THE IRAQI JOURNAL OF
VETERINARY MEDICINE
eISSN:2410-7409
pISSN: 1609-5693

OPEN ACCESS

Evaluation of Antibacterial and Antibiofilm Activity of Biogenic Silver Nanoparticles and Gentamicin Against *Staphylococcus aureus* Isolated from Caprine Mastitis

Ali H AL-Dujaily*[1], Alaa K Mahmood[2]

[1]Department of Veterinary Clinical Sciences, College of Veterinary Medicine, University of Kufa, Kufa, Iraq, [2]Department of Internal and Preventive Veterinary Medicine, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq

***Correspondence**:
Alih.aldujaily@uokufa.edu.iq

Received: 13 October 2021
Accepted: 8 January 2022
Published: 28 June 2022

DOI:
https://doi.org/10.30539/ijvm.v46i1.1309

Cite:
AL-Dujaily AH, Mahmood AK. Evaluation of antibacterial and antibiofilm activity of biogenic silver nanoparticles and gentamicin against *Staphylococcus aureus* isolated from caprine mastitis. Iraqi J. Vet. Med. 2022; 46(1): 10-16.

A B S T R A C T

The goal of this study was to assess the antibacterial efficiency of biogenic silver nanoparticles (AgNPs) and gentamicin against *Staphylococcus aureus* that can form biofilms. The characterization of AgNPs was confirmed by the scanning electron microscope (SEM) which was spherical and homogenous in form, with a diameter between 25 and 45 nm. The X-ray diffraction (XRD) presented the size of AgNPs to be 50 nm. Energy dispersive spectroscopy (EDS) was used to examine the presence of elemental silver. The three-dimensional structure of silver nanoparticles was discovered using an atomic force microscope (AFM), with a diameter of 47.18 nm on average. The minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of AgNPs and gentamicin against *S. aureus* isolated from caprine mastitis were determined using the microdilution assay. The checkerboard microdilution technique was utilized to inspect the synergistic antibacterial activity of AgNPs with gentamicin utilizing the fractional inhibitory concentration index (FICI). The antibiofilm capability of AgNPs was also investigated. The results indicate that AgNPs generated by biosynthesis are antibacterial against *S. aureus*. Moreover, AgNPs and gentamicin exhibit synergistic action. The study's findings suggest that biogenic AgNPs may act as anti-biofilm agents and treat mastitis caused by *S. aureus*. In conclusions biosynthesized AgNPs exhibit strong antibacterial and antibiofilm effectiveness and synergistic activity when combined with gentamicin.

Keywords: silver nanoparticle, synergistic, antibacterial, antibiofilm, *Staphylococcus aureus*

INTRODUCTION

Staphylococcus aureus (*S. aureus*) infection is of the mammary gland deserves special attention owing to the bacteria's diverse virulence factors, which induce together acute clinical mastitis in lactating goats (gangrenous mastitis) and subclinical mastitis (1).

One possible explanation for repeated infections is *S. aureus*' capacity to survive as a biofilm in mammary tissue. Biofilm-forming ability has been gradually recognized as an

effective virulence factor in staphylococci (2). Most pathogenic bacterial species aggregate, attach, reproduce, and create a three-dimensional (3D) composite grid on biotic or biotic surfaces. The term "biofilm" alludes to this complex three-dimensional multidimensional network (3). After maturation, inactive bacteria in biofilms might migrate to additional surface and create a new biofilm. Biofilm extracellular components, such as nucleic acids, polysaccharides, proteins, and fats, interrelate with one another and with the implanted bacterial cells (4).

The limits and inefficiency of the existing treatment for *S. aureus* biofilm implant-accompanying infections led research to alternate methods. Nanotechnology is a potential technique for treating biofilm-associated infections (5). Silver nanoparticles (AgNPs) have recently been investigated as substances capable of suppressing microorganism activity (6). Another benefit of AgNPs is that it has a broad spectrum of bactericidal or bacteriostatic action at low doses and do not activate bacterial resistance mechanisms (7). Although the precise mechanism of AgNPs' bactericidal effect is still unknown, various publications proposed mechanisms for AgNPs antibacterial action, the most common of which are ATP synthesis interruption, defects in DNA duplication, the generation of reactive oxygen species (ROS), the breakdown of the proton motivated power system, and direct cell membrane damage (8). The toxic effect of silver ion on wide range of microorganisms combined with minimum toxic effects on host cells. However, excessive silver consumption causes silver accumulation in skin tissues, which can lead to argyria or argyrosis when are exposed to sunlight. AgNPs have also been found to be toxic to fibroblasts, hepatocytes, osteoblasts, and bone marrow cells (9).

To the best of our knowledge, there are scanty studies on just the effect of AgNPs on S. aureus, that leads to biofilm-related infections associated with mastitis in local breed goats. This study's purpose was to assess the antibacterial activity of biogenic AgNPs and gentamicin versus *Staphylococcus aureus* biofilms.

