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Antibacterial Activity of Gold Nanoparticles against Bacteria Isolated from Diabetic Foot Infections

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Abstract: Diabetic foot ulcers (DFUs) are among the most important challenges in diabetes mellitus (DM) patients that are linked to a higher risk of morbidity and death in these patients. Specifically, antibiotic resistance in DFUs is essential for efficient management and treatment of infected wounds. The current study was aimed to evaluate the activity of gold nanoparticles (Au NPs) against multidrug-resistant bacterial isolates from diabetic foot infections in Najaf city. The study was carried out in the Al-Hakem hospital from January 2021 to July 2022. Totally, 150 samples were taken from diabetic foot infections of patients who were admitted to different hospitals (Al-Hakeem General Hospital and Al-Zahraa Teaching Hospital) in AL-Najif AL-Ashraf city. Diagnosis of bacteria and antibiotic susceptibility test was carried out for all isolates using Vitek 2 instrument. Au NPs were synthesized using the green synthesis method using bacteria Bacillus subtilis was cultured then incubated in shaking then centrifuged and 100 µl of 1 M HAuCl4 (Sigma Aldrich, USA) solution was added to 100 ml of the obtained microbial supernatant then incubated in a shaker incubator .The successful preparation of AuNPs was detected by Ultraviolet–visible spectroscopy(the AuCl4 had been reduced to gold nanoparticles, which can be distinguished by the peak at 650 nanometers detected in the spectrum, Atomic Force Microscopy (AFM)Also, the results indicated proper synthesis, and Fourier Transform Infrared Spectroscopy (FTIR).The FT-IR spectrum of the Au NPs shows different bands at 3418.39, 2088.60, 1639.93, 1407.05, 1084.52, 706.75 and 658.36 cc. The antibacterial activity of AuNPs was examined against three types of bacterial isolates including P. auerginosa, E.coli and Staph aureus. The concentrations that were used in this assay were 100 µg/mL, 50 µg/mL and 25 µg/mL (serial dilution) were used. The results showed the most bacteria isolated from foot diabetic infections were Staphylococcus aureus (64.8%), Pseudomonas aeruginosa (14.8%) and E.coli (14.8%). Antibacterial effects of Au NPs were established in the all concentration ranges of this study. Besides, the bactericidal potential of NPs were dose dependent where the highest and lowest antibacterial activity were observed in the NPs concentration of 100 µg/ml and 25 µg/ml, respectively. The findings indicated killing rate of 77.1%, 77.0%, 76.8% against isolated Pseudomonas auerginosa, E.coli and Staph aureus in 100 µg/ml of NPs, respectively. However antibacterial activity were 32.2%, 37.0%, and 28.1% in 25 µg/ml concentrations for the same order of bacterial species.25 µg/ml concentrations of Au NPs showed antibacterial activity between 32.2%, 37.0%, and 28.1% against isolated pseudomonas aeruginosa, E.coli and Staph aureus. The current study concluded the AuNPs antibacterial have higher activity against bacterial isolates Pseudomonas aeruginosa, E.coli and Staph aureus. The antioxidant activity to AuNPs is effective, and compared to other elements, gold also proved to be highly effective against oxidation, so it is considered an effective element in medical applications. The hemolytic activity of gold nanoparticles in whole blood (ex vivo) showed very low hemolytics.

Keywords: Gold Nanoparticles, Diabetic Foot infections, MDR, Antibiotic, Green synthesis.

INTRODUCTION

Nowadays, diabetes mellitus is one of the most serious problems in the health systems of all countries with high economic burden. Approximately 463 million people worldwide are affected by the illness, with 80 % of those living in low- and middle-income countries (LMICs) being affected by the condition [1]. A previous study referred to Diabetic foot ulcers (DFUs), are linked to a higher risk of morbidity and death [2].

