property of the tested nanoparticles against *S. aureus*. The highest zone of inhibition in disk diffusion assay was shown by copper nanoparticles formed at pH 7 (Moshalagae Motlatle et al., 2016). Further, the authors suggested that the difference was due to the pH that affected surface charges on copper nanoparticles (Moshalagae Motlatle et al., 2016). Copper-based nanoparticles have also been shown to in-

hibit biofilm formation and act against resistant *S. aureus* strains (Agarwala et al., 2014; Singh et al., 2015).

Zinc-based nanoparticles

Zinc-based nanomaterials are additional nanotherapeutics that many studies have used against *S. aureus* infection. For instance, Baek and An (2011) reported inhibition of *S. aureus* growth by zinc oxide nanoparticles (ZnO NPs) having a size range of 50 to 70 nm. Similarly, ZnO NPs of tiny size (3 nm) exhibited bacteriostatic and bactericidal activities toward *S. aureus*, with the MIC and MBC values of 0.5 mg/mL and 8 mg/mL, respectively (Emami-Karvani and Chehrazi, 2011). In a comparative antibacterial study against *S. aureus*, hexagonal-shaped ZnO NPs (19.89 ± 1.43 nm) showed maximum antibacterial activity followed by CuO NPs (29.11 ± 1.61 nm), and the minor antibacterial activity was shown by Fe₂O₃ NPs (35.16 ± 1.47 nm) (Azam et al., 2012). Pati and coworkers (2014) synthesized spherical shaped ZnO NPs of 500 nm size via wet chemical methods using zinc nitrate and sodium hydroxide as precursors and soluble starch as a stabilizing agent. The authors reported that ZnO NPs perturbed biofilm formation by both *S. aureus* and MRSA and substantially reduced the *S. aureus* bacterial load and inflammation in *vivo* skin infection mice model (Pati et al., 2014).

Similarly, in another study, ZnO NPs reduced the bacterial load and promoted wound healing in an experimental mice model infected with *S. aureus* (Daghdari et al., 2017). In Jesline et al.'s (2015) study, ZnO NPs of size less than 100 nm showed good antibacterial activity against biofilm-forming and non-biofilm forming MRSA strains. Salina and group (2015) reported that the antibacterial activity of ZnO NPs against *S. aureus* is pH and temperature-dependent. The authors observed that ZnO nanofluid formed by dissolving zinc oxide NPs in glycerol and ammonium citrate inhibited the growth of *S. aureus* in a concentration-dependent manner, and temperature increase and acidic pH can further improve the antibacterial effect of ZnO nanofluid on *S. aureus* (Saliani et al., 2015). ZnO NPs (20.4 nm)

synthesized by flame spray pyrolysis method showed antibacterial activity against *S. aureus*, with MBC value between 1-10 µg/mL (Aruoja et al., 2015).

Similarly, very small-sized (3 nm) ZnO NPs synthesized via the sol-gel method exhibited a prominent antibacterial effect at the exponential phase of *S. aureus* (Dadi et al., 2019). Reyes-Torres et al. (2019) synthesized spherical ZnO NPs of 15 nm size using the mixture of LiNO₃/NaNO₃ as inorganic media and found the nanoparticles, alone and with the antibiotic ampicillin, to be effective against *S. aureus*. In a new study, Kadiyala et al. (2018) showed the concentration-dependent killing of *S. aureus* by spherical and hexagonal shape ZnO NPs. The researchers found that the antibacterial activity of ZnO NPs against MRSA is not mediated by reactive oxygen species (ROS), as commonly reported, but by the regulation of energy metabolism pathways. Such as carbohydrate metabolism, amino acid biosynthesis, and pyrimidine biosynthesis pathway. Further, the authors also proposed that the changes in the energy metabolism of the bacteria could be due to the biomimetic role played by ZnO NPs in the bacterial cells (Kadiyala et al., 2018). In another new study, Choi and coworkers (2017) synthesized novel caffeic acid-conjugated ZnO nanoparticles that inhibited the growth of both *S. aureus* and MRSA. Patra et al. (2012) reported the microwave-assisted synthesis of ZnO NPs with hexagonal shape and size distribution of 18-20 nm. The authors further conjugated ZnO NPs with antibiotic ciprofloxacin and found an excellent antibacterial activity for the conjugated nanoparticles against the clinically isolated multidrug-resistant strain of *S. aureus* (Patra et al., 2012). De Souza et al. (2019) utilized the sonochemical method to synthesize rod-shaped ZnO NPs, with a length of 145.1 nm and a diameter of 97.2 nm that showed a zone of inhibition in agar well against *S. aureus*. Horky et al. (2019) investigated the efficacy against *S. aureus* and MRSA of zinc phosphate-based NPs that were prepared using different precursors: (NH₄)₂HPO₄, Na₂HPO₄·7H₂O, Na₄P₂O₇, and Na₅P₃O₁₀. Zinc phosphate-based NPs exhibited better antibacterial activity against *S. aureus* with IC₅₀ value ranging between 0.5 and 1.6 mmol/L, whereas against MRSA, the