MATERIALS AND METHODS

The biogenic silver nanoparticles were employed in this study were generated utilizing *Bacillus clausii* in a green environmentally friendly manner. Biosynthesized AgNPs were inspected using the scanning electron microscope (FEI, Netherland), AgNPs were round and uniform in shape, with a size range of 25 to 45 nm. The AgNPs had a diameter of 50 nm as was measured by X-ray diffraction (Shimadzu XRD-6000). Energy dispersive spectroscopy (Broker, Germany) was used to find out if there was any elemental silver. The atomic force microscope (Broker, Germany) was used to reveal the silver nanoparticles' three-dimensional structure, which averaged 47.18 nm in diameter.

Bacterial Isolates

Twenty-two *S. aureus* were isolated from milk of lactating local breed goats aged 1.5 to 5 years which suffered from clinical mastitis. All preliminary discovered *S. aureus* isolates were evaluated using the VITEK-2 Compact System (BioMeirieux, France) as per the company's guidelines. Finally, the findings were automatically evaluated and tallied by the gram-positive identification (GP-ID) library.

Antibacterial Activity

Gentamicin's antibacterial activity alone and in combination with AgNPs was evaluated employing the disc diffusion procedure. The Clinical and Laboratory Standards Institute recommends a discriminatory concentration of gentamicin of 10 µg (10). To make the bacterial suspension, a colony was cultured overnight in nutrient-rich broth (Sigma-Aldrich). McFarland Standard 0.5 was used to match turbidity. The colony was then grown on Mueller Hinton (MH) agar medium (Sigma-Aldrich) and six-millimeter disks were permeated with gentamycin and AgNPs at standardized concentrations. The disks were placed in the culture medium. The disks steeped in gentamicin at a standardized concentration which was used to make mixed disks containing 10 µg/mL of Nano silver. The plates were then incubated for 24 h at 37°C. The diameter of the disc was subtracted from the diameter of the growth inhibitory zone to estimate the diameter of the growth inhibitory zone. Experiments were performed in triplicate.

Minimal Bactericidal Concentration Determination

The minimal bactericidal concentration (MBC) of AgNPs was evaluated by using the colony forming unit (CFU) test. The microorganisms used in the experiment were cultured in freshly prepared MH medium at 37°C in a shaking incubator (Genex-USA) at 135 round per minute (rpm) for 18 h to achieve an optical density (OD) of 0.5 (corresponding to 10^8 cell/mL) at 600 nm. Different concentrations of AgNPs ranging from 1, 2, 4, 8, 16, 32, 64, 128, 256, 512, 1024 µg/mL were added to 10 mL of the aforementioned medium. Experiments were comprised control cultures that did not include nanoparticles, and the amount of colony- forming unit (i.e., the numeral of bacteria existing) recuperated following an 18 h incubation was counted by a set volume of media that were taken from the above-mentioned incubated cultures and serially saline-diluted (0.85% NaCl, w/v). Then incubation at 37°C for 18–24 h or until colonies emerged, was performed with samples (100 µL) distributed uniformly on MH agar plates, at which point the quantity of colony- forming unit was manually calculated. The number of viable colonies was multiplied by the dilution factor to get the final CFU. MBC

was determined to be the culture that demonstrated a 99.9% reduction in CFU following incubation (11, 12).

AgNPs with Gentamicin: MIC and Synergistic Effects

The synergistic impact of AgNPs and gentamicin was evaluated using the broth microdilution checkerboard technique. The fractional inhibitory concentration (FIC) is a communal way to express the degree of synergy between antibacterial drugs. The FIC is calculated by dividing the minimum inhibitory concentration (MIC) of the medication in conjunction with the MIC of the agent acting independently. Gentamicin was utilized to test the combination synergistic effects with prepared AgNPs against the pathogenic *S. aureus* bacteria that has been chosen. These compounds were produced as stock solutions in sterile Millipore water at concentrations ranging from 1, 2, 4, 8, 16, 32, 64, 128, 256, 512, 1024 µg/mL.

The microdilution "checkerboard" technique was used in microwell-containing plates to ascertain the fractional inhibitory concentration (FIC). The minimal inhibitory concentration (MIC) of both antibiotic and AgNPs alone, as well as their matched combinations, were determined (13). The test range for gentamicin and AgNPs was 1-1024 µg/mL. The produced microwell plates were tested for sterility by incubating them at 37°C for 24 h. The test organism was incubated on Muller-Hinton broth (MHB) for 18–24 h to produce bacterial inoculum. Then, the bacteria were collected and immersed in sterilized MHB to create turbidity, 0.5. Before inoculating the microwells with 0.01 mL of the 0.5 McFarland's solution, it was dissolved in freshly MH broth to reach a final colony- forming unit of 5×10^6. The microtiter plates with bacterial inoculation were incubated for 18 h at 37°C.

