Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 6814 - 6821
Received 16 February 2021; Accepted 08 March 2021.

Synthesis of Gold Nanoparticles and Evaluation the Synergistic Effect with Ceftriaxone against *Klebsiella pneumoniae*

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ABSTRACT

nanoparticles technologies are an sophisticated and utilized in numerous applications in biomedicine. The capability of metal nanoparticles as an microbial elimination which considered as alternative ways in treatment of MDR resistance bacteria. The current study discusses the novel approach in synthesis of gold nanoparticles using physical methods by laser source at 1064 nm with 500 mj and 1000 pulses. Synergism between gold nanoparticles and ceftriaxone against *Klebsiella pneumonia* had been appeared synergistic effect which analyzed using checkerboard technique.

Keywords: Gold nanoparticles, TE Microscope Synergism, *Klebsiella pneumoniae*,

INTRODUCTION

klebsiella pneumonia have become a worldwide health problem because of resistant isolates and developing of bacteria. It has made old style treatment of infectious diseases difficult, thus discovering new alternative classes of antimicrobial agents (nanomaterials) that can treat resistant isolates is predominant [1]. ESKAPE may also develop multidrug resistance and virulence[2]. Patients with surgical wounds or burns, as well as those with catheters, are at risk for life-threatening, untreatable infections in the hospital environment[3,4] . Nanoparticles have created as novel alternatives to broad range bacterial multi-drug resistance fortified worldly due to of misuse of the antibiotic; therefore, the creation of alternative new nanoparticle-antimicrobial agents that can treat resistant strains is paramount [5]. The antimicrobial specialists' nanoparticles exhibit various systems reduce bacterial resistance as the microbicidal nature of nanoparticles resulted from direct adjacency with the cell mass of microscopic organisms, without the need to get overcome cells [6]
. The gold nanoparticle (AuNP) is an excellent candidate to any medical application because of its size and shape dependent optical properties and physiological. gold nanoparticles are being formed by physical and chemical methods with controlled uniform dispersion and size. The Physical synthesis of NPs used to produce nanoscale gold nanoparticles with very high purity and sizes that can be controlled [7]

The nanomaterial has exhibited a wide spectrum anti-microbial efficacy toward Gram positive and negative microbes. AuNPs are displaying bacteriostatic or bactericidal activity to microbial cells. In any case, antibiotics combined AuNPs are shown to have strong bactericidal effect against the drug resistant bacteria. The AuNP has unique physical and chemical characteristics and has strong binding attraction to the disulfides, proteins, carboxylic acid, and thiol, which provide strong drug loading; drug is loaded on nano carriers by non-covalent interaction or through covalent conjugation with the help of prodrug, which is treated by cells [8].

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MATERIAL AND METHODS

Chemicals and cultures

In this study, chemicals were used such as Gold chloride ($HAuCL_4.4H2O$; chloroauric acid), Luria–Bertani broth and tri sodium citrate blood agar MacConkey agar, obtained from Oxoid Ltd., England, and sterile deionized water was employed in the current study.

Isolation of bacterial strains:

Bacterial strains isolated from the patients in Medical city in Anbar (Al Ramadi Teaching Hospital, Al-Ramadi Teaching Hospital for Women and Children). Specimens were cultured on MacConkey agar and blood agar and incubated for 19-24 h at 37 C°. after that, the growth, the specimens were identified. Using Vitek 2 compact system by card of GN (Gram Negative) depending on instructions of the manufacturer [9].

Synthesis of AuNPs

According to [10] with some modification (Physical method) was adopted for preparing the colloidal of the gold nanoparticles at a concentration of 75.0 µg/ml as follow:

Solution A:

Gold nanoparticle (AuNP) was synthesized by pulsed laser ablation of gold target in double distilled water the gold target (purity of 99.999%) fixed at bottom of glass vessel containing of 5ml of double distilled water the level of water up the target surface is 7 mm, the high of laser source is 14 cm, the ablation was achieved focused output of pulsed laser type (HUAFEI) Ablation is carried out with laser operating at 1064 nm wavelengths with (500) mj by (1000) pulses The absorbance e spectra o the nanoparticle s solution and the surface plasmon resonance peaks have been measured by UV-Vis spectroscopy type (SP8001) the concentrations of nanoparticles in the solutions were measured with atomic absorption spectrometer type (GBC 1000 pulse).

