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ENVIRONMENTAL REMEDIATION OF BACTERIAL POLLUTANTS USING SILVER NANOPARTICLES: A PROMISING APPROACH FOR HEALTH AND ENVIRONMENTAL SUSTAINABILITY

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Abstract

Numerous limitations in utilising traditional disinfectants appeared due to various limitations such as microbial resistance, disinfectant toxicity, and their environmental impacts. Therefore, the present study aimed to develop and evaluate a new disinfectant by merging the nanotechnology approach to explore a new disinfectant. Silver nanoparticles (AgNPs) were synthesised via the chemical reduction method and evaluated for their antibacterial effect against clinical isolates of Staphylococcus aureus, which is a major risk factor for human health. Different concentrations of AgNPs were prepared: 1, 5, 10, 50, and 100 µg/mL, and tested against the aforementioned bacteria via agar-well diffusion method. The results revealed that the bacterial growth was inhibited in a dose-dependent manner. The developed disinfectant has been compared to common antibiotics. These antibiotics included Gentamycin (GN, 10 µg), Cephalexin (KF, 30 µg), Erythromycin (E, 15 µg), Ampicillin (AM, 10 µg), and Amoxicillin (AMX, 10 µg), and were evaluated using the disc diffusion method against *S. aureus* isolates. The highest bacterial inhibition, with a zone diameter of 20 mm, was produced by the AgNPs at the highest tested concentration of 100 µg/mL. Their inhibition zone ranged from 3-14 mm in comparison with 6-20 mm of AgNPs dilutions. These findings revealed that the chemically synthesised silver nanoparticles had outperformed all of the tested antibiotics, which confirms their potency as a good disinfectant against *S. aureus*.

Keywords: AgNPs, Antibacterial, Antibiotics, Disinfection, Silver nanoparticles, Staphylococcus aureus.

1. Introduction

Infectious diseases pose a significant challenge to humanity due to the emergence of new strains and adaptations. To overcome this problem, a wide range of disinfectants was used to eradicate illnesses. Globally, hygiene is an increasingly important public health issue. Public health has long struggled with the issue of the harmful bacteria *Staphylococcus aureus*. However, over the past decade, numerous reports have been published about the increased epidemiological risk of this bacterium, especially about the acquisition of antibiotic resistance genes in these bacteria. Treatment-related problems occurred in approximately 11-53% of *S. aureus* bacteraemia cases, depending on the bacterial source [1].

The commensal bacterium *S. aureus* is a pathogen that colonises the nostrils and can lead to opportunistic infections of the skin, blood, soft tissues, wounds, osteomyelitis, septic arthritis, pneumonia, endocarditis, and sepsis. Many characteristics of *S. aureus* contribute to its high invasiveness, including the production of enzymes that cause cytolytic effects, such as lipases and proteases, and the ability to evade or eliminate host defences. In addition, it can bind to cells, produce toxins and exotoxins, and form endospores and biofilms [2].

The fundamental feature that contributes to *S. aureus's* high virulence and invasiveness is the synthesis of surface proteins, sometimes referred to as surface components that recognise adhesive matrix molecules (MSCRAMM). These proteins attach to fibronectin, collagen, and fibrinogen particles present in host tissues, enabling the organism to adhere firmly and cause various infections, such as intravascular infections, bone and joint infections, or those related to prosthetic devices. Furthermore, *S. aureus* has another virulence factor, including coagulase, lactoferrin, or a group of genes known as catabolic arginine mobile element [3].

The development of concurrent resistance to many antibiotics, particularly in methicillin-resistant *S. aureus* (MRSA), is the most significant problem with these bacteria. The methicillin-resistant *S. aureus* is a hallmark of several illnesses, such as epidermal and wound infections, blood poisoning, and sepsis, and can cause death. It is the most common cause of nosocomial infections [4]. However, it appeared that the problem with this bacterium extended beyond clinical settings (hospital-associated MRSA), and a steady increase in MRSA infections, particularly community-associated MRSA (CA-MRSA) and livestock-associated strains (LA-MRSA), has been observed in recent years [5].