The MIC values of the test agents, both alone and in combination, were determined as the lowest concentration during which time no observable growth development. The FIC was computed using the MIC of test ingredient A as well as the MIC of test ingredient A in conjunction with test ingredient B. As a result, the FIC of antimicrobial gentamicin= MIC of antimicrobial gentamicin in conjunction/MIC of antimicrobial gentamicin separately. The FIC of antimicrobial ingredient AgNPs was computed in the similar way and the summation of the two FIC ingredients was combined to create the ΣFIC index.

ΣFIC index = FIC of antimicrobial gentamicin+ FIC of antimicrobial AgNPs

The estimated FIC index was utilized to determine the kind of interplay between the two ingredients under test (gentamicin and AgNPs), which may be synergism, indifference, or antagonism type. The values from the American Society of Microbiology were utilized to identify the category of interaction FICI < 0.5 synergy, 0.5 ≤ FICI < 1 partial synergy, FICI = 1 additive, 1 < FICI < 4 indifferent, and FICI >4 denoting antagonism (14, 15).

Biofilm Formation Using Congo Red Agar Method (CRA)

The CRA screening procedure was used to determine whether or not the Staphylococcal isolates form biofilm. Congo red agar was a particularly prepared medium consisting of brain heart infusion (BHI) broth (37 g/L) added with sucrose (50 g/L), agar No1 (10 g/L) and Congo red (0.8 g/L). A concentrated aqueous CR stain solution was made and then autoclaved for 15 min at 12°C. Lastly, it was transferred to autoclaved BHI agar containing sucrose at 55°C. The *S. aureus* isolate was injected into the prepared CRA plates and then incubated at 37°C for 24 h in an aerobic environment. According to the development of black dry crystal-like colonies on the CRA plates, biofilm generation was taking place, whereas nonproducer colonies remained pink or red in hue (16).

Antibiofilm Activity of AgNPs

The activity of AgNPs in biofilm generation was evaluated utilizing a 96-wells microtiter plate method (17). Individual microtiter plate wells were stuffed with 180 µL of MH broth and 10 µL of a 24-hour period *S. aureus* growth culture were inoculated. The mixture received 10 µL of stock AgNPs, resulting in an ultimate concentration of nanoparticles varying amid 1, 2, 4, 8, 16, 32, 64, 128, 256, 512, 1024 µg/mL. Twenty-four hours were spent incubating the microtiter plates at 37°C. Following incubation, to exclude free-floating 'planktonic' bacteria, each well's contents was carefully removed then rinsed three times with 0.2 mL of phosphate buffer saline (PBS, pH 7.2). They used sodium acetate (2% w/v) to keep biofilms that were made by 'sessile' organisms from sticking to the walls of the plates. They also used crystal violet (0.1% w/v) to color the biofilms. Residual discoloration was removed from the plates by carefully washing them in sterile Millipore water and allowing them to dry. Following drying, the wells were filled with 200 µL of 95% (v/v) ethanol. An ELISA reader was used to measure the absorbance at 620 nm, and the results were interpreted as a sign of bacteria sticking to the well wall's surface and forming biofilms. Three replicated investigations were conducted, and the results were reported as absorption means. The following equation was used to calculate the percentage of biofilm inhibition:

Percent biofilm inhibition equals [1 – (OD620 of cells treated with AgNPs /OD620 of cells not treated with AgNPs) 100].

Statistical Analysis

The collected data was analyzed using SPSS (IBM SPSS; version 26.0, IBM Corp., Armonk, NY, USA) software. All data were subjected to one-way analysis of variance (ANOVA). Significant differences among group means were

tested using the least significant differences (LSD) test at $P \leq 0.05$.

RESULTS AND DISCUSSION

Antibacterial Activity

The results revealed that the disks' growth-inhibition zone impregnated with AgNPs plus gentamicin were greater than the zone around disks that have been alone impregnated with gentamicin. The diameters of *S. aureus* isolates' growth inhibition zones were measured (Figure 1).

