

# Studying the effect of Gold Nanoparticles on some bacterial species isolated from surgical wound infections

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## Abstract

The process of synthesis of gold nanoparticles biologically is a safe, environmentally friendly and inexpensive method and had an effect on the most common bacterial species in surgical inflammation, as the gold particles were synthesized in a biological way and no toxic substance was used, as secondary metabolites (*K. pneumoniae*) as a reducing agent of HAuCl<sub>4</sub>.4H<sub>2</sub>O chloric acid to gold nanoparticles.

Characterization of gold nanoparticles was carried out using ultraviolet spectroscopy, which showed that the absorption peak was at 572 nm. The infrared absorption spectrum showed several peaks ranging between (600-4000) evidence of the interference of gold nanoparticles with the particles contained in the bacterial suspension (leachate), and transmission electron microscopy (TEM) images showed that gold nanoparticles were spherical in shape, and X-ray diffraction examination showed that they are particles of spherical shape. Nano-gold compared to the reference card (ICDD card No.03-065-2870).

**Keywords:** nanoparticles; Nanographite oxide, Nanocomposites

## INTRODUCTION

Gold particles are very small particles of gold measured in nanometers smaller than the wavelength of light, as gold shows amazing properties when it is in nanometer sizes, as the color of gold changes at the nanoscale from golden to red, orange or blue, and this depends on The size, shape, and distances between gold nanoparticles, as the color changes from golden to orange when the distance between its grains is 80 nanometers, and when it becomes smaller within 20 nanometers, it becomes red (Capek, 2013), Due to its practical side, it is effectively successful as an antimicrobial aid that is somewhat healthy and non-toxic compared to antibiotics. In recent years, researchers have increased interest in the activity of gold particles as an antimicrobial agent, because it has less toxic effects on mammalian cells

compared to silver nanoparticles (Abdel Kareem&Zohri,2018). As there are many alternative techniques to chemical and physical methods for producing gold nanoparticles, the use of microorganisms is one of those environmentally friendly and inexpensive alternatives, as it uses natural products to manufacture nanoparticles such as polysaccharides, microbes (fungi - bacteria, yeast), algae, Plant extracts and plant tissues (leaves - fruits - peels - flowers - roots - stems - latex) (Hasan,2015), The physical and chemical methods include many defects, the most important of which is the use of carcinogenic materials and solvents that cause radioactive contamination and are highly toxic (Hu et al., 2020). The effectiveness of gold nanoparticles against microbes includes a variety of gold nanoparticles.

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### Access this article online

#### Quick Response Code:



**Website:**  
www.pnrjournal.com

**DOI:**  
10.47750/pnr.2022.13.04.106

Received date: 17 August 2022

Accepted: 10 September, 2022

Published: 11 October, 2022

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How to cite this article Latif Yassin A H, Faisal Subhi B, Studying the effect of Gold Nanoparticles on some bacterial species isolated from surgical wound infections, J Pharm Negative Results 2022;13(4):798-803

(gold - silver - zinc - titanium - copper) as these particles interfere with the process of protein biosynthesis and production processes (DNA, RNA), In addition to disrupting the functions of the bacterial cell membrane (Singh et al., 2018), the antimicrobial effect of gold nanoparticles is mainly summarized through two mechanisms, the first is by preventing the coupling of the ribosomal subunit with tRNA, and the second includes the breakdown of membrane components that inhibits ATPase enzymes and thus reduces Energy output (Cui et al., 2012).

