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Biological Study of Ampicillin Conjugated Silver Nanoparticles Against Staphylococcus Aureus (in Vitro Experiment)

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Abstract
The current work aimed to study the effects of ampicillin conjugated silver nanoparticles against S. aureus. S. aureus were collected and selected from 70 patients (strains were found in samples in the 17(24.3%)) with urinary tract infection who were admittable in Azadi Teaching Hospital, Kirkuk city during a period from September 2021 to January 2022. In vitro activity, Ampicillin conjugated silver nanoparticles against highly multi resistance strains were evaluated by inhibition zone testing method. The results demonstrated that the UV spectrum showed maximum absorption (λ max) at 422 nm. Samples were collected from patients' wounds and as shown in Table (1). A total of 150 samples were taken from UTI patients, with an average of 91 (60.7%) samples for males and 59 (39.3%) for females. The results found that the isolated samples showed positive bacterial growth that was grown on optimal culture media such as blood agar by 68(45.3%), while the percentage of negative growth was 82(54.7%). The susceptibility test for staphylococcus aureus showed a different inhibition zone where the diameter of the inhibition for Aztreonam was 11.3 mm, while the diameter of the inhibition for Tobramycin was 15.8 mm. As for Trimethoprim and Ampicillin only, the inhibition zone diameter was 4.2 and 3.1 mm, respectively. On the other hand, inhibition zone diameter after using ampicillin conjugated silver nanoparticles, reached 42.7 mm. Therefore, it is concluded from the current study that ampicillin conjugated silver has antibacterial effects against staphylococcus aureus.

Keywords: Silver nanoparticles; S. aureus; ampicillin; inhibition zone.

Introduction

Staphylococcus aureus (S. aureus) is a gram positive and commensal bacterium that colonizes 30% of healthy individuals from different body parts [1]. It contributes significantly to infections that range from minor to life-threatening in hospitals and the general population [1-3]. In terms of pathology, S. aureus is the most dangerous member of the staphylococci genus and the cause of a wide range of illnesses, including superficial skin abscesses, food poisoning, and life-threatening conditions such bacteremia, necrotic pneumonia in infants, and endocarditis [4-5]. Direct contact between individuals as well as interaction with contaminated things are two ways that the bacteria might spread (such mobile phones, handles of different types of door, tap faucets, the keyboards computer, the knife, the equipments that useed in medical filed, etc). Even inhaling contaminated droplets that are released during coughing or sneezing poses a risk of transmission [6-7]. Materials with two or more dimensions and a diameter between 1 and 100 nm are known as nanoparticles (NPs) [8]. The preparation of NPs and nanomaterials uses silver, the precious metal with the highest profit-orientedness. These are well-known due to their unusually enhanced physicochemical properties, like optical, thermal, electrical, and catalytic properties, compared to the bulk material, as well as their antibacterial, antiviral, antifungal, and antioxidant properties [9–11]. There are several ways to make silver nanoparticles, including physical, chemical, and biological ones. The chemical reduction approach was chosen and researched because it produces nanoparticles that don't aggregate, has a high yield, requires little effort to prepare, and is simple and gentle [12]. It is utilized as a more potent antibacterial than antibiotics because it forms a strong link with bacteria via the cell membrane and enters the bacterial cell. It is inert in its natural state, but the presence of water or tissue fluids causes it to ionize, which releases bioactive silver ions. Strong affinity exists between this substance and the sulfur groups in cell membranes. Because it depends on the shape, size, stability, and biocompatibility of nanoparticles, there are no negative effects on human health at low doses. However, prolonged exposure to silver can result in serious illnesses such argyrosis [13–14]. Therefore, the current work aimed to study the effects of ampicillin conjugated silver nanoparticles against *S. aureus.*

Materials & methods

Staphylococcus aureus

From September 2021 to January 2022, 150 male and female patients with urinary tract infections who were admissible at Azadi Teaching Hospital in Kirkuk city were gathered and selected for S. aureus testing. On the basis of their appearance and Gram-staining, the bacterial isolates were identified.

In a clean, wide-mouth container, one milliliter of midstream pee was taken. The maximum storage time for unpreserved specimens was 24 hours in the refrigerator or 2 hours in culture. [15].

