Review Article

A Systematic Review on the Synthesis and Mechanisms of Silver and Copper Nanoparticles against *Staphylococcus aureus*

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ABSTRACT

Antibiotic resistance among pathogenic bacteria impaired the purpose of antibiotics as different clinical conditions like *Staphylococcal* infections become more prevalent. With this, researchers studied different alternatives to address antibiotic resistance, including natural product research and nanomaterials. Like antibiotics, nanoparticles have shown excellent antibacterial properties against the physicochemical properties of bacteria to stop them from causing diseases. This study aims to evaluate the efficacy of silver nanoparticles (AgNPs) and copper nanoparticles (CuNPs) as antibacterial agents against *S. aureus,* a gram-positive bacteria, through a systematic review. Out of 4,382 studies obtained from PubMed and ScienceDirect, only 10 studies that passed the criteria were included. In this review, studies that were included discuss the characterization of nanoparticles and how each parameter affects them using TEM/SEM-EDX, DLS, XRD, UV-Vis, and zeta potential. Moreover, the studies that were gathered used a wet chemical method with sodium borohydride as the most common reducing agent to synthesize and stabilize the said nanoparticles. Results showed that both AgNPs and CuNPs presented increased zones of inhibition depending on their concentration. In contrast, AgNPs are better inhibitors of bacterial growth as compared to CuNPs; however, it should be noted that CuNPs are more effective for gram-positive bacteria, specifically *S. aureus*. Hence, both silver and copper nanoparticles could be considered good alternatives for antibiotics to fight against antibiotic-resistant bacteria.

Keywords: *Staphylococcus aureus,* Silver nanoparticles, Copper nanoparticles, Antibacterial property, Wet chemical method.

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INTRODUCTION

Staphylococcus aureus (S. aureus) is a commonly found gram-positive commensal bacterium that can induce many clinical conditions. It substantially increases the risk of infection in hospitals and communities, with clinical signs ranging from mild to severe.[1] *Staphylococcus aureus* is linked to various illnesses, including blood, respiratory tract, skin, and soft tissue infections.[2] It is also known to be capable of colonizing medical equipment.

Antibiotic-based medical and surgical treatment options are being performed to manage a variety of infections. Despite major advances in medicine, it continues to cause substantial morbidity and mortality rates in hospitals and the community due to the pathogenic bacteria *S. aureus*.[3] These medicines target important bacterial processes particularly cell wall synthesis, DNA synthesis, transcription, and translation.[4] However, multiple studies have revealed a continuous trend of increased antibiotic resistance of *S. aureus* to antibiotics such as methicillin, vancomycin, and daptomycin. Thus, this imposed a significant challenge in treating *S. aureus*

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infection.[3,5-7] Aside from improper use of antibiotics, bacteria can still develop resistance when they undergo genetic alterations such as mutations.[8]

Given the prevalence of antibiotic resistance to pathogens, researchers worldwide search for alternatives and new agents that will help them combat antibiotic resistance and the spread of infections. The common alternative treatments are antivirulence therapy, bacteriophages, and vaccines.[9] Antivirulence therapy works by inhibiting the virulence factors of bacteria that incite damage to the host.[10] Bacteriophage works by halting bacterial replication through injection of the viral genome. This technique is also selective to bacterial strains, but more clinical trials are needed to create effective administration guidelines.[11] The most effective treatment is the vaccine which stimulates the host's immunity to fend off pathogen infection.

Another emerging technique in addressing antibiotic resistance is the use of nanoparticles (NP). Nanoparticles are particles of sizes ranging from 1 – 100 nm and have a variety of uses in the field of optics, healthcare/ biomedical, and agriculture. In the field of healthcare, it is mainly used for drug delivery systems. Due largely to nanoparticles, chemotherapy drugs can be given directly to malignant growths and damaged arteries to treat cardiovascular disease. This technology is useful for protein research, cell and molecule separation and purification, DNA structure analysis, killing cancer cells with drugs or heat, MRI examinations, and pharmacokinetic studies.[12] With nanoparticles, pesticide and fertilizer distribution can be regulated with high site-specificity, reducing the potential for environmental impact. In recent years, the use of nanotechnology in agriculture has gained traction. (Mittal *et al.*, 2020).[13]

Nanoparticles work by altering the physicochemical property of bacterial cell membranes, which eventually induces cell death.[14] In addition, it is also reported that nanoparticles help inhibit the formation of bacterial biofilms. Biofilms are known to threaten the immune system by releasing superantigens that result in pathological conditions.[15] Some examples of nanoparticles with good antibacterial activity are gold NP, magnesium NP, zinc oxide NP, copper oxide NP, and silver NP.

