Biol. Stud. 2025, 19(2), 77–90 • doi: https://doi.org/10.30970/sbi.1902.833
www.http://publications.lnu.edu.ua/journals/index.php/biology

UDC: [579.86:576:577.1]:615.281

SYNERGISM EFFECT OF SILVER NANOPARTICLES AND CEFOTAXIME TO TREAT BIOFILM FORMATION OF *STAPHYLOCOCCUS AUREUS* CLINICAL ISOLATED

Safiya Saad Dhaif, Dhuha Badr Mahmood, Ghufran Salman Jawad, Lujain Ali Ghannawi, Haidar Fadhil Al-Rubaye

National Diabetes Center, Mustansiriyah University, Al-Qadisiyah, Baghdad 10011, Iraq

Dhaif, S. S., Mahmood, D. B., Jawad, G. S., Ghannawi, L. A., & Al-Rubaye, H. F. (2025). Synergism effect of silver nanoparticles and cefotaxime to treat biofilm formation of *Staphylococcus aureus* clinical isolates. *Studia Biologica*, 19(2), 77–90. doi:10.30970/sbi.1902.833

Background. *Staphylococcus aureus,* is a major pathogen causing infections in both hospital and community settings. Multi-drug-resistant strains, particularly Methicillin-resistant *Staphylococcus aureus* (MRSA), complicate treatment, as these strains can evade antibiotics. Biofilm formation by *S. aureus* protects bacterial cells from immune responses and antibiotics, making infections difficult to treat. This study evaluates the synergistic effect of silver nanoparticles (AgNPs) and cefotaxime in inhibiting biofilm formation by clinical *S. aureus* isolates, especially multi-drug-resistant strains.

Materials and Methods. Thirty clinical *S. aureus* isolates were obtained from patients with skin infections. Identification was confirmed using biochemical tests and the VITEK2 system. Antimicrobial susceptibility testing was performed using the disk diffusion method on antibiotics including ciprofloxacin, imipenem, amoxicillin-clavulanic acid, cefotaxime, and chloramphenicol. Minimum inhibitory concentrations (MICs) were also determined. Biofilm formation was quantified using crystal violet staining. Silver nanoparticles (AgNPs) were synthesized using sodium borohydride and characterized by atomic force microscopy (AFM) and field emission scanning electron microscopy (FE-SEM).

Results. All isolates were susceptible to ciprofloxacin and imipenem. Ninety percent were susceptible to amoxicillin-clavulanic acid, while 70% were susceptible to cefotaxime. All isolates were resistant to chloramphenicol. Biofilm formation assays showed variability in biofilm production. AgNPs alone demonstrated superior efficacy in inhibiting biofilm formation. The combination of AgNPs and cefotaxime exhibited the strongest inhibition, suggesting a synergistic effect.

Conclusion. This study suggests that AgNPs alone are more effective than cefotaxime in inhibiting biofilm formation. The combination of AgNPs and cefotaxime showed the most potent effect, providing a promising strategy for treating multi-drug-resistant *S. aureus* infections. AgNPs may serve as an adjunct to antibiotics in overcoming biofilm-associated infections.

Keywords: *Staphylococcus aureus*, biofilm, silver nanoparticles (AgNPs), cefotaxime

INTRODUCTION

Staphylococcus aureus is a Gram-positive, spherically shaped, facultative anaerobic bacterium known for its ability to cause a wide range of infections, including skin, respiratory, and bloodstream infections. *S. aureus* bacteria cause many infections and are widespread in both hospital and community settings; their management and treatment remain challenging because of the appearance of multi-drug-resistant strains such as MRSA (Methicillin-resistant *Staphylococcus aureus*) (Atta *et al*., 2023; Centers for Disease Control and Prevention, 2003; Boucher *et al*., 2008). *S. aureus* is commonly present in the environment and as a part of the normal human microbiota, particularly on skin and mucous membranes such as nasal area (Atta *et al*., 2023). *S. aureus* doesn't usually cause infection on healthy skin; though, these bacteria can cause many serious infections if allowed to enter the internal tissues or bloodstream (Atta *et al*., 2023). Transmission usually occurs through direct contact, although, other ways of transmission may also be involved in some infections (Rasigade *et al*., 2014). *S. aureus* is among the most prevalent bacterial pathogens in humans and is responsible for a variety of infections, such as infective endocarditis, bacteremia, skin and soft tissue infections (e.g., folliculitis, impetigo, carbuncles, furuncles, scalded skin syndrome, cellulitis, and more), septic arthritis, osteomyelitis, pulmonary infections (e.g., empyema and pneumonia), prosthetic device infections, meningitis, gastroenteritis, and UTI "urinary tract infections" (Tong *et al*., 2015) Silver has recently gained attention due to its well-known antibacterial properties. It has been used for centuries in the treatment of various conditions, ranging from burn wounds and typhoid to anthrax and bacterial conjunctivitis in infants (Amirulhusni *et al*., 2012, Bruna *et al*., 2021). Antibiotics are regularly used to treat all bacterial infections. However, the widespread emergence of multi-antibiotic-resistant bacteria has made it more and more difficult to treat these infections with conventional antibiotics (El-Fouly *et al*., 2015; Yonathan *et al*., 2022).

Metal or non-metal NPs such as silver, gold, copper, selenium, and metal oxide NPs such as ZnO, TiO_2, CaO, and MgO nanoparticles, often referred to as nano-antibiotics, have emerged as promising antimicrobial agents for the treatment and prevention of infectious diseases, demonstrating efficacy against antibiotic-resistant bacteria (Frei *et al*., 2023).