After being directly added to 5 ml of brain-heart infusion (BHI broth), a loopful of urine sample was transferred to plates of Mannitol Salt Agar, where it was incubated for 24 hours at 37 °C. Following subculture on brain-heart infusion (BHI) agar to purify all colonies from primary cultures, the colonies were re-inoculated onto MSA and incubated at 37°C for 24 hours. [16].

Gram stain, catalase, oxidase, tube coagulase, manitol salt agar, motility, and API staph system biochemical tests are used to confirm the diagnosis [17].

Synthesis of nanoprticles

The Tarkevisch method was used to create silver nanoparticles that were ampicillin conjugated [18]. By combining 21.23 mg of silver nitrate with 250 mL of double-distilled water, a 0.5 mM solution of the metal was created. Similar to this, 102.86 mg of ampicillin was dissolved in 250 mL of water to create a 0.5 mM solution. For the synthesis, sodium citrates and medication (which serves as a capping agent) were added after boiling a silver nitrate solution at 100°C. By combining various metal and ligand concentrations, the reaction was optimized. For the manufacturing of nanoparticles, the metal: ligand concentration ratio that produced the best absorbance value was chosen. Prepared nanoparticles were centrifugation at 12,000 rpm for 15 minutes to recover the nanoparticles, and then the residual reducing agent and antibiotic were washed away and the double water.

Antimicrobial Susceptibility Test

Disk diffusion testing against 5 antibiotics was utilized to determine and interpret the antibiotic sensitivity patterns of isolates to various antimicrobial drugs. The antibiotic disc (NCCLs) recommendation for Gram-positive bacteria was shown in Table 1.

Table (1): shows the antibiotic tablets against gram-positive bacteria

Antibiotics	Symbol	Concentratio n µg/disc	Inhibition zone /mm			Manufacturer
			R	I	S	
Ampicillin	AM	10	≤13	14-16	≥17	Bioanalyse
Aztreonam	ATM	30	≤12	13-14	≥12	=
Clindamycin	DA	10	≤14	-	≥15	=
Tobramycin	TOB	10	≤12	13-14	≥15	=
Trimethoprim	T	10	≤10	11-15	≥16	=

R: resistance, I: intermediate, S: sensitivity

Antibacterial activity

Using the inhibition zone testing method with (MHA) Mueller-Hinton agar [CLSI], the in vitro activity of ampicillin-conjugated AgNPs against highly multi-resistant bacteria was assessed. The inhibition zones were measured in cm after 24 hours of incubation at 37 C^0.

Characters of silver nanoparticle

UV–Visible Spectroscopy (UV–Vis)

The UV-Vis spectrophotometer (Double Beam Spectrophotometer T80 UV/Vis spectrometer) was used to measure the ultraviolet (UV) spectrum. An aliquot of the tested solution was putted in a cuvette, and monitored for wave length (200 to 1000) nm [19].

Scanning Electron Microscopy (SEM)

SEM was used to examine the morphology, shape, and size of the generated nanoparticles. SEM measurements were made at Babylon University's College of Pharmacy using the SEM (FEI QUANTA 450) instrument operating at (10,000 V) [20]

Result & Discussion

Samples distribution

Samples were collected from patients' UTI and as shown in Table (2). A total of 150 samples were taken from UTI patients, with an average of 91 (60.7%) samples for males and 59 (39.3%) for females. The results found that the isolated samples showed positive bacterial growth that was grown on optimal culture media such as blood agar by 68(45.3%), while the percentage of negative growth was 82(54.7%).

Table (2): shows the number and distribution of samples depending on bacterial (S. aureus) growth and gender

Results \ Gender	Male	Female	Total
Negative grpwth –ve	49(59.7%)	33(60.3)	82(54.7%)
Positive growth +ve	42(61.8%)	26(37.2)	68(45.3%)
Total	91 (60.7%)	59 (39.3%)	150(100%)

The results of the current study also showed above that the percentage of positive growth on the agar and primary isolate medium reached 45.3% and this is consistent with the results of Abdullah (2013) study, which indicated that the percentage of Staphylococcus aureus among 337 samples was a strain of S. aureus that was examined. that 140 (41.54%) were positive for bacterial growth. On the other hand, a high prevalence of MRSA was observed (68.89%) in a study conducted to determine the prevalence of antimicrobial resistance among pathogenic bacteria isolated from two major hospitals in Basra, and this is higher than in the current study.