This review investigates the antibacterial properties of silver and copper nanoparticles against *S. aureus*, emphasizing molecular mechanisms that induce cell death. In addition, it highlights the preparation of NPs and the reducing agents used, their effectiveness compared to common treatments, and recent progress in this field.

MATERIALS AND METHODS

The protocols of Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) is utilized to identify, select, and synthesize studies in writing this systematic review.

Literature Search

The search terms used were "silver nanoparticles," "copper nanoparticles," and "*Staphylococcus aureus*" to obtain the related literature from credible search engines such as PubMed and ScienceDirect. Additional key phrases such as "antimicrobial analysis" and "synthesis methods" were used to streamline the literature search.

Eligibility Criteria

Inclusion Criteria

In selecting eligible articles, the following criteria were considered: (1) studies published for the last five (5) years from 2016 to 2022, (2) experimental research articles, (3) articles from reliable sources such as Google Scholar, ScienceDirect, ResearchGate, and PubMed which fits the criteria for ISI/ SCOPUS requirement, (4) wet chemical synthesis method and different reducing agents, (5) assessment of antibacterial properties determined by minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC), (6) discuss the different strains of *Staphylococcus aureus*, and (7) the reactive oxygen species (ROS) production of bacteria with NPs.

Exclusion Criteria

In selecting eligible articles, the following criteria were considered: (1) studies published before the year 2016,

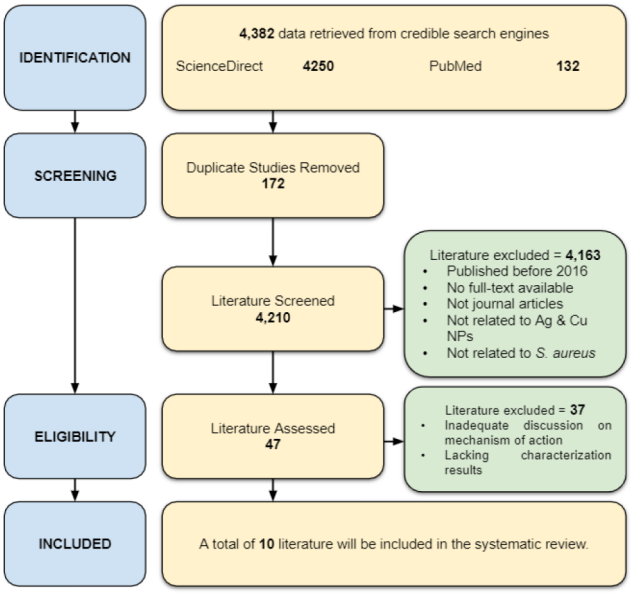

Figure 1: Literature Selection Process (PRISMA, 2020).

(2) articles from predatory sites, (3) articles that are not available in English, (4) review articles related to the topic, (5) does not include other pathogens that have resistance to antibiotics, (6) other nanoparticles, and (7) no in-depth discussion on the molecular interactions of bacteria and NPs.

Selection Strategy

In evaluating the journal articles, the researchers select and critically assess the studies that will be included. A web-based software platform Mendeley was used in screening eligible articles provided with the corresponding title, abstract, and full text.

Data Extraction

The selected journal articles for review were then analyzed for their study characteristics. As presented in Figure 1, the data extracted from the articles were assessed by considering the following criteria: (1) name of the author, (2) year of publication, (3) nanoparticle(s) used (4) results of the antibacterial assay by MIC and MBC, and (5) key findings of the research.

