In Vitro Investigation of Antibacterial Enhancement Using Silver and Gold Nanoparticles Against Multidrug-resistance *Staphylococcus aureus*

Bakhtyiar Shwan Azeez

Department of Veterinary, Shaqlawa Technical College, Erbil Polytechnic University, Erbil, Iraq.

*Corresponding authors E-mail: bakhtyiar.shwan@epu.edu.iq

https://doi.org/10.29072/basjs.20240107

ARTICLE INFO

Keywords

Multidrug, antibiotics, *S. aureus*, silver& gold nanoparticles.

ABSTRACT

The aim of this study is to investigate the antibacterial enhancement of silver and gold nanoparticles against multidrug-resistance *Staphylococcus*. A total of 75 multidrug-resistant *S. aureus were* isolated from 200 different clinical samples from patients in Arbil, Iraq. All isolates were tested for their resistance against ten different types of antimicrobials. Isolates were treated with silver nanoparticles (AgNPs) and gold nanoparticles (AuNPs) by the disc diffusion method. Treated isolates were tested for their minimum minimum inhibitory concentration (MIC) by using the broth microdilution method. The antibacterial activity of nanoparticles against isolates significantly increased the zone 3.6±36 mm for AuNPs and 1.78±25 mm for AgNPs. All isolates were sensitive to Imipenem, Ceftriaxone, and Ciprofloxacin, whereas highest rate of was seen among Trimethoprim, Azithromycin, Amikacin, Tetracycline, Aztreonam, Pipracycline, and Vancomycin. The fractional inhibitory concentration index (FICI) result revealed that 0.91 was from Azithromycin with AgNPs and 1 from Vancomycin with AgNPs, while the FICI results for AuNPs were 0.94 for Vancomycin with AuNPs, 1 for Imipenem. The synergistic effect between the Imipenem and AgNPs were as follows: the FICI value reached 0.45, and 0.62 Imipenem and AuNPs.

Received 7 Feb 2024; Received in revised form 17 Apr 2024; Accepted 22 Apr 2024, Published 30 Apr 2024

1. Introduction

Nanoparticles (NPs) have drawn a lot of attention lately as a potential solution to the problem of multidrug-resistant bacteria. NPs have recently been utilized as a substitute therapy for several multidrug-resistance bacterial infections [1-3]. Because of its low cytotoxicity, silver has long been used as an antibacterial therapy against a variety of microorganisms [4, 5]. Recent research has demonstrated that silver nanoparticles (AgNPs) and gold nanoparticles (AuNPs) are very efficient against methicillin-resistant *S. aureus* (MRSA) and have antibacterial activity against *S. aureus* [6, 7]. The wide range of action, cheap cost, and superior effectiveness of AgNPs make them advantageous for use as antimicrobial agents [8]. Antibiotics are known as organic compounds produced by certain types of microorganisms. Some microorganisms lack of these substance naturally [9]. The action of antibiotics against microbes including prevent the synthesis of the plasma membrane or cell wall by obstructing their structural integration, and the second is by preventing metabolic construction, such as the construction of nucleic acids or proteins that play a major role in the life of the bacterial cell [10]. Many antibiotic groups are present, such as β-lactams, based on the molecular structure having beta-lactam ring and are divided into four Penicillin, Cephalosporins, Monobactams, and Carbapenems[11]. These groups are more common and used due to their less of side effects when compared to other groups, as well as their high ability to eliminate bacteria [12]. The mechanism of bacterial resistance to antibiotics is through the production of special enzymes that break the beta-lactam ring and produce virulence factors [13]. Other health problems include urease and protease from toxins, as well as enzymes leading to the analysis of blood cells [14]. One of the most dangerous epidemiological phenomena for human society is the widespread resistant bacteria, so our study aims to evaluate the antibacterial efficacy of silver and gold nanoparticles against multidrug resistance *Staphylococcus aureus*.

2. Material and method

2.1 Bacterial strain

This investigation included 75 multidrug-resistant *S. aureus* isolated from 200 different clinical samples including wounds, urine, sputum, and burns collected from patients in local laboratories in Erbil City, Iraq.

2.2 Identification of *S. aureus*

The clinical isolated were cultured using the tube coagulase test and Mannitol salt agar, and all *S. aureus* strains were phenotypically identified using the VitekTM 2 compact system (BioMe′rieux, Paris, France).

2.3 Nanoparticles used in the study

Silver nanoparticles were prepared by adding 5 ml of aqueous extract of mint leaves with 45 ml of silver nitrate solution $AgNO_3$ (0.150M) prepared according to [15] continuous stirring by a magnetic stirrer at 60°C, for 30 minutes where the color changes to yellow and then brown [16].

Furthermore, gold nanoparticles were prepared by adding 5 ml of aqueous extract of mint leaves to 45 of gold chloride acid solution $HAuCl_4.3H_2O$ (12.1M) according to [17] continuous stirring by a magnetic stirrer at 80C°, for 30 minutes where the color changes to purple.

2.4 Antibiotic sensitivity test

The sensitivity of bacteria to antimicrobials was examined using the disc diffusion method [18]. Ten different class of antibiotics were used including Ceftriaxone, Azithromycin, Piperacillin, Amikacin, Ciprofloxacin, Imipenem, Aztreonam, Tetracycline, Vancomycin, and Trimethoprim. Forty isolates were subjected to a sensitivity test of antibiotics, and the results were determined by describing the bacteria as resistant by measuring the diameter of the inhibition zone and comparing that to standards as mentioned by [19].