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Isolation & Identification

Clinical S. aureus isolates were diagnosed based on culture characteristics, microscopy and biochemical examinations. The isolates of S. aureus showed yellow medium-sized colonies with yellow areas. Because it is a fermentation of mannitol on mannitol agar medium as shown in Figure (1).

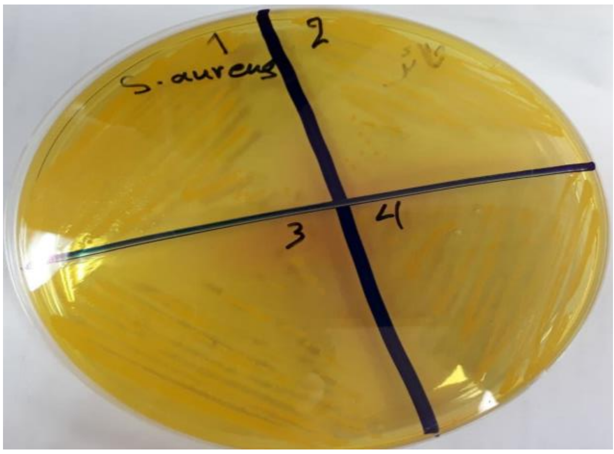

Figure 1: Shows the shape of S. aureus colonies on mannitol agar media.

Ampicillin conjugated silver nanoparticles

When drug (which serves as a capping agent) and sodium citrates were added to an aqueous mixture of AgNo3, the colorless solution changed to a pale-yellow color (fig. 2), indicating the creation of nanoparticles. AM-Ag-NPs was then created utilizing the chemical reduction procedure.

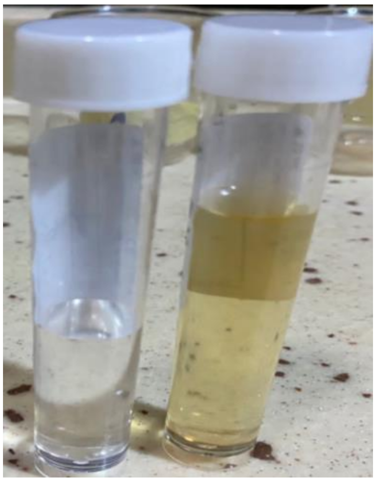

Figure (2): prepared nanoparticles (pale yellow solution) from silver nitrate (colorless solution)

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Preparation and characterization of Ampicillin conjugated silver nanoparticles was determining by using UV-Vis absorption spectrometer with wave length band about 200-1000 nm (fig: 3).