RESULTS

A total of 4,382 research papers were retrieved from various databases, including PubMed and ScienceDirect, in the initial search. Duplicate studies were then identified using Mendeley, a software used in managing research literature. Mendeley identified 172 duplicate studies, leaving 4,210 literature for screening. The authors evaluated the articles based on the title, abstract, and other earlier criteria, leaving 47 papers for eligibility assessment. Complete analyses of the potential eligible papers were done using Mendeley, and a total of 10 were used for the systematic review as shown in Table 1.

Synthesis of Silver and Copper Nanoparticles

Nanoparticles can generally be synthesized using a top-down and bottom-up approach. The top-down approach utilizes bulk materials, which are then physically or mechanically processed into nano-sized particles. On the other hand, the bottom-up approach uses atomic particles assembled into larger structures via chemical reduction techniques.[25] The studies reviewed in this paper utilized a bottom-up approach via the wet chemical method. The technique focuses on chemical reactions induced by different reagents and substances instead of the reactions themselves, making it distinct from other techniques such as biological synthesis, coprecipitation, hydrothermal synthesis, inert gas condensation, ion sputtering scattering, microemulsion, microwave, pulse laser ablation, sol-gel, sonomical, spark discharge, and template synthesis. Metal salts, reducing agents, and capping agents are three of the essential wet chemical reaction process components. The reducing agent will convert the metal salts into unstable metal nanoparticles, which the capping agent will then stabilize after they have been converted.[26]

Silver nanoparticles can be generated by reducing silver ions with a chemical agent, a plant extract, a biological agent, or irradiation. With an average particle size of 68 nm, the reducing agent trisodium citrate exhibits the effect of reductant concentration on particle size variation.[16] The reduction of AgNPs with different concentrations and gallic acid as the reducing agent proved successful. The size of the particles of AgNPs produced by the reduction of $AgNO_3$ with Gallic acid was 0.97–4.88 nm with a maximum diameter of 401.5–424.5 nm. Meanwhile, fresh $NaBH_4$ was mixed to an aqueous solution of 1 mM $AgNO_3$ and 6 mM trisodium citrate, and the color turns from colorless to yellowish-brown in thirty seconds.[17] A wet chemical procedure utilizing silver nitrate and sodium borohydride ($NaBH_4$) as a reducing agent produced efficient AgNPs. The appearance of three peaks in the dynamic light scattering (DLS) Zetasizer data demonstrated non-homogeneous AgNPs with an average particle size of 10.31 nm. The results suggested that the compound possessed favorable antibacterial characteristics.[19]

Copper has the advantage of being inexpensive and abundantly available, which makes obtaining CuNPs cost-effective. CuNPs' susceptibility to oxidation, when exposed to water environments, is one disadvantage. It entails using a reducing agent such as hydrazine, ascorbic acid, or sodium borohydride. Chemical reduction is frequently employed to create CuNPs due to its simplicity, high yield efficiency, and minimal equipment requirements. CuNps produced via chemical reduction can be utilized efficiently as antibacterial and antifungal agents in sodium borohydride as a reducing agent. The average crystalline size of nanoparticles determined using the Debye Scherer formula was less than 10 nm with a cubic structure, with a peak at 320 nm.[27] On the other hand, in the reducing agent ethylene glycol and the hydrazine hydrate, nanoparticles with an average particle size of roughly 40 nm and spherical morphologies were discovered.[28]

Nanoparticle Characterization

Particle Size and Morphology

High-magnification images with a wide depth of focus can be obtained by using the scanning electron microscope (SEM) in conjunction with Energy Dispersive X-ray analysis to identify individual crystals'

Table 1: Antibacterial property of AgNP and CuNP against some pathogens.