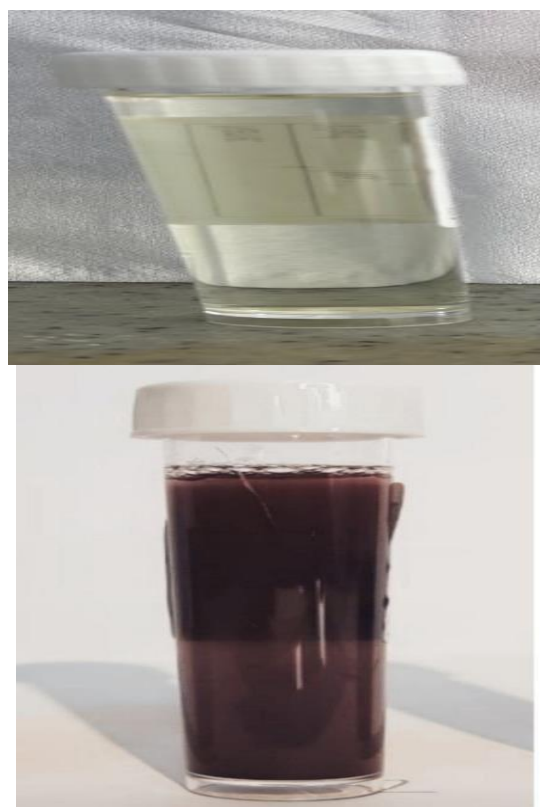
There are many studies that indicated the predominance of E.coli, K.pneumoniae bacteria in the hospital environment, and the reason may be due to its natural resistance to many common antibiotics for use in hospitals, as well as its ability to possess genes that are resistant to most antibiotics through the transmission of a substance A new genetic or mutation (Chabuck et al., 2011; Sood et al., 2008), S.aureus bacteria appeared in a percentage of (9.74%), as it is one of the most important opportunistic bacterial species, and its presence in the surgical site does not constitute any shock, as it is responsible for most cases of infections of wounds and ulcers, and this confirms what was indicated by (Bitrus et al., 2018).

### Materials and methods of work

In this study, (59) isolates were used from a total of (154) samples of Gram-negative and Gram-positive bacteria isolated from patients with inflammatory surgeries attending and inpatients at the Ramadi General Teaching Hospital and Women's and Children's Hospital in Ramadi City (Anbar Governorate). 4/10/2021 to 1/2/2022, the study included (Caesarean deliveries - appendix - tonsils - colon - hemorrhoids), for both sexes and at different ages, in order to investigate the presence of E.coli, K.pneumoniae, S.aureus bacteria in Surgical infections and isolates were distributed (25) E.coli, (19) K.pneumoniae, (15) S.aureus. Gram-negative bacterial isolates (E.coli, K.pneumoniae) were grown on MacConkay medium, which is one of the differential media. It contains crystal violet and yellow salts, thus allowing the growth of Gram-negative species (including the Enterobacteriaceae family) and inhibiting the growth of Gram-positive species, while Gram-positive isolates (S.aureus) grew on the medium of blood agar, which is It is one of the rich media, and the medium of mannitol salt agar, which is a differential media between S.aureus and other Staphlococcus spp..

### Biosynthesis of gold nanoparticles

The biosynthesis of gold nanoparticles was carried out by Klebsiella pneumoniae bacteria, as the suspension containing the bacterial culture was added after boiling gold chloride solution at a temperature of (150C) with stirring, after (30) minutes the color of the solution changed from pale yellow to violet, Fig. (1) .



**Figure 1:** shows the color change from pale yellow to purple

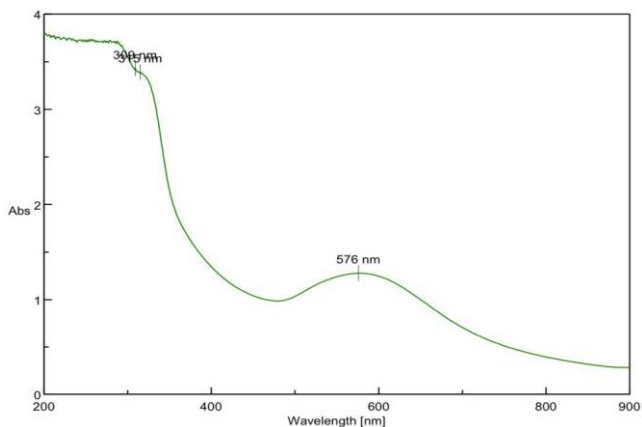
The appearance of the purple color indicates the reduction of the size of gold chloride to nanoparticles using a suspension of Klebsiella pneumoniae bacteria. The use of the biosynthesis method for nanoparticles allows the use of secondary metabolic products as a reducing agent, as the current study included the use of extracellular enzymes and biomolecules secreted by K. pneumoniae as a reducing agent for gold chloride solution instead of nitrate reaction, and this is in agreement with many studies (Al-Shaabani et al., 2020; Al-Tae et al., 2018).