Diabetic patients who are immunocompromised, on the other hand, are more prone to suffer from non-healing wounds. Approximately one-third of diabetic patients will develop a foot ulcer at some point in their lives, with half of these sores becoming infected. It is becoming more difficult to manage this problem because of the presence of multidrug-resistant (MDR) strains that cause diabetic foot infections [3].

Antibiotics are medications that may be used to both prevent and treat bacterial diseases. Antibiotic resistance boosts the expense of medical care, lengthens hospital stays, and death rate. However, bacterial resistance to antibiotics continues to pose a problem, despite the development of novel treatments and medications. Multidrug resistance is defined as the presence of bacterial species which are resistant to at least one antimicrobial medication from three or more antimicrobial groups [4]. Currently, treatment of MDR infections is a global health problem because of lack/low effectiveness of antibiotic regimes which result in higher death rate and more recovering costs for patients and health systems.

A new approach for Nanoparticles may help to increase the efficacy of antibiotics because they limit antibiotic exposure. Through the use of these nanostructures, it is possible to transfer antibiotics into the cytoplasm of bacteria [5].

NPs' form and size, as well as their unique optical and thermal properties, making them ideal for the treatment of wound infections. The researchers' goal was to discover a biocompatible and environmentally friendly alternative to current treatments. Nanoparticles may be able to increase the efficacy of medications because they limit antibiotic exposure. Through the use of these nanostructures, it is feasible to transfer antibiotics into the cytoplasm of bacteria [5].

Treatment as nanoparticles can play a significant role as a drug delivery system. Compared to conventional drugs, nanoparticle-based drug delivery has specific advantages, such as improved stability and biocompatibility, enhanced permeability and retention effect, and precise targeting [6].

In addition, inorganic antibacterial agents such as metal and metal oxides are advantageous compared to organic compounds due to their stability . Among these metals Au NPs has attracted special attention as an antibacterial agent [7], and Silver-zinc oxide [8] and many other types.

Assimilating green chemistry principles in nanotechnology is a developing area of nanoscience research nowadays. Thus, there is a growing demand to develop environmentally friendly and sustainable methods for the synthesis of nanoparticles that utilize nontoxic chemicals, environmentally benign solvents, and renewable materials to avoid their adverse effects [9].

Over the past decade, metallic nanoparticles have gotten a lot of interest as a way to stop diseases from spreading. These nanomaterials, in various forms, may attach to bacteria's cell surface, producing membrane damage, which may lead to changes in membrane potential and permeability, as well as cytoplasmic leakage and cellular damage. Metal nanoparticles also create a variety of intracellular reactive oxygen species (ROS), which damage the microbial membrane and other cellular components, resulting in cell death. Metal nanoparticles, such as gold and silver, have been shown to have antibacterial properties. Because of their tiny size and high surface-area-to-volume ratio, gold nanoparticles (AuNPs) .Previous study reported to be widely employed in treatments and diagnostics. [10].

Preparation of biogenic colloidal Au NPs using biological method is an efficient and cost-effective manner [11]. Au NPs might be employed in a variety of ways to kill harmful bacteria such as E.

coli, P. aeruginosa, and Salmonella typhi, as well as Bacillus subtilis and Candida albican, according to the researchers. It is more beneficial to utilize an antimicrobial drug in conjunction with AuNP because of the potential of AuNP to promote intracellular transport and diffusion [12]. In this study we will evaluate the activity of green synthesized gold nanoparticles (Au NPs) against MDR bacteria isolated from diabetic foot infections in Najaf city.

Materials and Methods

2.1 Samples Collections

150 samples were collected from diabetic foot infections in patients who were hospitalized to two separate hospitals in the city of AL-Najif AL-Ashraf (Al-Hakeem General Hospital and Al-Zahraa Teaching Hospital) for various reasons, with ages ranging from 50-80. Of the 122 samples, 81 (66.39 %) were females and 41 (33.6 %) were males .The research was conducted in the Al-Hakem hospital between January 2021 and July 2022. Swabs were collected using a transmission medium and then put into swab culture bottles, which were then incubated.

