



Gold nanoparticles effect on bacterial virulence factor formation with multiple drug resistance

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The study aims to investigate how the local *Pseudomonas aeruginosa* isolates' ability to form biofilms is impacted by gold nanoparticles. 47 samples are taken from burns (38%), urinary tract infections (7%), ear infections (4%), and wounds (51%). Fourteen isolates (29.78%) are found to be multidrug-resistant and to form biofilms. The sensitivity of isolates is evaluated using the Kirby-Bauer technique. According to the results, the largest percentages are found against levofloxacin, imipenem, ticarcillin, gentamicin, amikacin, piperacillin, ceftazidime, cefepime, and meropenem, respectively. Characterization of all isolates confirmed that they are *P. aeruginosa*. Only MDR are involved in this work. The quantitative evaluation of biofilm production is carried out using the microtiter plate technique (ELISA). The study also explores the green chemistry approach of creating gold nanoparticles using *Camellia sinensis*, or black tea leaf extract. The final nanoparticles pass all of the main investigations used to describe them, with SEM, FT-IR, XRD, and UV. The SEM images show spherical particles measuring 19 nm. The effect of the prepared gold nanoparticles as antibiofilm agents is studied at different concentrations (10, 20, 40, 80, 160) ppm compared to control sample. Analysis of variance shows that the highly significant effects ($p < 0.000$) on biofilm formation. The results show the mean $\pm$ S.D. of the different treatments; the control concentration is $0 \pm 0.104 \pm 0.033$, whereas the mean $\pm$ S.D. for the first concentration is as follows: 0.288 ± 0.075 , 0.312 ± 0.092 , 0.334 ± 0.119 , 0.380 ± 0.090 , 0.415 ± 0.0969 , and 0.305 ± 0.131 , respectively.

Keywords: Nanomaterials; MDR; Biofilm production; *P. aeruginosa*.

1. INTRODUCTION

Pseudomonas aeruginosa is found in a variety of biotic and abiotic environments, including soil, water, plants, animals, and insects [1]. It is also considered a major pathogen, especially in individuals with UTI, bacteremia, cystic fibrosis, and burns. Due to its innate resistance to many medications, *P. aeruginosa* often causes severe illness and death in immunocompromised people [2]. *P. aeruginosa* is an uncommon pathogen that can infiltrate nearly any tissue. Furthermore, *P. aeruginosa* has a global presence [3,4]. Because of its resistance to traditional antibiotic therapy, it presents a significant challenge in medical care [3,5]. This is particularly problematic for older nosocomial patients and those with weakened immune systems [4]. The accumulation of genes on R plasmids that individually confer resistance to specific antibiotics is frequently the origin of multidrug resistance in bacteria [6-8]. The bacterial aggregates, together with self-produced extracellular polymeric substances (EPS), are protected by the biofilm, a thick coating that the colony employs to adhere to a surface [9].

Biofilms are complex bacterial colonies that develop in a variety of environments, such as the human body, persistent diseases, and medical equipment. A safety model of extracellular polymeric materials that encircle the bacteria and present significant therapeutic and diagnostic hurdles is what defines biofilms [7,10]. In the setting of chronic infection, biofilms differ from free-floating bacteria in a number of ways [8,9]. Increased antibiotic resistance, persistent inflammation, tissue damage, and difficulties with diagnosis are some of the major treatment consequences of these alterations [9,11]. One of the most important impacts of bacterial biofilms is their ability to confer antibiotic resistance. Bacteria are extremely resistant to antibiotics because they are shielded from their effects by the protective biofilm matrix [11].

By decreasing the likelihood that the bacteria would be able to pass through immune cells and medications, this structure helps as a useful barrier to the immune system and the antibacterial, which might lead to continued colonization and treatment failure [10-12]. The biofilm is a bunker for antibiotic-tolerant persistent cells [13]. Mutations occurring in the biofilm environment make them withstand adversity, grow stronger, and become more tolerant to antimicrobial drugs. Nanotechnology, an emerging field of science, has many applications in different fields, including agriculture, electronics, and biomedicine, etc. The application of nanoparticles and their conjugates in antimicrobial therapy is being widely studied [11,13].

Gold nanoparticles (AuNPs) are tiny gold particles with dimensions 1 to 100 nm. Gold nanoparticles (AuNPs) have been used as antibacterial, anti-HIV, antitumor, antimalarial, and antibiofilm substances [12] besides being used in biosensors, DNA labeling, and vapor sensing [13-15]. Physical, chemical, and biological methods for the synthesis of nanoparticles have been developed. The biological method, or the green method, of synthesis is advantageous over the chemical and physical methods [14].

