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Article

The Combination Effect of Silver Nanoparticle and Some Antibiotics Against *Staphylococcus Aureus* and *E.Coli* Isolated From Wounds and Burns in Kirkuk City

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Abstract: The study included the isolation and identificstion of some species of pathogenic bacteria from wounds and in burn patients in Kirkuk city during the period from January 2023 to March 2024. 100 samples were collected from in Azadi Teaching Hospital in Kirkuk City The isolates were diagnosed by routine methods, and the diagnosis was confirmed by using the Vitik Compact system. The percentage of wound samples was 55% and the percentage of burn samples was 45%. The results of the culture showed that 15 isolates of *Staphylococcus aureus* appeared at a rate of 23% and 13 isolates of *Escherichia coli* at a rate of 20%. The highest bacterial infection rate was in the age groups (60-75) years with 25 isolates 25% and the age group (1-30) years recorded the least infection with 17 17.5%, while the infection rate of males was higher than females at 37% and 27% for females. The effectiveness of the manufactured nanoparticles was tested against bacterial isolates, as five different concentrations were used: 3, 6, 12, 25 and 50 micrograms per ml. All concentrations showed inhibiting the growth of most bacterial isolates. The results showed that silver nanoparticles recorded the highest inhibitory activity at a concentration of 50 micrograms on *E.coli* and *Staph. Aureus* with inhibition diameters (19 and 13) mm, respectively. The results Showed the synergism between the particles and ampicillin against *E.coli* and *S.aureus* bacteria with an inhibition diameter of 30 and 36 mm, respectively.

Keywords: Staphylococcus Aurous, Escherichia Coli, Silver Nanoparticles, Wounds, Burins

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1. Introduction

The skin performs many complex functions, including providing an ideal environment for deep tissues by isolating them from the external environment and at the same time ensuring communication with them through the exchange and reception of stimuli. In addition, it plays an important role in water balance (skin barrier and sweat glands) and lymphatic response. It is also an important sensory organ through free nerve endings. In addition, it participates in the process of homeostasis and is responsible for the removal, selective absorption and storage of substances [1]. Burns and wounds are rapidly colonized by microorganisms, including Gram positive bacteria, especially Staphylococcus aureus, as a result of the contaminated environment that the patient comes into contact with. Within hours to days, burns and wounds are colonized by Gram-negative bacteria. Early treatment of burns is important to prevent colonization by microorganisms [2] . In recent decades, the overuse and misuse of antibiotics has accelerated the spread of antibiotic-resistant microorganisms, rendering drug therapy ineffective against many strains and leading to the development of multi-antimicrobial resistance (MMR).

Elucidating the mechanisms of microbial resistance is a key step in the development of new antimicrobial drugs [3]. This has led to the rapid development of nanotechnology in recent times, which is known as the use of matter at the atomic, molecular and supra-molecular levels, due to the properties of these materials that depend on shape and size, as it has been used in various fields, including its use in the fields of health, medicine, energy, the environment and many other fields [4] . It was found that bacterial and fungal strains can develop resistance to antibiotics. Therefore, the use of nanoparticles instead of antibiotics was resorted to because they contain anti-microbial properties, including a large surface area compared to the size, which is (1-100) nanometers [5] . The study aimed to isolate and diagnose some species of bacteria from burns and wounds in different areas of the body in Kirkuk city, study the sensitivity of isolates to antibiotics and identify resistant species, study the effect of silver nanoparticles on bacterial isolates., study the synergistic effect of silver nanoparticles In combination with some antibiotics against bacterial isolates.

2. Materials and Methods

Samples Collection

Samples were collected for the period from (1-12-2023) to (1-3-2024). 100 samples were collected from patients with wound and burns infections from out patients and in patients in Azadi Teaching Hospital. Samples were collected from patients after consulting a specialist doctor and wearing personal protective equipment and using swabs containing Media Samples were taken from the purulent injury areas and transferred to the laboratory at Kirkuk University / College of Education for Pure Sciences, Department of Biology. After filling out the questionnaire form for all ages and both sexes, the samples were cultured on MacConkey Agar , blood agar and Mannitol Salt medium and incubated at 37 °C for 24 hours. After that, a microscopic examination was performed using Gram stain and diagnostic tests were performed, represented by IMVIC tests. , Oxidase, Catalase. Coagulase, Novobiocin test and then the diagnosis confermed using Vitek apparatus .