2.2. Bacteria Diagnosis and Antibiotic Susceptibility

The bacteria were diagnosed with antibiotic susceptibility using the VITEK® 2 device according to [13].

2.3. Gold Nanoparticles Synthesis

To produce AuNPs, *Bacillus subtilis* was obtained from the public health laboratory in the city of Najaf. *B. subtilis* was cultured in a flask containing 150 ml of sterile nutrient broth (NB). The bacterial culture was incubated in shaking conditions at 37°C, 150 rpm, for 24 h. After incubation, the media containing the microbial biomass were centrifuged at 6000 rpm for 10 min, and 100 µl of 1 M $HAuCl_4$ (Sigma Aldrich, USA) solution was added to 100 ml of the obtained microbial supernatant. Then flasks was incubated in a shaker incubator at 37°C, 200 rpm, for 24 h. Negative control flasks containing 100 ml of sterile NB with 100 µl of 1 M $HAuCl_4$ at a final concentration of 1 mmol were incubated under the same conditions [14].

2.4. Characterization of Gold Nanoparticles

2.4.1. UV-vis Spectra Analysis

The UV/vis spectrophotometer(Thermo Fisher Scientific Logo/UK), (200-800 nm) was operated at a resolution of one nm, and it was deemed a straightforward approach for obtaining information about particle concentration, size (diameter), and particle shape shift of the absorbance relay by measuring the absorbance shift.

2.4.2. Atomic Force Microscopy Analysis

The spm-AA300 (Angstrom Advanced Inc/ USA) from angstrom advanced Inc was utilized to define the topological surface of the gold nanoparticles, and the results were published in Nano Letters. This is accomplished by imaging the three-dimensional shape (topography) of a high resolution surface of a sample using the reaction force imposed on it by the sample between the probe and the forces.

It is carried out by raster scanning the location of the sample with respect to the tip of the probe and recording the height of the probe, resulting in continuous contact between the probe and the sample surface. Controlling the forces between the tip and sample may be accomplished by changing the sample parameters in a predictable manner. Atomic manipulation, lithography of scanning probes, and local cell stimulation are all examples of this kind of technology.

2.4.3. FT-IR analysis

A study on the potential properties for effective fixation and capping of the produced Au NPs was performed using Fourier transform infrared (FTIR) spectroscopy. The FT-IR spectrum of the Au NPs shows different bands at 3418.39, 2088.60, 1639.93, 1407.05, 1084.52, 706.75 and 658.36 cc..

2.4.4. X-ray Diffraction XRD

The X-ray diffraction (XRD) pattern of the Au NPs was determined using an X'Pert PRO analytical X-ray diffractometer operating at a voltage of 45 kV with a current of 40 mA with Cu K radiation using the X'Pert Highscore Plus software to determine the crystal structure. This test was performed in accordance with [15].

2.4.5. FESEM

Visualization of the nanoparticle's form and size was accomplished using a SEM (Scanning electron microscope). Preparation of slides was accomplished by putting tiny drops of nanoparticle suspension on the slides and allowing them to dry. Following the procedure, the slide was subjected to scanning electron microscopy (SEM) (JEOL-MODEL 6390 machine). The microscope was operated at varied magnifications with a speed up voltage ranging from 5 to 10 kV, with a low vacuum, a spot size of 4, and working distances ranging from 5 to 10 mm. This test is carried out in accordance with [16].

2.5. The Antibacterial Activity of Gold Nanoparticles

The antibacterial activity of Au NPs was done according to [17].

Result and Discussion

3.1. Bacteria Diagnosis

A total of 150 samples from diabetic foot infections in patients were collected. Positive bacterial growth was seen in 122 samples out of 150 samples, *Staphylococcus aureus* (64.8%), *Pseudomonas aeruginosa* (14.8%) and *E.coli* (14.8%) are the three most common pathogens responsible for diabetic foot infections (Figure 1). Foot infections are prevalent in diabetic patients, and they are linked with a high rate of morbidity and the possibility of lower limb amputation. Diabetic foot infections are classed as mild, moderate, or severe depending on the severity of the infection. Gram-positive bacteria, such as *Staphylococcus aureus* and beta-hemolytic streptococci, are the most prevalent pathogens in mild and moderate infection that have gone untreated for a long period of time before treatment [18].