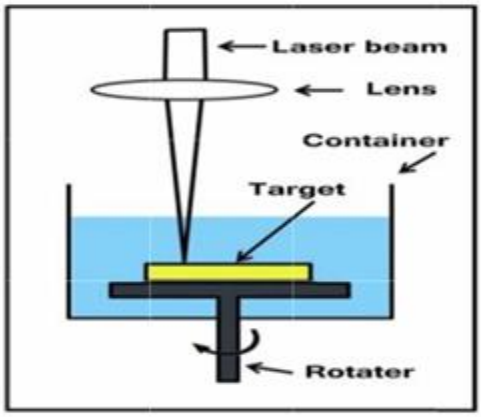

Fig. 1: LAP setup for nanoparticle synthesis

The diameter of nanoparticles has been measured by using the Transmission electron microscopy (TEM)

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Antibiotic sensitivity test

four antibiotics), Ceftriaxone (CRO), Ceftazidime (CAZ. Imipenem (IMP), and Ciprofloxacin (CIP), manufactured by Bioanalyze Company USA origin, were tested for efficacy against *Klebsiella pneumonia* bacteria depend on disk diffusion method described by [11].

Antibacterial activity of AuNPs against *Klebsiella pneumonia* and determination of MIC

The disk diffusion method was used to estimate the antibacterial efficacy of biosynthesized Nanoparticles, which suggested by[12]. The pure culture of the chosen bacteria has been subculturing separately. in tryptone soya broth at 37° C for 18-24h. About 20 milliliters of medium of Mueller Hinton Agar has been poured in each Petri dish and each strain has been uniformly swabbed in dishes with a sterile swab. The blank disks (5 mm) were pierced from Whatman No. 3, sterilized in an autoclave, and immersed in a gold nanoparticle solution for 1 hour before being dried. These disks were placed on each bacterium separately. inoculated dishes using a sterile loop. A strong zone of inhibition around the disks after overnight incubation at 37 °C has been presumed to suggest bactericidal efficacy. The minimum inhibitory concentration of gold nanoparticles was assumed using method of [13] by preparing serial concentrations of AuNPs (75,37.5, 18.75 and 9.37 µg/ml) and the lowest concentration inhibits the bacterial growth considered MIC.

Synergism effect of AuNPs and antibiotic against *Klebsiella pneumonia*

Synergism effect of gold nanoparticles and antibiotic against *Klebsiella pneumonia* was evaluated using disc diffusion method proposed by [14]. The bacterial suspension was made and compared with the standard McFarland No. 0.5. Five ml from tryptone soya broth inoculated with a loopful of *klebsiella pneumonia* from overnight culture grown on nutrient agar, incubated for 24h at 37 °C. About 20 milliliters of medium of Mueller Hinton Agar has been poured in each Petri dish and each strain has been uniformly swabbed in dishes using a sterile swab. Antibiotic disc soaked with the gold nanoparticle solution for one hours, then placed for drying. Using sterile plastic forceps, discs were placed onto each bacterium inoculated agar plate. A strong zone of inhibition around the disks after overnight incubation at 37 °C has been presumed to indicate bactericidal efficacy.

Statistical analysis

The Chi-square (Cross tabulation) or Mann-Whitney tests were used to evaluate all of the results. Microsoft Excel 2016 was used to create all of the study's graphics (dot map, bar chart, and scatter diagram).

RESULTS AND DISCUSSION

Isolation and Identification of *Klebsiella pneumonia*

Among 388 clinical specimens, 114 (32.6%) were identified as *Klebsiella pneumonia***.** The isolates obtained from MacConkey agar were identified by VITEK® 2 system. *klebsiella pneumonia* appeared on MacConkey agar (Figure 1)**.**

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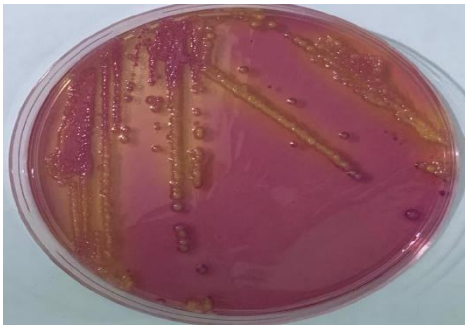

Figure1. The culture appearance of *Klebsiella pneumonia colonies* on MacConkey agar