Author and Year of Publication	Nanoparticle(s) used in the study	Pathogen Used	Antibacterial assay	Key findings
Ahari, H. *et al*., (2018).[16]	Silver	• *Staphylococcus aureus* (ATCC 6538) • *Escherichia coli* (ATCC 8739)	• The disc diffusion method was used to test the antibacterial characteristics of the nanocomposite film. Against *Escherichia coli (ATCC 8739)* and *Staphylococcus aureus (ATCC 6538),* the results revealed good antibacterial efficacy. • The antibacterial assay used MIC for *S. aureus* and *E. coli.*	• Surrounding the silver-coated films, there were no visible bacterial colonies as it was tested for antibacterial activity. • Results reveal that silver released from the coated film is sufficient to produce antibacterial activity in the solution.
Aminatum, Furqon, I., Hikmawati, D., and Abdullah, C. (2021).[17]	Silver	• *Staphylococcus aureus*	• Diffusion method was used. • Inhibition zone diameter of silver nanoparticles at different concentrations. ranged from 12-16mm. • Clear zones around the sample in correlation with the particle size are prominent.	• Silver nanoparticles are concluded as a good antibacterial agent against *Staphylococcus aureus*. • AgNP aids in the inhibition of bacterial growth and biofilm formation.
Amorim, A. *et al*., (2019).[18]	Copper	• *Staphylococcus aureus* (ATCC 29213)	• Antimicrobial testing revealed that CG-CuNPs were active against *Staphylococcus aureus* ATCC 29213 at a dose with a minimal inhibitory concentration of 0.64 mM.	• CG-CuNPs can be employed as an antibacterial agent with fewer cytotoxic effects than traditional antibiotics.
Awad, M., Awatif Hendi, Khalid Mustafa Ortashi, and M.S. Sharafeldin. (2016, April).[19]	Silver	• *Staphylococcus aureus* • *Bacillus subtilis* • *Escherichia coli* • *Klebsiella pneumoniae*	• The disc diffusion assay method was conducted. • Both gram-negative *E. coli* and *Klebsiella pneumonia,* and gram-positive *Staphylococcus* and *B. subtilis* bacteria were used. • The sterile discs were dipped in AgNP (5, 10, 15 g/mL) and placed in a nutrient agar plate for 1 day at 37 degrees Celsius. • Clear regions were seen surrounding the wells. The tests were carried out three times to get the mean values of zone diameter.	• The antibacterial activity of AgNPs was more evident in gram-negative bacteria than that of gram-positive bacteria. • Due to the high concentration of negative charges in gram-negative bacteria, these bacteria aid in the interaction of nanoparticles with the cell wall.
Esmaeillou, M., Zarrini, G., Ahangarzadeh Rezaee, M., Shahbazi Mojarrad, J., and Bahadori, A. (2017).[20]	Silver	• *Staphylococcus aureus* • *Enterococcus faecalis* • *Staphylococcus epidermidis* • *Pseudomonas aeruginosa* • *Escherichia coli*	• The minimum inhibitory concentration of silver nanoparticles (AgNP), vancomycin and vancomycin-capped AgNP against *Staphylococcus aureus* resulted to 0.8 µg/ml, >3.2µg/ml, and 0.05µg/ml respectively.	• Vancomycin capped silver nanoparticles showed enhanced antibacterial activity against gram-positive bacteria (*Staphylococcus aureus).* • The nanoparticles against gram-positive bacteria displayed higher antibacterial activity than gram-negative ones. • This mechanism may produce alternative agents against gram-positive bacteria.

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Table 1: Cont'd.