Antibiotic Susceptibility

Antibiotic susceptibility was tested according to the Kirby-Bauer method [6] and the diameters of inhibition were measured with a standard ruler and compared according to the standard tables [7].

Silver Nanoparticles Activity Against Bacteria

Five different concentrations were used(50, 25, 12, 6, 3) micrograms / ml on two types of bacteria(E.coli , S.aureus) to determine the ability of these concentrations to inhibit bacterial growth. The drilling diffusion method was used according to what was stated in [8].

Nanoparticle Characterization

Scanning Electron Microscope (SEM), Zeta Potential Analysis and X-Ray Diffraction (XRD) were performed to characterize silver nanoparticles and the tests were conducted in the Republic of Iran/Tehran University

Preparation of Nanoparticle Concentrations

The concentrations were prepared by dissolving 0.05 mg in 10 ml consisting of (5 ml deionized water + 5 ml of DMO) and the concentration was equal to 50 micrograms/ml and using the general dilution law C1V1=C2V2 The concentrations (25-12.5-6-3) micrograms/ml were prepared and sterilized using Millipore filter 0.22 micrometers.

Preparation of Antibiotics Concentration

The concentrations were prepared by dissolving 0.075 mg in 10 ml of distilled water, so the concentration was equal to 75 micrograms/ml, and using the general dilution law C1V1=C2V2, the concentrations (50-25) were prepared.

Preparation of mixture of antibiotics and silver nanoparticles solutions The mixing ratios of the solutions were prepared according to [9] to obtain the final concentrations of the solutions, as shown in Table 1.

Table 1. The ratios of incorporation of antibiotics with nanoparticles.

First percentage	Second percentage	Third percentage
Nanoparticels Antidiotic	Nanoparticels Antidiotic	Nanoparticels Antidiotic
3 : 1	1 : 1	1 : 3
Final concentration	Final concentration	Final concentration
μg /ml	μg /ml	μg /ml
75/25	50/50	25/75

Combination of silver nanoparticles and antibiotics effect

The test was conducted using the well diffusion method, where Mueller Hinton medium was prepared according to the manufacturer's instructions, then left to solidify, after which the bacterial(aged 18-24 hours)suspension was prepared and compared with the standard McFarland turbidity- standard solution at a concentration of (1.5 x 108) cells/ml. The bacterial isolates were spread using sterile cotton swabs on Mueller medium in all directions, then holes were made using a cork drill and 60 microliters of (75/25 - 50/50 - 25/75) micrograms/ml of the mixture of nanoparticles and antibiotics were placed in the holes, then placed in the incubator at a 37°C for 24 hours, and the resulting inhibition diameters for each concentration were recorded [10].

3. Results and Discussion

Collection and Diagnosis of Samples

After the samples were planted on the appropriate culture media, the bacterial isolates were diagnosed morphologically and microscopically, in addition to conducting biochemical tests. The number of positive bacterial isolates reached 64 isolates, representing 64%, with 35 wound samples, representing 54%, and 29 burn samples, representing 45%. The laboratory culture results showed that S.areus was obtained in numbers (15) at a rate of (23%) and E.coli in numbers (13) at a rate of (20%).. The percentage was higher among males at 58% and among females at 42%. This is consistent with the results of the two who showed that the infection rate among males was higher, as it was 59.1% and females at 40.9%.This is consistent with [11].

Antibiotic Sensitivity Test Results

The sensitivity of the isolates was tested to 16 types of antibiotics: Imipenim, Levofloxacin, Gentamicin, Amikacin, Azethromycin, Ampicilin, Tobromycin, Amoxicillin, Clarithromycin, Carbapnem, Clindamycin, Amoxicillin, Cefotaximase, Clavulanate, Ceftazidime, Cefoxitin, Ciprofloxacin. The results showed a high percentage of resistance of the bacterial isolates to antibiotics (Ampicillin, Clindamycin, Cefotaximase, Ceftazidime), while the rest of the antibiotics had good sensitivity. As shown in the figure 1. These results are similar to the researcher's results [12].

