

Effect of the Electric Field on the Antibacterial Activity of Au Nanoparticles on Some Gram-positive and Gram-negative Bacteria

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Abstract Metal nanoparticles are being extensively used in various biomedical applications due to their small size to volume ratio and extensive thermal stability. Gold nanoparticles (AuNPs) are an obvious choice due to their amenability of synthesis and functionalization, less toxicity and ease of detection. The synthesis and bioactivity of gold nanoparticles has been extensively studied. The present study was focused on method to increase the activity and the efficacy of the antibacterial activity of gold nanoparticles which produced by laser ablation of 1064 nm wavelength and three energy powers (400,500,600) mJ were applied to produced gold nanoparticles with different sizes on Gram-positive isolate (*Staphylococcus aureus*) and the Gram-negative isolate (*Pseudomonas aeruginosa*). It was found that using the agar well diffusion assay method which showed that the individually of AuNPs of 0.2 mg/ml concentration have no synergistic effect on the studied *Staphylococcus aureus* and *Pseudomonas*. So, a new modified technique was made on these AuNPs with the same concentration to increase their antibacterial activity, is exposure the gold nanoparticles colloidal to 1500 v/m applied electric field which resulting to be an effective AuNPs with inhibition properties against Gram-positive isolates (*Staphylococcus aureus*) contrary to nanoparticles that was not exposed to electric field with the same concentration.

Keywords: gold nanoparticles, Mie scattering, antibacterial activity, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, electric field, well diffusion

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1. Introduction

The emerging and developing immunity to the conventional antimicrobial agents and conventional disinfection methods in recent years by microorganisms enforce researchers to show much interest in synthesizing nanoparticles and study their size with various applications because of their unique physicochemical characteristics. Nanoparticles are clusters of atoms with sizes ranging between 1–100 nm, and due to their small size and large surface to volume ratio they displays a wide variety of chemical and physical properties (e.g. mechanical properties, biological and sterile properties, catalytic properties, thermal and electrical conductivity, optical absorption and melting points) compared to the bulk of same substance. Thus, by modifying and controlling the size and shape at nano metric level, nanoparticles exhibit interesting dependence of properties like biosensing, catalytic activity, optical activity, antimicrobial activity etc on the mentioned parameters [1].

Metal nanoparticles (Me-NPs) with a high specific surface area and a high fraction of surface atoms have

been studied extensively because of their unique physicochemical characteristics which include catalytic activity, optical properties, electronic properties, antimicrobial activity and magnetic [2]. Gold is among these metals which have been used for centuries as bactericidal and bacteriostatic agents, with different properties and spectra activity. The antibacterial gold metals activity depends on their contact surface; a nanoparticle's larger surface area that allows a broader gamut of interactions with other organic and inorganic molecules [3].

Mie theory plays a crucial role in describing the optical properties of metal nanoparticles, especially silver and gold particles. One of the important uses of this theory in describing the size dependence of localized surface plasmon resonances [4]. Mie theory easily describes red shifts and broadening of the dipole Plasmon resonance as particle size is increased, as well as the appearance of quadrupole and higher resonance contributions Figure 1.

When spherical metal particles are transformed into nanoscale rods [5], disks, or triangular prisms, the surface plasmon resonances are strongly affected, typically red-shifting and even splitting into distinctive dipole and quadrupole plasmon modes [6].

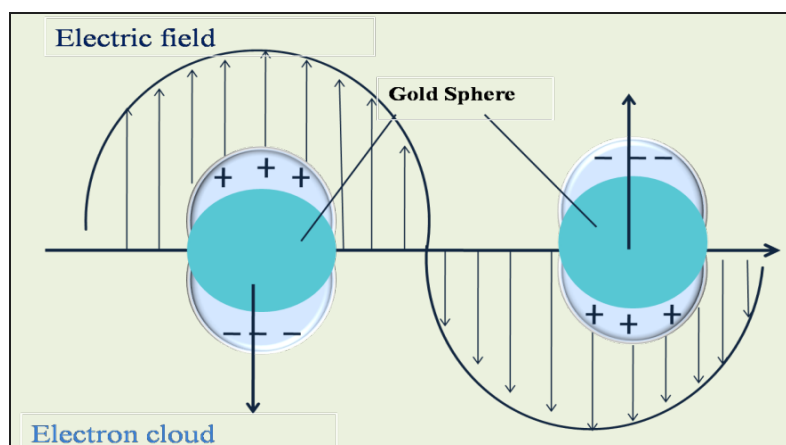


Figure 1. Resonances of gold spheres

This study investigated the effect of the electric field on the antibacterial activity of gold nanoparticles against hospital-acquired multiple drug-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa* clinical isolates.

