

## Characterization, antibacterial and antibiofilm evaluation of biosynthesized silver nanoparticles from *Pseudomonas aeruginosa* against drug resistant *Acinetobacter baumannii*

Talar Ibrahim Hasan\*, Akhter Ahmed Ahmed\*

\*Department of Biology, College of Science, Salahaddin University- Erbil, Iraq

Article Info:

Received Feb 2023

Accepted Mar 2023

Corresponding Author email:

Email: [talar.hassan@su.edu.krd](mailto:talar.hassan@su.edu.krd)

Orcid: <https://orcid.org/0000-0002-6076-6345>

DOI : <https://doi.org/10.32947/ajps.v23i3.1049>

### Abstract :

Antimicrobial resistance is regarded as one of the top three terrible events threatening the worldwide existence of humans Here of, *Acinetobacter baumannii* evolved as the most challenging pathogen threatening to initiate the post-antibiotic era.

Their ability to withstand antibiotics is attributed to a set of virulence determinants in particular biofilms which are known to enhance pathogenesis and drug resistance potency. Studies regarding green silver nanoparticles (AgNP)s as an alternative treatment modality to antibiotics increased over recent years. Considering these facts, we aimed to explore the antibiofilm effect of AgNPs in the multi-drug-resistant *Acinetobacter baumannii*. AgNPs were bio-fabricated by *Pseudomonas aeruginosa* and characterized via FTIR, UV-Vis, XRD, EDS, and SEM. Well-diffusion was used to screen the antimicrobial effects of AgNPs. Minimal-inhibitory concentrations of AgNPs were determined to study their antibiofilm effect at sub-inhibitory concentrations (SIC). Results showed that all isolates were biofilm producers and portrayed high resistance to the tested antibiotics. Characterization results supported the successful fabrication of crystalline nanoparticles. Exposure of the isolates to the bacteriogenic AgNPs resulted in pronounced inhibition zones and reduced biofilms at SICs values. These results indicate that *Pseudomonas aeruginosa* can be employed to produce AgNPs with an aptitude to disrupt biofilm development and growth in the multi-drug resistant *Acinetobacter baumannii*.

**Key words:** drug resistance, biofilm, *Acinetobacter baumannii*, biogenic silver nanoparticles.

**توصيف وتقييم تأثير جسيمات الفضة النانوية المصنوعة بواسطة *Pseudomonas aeruginosa* كمضاد للبكتيريا ومضاد لغشاء الحيوي ضد البكتيريا *Acinetobacter baumannii* المقاومة للأدوية**

تالار إبراهيم حسن\* , اختر احمد احمد\*  
قسم علوم الحياة – كلية العلوم – صلاح الدين – أربيل / العراق

### الخلاصة:

يتم تصنيف مقاومة المضادات الحيوية كواحدة من أكبر ثلاث ظواهر كارثية تهدد وجود البشرية في جميع أنحاء العالم. في هذا الصدد ، تطورت (*Acinetobacter baumannii*) باعتبارها أكثر مسببات الأمراض تحديًا والتي تهدد ببدء عصر ما بعد المضادات الحيوية. تُعزى قدرتها على تحمل المضادات الحيوية إلى مجموعة من عوامل الضراوة بالاختصاص الأغشية الحيوية التي يُعرف عنها أنها تعزز الأمراض وفعالية مقاومة الأدوية. زادت الدراسات المتعلقة بجسيمات الفضة النانوية الخضراء (AgNP) كطريقة علاج بديلة للمضادات الحيوية خلال السنوات الأخيرة. بالنظر إلى هذه الحقائق ، نهدف في

هذه الدراسة إلى استكشاف تأثير المضادات الحيوية للجسيمات النانوية المصنعة بواسطة البكتيريا (AgNPs) في بكتيريا (*Acinetobacter baumannii*) المقاومة للأدوية المتعددة. تم تصنيع AgNPs بيولوجيًا بواسطة (*Pseudomonas aeruginosa*) واستخدمت FTIR , UV-Vis , XRD , EDS لتوصيفها. تم استخدام طريقة الانتشار في الاغار لفحص التأثيرات المضادة للميكروبات لـ (AgNPs). تم تحديد تركيز المثبط الأدنى (MIC) وما تحته (Sub-MIC) لـ (AgNPs) لدراسة تأثيره على نمو الأغشية الحيوية. كانت جميع العزلات منتجة للغشاء الحيوي وقد أظهرت مقاومة عالية للمضادات الحيوية المختبرة. دعمت نتائج التوصيف التصنيع الناجح للجسيمات النانوية البلورية. أدى تعرض عزلات البكتيريا (AgNPs) إلى ظهور مناطق تثبيط واضحة وتقليل الأغشية الحيوية عند قيم تحت ال MIC تشير هذه النتائج إلى أنه يمكن استخدام (*Pseudomonas aeruginosa*) لإنتاج (AgNPs) قادرة على تعطيل نمو الأغشية الحيوية في البكتيريا مقاومة الأدوية المتعددة (*Acinetobacter baumannii*).

**الكلمات المفتاحية:** مقاومة الأدوية ، الأغشية الحيوية ، *Acinetobacter baumannii* ، جسيمات الفضة النانوية الحيوية.

## Introduction

Antibiotic resistance along with climate change and overpopulation represent the most paramount catastrophes menacing the existence of mankind worldwide (1). Unearthing of penicillin as the first antibiotic agent in 1928 marked the beginning of the golden antibiotics era (2). Over 7 decades later, antibiotics allowed major advances in several fields of medical practice, enabled the treatment of formerly fatal bacterial infections, extended the life expectancy of mankind, and saved countless lives (2-4). Nevertheless, adequate usage of antibiotics, dearth of developing new effective drugs, and inefficient infection control systems gave rise to the so-called antibiotic resistance phenomenon (2, 5). In 2019, a report was released by World Health Organization (WHO) where drug resistance was mentioned to account for 700000 annual global deaths and in case the present state is left unresolved that number is anticipated to reach 10 million annual deaths by 2050 (4, 6). Recently, *Acinetobacter baumannii* (*A. baumannii*) emerged as an ESKAPE pathogen of utmost significance in nosocomial settings where they account for 20% of the total ICU bacterial infections (7-9). According to (10), the estimated number of the reported annual *A. baumannii* infections exceed 1000,000 cases globally with mortality rates as high as 80%. Due to a shortage in treatment options, WHO placed these bacteria as the most critical in their

first priority list for antibiotic-resistant pathogens issued in 2017 (11). Moreover, *A. baumannii* was listed as an urgent pathogen in drug resistance threat reports (2013 and 2019) by the center for disease control and prevention (CDC) (12). What makes *A. baumannii* an alarming pathogen is a collection of virulence determinants and immense competence to withstand various antibiotic classes and harsh environmental settings (13). Infection frequency with these bacteria is lower than those caused by other gram-negative pathogens nevertheless the rate of drug resistance in *A. baumannii* exceeds those seen in other gram-negative bacteria by 4 folds (14). The issue is more aggravated owing to their ability to form biofilm on various abiotic and biotic planes which increases chances of getting infected by these bacteria in addition to enhancing their resistance profile against desiccation, extreme pH, host immune attacks, and various antibiotic agents by 1000 folds compared to their planktonic phenotypes (9, 15, 16). Considering these facts, without significant interference, fears proceed to increase that *A. baumannii* infections might become untreatable in the near future (17). Hence, there is a need to search or develop novel antibiotics or other treatment modalities urgently for these challenging pathogens (18, 19). In this regard nanomaterials in particular silver nanoparticles (AgNPs) have emerged as a possible approach to inhibit biofilm