nanoparticles were less potent with IC₅₀ value ranging from 1.2 to 4.7 mmol/L (Horky et al., 2019).

Additional transition metals

Physicochemically synthesized nanoparticles of transition metals other than silver, gold, iron, nickel, copper, and zinc have been less studied as antibacterial agents against *S. aureus*. Transition metals include titanium, palladium, cobalt, molybdenum, cadmium, vanadium, manganese, tungsten, zirconium, and platinum.

Ghosh et al. (2014), in a pyrolysis method using two different cobalt precursors, a coordination polymer and a dinuclear complex, synthesized two types of cobalt oxide nanoparticles (Co₃O₄ NP_s): square-shaped with a smaller size range of 10-25 nm and hexagonal-shaped with a more extensive size range of 100-150 nm. Both types of Co₃O₄ NP_s displayed bacteriostatic and bactericidal activity toward *S. aureus*. The MIC and MBC values were 128 µg/mL for square-shaped Co₃O₄ NP_s and 64 µg/mL and 128 µg/mL, respectively, for hexagonal-shaped Co₃O₄ NP_s (Ghosh et al., 2014). Similarly, Co₃O₄ NP_s of 11.5 nm size synthesized by flame spray pyrolysis method showed antibacterial activity against *S. aureus*, with an MBC value of 100 µg/ml (Aruoja et al., 2015). In a recent report, researchers fabricated cobalt nanosuspension from three different cobalt-based metallosurfactants -CoCTAC (bis-hexadecyltrimethylammonium cobalt tetrachloride), CoDDA (bis-dodecyl amine cobalt dichloride). Furthermore, CoHEXA (bis-hexadecylamine cobalt dichloride)-via microemulsion method without using any reducing agents and reported antimicrobial activities against *S. aureus* for all the three cobalt-based nanosuspension (Dogra et al., 2019).

Konieczny et al. (2013) demonstrated size-dependent inhibition of *S. aureus* growth by using a colony-reduction assay using PVP-coated platinum nanoparticles NPs (PtNPs). Similarly, small-sized pectin-capped PtNPs (2-5 nm) fabricated via chemical reduction method displayed excellent bacteriostatic effect at the MIC value of 31.2 µg/ml toward *S. aureus* (Ayaz Ahmed et al., 2016). Platinum nanoparticles disintegrate the cytoplasmic

membrane and cell wall of *S. aureus* and induce leakage of intracellular components (Chwalibog et al., 2010).

Recently, transition metal molybdenum has also generated interest due to its limited toxicity to humans, biodegradability, and fast elimination from the body (Hao et al., 2018). Irregularly shaped molybdenum oxide nanoparticles (MoO_3 NPs) of 46 nm size prepared via electrochemical reduction method were reported to be efficient in killing *S. aureus* in agar well diffusion assay (Dighore et al., 2017). Similarly, MoO_3 NPs synthesized in a one-step thermal decomposition method using ammonium heptamolybdate tetrahydrate as precursor inhibited the growth of both MSSA and MRSA at MIC value of 700 $\mu\text{g/ml}$ (Lopes et al., 2018). Desai and coworkers (2015) reported antibacterial action against *S. aureus* of MoO_3 NPs nanocrystals having hexagonal rods with sea urchin-like morphology prepared through the chemical bath deposition technique. The coating of MoO_3 NPs on the glass surface also exhibited good antibacterial activity against *S. aureus* (Piçarra et al., 2019).

