Iraqi JMS

Published by Al-Nahrain College of Medicine
P-ISSN 1681-6579
E-ISSN 2224-4719
Email: iraqijms@colmed.nahrainuniv.edu.iq
http://www.colmed-alnahrain.edu.iq
http://www.iraqijms.net
Iraqi JMS 2024; Vol. 22(1)

The Effect of Selenium Nanoparticles on the Expression of MexB Gene of *Pseudomonas aeruginosa* Isolated from Wound and Burn Infections

Maryam F. Salman[1] *BSc*, **Nada H. A. L. Al-Mudallal**[1] *PhD*, **Mais E. Ahmed**[2] *PhD*

[1]Dept. of Medical Microbiology, College of Medicine, Al-Iraqia University, Baghdad, Iraq, [2]Dept. of Biology, College of Science, University of Baghdad, Baghdad, Iraq

Abstract

Background The World Health Organization lists the *Pseudomonas aeruginosa* (*P. aeruginosa*) bacteria as a major antibiotic-resistant priority pathogen, which function as an opportunistic pathogen and causes nosocomial infections.

Objective To biosynthesize selenium nanoparticles (SeNP) using bacteriocin from *Acinetobacter bumannii* (*A. bumannii*) and to evaluate the effect on the expression of MexB gene that is responsible for the multidrug resistance (MDR) in *P. aeruginosa*.

Methods Using an agar well diffusion assay, the first screening process evaluated the effect of bacteriocin-like inhibitory substance (BLIS) derived from *A. baumannii* on MDR *P. aeruginosa*. SeNP were synthesized biologically then analyzed and characterized using several techniques. The biosynthesized nanoparticles were identified, and the chemical and physical characteristics of the final product were determined, using atomic force microscope (AFM), scanning electron microscope (FESEM), energy dispersive X-ray (EDX), X-ray diffraction (XRD), and UV visible spectrometry.

Results The findings revealed a 24 mm substantial inhibitory zone of peptone water was shown to be the most appropriate supply of nitrogen, and glucose to be the best carbon source under the ideal circumstances for the synthesis of bacteriocin-like BLIS. The hexagonal structure was confirmed by XRD, and the dispersion and roughness of the nanoparticles were shown by AFM investigation revealed the 81.23 nm size range. MexB gene were found in each of the nine strains. The effect of the SeNP on the expression of the MexB gene demonstrated a considerable downregulation of *Mex*B expression in these isolates. This can be a useful antibacterial agent against multiple drug resistance *P. aeruginosa* infection.

Conclusion SeNP showed a major down-regulation in *Mex*B expression in *P. aeruginosa* isolates.

Keywords BLIS, Sodium selenite, SeNps, MexB gene

Citation Salman MF, Al-Mudallal NHAL Ahmed ME. The effect of selenium nanoparticles on the expression of MexB gene of *Pseudomonas aeruginosa* Isolated from wound and burn infections. Iraqi JMS. 2024; 22(1): 79-92. doi: 10.22578/IJMS.22.1.10

List of abbreviations: *A. baumannii* = *Acinetobacter bumannii*, AFM = Atomic force microscope, AWD = Agar well diffusion, BHIB = Brain heart infusion broth, BLIS = Bacteriocin-like inhibitory substance, EDX = Energy dispersive X-ray, MDR = Multiple drug resistance, P. aeruginosa = Pseudomonas aeruginosa, SEM = Scanning electron microscope, SeNP = Selenium nanoparticles, XRD = X-ray diffraction

Introduction

Microbial infection of severe burns and wounds is a major medical problem. Of the infections caused by bacteria that can colonize such injuries, *Pseudomonas aeruginosa* (*P. aeruginosa*) is one of the most

severe, putting a lot of time and effort into the recovery of burn patients or even resulting in their death. Moreover, consumed wound exudates (BWEs) stimulate the declaration of destructiveness factors in *P. aeruginosa* [1]. *P. aeruginosa* is impervious to antimicrobials through different systems; for example, chromosomal components of anti-infection opposition prompted efflux siphon frameworks, have been accounted for in the film of this microorganism [2]. The efflux pump *Mex*-type multidrug resistance is one of 12 different types of multidrug leakage pumps that *P. aeruginosa* may express. Five named *Mex*AB-oprM, *Mex*XYoprM, *Mex*EF-oprN, *Mex*CD-oprT, and *Mex*JK-oprM are the main variables of protection from anti-infection agents [3]. The MexB gene is an essential component of the *mex*AB-oprM efflux pump system, which is involved in the active transport of antimicrobial substances out of the cell in *P. aeruginosa*. *Acinetobacter bumannii* (*A. baumannii*) has become one of the most successful pathogens in modern healthcare because of its surprising ability to gain antibiotic resistance. Several strains of *A. baumannii* are extremely resistant to most clinically available antibiotics [4]. Bacteriocin is a ribosomally synthesized proteins or short polypeptides that have antibacterial activity against those closely related to the producing strain or other bacteria [5]. Application of bacterocin in many felids involves using *Salmonella spp*. bacteria isolated from various food products to produce bacteriocin and studying the effectiveness of crude bacteriocin against Gram positive and negative bacterial isolates from tap water in various parts of Basra, Iraq [6]. Any technology that is based on nanoscale materials and has several real-world applications is referred to as nanotechnology. It includes the creation and application of physical, chemical, and biological systems that use submicron-sized materials, ranging from individual molecules to individual atoms, into nanoscale materials. According to Nasrollahzadeh et al. (2019) [7], technology meets our needs and finds solutions to global problems. It covers a wide range of applications in medicine, pharmacology, agriculture, and other fields [8]. The green synthesis of selenium nanoparticles (SeNP) has attracted a lot of attention due to its promotion of alternative, long-lasting, safer, eco-friendly methods, less toxic and large-scale [9]. Nanoparticales of focus synergistic bacterocin are produced by certain archaea and bacteria to prevent the growth of related or similar bacterial strains. These compounds' ability to combat pathogenic and decaying microorganisms through antimicrobial action validates their potential for use in biotechnology [10]. Green synthesis of SeNP in this study done by Sodium selenite salt (Na_2SeO_3) was added to the volume of *A. bumannii* bacteriocin-like inhibitory substance bacteriocin-like inhibitory substance (BLIS) solution (baumanniicin).