Synthesis of AuNPs

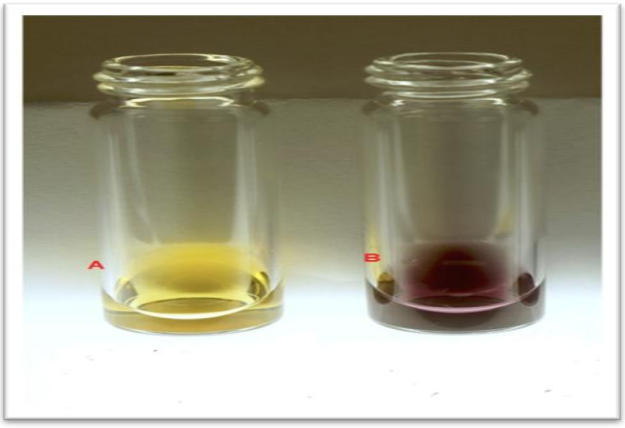

Figure 2. Changing color of solution of gold chloride and Synthesis of gold nanoparticles

UV-vis spectra of AuNPs

UV-visible spectrum is one of the most important techniques for determining the stability and formation of gold nanoparticles in watery solution, with the absorption peak occurring at 522 nm (Fig. 3), which depicts the gold absorption peak. The present study corroborates the findings of many other studies[15].

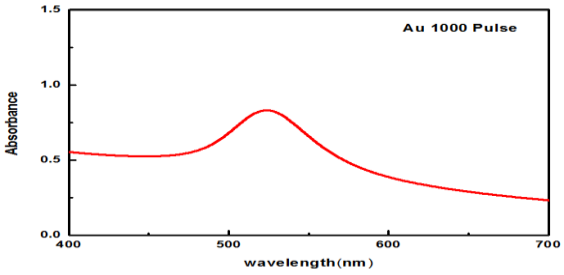

Figure 3. UV- visible spectrum of solution of gold nanoparticles

Transmission electron microscope analysis

Analysis of TEM (Transmission Electron Microscope) was achieved to define the size and shape of the bio-synthesized gold nanoparticles using *K. pneumoniae* and distribution of gold

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nanoparticles. The present study revealed that this gold nanoparticles is mono-dispersed and its size range was (1.07-nm 14.21nm) (Figure 4)

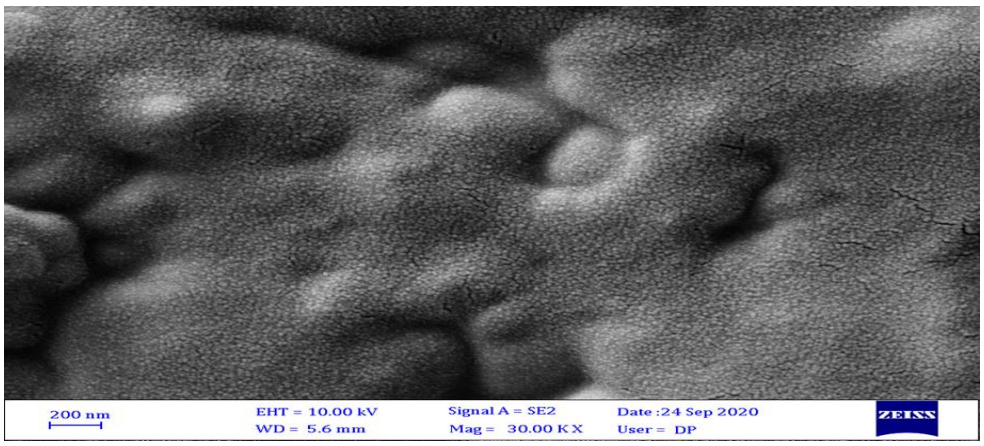

Figure 4. Transmission electron micrograph of gold nanoparticles

Antibacterial activity of AuNPs against *klebsiella pneumonia*

The results illustrated in Table 1 revealed that gold nanoparticles were highly effective toward *Klebsiella pneumonia* (Figure 7) that exhibits the decreasing in diameters of zone of the inhibition (18, 16, 13, and 9 mm) with the decreasing AuNPs concentrations (75, 37.5, 18.75, and 9.37 µg/ml, respectively). Similarly, (1) stated that the effect of gold nanoparticles was dose dependent.