development in these superbugs due to their unique physicochemical features, low cost, non-toxicity, and elevated antimicrobial performance (20-22). Conventionally, various physicochemical routes can be employed for the fabrication of AgNPs however these techniques are accompanied by high cost, energy consumption, toxic chemicals, environmental hazards and are quite complex. Hence, green or biological origins are gaining popularity because to their easiness, non-toxicity, cost-effectiveness, biodegradability, and low energy requirements. Bacteria are described as the elite candidate amongst the biological means by virtue of their remarkable aptitude to reduce heavy metal ions, low production cost, high abundance, and adaptability (23, 24). A number of studies reported antimicrobial and antibiofilm effect of AgNPs (25-28). However, few reports exist regarding the antibiofilm effect of AgNPs on *A. baumannii*. The current study was carried out to investigate antibacterial and antibiofilm effect of bacteriogenic AgNPs on the multi-drug resistance (MDR) *A. baumannii*.

## Materials and methods

### Collection and identification of *Acinetobacter baumannii* isolates

A sum of twenty-six non-duplicate *A. baumannii* isolates were gathered from multitude laboratories of bacteriology located in the capital of Iraqi Kurdistan, Erbil. *A. baumannii* ATCC 19606 was acquired from the Medya Diagnostic Center and employed as control during the research. The collected isolates were re-cultured onto MacConkey (MA, Merck, Germany) agar plates and incubated under aerobic circumstances at 37°C for 24 hours. Several conventional and biochemical diagnostic procedures were performed to identify the distinct colonies as *A. baumannii*, as previously described

by (29). Identification of the clinical isolates were confirmed via the automated Vitek-II system (Biomérieux, France). For future studies, pure cultures were stored at -70 °C as glycerol stocks.

### Antimicrobial susceptibility analysis

Taking Clinical and Laboratory Institute (CLSI) recommendations into account, antibiotic agents were selected to examine the sensitivity of the isolates via disc diffusion method (30). Bacterial suspensions with a turbidity of 0.5 McFarland were prepared and cultured on the Muller Hinton (MHA, Biomark Laboratories, India) agar plates. Following antibacterial agents were tested: Ceftazidime, piperacillin/tazobactam, gentamicin, ceftriaxone, ampicillin/sulbactam, levofloxacin, tobramycin, ciprofloxacin, amikacin, imipenem, trimethoprim/sulfamethoxazole, meropenem, cefepime, and doxycycline. Utmost resistant isolates were chosen to evaluate the inhibitory activity of AgNPs.

### Static biofilm analysis

The biofilm formation wells of the identified strains was examined via microtiter plate assay outlined previously by (31) with minor alterations. Concisely, overnight cultures of the selected bacteria were added into the cavities of the microplate (MTP, Cito-test Labware, China) containing sterilized Nutrient-broth (NB, Neogen, USA) enriched with 2% glucose. As control, wells housing NB were employed. Following the 24 hours of incubation of the inoculated microplate in a static state, the wells were cleared from the supernatant and washed threefold with sterilized phosphate buffer-saline (PBS). Then, the wells of the MTP were stained via 1% crystal-violet solution and rinsed gently threefold with PBS to remove excess dye. Once dried, the wells were supplemented with 95% ethanol solvent to spectrophotometrically measure the produced biofilms at a wavelength of 490 nm via Elisa (Bio-Tek Instruments, USA).

Most potent biofilm developers were hand-picked to analyze the antibacterial activity of the AgNPs. Triple biological replicates were measured and standard errors were determined.

### **Bio-fabrication of bacteriogenic silver nanoparticles**

*P. aeruginosa* ATCC strain 50126 was employed for the bio-fabrication of AgNPs according to the protocol detailed by (32) with minor alterations. The cell-free broth was obtained via high-speed centrifugation of the overnight culture of *P. aeruginosa* inoculated into sterile NB. Then a mixture of 500 mL of each of the cell-free extract and 4mm silver nitrate was prepared and incubated at a temperature of 60 °C for three days in the absence of light. As incubation coarse terminated, the color of the reaction solution changed to deep brown and this was viewed as an initial sign for formation of AgNPs.

### **Characterization of bacteriogenic silver nanoparticles**

Ultraviolet-visible (UV-vis) spectroscopy (UV-1900i, Shimadzu, Japan) was employed to confirm the bio-synthesis of the AgNPs. X-ray Diffractometer (XRD, PAN-analytical powder X-Pert, Holand)), Fourier-Transform Infrared Spectroscopy (FTIR) (Jasco, Japan), Energy-Dispersive X-Ray spectroscopy (EDX, Bruker, Germany), and Field Emission Scanning Electron Microscopy (FESEM-Quanta 450, USA) were operated at the Scientific Research Centre of Soran University for additional characterization of the harvested nanoparticles (33).

### **Screening antimicrobial impact of AgNPs**

The antimicrobial effect of the green AgNPs was assessed via well-diffusion technique (34). The turbidity of the overnight *A. baumannii* cultures was adjusted to 0.5 McFarland (OD adjusted to

0.5 at 550 nm) and plated onto Mueller Hinton Agar plates. Then, 6mm wells were bored and 150 µL of nanoparticle solution was placed into the wells. Following this, the plates were placed in the incubator for 24 hours at 37 °C. A ruler was employed to measure the diameter of the formed inhibition zones around the wells.

### **Calculation of Minimum and sub-minimum inhibitory concentrations of AgNPs**

Minimum inhibitory concentration (MIC) values of the harvested nanoparticles in opposition to *A. baumannii* ATCC strain and ten of the most resistant and potent biofilm producer clinical isolates were assessed via broth microdilution technique based on CLSI instructions (35). In brief, 10 µL of the selected overnight grown *A. baumannii* strains equilibrated to 0.5 Mcfarland were added into the wells of the microplate containing 200 µL of various concentrations of NB and AgNPs mixture. Following this, the plates were placed in a rotating incubator at 37 °C for 24 hours. Minimal concentrations with no apparent growth were labeled as MICs. Measures below MICs were indicated as subinhibitory concentration (SICs) values and were used to evaluate the anti-biofilm effect of the nanoparticles.