Silver nanoparticles (AgNPs) have garnered significant attention for their antimicrobial properties, with research indicating that the development of silver resistance in microorganisms is less likely due to AgNPs' ability to interact with various cellular targets (Jaber *et al*., 2023; Yonathan *et al*., 2022). While the precise mechanisms of action are still being explored, several studies suggest that AgNPs exert their antibacterial effects through multiple pathways, including the generation of reactive oxygen species (ROS),

disruption of bacterial cell membranes, and interference with DNA replication and protein synthesis (Yin *et al*., 2020; Jaber *et al*., 2023).

The aim of this study was to investigate the synergistic effect of silver nanoparticles (AgNPs) and cefotaxime in inhibiting biofilm formation by *Staphylococcus aureus* clinical isolates, particularly those resistant to multiple drugs, to improve treatment strategies for biofilm-related infections.

MATERIALS AND METHODS

Collection of *Staphylococcus aureus* isolates. Isolates (not stored in a collection) were gathered from Al-Yarmook and Al-Kindi Teaching Hospitals, also from Baghdad Medical City laboratories, then cultivated on MacConkey agar and incubated for 18 to 24 hours at 37 °C.

Identification of bacteria by cultural characteristics and biochemical tests. Various culture media, including mannitol salt agar and nutrient agar, were inoculated with isolates and incubated at 37 °C for 18 to 24 hours. Suspected colonies were identified using morphological and biochemical methods. Also Gram staining was preformed to examine the potential colonies and observe their characteristic bacterial morphology under a light microscope. Additionally, catalase and oxidase tests were conducted (Benson, 2002).

Using Vitek-2 system for bacterial identification. Vitek-2 system identified and validated bacterial isolates as *Staphylococcus aureus* after they had been positively identified by a preliminary biochemical test, assessing their susceptibility to antibiotics.

Antibiotic susceptibility test. The *S. aureus* antimicrobial susceptibility profiles were assessed according to Kirby Bauer's approach and the Clinical and Laboratory Standards Institute (CLSI) recommendations. The disk diffusion test (DDT) was preformed to assess the antibiotic resistance profiles of *Staphylococcus aureus* isolates (Wayne, 2015).

Synthesis of AgNps. The chemical reduction method is commonly used to synthesize silver nanoparticles (AgNPs) by reducing silver ions (Ag^+) to metallic silver (Ag^0) using reducing agents such as sodium citrate, sodium borohydride, and others. These agents cause silver to agglomerate into clusters, which form colloidal nanoparticles. Stabilizing agents, such as surfactants with thiol, amine, or alcohol groups, are added to prevent aggregation and maintain nanoparticle dispersion (Iravani *et al*., 2014).

Characterization of AgNPs by atomic force microscope (AFM). This method was used to assess the topography of silver nanoparticle surfaces. AFM was performed using a Bruker Dimension Icon. Silicon cantilever probes (e.g., Bruker RTESPA) with a spring constant of 40 N/m were used. Scans were conducted in tapping mode at a scan size of 5 μm, scan rate of 1 Hz, and resolution of 512 pixels per line. On the cover slide, a few drops of silver nanoparticles (AgNPs) solution were applied, and they were then allowed to dry at room temperature in the dark; the glass plate was then scanned with the AFM (Agnihotri *et al.*, 2013).

Field emission scanning electron microscope (FE-SEM). The diameter and form of nanoparticles were measured using scanning electron microscopy. SEM imaging was conducted with a JEOL JSM-7100F. Samples were sputter-coated with gold. Imaging was performed at an accelerating voltage of 10 kV and pressure of 10^{-6} Pa,

with magnifications ranging from 1,000x to 50,000x. A little drop of the sonicated AgNP solution sample was put on a glass slide and left to dry after being sonicated with distilled water (Vladár & Hodoroaba, 2020).

Biofilm formation assay. *Staphylococcus aureus* bacteria strains, which show greater resistance to antibiotics, were studied to evaluate their ability to form biofilms according to (Najim *et al.*, 2024). Biofilm production was assessed by standardizing a bacterial suspension to a 0.5 McFarland using sterile tryptic soya broth (TSB). Subsequently, 300 µL of the suspension was introduced into three wells of a 96-well microtiter plate, with three additional wells containing only sterile TSB serving as negative controls. After a 4-hour incubation period, the wells were washed, and fresh TSB medium was added. This procedure was repeated after additional 24-hour incubation. The wells were then fixed with methanol, stained with a 0.1% crystal violet solution, and the dye was resolubilized using 30% acetic acid. Biofilm production was quantified by measuring absorbance at 590 nm using a spectrophotometer.

Determination of minimum inhibitory concentration (MIC). A 96-well microtiter plate approach was used to assess the MIC of the antibiotic, silver nanoparticles, and their synergism against *S. aureus* (Kowalska-Krochmal *et al.*, 2021). A suspension of $5 \cdot 10^5$ CFU/mL (dilution of 0.5 McFarland suspension (9.9 mL broth + 0.1 mL 0.5 McFarland suspension) 100× to give a final concentration of $1 \cdot 10^6$ CFU/mL) was used to determine the minimum inhibitory concentration (MIC) using the broth microdilution method. This suspension is subsequently inoculated in microtiter wells containing a range of antibiotics by mixing 50 µL of the bacterial inoculum with 50 µL of antibiotic solution or by adding 10 µL of the inoculum to 100 µL of diluted antibiotic. Stock solutions of antibiotics are diluted twice and added into the wells (for assessing bacterial growth in the presence of different antibiotic concentrations). The microtiter plate can be used immediately for MIC testing or stored in plastic bags at ≤ -60 °C for up to three months.