Titanium nanoparticles have also been explored for their antibacterial properties against *S. aureus* (Roy et al., 2010; Jesline et al., 2015; Aruoja et al., 2015). The titanium nanoparticles are mainly utilized as an antibacterial coating on implants to keep them safe from infection after surgery (Orapiriyakul et al., 2018). Roy and group (2010) synthesized titanium dioxide NPs (TiO_2 NPs) via the sol-gel method using citric acid as a reducing agent and α -Dextrose saturated solution as a surfactant. The researchers observed that TiO_2 NPs could enhance the effectiveness of common antibiotics (β -lactam, cephalosporins, aminoglycosides, glycopeptides, fluoroquinolones, azalides, macrolides, lincosamides, and sulphonamides) against MRSA (Roy et al., 2010). Jesline's group (2015) reported the efficacy of commercially synthesized TiO_2 NPs (<50 nm) against biofilm-forming MRSA strains. TiO_2 NPs (12.2 nm) synthesized by flame spray pyrolysis method showed antibacterial activity against *S. aureus*, with an MBC value of >100 $\mu\text{g/mL}$ (Aruoja et al., 2015). TiO_2 is commonly found in three different phases: anatase, brookite, and rutile. Nanoparticles of all

the three-phase variants have been studied as antibacterial agents versus *S. aureus*. Haq et al. (2018) prepared TiO₂ NPs via the chemical precipitation method at room temperature and investigated the effect of temperature on the antibacterial activity of the nanoparticles against *S. aureus*. Researchers found that TiO₂ NPs at 120°C consisted of anatase phase with tetragonal morphology and were toxic for *S. aureus*, whereas TiO₂ NPs at 900°C contained only rutile phase with tetragonal geometry and exhibited no antibacterial activity against the bacteria (Haq et al., 2018). On the contrary, Fei and group (2006) found that cotton fabrics treated with rutile phase TiO₂ nanocrystals of less than 10 nm size prepared at room temperature showed bactericidal activity toward *S. aureus*. Similarly, in another study, thin films of anatase TiO₂ NPs on glass and titanium surfaces in the presence of UV light showed photocatalytic bactericidal activity against *S. aureus* (Shiraishi et al., 2009). The biphasic brookite-anatase TiO₂ NPs in combination with UV light showed better antibacterial activity than either TiO₂ NPs or UV light alone against the drug-resistant strain of *S. aureus* (Shah et al., 2008).

Palladium is another transition metal whose nanoparticles have been exploited for their antibacterial activity toward *S. aureus*. Adams et al. (2014) fabricated spherical palladium nanoparticles (PdNPs) from the precursor palladium acetate via a modified pyrolysis reaction. The researchers obtained PdNPs in three sizes (2.0±0.1 nm, 2.5±0.2 nm, and 3.1±0.2 nm) and found that PdNPs at a concentration as low as 10⁻⁹ M could kill *S. aureus* (Adams et al., 2014). Moreover, the small-sized PdNPs (2.0±0.1 nm) were more toxic than the PdNPs with sizes of 2.5±0.2 nm and 3.1±0.2 nm to *S. aureus* (Adams et al., 2014). Similarly, PdNPs having a size of 15.1 nm synthesized by flame spray pyrolysis method showed antibacterial activity against *S. aureus*, with MBC value in the range between 10 and 100 µg/mL (Aruoja et al., 2015). Recently in a fascinating study, authors reported the shape dependence of PdNPs on its antibacterial activity toward drug-resistant *S. aureus* (Fang et al., 2018). The authors used the hydrothermal method to prepare cube-

shaped and octahedron-shaped PdNPs. The cube-shaped PdNPs were more effective than octahedron-shaped PdNPs in killing drug-resistant *S. aureus*. Further, according to Fang et al. (2018), oxidase- and peroxidase-like properties of PdNPs generate reactive oxygen species that damage *S. aureus*.