Like other species in the genus *Staphylococci, S. aureus* is known for its remarkable ability to survive in dry environments. It can thrive on surfaces or other materials that are often found in houses, and it is able to survive under unfavourable conditions for long periods, maybe months, remaining contagious for exposed humans [6]. Therefore, the settled dust can be a source of these bacteria, and the dust re-aerosolization causes an increase in the risk of bacterial inhalation [7]. It is difficult to estimate the expenses of therapy, prolonged hospitalisations, and hindering the spread of *S. aureus*. A previous study recorded that costs amount to billions of EUR annually.

Nanoparticles might provide a means of reducing expenses and the use of plenty of disinfectants by incorporating the antibacterial properties into medical and industrial devices [8]. A previous study showed a strong antimicrobial effect of nanoparticles toward many bacterial species. The International Organisation

for Standardisation states that nanomaterials could have exterior dimensions between 1 and 100 nm. Many nanomaterials have been used as efficient disinfectants by enhancing their physicochemical properties. Therefore, the new research tends to manufacture nanomaterials that have multifunctional effects and carry the potential disinfection ability [9].

Nanotechnology has played an important role in the advancement of numerous industries. Nanoparticles are widely applied in areas like biomedicine and engineering. They are used in a wide range of applications, including disinfection of water, preservation of food, prevention of hospital-acquired infections, and enhancement of medical devices. The nanoparticles are made up of three layers and are not just simple molecules. (A) The surface layer, which may be functionalised with a variety of small molecules, polymers, metal ions, and surfactants; (B) the shell layer, which differs from the core regarding chemical compounds, and (C) the core, which is the centre of the nanoparticles and refers to the nanoparticles themselves [10].

Various methods could synthesise nanoparticles, but in general, these methods could be classified into two categories: bottom-up and top-down approaches. Furthermore, previous approaches could be subdivided into different subclasses according to the operation, protocols, and reaction conditions. Because of its antibacterial properties, silver nitrate was used for a very long time. According to recent research, the physicochemical characteristics of silver nanoparticles provide them with greater antibacterial efficacy.

The AgNPs have a larger surface-to-volume ratio, which provides greater surface exposure to microorganisms and improves antibacterial action. Also, the unique characteristics of AgNPs, such as size, phases, and shape, play a major role in the killing or inactivation. The NPs can penetrate the bacteria's cell wall and create gaps in the membrane, resulting in the release of free radicals that have the potential to damage the cell membrane. Furthermore, the ions produced by the nanoparticles can produce reactive oxygen species (ROS) and disrupt the synthesis of enzymes. Furthermore, NPs have been demonstrated to have an impact on DNA transcription [10].

However, previous studies have demonstrated that certain nanoparticles may be effective against particular bacterial species and have limited or no effect on other species. It is ideal to use a broad spectrum of NPs to target multiple species of bacteria in devices to prevent contamination. The use of combinations of metallic nanoparticles could target a broad spectrum of pathogens and may be an effective solution for devices that are used in healthcare [11].

The current research aims to study the efficiency of silver nanoparticles as a disinfectant against *S. aureus*. The novelty of this paper lies in testing different concentrations of a silver nanoparticle solution against *S. aureus* that have been isolated from different sources in Baghdad. Furthermore, this study intended to evaluate the effectiveness of Ag NPs against several commonly utilised antibiotics.

2. Materials and Methods

2.1. Collection of clinical specimens

The current study included the isolation of *S. aureus* from several clinical samples in Baghdad. Sixty patients were suspected of having an infection with a urinary

tract infection (UTI). Also, forty nasal swabs have been taken. The sample collection was from December 2019 to March 2020. Urine samples were taken from the patient in the morning; each midstream urine was kept in a dry and sterile labelled urine cup with a screw cover. All of these samples were cultured on blood, MacConkey, and nutrient agar at 37 °C overnight. Dry swabs were used to obtain nasal specimens from the nares. The swabs were rolled five times in each nostril and placed about 2.56 cm in the nares. All nasal swabs and UTI specimens were cultured [12]. The study flow chart is illustrated in Fig. 1.