Author and Year of Publication	Nanoparticle(s) used in the study	Pathogen Used	Antibacterial assay	Key findings
Gamboa, S., Rodriguez, R., Martinez, V., Vega-Baudrit, J. (2019).[21]	Silver	• *Staphylococcus aureus* • *Bacillus cereus* • *Bacillus luteus* • *Bacillus subtilis* • *Listeria monocytogenes* • *Escherichia coli*	• Disc diffusion technique was used to determine the antibacterial activity of AgNP stabilized with dextran sulfate. • The bacterial suspensions included 108 colony-forming units per milliliter, while the concentrations utilized for the test were 0.25mg/mL, 0.5mg/mL, and 1 mg/mL in 9-mm discs, incubating the strains for one day at 37 degrees Celsius. The radial diameter of inhibition zones for tested *S. aureus* in each concentration are 17, 18, and 19, respectively. • It has been discovered that the concentration of nanoparticles is directly related to its antibacterial activity, because of the greater diameter of the inhibition zone.	• The generation of reactive oxygen species (ROS) that disrupt bacteria, DNA, proteins, and the cell membrane is one probable mechanism for AgNP's bactericidal activity.
Gouyau, J., Duval, R. E., Boudier, A., and Lamouroux, E. (2021).[22]	Silver	• *Escherichia coli* • *Staphylococcus aureus*	• For the determination of antibacterial activity, the broth microdilution method was utilized with Cation-Adjusted Mueller Hinton Broth (CA-MHB). In the study, no minimum inhibitory concentration (MIC) is reached.	• Some of the Ag+ ions should be removed from the nanoparticles and improve their antibacterial activity. • There is a suggestion that in CA-MHB, the size and shape of the AgNPs should be light enough to be undetectable.
Kubo, A.-L., Capjak, I., Vrček, I. V., Bondarenko, O. M., Kurvet, I., Vija, H., Ivask, A., Kasemets, K., and Kahru, A. (2018).[1]	Silver	• *Escherichia coli* • *Staphylococcus aureus*	• Spot assay is used to determine the MBC or minimal bactericidal concentrations of silver nanoparticles against the different strains of *Staphylococcus aureus* and *Escherichia coli*. • In terms of pure coatings, a bacterial growth inhibition assay was used.	• Silver nanoparticles are proven to be effective antimicrobials. • The MIC and MBC results show that silver nanoparticles can cause slight toxicity to the strains of *Staphylococcus aureus* and *Escherichia coli*. • Silver nanoparticles need more time to develop antibacterial action towards gram-negative bacteria compared to gram-positive bacteria due to the components of their cell walls. • The net charge of nanoparticles cannot guarantee their antibacterial capacity as these positively charged silver nanoparticles show weaker antimicrobial properties than those that are negative in charge.

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Table 1: Cont'd.

Author and Year of Publication	Nanoparticle(s) used in the study	Pathogen Used	Antibacterial assay	Key findings
Wolny-Koładka, K. A., and Malina, D. K. (2018).[23]	Silver	• *Staphylococcus spp.* (*S. xylosus*, *S. warneri*, *S. sciuri*, *S. delphini*, *S. Vitulinus* and *S. aureus*) • *Escherichia coli*	• Disk diffusion method was used to determine bacterial susceptibility. • 22 strains were detected to be resistant to ampicillin. • 16 of them showed no inhibition growth around the antibiotic disk. • 6 strains have a growth inhibition size of less than 14 mm.	• The size of the nanoparticle affects its biological activity; if the diameter of the AgNPs is smaller, its activity will be higher.
Zia, R., Riaz, M., Farooq, N., Qamar, A., Anjum, S. (2018).[24]	Silver and Copper	• *Staphylococcus aureus* • *Escherichia coli*	• As the nanoparticles are assessed statistically, it is discovered that the greater the concentration of Ag and Cu nanoparticles, the more potent they can be to both gram-positive and negative bacteria.	• The more spherical and smaller silver and copper nanoparticles are, the more they can kill microorganisms. • Silver nanoparticles can actively inhibit bacterial growth compared to copper nanoparticles as they have a greater surface to the ratio that directly interacts with the bacterial cell wall. • Copper nanoparticles show greater effects on *S. aureus* than *E. coli*.

[a]Ahari, H. *et al.*, (2018), [b]Aminatun, *et al.*, (2021), [c]Amorim, A. *et al.*, (2019), [d]Awad, M. *et al.*, (2016, April), [e]Esmaeillou, M. *et al.*, (2017), [f]Gamboa, S. *et al.*, (2019), [g]Gouyau, J. *et al.*, (2021), [h]Kubo, A.-L. *et al.*, (2018), [i]Wolny-Koładka, K. A., and Malina, D. K. (2018), and [j]Zia, R. *et al.*, (2018).

compositions and impurities. It could also estimate their relative concentrations on the specimen's surface.

In the following studies, the AgNPs were further analyzed by TEM to obtain additional information about their size and shape. The TEM analysis showed that the synthesis has given similar results, which are spherical in shape and particle sizes ranging from 5-50 nm, with the exception[29] which gave a higher result. SEM-EDX analysis was utilized to determine the morphology and composition of the AgNPs and CuNPs. SEM-EDX analysis confirmed that there were AgNPs formed in the following studies.