### Characterization of the structure of gold nanoparticles

UV-vis spectroscopy, transmission electron microscopy (TEM), infrared spectroscopy and X-ray diffraction were used to examine the morphological features of gold nanoparticles.

### UV-Visible spectroscopy of AuNPs

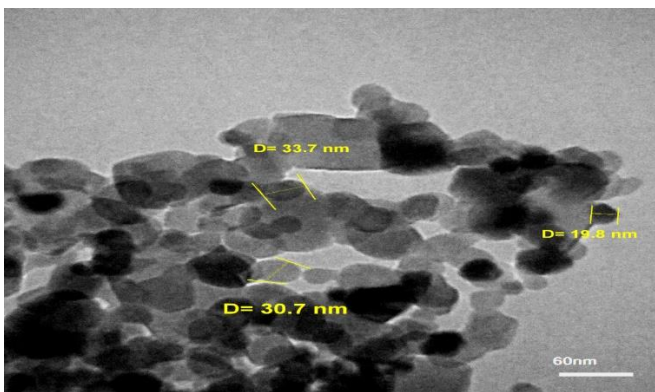
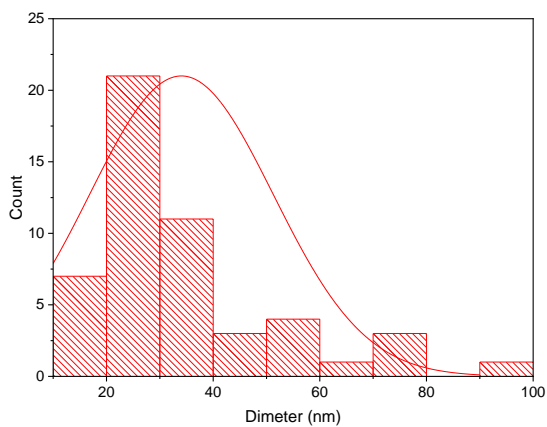
In order to reveal the optical properties of the bio-produced gold nanoparticles (AuNPs), UV-visible spectroscopy was used depending on the value of the UV-visible spectroscopic absorption at wavelengths (400-700) nanometers, figure (2), which indicates the absorption atom Gold, as the result of the current study agrees with (Al-Shaabani et al., 2020; Al-Tae, 2018).



**Figure 2:** UV visible spectrum of a solution of gold nanoparticles

**Transmission Electron Microscope analysis(TEM)**

Using transmission electron microscopy (TEM) to identify the shape and size of gold nanoparticles produced by Klebsiella pneumoniae bacteria and their distribution method. to an oval at a rate of 29 nanometers.

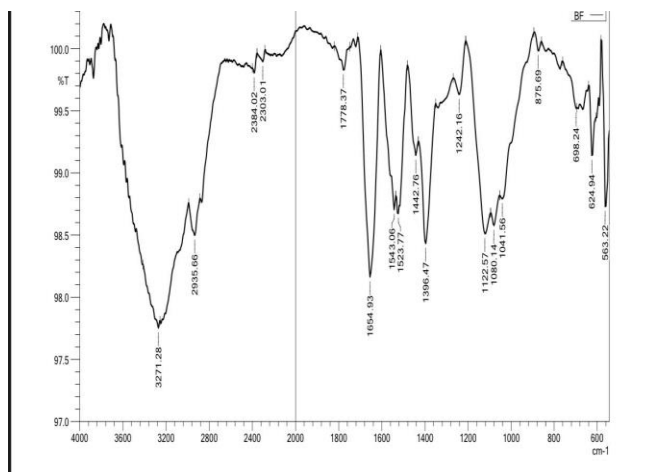


**Figure 3:** Gold nanoparticles by transmission electron microscopy (TEM)

Spherical nanoparticles are often formed at the beginning of the reaction and assemble with each other, and this is due to the presence of a large amount of reducing agent in the bacterial suspension of *K.pneumoniae*, and the result of this study came with (Al-Shaabani et al.,2020;Al- Taeq et al., 2018).