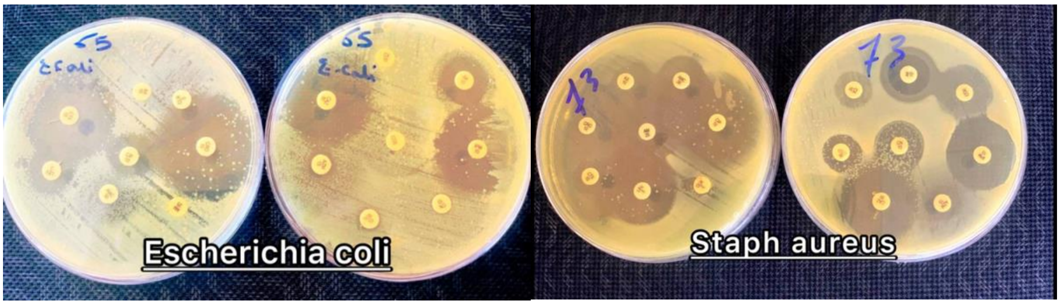

Figure 1. Antibiotic results on bacterial isolates.

Results of Nanoparticles Test

FE-SEM Analysis

FE-SEM was used to examine the shape and size of silver particles. Figure (2) shows FE-SEM images of silver powders synthesized by sol-gel chemical method and at different magnification powers (10μm, 1μm, 500 nm, 200nm), and the product mostly contains regularly arranged spherical nanoparticles with few clusters with an average diameter of (44.6865nm), as shown in Table 2 [13].

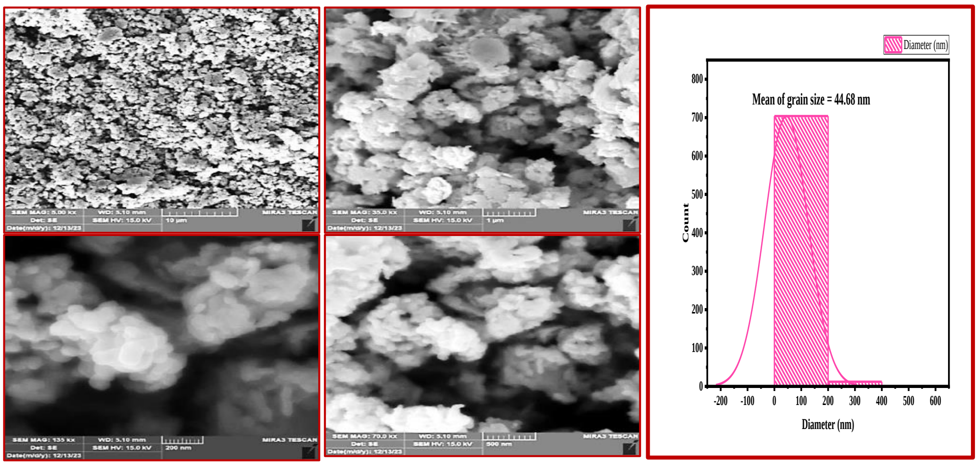

Figure 2. FE-SEM images and graph of grain sizes of silver nanoparticles.

Table 2. Average particle size and standard deviation of silver nanoparticles.

	N total	Mean	Standard Deviation	Sum	Minimum	Median	Maximum
Diameter (nm)	717	44.6865	80.7997	32040.237	15	30	1875

Zeta Potential Test

In this study, the repulsion between the particles was confirmed by the significantly high negative value of the zeta potential, which contributes to the stability of the formulation. The zeta potential value of the sample was found to be (-80.1mV) as shown in Figure 3 The negative value indicates the stability of the nanoparticles and their evasion of nanoparticle agglomeration. The negative potential value may be a result of the current blocking action by the silver nanoparticles.