A previous research discussed the various microbiology of diabetic foot infections; however *S. aureus* was shown to be the most common. In this study, the relationship between the prevalence of Gram positive and Gram negative organisms was shown [19]. Another study [20] showed that *Staphylococcus aureus* was the most prevalent organism constituting 23.16% (n=22) of the isolated organisms; *Escherichia coli* with 17.89% (n=17) and *Klebsiella sp.* with 12.63% (n=12) followed. Males presented more with diabetic foot (n=52) out of 95 patients. That is agreed with current study.

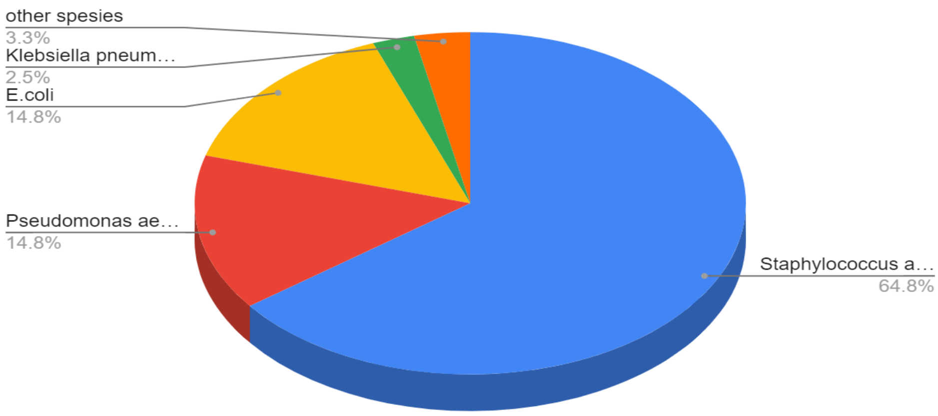


Figure 1: The prevalence of bacteria that causes diabetic foot infection.

3.2. Characterization of Gold Nanoparticles

3.2.1. UV-vis Spectra Analysis

Beyond the color change, ultraviolet (UV) spectroscopy confirmed that AuCl4 had been reduced to gold nanoparticles, which can be distinguished by the peak at 650 nanometers detected in the spectrum as shown in Figure 2. The peak position of gold nanoparticles generated in a variety of media changes with the medium [21].

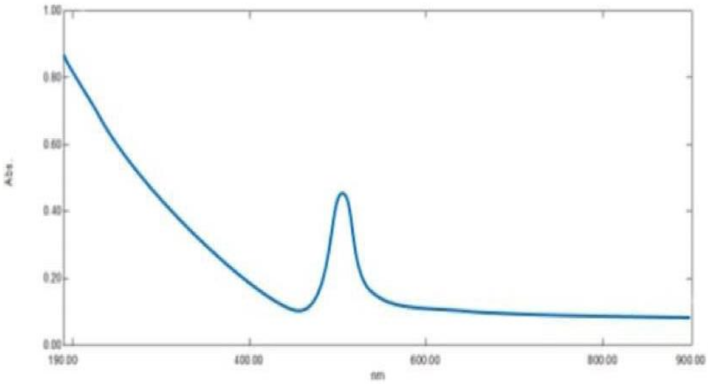


Figure 2: Spectrum analysis of biosynthesized gold nanoparticles

3.2.2. Fourier Transforms Infrared Spectroscopy analysis (FTIR)

An investigation of the probable biomolecules in responsible for the effective stabilization and capping of produced Au NPs was carried out using Fourier transform infrared spectroscopy (FTIR). FT-IR spectrum of Aloe Vera aqueous extract shows different bands positioned at 3418.39, 2088.60,1639.93,1407.05,1084.52,706.75 and 658.36 cm^{-1} bands as shown in Table 1 .