2.5 Antibacterial activity of nanoparticles

The antibacterial activity of AgNPs and AuNPs against multidrug-resistant *S. aureus* were performed by using the disk diffusion method with replacing antibiotic tablets with sterile filter tablets saturated with silver and gold nanoparticles. However, the synergistic antibacterial effect of the combination of antibiotic and nanoparticles was also evaluated by same method. The antibiotic discs with previously prepared nanoparticle solution were placed on the surface of the media with equal dimensions and the tablets were gently pressed by using sterile forceps and leaving for several minutes to soak in the medium, then the plates were incubated for 24 hours at 37 C° degrees. Then, the zone diameters around the discs were measured by a ruler [6]. FICI is measured by this equation:

$$FICI = \frac{\text{Antibiotic inhibitory diameter alone} + \text{Nanoparticles inhibitory diameter alone}}{\text{A mixture of antibiotic and nanoparticles inhibitory diameter}}$$

The result calculated like partial the synergistic effect is evaluated at (FICI ≤0.5) and partial synergy is evaluated at (FICI <1). While (FICI >1), is considered non-insignificant [20, 21]. Using the broth microdilution method, the minimum inhibitory concentration (MIC) of AgNPs and AuNPs against multidrug-resistant *S. aureus* was measured. Each inoculum received 100 µl, and incubated for 24-hour at 37°C. The MICs value of each isolate were measured by using the optical density technique (OD600) to assess the antibacterial effects of AgNPs and AuNPs on bacterial growth [21, 22]. A 50 aliquot without turbidity was transferred to Tryptose Soya Agar (TSA) plates without NP to quantify the minimal bacterial concentration (MBC). The plates were then incubated at 37°C for 24 hours. Every plate was examined both before and after incubation to see whether any bacteria had grown on it. Plates devoid of any bacterial growth demonstrate how lethal the NP concentration was. Viability counts were employed to ascertain the quantity of organisms that managed to endure. The lowest dose of NP that kill 99.99% of *S. aureus* from growing was known as MBC.

2.6 Instruction of *S. aureus* resistance to AgNPs and AuNPs

In this study, twenty isolates that had previously been treated with AgNPs and AuNPs were used [41]. Minimum inhibitory concentration (MIC) was selected to promote isolate resistance. Resistance induction for *S. aureus* was tried by subjecting the bacteria to growth media with sublethal doses of NPs below the MIC at concentrations where the strains were still able to flourish. Thus, in the current experiment, the concentrations of AgNPs (10-nm), AgNPs (20-nm). For each NP type, the MICs were compared before and after several passaged. The isolates were passaged ten times in TSB under completely sterile conditions using sublethal doses of AgNPs and AuNPs every three days. The MIC values of every isolate after the tenth passage were noted and compared with the MIC values before to passage. By streaking the isolates onto Baird-Parker Agar, a medium designed specifically for S. aureus, the purity of the culture was ascertained. The resistant isolates were subcultured daily in NP-free nutrient broth for 10 passes to evaluate the stability of NP resistance. The ultimate MIC was determined following the tenth passage. Testing for culture purity was done for this experiment [22].

2.7 Synergistic testing for NPs and antimicrobials

Cross-resistance to sensitive antibiotics was evaluated using the broth microdilution method (TREK Diagnostic Systems, UK) [33]. After streaking the isolates onto Mueller-Hinton agar plates (Sigma Aldrich, USA), the plates were cultured for 18 to 24 hours at 37°C. Five milliliters of 0.9% NaCl solution were added to sterile tubes containing four to five colonies. With the help of the Sensititre Nephelometer (TREK Diagnostic Systems, UK), the turbidity of the expanding broth culture was managed. Then, 20 ml of the combination was poured into each well of the sensitizer plates, combining 11 ml of Mueller-Hinton broth with 15 ml of the modified TSB. The plates were sealed with foil and left to incubate for a full day at 37°C. The sensitive automated reader (TREK Diagnostic Systems, UK) was used to read the plates.

2.8 Statistical analysis

All data recorded from antimicrobial sensitivity tests were entered into Statistical Package for the Social Sciences (SPSS) version 20.0 (SPSS Inc., Chicago, IL, United States).

3. Results and Discussion

The results of the culture examination of colonies growing on mannitol agar medium were shown to obtain large, bright yellow circular colonies with edges smooth, high, and surrounded by a bright halo, due to its consumption of mannitol sugar and the production of acid (]igure 1). The color of the phenol red indicator changes to yellow, and the mannitol salt medium is considered one of the media selectivity for *S. aureus*.