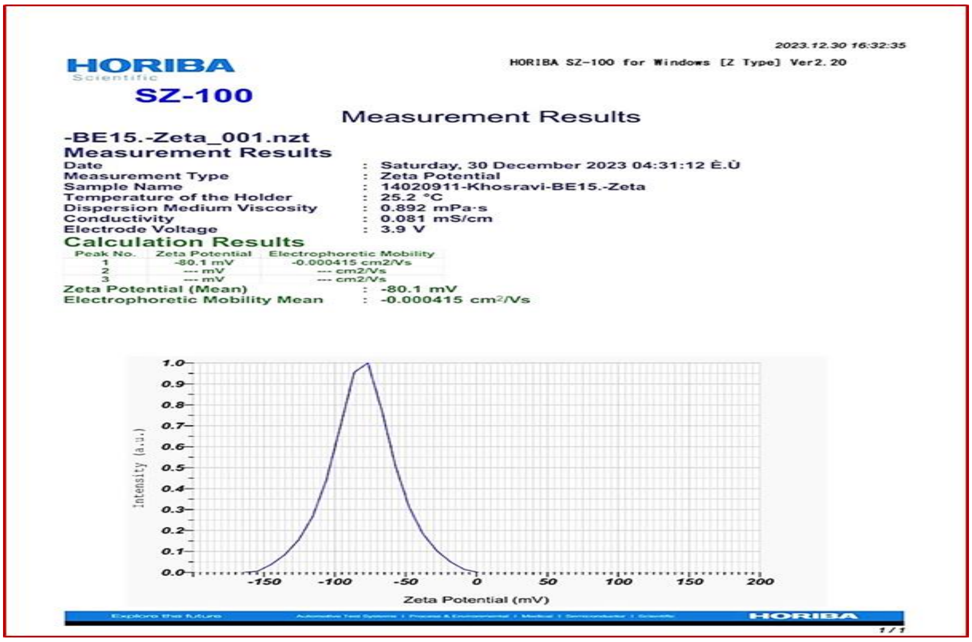

Figure 3. Zeta stress test result.

X-ray Diffraction Analysis

The sample under study was analyzed using a (Philips PANalytical X'Pert) XRD system. The X-ray source used was CuKα radiation, which has a wavelength of 1.54 Å). The sample was analyzed using a scanning technique with an angle difference of 0.1°, covering the 2θ range (from 10° to 80°). The diffraction peaks are prominent and highly concentrated. This showed that the materials have a clear crystalline structure. The peaks showed slight variation, indicating that the crystalline material was free of impurities. When the results were compared with the internationally recognized JCPDS standard tables with the sequence (96-110-0137), it was noted from Figure 4 that the silver nanoparticles showed the (Ag4) phase with a cubic crystalline structure. These results are consistent with the results of [14].

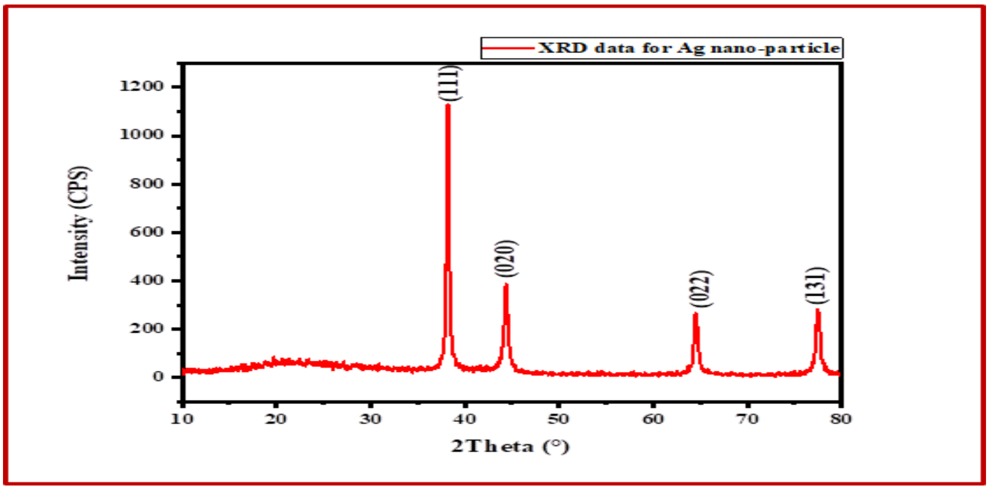

Figure 4. XRD graph of silver nanoparticles.