Estimation of sub-MIC effect of cefotaxime, silver nanoparticles, and synergism between them on biofilm formation. The choice of sub-minimum inhibitory concentration (Sub-MIC) synergism between cefotaxime and silver nanoparticles was based on previous studies that suggest combining sub-inhibitory concentrations of antibiotics with silver nanoparticles can enhance their antimicrobial effects without promoting resistance. This approach is often used to explore the potential for synergistic interactions that improve efficacy while minimizing side effects or resistance development (Malawong *et al.*, 2021). The assay for biofilm formation followed the same technique. On the other hand, tryptic soy broth included cefotaxime and silver nanoparticles and synergistic interactions between them at their sub-MICs. The plates were incubated at 37 °C for 24 hours. Each well was washed, stained, and then read at 630 nm. The positive controls were prepared by adding 200 mL of fresh bacterial solution, free of cefotaxime, silver nanoparticles, and cefotaxime in accordance with the 0.5 McFarland standards.

Estimation of biofilm inhibition. As mentioned by (Chen *et al.*, 2020), cefotaxime, silver nanoparticles, and their synergism as antibiofilm agents were tested on *S. aureus* biofilm using a sterile 96-well microtiter plate method.

Statistical analysis. The statistical analysis used in this study included descriptive statistics, correlation coefficients (Pearson), and graphical representation to evaluate antibiotic susceptibility, biofilm formation, and the synergistic effects of cefotaxime and silver nanoparticles, with no significant correlations found between treatments and biofilm reduction, and data analysis was performed using Excel 2019.

RESULTS AND DISCUSSION

Samples isolation and identification. All of the isolates grown on mannitol salt agar were utilized*, S. aureus* ferments mannitol, causing the medium to turn yellow, while most non-pathogenic staphylococci do not ferment mannitol (Aryal, 2016). The composition of mannitol salt agar includes beef extract and peptones, which provides the necessary nitrogen, vitamins, minerals, and amino acids for bacterial growth. The 7.5% sodium chloride concentration inhibits the growth of most bacteria except for staphylococci. Sodium chloride also provides critical electrolytes for osmotic balance and transport. As mannitol, a fermentable carbohydrate, produces acid, which if detected by the phenol red indicator, it will help to differentiate staphylococcal species (Selim *et al*., 2014). When the oxidase and catalase activity of all bacterial isolates grown on this medium was examined, the results showed that catalase activity was positive, while oxidase activity was negative.

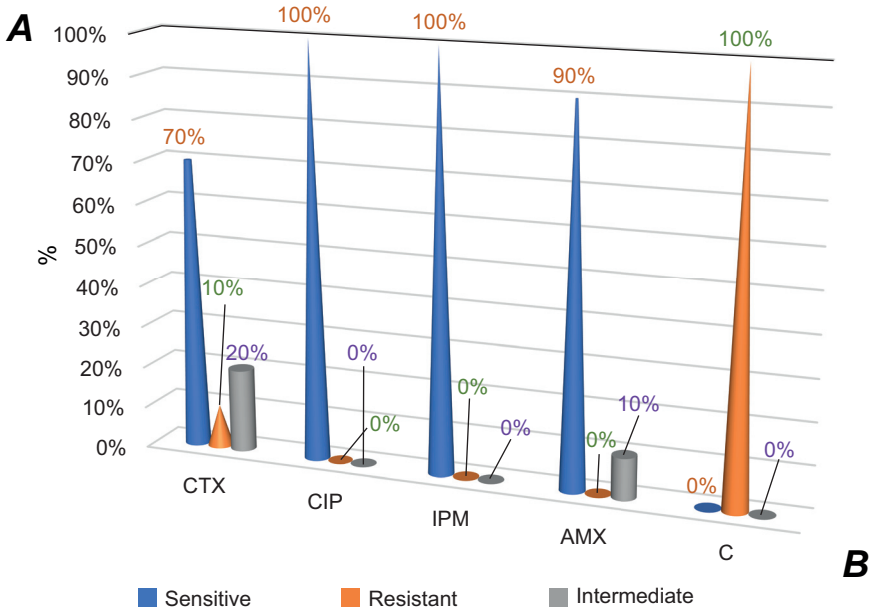
Identification of bacteria by Vitek-2 compact system. The Vitek-2 compact system was employed for the identification of each isolate, and the results confirmed that all isolates were *Staphylococcus aureus* **Table 1**.