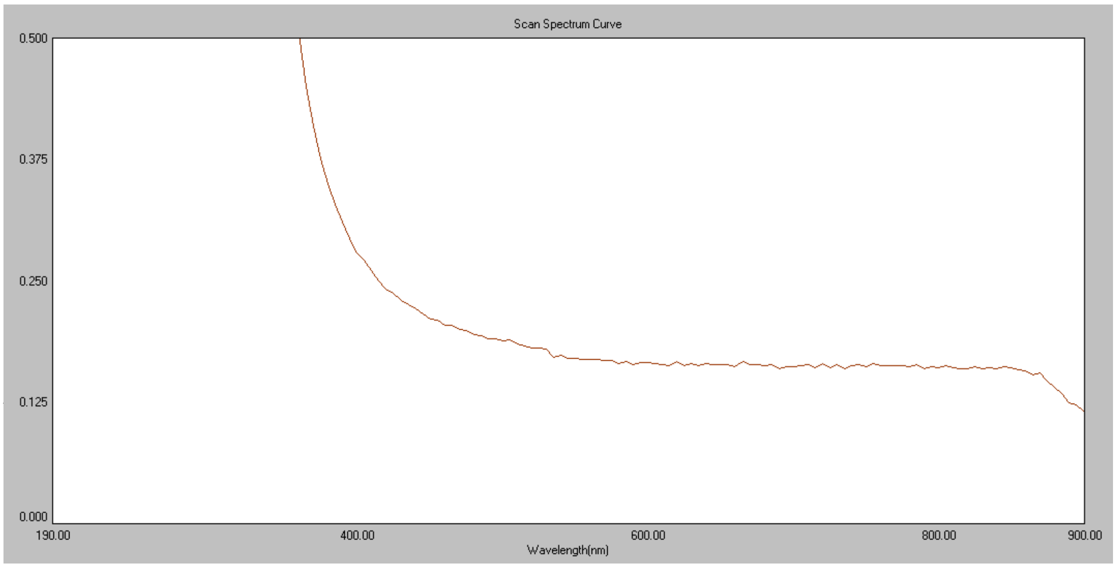

Figure (3): Absorbance band of Ampicillin conjugated silver nanoparticles.

SEM analysis

To investigate the morphological characteristics of AM-Ag-NPs, SEM examinations were carried out. The morphological properties of Rif-Ag-NPs are displayed in Figure 4. The "minimum diameter" and "highest diameter" of each particle must be measured because the particles were irregular rather than circular. We utilized the average area-weighted diameter (as shown in Figure 4).

Susceptibility test

The susceptibility test for staphylococcus aureus showed a different inhibition zone where the highest diameter of the inhibition for Aztreonam was 11.3 mm, while the diameter of the inhibition for Tobramycin was 15.8 mm. As for Trimethoprim and Ampicillin only, the inhibition zone diameter was 4.2 and 3.1 mm, respectively, as shown in table (3) and Figure (5). On the other hand, inhibition zone diameter after using ampicillin conjugated silver nanoparticles, reached 42.7 mm, as in the figure (6).

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Table (3): the diameter of inhibition zone of studied antibiotic and ACS nanoparticles

Isolates	Inhibition zone (mm)					
	ATM (30ug)	AM (10ug)	TOB (10ug)	T (10ug)	DA (10ug)	AM-Ag nanoparticles
S1	8.1	1.4	11.4	3.8	3.9	40.9
S2	7.4	2.1	9.8	2.9	2.3	35.3
S3	11.3	2.9	14.2	2.1	3.4	29.4
S4	6.4	1.8	14.9	4.2	2.5	37.5
S5	10.8	3.1	12.3	3.4	3.5	41.6
S6	9.5	1.7	9.2	2.4	4.1	32.8
S7	11.2	2.8	15.8	2.7	2.4	42.7
S8	8.5	1.9	12.5	4.1	2.8	36.5
S9	6.3	2.3	10.4	3.4	1.9	39.1
S10	10.1	1.5	14.9	2.1	1.3	28.4
Sensitivity	R	R	I	R	R	S

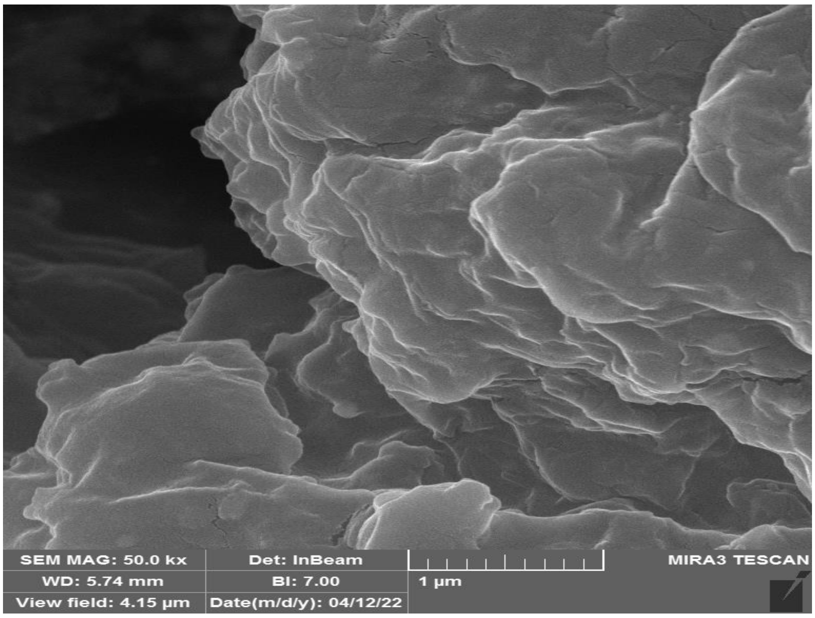

Figure (4): SEM images of ampicillin conjugated silver (AM-Ag) nanoparticles.