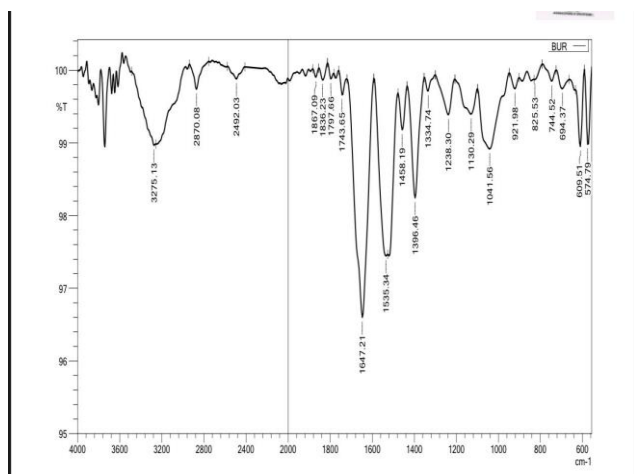
**Infrared spectroscopic analysis**

Figure (4) shows obtaining absorption bands with different positive numbers and functional groups related to the biomolecules of gold nanoparticles. (FTIR) is a useful spectroscopic technique for studying different molecular vibration patterns and a powerful tool for determining the types of chemical bonds in a molecule by producing the absorption spectrum of radiation. Infrared, which resembles a (molecular fingerprint).



**Figure 4:** FTIR Biosynthetic Gold Nanoparticles

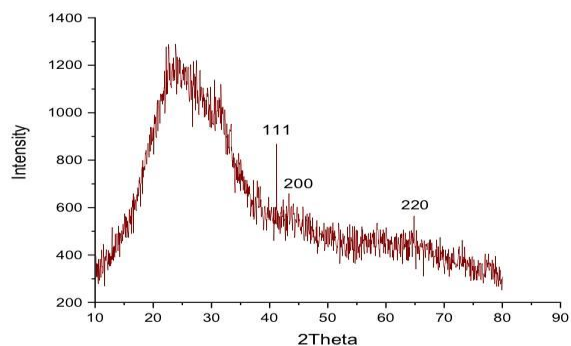
Infrared spectroscopy of the bacterial filtrate proved the presence of phatic nitrogen compounds such as  $\text{NO}_2$ , which indicate the presence of symmetrical bonds that appear during the survey, as it showed in the range (1543.06  $\text{cm}^{-1}$ ), and long-range bonds (1654.93  $\text{cm}^{-1}$ ) which refer to compounds (Alkenes) with bonds (C = C), as well as compounds with bonds (O = C) that indicate the presence of carboxyl groups, which appear within the range (1778.37  $\text{cm}^{-1}$ ) and the group of alcohols that appeared in the range (3271.28  $\text{cm}^{-1}$ ). -1) Which contains the (H-O) sphincter (Stuart, 2004). When comparing the FTIR results of the solution containing the bacterial filtrate, it is possible to observe the appearance of some bonds and the disappearance of some, and that some of the absorbance has decreased due to its consumption as a capping agent during the synthesis process (Srikar et al., 2016). Thus, the bacterial filtrate was distinguished as being rich in aggregates. It is a rich source of capping agents that stabilize nanoparticles, and this competes with chemical and physical methods that use toxic, harmful and expensive materials (Pavia et al., 2008).



**Figure 5:** FTIR of K.pneumoniae . bacterial filtrate

### X-Ray diffraction

An X-Ray diffraction device was used to measure the size of gold nanoparticles and study their characterization and crystalline nature. The rays have a high transmittance property for materials with a specific absorption capacity that depends on the density of the material, making it more accurate in measuring the size of nanoparticles (Bunaciu et al., 2015).