Table 1. **Identification of *Staphylococcus aureus* using the VITEK system**

Biochemical details											
AMY	-	PIPLC	-	dXYL	-	ADH1	+	BGAL	-	AGLU	+
APPA	-	CDEX	-	AspA	-	BGAR	-	AMAN	-	PHOS	-
LeuA	-	ProA	-	BGURr	-	AGAL	-	PyrA	+	BGUR	-
AlaA	-	TyrA	-	dSOR	-	URE	+	POLYB	-	dGAL	-
dRIB	-	ILATk	+	LAC	-	NAG	+	dMAL	+	BACI	+
NOVO	-	NC6.5	+	dMAN	+	dMNE	+	MBdG	+	PUL	-
dRAF	-	O129R	+	SAL	-	SAC	+	dTRE	+	ADH2s	-
OPTO	+										
Selected organisms – 96%; Probability – *Staphylococcus aureus*; Bionumber – 050002012763231											

Notes: AMY – amylase; PIPLC – phosphatidylinositol-specific phospholipase C; dXYL – D-xylose; ADH1 – alcohol dehydrogenase; BGAL – beta-galactosidase; AGLU – alpha-glucosidase; APPA – aminopeptidase A; CDEX – cyclodextrin; AspA – aspartase; BGAR – beta-glucuronidase; AMAN – alpha-mannosidase; PHOS – phosphatase; LeuA – leucine aminopeptidase; ProA – proline aminopeptidase; BGURr – beta-glucuronidase, resistant; AGAL – alpha-galactosidase; PyrA – pyrroline-5-carboxylate reductase; BGUR – beta-glucuronidase; AlaA – alanine aminopeptidase; TyrA – tyrosine aminopeptidase; dSOR – D-sorbitol; URE – urease; POLYB – poly-beta-hydroxybutyrate; dGAL – D-galactose; dRIB – D-ribose; ILATk – inositol lactate kinase; LAC – lactose; NAG – N-acetylglucosamine; dMAL – D-maltose; BACI – bacitracin; NOVO – novobiocin; NC6.5 – specific strain used in certain tests; dMAN – D-mannose; dMNE – D-mannitol; MBdG – methyl-β-D-glucuronide; PUL – pullulanase; dRAF – D-raffinose; O129R – resistant to O129; SAL – salicin; SAC – sucrose; dTRE – D-trehalose; ADH2s – alcohol dehydrogenase 2; OPTO – optochin sensitivity

Antibiotic susceptibility testing for *S. aureus.* A total of thirty *S. aureus* isolates underwent susceptibility testing for five antibiotics (imipenem, ciprofloxacin, cefotaxime, chloramphenicol, and amoxicillin-clavulanic acid) using disc diffusion technique, following the guidelines set by the Clinical Laboratory Standards Institute (CLSI, 2013). The results showed that (100%, 100%, 90% and 70%) of the isolates were sensitive to (ciprofloxacin, imipenem, amoxicillin clavulanic acid and cefotaxime), respectively. In contrast, all isolates were resistant to chloramphenicol as shown in **Figure**.



The percentage of antibiotic susceptibility against *S. aureus* isolates (CTX – cefotaxime; CIP – ciprofloxacin; IMP – imipenem; AMC – amoxicillin-clavulanic acid; C – chloramphenicol). ***A*** – sensitivity percentage %; ***B*** – types of antibiotics

Characterization of AgNPs. Atomic force microscopy (AFM) characterized silver nanoparticles (AgNPs). The generation of AgNPs was confirmed when the predicted size and shape of the AgNPs were examined using a scanning electron microscope (SEM). The SEM revealed that the nanoparticles were spherical, and their average size was 29.17 nm. A. Daphedar and T. C. Taranath in 2018 showed that the AgNPs are spherical, with an average size of 10 to 25 nm (Daphedar & Taranath, 2018). Another study in 2016 by Ali *et al.* estimated that the average size of the AgNPs was 30.25±5.26 nm (Ali *et al*., 2016).

Biofilm formation assay. All bacterial isolates were chosen for the biofilm assay because biofilm formation is thought to be a sign of virulence. Several new methodologies have recently been created or modified for biofilm studies, which have helped to provide deeper insight into physiology, structure, and composition (Sharma *et al*., 2023). In this study, 96-well (pre-sterilized) polystyrene microtiter plates, which are commonly used for assessing biofilm biomass, were employed to assess the biofilm-forming ability of *S. aureus* isolates. A microplate reader measured absorbance at 630 nm to evaluate the biofilm concentration. Consequently, the absorbance readings indicated the degree of biofilm formation by the isolates on the surface of the microtiter wells (Chen *et al*., 2020). Our findings were classified into three groups: seven isolates as strong biofilm producers, eighteen isolates as moderate, and five isolates as weak, based on the criteria outlined in **Table 2**.

Based on the criteria outlined in **Table 2**, the present study found that 7 (23.30%) of the *S.aureus* isolates were strong biofilm producers, while 18 (60%) were moderate producers and 5 (16.70%) were weak producers. Variations in biofilm thickness

may be attributed to differences in isolates' capabilities. Signaling molecules named "Autoinducer" produced by each isolate play crucial roles in biofilm development. Differences may arise from the initial number of cells that successfully adhered, as well as variations in the quality and quantity of quorum sensing (Sharma *et al*., 2023).