2. Experimental Set Up

2.1. Preparation of Gold Nanoparticles

A gold nanoparticle was prepared by pulsed laser ablation (Nd:YAG) of gold plate (with purity of 99.99%) immersed in deionized water. The gold plate was fixed at

the bottom of a glass containing 2.5 ml deionized water, and ablation was carried out using focusing beam output of pulsed laser at 1064 nm and different laser energies (400,500,600) mJ, respectively, with a repetition rate of 6 Hz per second and pulse width of 10 ns. Such energies have been applied in this work to obtain tolerable and satisfactory average size of gold nanoparticles. The laser beam was focused on the gold target using convex lens of 11 cm focal length. The typical laser beam diameter on the target was 2mm in diameter by changing the distance between the focusing lens and the Au target and that shown in (Figure 2).

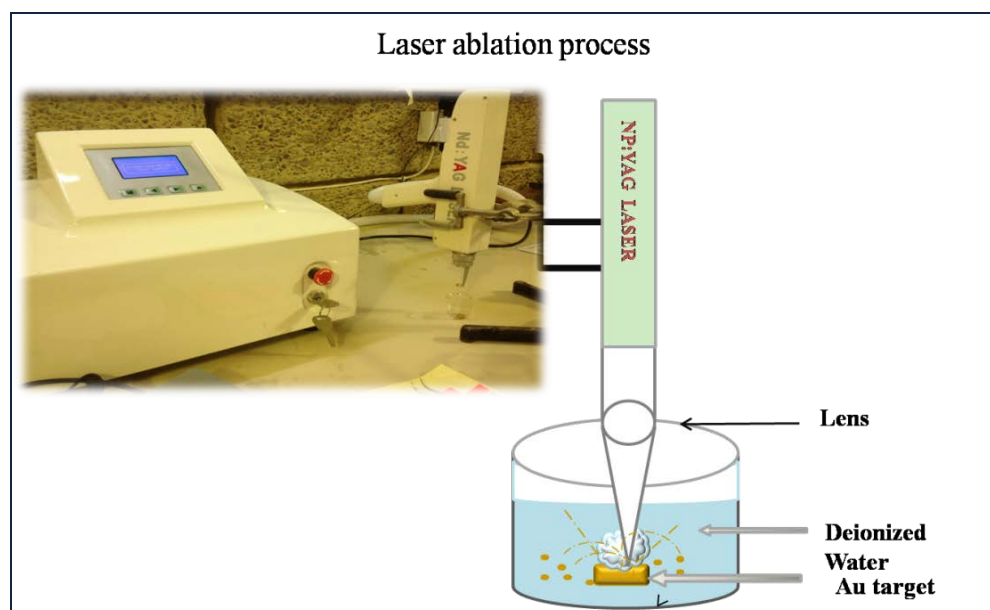


Figure 2. at left is apparatus of laser ablation system, while on right is schematic of laser ablation system

Gold nanoparticles concentrations were estimated by net weight (mg/ml) (weight of the target before ablation – weight of the target after ablation) and it was 0.2mg/ml. The optical absorbance spectrum of the produced nanoparticles was recorded using spectrophotometer.

2.2. UV- Visible Spectral Analysis of Gold Nanoparticles Colloidal

The optical absorbance spectrum of the produced nanoparticles using different laser energy (400,500,600)

mJ of laser shots 100 pulses for each, was recorded using UV-Vis spectrophotometer (Metrtech SP-8001) which has been proved to be a useful diagnostic tool [7].

2.3. Transmission Electron Microscope

The morphology and size distributions of gold nanoparticles were analyzed using Transmission electron microscope (TEM) using a CM10 pw6020, Philips-Germany at college of medicine Al-Nahrain University as well as the crystallization of the produced nanoparticles by electron diffraction technique.