P. aeruginosa infections are antibiotic-resistant generally difficult to treat and the number of antibiotics available to treat them is limited. The increased resistance of this bacteria to antibiotics, particularly in its multidrug form, leads to numerous therapeutic problems. At the moment nanoparticles are considered a suitable alternative to antibiotics. This study was aimed to investigate the effect of SeNP on the expression of MexB gene in *P. aeruginosa*, which has multifactorial antibiotic resistance that includes acquired, adaptive, and inherent resistance mechanisms.

A. bumannii and P. aeruginosa isolation and identification

In this study, a total of 200 burn and wound infection samples were collected during period between September 2023 to December 2023. Samples were collected from Medical City and the Martyr Gazi Al-Hariry Hospital in Baghdad.

One hundred and fifty specimens were cultured on *Acinetobacter* agar (HiMedia, India) and 50 specimens were cultured on Cetrimide agar (HiMedia, India) then incubated for 24 hr at 37°C under aerobic conditions, suspected colonies were identified morphologically and

biochemically by oxidase test, confirmed *A. baumannii* by using the Vitek 2 compact system (BioMe`rieux/France), following the instructions provided by the manufacturer [11].

A. bumannii and _P. aeruginosa_ antibiotic sensitivity test

To determine the values of the minimum inhibitory concentration (MIC) for Gram negative isolates of *P. aeruginosa*, Antibiotic Sensitivity Test Card (ASTC) AST-N222 Supplement, was performed on each and every isolate that was confirmed to have been detected by the VITEK-2 compact system. ASTC has between 18 and 20 antibiotics distributed across 64 holes. Following the growth of the bacteria, the device recorded changes in turbidity. This was done in accordance with BioMerieux's instructions. Isolates were categorized into three groups based on the results of a sensitivity test; multidrug-resistant (MDR), extremely drug-resistant (XDR), and pan-drug-resistant (PDR). Ten *P. aeruginosa* MDR strain were chosen to detect of MexB gene and isolates of *P. aeruginosa* rename them from 1 to 10.

Biosynthesis of bacteriocin-like inhibitory substance BLIS:

Using the Agar Well Diffusion Assay (AWD) method, all *A. baumannii* isolates were screened for their inhibitory activity towards a series of indicator strains in order to identify the isolate that produced the highest amount of bacteriocin. Gram-positive and Gram-negative bacteria, as well as fungi, were among the indicator strains [12].

The following criteria were used to assess the isolates' bacteriocin production using the AWD method: The McFarland standard ($1.2*10^8$ colony forming unit (CFU)/ml) of an overnight culture of the producer isolate was added to tubes containing 10 ml of brain heart infusion broth (HiMedia, India). After that, tubes were incubated at 37°C for 24 hr. Following incubation, the cultured broth was centrifuged for 15 min. at 6000 rpm to extract the cell-free supernatant (CFS). Next, CFS was filtered using sterile 0.22 µm millipore filter paper. Using the AWD method, the CFS of each isolate was tested for the presence of bacteriocin as follows: 0.1 milliliters of growth overnight growth of indicator bacterium culture ($1.2*10^8$ CFU/ml) was spread over the Mueller Hinton agar plate surface for 24 hr. A cork borer was used to cut a circular well in the plate that measured 5 mm in diameter. Volume of 100 µl of CFS was then added to the wells, and the plate was incubated for another 24 hr [13].

Optimization of crud bacteriocin production

Synthesis of bacteriocins is optimized; numerous factors were examined to determine the ideal settings for high-level bacteriocin synthesis, and these are [14], determination of optimum pH of different pH values of 4, 6, 7, and 8 and determination of optimum incubation time in different durations (18, 24, 48, and 72 hr) at 37°C under aerobic conditions. And Effect of nitrogen source and carbon source (sugars) included (peptone water, yeast extract, lactose, and glucose) with 1% concentrations were used of each one of them added to brain heart infusion (BHI) broth.