Table 2. **Biofilm forming capacity of *Staphylococcus aureus* isolates**

Strong biofilm isolates	Mean OD630	Moderate biofilm isolates	Mean OD630	Weak biofilm isolates	Mean OD630
S9	0.295	**S1**	0.198	**S2**	0.115
S14	0.310	**S3**	0.194	S17	0.09
S16	0.342	**S4**	0.196	S18	0.083
S20	0.395	**S5**	0.159	S26	0.091
S23	0.289	**S6**	0.181	S30	0.073
S25	0.421	**S7**	0.199		
S28	0.314	**S8**	0.197		
C	0.038	**S10**	0.211		
		S11	0.152		
		S12	0.149		
		S13	0.131		
		S15	0.182		
		S19	0.191		
		S21	0.129		
		S22	0.158		
		S24	0.112		
		S27	0.151		
		S29	0.160		

S – *Staphylococcus aureus*; C – control; Cut off value 0.05324924

Minimal inhibitory concentration test of (cefotaxime, silver nanoparticles) for *S. aureus.* The results showed that the MICs of cefotaxime against *S. aureus* were distributed between 8 and 32 g/mL, while MICs of silver nanoparticles were between 4 and 63 g/mL. Seven strong biofilm-producing *S. aureus* isolates were tested to determine the MIC for cefotaxime and silver nanoparticles using the microdilution method, as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2013) as shown in **Table 3**. D. Swolana and R. D. Wojtyczka in 2022, reported that the MIC of silver nanoparticles against *Staphylococcus aureus* was 10 µg/mL (Swolana & Wojtyczka, 2022), while K. E. Aldridge (1995) reported that cefotaxime is the stable third-generation cephalosporin against the staphylococcal β-lactamases (Aldridge, 1995).

Table 3. **Minimum inhibitory concentrations of cefotaxime, silver nanoparticles, and synergism between them against *Staphylococcus aureus***

Isolates	MIC cefotaxime	MIC silver nanoparticles	MIC synergism between cefotaxime and silver nanoparticles	
S9	9	31	Cefotaxime	1.5
			Ag NPs	10.2
S14	8	63	Cefotaxime	2.7
			Ag NPs	15.5
S16	8	4	Cefotaxime	1.1
			Ag NPs	2.2
S20	32	8	Cefotaxime	8.0
			Ag NPs	2.6
S23	33	31	Cefotaxime	7.8
			Ag NPs	1.8
S25	16	8	Cefotaxime	2.2
			Ag NPs	1.0
S28	8	62	Cefotaxime	3.2
			Ag NPs	3.9

The impact of sub-minimum inhibitory concentrations (sub-MIC) of cefotaxime, silver nanoparticles, and their combined effect on biofilm formation by *S. aureus*. Due to multidrug resistance and the limited number of alternative medicines available, treating infections is becoming increasingly difficult. The condition becomes more problematic when multidrug-resistant organisms build a 3D structure called a biofilm. Most infections develop biofilms, particularly chronic infections, which are challenging to treat with standard methods (Chhibber *et al*., 2017; Rasool *et al*., 2025). In this investigation, strong biofilm isolates of *S. aureus* were treated with sub-MIC concentrations of cefotaxime and silver nanoparticles, and synergism between them as presented in **Table 4** altered the isolates' ability to create thick biofilms. The findings showed that this weakened their biofilms and reduced their ability to build biofilms, as presented in **Table 5**. At the same time, it is evident that the highest inhibition effect was achieved after treating isolates with a combination of sub-MICs of cefotaxime and silver nanoparticles. The combination of them gave a synergistic action which inhibited biofilm formation even when it reached 100%; the biofilm inhibition under the effect of silver nanoparticles sub-MIC was higher than it under cefotaxime sub-MIC in most isolates, as shown in **Table 5**.

The correlation coefficients for biofilm reduction after treatment with sub-MICs of cefotaxime, silver nanoparticles, and their combination on S9, S14, S16, S20, S23, S25 and S28 isolates, shows that all correlations are weak and insignificant, **Table 6**.

Table 4. **Sub-minimum inhibitory concentration of (cefotaxime, silver nanoparticles) and synergism between them on biofilm formation by *Staphylococcus aureus***

Isolates	Sub MIC cefotaxime	Sub MIC silver nanoparticles	Sub-MIC synergism between cefotaxime and silver nanoparticles	
S9	2	16	Cefotaxime	1.1
			Ag NPs	7.8
S14	4	31	Cefotaxime	1.9
			Ag NPs	6.8
S16	4	4	Cefotaxime	1.6
			Ag NPs	1.2
S20	16	4	Cefotaxime	3.2
			Ag NPs	2.1
S23	16	4	Cefotaxime	1.5
			Ag NPs	2.0
S25	16	16	Cefotaxime	2.2
			Ag NPs	4.6
S28	4	31	Cefotaxime	1.1
			Ag NPs	3.4

Table 5. **The biofilm formation reduction in *Staphylococcus aureus***

Isolates	Biofilm reduction after being treated with cefotaxime	Biofilm reduction after being treated with silver nanoparticles	Biofilm reduction after being treated with a combination of cefotaxime and silver nanoparticles
S9	34%	21%	69%
S14	25%	43%	100%
S16	44%	62%	64%
S20	63%	75%	78%
S23	49%	67%	75%
S25	35%	76%	97%
S28	44%	69%	74%

Table 6. **Correlation matrix**

Correlations				
Correlations		Biofilm reduction after being treated with cefotaxime	Biofilm reduction after being treated with silver nanoparticles	Biofilm reduction after being treated with a combination of cefotaxime & silver nanoparticles
Biofilm reduction after being treated with cefotaxime	Pearson correlation	1	0.580	-0.488
	Sig. (2-tailed)	–	0.172	0.267
	N	7	7	7
Biofilm reduction after being treated with silver nanoparticles	Pearson correlation	0.580	1	0.138
	Sig. (2-tailed)	0.172	–	0.768
	N	7	7	7
Biofilm reduction after being treated with a combination of cefotaxime and silver nanoparticles	Pearson correlation	-0.488	0.138	1
	Sig. (2-tailed)	0.267	0.768	–
	N	7	7	7