2.4. Antibacterial Activity Test

Both of *Staphylococcus aureus* and *Pseudomonas aeruginosa* clinical isolates were used as bacterial model for evaluating the antibacterial activity of gold nanoparticles.

A standard agar well diffusion method was used, when pure cultures of each bacterial isolates were subcultured in nutrient broth for 24 hours at 37°C. After 24 hours, the inoculums (a turbidity of 0.5 MacFarland standard 10^8 CFU per ml) were spread with sterile cotton swab on

Mueller Hinton agar plates. Wells of 6 mm diameter were made using sterile cork borer and 50 μ l of each nanoparticles suspension were poured into each well of plates. The plates were incubated overnight at 37°C and results were recorded by measuring the diameter of inhibition zone (mm) [8,9]. The same procedure was repeated after expose the gold nanoparticles colloidal to 1500v/m a d.c. electrical field for 5, 15 and 30 minutes by adjusting the voltage using two copper quadrate parallel electrodes with 1cm distance between them as in the Figure 3.

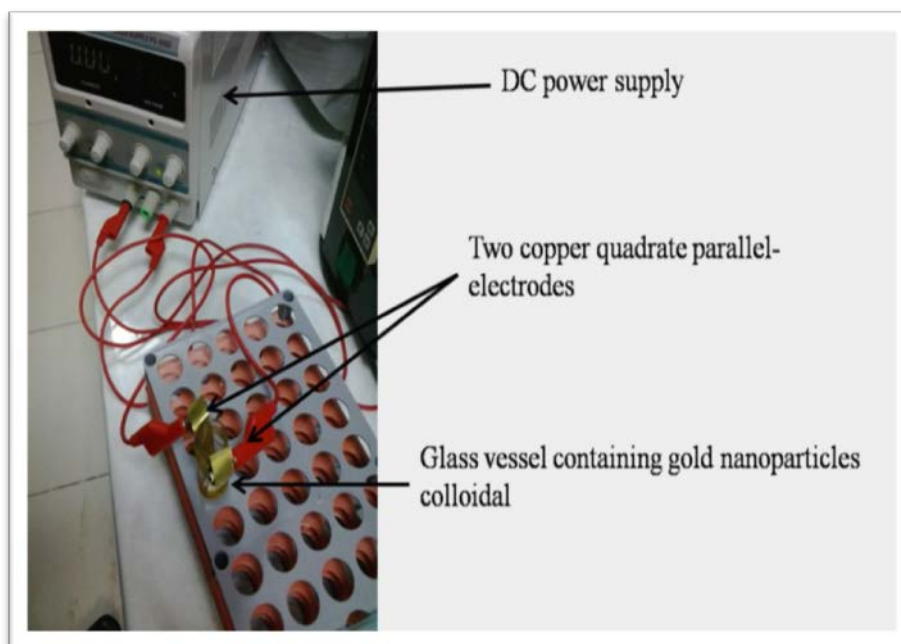


Figure 3. shows the process of apply external dc electric field to the gold nanoparticles colloidal

3. Results and Discussion

3.1. Characterization of Gold Nanoparticles

Laser ablation of bulk target immersed in liquid environment which is simple method, recently has attracted much attention for gold nanoparticles formation, and these nanoparticles have a wide range of applications

in areas such as catalysis, medical, diagnostics, and biological imaging[10]. Our results showed that the color of the gold colloidal started to change to wine color due to the reduction of metal ions which arises from excitation of surface plasmon vibration in the metal nanoparticles. So, the formation of wine red colloids is the characteristic of gold nanoparticles as in Figure 4.

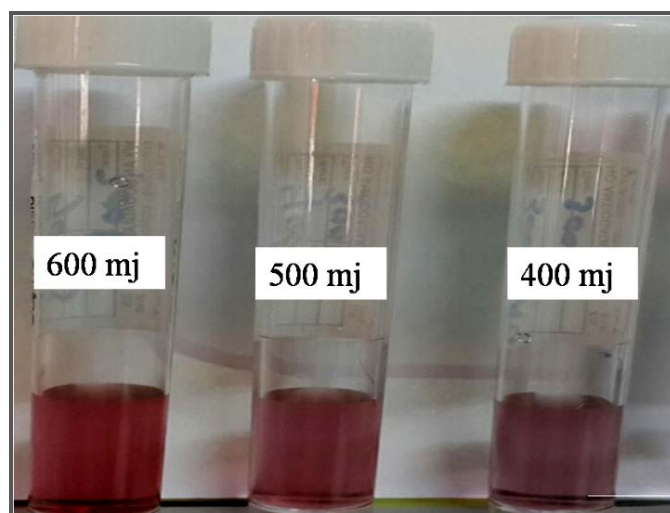


Figure 4. the color of the three nanoparticles colloidal obtained by laser ablation of metal plate immersed in DDDW with different laser energies (400,500,600) 600 mJ, laser shots of 10 pulses and wave length is 1064 nm of Nd-YAU