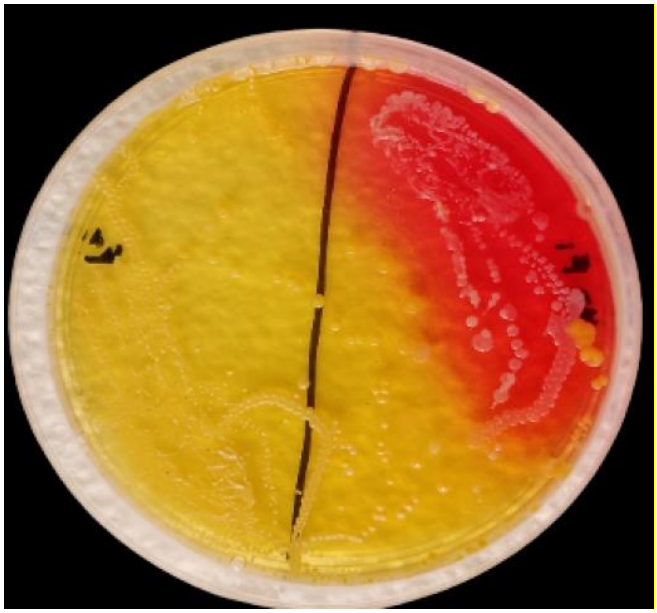

Figure 1: Growth of *S. aureus* on the mannitol saline agar medium.

The antimicrobial resistance results showed a clear difference in the extent to of the isolates being studied responded to the antibiotics. The most sensitive rate was found in Imipenem 10%, Ceftriaxone 13%, and Ciprofloxacin 15%. While, the highest resistance rate was found in Trimethoprim 58%, Azithromycin 58%, Amikacin 55%, Tetracycline 50%, Aztreonam 48%, Pipracycline 35%, and Vancomycin 22%,

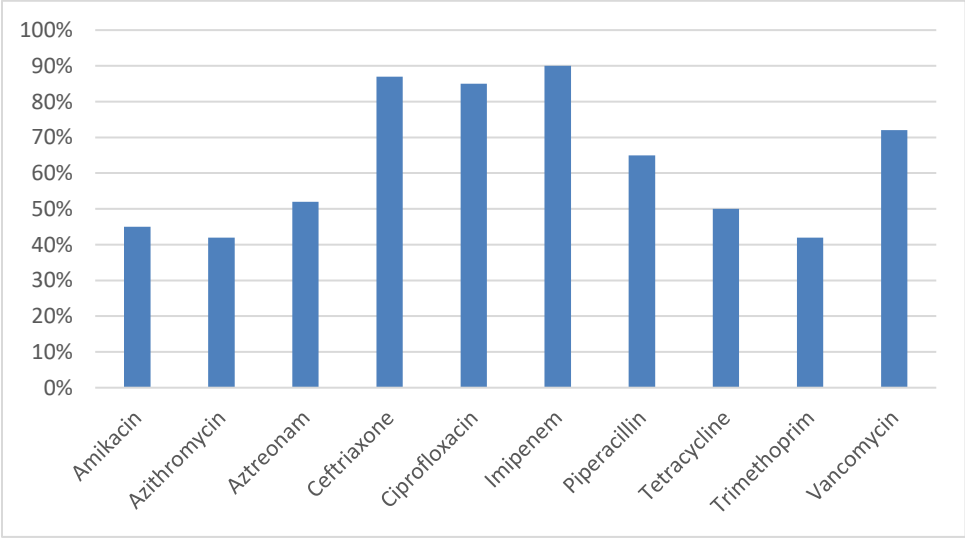

Figure2: Antimicrobial sensitivity test against isolates

It is outstanding that the isolates were resistant to the antibiotic Vancomycin. It is noteworthy that the isolate's resistance was close to what the researcher found [21, 23], the majority of isolates have higher resistance as found by [24, 25]. The reason for resistance may be due to changes in the process of synthesis the cell wall and maybe this type of pathogen is week against the antibiotic, leading to an increase or decrease in its thickness cross-linking or increasing the peptide end D-Ala inducing a change in the target site of the anti-D-Ala cross-links or increasing the peptide end to Vancomycin, or resistance may be the result of the transmission of genes encoding the resistance trait to this antibiotics carried on plasmids or jumping genes from other bacterial strains and species to *S. aureus,* this gives it the characteristic of resistance to this antibiotic [26, 42]. The resistance of isolates to antibiotics such as Azithromycin that type of the family macrolides that work to inhibit protein synthesis. This is because these isolates produce it may be the result of having isolates to resistance enzymes and binding of macrolides to the ribosomal secondary unit (50 S) that RNA methylase [27, 28]. Imipenem is a semisynthetic antibiotic from the carbapenem group. Isolations have been proven sensitive to this antibiotic, and this result matched with what was recorded by [29, 30]. The antibiotic compared to other antibiotics, in addition to the effectiveness and ability of the antibiotic imipenem, penetrates bacterial membranes in addition to its high stability towards most beta-lactamase enzymes. It is effective against infections, and the reason for the sensitivity of bacteria to this antibiotic is attributed to the fact that this is rarely used in comparison with others. The isolates also showed sensitivity to Trimethoprim, which inhibits bacterial dihydrofolate reductase subsequently attributed to its interference with the enzymes responsible for the cloning process of DNA, thus, it leads to preventing the survival of bacteria [24, 31]. In this study, AgNPs and AuNPs nanoparticles were used as antibacterial activity against isolates under study by using the disc diffusion method on Muller Hinton agar and the results were 1.78 ± 25 for AgNPs and 3.6 ± 26 for AuNPs of damping zone diameter (mm) Table 1.