Nowadays, more and more nanoparticles are being synthesized for usage in vital applications, with virulence factors and biofilm inhibitor agents [14,31,36]. They are often referred to as colloidal gold [13,14,34]. Because of their large surface area, low cell toxicity, and durability [15], gold nanoparticles are being used in hair tonics and cosmetics [14] and have been effective gene carriers. Moreover, they can evade lysosomal digestion, protecting the genes or DNA they carry [16]. Gold nanoparticles inhibited the formation of biofilms and had no detrimental effect on *P. aeruginosa* [17,35].

Nanoparticles (NPs) can offer an alternative solution to battle MDR pathogens. Gold nanoparticles affect many virulence features by interacting with the bacterial cellular communication networks [18,19]. The study is designed to assess the use of gold nanoparticles as an anti-biofilm agent and the connection

between the capacity of multidrug-resistant isolates to produce biofilms and their sensitivity to antibiotics.

2. EXPERIMENTAL

The gold nanoparticles are prepared according to Al-Essawi and Mahmood [25], as follows:

A-Preparation stock solution (12 mM) of HAuCl₄

One gram of HAuCl₄ (99% Germany) is thawed in 200 ml of D.W. to give HAuCl₄ III (12 mM), and stored in a brown bottle.

B-Preparation of black tea leaves solution

Black tea leaves are prepared by dissolving 0.34 gram in 100 ml of D.W. and put on a magnetic hot plate for 15 min at a temperature of 70-80 °C, then clarified with Whatman filter paper No.1. Standard AuNPs are prepared by reducing HAuCl₄ to Au. 100 ml (12 mM) of HAuCl₄ solution is mixed with the leaf extract (100 ml) on a hotplate at 80 °C. The color is becoming purple within 10-15 minutes, confirming AuNPs, then collected by centrifugation at 12000 rpm for 20 min, washed three times, and dried at 40 °C for 48 hours [25]. According to a prior work by Al-Essawi and Mahmood [25], the nanoparticles are produced and characterized using a variety of techniques to determine the physicochemical characteristics, including UV-visible, SEM, FTIR, and XRD analysis. The Kirby-Bauer and NCCLS recommendations are applied to assess the resistance of *P. aeruginosa* isolates to nine antibiotic-containing discs.

A microtiter plate approach is based on the ELISA technique [13]. The microtiter plate wells are filled with 180 µl of nutrient broth (NB) and 1% glucose. The well is supplemented with 20 µl of previously produced bacterial suspensions. The cultures are maintained at 37°C for 24 hours before being eliminated. Following the pouring of the cells, sterile PBS is used for three rounds of washing. After 20 minutes of methanol fixation, drying at room temperature and 25°C, and staining with 0.1% safranin, the results are obtained. One milliliter of 99% methanol solution is used to dissolve the safranin that had adherent cells attached to it. Lastly, an ELISA reader is used to read the microtiter plates at 630 nm (A630).

The data are analyzed using an ANOVA test to search for the effect of treatment with gold nanoparticles, and LSD is used to compare the means of different treatments. Analysis is carried out using the Statistical Package of Social Sciences (SPSS) program version 26VA, which is used for the analysis of the effect of treatments of gold nanoparticles as an antibiofilm agent.

3. RESULTS AND DISCUSSION

The current investigation has analyzed 47 clinical specimens, including catheter-acquired urine, wound infections, burns, cystic fibrosis, and otitis media. The isolates are obtained from patients admitted to Al-Ramadi Teaching Hospital (Ramadi, Iraq).

Various types of media and chemical characteristics are used to isolate and identify *Pseudomonas aeruginosa*. The isolates are first identified as *Pseudomonas aeruginosa*, which appeared as Gram-negative-negative rod-shaped cells that could not produce spores [26,27,30]. All isolates test positive for oxidase and catalase and are able to produce catalase. The colonies vary in size and medium of infection, ranging from tiny to large mucosal, and positive oxidase findings are linked to the capacity to manufacture cytochrome oxidase [34]. After all confirmation tests to identify *Pseudomonas aeruginosa*, a total of forty-seven isolates reveal that the highest percentage of isolates is from wounds (51%) and the lowest percentage is from ear swabs (4%). Hamid et al, 2017 [28] find the isolation rate of ear

infections to be 11.6%. Other factors contributing to this discrepancy include the category of sterilization and disinfectants used in hospitals and other sterilization techniques, as well as variations in isolation sites or the quantity, kind, and distribution of various types by location [c29, 32, 33]. As recommended by CLSI (2022), the isolates from burns, wounds, urine, and ear swabs are tested. The Isolates' antigram reveals varying antibiotic resistance.