Partial purification of bacteriocin-like inhibitory substance BLIS

Partial purification of BLIS was prepared via precipitation with ammonium sulfate at different concentrations, by gradually adding ammonium sulfate to the crude enzyme with continuous stirring on ice at different degrees of saturation then centrifuged at 6000 rpm for 20 min at 4°C. Ammonium sulfate precipitate obtained from previous step was dialyzed in a dialysis membranes (wide flat 34MM – MwCO 8000-14000D) cut off against potassium phosphate buffer PH 7 for 24 hr under cooling condition (4°C), then the enzyme activity and protein concentration were measured [15], and then estimation of protein concentration according to Bradford [16].

Biosynthesis of SeNP

For green synthesis of SeNP, 2 g of sodium selenite (Na_2SeO_3) was added to a volume of 10 ml of *A. baumannii* BLIS solution (baumanniicin) at concentration 10.65 µg/ml,

the flasks were incubated at 30°C to 35°C for 3 days, and each color change was recorded. After incubation, the reaction mixture was centrifuged at 6000 rpm for 25 min at 4°C to remove the supernatant. The supernatant was then replaced with deionized water and the centrifugation process was repeated three more times under the same conditions to remove the remaining supernatant. The collection of nanoparticles in the form of pellets was transferred to a hot air oven set at 120°C to evaporate all liquid contents. The dried powder was carefully collected and stored for further analysis [17].

Characterization of biosynthesized SeNP

The morphology and size of the SeNP were investigated. SeNP were measured at (200-600 nm) using a UV–Vis spectrophotometer (UV/Vis-1800, Shimadzu, Kyoto, Japan). Vibrational and structural characterization of SeNP were characterized using Fouriertransform infrared spectroscopy (FTIR, Bruker Co., Ettlingen, Germany). SeNP were performed by scanning the wavelength range of 400–4000 cm^{-1}. The images were taken with a scanning electron microscope (SEM) with a resolution of 500 nm (Hitachi s-3400N). Atomic force microscopy (AFM) was used to determine the size and surface morphology of SeNP [18]. Energy-dispersive X-ray analysis (EDX) analysis was used to identify the qualitative and quantitative state of elements that may be involved in the formation of nanoparticles [19]. Crystalline nature of SeNPs was measured by XRD (Shimadzu/Japan). Powdered sample slurry/smear slide mount for small sample amounts [20].

SeNP MIC against *P. aeruginosa* strains

The MIC of synthesized SeNP against *P. aeruginosa* strains were determined using broth microdilution method in culture broth via making serial dilutions (1000, 500, 250, 125 and 62.5 µg/ml) as follows:

1. One ml of stock solution (1000 mg/ml) of SeNP was prepared, transfer 100 µl of diluted test material to the first line of wells.
2. 100 µl of Müller Hinton broth each well from 1 to 10. Two-fold dilution by transferring 100 µl from the first to the 10^{th} well, well G were positive control and well H were used as negative control.
3. All well were inculcated with 10 µl of bacterial suspension adjusted to McFarland ($1.5*10^8$ CFU\ml) using densicheck except for the negative control.
4. The microtitre plate were incubated at 37°C for 24 hr. Then the growth was measured by determining OD450 using microliter plate reader.
5. Wells that appeared turbid are indicated of bacterial growth while wells that remained clear indicated no growth. The MIC of the SeNP is the lowest concentration that does not show any growth [21].

Detection of MexB gene

Genomic DNA was isolated from bacterial growth according to the protocol of ABIO pure extraction. Primer's sequence shown in table (1), PCR program shown in table (2) and real time PCR program shown in table (3).

Ethical statement

This research was approved by the Committee of Ethical Standards in College of Medicine, Al-Iraqia University. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee according to the document number FM.SA/308 dated December 25, 2023.

Table 1. Primer's sequence (These primers were supplied by Macrogen 226 Company in a lyophilized form) [22]

Primer Name	Sequence 5`-3`	Annealingtemp. (˚C)
*Mex*B-F	GTGTTCGGCTCGCAGTACTC	60
*Mex*B-R	AACCGTCGGGATTGACCTTG	

Table 2. PCR Program

Steps	°C	m:s	Cycle
Initial Denaturation	95	05:00	1
Denaturation	95	00:30	30
Annealing	60	00:30	
Extension	72	00:30	
Final extension	72	07:00	1
Hold	10	10:00	

Table 3. Real-time PCR program

Steps	°C	m:s	Cycle
RT. Enzyme Activation	37	15:00	1
Initial Denaturation	95	05:00	
Denaturation	95	00:20	40
Annealing	60	00:20	
Extension	72	00:20	

Results

Isolation of *A. baumannii*

In this study, one hundred and fifty specimens from burns and wounds infections samples as shown in figure (1), were examined and only 44 isolates were identified *A. baumannii* as shown in table (4).

Isolation of *P. aeruginosa* strains

In this study, fifty specimens from burns and wounds infections samples as shown in figure (2), were examined and only 45 isolates were identified *P. aeruginosa* as shown in table (5).