### **Anti-biofilm impact of nanoparticles**

The competency of the bacteriogenic nanoparticles to inhibit biofilm development at SIC was assessed against a selection of 10 strongest biofilm producers and MDR isolates along with an ATCC strain of *A. baumannii* following the protocol formerly illustrated by (36). In brief, 200µL of sterile NB enriched with 2% glucose was added into the wells of flat-bottom MTP. Then, 15 µL from the SIC wells of the former procedure (MIC) were added into the wells and incubated overnight at 37 °C under a static state. For control, wells with bacterium inoculum and NB solely were considered. Liquid cultures were removed from the wells after

incubation, followed by three PBS rinses and 1% crystal violet staining. After the staining process, PBS was employed to rinse the excess dye from the wells, 95% ethanol solution was used for elution and an Elisa reader at the wavelength of 490 nm was considered for quantification of the formed biofilms.

### Statistical analysis

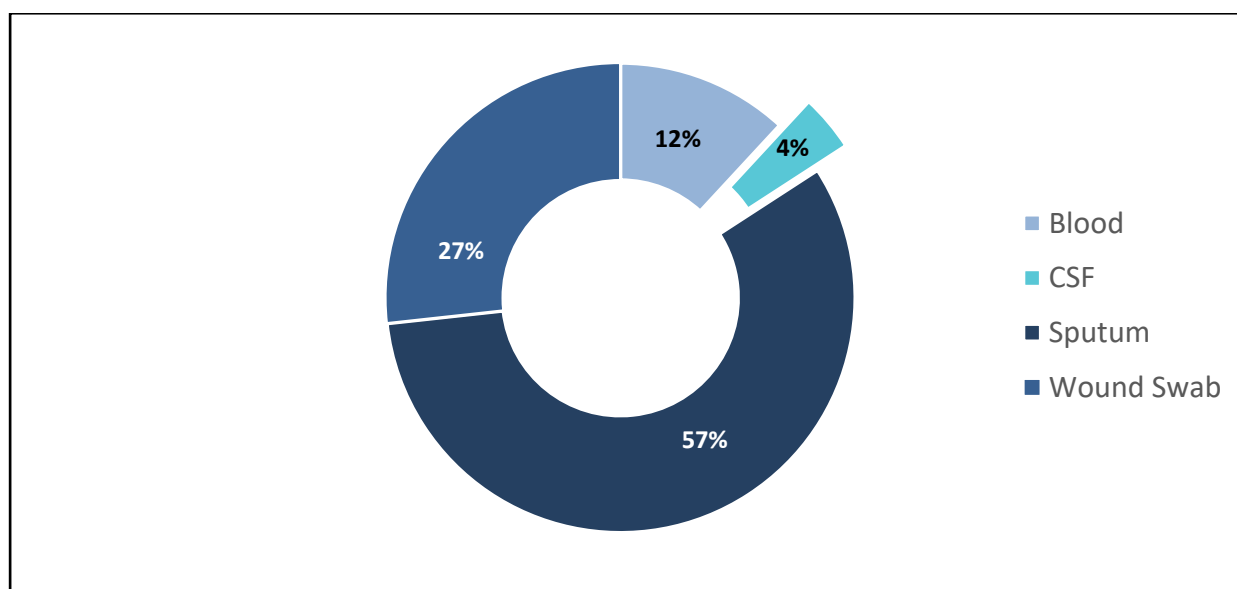
Graphpad Prism 8.0 software was used for the statistical analysis of the obtained results. For multiple comparisons, a two-way analysis of variance (ANOVA) test was employed. Data interpreted as mean $\pm$ SE.

## Results and discussion:

### *A. baumannii* isolates distribution and Antibiotic resistance pattern

A total sum of 27 non-duplicate *A. baumannii* isolates together with ATCC strain (19606) were procured from various infirmaries in the Erbil governorate to carry out the current research. The clinical

isolates were identified as catalase-positive, indole-negative, oxidase-negative, citrate-positive, and gram-negative non-fermenting coccobacilli suited for growth on the MA culture medium. Vitek II automatic system was operated for validation of their identification as *A. baumannii* with 96 to 99% probability rates. As for specimen types it was found that sputum accounted for (58%) of the recovered isolates followed by (27%), (12%), (4%) from wounds, blood, and CSF as illustrated in figure (1). Similarly, recent studies from Thailand and Iraq reported 80% and 32% of the *A. baumannii* isolates were recovered from sputum (37, 38). Their tendency to target the respiratory tract could explain isolation of these pathogens at a higher rate from sputum (39). Isolation of *A. baumannii* strains from wound, blood, and CSF mirror their proficiency to affect various sites such as septicemia, wound infections, meningitis, and urinary tract infections (13, 40).



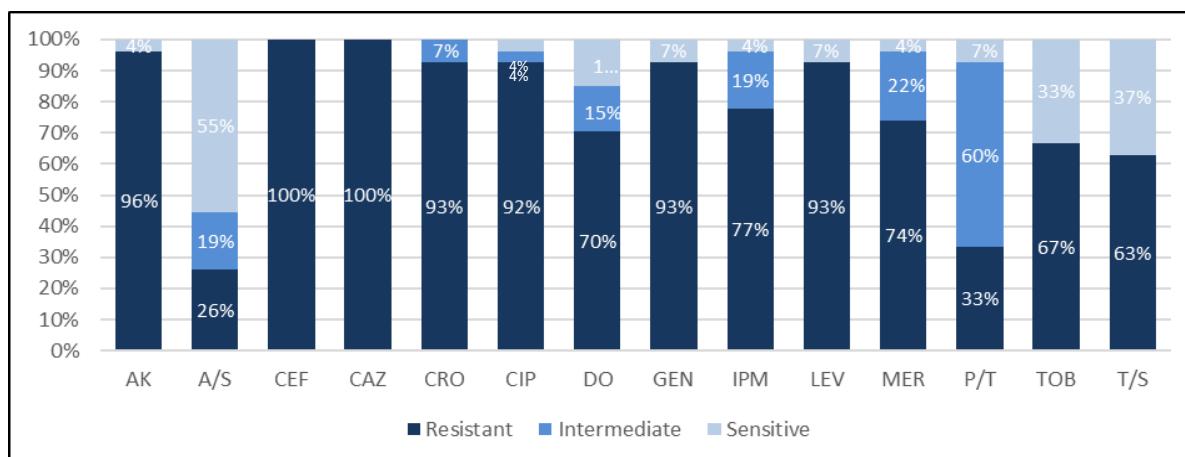
**Figure 1: Diverse clinical samples retrieved from *A. baumannii* patients viz wound, CSF, sputum, and blood.**

Kirby-Bauer assay was operated to assess the sensitivity of our isolates to the selection of 14 antibiotics chosen for this

study. As observed in figure (2), all isolates exhibited minimum

resistance (26%) against ampicillin/sulbactam whilst maximum resistance was shown against ceftazidime

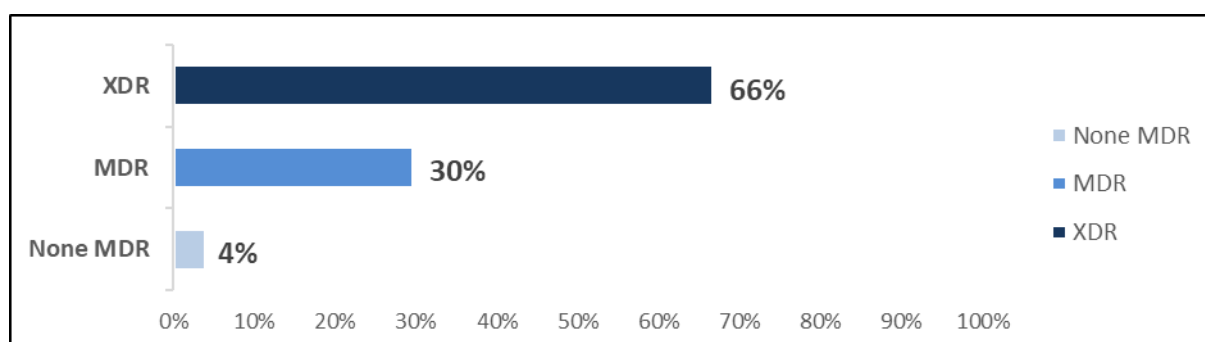
and cefepime by 100% followed by 96% amikacin resistance.