Ceftazidime (CAZ), Imipenem (IPM), Piperacillin-tazobactam (PRL), Levofloxacin (LEV), Meropenem (MEM), Gentamicin (GN), Colistin (CO), Amikacin (AK), and Cefepime (FEP/CPM) are among the nine antimicrobial agents whose susceptibility is examined. Every antibiotic we employ in our investigation is ineffective against the isolates (Figure 1). According to the criteria published by CLSI (2024), *P. aeruginosa* is categorized as MDR. The isolates are classified as multidrug resistant according to the resistance to more than three groups of the used antibiotics.

Under various experimental conditions, a spectrophotometric approach is employed to quantitatively measure biofilm production. The isolate producers are categorized according to the biofilm intensities into strong (S), intermediate (I), and weak (W) based on the findings of the quantitative biofilm formation test by using the ELIZA technique in comparison to the control. According to our findings, 14 (29.7%) are biofilm-producing, with the remaining 14 (29.7%) being intermediate biofilm producers. Of the 47 isolates, 51% are from wounds, burns (38%), 7% from urinary tract infections, and 4% from otitis media. The highest percentages are biofilm producers: 14 isolates produced intermediate biofilms. After the preparation and the physicochemical characterizations of the gold nanoparticles by Al-Essawi and Mahmood (2024) [25], and the treatment with (10,20,40,60, and 80) ppm of AuNPs.

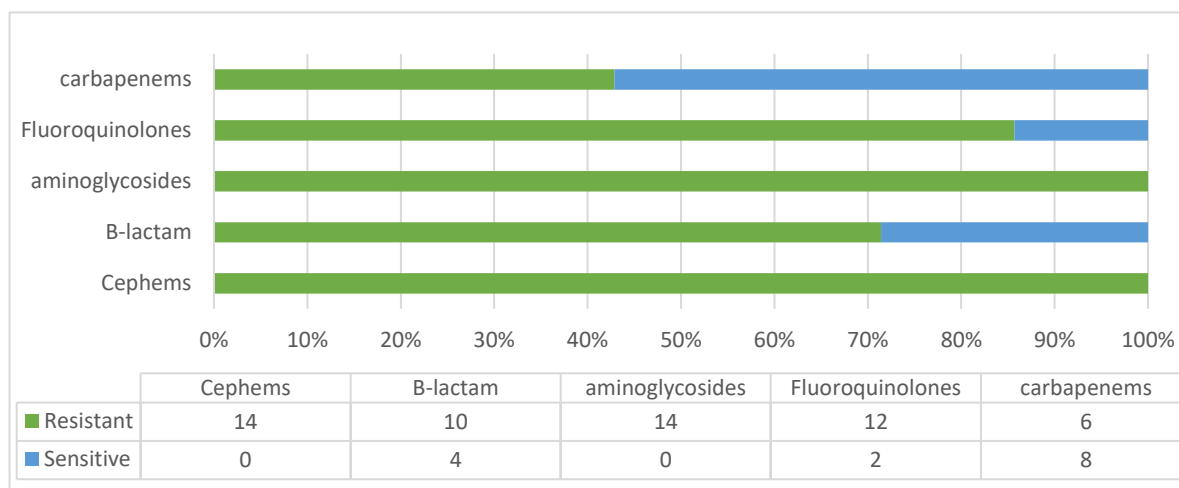


Figure 1 Clinical *P. aeruginosa* antigenogram used in the study.

A significant treatment is thought to be the rise of bacterial resistance to several antibiotics. The findings reveal that the antibiotic Meropenem (MEM) had the lowest percentage of resistance (8.5%), and Ceftazidime (CAZ) had the greatest rate (87.3%).

Table 1 indicates that 43(91.5%) isolates have the highest antibiotic sensitivity to Meropenem (MEM), with no significant differences ($p > 0.07$), whereas 6 (12.7%) isolates have the lowest antibiotic resistance ($p < 0.000$) to Amikacin (AK). ANOVA analysis exposes that the gold nanoparticle concentrations have a highly significant effect ($p < 0.000$) on the biofilm formation.