This study showed a notable decrease in biofilm formation in all bacterial isolates, especially under the synergistic effect of cefotaxime and silver nanoparticles. Before treatment, the isolates formed biofilm, and the results showed that seven isolates were strong, eighteen moderate, and five isolates were weak. In contrast, the biofilm was reduced after synergistic treatment, and all isolates were weak. Silver nanoparticles alone, as well as in combination with gentamicin, were effective in eliminating biofilm of *Klebsiella pneumoniae*. Sliver nanoparticles functionalized with amino acid effectively broke down the biofilm in vitro and enabled a reduced gentamicin dosage when used together. This aligned with our findings, where the use of silver nanoparticles also contributed to a decreased antibiotics dosage (Taraszkiewicz *et al*., 2013). AgNPs exhibited excellent antibacterial activity at 125 µg/mL for MF953599 while for MF953600 at 62.5 µg/mL. AgNPs exhibited antibiofilm activity against two MDR strains MF953599 and MF953600. Therefore, the nanoparticles might be useful for various purposes such as pharmaceutical product development, water purification, drug delivery, and many other commercial processes (Siddique *et al*., 2020). A bacterial biofilm referred to a community of bacteria encased in a self-produced polymeric matrix, adhering to either inert or living surfaces (Hemati *et al*., 2016). Biofilm-forming bacteria generally exhibit lower antibiotic sensitivity compared to planktonic cells. This reduced sensitivity is thought to results from the exopolysaccharide structure and a decreased metabolic activity (Sharma *et al*., 2020). While antibiotics can reduce biofilm formation, some studies have shown that antibiotics may actually stimulate biofilm formation, depending on the antibiotic class and the bacterial strain (Hemati *et al*., 2016). High level of biofilm formation may be associated with specific phenotypes traits and gene complexes in biofilm, such as capsules, lipopolysaccharides, and fimbriae (Zhao *et al*., 2023). The ability

of low concentrations of natural compounds to inhibit biofilm formation is likely due to their interference with receptors and molecules found in the quorum sensing pathway, which is essential for biofilm development (Shariati *et al*., 2024).

CONCLUSION

In conclusion, the study demonstrated that sub-MIC concentrations of cefotaxime, ciprofloxacin, imipenem, amoxicillin-clavulanic acid, and silver nanoparticles significantly reduced biofilm formation in *Staphylococcus aureus*. The combination of silver nanoparticles (ranging from 4–63 µg/mL) with cefotaxime (8–32 µg/mL) showed the most effective biofilm inhibition, with up to 100% reduction in some isolates. This synergistic effect indicates that silver nanoparticles can enhance the efficacy of antibiotics such as cefotaxime, potentially allowing for reduced antibiotic dosages while effectively combating biofilm-related infections. The correlation coefficients for biofilm reduction after treatment with sub-MICs of cefotaxime, silver nanoparticles, and their combination on S9, S14, S16, S20, S23, S25 and S28 isolates, shows that all correlation are weak and insignificant.

CONFLICT OF INTERESTS

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

FUNDING

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

COMPLIANCE WITH ETHICAL STANDARDS

Human Rights: The bioethics protocol for collecting isolates from Al-Yarmook, Al-Kindi, and Baghdad Medical City ensures patient confidentiality, informed consent, and adherence to ethical standards. All samples were gathered following ethical approval, with strict guidelines for handling and processing. The protocol prioritizes privacy, safety, and transparency throughout the research, maintaining responsibility in laboratory practices. Additionally, all procedures performed in studies involving human participants are in accordance with the ethical standards of the institutional and national research committees, and comply with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

AUTHOR CONTRIBUTIONS

Conceptualization, [S.S.D.]; methodology, [S.S.D.]; validation, [D.B.M.]; formal analysis, [D.B.M.].; investigation, [S.S.D.; D.B.M.]; resources, [G.S.J.]; data curation, [L.A.G.]; writing – original draft preparation, [L.A.G.]; writing – review and editing, [L.A.G.]; visualization, [S.S.D.; L.A.G.] supervision, [H.F.]; project administration, [G.S.J.]; funding acquisition, [S.S.D.].

All authors have read and agreed to the published version of the manuscript.

REFERENCES

Agnihotri, S., Mukherji, S., & Mukherji, S. (2013). Immobilized silver nanoparticles enhance contact killing and show the highest efficacy: elucidation of the mechanism of bactericidal action of silver. *Nanoscale,* 5(16), 7328–7340. doi:10.1039/c3nr00024a
Crossref ● PubMed ● Google Scholar

Aldridge, K. E. (1995). Cefotaxime in the treatment of staphylococcal infections: comparison of *in vitro* and *in vivo* studies. *Diagnostic Microbiology and Infectious Disease,* 22(1-2), 195–201. doi:10.1016/0732-8893(95)00051-b
Crossref ● PubMed ● Google Scholar

Ali, Z. A., Yahya, R., Sekaran, S. D., & Puteh, R. (2016). Green synthesis of silver nanoparticles using apple extract and its antibacterial properties. *Advances in Materials Science and Engineering,* 2016(1), 4102196. doi:10.1155/2016/4102196
Crossref ● Google Scholar