Azhir et al. (2015) investigated the effect of hausmannite manganese oxide nanoparticles (Mn_3O_4 NPs) (10–30 nm) prepared via precipitation method on *S. aureus*. The Mn_3O_4 NPs inhibited *S. aureus* concentration-dependent with MIC and MBC values of 625 and 1250 $\mu\text{g/ml}$ (Azhir et al., 2015). Aurora and group (2015) utilized the flame spray pyrolysis method to synthesize Mn_3O_4 NPs (15.2 nm), which showed antibacterial activity against *S. aureus* with MBC value 10–100 $\mu\text{g/ml}$. Cherian et al. (2016), using the co-precipitation method, fabricated spherical-shaped manganese dioxide nanoparticles (Mn_3O_4 NPs) have sizes 40.5–70 nm and found the nanoparticles toxic to *S. aureus*.

Nanoparticles of cadmium oxide, another transition metal, were synthesized by Salehi and group (2014) using a chemical method including cadmium sulfate as a precursor and CTAB as the surfactant. Cadmium oxide NPs (CdO NPs) inhibited the growth of *S. aureus* in a concentration-dependent manner, and at 20 $\mu\text{g/ml}$, it ultimately killed *S. aureus* within 25–30 hours (Salehi et al., 2014). Similarly, Nandhini and group (2018) used the precipitation method to synthesize CdO NPs, effective against *S. aureus*. Abd et al. (2018) prepared a thin film of CdO NPs with (50–110 nm) antibacterial property against *S. aureus*. Rectangle shape CdO NPs prepared in a microwave-assisted hydrothermal method inhibited *S. aureus* growth in agar well diffusion assay (Karthik et al., 2019).

Vanadium is another transition metal whose nanoparticles have been utilized against *S. aureus*. For instance, via a hydrothermal method, Natalio et al. (2012) prepared vanadium pentoxide nanowires (V_2O_5 NWs) of 300 nm length and 20 nm width. The nanowires of V_2O_5 were found to mimic the role of enzyme vanadium haloperoxidases and cause significant reduction (96%) in *S. aureus* growth (Natalio et al., 2012). Wang and coworkers

(2017A) prepared metallic vanadium NPs, V_2O_3 NPs, VO_2 NPs, and V_2O NPs films by depositing them on quartz glass via the magnetron sputtering method. All nanofilms proved to be effective against MRSA and were further found to be non-toxic to mammalian cells, thus holding promises for therapeutic application in controlling implant-related infection caused by *S. aureus* (Wang et al., 2017A).

Zirconium nanoparticles represent another transition metal-based approach against *S. aureus*, although multiple studies on the antibacterial effect of zirconium-based nanoparticles toward *S. aureus* have shown contradictory results. For example, Ravikumar et al. (2012) did not find any anti-*S. aureus* activity for commercial zirconium dioxide nanoparticles (ZrO_2 NPs) with less than 100 nm size either in well diffusion assay or broth dilution assay. Similarly, neither Jangra et al. (2012) found any antibacterial activity against *S. aureus* for ZrO_2 NPs of two size ranges (5–30 nm and 15–20 nm), both synthesized by hydrothermal method. On the other hand, in the study of Fathima and group (2017), chemically-synthesized spherical-shaped ZrO_2 NPs (15–21 nm) showed antibacterial activity versus *S. aureus* in disc diffusion assay. Similarly, agglomerated ZrO_2 NPs of 30 nm size synthesized by the sol-gel method exhibited good antibacterial activity on *S. aureus* (Sultana et al., 2015). Thakare and group (2016) utilized the sol-gel method to fabricate tetragonal ZrO_2 NPs (35–60 nm). The nanoparticles were capable of arresting the growth of *S. aureus* bacteria (Thakare et al., 2015).