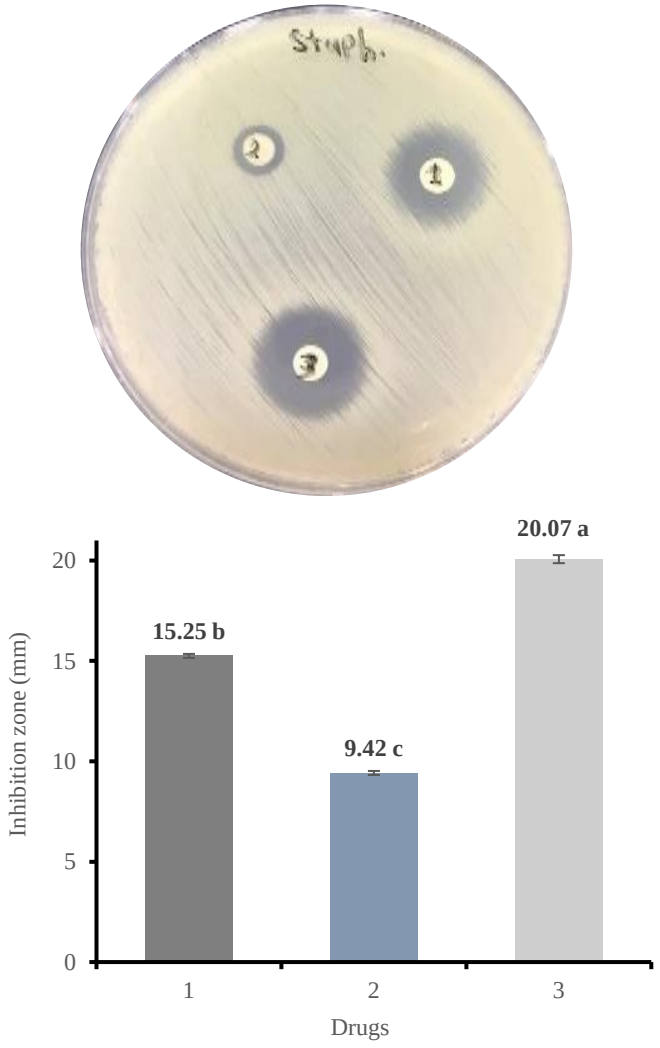

Figure 1. The discs' growth inhibition zone (mm). 1=gentamicin 10 µg; 2=silver nanoparticles (AgNPs) 10 µg/mL, 3=gentamicin+AgNPs aginst *Staphylococcus aureus*

Scientists have so far established the mechanism of action of gentamicin (18). Several mechanisms of AgNPs have been proposed. According to some hypotheses, these particles disturb the cellular respiration chain via interacting with oxygen. Furthermore, the interaction of AgNPs with the bacterial cellular membrane whether by binding to respiratory enzymes or lowering intracellular ATP levels, can cause the bacteria's cell wall to break down and resulting in cell death (19). According to certain research, the antibacterial action of AgNPs might be exhibited through the binding to DNA and causing damage (8). In general, all major models proposed include disruption to cell membranes and intracellular structures as a result of direct contact with NPs, as well as cationic silver release from NPs within and outside the cells, which initiates a chain reaction of intracellular reactions that results in the generation of ROS, enzyme inactivation, inhibition of DNA replication, and protein synthesis (20).

The interaction of gentamicin with AgNPs can be described as previously stated, AgNPs and gentamicin may all kill bacteria via various methods. If a strain of bacteria develops resistance to one or more of the medicines, a novel antimicrobial agent may be able to eliminate them via an entirely different mechanism. This sort of medicines mixture is commonly utilized, particularly when germs develop resistant. Gentamicin has active hydroxyl and amide groups. These groups react quickly with and attach to Nano silver. It was known whenever an antibiotic agent enters the bacterial cell; its action becomes increasingly apparent. AgNPs medication delivery to the cell may be responsible for the synergistic impact. Hydrophobic groups are well-known to exist in biological membranes. Gentamicin is a hydrophilic compound, whereas AgNPs are a hydrophobic substance. As a result, unlike antibiotics, AgNPs may easily penetrate through cellular membranes. It is possible to deduce that combination of silver-nanoparticle bound antibiotics may be administered to the cell with ease. Some researchers also stated that silver prevents DNA unraveling. When AgNPs interact with DNA, nanoparticles-bound antimicrobial compounds hinder DNA unwinding owing to spatial constraints, resulting in more severe cell damage (21).

Determination of MIC, MBC, and Synergistic Effects of AgNPs and Gentamicin

Antimicrobial properties of biogenic AgNPs and gentamicin against *S. aureus* were assessed utilizing MIC and MBC values. The MIC of biosynthesized AgNPs and gentamicin against *S. aureus* was 16.1 µg/mL and 8.3 µg/mL, respectively (Figure 2), whereas MBCs were 32.2 µg/mL and 16.2 µg/mL, respectively. The outcomes of the synergistic impact in the term of ΣFIC are exhibited (Figure 2).

The MIC and MBC of our results are in close agreement with certain previous studies, such as (22) and (23), but differ with others, such as (24) and (25), Because of using different manufacturing methods, different nanoparticle sizes, and different nanoparticle shapes, many products often are referred to that physical and chemical properties of nanoparticles might completely be different preparations (26). Our findings are consistent with the

findings of a previous research (27) in which, it was discovered the synergistic impact of AgNPs with various antibiotics against *S. aureus* (28). It was also investigated the combination antibacterial impact of antibiotics and AgNPs, hypothesizing that the enhancement of the synergistic action might occur owing to the linking interaction involving antibiotics and AgNPs. Furthermore, (29) discovered that when antibiotics such vancomycin, gentamycin, streptomycin, ampicillin, and kanamycin were combined with AgNPs, they acted synergistically against Pseudomonas aeruginosa, *Staphylococcus aureus*, and Escherichia coli. Several studies have discovered that AgNPs can increase the efficiency of various antibiotics, including vancomycin, gentamicin, rifampicin, and levofloxacin (30).