The size, shape, and morphology of the CuNPs were studied by utilizing TEM and SEM-EDX analysis. In the TEM technique, the studies showed similarity in their shapes with varying sizes of 2-80 nm. TEM and SEM-EDX studies gave similar results for the shape and size range of the CuNPs. SEM-EDX analysis confirmed the formation of CuNPs in the following studies.

In addition, Dynamic Light Scattering (DLS), known as "photon correlation spectroscopy" or "quasi-elastic light scattering" employs the light scattering principle to measure the size and surface charge of nanoparticles.[30] Dynamic light scattering applies the properties of colloid dispersion to get the hydrodynamic diameter and potential difference of nanoparticles. On the other hand, X-ray Diffraction or XRD characterizes the properties of nanoparticles such as structure, size, and strain. X-ray diffraction applies the principle of Bragg's law, which states that when the X-ray beam passes through the crystal surface, a reflection of the angle of incidence will happen and have the same angle of scattering.[31] To stabilize the 4-12 nm size range of AgNPs the presence of capping agents namely cetyltrimethylammonium bromide (CTAB), di-n-dodecyldimethylammonium bromide (DDAB), sodium dodecyl sulfate (SDS), dioctyl sodium succinate (DOSS), 2-mercaptoethanesulfonate sodium (MES) are being utilized.[32]

The normal particle size of copper nanoparticles that are synthesized by wet chemical methods ranges from 40-50 nm.[33] In the case of copper nanoparticles, only one capping agent is used, sodium dodecyl sulfate (SDS). In terms of X-ray diffraction, silver nanoparticles peak at 2θ with the values of 38.45°, 44.67°, 64°, 77°, and 81°. Here the average particle size of silver nanoparticles is 18.63 nm, and the crystallization is acquired at the temperature of below 30° Celsius.[34] Copper nanoparticles peak at 2θ with the values of 43.39°, 50.49°, and 74.18°. The average

particle size of copper nanoparticles is 28.73 and this is obtained at the temperature of 25° Celsius.[35]

Spectral characteristics

The following studies used different UV–vis spectrum analyses to investigate the stability and the formation of absorption bands proving the presence of silver nanoparticles. Silver nanoparticles (AgNPs) of two different sizes, 10nm, and 50 nm, were presented at 390-395 nm and 400-440 nm correspondingly.[1] In another research, AgNPs displayed a wavelength around 418 to 425 nm.[23] Lastly, the UV-vis spectrum of the same nanoparticles resulted at 392.37 nm.[19]

The presence of copper nanoparticles was confirmed using UV–Vis absorption spectroscopy. The optical properties of CuNPs were investigated at wavelengths ranging from 300 nm to 800 nm.[36] The UV-vis spectra of chemically produced CuNPs displayed peaks at 377 nm, 552 nm, and 775 nm. Peak wavenumber changes implies the presence of NPs agglomerates of various sizes. Furthermore, there was no absorption band seen at 320 nm, indicating that no copper nanoparticles were synthesized.[28] Finally, in the 200–700 nm wavelength range, a significant surface plasmon resonance peak at 275 nm and a low intensity peak at 610 nm were recorded.[29]

Particle stability

Different concentrations of silver nanoparticles (AgNPs) presented -36mv, -32 mv, and -26 mv as the corresponding zeta potential values of AgNPs-1, AgNPs-2, and AgNPs-3.[37] The principle for this concept is that the more the diameter of a nanoparticle increases, the more its stability will decrease. A two-day experiment was done to analyze further and confirm the zeta potential of CuNPs.[38] During the first day of the experiment, the initial zeta potential value of CuNPs increased from 14 mV to 17 mV. On the second day, the zeta potential value decreased to 12 mV. The decreased in zeta potential was said to be due to the increase in the hydrodynamic diameter of the CuNPs, which causes them to aggregate or clump together. Furthermore, a nanoparticle reaching a zeta potential value above –30 mV or +30 mV depicts good stabilization of the specific nanoparticle. With these, the zeta potential of nanoparticles is a good indicator to characterize nanoparticles and their possible application against specific bacteria.