Table1: Inhibition zone diameters (mm) of silver and gold nanoparticles against bacteria

Type of organism	Damping zone diameter (mm)	
	AgNPs	AuNPs
S. aureus	1.78± 25	3.6± 26*

* Mean ± standard deviation

Nanoparticles work to change the ionic interaction in the bacterial cell membrane. The mechanism of nanoparticles interacts with bacterial cells, the bacterial cell carries negative charges, while nano-metallic oxides carry a positive charge, which creates an attraction. Electromagnetically between the bacteria and the surfaces of the particles, the particles release ions that interact with the thio group for proteins that transport nutrients that protrude from the bacterial cell membrane and it will reduce the permeability of the membrane, leading to the death of the bacterial cell [17, 32]. Mixing silver nanoparticles with antimicrobials has increased the effectiveness. This increase varied from slight, as in the case of *S. aureus* inhibitory action against bacteria with Ceftriaxone, the inhibition diameter increased from (35 to 38 mm) for the combination of the antimicrobial with nanosilver leading to a significant increase as in the antimicrobial. Trimethoprim was recorded (at 8 mm) as the diameter of inhibition increased from (17 mm) for the antimicrobial alone and became (25 mm) for the antimicrobial mixture with nanosilver (Table 2). The results of calculating the FICI showed the existence of a partial synergy between the antibiotic Azithromycin with silver nanoparticles reaching a value of FICI (0.91) considered the best result of inhibitory effectiveness against isolates. The effect of adding Vancomycin with silver nanoparticles where it was valuable of FICI is reaching (1), where other antimicrobials did not show any synergistic relationship when mixed with silver nanoparticles (Table 2). The mechanism of gold nanoparticles inhibits the ability of DNA to replication and gene expression of proteins as well as various cellular proteins and enzymes. Therefore, it becomes ineffective, which leads to the death of bacterial cells [33]. Showed that AuNPs and AgNPs inhibit the growth of resistant pathogens. These results were consistent with the results of [6, 21, 30, 34]. The inhibition zone appeared for both samples with different inhibition diameters depending on the type of bacteria. This indicates that some bacteria possess methods and materials through which they can withstand these nanoparticles. The researchers pointed out the strong effectiveness of gold nanoparticles in inhibiting the growth of a group of bacteria those having multiple resistance to antibiotics [17, 30, 35, 36]. The results of calculating the FICI showed the existence of a partial synergy between the antibiotic Azithromycin with silver nanoparticles reaching a value of FICI (0.91) considered the best result of inhibitory effectiveness against isolates. The effect of adding Vancomycin with silver nanoparticles where it was valuable of FICI is reaching (1), where other antimicrobials did not show any synergistic relationship when mixed with silver nanoparticles (Table 2).

Table2: Inhibitory effectiveness of antibiotic combination with AgNPs and indicator partial inhibition concentration of *S. aureus*

Antimicrobial	Antagonist alone	Mixed	FICI	Type of effect
Ceftriaxone	35	38	1.50	None
Azithromycin	17	20	0.91	Partially effect
Piperacillin	26	27	1.11	None
Amikacin	18	25	1.19	None
Ciprofloxacin	34	37	1.70	None
Imipenem	36	38	1.55	None
Aztreonam	21	27	1.60	None
Tetracycline	20	22	1.40	None
Vancomycin	29	33	1.00	Added
Trimethoprim	17	25	1.95	None

In general, it was found that the antibacterial activity of nano-gold particles was higher than the activity of silver nanoparticles, as the maximum diameter of the inhibition zone reached (40 mm) and (39 mm) for nano-gold nanoparticles against isolates respectively, compared to the activity of nano-silver, the diameters of the inhibition zone ranged from 37-38mm from the same type of isolates. The synergistic activity of gold nanoparticles with a group of antimicrobials was examined to visualize the diameter of the inhibition zone for antimicrobials against isolates. A mixture of gold nanoparticles with antimicrobials was used in the study and the index to determine the nature of the inhibitory relationship of the antibiotic mixture and FICI with nanoparticles against bacteria. The results showed a clear difference in the sensitivity of bacteria to different vital signs, where it was found that the highest diameter of inhibition (30 mm) was for Imipenem, followed by Ceftriaxone had an inhibition diameter (29 mm), while Piperacillin, Amikacin, and

Tetracycline have the least activity against bacteria, as shown in the (Table 3). Mixing gold nanoparticles with antibiotics has increased the effectiveness of inhibition towards *S. aureus*. This increase varied from slight, as in the case of Imipenem, as the diameter of inhibition increased from (30 mm) for the antibiotic alone to (38 mm) for the antibiotic mixture with gold nanoparticles (Table 3). The results of calculating the FICI showed the presence of a partial synergy between the antibiotic Vancomycin with gold nanoparticles reached a value of FICI (0.94) considered the best result of inhibitory effectiveness against isolates. The effect of adding Imipenem with gold nanoparticles where it was valuable of FICI reach (1), where other antimicrobials did not show any synergistic relationship when mixed with silver nanoparticles (Table 3).