Nanoparticles of transition metal tungsten can be effective against *S. aureus*. Aruoja et al. (2015) reported the efficacy of tungsten trioxide nanoparticles (WO_3 NPs) of 10.6 nm size synthesized by flame spray pyrolysis method against *S. aureus*, with an MBC value of more than 100 $\mu\text{g/mL}$ (Aruoja et al., 2015). Similarly, in another study, tungsten nanoparticles (SNPs) (8.1 ± 2.8 nm) at the MIC value of 1500 $\mu\text{g/mL}$ inhibited the growth of *S. aureus* in direct spotting method and cup diffusion method (Syed et al., 2010). Bankier et al. (2019) did not find any antibacterial effect for tungsten carbide nanoparticles (250 nm, hex-

agonal) on *S. aureus* in flow cytometry dead assay. A recent study showed that tungsten oxide (WO_{3-x}) nanodots could inhibit *S. aureus* in a concentration- and a time-dependent fashion (Duan et al., 2019).

Post-transition metals

Few studies have explored physicochemically synthesized nanoparticles of pure post-transition metals such as aluminum, bismuth, and tin against *S. aureus*.

Ravikumar et al. (2012) prepared aluminum oxide nanoparticles (Al_2O_3 NPs) and found antibacterial activity for the nanoparticles against *S. aureus* only in well diffusion assay but not in broth dilution assay. Aruoja et al. (2015) used the flame spray pyrolysis method to synthesize Al_2O_3 NPs of (11.4 nm) that showed anti-*S. aureus* antibacterial activity with an MBC value of more than 100 $\mu\text{g/ml}$. Similarly, spherical Al_2O_3 NPs (9.5 nm) arrested the growth and reproduction of clinically-isolated *S. aureus* strains, MRSA, and MSSA (Ansari et al., 2013).

Campos and group (2018) compared antibacterial activities of bismuth sulfide nanoparticles (Bi_2S_3 NPs), metallic bismuth nanoparticles (BiNPs), bismuth oxide nanoparticles (Bi_2O_3 NPs), and silver nanoparticles (AgNPs) on *S. aureus*. In the study, both BiNPs and Bi_2O_3 NPs showed suitable antibacterial activities, whereas Bi_2S_3 NPs exhibited minor antibacterial activity (Campos et al., 2018). Kadhim (2018), using laser ablation techniques, synthesized spherical-shaped BiNPs, and reported their effectiveness against *S. aureus*. Very recently, Vazquez-Munoz and coworkers (2020) synthesized PVP-coated BiNPs with sizes ranging from 1.7 nm to 44.4 nm via a chemical reduction method. The nanoparticles exhibited excellent antibacterial activity against planktonic *S. aureus* cells at the MIC value of 1 $\mu\text{g/ml}$, and they also inhibited biofilm formation by *S. aureus* (Vazquez-Munoz et al., 2020). Amininezhad and group (2015) prepared spherical-shaped tin oxide nanoparticles (SnO_2 NPs) by the solvothermal method and showed their antibacterial potential toward *S. aureus*.

Similarly, Kumar et al. (2017A) also showed tetragonal rutile nanocrystals of SnO₂ to have bactericidal activity on *S. aureus* bacteria.

Alkaline earth metals

Alkaline earth metals like calcium and magnesium at a nanoscale size have been found to possess the ability to kill *S. aureus*. For example, magnesium oxide nanoparticles (MgO NPs) (13.6 nm) synthesized by flame spray pyrolysis method showed antibacterial activity against *S. aureus* at the MBC value of more than 100 µg/mL (Aruoja et al., 2015). On the contrary, in Ravikumar et al.'s (2012) study, MgO NPs of less than 50 nm size did not show any activity against *S. aureus* in healthy diffusion or broth dilution method. In another study, Bindhu et al. (2016) reported the synthesis of well-dispersed spherical nanoparticles of magnesium oxide through a wet chemical reaction method. The magnesium oxide nanoparticles displayed antibacterial properties toward *S. aureus* (Bindhu et al., 2016). Similarly, in the study of Nguyen et al. (2018), MgO NPs (size 23±5 nm) exhibited bacteriostatic and bactericidal activities against *S. aureus* and MRSA. The MIC and MBC were 0.7 and 1.4 mg/ml against *S. aureus*; and 1.0 and 1.4 mg/ml against MRSA (Nguyen et al., 2018). In other studies, magnesium oxide nanoparticles were prepared from the wet chemical method (Sundrarajan et al., 2012; Krishnamoorthy et al., 2012), and microwave-assisted synthesis (Makhluf et al., 2005) were found to control the growth of *S. aureus*.