Antibacterial properties of AgNP and CuNP

Mueller Hinton Broth (MHB) was used to determine the minimal inhibitory concentration (MIC) of AgNPs. Based on the MIC value, the inhibitory effects of the AgNPs against *S. aureus* differ according to their concentrations. Further, synthesized AgNPs exhibited effective antibacterial activity against different strains of *S. aureus*.[5,23,39]

Copper nanoparticles were examined in Mueller Hinton Broth (MHB) to determine their minimal inhibitory concentration (MIC). CuNPs' inhibitory effects on *Staphylococcus aureus* differed according to the MIC value used to determine the CuNPs' concentration. In the isolation of multidrug-resistant bacteria, *S. aureus* was resistant to multiple antibiotics such as gentamicin, ceftazidime, ciprofloxacin, erythromycin, vancomycin, cephalexin, penicillin, and aztreonam. The inhibition level depends on the dosage.[40] Moreover, the findings revealed that copper nanoparticles might be utilized as an alternate technique against bacterial contamination and as a potential new antibacterial agent against a variety of *S. aureus* strains.[18,40,41]

Mechanism of Antibacterial Action

Cellular membrane damage

Silver nanoparticles have synergistic antimicrobial effects once combined with conventional antibiotics like Kanamycin and Chloramphenicol.[42] The most common effects of this synergistic combination are bacterial cell wall disruption and increased bacterial cell membrane permeability. In this case, the sub-lethal concentration of silver nanoparticles, 6-7 $\mu g.mL^{-1}$, and its combination with different antibiotics cause damage to the bacterial membrane, leading to its increased permeability, which will cause the cell to lyse. The same study also elaborated that silver nanoparticles can never disrupt or damage the bacterial cell wall. However, they can cause depolarization and destabilization of the bacteria, which still makes them good alternatives for antibiotics.

On the other hand, another study stated that even without antibiotics, silver nanoparticles could disrupt the bacterial cell wall and cause increased cell permeability.[43] Here, the silver nanoparticles release silver ions with high electrostatic attraction and affinity with sulfur proteins, attaching them to the bacterial cell wall. Once the silver ions adhere to the cell wall, it enhances the permeability of the cell membrane, which again will lead to cell disruption. Deactivation of enzymes, generation of reactive oxygen species, and interruption of adenosine triphosphate production occur after the silver ions are ingested in the cells. Reactive oxygen species are principal agents in disrupting the cell membrane and DNA modification. Other than releasing silver ions, silver nanoparticles can also kill bacteria. Due to their

nanoscale sizes, they can directly penetrate the cell wall and cause changes in its structures or organelles.

Copper nanoparticles affect the bacterial cell wall due to their affinity with the carboxyl groups located in the cell membrane. Like silver nanoparticles that release silver ions, copper nanoparticles also release copper ions.[44] Reactive oxygen species are generated once the cell wall absorbs these copper ions. ROS production leads to the toxicity of certain nanoparticles as well as the modification of cellular processes such as apoptosis, differentiation, signaling, and survival.[45] Furthermore, after ROS production, the cell membrane will lose its integrity as the electrochemical potential inside the cell increases.[46] Lastly, copper nanoparticles cause more harm to gram-positive bacteria like *S. aureus* since there are significant amounts of amino and carboxyl groups in the cell membranes of these types of bacteria. In addition, silver nanoparticles are more effective against gram-negative bacteria.

Oxidative Stress

The overall mechanism of action of the characterized nanoparticles demonstrates the binding of the surface of the nanoparticles to the bacteria, ion release, and the production of significant oxidative stress.[47] Bacteria exposed to oxidative stress may lead to the destruction in the lipid membrane, leakage of proteins in the bacterial cell, and DNA oxidation, which can lead to the demise of microorganisms.[48] Multiple studies have revealed that the strong affinity of silver nanoparticles causes oxidative stress in *Staphylococcus aureus*, causing the electron transport chain within the cell membrane to be disrupted.[49,50] Furthermore, it was observed in these publications that silver nanoparticles produced an oxidative mechanism in *Staphylococcus aureus*, which was caused by an increase in reactive oxygen species (ROS), and was associated with improved antimicrobial properties.