**Figure 2** resistant, intermediate and sensitive percentage of *A. baumannii* isolates granted by antibiotic susceptibility test: AK (Amikacin); A/S (ampicillin/sulbactam); CEF (cefepime); CAZ (ceftazidime); CRO (ceftriaxone); CIP (ciprofloxacin); DO (doxycycline); GEN (gentamicin); IPM (imipenem); LEV (levofloxacin); MER (meropenem); P/T (piperacillin/tazobactam); TOB (tobramycin); T/S (trimethoprim/sulfamethoxazole).

Moreover, although infection rate by *A. baumannii* is relatively lower than other gram-negative pathogens such as *P. aeruginosa*, the rate of multi drug resistance among them is believed to reach 45% which exceeds those observed in other gram-negative bacteria by four-folds (14, 41). In this respect, we arranged *A. baumannii* isolates into three categories

(non-MDR, MDR and XDR) according to the results of the antibiotic-susceptibility assay. Biggest share (67%) of the isolates were XDR while 30% and 4% were MDR and non-MDR (Figure 3). In parallel with our results, Maspi *et al* reported a similar susceptibility pattern to ours where 71% of the isolates were designated as XDR *A. baumannii* (42).



**Figure 3:** Classification of *A. baumannii* isolates based on their resistance pattern.

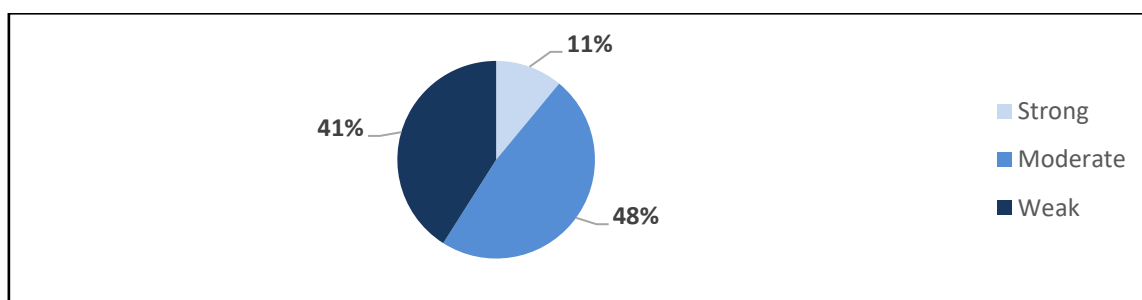
### Biofilm assay

Biofilms are well-known for their potential to promote pathogenesis and antimicrobial resistance, as bacteria living in these microbial structures are 1000 times more

resistant to antibiotics than their planktonic or free-living counterparts (43). According to (16), the rate of biofilm development in *A. baumannii* is comparatively higher than in other species. In this sense, all of the

isolates exhibited varying degrees of biofilm production. As illustrated in figure (4), 11% of the isolates were labeled as

strong biofilm developers, and 41%, and 48% were recorded as weak and moderate biofilm creators.

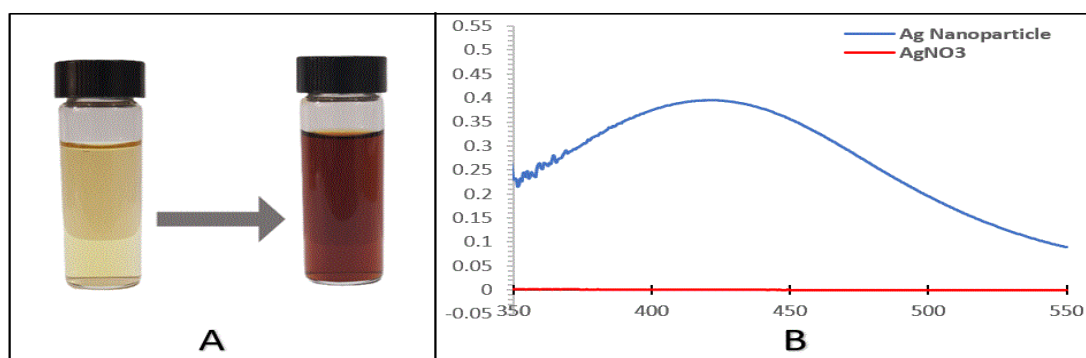


**Figure 4: Biofilm development rate of *A. baumannii* isolates in relation to the results acquired from the microplate analysis.**

### Bio-fabrication and characterization of bacteriogenic Nano-silvers

*P. aeruginosa* ATCC (50126) strain was considered for the fabrication of AgNPs extracellularly. According to (33), enzymes in particular nitrate reductase along with silver-resistant genes, C-type cytochromes, peptides, and reducing cofactors warrant the bacteria ability to reduce/stabilize AgNPs. Following incubation of the reaction mixture, we inspected an alteration in the color of the solution to deep brown as illustrated in figure (5A). This color change is believed to be caused by an incident named surface

plasmon resonance and is viewed as a preliminary sign of the reduction of silver ions to nano-silvers (44). Bio-reduction of silver nitrate to nano-sized silver was validated via UV-Vis technique. 2 to 100 nm sized spherical AgNPs would be anticipated if a single UV peak was detected between 440 and 400nm (45). In the current study, the UV band for silver nitrate at a wavelength of 268 nm vanished following incubation with the cell-free extract for 72 hours and a new absorption peak occurred at a wavelength of 420nm suggesting bio-formation of spherically built nano-silvers (Figure 5B).



**Figure 5: A) a change in color from pale yellow to dark brown indicated biosynthesis of nano silvers.**

**B) UV-Vis absorption spectra at 420 nm of bacteriogenic AgNPs.**

### FTIR analysis

FTIR measurements were operated to analyze the possible functional groups that could be accountable for the bio-reduction/capping of silver-ions. FTIR spectra observed in figure (6) displayed 6 peaks at 1218, 1369, 1635, 1735, 2136, and 3266  $\text{cm}^{-1}$  which are equivalent to the stretching vibrations of C-O for (alcohols, carboxylic acids, esters, ethers), C-H (alkane), C double bond (alkene), C=O

(carbonyl),  $\text{C}\equiv\text{C}$  (alkyne) and O-H (alcohol) (46). Our results in line with findings of (47) who revealed presence of carbonyl and alkene groups following FTIR spectrum analysis of AgNPs synthesized by means of *P. aeruginosa*. Altogether, the FTIR technique confirmed the existence of several biomolecules that could be involved in the reduction/capping process of the green nano-silvers (48).