Optimum conditions for bacteriocin production

The ideal settings for high-level bacteriocin synthesis, optimum pH value 4, optimum incubation time was 48 hours at 37°C under aerobic conditions. and optimum nitrogen source was peptone water while glucose optimum carbon source.

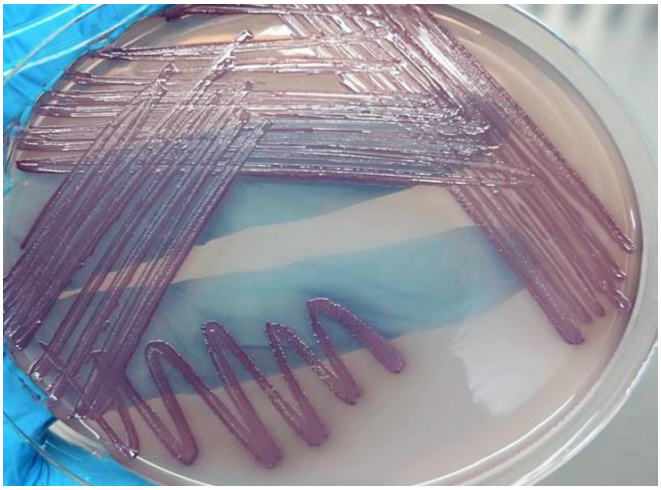

Figure 1. *A. baumannii* on Acinetobacter agar base

Table 4. Prevalence of *A. baumannii* isolates among clinical samples

Source of Samples	Other gram-negative bacteria Isolates No.	*A. baumannii* No. (%)
Burns	66	27 (61.36%)
Wounds	40	17 (38.63%)
Total	106 (70.66%)	44 (29.33%)

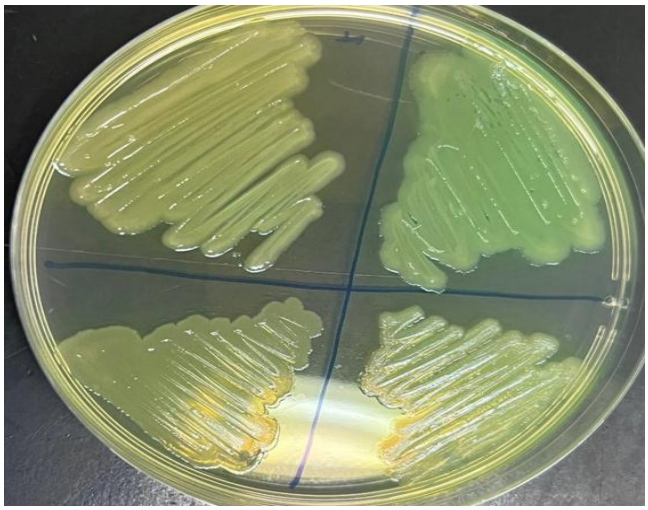

Figure 2. *P. aeruginosa* cultured on Cetrimide agar after 24 hr of Incubation at 37°C

Table 5. Prevalence of *P. aeruginosa* isolates among clinical samples

Source of Samples	Other *Pseudomonas spp*. Isolates No.	*P. aeruginosa* No. (%)
Burns	2	20 (44.44%)
Wounds	3	25 (55.55%)
Total	5 (10%)	45 (90%)

Ammonium sulfate precipitation and dialysis

Purification of bacteriocin-like inhibitory substance from *A. baumannii* was achieved by gradually add ammonium sulfate to the crude BLIS, stirring constantly on ice. It was found that this ratio gave proteins concentration is increased to 60% saturation. Proteins concentration was increased to reach 6.524 mg/ml compared with that of the crude extract 4.343 mg/ml. Then dialysis by dialysis membranes (wide flat 34MM – MwCO 8000-14000D) proteins concentration was increased to reach 10.65 mg/ml.

Biosynthesis of SeNP

SeNP were biosynthesized from bacteriocin-like inhibitory substance MDR *A. baumannii* in isolated from burn. It was considered the first time to use this bacterium in biosynthesis of SeNP. The formation of nanoparticles was investigated by color change of the reaction mixture from yellow to red color after 96 hr in dark room, after centrifuged at 6000 rpm for 25 min. The precipitate was shown red that means selenium reduction and production of SeNP.

Characterization of biosynthesized SeNP

Biosynthesized SeNP displayed a highest peak (absorption peak) at 200 nm to 800 nm [23] as shown in figure (3), AFM was used to identify and characterize nanoparticle size of SeNP which have an average diameter of 81.23 nm, the spherical shape of SeNP at 40000 magnification power and 3000 kv as shown in figure (4). The EDX spectra together with the major elemental peak at 22 keV that is unique to the se metal and the composition percentage as shown in figure (5).

SEM pictures showed that, with a diameter ranging from 34 to nm, it was basically uniformly spherical in appearance as evidenced in figure (6). The results showed the EDX spectra together with the major elemental peak at 22 keV that is unique to the se metal and the composition percentage. Other additional minor peaks for A. baumannii biomolecules were detected, as demonstrated in figure (7). As seen in Figure (8), FTIR analysis of the chemical bonds in the prepared SeNP was carried out by scanning in the 400–4000 cm-1 wavelength range.