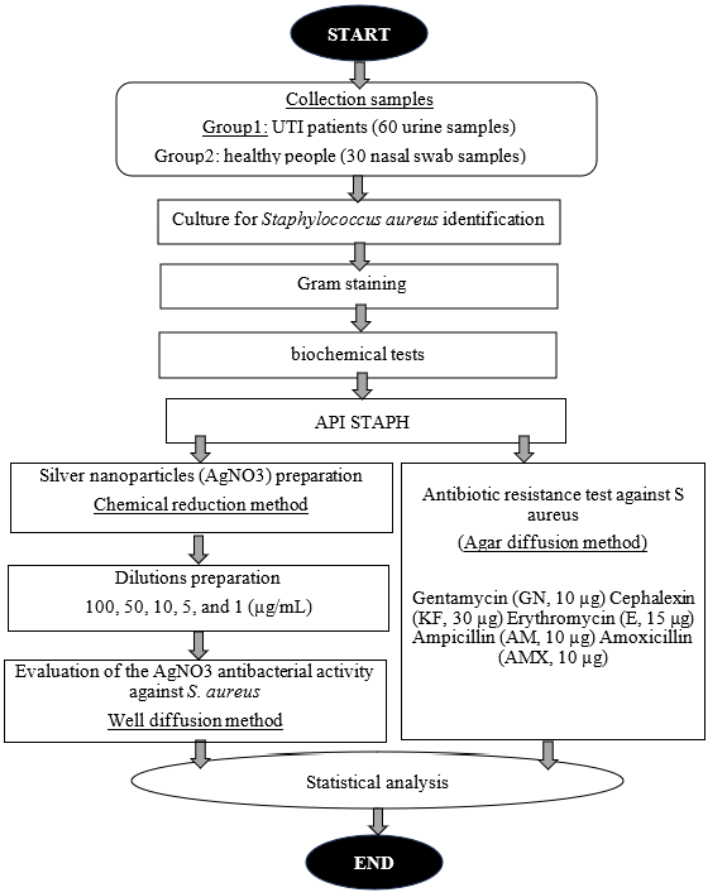

Fig. 1. Flow chart of the current study.

2.2. Bacterial growth conditions and culture media

Following the manufacturer's instructions, mannitol salt agar, blood agar, nutrition agar, and brain heart infusion broth (BHIB) were prepared and autoclaved at 121 °C for 15 minutes at 15 pounds per square inch. After that, the melted media was transferred into sterile Petri plates and allowed to solidify at room temperature. The media were incubated overnight at 37 °C to ensure optimal sterility. The nutrient broth medium was prepared by dissolving 13 grams of its powder in one litre of distilled water, adjusting the pH to 7.2, and then autoclaving. The

physiological preparation of the physiological saline solution (normal saline) was done by dissolving 0.85 grams of sodium chloride in 100 millilitres of distilled water and then adjusting the pH to 7.0 [13].

2.3. Chemicals and reagent preparation

2.3.1. Solution of silver nitrate ($AgNO_3$)

The preparation of a metal salt precursor, AgNO3, with a molarity concentration of 0.001 M was performed by taking 0.01g of AgNO3 powder and dissolving it in 100 millilitres of double-distilled deionised water within a volumetric flask. The resulting solution was then stored in a dark, sterile container [14].

2.3.2. Tri-Sodium Citrate Preparation

To prepare a reducing agent of Tri-Sodium citrate, $C_6H_5O_7Na$, with a molarity concentration of 0.001 M, approximately 0.25 g of $AgNO_3$ powder was dissolved in 100 mL of double-distilled deionised water in a volumetric flask, and then the solution was kept in a sterile and dark container [14].