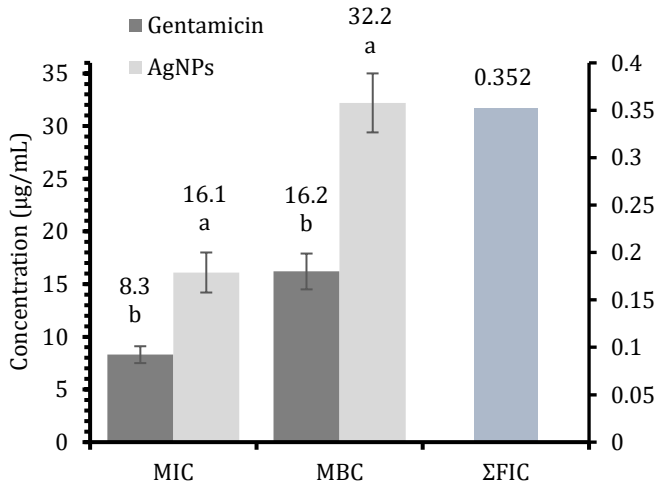

Figure 2. MIC, MBC and ΣFIC index for gentamicin and AgNPs

The current study found that mixing gentamicin with biologically synthesized AgNPs offered a beneficial therapeutic method for *S. aureus* treatment, which agreed with the results of (31) and (32), who revealed that synergistic effects of silver nanoparticles and ampicillin or gentamicin on *S. aureus* and *P. aeruginosa* when they were combined. Synergistic effects of NPs and antibiotics were almost certainly circumvent bacterial antimicrobial resistance mechanisms, whereas antimicrobial nano-drug vehicles, like AgNPs, did not only shield antibiotics against molecular resistance mechanisms but also conferred a complex synergistic antibacterial effect between the nano-drug delivery system and antibiotics (33).

Formation of Biofilms by the Congo Red Agar method (CRA)

All of the S. aureus in this study were grown on Congo red agar for 24 hours at 37°C, which demonstrated the capacity of creating biofilms by converting the red colony to a black colony (Figure 3). The development of *S. aureus* biofilm is in consistent with earlier studies (34) showed that S. aureus was known to generate biofilms under a range of clinical circumstances

Figure 3. *Staphylococcus aureus* biofilm formation (black colony) on congo red agar

It is a thick, sticky substance in which bacteria are embedded, known as biofilm. One of *S. aureus'* defensive mechanisms is biofilm formation, which is defined as surface-adhered phenotypically heterogeneous communities of microorganisms which could be observed in vitro and in vivo in infected tissues (35).

Antibiotics have little effect on established biofilms, and they can avoid immune response, moreover, biofilm can serve as infection reservoirs that are difficult to eradicate, which leading to treatment failure and recurrent disease episodes (36). Since, there were no appropriate anti-biofilm antibiotics, nanoparticles were utilized as a prospective anti-biofilm material, which exhibit anti-biofilm effectiveness (37, 38).

Anti-biofilm activity

The results showed that the AgNPs were used to inhibit and eliminate biofilm activity in S. aureus. AgNPs have anti-biofilm action; with ≥50 percent biofilm formation inhibitory effect was observed at a concentration16 µg/ml of S. aureus.

According to these findings, biogenic AgNPs were superior anti-biofilm agent against *S. aureus*. Current findings are in consistent with (39), who investigated AgNPs-mediated biofilm eradication and discovered 89% inhibition of *S. aureus* at 15 µg/mL, Furthermore, (40) revealed that AgNPs had anti-biofilm potential against G+ve bacteria; *S. aureus* biofilm formation was virtually completely reduced when AgNP-coated catheters were studied in vitro.

Anti-biofilm activity has become a required and significant assessment in the development of nano

antibiotics because it is a key virulence factor that merits the creation of molecules with better penetrating capacity and the ability to inhibit biofilm (41).

AgNPs had direct antimicrobial action because they had the ability to interact with a diverse array of planktonic microorganisms and biofilm constituents. They impaired the metabolism of microorganisms and impeded biological functioning on the outside through these interactions (42). Overall antibacterial activity of AgNPs might be due to a blend of cell wall disintegration, instabilities of structural proteins, inactivation of membrane proteins, inactivation of an enzyme, inhibition of the electron transport chain (ETC), nucleic acid degradation, and oxidative damage that induced by ROS (43). Hydroxyl radicals have the ability to depolymerize polysaccharides, cause DNA disruptions, and inhibit enzyme activity, all of which might jeopardize the biofilm architecture's extracellular polymeric substances (EPS) matrix (44, 45).