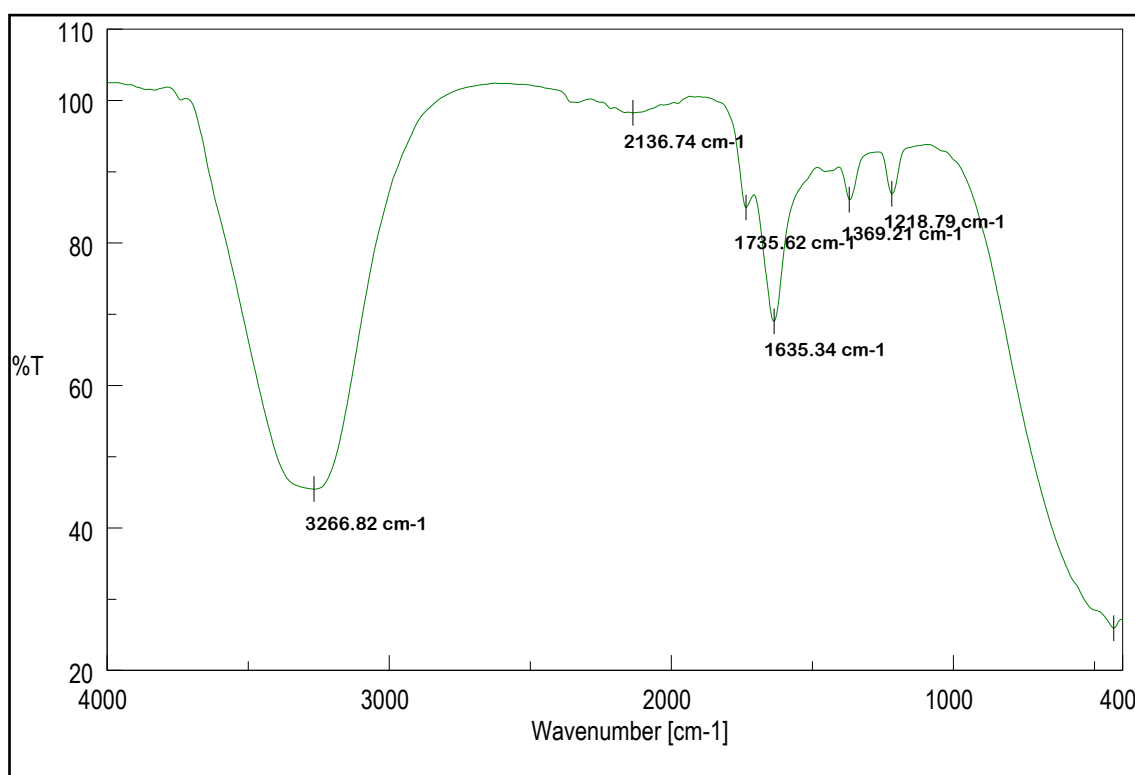
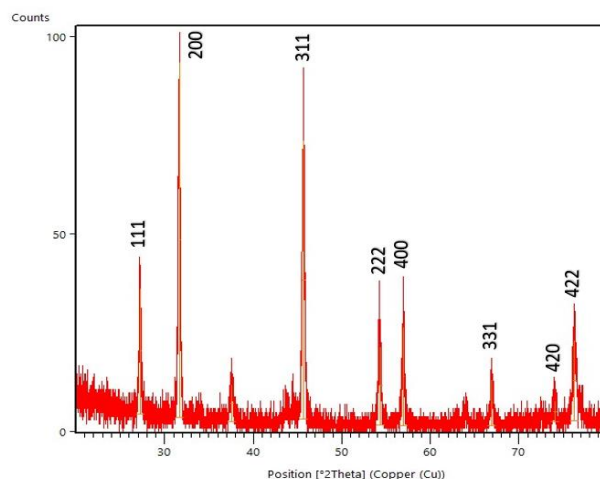


Figure 6: FTIR spectra of bacteriogenic silver nanoparticles.

### XRD, SEM and EDX

XRD technique was employed to validate the crystallin-nature of the harvested nanoparticles. The XRD pattern seen in figure (7) exhibits 8 sharp peaks equivalent to the planes of face-centered cubic crystals of silver-nanoparticles. Standard data provided by the international center for diffraction data (ICDD) (JCPDS no. 98-005-6538) was considered to support these findings. The intense distinct peaks are believed to result from the capping

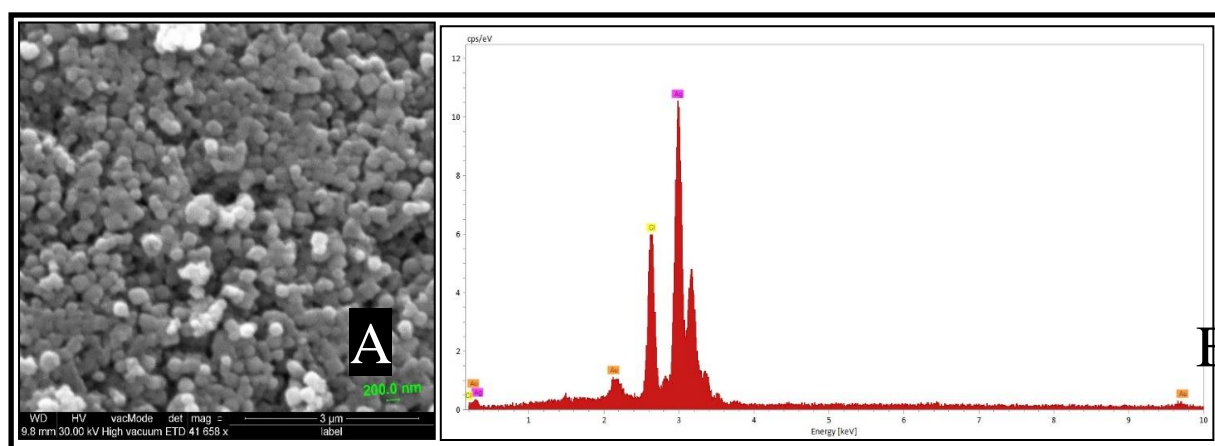
agents that aided in the stabilization of the green nanoparticles (49). Furthermore, with aid of XRD data, we managed to calculate an average crystallite size equivalent to 28.32 nm via the Dybe-Scherer equation ( $D = K\lambda / \beta \cos\theta$ ) (22). Average crystallite size in this study was much smaller compared to previous reports that made use of Scherrer formula to calculate crystallite size (50, 51).