Rare-earth metals

Rare earth metals-based nanoparticles are gaining widespread importance in biomedical applications because of their low toxicity and high chemical and thermal stability (Escudero et al., 2017). Limited studies have utilized pure rare-earth metals nanoparticles to destroy *S. aureus*. Studies have used them as a dopant to improve upon the various properties of other metal nanoparticles (Daksh and Agarwal, 2016).

The studies on cerium oxide nanoparticles (CeO₂ NPs) as an antibacterial agent against *S. aureus* have shown conflicting results. Ravishankar et al. (2015) synthesized

spherical-shaped CeO₂ NPs by solution combustion technique using ceric ammonium nitrate as an oxidizer and ethylenediaminetetraacetic acid (EDTA) as fuel at a high temperature of 450°C. The researchers did not find any activity for CeO₂ NPs up to the concentration of 1000 µg/50 µl against *S. aureus* either in agar well diffusion assay or in broth dilution assay. On the other hand, Ravikumar et al. (2012) reported inhibition of *S. aureus* by commercially available CeO₂ NPs in agar healthy diffusion test. However, the authors did not find any antibacterial activity of CeO₂ NPs in the broth dilution method. In another interesting study, Masadeh and group (2014) reported that the spherical-shaped CeO₂ NPs have a size in the range from 25 to 50 nm significantly reduced antibacterial activity of the antibiotic ciprofloxacin against MSSA and MRSA. The MIC values for ciprofloxacin against MRSA and MSSA planktonic culture were 0.10±0.04 and 0.40±0.20 µg/mL. However, in the presence of CeO₂ NPs, the MIC values increased many folds indicating a decrease in efficacy of the antibiotic against both MRSA and MSSA (Masadeh et al., 2014).

Balusamy et al. (2012), in a comparative study, reported that spherical lanthanum oxide nanoparticles (La₂O₃ NPs) of size 100 nm showed antibacterial activity against *S. aureus*, whereas La₂O₃ bulk material having a size of 1 µm was not effective against the bacteria. In another study, Dědková and group (2017) prepared nanoparticles of gadolinium oxide (Gd₂O₃), samarium oxide (Sm₂O₃), and erbium oxide (Er₂O₃) in a simple thermal decomposition reaction and observed that all tested rare earth metals nanoparticles could inhibit the growth of *S. aureus*.