Grace and Pandian [11] reported that the formation of wine red colloids is the characteristic feature of gold nanoparticles.

The surface plasmon resonance spectra of AuNPs in samples were capable in identifying the size evolution of AuNPs based on localized surface plasmon resonance band exhibiting at different wavelengths Figure 5.

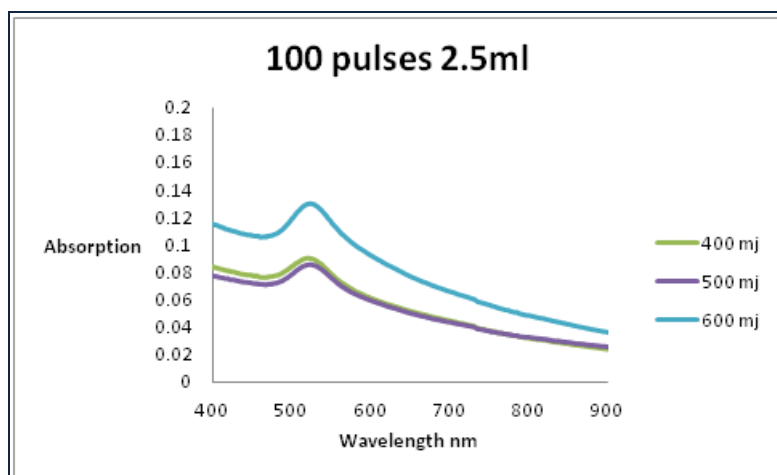


Figure 5. UV-VIS spectrum of the (400,500,600) mJ colloidal of AuNPs

The figure showed broad surface plasmon absorption peak for AuNPs around (521,523,524) nm for (400,500,600) mJ, respectively, which can be assigned to gold nanoparticles. According to Henglein [7], UV-VIS spectrum is commonly used as an analytical tool in analyzing AuNPs because free electrons in metal nanoparticles gave rise to surface plasmon resonance absorption bands. Moreover, it was also being reported that ultraviolet region is the region of absorption for bulk plasmon frequency in various metal nanoparticles. Hence,

bioreduction of AuNPs can be identified using UV-vis spectrum.

The morphology of the synthesized gold nanoparticles was observed under transmission electron microscopy (TEM). Figure 6 and Figure 7 reveals that the particles are spherical in nature and it did not appear as discrete one but form much larger dendritic flocs. The aggregation is attributed due to the Vander Waals forces and magnetic interactions among the particles. This TEM results coincides with the earlier report of Sobczak-Kupiec *et al* [12].

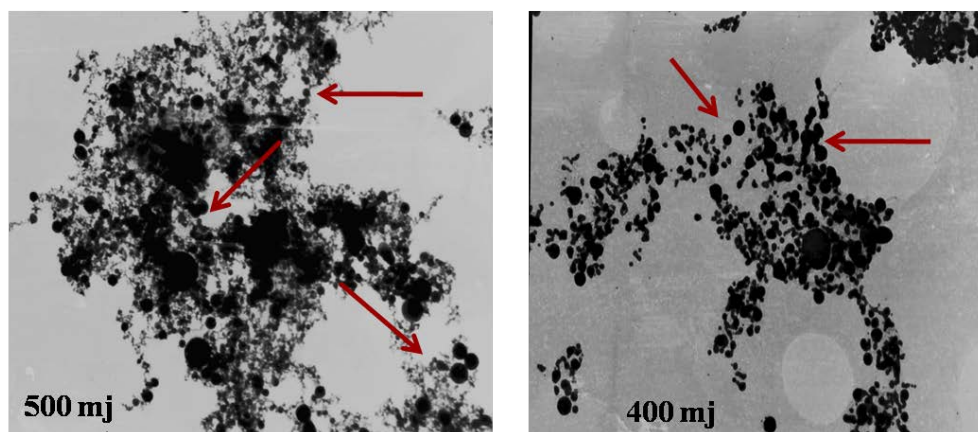


Figure 6. TEM images and the corresponding size distributions of gold nanoparticles for (400 and 500 mJ)