Table3: Inhibitory efficacy of antimicrobial mixture with AuNPs and partial inhibitory concentration index of *S. aureus*

Antimicrobials	Antagonist alone	Mixed	FICI	Type of effect
Ceftriaxone	29	39	1.23	None
Azithromycin	22	25	0.85	None
Piperacillin	17	20	1.50	None
Amikacin	18	20	1.44	None
Ciprofloxacin	25	28	1.30	None
Imipenem	30	40	1.00	Added
Aztreonam	21	24	1.70	None
Tetracycline	19	22	1.10	None
Vancomycin	26	35	0.94	Partially effect
Trimethoprim	20	24	1.45	None

The synergistic activity of gold nanoparticles with some antimicrobials against isolates by measuring the minimum inhibitory concentration of antibiotics, nanoparticles, and their mixture

using the checkerboard testing method and calculating the partial inhibitory concentration index FICI to determine the type of inhibitory effect of the mixture of antimicrobials and nanoparticles against bacteria. The best two antimicrobials were selected that gave the highest synergistic effect in the way the disc spreads. The results shown in Table 4 showed that the MIC of Ceftriaxone reached (22 µg/ml) against *S.aureus*. The minimum inhibitory concentration of Imipenem in the mixture decreased to reach (48 µg/ml), and it was found that the leaving value of the minimum retarder of silver nanoparticles was (6.22 µg/ml), while its concentration is reached (1.63 micrograms/ml). It has been shown that the value of the inhibitory concentration index. The minimum for the mixture of Ceftriaxone with nanosilver is 0.55, and therefore there is a synergistic relationship between Ceftriaxone and nanosilver. The results also showed a synergistic relationship between the antimicrobial Imipenem and nanosilver, as the FICI value reached 0.45 (Table4).

Table4: MIC of antibiotics, nano silver and their mixture, and FICI value of *S. aureus*

Transaction	*S. aureus*	
	Ceftriaxone	Imipenem
Antibiotic alone	22	48
Antibiotics in the mix	7.5	4.60
Nanosilver alone	6.22	3.78
Nanosilver in the mix	1.63	0.48
FICI	0.55	0.45
Type of effect	Synergy	Synergy

The additive or synergistic antibacterial effect of the combination of antibiotics was evaluated. All antibiotics demonstrated AuNPs and AgNPs with ten tested (amikacin, azithromycin, aztreonam, ceftriaxone, ciprofloxacin, imipenem, piperacillin, tetracycline, trimethoprim, and vancomycin) their effects against isolates in varying proportions. The inhibitory effectiveness of all antibiotics was increased by mixing with nanoparticles in all tested bacterial strains. These results indicate the presence of differential sensitivity between isolates for the type of antibacterial

agent that is combined with nano silver or nano gold. These differences may relate to the composition of the cell wall of each isolate. The results of the tables showed the synergistic action of silver and gold nanoparticles with various antibiotics has better antimicrobial effects than nanoparticles alone or free antibiotics [2, 4, 10]. It was found that the effect of using antibiotics mixed with silver and gold nanoparticles is more effective against isolates than if they were applied separately. In this way, the consumption of treatment quantities can be reduced. In general, the side effects are greatly reduced, by directing the activity in the area exposed to harm only and without any higher dose than needed [37]. Among the widely used nanomaterials are nano-metals and their oxides due to their antibacterial activity, such as AgNPs, and AuNPs [38]. Therefore, the mechanisms of nano-metal MNPs as antibacterial activity of two proposed reasons: First, when the metals are transformed into nano-metals and these NPs are formed, a larger metal ion displaces them, and a fatal toxicity to the cell is formed. Second, the MNPs form reactive oxygen (ROS) on the surface of the NPs, which leads to oxidation and stress inside the bacterial cell, which leads to its death [12, 34]. The results shown in Table 5 showed that the MIC of the antimicrobial Ceftriaxone reached (45 µg/ml) against the isolates. The inhibitory concentration of Ceftriaxone in the mixture decreased to reach (14.5 µg/ml), and it was found that the leaving value of the minimum retarder of gold nanoparticles was (2.13 µg/ml), while its concentration in the mixture reached (1.13µg/ml). It has been shown that the value of the inhibitory concentration index. The minimum for the mixture of Ceftriaxone with nanogold is 0.45 therefore there is a synergistic relationship between Ceftriaxone and nanogold. Furthermore, the results of MIC of Imipenem reached (33 µg/ml) against the isolates. The inhibitory concentration of Imipenem in the mixture decreased to (8.25 µg/ml), minimum retarder of gold nanoparticles was (1.50 µg/ml) and with its concentration in the mixture reached (0.44µg/ml). While, the results also showed a synergistic relationship between the Imipenem and nano gold as the FICI value reached (0.62 µg/ml).

Table 5: MIC of antibiotics, nano gold and their mixture, and FICI value of *S. aureus*

Transaction	*S. aureus*	
	Ceftriaxone	Imipenem
Antibiotic alone	45	33
Antibiotics in the mix	14.5	8.25
Nanogold alone	2.13	1.50
Nanogold in the mix	1.13	0.44
FICI	0.45	0.62
Type of effect	Synergy	Synergy

There are many explanations for the mechanism of action of the antimicrobial activity of AgNPs against bacteria. Some researchers have suggested that the outer bacterial membrane could be damaged by AgNPs [30, 36]. Others suggested that the segmentation of bacterial cells could depend on treatment with micronutrients on the gaps and pits in the cell membrane of the bacteria [39, 40].

Conclusions

The current investigation pointed out that isolates understudy have multi-drug resistance which establishes a major therapeutic challenge. Bacterial growth was greatly influenced by the inhibitory effectiveness by using of silver and gold nanoparticles, as they were highly effective against isolates. Nanoparticles have excellent antibacterial activity and enhance inhibiting of pathogenic bacteria. However, nanoparticles have potential synergy effective when used with antibiotics that target the isolates pathogens.