The formation of ROS by silver nanoparticles was dependent on time and concentration.[49] The enhanced generation of ROS in *S. aureus* after a one-hour incubation with AgNPs (1.29-12.9 pM).

Oxidative stress caused by the copper nanoparticles plays a big role in triggering cell damage of bacteria.[51] Copper nanoparticles showed increased sensitivity against *Staphylococcus aureus,* as stated in different studies. Furthermore, there is a correlation between the nanoparticle size and its penetration into the bacteria leading to cell death.

CONCLUSION AND RECOMMENDATION

Silver and copper nanoparticles are among the most significant and useful metal nanoparticles. They are widely used in the biomedical field because of their antibacterial properties. In this review, various studies have revealed that silver and copper nanoparticles can be synthesized using the wet chemical method. In this method, reducing agents were used. Two or more reactants were combined to serve as reducing agents. This method was proved to be one of the most reliable and cost-effective to synthesize a diverse range of metal nanoparticles without the need for complicated equipment.

Considerable characterization of silver and copper nanoparticles have been performed using the TEM/SEM-EDX, DLS/XRD, and UV-Vis. The size and morphology of the nanoparticles are critical for the purpose of the nanoparticle. Studies on the synthesis of copper and silver nanoparticles proved their effectiveness against the spectrum of pathogenic microorganisms, including *Escherichia coli, Staphylococcus aureus*, etc.

Several studies have been reviewed for the benefit of future researchers. It is recommended to investigate how metal-doped and metal oxide nanoparticles can enhance the antibacterial characteristics of copper and silver nanoparticles, which are beneficial in a range of sectors.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

AgNPs: Silver nanoparticles; **$AgNO_3$:** Silver nitrate; **CA-MHB:** Cation-Adjusted Mueller Hinton Broth; **CTAB:** Cetyltrimethylammonium bromide;

CuNPs: Copper nanoparticles; **DDAB:** di-n-dodecyldimethylammonium bromide; **DLS:** dynamic light scattering; **DOSS:** dioctyl sodium succinate; **DNA:** Deoxyribonucleic acid; **MBC:** Minimum Bactericidal Concentration; **MES:** 2-mercaptoethanesulfonate sodium; **MIC:** Minimum Inhibitory Concentration; **MHB:** Mueller Hinton Broth; **MRI:** Magnetic resonance imaging; **NP:** Nanoparticle; **PRISMA:** Preferred Reporting Items for Systematic Reviews and Meta-Analyses; **ROS:** Reactive Oxygen Species; **SDS:** Sodium dodecyl sulfate; **SEM-EDX:** Scanning Electron Microscopy with Energy Dispersive X-Ray Analysis; **TEM:** Transmission electron microscopes; **UV-Vis:** Ultraviolet–visible spectroscopy; **XRD:** X-ray diffraction analysis.

SUMMARY

Nanotechnology is a field of science with enormous medical potential. Metal nanoparticles, specifically copper and silver, have shown a wide range of antibacterial activity against various microorganisms. In this review, various sizes of copper and silver nanoparticles were synthesized using the wet chemical method and were characterized using TEM/SEM-EDX, DLS/XRD, and UV-VIS. Various results showed that depending on the concentration of copper nanoparticles and silver nanoparticles, the zones of inhibition increased. Additionally, copper nanoparticles are effective against gram-positive bacteria such as the *S. aureus* while silver nanoparticles are more effective at inhibiting bacterial growth than copper nanoparticles. As a result, both silver and copper nanoparticles could be used as antibiotic alternatives to treat antibiotic-resistant microorganisms.

Authors' Contributions

Three authors (A.B.B., J.R.C., and D.I.S.) performed the screening of articles, while the data extraction was done by all authors (A.B.B., H.R.C., J.R.C., S.S.M., L.C.M., E.M.M., and D.I.S.). Writing of the first draft of the manuscript and manuscript revisions were done by all authors. All authors contributed to the analysis of the study and literature searches. All authors agreed to be held accountable for the content of the manuscript and approved the final version.

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