The results of this work showed that at therapeutically feasible AgNPs can efficiently and quickly remove biofilm formed by *S. aureus*. This suggests that these AgNPs might be used as biofilm disruptors. The biosynthesized AgNPs exhibited strong antibacterial and anti-biofilm activity against *S. aureus*, which was clinically relevant in caprine mastitis. The synergistic activity between gentamicin and AgNPs could be proposed to lessen the dosage rate of gentamicin, but it needs a large scale of in vivo toxicological studies. This study demonstrates how gentamicin and silver nanoparticles work together to increase bactericidal action and lessen the requirement for large dose of each drugs reducing the possibility of their side effect.

ACKNOWLEDGEMENTS

The researchers would like to express their appreciation to the staff of Al-Najaf Veterinary Teaching Hospital for establishing the first diagnosis and obtaining samples, as well as to the entire diagnostics laboratory staff at the College of Veterinary Medicine, University of Baghdad, and University of Kufa.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Rainard P, Gitton C, Chaumeil T, Fassier T, Huau C, Riou M, et al. Host factors determine the evolution of infection with *Staphylococcus aureus* to gangrenous mastitis in goats. Vet Res. 2018; 49(1):1–17.
2. Angelidis AS, Komodromos D, Giannakou R, Arsenos G, Gelasakis AI, Kyritsi M, et al. Isolation and characterization of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) from milk of dairy goats under low-input farm management in Greece. Vet Microbiol. 2020; 247:108749.
3. Roy R, Tiwari M, Donelli G, Tiwari V. Strategies for combating bacterial biofilms: A focus on anti-biofilm agents and their mechanisms of action. Virulence. 2018; 9(1):522–554.
4. Jiang Y, Geng M, Bai L. Targeting biofilms therapy: current research strategies and development hurdles. Microorganisms. 2020; 8(8):1222.
5. Malaekeh-Nikouei B, Bazzaz BSF, Mirhadi E, Tajani AS, Khameneh B. The role of nanotechnology in combating biofilm-based antibiotic resistance. J Drug Deliv Sci Technol. 2020; 101880.
6. Salih ANA, Eesa MJ. Antibacterial activity of biosynthesized silver nanoparticles against *Pseudomonas aeruginosa* in vitro. Iraqi J. Vet. Med. 2017; 41(1):60–65.
7. Wang J, Li J, Guo G, Wang Q, Tang J, Zhao Y, et al. Silver-nanoparticles-modified biomaterial surface resistant to *Staphylococcus*: new insight into the antimicrobial action of silver. Sci Rep. 2016; 6(1):1–16.
8. Murphy M, Ting K, Zhang X, Soo C, Zheng Z. Current development of silver nanoparticle preparation, investigation, and application in the field of medicine. J Nanomater. 2015; 2015.
9. Gaiser BK, Hirn S, Kermanizadeh A, Kanase N, Fytianos K, Wenk A, et al. Effects of silver nanoparticles on the liver and hepatocytes in vitro. Toxicol Sci. 2013;131(2):537–547.
10. Institute C and LS. Performance standards for antimicrobial susceptibility testing. CLSI supplement M100. Clinical and Laboratory Standards Institute Wayne, PA; 2017.
11. Bär W, Bäde-Schumann U, Krebs A, Cromme L. Rapid method for detection of minimal bactericidal concentration of antibiotics. J Microbiol Methods. 2009; 77(1):85–89.
12. Owuama CI. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using a novel dilution tube method. African J Microbiol Res. 2017; 11(23):977–980.
13. Humphries RM. Testing: broth microdilution checkerboard and broth macrodilution methods. Clin Microbiol Proced Handbook Washington, DC Am Soc Microbiol. 2016; 5:1–5.
14. Botelho MG. Fractional inhibitory concentration index of combinations of antibacterial agents against cariogenic organisms. J Dent. 2000; 28(8): 565–570.
15. Meletiadis J, Pournaras S, Roilides E, Walsh TJ. Defining fractional inhibitory concentration index cutoffs for additive interactions based on self-drug additive combinations, Monte Carlo simulation analysis, and in vitro-in vivo correlation data for antifungal drug combinations against *Aspergillus fumigatus*. Antimicrob Agents Chemother. 2010;54(2):602–609.
16. Ruchi T, Sujata B, Anuradha D. Comparison of phenotypic methods for the detection of biofilm production in uro-pathogens in a tertiary care hospital in India. Int J Curr Microbiol App Sci. 2015; 4(9):840–849.
17. Singh P, Pandit S, Garnæs J, Tunjic S, Mokkapati VRSS, Sultan A, et al. Green synthesis of gold and silver nanoparticles from *Cannabis sativa* (industrial hemp) and their capacity for biofilm inhibition. Int J Nanomedicine. 2018; 13:3571.
18. Greenwood D, Finch R, Davey P. Antimicrobial chemotherapy. Oxford university press, USA; 2007.
19. Rai M, Yadav A, Gade A. Silver nanoparticles as a new generation of antimicrobials. Biotechnol Adv. 2009; 27(1):76–83.
20. Jiravova J, Tomankova KB, Harvanova M, Malina L, Malohlava J, Luhova L, et al. The effect of silver nanoparticles and silver ions on mammalian and plant cells in vitro. Food Chem Toxicol. 2016;96:50–61.
21. Batarseh KI. Anomaly and correlation of killing in the therapeutic properties of silver (I) chelation with glutamic and tartaric acids. J Antimicrob Chemother. 2004; 54(2):546–548.
22. Elbehiry A, Al-Dubaib M, Marzouk E, Moussa I. Antibacterial effects and resistance induction of silver and gold nanoparticles against *Staphylococcus aureus*-induced mastitis and the potential toxicity in rats. Microbiologyopen. 2019; 8(4):e00698.
23. Ipe DS, Kumar PT, Love RM, Hamlet SM. Silver nanoparticles at biocompatible dosage synergistically increases bacterial susceptibility to antibiotics. Front Microbiol. 2020; 11:1074.
24. Sharifi-Rad J, Hoseini-Alfatemi SM, Sharifi-Rad M, Iriti M. Antimicrobial synergic effect of Allicin and silver nanoparticles on