2.4. Preparation of nanoparticle solution

Silver nanoparticles were synthesised by chemical reduction assay. Fifty millilitres of $AgNO_3$ with a molarity of 0.001 M was heated to boiling, then 5 ml of 1% trisodium citrate was added to it drop by drop. Then, the solution was vigorously mixed and heated until a change in the solution colour to pale brown occurred. The resulting mixture was stirred further until it reached room temperature. Double-distilled water was used to prepare all of the reactive material solutions. To obtain a powder form of the silver nanoparticles, we air-dried the aqueous solution for up to 3 days. To create a suspended stock solution, one gram of silver nanoparticle powder was dissolved in 100 millilitres of sterile, ultra-pure water and then mixed using an auto vortex for six hours at room temperature. Silver nanoparticle solution was serially diluted (100, 50, 10, 5, and 1 μg/ml) [15, 16].

2.5. Bacterial identification

All clinical specimens were inoculated on Blood agar medium, nutrient agar, and a selective medium, Mannitol salt agar, and incubated overnight at 37 °C. The bacterial isolates were checked by observing the morphologies and Gramme stain. The identification of these bacteria was done on the results of conventional biochemical tests and confirmed by the API system, such as the API STAPH test. To perform the catalase test, a single drop of 3% catalase reagent was applied to a clean, dry slide. Subsequently, the bacterial colony was mixed with the reagent. The formation of bulbs on the slide is an indicator of a positive result.

The oxidase test was performed by adding a few drops of the oxidase reagent onto a filter paper; then, the colonies of the bacteria were added by using an applicator stick. After 10-60 seconds, the change of the filter paper colour to violet is an indicator of a positive result. Urease production test was carried out by inoculating bacterial growth in slants containing urea medium, then incubating and checking for any colour change.

The fermentation of sugars and production of H_2S test was performed using TSI agar tubes. They were inoculated with bacteria isolates by streaking and stabbing the butt, then incubated, and the changes in the butt and slants were recorded.

Indole, MRVP, and citrate tests were performed as part of the IMVIC test. Tubes filled with a peptone water medium were used to conduct the Indole Test. The bacterial isolates were added to the medium and allowed to incubate. Each bacterial growth tube was then filled with 0.5 ml of Kovac's reagent. Any alterations to the surface of the medium were observed.

The MR-VP test was conducted by utilising the MR-VP medium tubes that were inoculated with a bacterial isolate, incubated, and then divided into two tubes.

Methyl red reagent (0.5 ml) was added to the first tube. 0.6 ml and 0.2 ml of VP-1 and VP-2 reagents, respectively, were added to the second tube. These tubes were checked for any colour changes. The Citrate utilisation was done by inoculating a bacterial culture using streaked slant tubes containing Simmons citrate medium. Then, any colour change in the incubated medium was checked [17].

2.6. Evaluation of the antibacterial activity

To test the antibacterial properties of silver nanoparticles, five serial dilutions (100, 50, 10, 5, and 1 µg/ml) were made. The antibacterial activity was assessed using Muller-Hinton agar medium with the agar well diffusion technique. To create a bacterial suspension, a single isolated colony was taken and inoculated into tubes containing five millilitres of Brain Heart Infusion broth (BHIB). These tubes were then incubated for 4 to 6 hours at 37 °C until inoculum turbidity (measured at 620 nm) was > 0.1 OD. This was followed by streaking each of these bacterial isolates on a Muller-Hinton agar plate to yield around 1×10^7 CFU/ml compared to the McFarland standard. The solid agar was then drilled using a sterile glass borer to create wells that were 6 mm in diameter and 5 mm deep. Each dilution was loaded into the wells in quantities of twenty microliters. The last step was to incubate these plates overnight at 37°C, then measure the diameter of the inhibition zone of each silver nanoparticle dilution for each bacterial isolate [18].