Inhibitory Effect of Silver Nanoparticles on Bacterial Isolates

Table 3 and Figure 5 show the results of the inhibitory effect of silver nanoparticles on two types of pathogenic bacteria, namely (S.aureus and E.coli). The results of the current study showed the inhibitory effectiveness of silver nanoparticles towards E.coli bacteria with an average diameter of inhibition at concentrations (50, 25, 12, 6, 3) micrograms / ml being (19, 16, 13, 6, 2 mm), respectively. This result is close to the result of the researcher [15], as the average diameter of inhibition at the same concentrations was around (17-3) mm. As for S.aureus, the rate of inhibition diameter appeared at the concentrations mentioned above (13, 11, 10, 7.6, 2.8) mm, and this result is close to the result of [16]. Which is around (5-17mm). To explain the mechanism of action of nanoparticles on bacteria, it may be due to the fact that silver nanoparticles have physical, chemical and biological properties that differ from ordinary silver ions, as they can concentrate on the bacterial cell wall after adhering to the cell wall and penetrate the cell membrane and the nanoparticles enter the bacteria. There is an antibacterial effect that depends on the size of the nanoparticles, i.e. smaller nanoparticles have a large surface area that contacts bacterial cells and can reach the cytoplasm more than large nanoparticles. This procedure will lead to physical changes in the bacterial membrane, such as membrane damage, which can lead to leakage of cellular contents and death of bacteria [17]. Since the bacterial cell membrane has a negative charge due to the presence of carboxyl, phosphate and amino groups, the positive charge of silver nanoparticles gives them the ability to attract towards the negatively charged cell membrane of the bacteria, thus facilitating the binding of AgNP to the bacterial cell membranes [18].

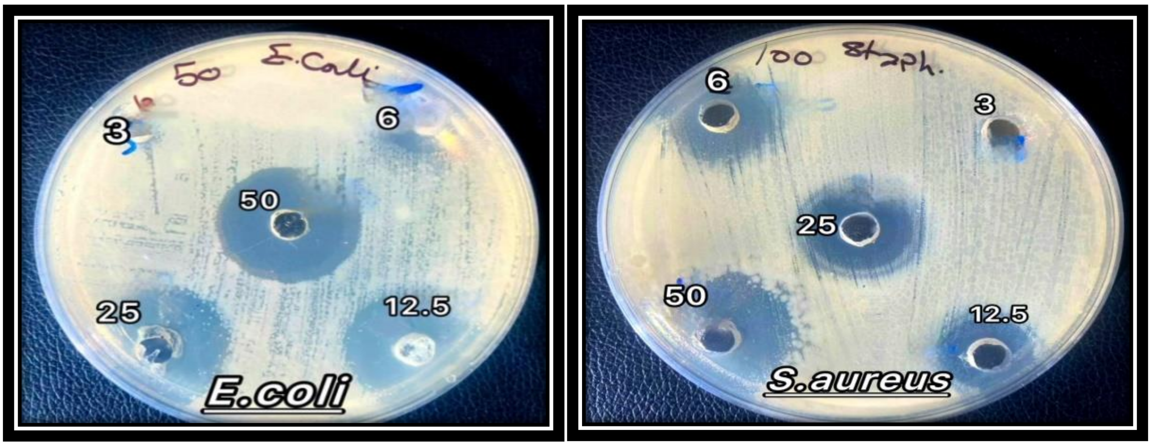

Figure 5. shows the effect of silver nanoparticles on bacterial isolates.

Table 3. shows the average inhibition diameters of silver nanoparticles on bacterial isolates.