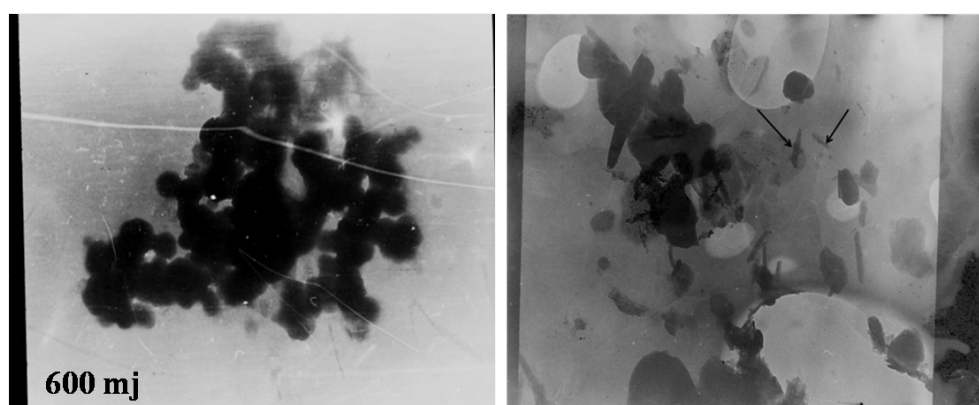


Figure 7. TEM images and the corresponding size distributions of gold nanoparticles for (600mJ)

The gold nanoparticles obtained were spherical except for 600mJ where clusters and variety of shapes especially the spindles are exist due to the high energy. The average size of gold nanoparticles were (18, 35, 50) nm in deionized water solution for the three colloidal of energy (400,500,600) mJ, respectively. The origin of the surface morphology of the irregularly shaped particles sizes and the size distribution broadens can be explained by

absorption defects and thermally induced pressure pulses which cause cracking [13].

It is clear from the image that the size of the nanoparticles increase as the energy increased and at high energy not only the size but also the shape differs the electron diffraction for gold nanoparticles which shows that they have a polycrystalline structure as in the [Figure 8](#).

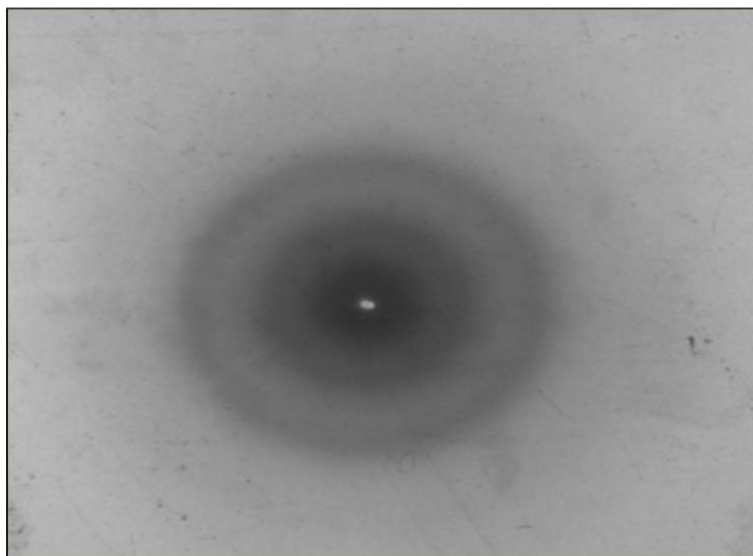


Figure 8. TEM electron diffraction image of AuNPs

3.2. Antimicrobial Efficiency of Gold Nanoparticles

Antibacterial activities of gold nanoparticles were studied against *Pseudomonas aeruginosa* a Gram-negative bacteria and *Staphylococcus aureus* a Gram-positive bacteria. The diameter of inhibition effect of the AuNPs (gold nanoparticles) colloidal solutions of 0.2mg/ml concentration synthesized by different laser

energies (400,500,600) mJ, and applied to be tested against *Staphylococcus aureus* and *Pseudomonas aeruginosa* isolates were illustrated in [Figure 9](#). The results and images of inhibition zones were found that AuNPs nanoparticles have no synergistic effect on the studied Gram-positive isolate (*Staphylococcus aureus*) and on the Gram-negative isolate *Pseudomonas* upon this a low specific concentration.