**Figure 7: X-ray diffraction spectra of bacteriogenic silver nanoparticles.**

SEM instrument was utilized to study the surface morphology and size of PA-AgNPs which appeared to be spherically shaped and polydispersed with a size range of 45nm to 50 nm (Figure 8A). Our findings reveal smaller particle sizes and better dispersity compared to (47) where majority of nanoparticles had size range of 50 to 100nm. According to (33), although green methods are biocompatible and safe, here a number of factors such as amino acids, cofactors, enzymes, and media components interact with and reduce the silver-ions hence resulting in polydispersity

bacteriogenic nano-silvers. Moreover, the elemental composition of the harvested AgNPs was studied via the EDX technique. The absorption peak observed at 3keV in figure (8B) validates the biosynthesis of AgNPs as stated by (52). The elemental weight of silver and chlorine were deemed to be 84.15% and 15.85% respectively indicating a higher abundance of silver. According to (53), the chlorine peak observed could be attributed to the radiations emitted from proteins or enzymes within the cell-free supernatant.



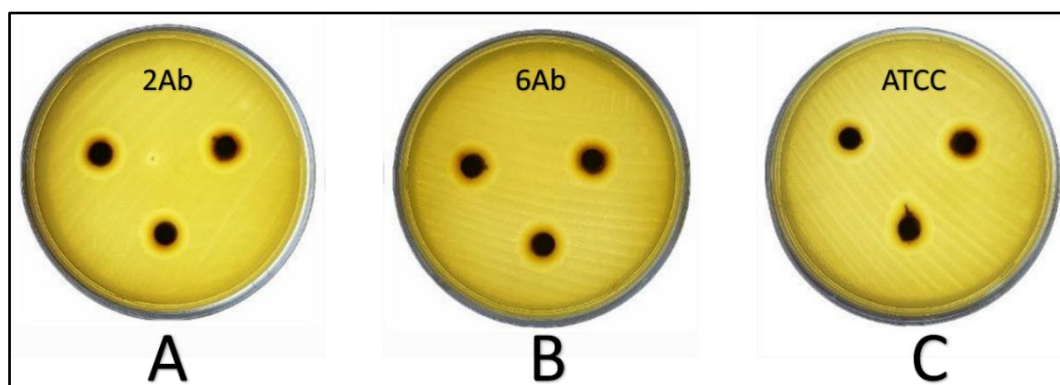
**Figure 8: A) SEM photomicrograph of green synthesized AgNPs B) EDX data profile of bacteriogenic AgNPs**

### Screening of antibacterial effect of AgNPs

An ATCC strain along with an MDR and XDR isolates were selected to evaluate the inhibitory potency of the obtained AgNPs via the agar-well diffusion technique.

Three biological replicates were considered and the mean of the clear zones

established around the wells was calculated. Exposure to 20mg/ml of the harvested AgNPs led to the formation of pronounced inhibition zones with a mean diameter of 16.3mm in the tested isolates after incubation at 37 °C overnight as seen in figure (9).



**Figure 9: Antibacterial effect of AgNPs against A) XDR, B) MDR, C) ATCC.**

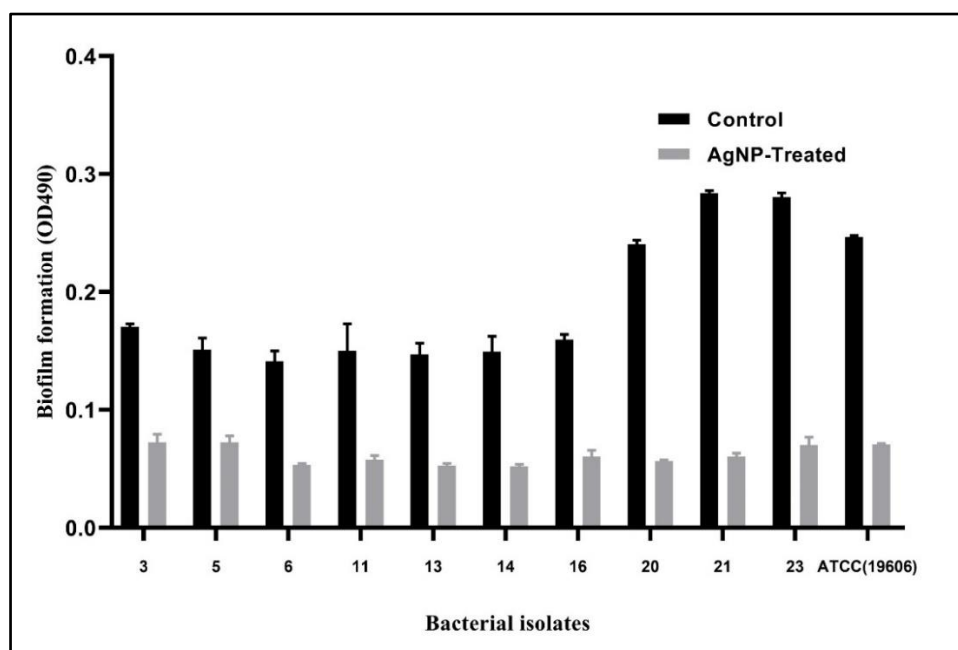
### Antibiofilm activity of bacteriogenic silver nanoparticles at Sub-MIC

MIC values for the bio-fabricated silver nanoparticles were first unmasked to assess their ability to reduce biofilm development in the tested isolates at Sub-

MIC values. In this respect, an ATCC strain along with 10 most potent biofilm-developers were selected and the results revealed MIC range from 10-20 mg/ml along with corresponding Sub-MIC values of 5-10 mg/ml as observed in table (1).

**Table 1: Minimal inhibitory concentrations (MICs) and Sub-MICs values of bacteriogenic AgNPs against MDR *A. baumannii*.**

Bacterial isolates	MIC (mg/ml)	Sub- MIC (mg/ml)
1	10	5
2	20	10
3	20	10
4	20	10
5	20	10
6	10	5
7	10	5
8	20	10
9	20	10
10	20	10
ATCC (19606)	20	10



**Figure 10: Quantitative data of *A. baumannii* biofilm depletion by Sub-MIC of bacteriogenic AgNPs. Data are depicted as mean $\pm$ SE. complete data are statistically significant at  $P < 0.0001$ .**

Antibiofilm activity of the bio-fabricated nano-slivers at Sub-MIC values was tested via microplate technique and the results implied that subjection to the bacteriogenic AgNPs decreased biofilm-production ability of the tested isolates significantly as demonstrated in figure (10). The ability to penetrate the exopolymeric layer (EPS) along with depletion of ATP, ROS production, cell-membrane disruption, and interaction with extracellular DNA (eDNA), proteins, and lipids represent the antibacterial mechanism of AgNPs that is also believed to be associated with less bacterial resistance since the bacteria must develop several mutations at once to conquer AgNPs effect (15, 16). Moreover, unlike antibiotics, AgNPs can penetrate into deeper layers of the biofilm due to their minute sizes suggesting their efficiency for biofilm eradication (33). According to (54), the expression of various virulence determinants including biofilms are under the control of quorum sensing (Q) machinery. Therefore interference with this system might lower

their biofilm formation potency and increase their susceptibility without enforcing evolutionary tension (55, 56). Henceforth, with the remarkable antibacterial and antibiofilm results obtained in our study, we suggest employing bacteriogenic AgNPs as a possible quorum quenching agent against drug-resistant bacteria such as *A. baumannii* which might provide an avenue to overcome drug resistance crisis in the post-antibiotic era.