2.7. Assessing the bacterial sensitivity to antibiotics

The disk agar diffusion method was used for the antibiotic bacterial sensitivity test. The bacterial inoculum was prepared by dissolving a few pure bacterial colonies in regular saline. The inoculum turbidity was then compared to the standard McFarland tube (no. 0.5), which is 106 CFU/ml. After spreading 0.1 ml of the bacterial solution onto Muller-Hinton agar medium plates, they were left to dry at room temperature. Gentamycin (GN), cephalexin (KF), erythromycin (E), ampicillin (AM), and amoxicillin (AMX) are the five common antibiotics we evaluated. Their respective concentrations were 10, 30, 15, 10, and 10 mcg. Each antibiotic disc was placed on the inoculated plates and incubated inverted for 24 hours at 37 °C. The diameters of the entire inhibition zone after the findings during incubation were measured in Millimetres (mm) [19].

2.8. Research variables and values

The research variables and their values that were adopted in this investigation are illustrated in Table 1.

Table 1. The research variables and values of this study.

Variable	Variable type	Description	Values Adopted
Samples source	Independent	Clinical source of *S. aureus* isolates	Urine (UTI patients), Nasal Swabs
Clinical samples NO.	Descriptive	total number of the collected samples	60 urine, 40 nasal
Bacterial type	Dependent	Targeted organism	*Staphylococcus aureus*
Media used in culturing.	Controlled	Media for isolation and growth	Blood agar, Mannitol Salt Agar, BHIB
Concentrations of AgNPs	Independent	Different concentrations prepared	1, 5, 10, 50, 100 µg/mL
Inhibition Zone of (AgNPs)	Dependent	Antibacterial effect measured as an inhibition zone	6-20 mm (mean values per concentration)
Antibiotics	Independent	Type of antibiotic used against *S. aureus*	GN, KF, E, AM, AMX
Inhibition Zone of the antibiotics	Dependent	The inhibition zone caused by the effect	3-14 mm (mean values per antibiotic)
AgNPs synthesis method	Controlled	Synthesis technique of AgNPs	Wet chemical reduction by utilising trisodium citrate
Testing Method	Controlled	Antibacterial activity test	Agar well diffusion
Identification Method	Controlled	Bacterial characterisation and identification protocol	Gram staining Biochemical tests API STAPH

3. Results and Discussion

Based on the results of biochemical testing, API STAPH, culturing, and Gramme staining, the *S. aureus* isolates were identified.

3.1. Isolation and identification

The results of bacterial isolation of urine samples from UTI and nasal swab samples showed that *S. aureus* was isolated with a percentage of 30% and 60%, respectively. The appearance of bacterial colonies on blood agar medium was gold in colour, 2-3 mm in diameter, with blood hemolysis. The *S. aureus* colonies appeared on the Mannitol salt agar with a yellow colour that had a 2-3 mm in a diameter.

3.2. Results of biochemical tests

Biochemical assays have been used to identify the bacterial isolates. Table 2 provides illustrations of the findings.

Table 2. The biochemical test results of *S. aureus.*

Characteristic Cell shape	*S. aureus*[$] Cluster shape
Gram stain	+
catalase	+
oxidase	-
Motility	+

H_2S (TSI*)	-
Gas (TSI*)	-
Fermentation of sugars (TSI*)	A/A
Indole	-
Methyl red	-
Voges- Proskauer	-
Citrate Utilization	-
Urease	-
Type of blood hemolysis	Â
Type of pigment	Golden

[$] (+) indicates that this factor or reaction should be seen; (-) indicates that the reaction should not appear. (A) Acidic; *(TSI): Triple sugar iron; H2S: refers to hydrogen sulphide production; Gas denotes gas production (typically CO_2) from sugar fermentation.

3.3. The results of the API STAPH test

Rapid identification methods API STAPH **test** was used to confirm *S. aureus*. As illustrated in Table 3. The (+) refers to a positive result, and the (-) refers to a negative result.