References

[1] AJ Kora, L Rastogi. "Enhancement of antibacterial activity of capped silver nanoparticles in combination with antibiotics, on model gram-negative and gram-positive bacteria, Bioorg Chem (2013). https://doi.org/10.1155/2013/871097

[2] M.R Bindhu, M. Umadevi, Silver and gold nanoparticles for sensor and antibacterial applications, Spectrochimica Acta Part; SAA., 128(2014)37-45, https://doi.org/10.1016/j.saa.2014.02.119

[3] T.T Tsai, TH Huang, C.J Chang, N. Yi-Ju Ho, Y.T Tseng, C.F Chen, Antibacterial cellulose paper made with silver-coated gold nanoparticles, Scientific Reports, 7(2017)3155, http://dx.doi.org/10.1038/s41598-017-03357-w

[4] T. Bruna, F. Maldonado-Bravo, P. Jara, N. Caro, Silver nanoparticles and their antibacterial applications, Int. J. Mol. Sci., 22(2021)7202, https://doi.org/10.3390/ijms22137202

[5] G. Franci, A. Falanga, S. Galdiero, L. Palomba, G. Rai, M. Morelli, M. Galdiero, Silver nanoparticles as potential antibacterial agents, Molecules, 20(2015)8856-74, https://doi.org/10.3390/molecules20058856

[6] A.K Yaqubi, S.D Astuti, A.H Zaidan, A. Syahrom, D.Z Nurdin, Antibacterial effect of red laser-activated silver nanoparticles synthesized with grape seed extract against Staphylococcus aureus and Escherichia coli, Laser Med Sci., 39(2024)1-3, https://doi.org/10.1007/s10103-024-03991-7

[7] A.N Grace, K. Pandian, Antibacterial efficacy of aminoglycosidic antibiotics protected gold nanoparticles-A brief study, Colloids and Surf. A: Physicochem. Eng. Asp., 297(2007)63-70, https://doi.org/10.1016/j.colsurfa.2006.10.024

[8] G.A Martínez-Castañon, N. Nino-Martinez, F. Martinez-Gutierrez, J.R Martínez-Mendoza, F. Ruiz, Synthesis and antibacterial activity of silver nanoparticles with different sizes. J. Nanopart. Res.10(2008)1343-8. https://doi.org/10.1007/s11051-008-9428-6

[9] B. Le Ouay, F Stellacci, Antibacterial activity of silver nanoparticles: A surface science insight. Nano Today, 10(2015):339-54. https://doi.org/10.1016/j.nantod.2015.04.002

[10] C.M Crisan, M. Mocan, T. Manolea, L.I Lasca, F.A Tăbăran, L. Mocan, Review on silver nanoparticles as a novel class of antibacterial solutions, Appl Sci.,11(2021)1120, https://doi.org/10.3390/app11031120

[11] F. Haghi, N. Keramati, F. Hemmati, H. Zeighami, Distribution of integrons and gene cassettes among metallo-β-lactamase producing Pseudomonas aeruginosa clinical isolates, Infect Epid Micr., 3(2017)36-40, http://dorl.net/dor/20.1001.1.25884107.2017.3.2.6.5

[12] J. Tang, S. Zheng, Antibacterial activity of silver nanoparticles: structural effects, Adv. Healthc Mater., 7(2018)1701503, https://doi.org/10.1002/adhm.201701503

[13] J. Davies, D. Dorothy, Origins and evolution of antibiotic resistance, Microbiol Mol Biol R, 74,(2010): 417-433. https://doi.org/10.1128/mmbr.00016-10

[14] A. MacGowan, E. Macnaughton, Antibiotic resistance, Medicine, 45(2017)622-8, https://doi.org/10.1016/j.jiph.2016.08.007

[15] Á.M Gabriela, M.D Gabriela, A.M Luis, P.R Reinaldo, H.M Michael, G.P Rodolfo, V.B Roberto, Biosynthesis of silver nanoparticles using mint leaf extract (Mentha piperita) and their antibacterial activity, Adv Sci Eng Med., 9(2017)914-23, https://doi.org/10.1166/asem.2017.2076

[16] Y. Torres-Martínez, E. Arredondo-Espinoza, C. Puente, O. González-Santiago, N. Pineda-Aguilar, I. Balderas-Rentería, I. López, M.A Ramírez-Cabrera, Synthesis of silver nanoparticles using a Mentha spicata extract and evaluation of its anticancer and cytotoxic activity, Peerj, 7 (2019)e8142. https://doi.org/10.7717/peerj.8142

[17] M.A Morsi, A.H Oraby, A.G Elshahawy, R.M Abd El-Hady, Preparation, structural analysis, morphological investigation and electrical properties of gold nanoparticles filled polyvinyl alcohol/carboxymethyl cellulose blend, J Mater Res Techno., 8(2019)5996-6010, **https://doi.org/10.1016/j.jmrt.2019.09.074**

[18] W.L Drew, A.L Barry, J.C O'.Toole, R. Sherris. Reliability of the Kirby-Bauer disc diffusion method for detecting methicillin-resistant strains of Staphylococcus aureus, Appl Microbiol, 24(1972)240-7, https://doi.org/10.1128/am.24.2.240-247.1972