skin infection caused by methicillin resistant *Staphylococcus aureus* spp. Ann Med Health Sci Res. 2014; 4(6):863–868.
25. Yuan Y-G, Peng Q-L, Gurunathan S. Effects of silver nanoparticles on multiple drug-resistant strains of *Staphylococcus aureus* and *Pseudomonas aeruginosa* from mastitis-infected goats: an alternative approach for antimicrobial therapy. Int J Mol Sci. 2017; 18(3):569.
26. Pal S, Tak YK, Song JM. Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram-negative bacterium *Escherichia coli*. Appl Environ Microbiol. 2007; 73(6):1712–1720.
27. Barapatre A, Aadil KR, Jha H. Synergistic antibacterial and antibiofilm activity of silver nanoparticles biosynthesized by lignin-degrading fungus. Bioresour Bioprocess. 2016; 3(1):1–13.
28. Fayaz AM, Balaji K, Girilal M, Yadav R, Kalaichelvan PT, Venketesan R. Biogenic synthesis of silver nanoparticles and their synergistic effect with antibiotics: a study against gram-positive and gram-negative bacteria. Nanomedicine Nanotechnology, Biol Med. 2010; 6(1):103–109.
29. Birla SS, Tiwari V, Gade AK, Ingle AP, Yadav AP, Rai MK. Fabrication of silver nanoparticles by Phoma glomerata and its combined effect against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Lett Appl Microbiol. 2009; 48(2):173–179.
30. Smekalova M, Aragon V, Panacek A, Prucek R, Zboril R, Kvitek L. Enhanced antibacterial effect of antibiotics in combination with silver nanoparticles against animal pathogens. Vet J. 2016; 209:174–179.
31. Van Dong P, Ha CH, Kasbohm J. Chemical synthesis and antibacterial activity of novel-shaped silver nanoparticles. Int Nano Lett. 2012; 2(1):1–9.
32. Saha S, Malik MM, Qureshi MS. Study of synergistic effects of antibiotics and triangular shaped silver nanoparticles, synthesized using UV-light irradiation, on *S. aureus* and *P. aeruginosa*. Mater Today Proc. 2019; 18:920–927.
33. Arul Selvaraj RC, Rajendran M, Nagaiah HP. Re-potentiation of β-lactam antibiotic by synergistic combination with biogenic copper oxide nanocubes against biofilm forming multidrug-resistant bacteria. Molecules. 2019; 24(17):3055.
34. Archer NK, Mazaitis MJ, Costerton JW, Leid JG, Powers ME, Shirtliff ME. *Staphylococcus aureus* biofilms: properties, regulation, and roles in human disease. Virulence 2: 2011 445–459.
35. Yarwood JM, Bartels DJ, Volper EM, Greenberg EP. Quorum sensing in *Staphylococcus aureus* biofilms. J Bacteriol. 2004; 186(6):1838–1850.
36. Otto M. Staphylococcal biofilms. Bact biofilms. 2008: 207–28.
37. Faller M, Kohler T. The status of biofilms in penile implants. Microorganisms. 2017; 5(2):19.
38. Aldujaili NH, Alrufa MM, Sahib FH. Antibiofilm antibacterial and antioxidant activity of biosynthesized silver nanoparticles using pantoea agglomerans. J Pharm Sci Res. 2017; 9(7):1220.
39. Goswami SR, Sahareen T, Singh M, Kumar S. Role of biogenic silver nanoparticles in disruption of cell–cell adhesion in *Staphylococcus aureus* and *Escherichia coli* biofilm. J Ind Eng Chem. 2015; 26:73–80.
40. Martinez-Gutierrez F, Boegli L, Agostinho A, Sánchez EM, Bach H, Ruiz F, et al. Anti-biofilm activity of silver nanoparticles against different microorganisms. Biofouling. 2013; 29(6):651–660.
41. Sharma VK, Sayes CM, Guo B, Pillai S, Parsons JG, Wang C, et al. Interactions between silver nanoparticles and other metal nanoparticles under environmentally relevant conditions: A review. Sci Total Environ. 2019; 653:1042–1051.
42. Yin IX, Zhang J, Zhao IS, Mei ML, Li Q, Chu CH. The antibacterial mechanism of silver nanoparticles and its application in dentistry. Int J Nanomedicine. 2020; 15:2555.
43. Singh P, Pandit S, Beshay M, Mokkapati V, Garnaes J, Olsson ME, et al. Anti-biofilm effects of gold and silver nanoparticles synthesized by the Rhodiola rosea rhizome extracts. Artif cells, nanomedicine, Biotechnol. 2018; 46(sup3):S886–S899.
44. Applerot G, Lellouche J, Perkas N, Nitzan Y, Gedanken A, Banin E. ZnO nanoparticle-coated surfaces inhibit bacterial biofilm formation and increase antibiotic susceptibility. Rsc Adv. 2012;2(6):2314–21.
45. Sadekuzzaman M, Yang S, Mizan MFR, Ha SD. Current and recent advanced strategies for combating biofilms. Compr Rev Food Sci Food Saf. 2015; 14(4):491–509.