SeNP minimum inhibitory concentration

The MIC of the SeNP is the lowest concentration that does not show any growth, 10 multidrug resistant strain of *P. aeruginosa* MIC was 500 µg/ml for all the ten isolates, so Sub-MIC was 250 µg/ml.

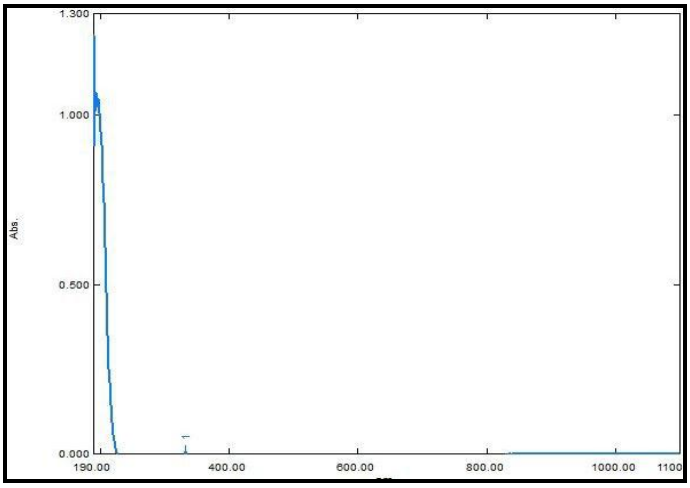

Figure 3. UV–Vis Spectrophotometry of synergistic SeNP [22]

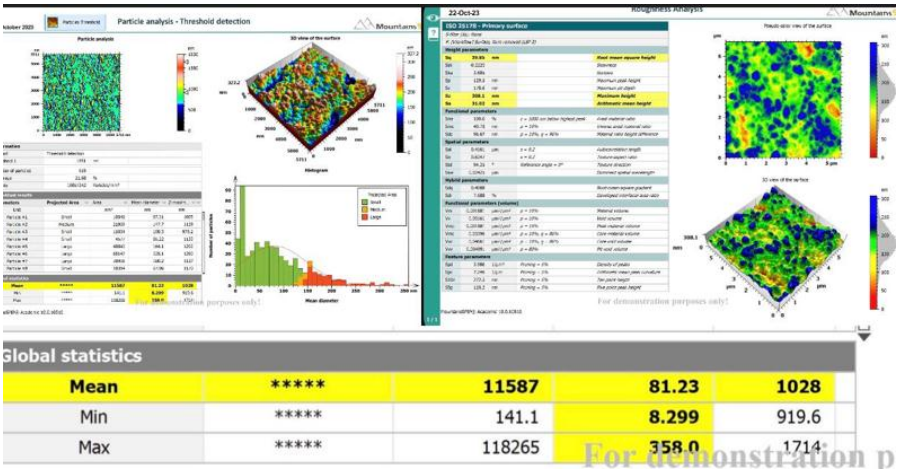

Figure 4. Atomic force microscopy (AFM) analysis

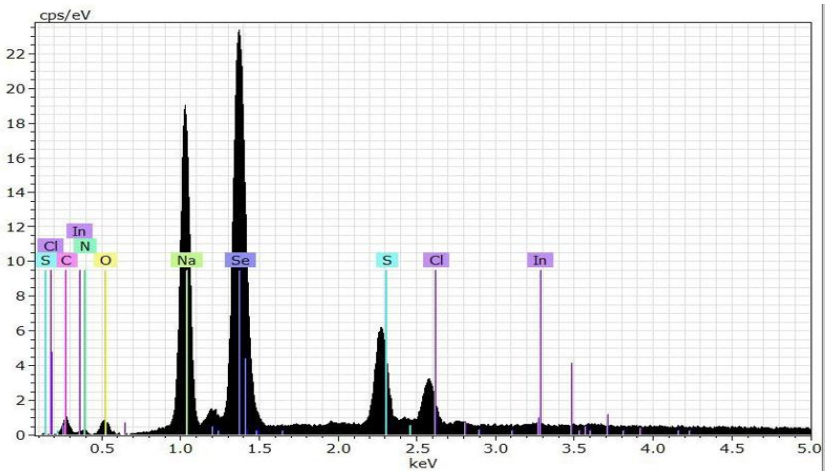

Figure 5. Energy dispersive X-ray spectrometry of SeNP

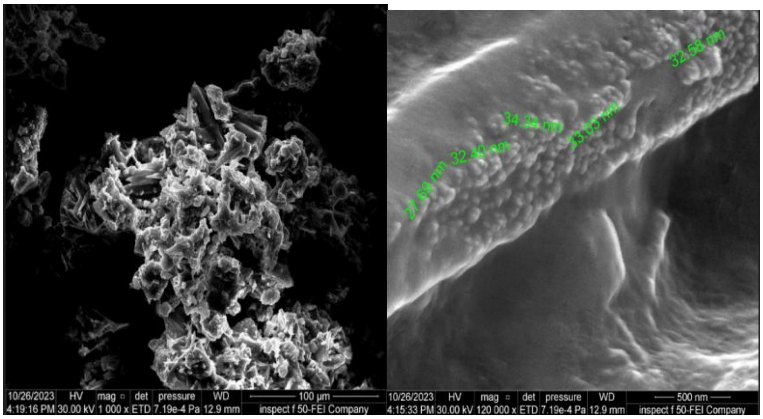

Figure 6. Field Emission Scanning Electron Microscope (FESEM)