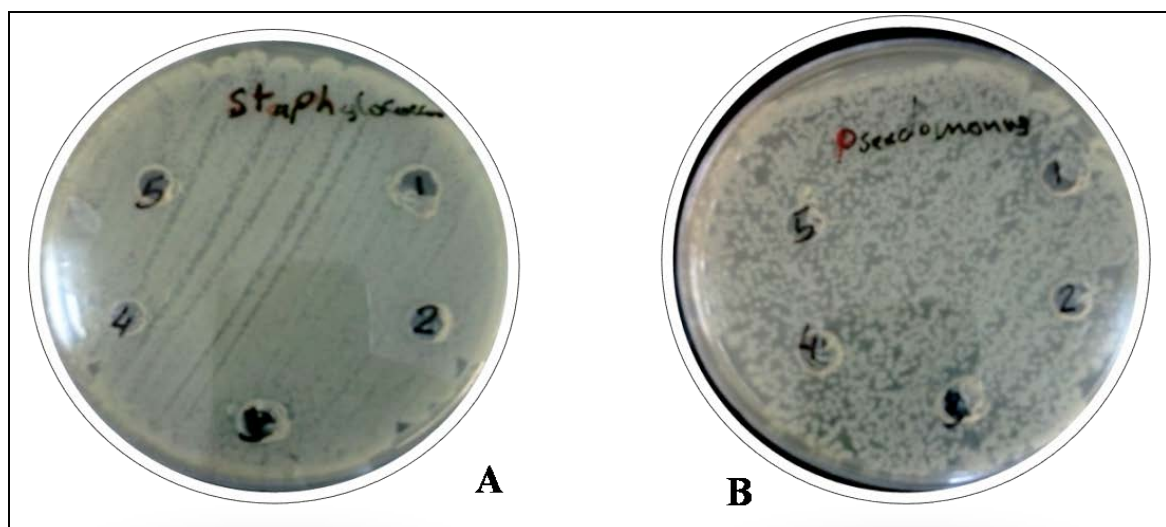


Figure 9. no inhibition zone of AuNPs on the growth of: A-*S.aureus* and B- *P.aeruginosa*

These result were in contrary to those [1,2] who found that gold nanoparticles have high potential towards antimicrobial and antifungal activity. The antibacterial efficacy of gold nanoparticles increases because of their larger total surface area per unit volume.

Bacteriological tests showed either bacterial growth inhibition or cell death occurred, depending on the concentration of gold nanoparticles and the type of bacteria, where a little concentration of gold nanoparticles there is not significant effect.

While, results showed that when Au nanoparticles colloidal (400,500,600) mJ were applied to 1500 v/m dc electric field at different exposure time (5, 15, 30) minutes the activity of the gold nanoparticle in solution against bacteria increases with increasing time of exposure to electrical field, size and shape as in Table 1 and Figure 10. The diameter of inhibition zone of (*Staphylococcus aureus*)

was null for 5 minutes of exposure for the three energies (400,500,600) mJ due to adequate time of exposure to induce the dipole moment, while the diameter of inhibition zone for 400mJ and 500mJ became 10.5 mm and 10.1, respectively at 15 minutes of exposure but no inhibition zone for 600mJ at the same time of exposure.