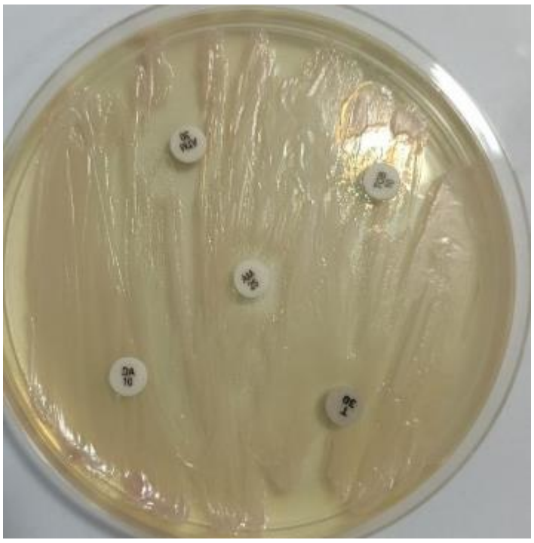

Figure (5): ATM= Aztreonam (30ug), AM= Ampicillin (10ug), TOB= Tobramycin (10ug), T= Trimethoprim

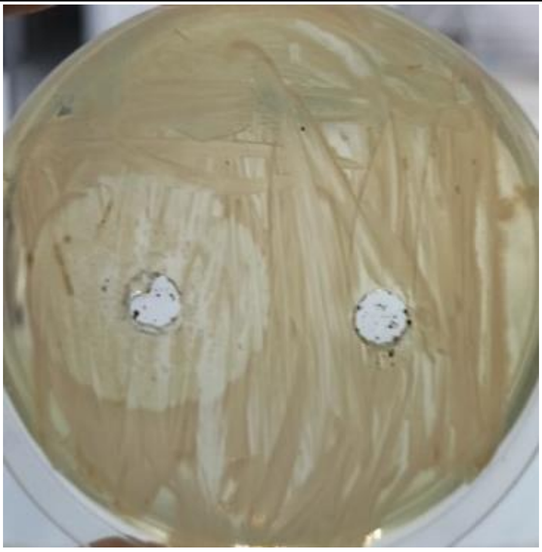

Figure (6): ampicillin conjugated silver nanoparticles

Antibiotics and silver nanoparticles have a well-known synergistic impact [22–24]. Additionally, it has been demonstrated that the only way to achieve a beneficial outcome is to apply silver nanoparticles and antibiotics simultaneously [25]. According to Brown et al. [26], ampicillin-resistant bacterial strains such P. aeruginosa, E. aerogenes, and S. aureus are vulnerable to silver nanoparticles functionalized with this antibiotic. Even more active than silver nanoparticles by themselves, they were. The interaction of AgNPs (drug carriers) with microbial membranes results in the release of antibiotics into the cell, which is likely what causes these synergistic effects [27]. However, compared to their unmodified counterparts, NPs functionalized with ampicillin have been less effective against K. pneumoniae and S. aureus, with E. coli being the lone exception. The amoxicillin-resistant strain of S. aureus was shown to exhibit a similar behavior. The concurrent use of amoxicillin and silver nanoparticles in this case demonstrated an antagonistic impact [28]. Nanoparticles of several pharmaceuticals and organic substan-ces have demonstrated improved biofilm-eradicating capabilities [29]. Our findings are also compared to those of other research, which similarly demonstrated that nano-conjugates have a greater ability to penetrate biofilms than a single drug [30].

The outcomes seen in this investigation are comparable to those with drug-sensitive strains of S. aureus reported in the literature [31-32]. Differences in the attributes of the used bacterial strains and nanoparticles could be the cause of variations in the actual MIC values. Silver nanoparticles were used at significantly lower quantities than those described by Chien et al. [33] to kill bacteria. Silver nanoparticles coated in PVP are demonstrated to be active at lower concentrations when compared to the results obtained here and the antibacterial activity of selenium nanoparticles or chitosan/ silver nanoparticles against MRSA [34-35].

Conclusions

According to the results of current study, it is concluded from the current study that ampicillin conjugated silver has antibacterial effects against staphylococcus aureus.

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