تقييم النشاط المضاد للبكتيريا والغشاء الحيوي لجسيمات الفضة النانوية الحيوية والجنتاميسين ضد المكورات العنقودية الذهبية المعزولة من التهاب الضرع للماعز

علي حسين الدجيلي[1]، علاء كامل محمود[2]

[1]قسم العلوم السريرية البيطرية، كلية الطب البيطري، جامعة الكوفة، الكوفة، العراق، [2]فرع الطب الباطني والوقائي، كلية الطب البيطري، جامعة بغداد، بغداد، العراق

الخلاصة

كان الهدف من العمل الحالي هوتحديد الفعالية المضادة للبكتيريا لجسيمات الفضة النانوية الحيوية والجنتاميسين ضدالمكورات العنقودية الذهبية التي يمكن أن تشكل الأغشية الحيوية. تم تأكيد توصيف الجسيمات النانوية الفضية بواسطة المجهرالإلكتروني الماسح الذيك ان لها شكل كرويومتجانس،وتراوح حجمها من 25 إلى 45 نانومتر. كان حجم جسيمات الفضة النانوية في حيودالأشعة السينية 50 نانومتر.تم استخدام التحليل الطيفي المشتت للطاقة لفحص وجود عنصرالفضة.كشف مجهرالقوةالذريةعن البنية ثلاثية الأبعاد لجسيمات الفضةالنانوية،التي يبلغ قطرها 47.18 نانومترفي المتوسط. تم استخدام اختبار التخفيف الدقيق لتقييم الحدالأدنى للتراكيزالمثبطة والحدالأدنى من التراكيزالقاتلة للجراثيم من جسيمات الفضة النانوية والجنتاميسين ضدالمكورات الذهبية العنقودية المعزولة من التهاب الضرع للمعز. تم استخدام تقنية التخفيف الدقيق للوحة الشطرنج للتحقيق في النشاط التآزري المضاد للبكتيريا لجسيمات الفضة النانوية مع الجنتاميسين باستخدام مؤشرالتركيزالمثبط الجزئي. كما تم التحقيق في إمكانات جسيمات الفضة النانوية في منع تكوين الاغشية الحيوية. أظهرت النتائج أن جسيمات الفضة النانوية الحيوية لها نشاط مضاد للجراثيم ضد المكورات العنقودية الذهبية. يُظهرالبحث المضاد للبكتيريا تأثيرًا تآزريًا بين جسيمات الفضة النانوية والجنتامايسين.تشير نتائج الدراسة إلى أن جسيمات الفضة النانوية الحيوية المنشأ لديها القدرة على العمل كعوامل مضادة للاغشية الحيوية وعلاج التهاب الضرع الناجم عن المكورات العنقودية الذهبية. تظهرالنتائج ان جسيمات الفضة النانوية الحيوية لها الفعالية القوية المضادة للجراثيم والنشاط التآزري عندما تقترن مع الجنتامايسين.

الكلمات المفتاحية: جسيمات الفضة النانوية، النشاط التآزري المضاد للبكتيريا، نشاط المضاد للاغشية الحيوية، المكورات العنقودية الذهبية