[19] R. Humphries, A.M Bobenchik, J.A Hindler, A.N Schuetz, Overview of changes to the clinical and laboratory standards institute performance standards for antimicrobial susceptibility testing, M100, J. Clin. Microbiol., 59(2021)10-128, https://doi.org/10.1128/jcm.00213-21

[20] K.T Hassan, I.J Ibraheem, O.M Hassan, A.S Obaid, T.A Ali, H.H Salih, M.S Kadhim, Facile green synthesis of Ag/AgCl nanoparticles derived from Chara algae extract and evaluating their antibacterial activity and synergistic effect with antibiotics, J. Environ. Chem. Eng. 9(2021)105359, https://doi.org/10.1016/j.jece.2021.105359

[21] A. Elbehiry, M. Al-Dubaib, E. Marzouk, I. Moussa, Antibacterial effects and resistance induction of silver and gold nanoparticles against Staphylococcus aureus-induced mastitis

and the potential toxicity in rats, Microbiol Open, 8(2019):e00698. https://doi.org/10.1002/mbo3.698

[22] S.M Daly, C.R Sturge, D.E Greenberg, Inhibition of bacterial growth by peptide-conjugated morpholino oligomers, Morpholino Oligomers, Methods Pro.,1565(2017)115-22, https://doi.org/10.1007/978-1-4939-6817-6_10

[23] A.N Al-Khafaji, Isolation and identification of methicillin resistance Staphylococcus aureus and detection their ability to the production of virulence factors, J Pure Appl., 26(2018)100-11, http://dx.doi.org/10.29196/jubpas.v26i8.1675

[24] K. Vanamala, K. Tatiparti, S. Bhise, K. Sau, M.H Scheetz, M.J Rybak, D. Andes, A.K Iyer, Novel approaches for the treatment of methicillin-resistant Staphylococcus aureus: Using nanoparticles to overcome multidrug resistance, Drug Discov Today, 26(2021)31-43, https://doi.org/10.1016/j.drudis.2020.10.011

[25] A. Srinivasan, J.D Dick, T.M Perl, Vancomycin resistance in staphylococci, Clin. Microbiol. Rev.15(2002)430-8, https://doi.org/10.1128/cmr.15.3.430-438.2002

[26] S. Gardete, A. Tomasz, Mechanisms of vancomycin resistance in Staphylococcus aureus, J Clin Investig., 124(2014)2836-40, https://doi.org/10.1172/JCI68834

[27] C.R Hansen, T. Pressler, N. Hoiby, H.K Johansen, Long-term, low-dose azithromycin treatment reduces the incidence but increases macrolide resistance in Staphylococcus aureus in Danish CF patients, J. Cyst Fibros., 8(2009)58-62, https://doi.org/10.1016/j.jcf.2008.09.001

[28] L Omran, E Askar. "Antibiotic sensitivity patterns of the most common bacteria isolated from al-mouwasat university hospital in 2015, Syria." *Int J PharmTech Res*. 9(2016): 165-170. DOI: 10.9790/3008-1104024448

[29] S. Kidwai, C.Y Park, S. Mawatwal, P. Tiwari, M.G Jung, T.P Gosain, P. Kumar, D. Alland, S. Kumar, A. Bajaj, Y.K Hwang, Dual mechanism of action of 5-nitro-1, 10-phenanthroline against Mycobacterium tuberculosis, Antimicrob Agents Chemoth., 61(2017)10-128, https://doi.org/10.1128/aac.00969-17

[30] H.M Sharqi, O.M Hassan, A.S Obaid, Investigation of the Antibiotic-Resistant ESKAPE Pathogens in Ramadi Hospitals, Iraq., Indian J Med Toxicol**.,** 15(2021)3306-13, http://dx.doi.org/10.37506/ijfmt.v15i4.17219

[31] M.M Masadeh, K.H Alzoubi, S. Al-Azzam, O.F Khabour, A.M Al-Buhairan, Ciprofloxacin-induced antibacterial activity is atteneuated by pretreatment with antioxidant agents, Pathogens, 5(2016) 28, https://doi.org/10.3390/pathogens5010028

[32] Y. Tauran, A. Brioude, A.W Coleman, M. Rhimi, B. Kim, Molecular recognition by gold, silver and copper nanoparticles, World J. of Biol Chem., 4(2013)35, https://doi.org/10.4331%2Fwjbc.v4.i3.35

[33] B. Wang, X. Zhou, D. Yao, X. Sun, M. He, X. Wang, X. Yin, H. Liang. Contribution of gold nanoparticles to the catalytic DNA strand displacement in leakage reduction and signal amplification**,** Chem Commun., 53(2017)10950-3,https://doi.org/10.1039/C7CC05832B

[34] H.N Verma, P. Singh, R. M Chavan, Gold nanoparticle: synthesis and characterization, Vet World, 7(2014) 72, DOI:10.14202/vetworld.2014.72-77

[35] A.K Khan, R. Rashid, G. Murtaza, A.J Zahra, Gold nanoparticles: synthesis and applications in drug delivery, Tropical J. Pharm. Res, 13(2014)1169-77, https://doi.org/10.4314/tjpr.v13i7.23