Table 3. The results of the API STAPH test for *S. aureus*.

Test bacteria	GLU	FRU	MNE	MAL	LAC	TRE	MAN	XLT	MEL	NIT	PAL	VP	RAF	XYL	SAC	MDG	NAG	ADH	URE
***S. aureus* results**	+	+	+	+	+	+	+	-	-	+	+, -	+	-	-	+	-	+	+	-

3.4. Antibiotic bacterial sensitivity test

The susceptibility of *S. aureus* isolates to five common antibiotics: gentamycin, cephalexin, erythromycin, ampicillin, and amoxicillin has been investigated. The results revealed that gentamycin and cephalexin were the best antibiotics that were effective against *S. aureus* compared to the other antibiotics. Meanwhile, *S. aureus* showed a high resistance pattern, as shown in Fig. 2.

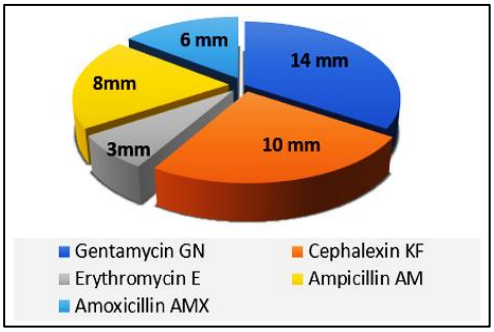

Fig. 2. Pie chart of the means of the inhibition zones diameters of the tested antibiotics: Gentamycin, Cephalexin, Erythromycin, Ampicillin, and Amoxicillin, against *S. aureus*.

3.5. Evaluation of the antibacterial activity of silver nanoparticles against *S. aureus*

The antibacterial activity of silver nanoparticles against *S. aureus* was assessed using serial dilutions, and the results are shown in Fig. 3.

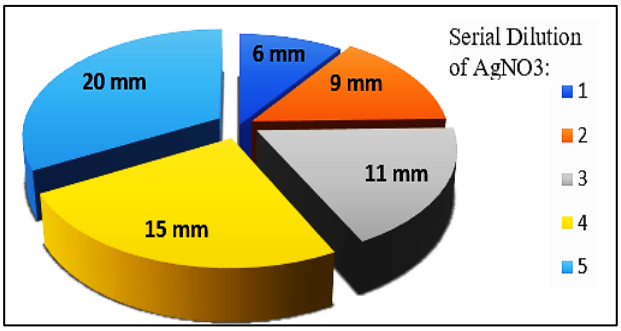

Fig. 3. Pie chart of the inhibition zones' diameter means of the serial dilutions of silver nanoparticles: 1, 2, 3, 4, and 5, which represent the concentrations:1, 5, 10, 50, and 100 µg/ml, tested against *Staphylococcus aureus.*

The results of this research showed that silver nanoparticles exhibited a strong antimicrobial impact against *S. aureus* in comparison with the antibiotics tested. As shown in Figs. 4, 5, and 6, the highest dilution, 100 µg/ml of the silver nanoparticle solution, yielded the largest inhibition zone against S. aureus, which is consistent with other studies that reported a similar antimicrobial effect; this inhibition zone was greater than that of all antibiotics tested. This finding could encourage the idea of using these nanoparticles as an effective disinfectant to reduce nosocomial infection with *S. aureus*. The findings of the present study are consistent with other previous research that found that silver nanoparticles and even silver ions had lethal and inhibitory effects on a variety of bacterial species, including *S. aureus* and Escherichia coli, as well as an inhibitory impact on yeasts [20, 21].