Table 1: Fourier Transforms Infrared Spectroscopy analysis (FTIR)

Peak Number	X (cm-1)	Y (%T)
1	3418.39	27.98
2	2088.60	82.79
3	1639.93	46.67
4	1407.05	80.51
5	1084.52	89.22
6	706.75	74.78
7	658.36	75.29

3.2.3. FESEM

FESEM was performed on samples that had already been processed. Neither nan rods nor other 1D morphologies, which are unrelated to these two plasmonic features, grew on the surface of the substrate, which had been entirely coated with high density networked string-like structures of gold nanoparticles. Figure 3 shows photos of photoluminescence and FESEM experiments. As a whole they seemed to be an aggregate rather than a networked structure, even in the majority of situations in catalysis and sensing applications, such tiny 1D nanostructure may have the potential to provide unique features as a consequence of anisotropic morphology and quantum effect.

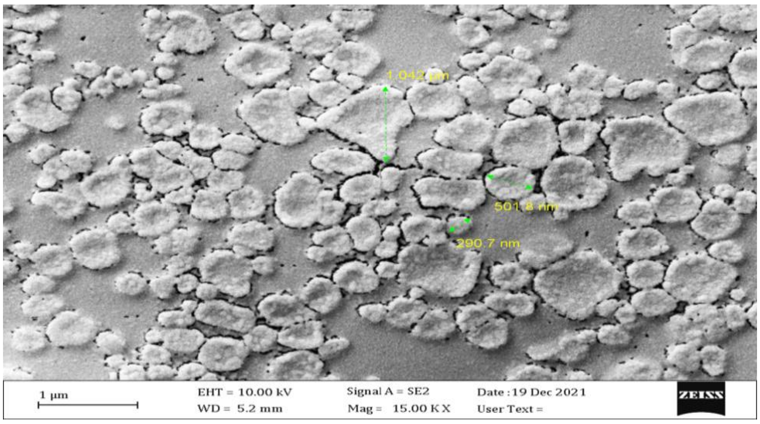


Figure 3: FESEM images and high magnification of gold nanoparticles, this assay was applied to determine the dimension and shape of NPs. According to the image, it is obvious that gold NPs have a nearly flatenet spherical and triangular morphology with an excellent distribution of particles with sizes between 300 and 1000 nm.

3.2.4. X-ray Diffraction XRD

Figure 4 shows the X-ray diffraction patterns of the produced Au NPs. The face-centered cubic shape of the Bragg reflection peaks at (30-32)° indicates that they correspond to 900 planes of pure Au (JCPDS, file No. 04-0783) . The XRD results clearly show that the Au nanoparticles synthesized.