**Figure 6:** XRD Synergistic effect of gold nanoparticles and ceftriaxone against E.coli, K.peumoniae and S.aureus

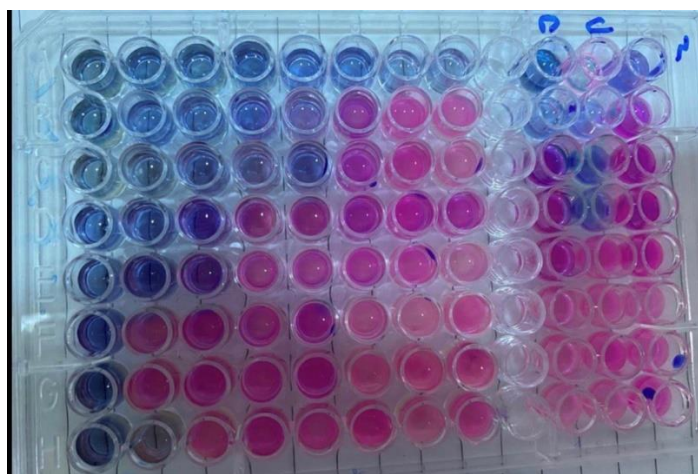
Ceftriaxone antagonist was used in detecting the synergistic effect of gold nanoparticles because the bacterial isolates used in the study had proven their high resistance to this antagonist, so it was chosen for this study.

The microdilution method Checkerboard was used to study the minimum inhibitory concentration (MIC) of gold nanoparticles and antibiotics individually and in combination, and thus calculate the value of the partial inhibitory concentration index (FIC) for gold nanoparticles and anti-Ceftriaxone individually, as the concentration of the antibody used was (100,000), and the concentration of Colloidal gold solution (98).

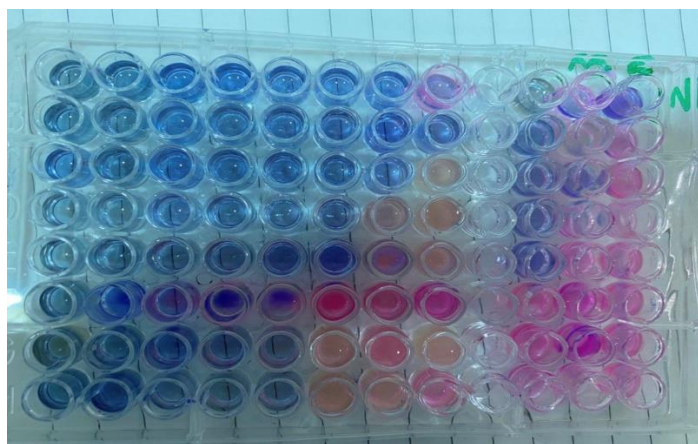
The results of the current study showed that the minimum inhibitory concentration (MIC) of ceftriaxone for E.coli was 3,125, and (MIC) for gold nanoparticles (49), while the synergistic effect was between (MIC) of the antibiotic and (MIC) of gold nanoparticles (AuNPs) for (FIC) is less than (0.5) as it reached (0.3), as shown in Figure (7).

As for the bacteria K.pneumoniae, the MIC of the antibody reached (25), the concentration of the (MIC) for AuNPs was (49), while the value of the molecular inhibitor concentration index (FIC) for the synergistic effect between the MIC of the antibody and (MIC) of AuNPs was (0.5) As shown in Figure (8).