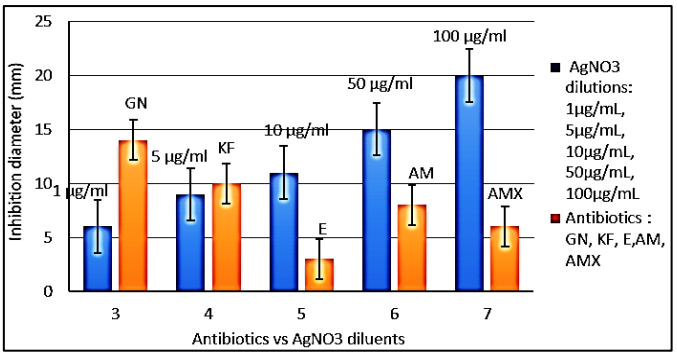

Fig. 4. Comparison of antibacterial effects between serial dilutions of antibiotics and silver nanoparticles against *Staphylococcus aureus*. GN, KF, E, AM, and AMX: gentamycin, cephalexin, erythromycin, ampicillin, and amoxicillin, respectively.

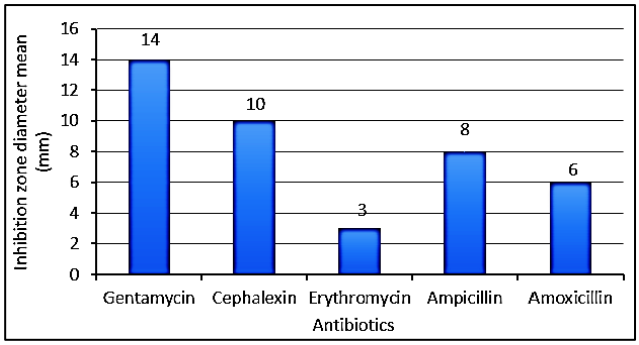

Fig. 5. Mean plot of the inhibition zone of the antibiotics tested: gentamycin, cephalexin, erythromycin, ampicillin, and amoxicillin, against *Staphylococcus aureus*.

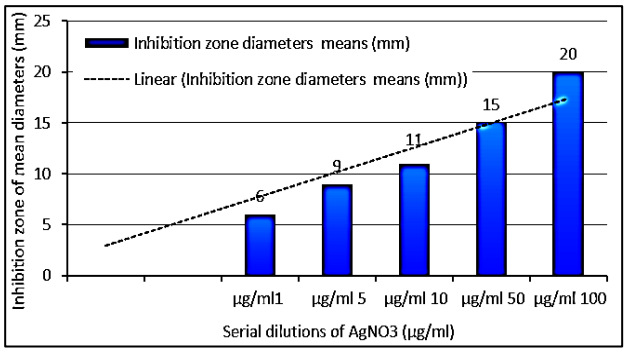

Fig. 6. Mean plot of the serial dilutions of inhibition zone of the silver nanoparticles: 1, 5, 10, 50, and 100 µg/ml, against *Staphylococcus aureus*.

3.6. Comparison with the literature

The inhibition effect of silver is probably due to the accumulation of various mechanisms of action. Many other investigations indicated that silver ions could inactivate the bacteria by interfering with their SH groups of proteins [22]. Even a low concentration of silver nanoparticles, reaching a few microns, could cause uncoupling of the respiratory electron transport from the oxidative phosphorylation, and inhibit respiratory chain enzymes, or could corrupt the membrane permeability of the bacteria. The impact of silver ions may be limited in gram-positive bacteria because of their thicker peptidoglycan coating than in gram-negative bacteria. Other studies reported that after silver ions were administered to bacteria, sulphur and silver ions were detected in the electron-dense granules. They proposed that the interaction between silver ions and nucleic acids caused the replication of DNA to be disrupted [23].

According to another earlier study, the antibacterial activity of silver nanoparticles is somehow correlated with the production of free radicals. Bacterial death may result from the ability of these free radicals to weaken and permeate the cell membrane of the bacteria. Also, oxidative stress was recorded in these bacterial cells after exposure to silver nanoparticles. However, the role of free radicals in the antibacterial activity of

silver nanoparticles was somewhat different in another study. Consequently, more studies are needed to clarify the precise function of free radicals [22].