## Conclusions

Experimental data of this study reinforce employing *P. aeruginosa* efficiently for bio-fabrication of nano-sized silver in an economical green manner. All of our clinical isolates produced biofilms at various extents and majority exhibited high resistance to the tested antibiotics. Isolates exposed to the harvested AgNPs portrayed marked inhibition zones and their biofilm formation ability was significantly reduced at SIC values. To our knowledge this is the first report regarding antibacterial and

antibiofilm effect of AgNP synthesized via *P. aeruginosa* against *A. baumannii*. Furthermore, since biofilms and various virulence factors are controlled by QS we recommend studying anti-QS activity of green AgNPs in drug-resistant pathogens such as *A. baumannii* which might serve as a new avenue to conquer drug-resistance catastrophe.

## References

- 1- Boucher HW. Bad Bugs, No Drugs 2002-2020: progress, challenges, and call to action. Transactions of the American Clinical and Climatological Association, 2020;131:65. 2020;131:65-71.
- 2- Ventola CL. The antibiotic resistance crisis: part 1: causes and threats. Pharmacy and Therapeutics. 2015;40(4):277-83.
- 3- Laws M, shaaban A, Rahman KM. Antibiotic resistance breakers: current approaches and future directions. FEMS Microbiology Reviews, 2019;43(5):490-516.
- 4- Uddin TM, Chakraborty AJ, Khusro A, Zidan Bmrm, Mitra S, Emran TB, et al. Antibiotic resistance in microbes: history, mechanisms, therapeutic strategies and future prospects. Journal of Infection and Public Health. 2021;14(12):1750-66.
- 5- Aslam B, Khurshid M, Arshad MI, Muzammil S, Rasool M, Yasmeen N, et al. Antibiotic resistance: one health one world outlook. Frontiers in Cellular and Infection Microbiology, 2021;11:771510.
- 6- WHO. New report calls for urgent action to avert antimicrobial resistance crisis. World Health Organization. 2019.
- 7- Hamed S.L. Detection of insertion sequence isab1 among clinical isolates of Carbapenems-resistant *Acinetobacter baumannii*. Al Mustansiriyah Journal of Pharmaceutical Sciences. 2014;14(1):128-35.
- 8- Lee CR, Lee JH, Park M, Park KS, Bae IK, Kim YB, et al. Biology of *Acinetobacter baumannii*: pathogenesis, antibiotic resistance mechanisms, and prospective treatment options. Frontiers in Cellular and Infection Microbiology, 2017;7, 55.
- 9- Singh R, Vora J, Nadhe SB, Wadhvani Sa, Shedbalkar UU, Chopade Ba. Antibacterial activities of bacteriogenic silver nanoparticles against nosocomial *Acinetobacter baumannii*. Journal of Nanoscience and Nanotechnology. 2018;18(6):3806-15.
- 10- Havenga B, Reyneke B, Waso-Reyneke M, Ndlovu T, Khan S, Khan W. Biological control of *Acinetobacter baumannii*: in vitro and in vivo activity, limitations, and combination therapies. Microorganisms. 2022;10(5).
- 11- Sarshar M, Behzadi P, Scribano D, Palamara At, Ambrosi C. *Acinetobacter baumannii*: an ancient commensal with weapons of a pathogen. Pathogens. 2021;10(4).
- 12- CDC. Antibiotic resistance threats in the united states. CDC, 2019.
- 13- Ayoub Moubareck C, Hammoudi Halat D. Insights into *Acinetobacter baumannii*: a review of microbiological, virulence, and resistance traits in a threatening nosocomial pathogen. Antibiotics (basel). 2020;9(3).
- 14- Upmanyu K, Haq QR, Singh R. Factors mediating *Acinetobacter baumannii* biofilm formation: opportunities for developing therapeutics. Current Research in Microbial Sciences. 2022;3:100131.
- 15- Singh R, Nadhe S, Wadhvani S, Shedbalkar U, Chopade BA. Nanoparticles for control of biofilms of *Acinetobacter* species. Materials (Basel). 2016;9(5).
- 16- Roy S, Chowdhury G, Mukhopadhyay AK, Dutta S, Basu S. Convergence of biofilm formation and antibiotic resistance in *Acinetobacter baumannii*

- infection. *Frontiers in Medicine*, 2022;9:793615.
- 17- Morris FC, Dexter C, Kostoulis X, Uddin MI, Peleg AY. The mechanisms of disease caused by *acinetobacter baumannii*. *Frontiers in Microbiology*. 2019;10:1601.
- 18- Salih T, Salih HA. In silico design and molecular docking studies of carbapenem analogues targeting *acinetobacter baumannii* pbp1a receptor. *Al Mustansiriyah Journal of Pharmaceutical Sciences*. 2020;20(3):35-50.
- 19- Jalali Y, Jalali M, Payer J. *Acinetobacter baumannii*: emergence of a superbug, past, present, and future. In: Guillermo T-I, editor. *The Global Antimicrobial Resistance Epidemic*. Rijeka: IntechOpen; 2022. Ch. 13.
- 20- Lahiri D, Nag M, Sheikh HI, Sarkar T, Edinur HA, Pati S, et al. Microbiologically-synthesized nanoparticles and their role in silencing the biofilm signaling cascade. *Frontiers in Microbiology*. 2021;12.
- 21- Mateo EM, Jiménez M. Silver nanoparticle-based therapy: can it be useful to combat multi-drug resistant bacteria? *Antibiotics (basel)*. 2022;11(9).
- 22- Almatroudi a. Silver nanoparticles: synthesis, characterisation and biomedical applications. *Open Life Science*. 2020;15(1):819-39.
- 23- Busi Ss, Rajkumari J. Chapter 15 - microbially synthesized nanoparticles as next generation antimicrobials: scope and applications. In: grumezescu am, editor. *Nanoparticles in pharmacotherapy*: william andrew publishing; 2019. P. 485-524.
- 24- Mukherjee S, Nethi SK. Biological synthesis of nanoparticles using bacteria. In: Panpatte DG, Jhala YK, editors. *Nanotechnology for agriculture: advances for sustainable agriculture*. Singapore: Springer Singapore; 2019. P. 37-51.
- 25- Gondil VS, Kalaiyaran T, Bharti VK, Chhibber S. Antibiofilm potential of seabuckthorn silver nanoparticles against *Pseudomonas aeruginosa*. *3 Biotech*. 2019;9(11):402.
- 26- Swidan NS, Hashem YA, Elkhatib WF, Yassien MA. Antibiofilm activity of green synthesized silver nanoparticles against biofilm associated enterococcal urinary pathogens. *Scientific Reports*. 2022;12(1):3869.
- 27- Dias DE Emery B, Zottis Chitolina G, Qadir MI, Quedi Furian T, Apellanis Borges K, De Souza Moraes HI, et al. Antimicrobial and antibiofilm activity of silver nanoparticles against *salmonella enteritidis*. *Brazilian Journal of Microbiology*. 2022.
- 28- Ahamad I, Bano F, Anwer R, Srivastava P, Kumar R, Fatma T. Antibiofilm activities of biogenic silver nanoparticles against *Candida albicans*. *Frontiers in Microbiology*. 2021;12:741493.
- 29- Tille PM. *Bailey & Scott's Diagnostic Microbiology*, 15th edition. 2021:1184.
- 30- Wayne p. Performance standards for antimicrobial susceptibility testing, 30th ed. CLSI, 2020.
- 31- Limban C, Marutescu L, Chifiriuc MC. Synthesis, spectroscopic properties and antipathogenic activity of new thiourea derivatives. *Molecules*. 2011;16(9):7593-607.
- 32- Nirmala C, Sridevi M. Characterization, antimicrobial and antioxidant evaluation of biofabricated silver nanoparticles from endophytic *pantoea anthophila*. *Journal of Inorganic and Organometallic Polymers and Materials*. 2021;31(9):3711-25.
- 33- Singh R, Shedbalkar UU, Wadhwani SA, Chopade BA. Bacteriogenic silver nanoparticles: synthesis, mechanism, and applications. *Applied Microbiology and Biotechnology*. 2015;99(11):4579-93.
- 34- Hetta HF, Al-kadmy IM, Kkhazal SS, Abbas S, Suhail A, El-Mokhtar MA, et