Table 1. Impact of AuNPs with various concentrations on growth of *Klebsiella pneumonia* growth (n= 20)

AuNPs concentration (µg/mL)	Mean of Inhibition zone ± standard deviation (mm)
75	18
37.5	16
18.75	13
9.37	9

Similarly, study by [8] and co-worker recorded that the maximum anti-bacterial efficacy in 300 µl/ml concentrations of the biosynthesized gold nanoparticles was 19 mm for Gram negative pathogens such as *E. coli*, 16 mm for Gram positive pathogens such as *B. subtilis* 17 mm for *P. aeruginosa*, and; by use different concentrations of 100, 200, and 300 µl/ml.

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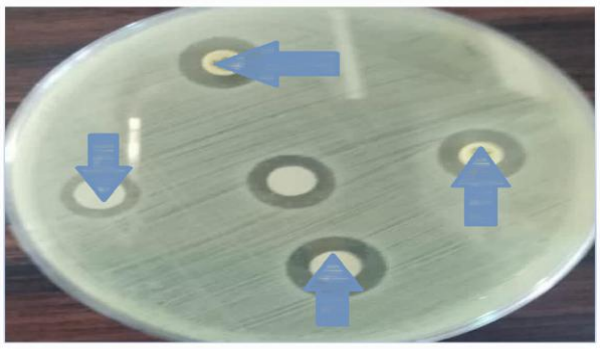

Figure 7. The inhibitory effect of AuNPs with difference concentrations 75, 37.5, 18.75 and 9.36 µg/ml) against *Klebsiella pneumonia* isolates

Synergism effect of AuNPs and antibiotic against *Klebsiella pneumonia* isolates

The results summarized in Table 2 showed that significant influence (P<0.05) of synergistic effect of gold nanoparticles and antibiotic against *klebsiella pneumonia* of each isolate in terms of between groups, within groups, and location at (37. 5 µg/ml) MIC of gold nanoparticles.

Table 2. Effect of AuNPs and antibiotic against *Klebsiella pneumonia* isolates

Antibiotics CEFTRIAXONE	without AuNPs		with AuNPs			Perce. excess of synergism (%)
	Mean of Inhbition zone (mm)	Std. Error	Mean of Inhbition zone (mm)	Std. Err		
	9.06	1.44	11.36	1.80		

The study by [8] showed that the ceftriaxone- gold nanoparticles were highly effective against *Klebsiella pneumonia* strains compared to ceftriaxone and gold nanoparticles alone. *Klebsiella pneumonia* showed zones of inhibition of 6 mm for ceftriaxone, 6 and 8 mm respectively, for gold nanoparticles, and 26 mm and 28 mm inhibition zones respectively, for ceftriaxone- gold nanoparticles. However, a research by [16] found that cefotaxime-coated gold nanoparticles have a common mechanism of action against drug-resistant bacteria that produce extended spectrum -lactamase. Another study [17] found that combining Amoxicillin with gold nanoparticles increased wide spectrum bactericidal efficacy against Gram-negative and Gram-positive microbes.

antibiotics and their synergistic efficacy(mm).

However, antibiotics combined to gold nanoparticles antibiotics combined with gold nanoparticles pass to the bacterium and easily stop all regulatory functions of the membrane, demonstrating an increased and more targeted local concentration (of antibiotics) and helping

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destroy microbes more efficiently than antibiotics alone. effectively inactivating the bioactive blockage of protein synthesis, sulfur including proteins, and interaction with the phosphorous element in the nucleic acid structure, combination of ampicillin, streptomycin, and kanamycin to AuNPs made these drugs more heat tolerant and stable, also reduced their MICs to *Staphylococcus aureus*, and *E. coli*, and *Micrococcus luteus* (The percentage of excess after synergism measured according to[18] .The percentage of inhibition and synergistic effect were determined using the following: formula B _ A/A * 100.

A= Inhibition zone of antibiotic

B= Inhibition zone synergism

CONCLUSION

This study concluded that the simple green approach with some modification using *K. pneumoniae* is a good method to produce AuNPs. The current study concluded that the AuNPs were highly effective toward *klebsiella pneumoniae* isolates in different concentrations. The current study concluded that the highlights synergistic influence of wide spectrum antibiotics with NPs resulting in increased fold efficacy toward drug resistant microbes conferring the emerging strategy to resist multi-drugs resistant bacteria.

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