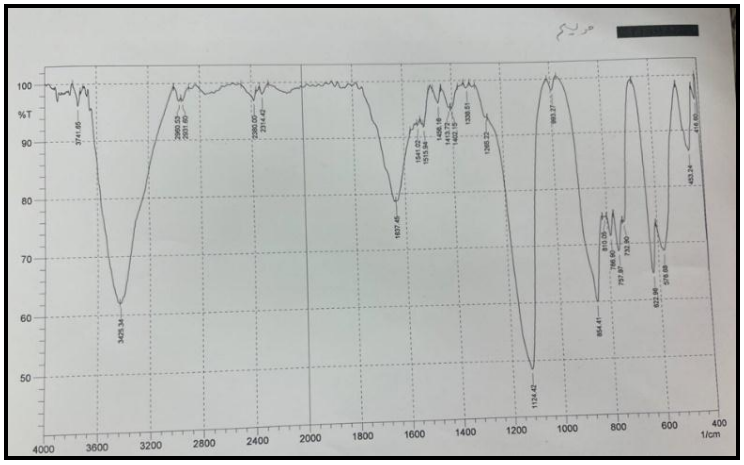

Figure 7. Fourier transform infrared (FTIR) spectroscopy

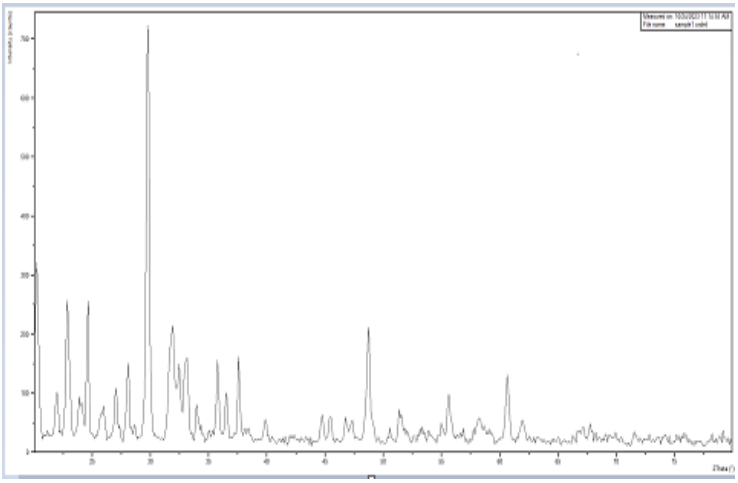

Figure 8. X-Ray Diffraction analysis of biosynthesized SeNP

Polymerase chain reaction (PCR) technique

The PCR results identified nine isolates of *P. aeruginosa* with the MexB gene, selected based on their MDR. The positive gene result was subsequently confirmed through electrophoresis on a 1.5% agarose gel stained with ethidium bromide, electrophoresed at 75 volts for 50 minutes, and visualized under an ultraviolet (UV) transilluminator. The present study revealed the presence of a sharp, singular, and non-dispersed 180 bp MexB gene band, which was clearly distinguished from the DNA ladder, as demonstrated in figure (9). Notably, there was no evidence of DNA degradation, as indicated by the absence of any smearing of the gene band.

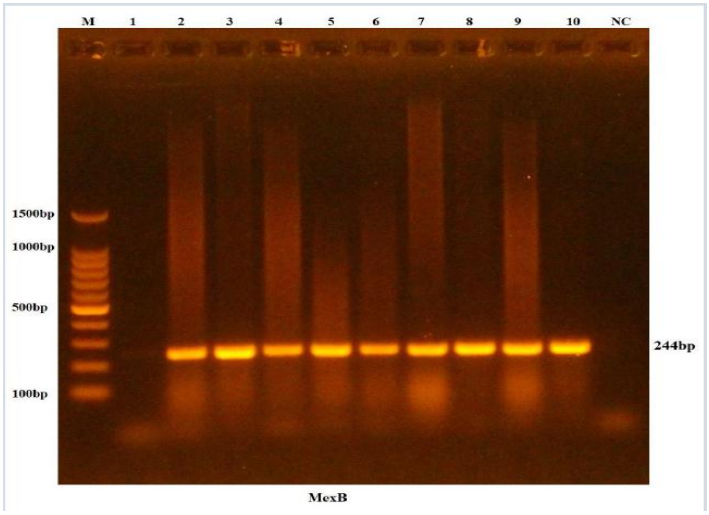

Figure 9. Pseudomonas aeruginosa bacterial strains were fractionated on 1.5% agarose gel electrophoresis and stained with Eth.Br. M: 100 bp ladder marker as a result of the MexB gene amplification. Z1-79 lanes have 244 bp PCR products in common

To estimate the effect oF SeNP at concentrations 250 µg/ml, two Real time PCR reveals a major down regulation in *Mex*B expression after the exposure to SeNP compared to normal gene expression in bacterial. Fold change in gene expression reveals that *Mex*B was down regulated in response to SeNP CFS in 5 isolates of *P. aeruginosa*, as entailed in tables (6 & 7). Mechanical exploration different antimicrobial substance was performed with loss of functional genes as demonstrated in figures (10 and 11).