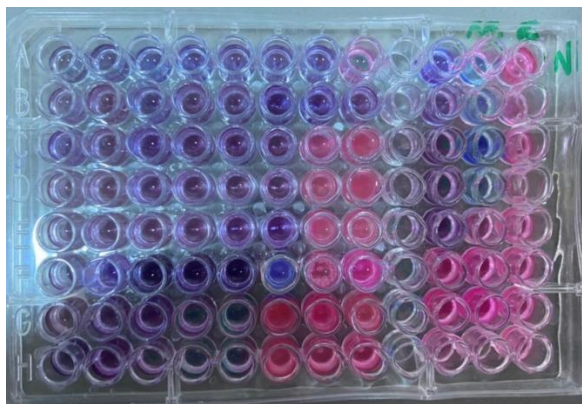
As for S.aureus bacteria, the results showed that it was not affected by the same force as E.coli and K.pneumoniae bacteria, meaning that the extent of their effect did not result in killing the bacteria, but rather affected the genetic level as shown in Figure (9).



**Figure 7:** shows the synergistic effect of K.pneumoniae by the Checkerboard method



**Figure 8:** The synergistic effect of E.coli bacteria is placed by the Checkerboard method



**Figure 9:** S.aureus bacteria by Checkerboard method

Antibiotics conjugated with gold nanoparticles showed a higher effect than if they were alone, as the antibiotics when combined with gold nanoparticles are easier to penetrate into the bacterial cell wall, thus disrupting all regulatory activities of the cell membrane and inhibiting the protein synthesis process, as they interact with phosphorous compounds. In the structure of bacterial DNA, while the fusion of gold nanoparticles with each of (Ampicillin - Streptomycin - Kanamycin) more stable and resistant to the effect of heat, which helped reduce the (MIC) of these drugs against E.coli, S.aureus-, Micrococcus luteus (Gad EL-Rab et al., 2018)

The result of this study was consistent with what the researcher (Al-fahdawi, 2021) reached, about the synergistic effect of gold nanoparticles with Ceftriaxone, despite the difference in the way to determine the synergistic effect, the type of bacterial isolates under study, and the difference in the types of antibiotics used.

### RNA extraction of bacterial isolates

RNA was extracted from S.aureus, E.coli and K.pneumoniae bacteria before and after treatment with gold nanoparticles and isolated from surgical inflammation, and one extraction before treatment and four after treatment with materials for each bacterial isolate and for each gene, RNA was obtained with high concentration and purity For both types of bacteria, the RNA concentration of S.aureus and K.pneumoniae was between 148 to 189 ng/microliter, while the measured purity of (280/260) was between 1.82 to 2.18, which was measured by the Nanodrop device.

9-3: Quantification of biofilm gene expression before and after treatment with gold nanoparticles using qRT-PCR technique:

Gene expression was measured using qRT-PCR technique for biofilm genes, specifically for icaA, KPHS, Flu genes. The Ct (Cycle Threshold) threshold is the basic rule for the amount of gene expression in qRT-PCR technique (Fig. 10) which is the cycle that The fluorescence in it reaches its maximum represented by the curve expressing the amount of gene expression that was measured by the two-step method, in which RNA is converted into cDNA in one tube

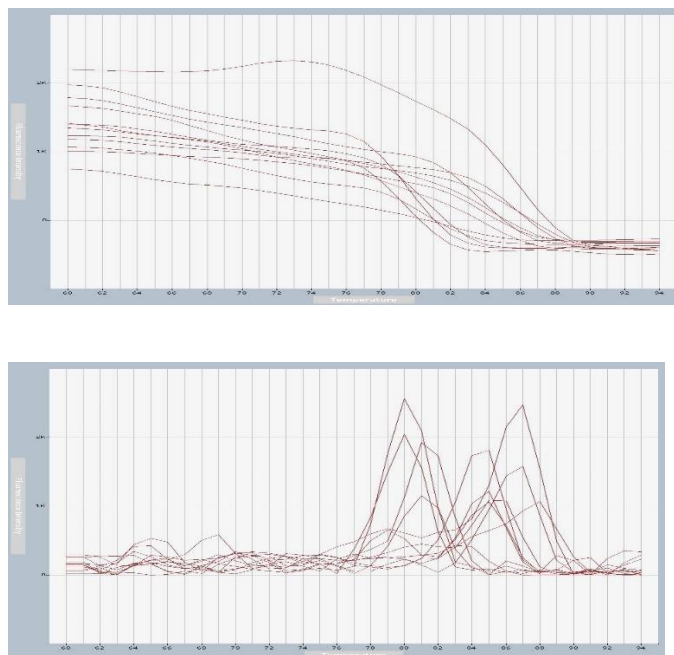
using random primers and gene expression is multiplied in a separate tube for qRT-PCR technique. In both samples, the results of the Ct results for each isolate treated with gold nanoparticles are also compared with the results of the Ct results for the gene house keeping gene.