A previous study mentioned that the combination of metallic nanoparticles was more effective against *S. aureus* than using single metallic nanoparticles. A very potent antibacterial effect was recorded against *P. aeruginosa.* Furthermore, they discussed the possibility of a synergistic impact of nanoparticles for a wide range of Gram-positive and Gram-negative bacteria when used in combination rather than as a single type [24]. On the other hand, there is an increasing demand to explore new water disinfectants to reduce pollution levels in water resources, especially in developing countries such as Iraq, which have suffered from serious problems in the health and environmental sectors [25].

The environmental effects on public health have received great attention in global research. The presence of pollutants, such as inorganic arsenic, was linked to the development of several diseases, such as diabetes, which is one of the leading chronic diseases worldwide [26]. Many other diseases are associated with the consumption of polluted water, such as cardiovascular disease, neurological disorders, skin lesions, nephropathy, and even cancer [27-29]. Therefore, exploring new antimicrobial materials could be the rescue for countries to tackle the microbial pollution problem. This current study is one of the attempts to find a solution to the pollution issue.

4. Conclusions

Nanotechnology is a modern approach that could help to develop new formulations of nanoparticles with antimicrobial activity. This may be useful in discovering new disinfectants and reducing the use of antibiotics and other traditional disinfectants. In addition, the science of nanomaterials can help healthcare practitioners and engineers develop new pharmaceutical disinfectants that could target a wide range of pathogenic microorganisms. Silver nanoparticles showed sufficient antibacterial action against one of the dangerous bacteria that is responsible for the majority of nosocomial infections, *Staphylococcus aureus.* The results of the current study revealed the potent antibacterial activity of AgNPs against the tested bacteria.

Moreover, among the five dilutions of AgNPs that had been tested, the concentration of 100 µg/mL caused the highest inhibition of the bacteria (20 mm), in comparison with the five tested antibiotics, which gave lower inhibition zones, even for the strongest antibiotic that was tested (gentamicin), which gave an inhibition zone diameter of 14 mm. The current findings indicate that AgNPs could be a successful alternative to various antibiotics and could be used as a disinfectant agent in hospital sanitation and eliminate bacterial contamination from medical devices, surfaces, and even polluted water, to reduce environmental and nosocomial infections.

In light of the current outcomes, the current research recommends conducting in vivo studies to evaluate the toxicity pattern and the biocompatibility of AgNPs. Also, a molecular study could be useful to elucidate the exact antibacterial mechanism. Future research has to focus on studying the synergistic effect between AgNPs and other chemicals, antibiotics, and other nanoparticles to enhance the antibacterial efficiency. Finally, attempts should be made to scale up and broaden the applications of AgNPs as a disinfection product in industrial, environmental, and medical fields.

Nomenclatures

A	Acidic
ADH	Arginine dihydrolase
AgNPs	Silver nanoparticles
API Staph	a standardised identification system of *Staphylococcus*, *Micrococcus*, and related genera
BHIB	Brain Heart Infusion Broth
CFU	Colony-forming units
FRU	Fructose
GLU	Glucose
LAC	Lactose
MAL	Maltose
MAN	Mannitol
MDG	Methyl-α-D-glucopyranoside
MEL	Melibiose
MNE	Mannose
MRSA	methicillin-resistant *S. aureus*
MSCRAMM	Microbial surface components recognising adhesive matrix molecules
NAG	N-acetyl-β-D-glucosaminidase
NIT	Nitrate reduction
PAL	P-nitrophenyl-α-D-galactopyranoside
RAF	Rhamnose
RAF	Rhamnose
S. aureus.	*Staphylococcus aureus*.
SAC	Sucrose
SAC	Sucrose
TRE	Trehalose
TRE	Trehalose
TSI	Triple sugar iron.
URE	Urease
URE	Urease
UTI	Urinary tract infection
VP	Voges-Proskauer
VP	Voges-Proskauer
XLT	Xylitol
XLT	Xylitol
XYL	Xylose
XYL	Xylose

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