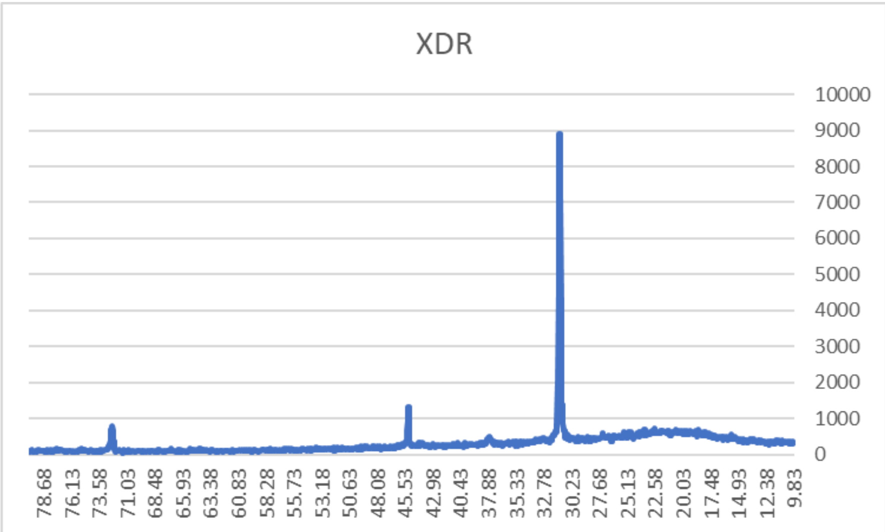


Figure 4: X-ray Diffraction XRD assays for Au NPs

3.3. Antimicrobial Activity

The antibacterial activity of AuNPs was examined against three types of isolated bacteria including *P. aeruginos, E.coli* and *Staph aureus*. The concentrations that were used in this assay were 100 µg/mL, 50 µg/mL and 25 µg/mL (serial dilution), as shown in Figure 5. The results showed that the gold NPs concentration (100 µg/ml) gave the highest antibacterial activity against isolated *P. aeruginosa, E.coli* and *Staph aureus* by killing about 77.1%, 77%, 76.85% respectively, While, the lowest concentration (50µg/ml) gave also a good antibacterial activity by killing about 47%, 50%, 43% respectively among isolated bacteria. While 25 µg/ml concentration gave antibacterial activity ranging between (32.2%, 37%, 28%), among *pseudomonas aeruginosa, E.coli* and *Staph aureus*. So, among these different concentrations, 100 µg/ml NPs concentration was considered as the best antibacterial concentration.

A broad variety of biological issues may be solved with gold nanoparticles (AuNPs). The biological process ("green synthesis") used to create gold nanoparticles is environmentally benign and reduces the number of hazardous chemicals and poisonous byproducts [22]. A growing challenge in clinical medicine is antibiotic resistance, which may be addressed by using nanoparticles of different metals, particularly gold nanoparticles (AuNPs), in a variety of applications. The antibacterial characteristics of biosynthesized gold nanoparticles are influenced by the size, shape, and concentration of the nanoparticles ([23].

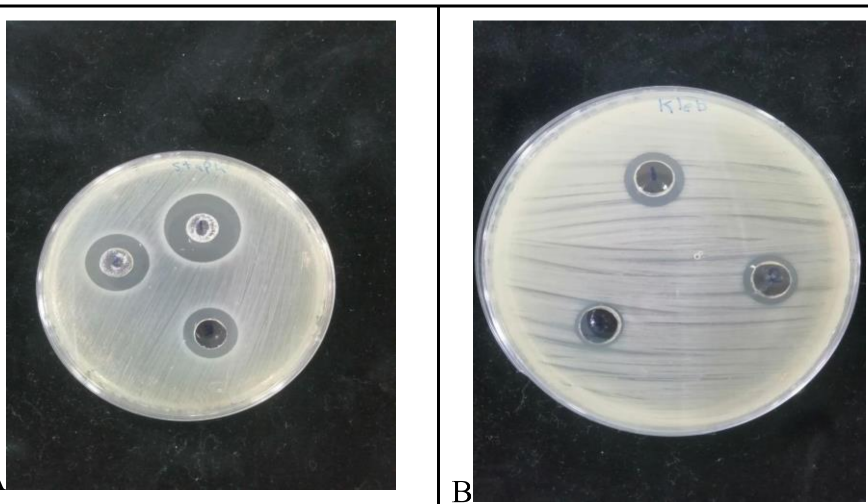
Gold nanoparticles have been proven to have photo thermal effects, and modified gold nanoparticles may be a good medium for photo thermal treatment to destroy bacteria. However, further research is needed. Using gold nanoparticles that have been functionally changed, it may be possible to impart antibacterial properties to many materials that now lack them. There are a variety of methods in which gold nanoparticles may be combined with cations and other antibacterial chemicals to produce antibacterial effects. In conclusion, the antibacterial capabilities of functionalized gold nanoparticles demonstrate that they have great practical application value and provide fresh ideas for addressing antibacterial issues [24]. The integrity and stability of the cytoplasmic membrane may be compromised by gold nanoparticles adhering to the cell wall and penetrating through the cell membrane, resulting in the death of bacteria. Gold nanoparticles adhere to the cell wall and penetrating through the cell membrane [25]. Taking action is feasible and/or possible via the interaction of multiple cellular organelles and/or DNA [26]. When AuNPs were applied to bacteria such as *E. coli* and *S. aureus*, it was discovered that they cause obvious cell surface damage, flagella lost, cell walls weaken, cytoplasm shrink and cellular components release [27]. Nanoparticles with different shapes have different antibacterial properties. Shape, components linked to surface, and surface charge are all factors that may contribute to AuNPs antibacterial action. The physical mutilation of bacterial cells, as shown and corroborated by microscopic examination and nucleic acid leaking, is the primary cause of the harm caused by AuNPs [28].