Table 1 Antibiotic Resistance percentages by the *Pseudomonas aeruginosa*.

Antibiotic disc	No. (%) of resistant	No. (%) of Sensitive	P Value
Amikacin (AK)	30(63.8%)	17(36.2%)	0.000
Cepfepime (FEP)	31(65.9%)	16(34.1%)	0.280
Ceftazidime (CAZ)	41(87.3%)	6(12.7%)	0.000
Gentamicin (GN)	27(57.4%)	20(42.6%)	0.162
Imipenem (IPM)	12(25.5%)	35(74.5%)	0.003
Levofloxacin (LEV)	12(25.5%)	35(74.5%)	0.549
Meropenem (MEM)	4(8.5%)	43(91.5%)	0.072
Piperacillin (PRL)	20(42.5%)	27(57.5%)	0.001
Ticarcillin (TC)	7(14.8%)	40(85.1%)	0.162

To prove that our approach is environmentally friendly, we use a medicinally significant *Camellia sinensis*, or black tea leaf extract, to synthesize AuNPs and demonstrated their efficacy against the *P. aeruginosa* biofilm.

The findings show that the mean $\pm$ S.D. of the different treatments (Table 2), the control concentration is $0 \pm 0.104 \pm 0.033$, whereas the mean $\pm$ S.D. for the following concentrations (10, 20, 40, 60, and 80) ppm are

Exp. Theo. NANOTECHNOLOGY 10 (2026) 1625-1632
as follows: 0.288±0.075, 0.312±0.092, 0.334±0.119, 0.380± 0.090, 0.415±0.0969, 0.305±0.131respectively.

Table 2 Biofilm-producing isolates treated with different AuNPs concentrations.

Descriptive Statistics		
Dependent Variable: Conc.		
Treat	Mean	Std. Deviation
Control	.104357143	.0334907729
1 st treatment (10) ppm	.288642857	.0759601182
2 nd treatment (20) ppm	.312571429	.0925017374
3 rd treatment(40) ppm	.334500000	.1193564474
4 th treatment (60) ppm	.380000000	.0909175451
5 th treatment (80) ppm	.415428571	.0969787560

These findings agree with a previous study by Ali et al. (2022), who obtains that biofilm formation is reduced at all three doses for both PAO1 and clinical isolates of *P. aeruginosa*. TG-AuNPs at 150 µg/mL reduced biofilm formation by 59.09% for PAO1, and by 49.1% to 64.7% of *P. aeruginosa*.

Our findings are also consistent with recent investigations by Elshaer and Shaaban [38], who finds that gold nanoparticles at ½ and ¼ MIC values decreased biofilm development by 26-68% and 21-37%, respectively. Because NPs' antibacterial action is dose-dependent, using larger doses of NPs above the MIC is necessary to get strong therapeutic outcomes. More focus is being paid to factors including form, size, concentration, and coating agent that determine the antibacterial effects of Au NPs. Au NPs are given increased study because of their electrical and optical characteristics. One of its most notable properties is the localized surface Plasmon resonance (LSPR), which is critical for many applications, including biosensors. This occurs when electrons on the surface of noble metal nanoparticles react with electromagnetic radiation, resulting in LSPR. As a result, metal nanoparticles cause considerable extinction, which is beneficial in a variety of applications [39]. It is generally known that Au NPs have unique properties.

4. CONCLUSIONS

The findings of this study demonstrated the ability of using black tea extract as a reducing agent for gold nanoparticles formation, also exhibited substantial effectiveness of the various gold nanoparticle treatments on the severity of biofilm formation by *Pseudomonas aeruginosa* isolates. Analysis of variance showed that green synthesized gold nanoparticles have highly significant effects (p<0.000) on biofilm formation. The antibacterial effect of NPs was dose-dependent and requires the usage of elevated concentrations of NPs above the MIC to reach strong treatment results, along with possible toxicity.

Conflict of Interest

Regarding the publishing of this study, the authors affirm that they have no conflicts of interest.

Ethical Considerations

The ethical guidelines are strictly followed throughout the study. No data fabrication, falsification, plagiarism, or research misconduct has occurred. Ethical approval is granted by the Research Ethics Committee / University of Anbar (dated June 30, 2024).

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Exp. Theo. NANOTECHNOLOGY 10 (2026) 1625-1632

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