Average Inhibition zone	Bacteria species
8.9867 a	*S.aureus*
11.4769 b	*E.coli*

Effect of Combining Antibiotics With Silver Nanoparticles on Bacterial Isolates

The results of the study showed an increase in the effectiveness of the antibiotic when combined with AgNPs against all bacterial isolates, as the highest inhibitory effectiveness was recorded when combining the antibiotic Ampicillin with AgNPs for E.coli and S.aureus at concentrations (25:75) 30, 36 mm, and at concentration (75:25) the average diameter of inhibition reached 26, 27, 30, 32 mm, while at concentration (50-50) the average diameter of inhibition reached 23, 27 mm. These results are close to the results of [19], while when combining the antibiotic Ciprofloxacin, the average diameter of inhibition at a concentration of (25:75) was 28, 36 mm, while the average diameter of inhibition at a concentration of (75:25) was 24, 25 mm, and at a concentration of (50:50) 19, 20 mm. These results are close to the results of [20]. As for the antibiotic Cefotaxim, when combined with AgNPs, the average diameter of inhibition at a concentration of (75:25) was 27, 29 mm, and at a concentration of (25:75) 24, 25 mm, and at (50:50) 20, 20 mm. as show in the Figure (6) . These results were close to the results of [21]. The results of [22] support the coupling of AgNPs with topical antibiotics including ampicillin, ciprofloxacin and cefotaxime as they have great potential in topical preparations when treating resistant bacterial infections. When AgNPs were mixed with these antibiotics, an increase in the rate of inhibition diameters was shown, meaning that silver nanoparticles increased the effectiveness of antibiotics. The possible mechanism involved in the pharmacodynamic interaction between antibiotics and AgNPs is through increasing the level of ROS and membrane damage after protein release and leakage of K+ and inhibition of the bio-manufactured membrane. It is more closely associated with the surface of the bacterial cell and enters the bacteria, which leads to DNA replication and interruption of ATP production and directly affects the cell structure. It is likely that the bactericidal effect of AgNPs is related to inhibition of Phosphomannose isomerase, which is involved in the transfer of mannose-6-phosphate to fructose-phosphate, which is the main control of glycolysis and a common sugar catabolism mechanism in living organisms [23] and Table 4 shows the statistical results of antibiotics combined with nanoparticles on bacterial isolates in general, as it was found that there is a significant difference between Ciprofloxacin + and Cefotaxim, and there is no significant difference between Ampicillin and Ciprofloxacin at a probability level of $0.05 \geq P$.

Table 4. Statistical results of antibiotics combined with nanoparticles on bacterial isolates in general.

Average Inhibition zone	Antibiotic
28.6000 a	Ampicillin+Ag NP
28.1167 a	Ciprofloxacin+ Ag NP
26.1667 b	Cefotaxim+ Ag NP

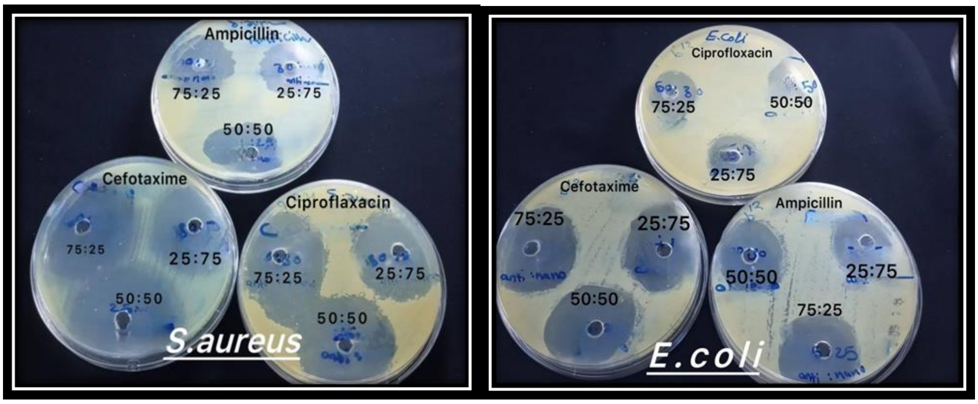

Figure 6. Effect of silver nanoparticles with some antibiotics on bacterial isolates.

4. Conclusion

The study showed that the infection rate among males was the highest, while the infection rate among females was the lowest. The infection rate with bacteria was in the age groups (40-06) compared to other age groups, and the lowest infection rate was in the age group (1-30). It also showed that the infection rate in wounds was higher than in burns, while silver nanoparticles showed a clear inhibitory effect on bacterial isolates. When antibiotics and silver nanoparticles were combined, the inhibitory efficacy increased.

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