Amirulhusni, A. N., Palanisamy, N. K., Mohd-Zain, Z., Ping, L. J., & Duraira, R. (2012). Antibacterial effect of silver nanoparticles on multi-drug resistant *Pseudomonas aeruginosa*. *International Journal of Medical Science and Public Health,* 6(7), 291–294. doi:10.5281/zenodo.1329578
Crossref ● Google Scholar

Aryal, S. (2016). Mannitol salt agar for the isolation of *Staphylococcus aureus*. *Microbiology Info*. 5–8.
Google Scholar

Atta, S. E., Ghannawi, L., Shakir, O. Y., & Gharab, K. M. (2023). Molecular investigation of *gyrA* mutations in clinical isolates of methicillin-resistant *Staphylococcus aureus* derived from diverse sources. *Al-Rafidain Journal of Medical Sciences*, 5(1), S64–S70. doi:10.54133/ajms.v5i1S.282
Crossref ● Google Scholar

Benson, H. J. (2002). *Microbiological applications: a laboratory manual in general microbiology*. McGraw-Hill.
Google Scholar

Boucher, H. W., & Corey, G. R. (2008). Epidemiology of methicillin-resistant *Staphylococcus aureus*. *Clinical Infectious Diseases,* 46(5), S344–S349. doi:10.1086/533590
Crossref ● PubMed ● Google Scholar

Bruna, T., Maldonado-Bravo, F., Jara, P., & Caro, N. (2021). Silver nanoparticles and their antibacterial applications. *International Journal of Molecular Sciences*, 22(13), 7202. doi:10.3390/ijms22137202
Crossref ● PubMed ● PMC ● Google Scholar

Centers for Disease Control and Prevention (CDC). (2003). Outbreaks of community-associated methicillin-resistant *Staphylococcus aureus* skin infections – Los Angeles County, California, 2002–2003. *MMWR. Morbidity and Mortality Weekly Report*, 52(5), 88.
PubMed ● Google Scholar

Chen, Q., Xie, S., Lou, X., Cheng, S., Liu, X., Zheng, W., Zheng, Z., & Wang, H. (2020). Biofilm formation and prevalence of adhesion genes among *Staphylococcus aureus* isolates from different food sources. *MicrobiologyOpen*, 9(1), e00946. doi:10.1002/mbo3.946
Crossref ● PubMed ● PMC ● Google Scholar

Chhibber, S., Gondil, V. S., Sharma, S., Kumar, M., Wangoo, N., & Sharma, R. K. (2017). A novel approach for combating *Klebsiella pneumoniae* biofilm using histidine functionalized silver nanoparticles. *Frontiers in Microbiology,* 8, 1104. doi:10.3389/fmicb.2017.01104
Crossref ● PubMed ● PMC ● Google Scholar

Daphedar, A., & Taranath, T. C. (2018). Characterization and cytotoxic effect of biogenic silver nanoparticles on mitotic chromosomes of *Drimia polyantha* (Blatt. & McCann). *Toxicology Reports,* 5, 910–918. doi:10.1016/j.toxrep.2018.08.018
Crossref ● PubMed ● PMC ● Google Scholar

El-Fouly, M. Z., Sharaf, A. M., Shahin, A. A. M., El-Bialy, H. A., & Omara, A. M. A. (2015). Biosynthesis of pyocyanin pigment by *Pseudomonas aeruginosa*. *Journal of Radiation Research and Applied Sciences*, 8(1), 36–48. doi:10.1016/j.jrras.2014.10.007
Crossref ● Google Scholar

Frei, A., Verderosa, A. D., Elliott, A. G., Zuegg, J., & Blaskovich, M. A. T. (2023). Metals to combat antimicrobial resistance. *Nature Reviews Chemistry*, 7(3), 202–224. doi:10.1038/s41570-023-00463-4
Crossref ● PubMed ● PMC ● Google Scholar

Hemati, S., Sadeghifard, N., Ghafurian, S., Maleki, F., Mahdavi, Z., Hassanvand, A., Valadbeigi, H., Hemati, S., & Hatami, V. (2016). The association of biofilm formation and sub-minimal inhibitory concentrations of antimicrobial agents. *Journal of Basic Research in Medical Sciences,* 3(1), 26–30.
Google Scholar

Iravani, S., Korbekandi, H., Mirmohammadi, S. V., & Zolfaghari, B. (2014). Synthesis of silver nanoparticles: chemical, physical and biological methods. *Research in Pharmaceutical Sciences*, 9(6), 385–406.
PubMed ● PMC ● Google Scholar

Jaber, G. S., Dhaif, S. S., Abdul Hussian, T. A., Ibrahim, N. A., & Arifiyanto, A. (2023). Enhancing the prodigiosin pigment by adding Ag/TiO_2 synergism for antibacterial activity. *Biocatalysis and Agricultural Biotechnology*, 54, 102900. doi:10.1016/j.bcab.2023.102900
Crossref ● Google Scholar

Kowalska-Krochmal, B., & Dudek-Wicher, R. (2021). The minimum inhibitory concentration of antibiotics: methods, interpretation, clinical relevance. *Pathogens*, 10(2), 165. doi:10.3390/pathogens10020165
Crossref ● PubMed ● PMC ● Google Scholar

Malawong, S., Thammawithan, S., Sirithongsuk, P., Daduang, S., Klaynongsruang, S., Wong, P. T., & Patramanon, R. (2021). Silver nanoparticles enhance antimicrobial efficacy of antibiotics and restore that efficacy against the melioidosis pathogen. *Antibiotics,* 10(7), 839. doi:10.3390/antibiotics10070839
Crossref ● PubMed ● PMC ● Google Scholar