The other important factors are the concentration and size of the nanoparticles, which play a vital role in the antibacterial mechanism. The antibacterial mechanism of gold NPs on gram-positive and gram-negative bacteria depends on the concentration of the gold NPs [29]. It was demonstrated that the catalytic and antibacterial mechanism of the gold NPs increases with a decrease in average size [30].

The electrostatic interaction between positively charged nanoparticles and a negatively charged cell wall results in the breakdown of the cell's outer layer. Toxicity may be produced by nanoparticles that have been adhered to the cytoderm and have entered the cell after being released.

Table 3 Au NPs activity against three species of bacteria with different concentration of NPs.

Au NPs Concentration	**25 µg/mL**	**50 µg/mL**	**100 µg/mL**	**P.value**
P. auerginosa	32.2%	47%	76.85%	0.003
E.coli	37%	50%	77%	0.000
Staph aureus	28%	43%	77.1%	0.001



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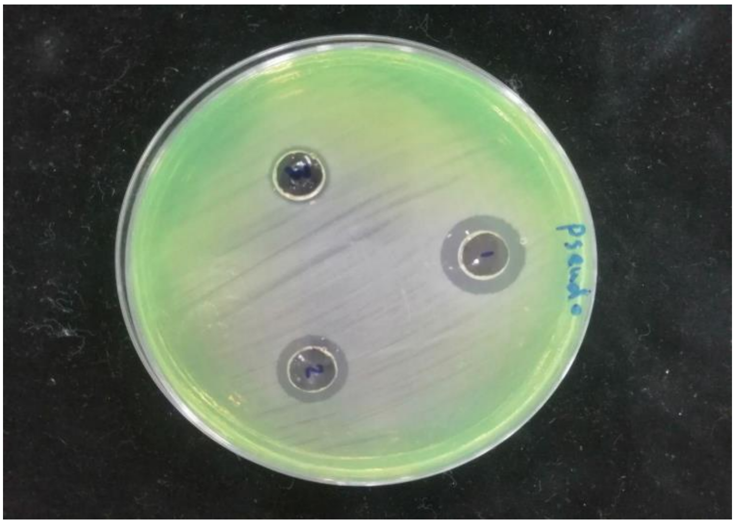

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Figure 5: Antibacterial activity of gold nanoparticles against (A): *Staph aureus (*B):*E.coli* (C): *Pseudomonas aeruginosa* using different concentrations of AuNPs.

Conclusion

The most common bacterial species detected in diabetic foot infections samples was *Staphylococcus aureus*, followed by *Pseudomonas aeruginosa*. The antibacterial activity of AuNPs in concentration (100 µg/ml) gives the highest antibacterial activity against bacterial isolates *Pseudomonas aeruginosa, E.coli* and *Staph aureus*.The study also concluded that the green synthesis of gold nanoparticles was effective and has unique characteristics that have the ability to kill microbes that were isolated from clinical samples and at very low concentrations. Therefore, the current study recommends testing other synthesis methods with different concentrations as well.

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