Table 6. Gene expression in burn isolates before and after treatment with SeNP

samples Isolate From Burn	house keeping (fbp1)	mexB	DCT	DDCT	folding
2 Before treatment	21.67	16.16	-5.6	0	1
2 After treatment	19.76	15.14	-4.62	0.98	0.51
10 Before treatment	21.76	17.58	-4.17	0	1
10 After treatment	19.76	16.17	-3.59	0.59	0.67
7 Before treatment	19.15	18.65	-0.5	0	1
7 After treatment	19.61	21.16	1.55	2.04	0.24

Table 7. Gene expression in wound isolates before and after treatment with SeNP

samples Isolate From Wound	house keeping (fbp1)	mexB	DCT	DDCT	folding
3 Before treatment	27.67	27.08	-0.59	0	1
3 After treatment	24.68	22.65	-2.02	-1.44	2.71
5 Before treatment	21.76	17.07	-4.69	0	1
5 After treatment	19.76	15.15	-4.61	0.08	0.95
9 Before treatment	25.29	24.41	-0.89	0	1
9 After treatment	21.42	21.08	-0.35	0.54	0.69

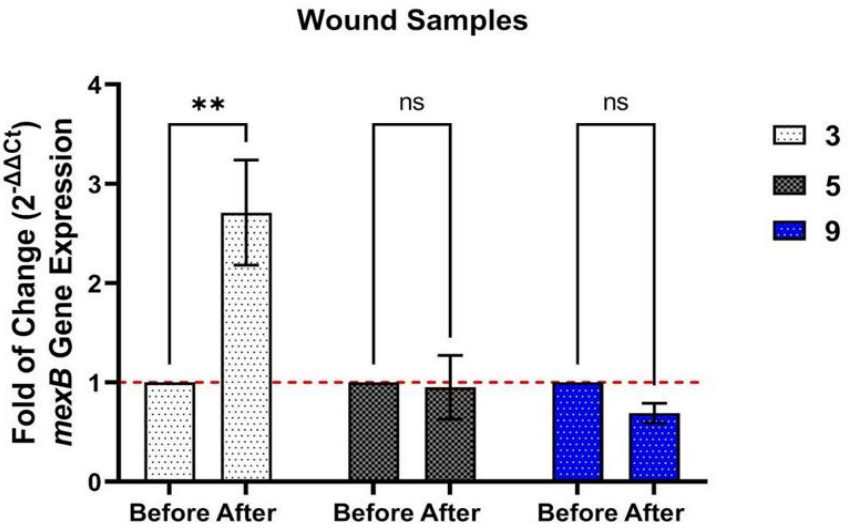

Figure 10. Mean (±SD) fold of change of mexB gene expression in *P. aeruginosa* (3,5,9) before and after treatment with SeNp compared with untreated cells: standard deviation, (n = 3) in wound isolates

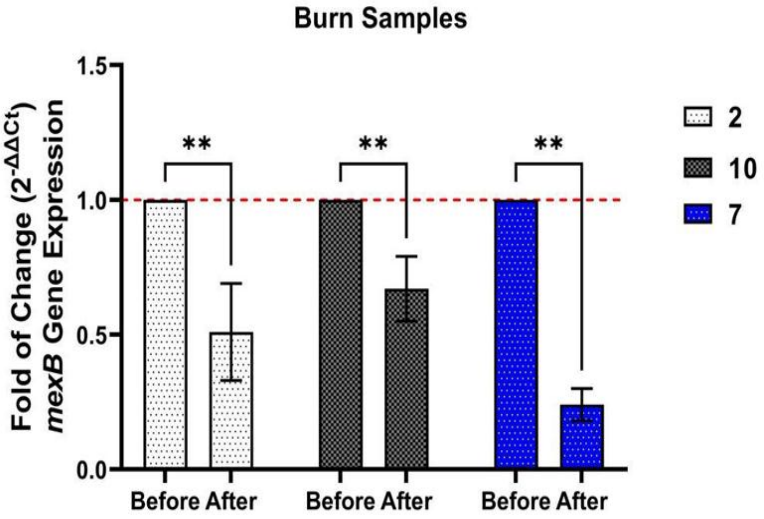

Figure 11. Mean (±SD) fold of change of mexB gene expression in *P. aeruginosa* (2,10,7) before and after treatment with SeNp compared with untreated cells: Standard deviation, (n = 3) in burn isolates

Discussion

The isolation of bacteria from wound infections agrees with [24]. *A. baumannii*, is a significant bacteria associated with infections of burns and wounds that are MDR. The production of biofilms complicates treatment using commercially available antibiotics. The present study involved the isolation of the *A. baumannii*-specific. Simile study by [25] produced numerous bacteriocin types that possess antibacterial properties against closely related species are produced by *Klebsiella pneumoniae*; nevertheless, the distribution of bacteriocins throughout the *Klebsiella* population has not been thoroughly documented in many investigations.