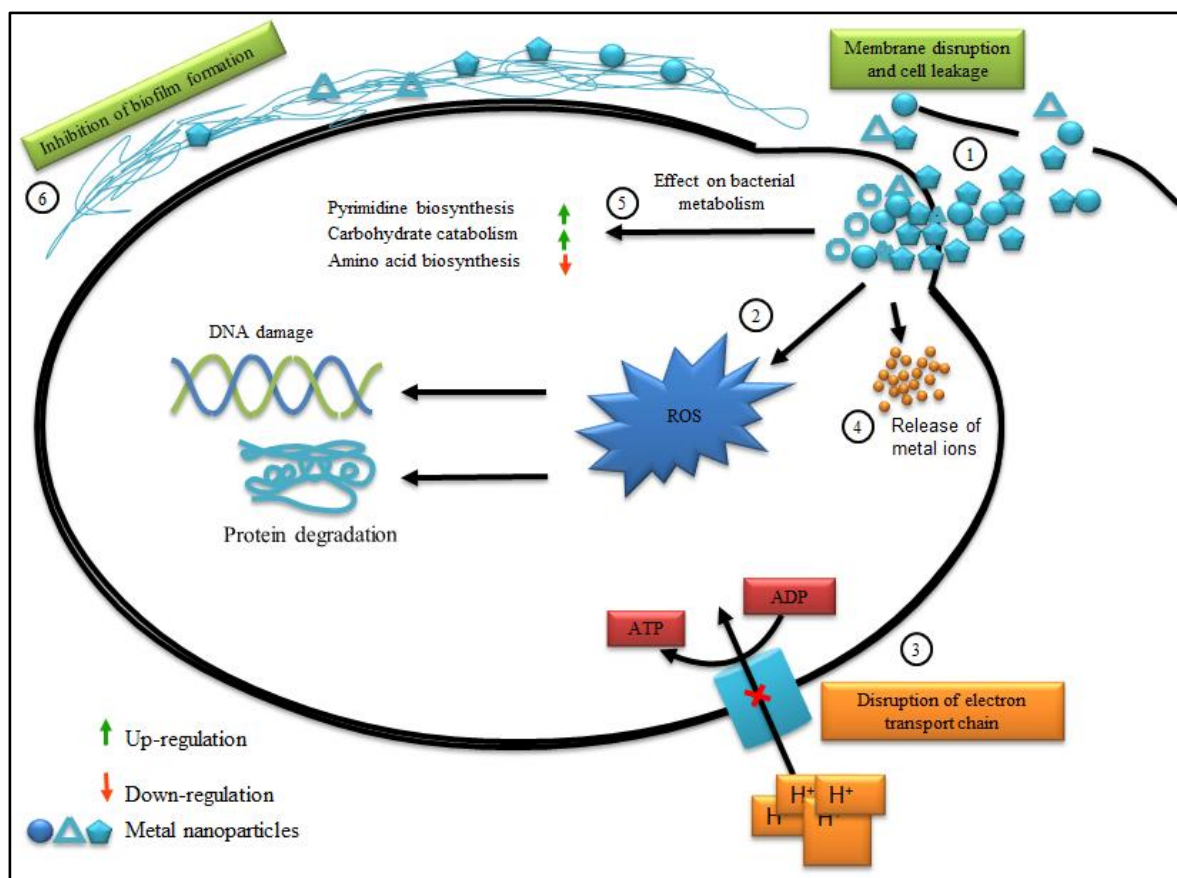


Figure 3: Schematic representation of the various antibacterial mechanisms of metal nanoparticles against *Staphylococcus aureus*. These include (1) disruption of bacterial membrane and leakage of intracellular content, (2) reactive oxygen species (ROS) generation that cause break in DNA, protein degradation, etc. (3) disruption of electron transport chain, (4) release by certain meta nanoparticles of metal ions that further wreak havoc inside the cell, (5) alteration in the bacterial metabolic pathway, (6) inhibition of biofilm formation.

Conclusion

Staphylococcus aureus causes various infections responsible for a significant number of deaths worldwide. The emergence of many drug-resistant strains of *S. aureus* has made treating the associated infections very difficult. The failure of conventional antibiotics has led to novel therapeutic approaches, such as nanosize structures. In this review, we have highlighted the application of metal nanoparticles prepared through chemical and physical methods against *S. aureus* strains. Large metal nanoparticles can be synthesized through diverse chemical and physical methods. Although research in nanoparticle-based therapeutics for infectious diseases is growing at a breakneck pace, it is still far behind the use of nanoparticles for cancer therapy. Similarly, despite reports of the effectiveness of metal nanoparticles against *S. aureus*' drug-resistant strains, the recent reports that the

bacteria can develop resistance against nanoparticles are a significant concern. Additionally, there is the issue of metal nanoparticle toxicity to multiple organs such as kidney, brain, muscle, bone, skin, liver, heart, spleen, etc. The other major challenge in nanotherapeutics research is the clinical translatability of published research findings. Therefore, there is opportunity for further research to make nanoparticles-based therapeutics a viable and long-term solution for *S. aureus*-associated infections.

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