[36] D. Pissuwan , C.H Cortie, S.M Valenzuela, M.B Cortie, Functionalised gold nanoparticles for controlling pathogenic bacteria, Trends Biotechnol, 28(2010)207-13, https://doi.org/10.1016/j.tibtech.2009.12.004

[37] W.H Chen, X.D Xu, H.Z Jia, Q. Lei, G.F Luo, S.X Cheng, R.X Zhuo, X.Z Zhang. Therapeutic nanomedicine based on dual-intelligent functionalized gold nanoparticles for cancer imaging and therapy in vivo, Biomaterials, 34(2013)8798-807, https://doi.org/10.1016/j.biomaterials.2013.07.084

[38] Y.N Slavin, J. Asnis, U.O Hńfeli, H. Bach, Metal nanoparticles: understanding the mechanisms behind antibacterial activity, J Nanotechnol., 15 (2017)1-20, https://doi.org/10.1186/s12951-017-0308-z

[39] Q. Yu, J. Li, Y. Zhang, Y. Wang, L. Liu, M. Li, Inhibition of gold nanoparticles (AuNPs) on pathogenic biofilm formation and invasion to host cells, Sci Rep., 6(2016)26667, https://doi.org/10.1038/srep26667

[40] H. Yun, J.D Kim, H.C Choi, C.W Lee, Antibacterial activity of CNT-Ag and GO-Ag nanocomposites against gram-negative and gram-positive bacteria, Bull. Korean Chem. Soc.34(2013)3261-4, http://dx.doi.org/10.5012/bkcs.2013.34.11.3261

[41] A. Elbehiry, E. Marzouk, I. Moussa, S. Anagreyyah, A. AlGhamdi, A. Alqarni, A. Aljohani, H.A Hemeg, A.M Almuzaini, F. Alzaben, A Abalkhail, Using protein fingerprinting for identifying and discriminating methicillin resistant Staphylococcus aureus isolates from inpatient and outpatient clinics, Diagnostics 13(2023) 2825, https://doi.org/10.3390/diagnostics13172825

[42] H K Ibraheim, K S Madhi, G K Baqer, and H A Gharban. Effectiveness of raw bacteriocin produced from lactic acid bacteria on biofilm of methicillin-resistant Staphylococcus aureus. VET ROLRD, 16(2023), 491. https://doi.org/10.14202%2Fvetworld.2023.491-499

دراسة مخبرية للتعزيز المضاد للبكتيريا باستخدام الجسيمات النانوية الفضية والذهبية ضد المكورات العنقودية الذهبية المقاومة للأدوية المتعددة

بەختیار شوان عزیز

قسم البيطره، كلية شقلاوة التقنية، جامعة أربيل التقنية

المكورات العنقودية الذهبية (S. aureus) هي مسببات الأمراض البكتيرية المنتشرة على نطاق واسع والتي تسبب مجموعة متنوعة من الالتهابات المختلفة لدى البشر ويمكن أن تصيب الأفات السطحية والأمراض الجهازية في جميع أنحاء العالم. تعتبر مقاومة المكورات العنقودية الذهبية للمضادات الحيوية في الوقت الحاضر قضية جديدة بالنسبة لصحة الأفراد. ولذلك، فإن الهدف من هذه الدراسة هو دراسة التعزيز المضاد للبكتيريا للجسيمات النانوية الفضية والذهبية ضد المكورات العنقودية المقاومة للأدوية المتعددة. تم عزل ما مجموعه 75 بكتريا المكورة العنقودية الذهبية المقاومة للأدوية المتعددة من 200 عينة سريرية مختلفة من المرضى في أربيل، العراق. تم اختبار مقاومة جميع العزلات لعشرة أنواع مختلفة من مضادات الميكروبات. عولجت العزلات بجسيمات الفضة النانوية (AgNPs) وجسيمات الذهب النانوية (AuNPs) بطريقة الانتشار القرصي. تم اختبار العزلات المعالجة لمعرفة الحد الأدنى للتركيز المثبط الأدنى (MIC) باستخدام طريقة التخفيف الدقيق للمرق. أدى النشاط المضاد للبكتيريا للجسيمات النانوية ضد العزلات إلى زيادة كبيرة في المنطقة 3.6 ± 36 مم لـ AuNPs و 1.78 ± 25 مم لـ AgNPs. كانت جميع العزلات حساسة لمضادات الإيميبينيم، سيفترياكسون، والسيبروفلوكساسين، في حين شوهدت أعلى نسبة حساسية بين تريميثوبريم، أزيثروميسين، أميكاسين، تتراسيكلين، أزتريونام، بيبراسيكلين، وفانكومايسين. كشفت نتيجة مؤشر التركيز المثبط

الكسري (FICI) أن 0.91 كان من أزيثروميسين مع AgNPs و1 من فانكومايسين معAgNPs ، في حين كانت نتائج FICI لـ 0.94 AuNPs للفانكومايسين معAuNPs ، 1 للإيميبينيم. وكان التأثير التآزري بين Imipenem و AgNPsعلى النحو التالي: وصلت قيمة FICI إلى 0.45، و0.62 Imipenem و .AuNPsيحدد هذا الاكتشاف أن NPs يعزز بشكل كبير التأثير المضاد للبكتيريا ضد مقاومة الأدوية المتعددة المكورات العنقودية الذهبية.