for bacteria (E.coli, K.pneumoniae, S.aureus) respectively, as the gene expression values were extracted according to (Livak and Schmittgen, 2001) equation as follows:

$$\Delta Ct(\text{treated sample}) = CtGOI - Ctnorm$$

$$\Delta Ct(\text{calcolator}) = CtGOI - Ctnorm.$$

$$\text{Fold1} = 2^{-\Delta Ct/\text{Control group}}$$



**Figure 10:** Curves of Ct values for biofilm genes and service genes for S.aureus and K.pneumoniae E.coli before and after treatment with gold nanoparticles

Gene expression level of icaA, KPHS, Flu genes of S.aureus, K.pneumoniae, and E.coli bacteria before and after treatment with gold nanoparticles.

The results in Table (1) showed a decrease in the amount of gene expression of the icaA gene of S.aureus bacteria after treatment with gold nanoparticles at the minimum inhibitory concentration compared to the untreated control group, as the gold particles had a significant effect on reducing the amount of gene expression of the icaA gene, which has a role In the process of adhesion and biofilm formation, the rate of gene expression decrease was 0.21 and the icaA gene is responsible for the biosynthesis of intercellular adhesion polysaccharide containing N-acetylglucosamine and essential in the accumulation stage of biofilm formation, as it plays a vital role in the virulence of S. aureus ( Arciola et al., 2001).

Gene expression level of K.pneumoniae gene (Kphs) before and after treatment with gold nanoparticles

The results showed a decrease in the amount of gene expression of Kphs gene for K.pneumoniae bacteria after treatment with gold nanoparticles at a concentration under the minimum inhibitor using real-time PCR. 49 pp with an incubation period of 24 hours at a temperature of 37°C, where the rate of gene expression reduction was 0.15, compared to the control group by 1.

3-9-3: Gene expression level of E.coli gene (Fiu) before and after treatment with gold nanoparticles:

The results showed in Table (1) the level of decrease in the amount of gene expression of the Fiu gene of E.coli bacteria after treatment with gold nanoparticles at the concentration under the minimum inhibitor using real-time PCR.

The particles were effective in decreasing the gene expression of the Fiu gene, as the amount of gene expression decreased by 0.57 at the concentration under the lowest inhibitor 49, and these particles had less effect on the Fiu gene of E.coli bacteria after incubation for 24 hours at a temperature of 37 °C.

**Table 1:** Gene expression of icaA gene, KPHS, Flu) of S.aureus, K.pneumoniae, and E.coli bacteria using qRT-PCR technique

Sample	CT Treatemnt	CT Untreated	$\Delta$ CTE	$\Delta$ CTC	$\Delta\Delta$ Ct	$2^{-\Delta\Delta$ Ct	Expression Fold Change
Control	0	0	0	0	0	1	
E. coli	32.24	26.09	10.57	9.76	0.81	0.570381858	
K. pneumoniae	32.08	24.16	7.53	4.87	2.66	0.158219574	
S.aureus.	28.22	24.51	5.94	3.72	2.22	0.214641359	

## CONCLUSION

The results showed a synergistic effect between gold nanoparticles and the antibiotic Ceftriaxone on E.coli and K.pneumoniae bacteria, and no effect was shown on S.aureus, but gold nanoparticles had an effect on gene expression, where the results of gene expression for icaA gene showed a decrease in gene expression For S.aureus bacteria, gene expression decreased to (0.21), and a decrease in gene expression of E.coli flu gene showed, where expression decreased to (0.57), and K.pneumoniae gene expression decreased to (0.07). 15th.

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