Table 1. *S.aureus* and *P.aeruginosa* isolates with the inhibition zone(mm) of Au-NPs 400, 500 and 600 mJ after 5, 15 and 30 min of exposure to electrical field

Pathogen isolates	Time (min.) of exposure to electrical field	Inhibition zone(mm) of different Au-NPs laser Energy powers(mJ)		
		400	500	600
<i>Staphylococcus aureus</i>	5	–	–	–
<i>Staphylococcus aureus</i>	15	10.5	10.1	–
<i>Staphylococcus aureus</i>	30	–	10.8	16
<i>Pseudomonas aeruginosa</i>	5	–	–	–
<i>Pseudomonas aeruginosa</i>	15	–	–	–
<i>Pseudomonas earuginosa</i>	30	–	–	–

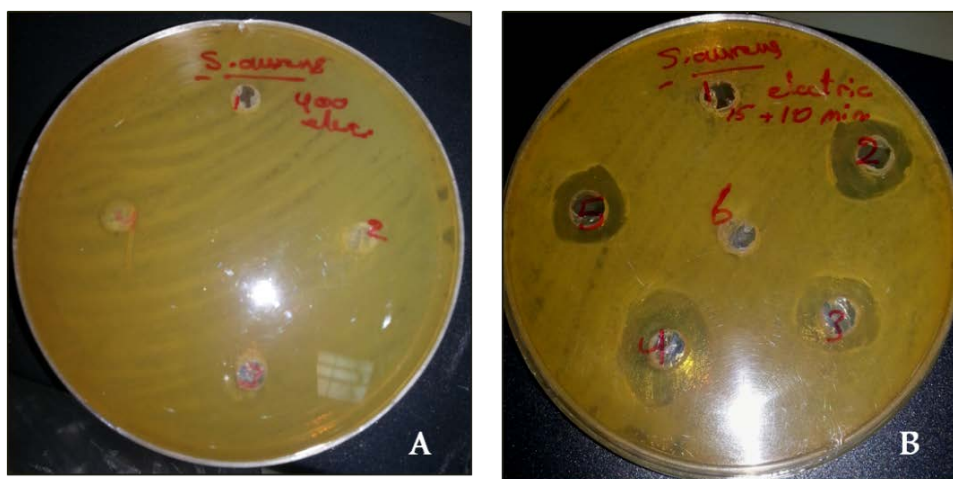


Figure 10. A- *S.aureus* after exposure 15 and 30 min. with AuNps B- *S.aureus* after 5 min. of exposure

The reason may be that the time of resonance didn't enough, but the inhibition zone become 16mm when the time increased to 30 minutes as well as 10.8 mm for the 500 mJ nanoparticles which is obvious that the inhibition zone increased with time of exposure. In spite of the size of nanoparticles produced by the laser energy of 600 mJ they have highest activity against bacteria which may attributed to the shape of nanoparticles especially the

spindles which acuminate and having a sharp tip consequently, hence have concentrated charge and more dipole moment(p) ($p=qd$) where q is the charge value (coulomb) and d is the distance (meter) between the opposite charges) and increases with the exposure time. In contrast there were no inhibition zones for all of the Au-NPs at (5, 15, and 30) minutes of exposure for *Pseudomonas aeruginosa* as shown in Table 1 and (Figure 11).



Figure 11. A- *P.aeruginosa* after exposure 15 and 30 min. with Au-Nps B- *P.aeruginosa* after 5 min. of exposure

Depending on definition of nanomaterial, the surface area to the volume will be increased sharply. Hence the surface energy of the AuNPs will be vast increasing. Accordingly, the interaction effects of the AuNPs will be increasing especially when exposed to the electrical field where the Fermi sphere moved from its equilibrium states because the electrons on the outer shells will be drastically moving around their equilibrium states. Hence the potential energy of the AuNPs becomes more affecting against the bacteria. As well as, gold nanoparticles generates holes in the cell wall due to surface ionization, resulting in the leakage of the cell contents & cell death. It is also possible that gold nanoparticles bind to the DNA of the bacteria & inhibit uncoiling & transcription of DNA. [14]. Gram-positive bacteria usually have much higher amount of peptidoglycan than gram negative bacteria. The gram negative cell wall is more complex in morphology because it contains outer membrane that surrounded a thin underlying of peptidoglycan. It is constructed by specialized type of polysaccharide and protein. This outer membrane serves as an impermeable barrier to prevent or slows the entry of gold nanoparticles that killed bacteria. But the cell wall of gram positive bacteria would be easily destroyed by gold nanoparticles for the reason of easy contact with cell membrane due to lack of extra protective membrane.

4. Conclusion

The electrical field assisted method was found to be the successful way to enhance and increase the effectiveness of gold nanoparticles that have no synergistic effect on the studied Gram-positive isolates (*Staphylococcus aureus*) because of low specific concentration or have relatively large size.

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