Najim, R. S., Risan, M. H., & Al-Ugaili, D. N. (2024). Antimicrobial efficacy of quercetin against biofilm production by *Staphylococcus aureus*. *Al-Mustansiriyah Journal of Science*, 35(3), 74–80. doi:10.23851/mjs.v35i3.1367
Crossref ● Google Scholar

Rasigade, J.-P., & Vandenesch, F. (2014). *Staphylococcus aureus*: a pathogen with still unresolved issues. *Infection, Genetics and Evolution*, 21, 510–514. doi:10.1016/j.meegid.2013.08.018
Crossref ● PubMed ● Google Scholar

Rasool K, H., Abad, W. K., & Abd, A. N. (2025). Preparation of ZnO nanoparticles from *Juglans regia* dry husk extract for biomedical applications. *Journal of Biosafety and Biosecurity*, 7(1), 1–8. doi:10.1016/j.jobb.2024.10.004
Crossref ● Google Scholar

Selim, S. A., Adam, M. E., Hassan, S. M., & Albalawi, A. R. (2014). Chemical composition, antimicrobial and antibiofilm activity of the essential oil and methanol extract of the Mediterranean cypress (*Cupressus sempervirens* L.). *BMC Complementary and Alternative Medicine*, 14(1), 179. doi:10.1186/1472-6882-14-179
Crossref ● PubMed ● PMC ● Google Scholar

Shariati, A., Noei, M., Askarinia, M., Khoshbayan, A., Farahani, A., & Chegini, Z. (2024). Inhibitory effect of natural compounds on quorum sensing system in *Pseudomonas aeruginosa*: a helpful promise for managing biofilm community. *Frontiers in Pharmacology*, 15, 1350391. doi:10.3389/fphar.2024.1350391
Crossref ● PubMed ● PMC ● Google Scholar

Sharma, S., Mohler, J., Mahajan, S. D., Schwartz, S. A., Bruggemann, L., & Aalinkeel, R. (2023). Microbial biofilm: a review on formation, infection, antibiotic resistance, control measures, and innovative treatment. *Microorganisms*, 11(6), 1614. doi:10.3390/microorganisms11061614
Crossref ● PubMed ● PMC ● Google Scholar

Siddique, M. H., Aslam, B., Imran, M., Ashraf, A., Nadeem, H., Hayat, S., Khurshid, M., Afzal, M., Malik, I. R., Shahzad, M., Qureshi, U., Khan, Z. U. H., & Muzammil, S. (2020). Effect of silver nanoparticles on biofilm formation and EPS production of multidrug-resistant *Klebsiella pneumoniae. BioMed Research International*, 2020(1), 6398165. doi:10.1155/2020/6398165
Crossref ● PubMed ● PMC ● Google Scholar

Swolana, D., & Wojtyczka, R. D. (2022). Activity of silver nanoparticles against *Staphylococcus* spp. *International Journal of Molecular Sciences*, 23(8), 4298. doi:10.3390/ijms23084298
Crossref ● PubMed ● PMC ● Google Scholar

Taraszkiewicz, A., Fila, G., Grinholc, M., & Nakonieczna, J. (2013). Innovative strategies to overcome biofilm resistance. *Biomed Research International,* 2013(1), 150653. doi:10.1155/2013/150653
Crossref ● PubMed ● PMC ● Google Scholar

Tong, S. Y., Davis, J. S., Eichenberger, E., Holland, T. L., & Fowler, V. G. (2015). *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. *Clinical Microbiology Reviews*, 28(3), 603–661. doi:10.1128/cmr.00134-14
Crossref ● PubMed ● PMC ● Google Scholar

Vladár, A. E., & Hodoroaba, V.-D. (2020). Characterization of nanoparticles by scanning electron microscopy. In: V.-D. Hodoroaba, W. E. S. Unger, & A. G. Shard (Eds.), *Characterization of nanoparticles* (pp. 7–27). Elsevier. doi:10.1016/b978-0-12-814182-3.00002-x
Crossref ● Google Scholar

Wayne, P. A. (2015). *Clinical and laboratory standards institute* (*CLSI*) *performance standards for antimicrobial susceptibility testing.*
Google Scholar

Yin, I. X., Zhang, J., Zhao, I. S., Mei, M. L., Li, Q., & Chu, C. H. (2020). The antibacterial mechanism of silver nanoparticles and its application in dentistry. *International Journal of Nanomedicine*, 15, 2555–2562. doi:10.2147/ijn.s246764
Crossref ● PubMed ● PMC ● Google Scholar

Yonathan, K., Mann, R., Mahbub, K. R., & Gunawan, C. (2022). The impact of silver nanoparticles on microbial communities and antibiotic resistance determinants in the environment. *Environmental Pollution*, 293, 118506. doi:10.1016/j.envpol.2021.118506
Crossref ● PubMed ● Google Scholar

Zhao, A., Sun, J., & Liu, Y. (2023). Understanding bacterial biofilms: from definition to treatment strategies. *Frontiers in Cellular and Infection Microbiology*, 13, 1137947. doi:10.3389/fcimb.2023.1137947
Crossref ● PubMed ● PMC ● Google Scholar

Received / Одержано	Revision / Доопрацьовано	Accepted / Прийнято	Published / Опубліковано
27 December, 2024	02 February, 2025	03 June, 2025	25 June, 2025