- al. Antibiofilm and antivirulence potential of silver nanoparticles against multidrug-resistant *Acinetobacter baumannii*. *Scientific Reports*. 2021;11(1):10751.
- 35- CLSI. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard. 10th ed. 2018.
- 36- Ahmed AA, Salih FA. Quercus infectoria gall extracts reduce quorum sensing-controlled virulence factors production and biofilm formation in *Pseudomonas aeruginosa* recovered from burn wounds. *BMC Complementary and Alternative Medicine*. 2019;19(1):177.
- 37- Thirapanmethee K, Srisiri-A-Nun T, Hongsaitong J, Montakantikul P, Khuntayaporn P, Chomnawang M. Prevalence of oxa-type  $\beta$ -lactamase genes among carbapenem-resistant *Acinetobacter baumannii* clinical isolates in thailand. *Antibiotics*. 2020;9:864.
- 38- Muhsin SS, Bakir WA, Sabbah MA. Identification and sequencing of ISAb2 of *Acinetobacter baumannii* isolated from baghdad hospitals. *Mustansiriya Medical Journal*, 2022, 1;21(1):30.
- 39- Li YJ, Pan CZ, Fang CQ, Zhao ZX, Chen HL, Guo PH, et al. Pneumonia caused by extensive drug-resistant *Acinetobacter baumannii* among hospitalized patients: genetic relationships, risk factors and mortality. *Bmc Infectious Diseases*. 2017;17(1):371.
- 40- Buchhorn DE Freitas S, hartwig DD. Promising targets for immunotherapeutic approaches against *Acinetobacter baumannii*. *Microbial Pathogenesis*. 2022;173:105855.
- 41- Vázquez-lópez R Solano-Gálvez SG, Juárez Vignon-Whaley JJ, Abello Vaamonde JA, Padró Alonzo LA, Rivera Reséndiz A, et al. *Acinetobacter baumannii* resistance: a real challenge for clinicians. *Antibiotics* (Basel). 2020;9(4).
- 42- Maspi H, Mahmoodzadeh Hosseini H, Amin M, Imani Fooladi AA. High prevalence of extensively drug-resistant and metallo beta-lactamase-producing clinical *Acinetobacter baumannii* in iran. *Microbial Pathogenesis*. 2016;98:155-9.
- 43- Hall CW, Mah TF. Molecular mechanisms of biofilm-based antibiotic resistance and tolerance in pathogenic bacteria. *FEMS in Microbiology Reviews*. 2017;41(3):276-301.
- 44- Haji SH, Ali FA, Aka ST. Synergistic antibacterial activity of silver nanoparticles biosynthesized by carbapenem-resistant Gram-negative bacilli. *Scientific Reports*, 2022, 9;12(1):15254.
- 45- He R, Qian X, Yin J, Zhu Z-Kjjomc. Preparation of polychrome silver nanoparticles in different solvents. *Journal of Materials Chemistry*. 2002;12(12):3783-6.
- 46- Bhatt D, Gupta E, Kaushik S, Srivastava VK, Saxena J, Jyoti A. Bio-fabrication of silver nanoparticles by *Pseudomonas aeruginosa*: optimisation and antibacterial activity against selected waterborne human pathogens. *IET nanobiotechnology*. 2018;12(7):981-6.
- 47- Peiris MK, Gunasekara CP, Jayaweera PM, Arachchi ND, Fernando N. Biosynthesized silver nanoparticles: are they effective antimicrobials? *Memórias do Instituto Oswaldo Cruz*.. 2017;112(8):537-43.
- 48- Jain as, pawar ps, sarkar a, junnuthula v, dyawanapelly s. Bionanofactories for green synthesis of silver nanoparticles: toward antimicrobial applications. *Intenational Journal of Molecular Sciences*. 2021;22(21).
- 49- Ahmed FY, Farghaly Aly U, Abd El-Baky RM, Waly NG. Comparative study of antibacterial effects of titanium dioxide nanoparticles alone and in combination with antibiotics on

- mdr pseudomonas aeruginosa strains. International Journal of Nanomedicine. 2020.
- 50- Sobha K, Dmp P, Anantha R, Verma M, Surendranath K. Evaluation of therapeutic potential of the silver/silver chloride nanoparticles synthesized with the aqueous leaf extract of rumex acetosa open. Scientific Reports. 2017;7.
- 51- Abootalebi s, mousavi sm, hashemi sa, shorafa e, omidifar n, gholami a. Antibacterial effect of green synthesized silver nanoparticles using ferula assafoetida against acinetobacter baumannii isolated from the hospital and their cytotoxicity on the human cell line. Journal of Nanomaterials. 2021;2021.
- 52- Ibrahim E, Fouad H, Zhang M, Zhang Y, Qiu W, Yan C, et al. Biosynthesis of silver nanoparticles using endophytic bacteria and their role in inhibition of rice pathogenic bacteria and plant growth promotion. RSC Advances. 2019;9(50):29293-9.
- 53- Kumar CG, Mamidyala SK. Extracellular synthesis of silver nanoparticles using culture supernatant of pseudomonas aeruginosa. Colloids and Surfaces B: Biointerfaces. 2011;84(2):462-6.
- 54- Zhong S, He S. Quorum sensing inhibition or quenching in Acinetobacter baumannii: the novel therapeutic strategies for new drug development. Frontiers in Microbiology, 2021;12:558003.
- 55- Santajit S, Sookrung N, Indrawattana N. Quorum sensing in escape bugs: a target for combating antimicrobial resistance and bacterial virulence. Biology (Basel). 2022;11(10).
- 56- Sionov RV, Steinberg D. Targeting the holy triangle of quorum sensing, biofilm formation, and antibiotic resistance in pathogenic bacteria. Microorganisms, 2022; 10(6).