On the other hand, result agrees biosynthesis of SeNP with [26]; the surface of biogenic SeNP is crucial for the stability and capping of SeNP, as the FTIR study amply illustrated. Additionally, spectroscopic analysis employing FTIR, XRD, and Raman spectroscopy was used to characterize these SeNP demonstrated the particle size range of from 10 to 700 nm, its amorphous nature, stability, and oxidation state. According to biochemical research, selenite may be significantly reduced into elemental selenium by the membrane-bound reductase enzyme. As a result, Bacillus paramycoides strain MF-14 may be effectively used for bioremediation of environmental sites contaminated. The synthesized SeNP were studied for their crystalline structure, particle sized distribution, and morphology using a variety of techniques, including UV–Vis, X-ray diffraction, dynamic light scattering analyses, high resolution transmission electron microscopy, and FTIR. Additionally, the antioxidant and antibacterial properties in vitro were assessed. Strong antifungal and antibacterial properties were demonstrated by the SeNPs produced by the four strains against various human and phytopathogens [27]. Resistance to a variety of antibiotics is offered by efflux pumps. Regularly identifying *P. aeruginosa's* molecular pathways of resistance will aid in the fight against the global development of antibiotic resistance [28]. The result agrees with [29]; efflux pumps are crucial in the development of P. aeruginosa strains that are resistant to antibiotics. The current investigation sought to evaluate the expression of the efflux pumps *Mex*AB-OprM, *Mex*CD-OprJ, *Mex*EF-OprN, and *Mex*XY-OprM in strains of *P. aeruginosa* which were resistant to carbapenem and MDR bacteria that were recovered from clinical specimens. When looking at the effects of NPs on drug resistance in *P. aeruginosa*, several nanostructures with distinct antibacterial qualities were discovered. According to our study's findings, NPs can be a practical substitute for treating *P. aeruginosa's* microbiological resistance by obstructing flux pumps and preventing the growth of biofilms [30].

According to quantitative PCR was used to assess the antibacterial synergism of Ag–TSC NPs with ciprofloxacin against *P. aeruginosa* in addition to any potential effects of the NPs on the expression of the MexA and MexB genes. When combined with ciprofloxacin at a sub-MIC concentration, Ag-TSC NPs (concentration ≥32 µg/ml) exhibited synergistic effects, which successfully inhibited the growth of bacteria. When compared to the controls, the bacteria that were concurrently treated to ciprofloxacin and Ag-TSC NPs had lower expression of the mexA and mexB genes by 6.0 and 2.75 folds, respectively [31]. While results of [32] showed CuO NPs' antibacterial qualities and the expression of *mex*AB-oprM in *P. aeruginosa* clinical samples were assessed using the Microdilution Broth Method and real-time polymerase chain reaction analysis, respectively.

In conclusion, the findings showed that after being exposed to SeNP, burn isolates had a significant down-regulation in *Mex*B expression. Three *P. aeruginosa* isolates' fold change in gene expression indicated that *Mex*B was down-regulated in response to SeNP.

Following exposure to SeNPs, wound identifies a significant down-regulation in *Mex*B expression. *Mex*B was found to be down-regulated in two *P. aeruginosa* isolates and up-regulated in one isolate upon exposure to SeNP, according to the fold change in gene expression. These results imply that SeNP may inhibit the expression of the MexB gene, which

could result in the loss of genes essential to the efflux pump system.

Acknowledgement
None.

Author contribution
Salman: performed the clinical isolation of bacteria, prepared the nanoparticles and wrote the manuscript. Dr. Ahmed and Dr. Al-Mudallal designed and performed the experiments with making final revision of the manuscript.

Conflict of interest
None.

Funding
Self-funding.

References
1. Gonzalez MR, Fleuchot B, Lauciello L, et al. Effect of human burn wound exudate on *Pseudomonas aeruginosa* virulence. Msphere. 2016; 1(2), e00111-15. doi: https://doi.org/10.1128/msphere.00111-15.
2. Munita JM, Arias CA. Mechanisms of antibiotic resistance. Microbiol Spectr. 2016; 4(2). doi: 10.1128/microbiolspec.VMBF-0016-2015.
3. Goli HR, Nahaei MR, Rezaee MA, et al. Role of MexAB-OprM and MexXY-OprM efflux pumps and class 1 integrons in resistance to antibiotics in burn and Intensive Care Unit isolates of *Pseudomonas aeruginosa*. J Infect Public Health. 2018; 11(3): 364-72. doi: 10.1016/j.jiph.2017.09.016.
4. Lee CR, Lee JH, Park M, et al. Biology of *Acinetobacter baumannii*: Pathogenesis, antibiotic resistance mechanisms, and prospective treatment options. Front Cell Infect Microbiol. 2017; 7: 55. doi: 10.3389/fcimb.2017.00055.
5. Bindiya ES, Bhat SG. Marine bacteriocins: A review. J Bacteriol Mycol Open Access. 2016; 2(5): 140-7. doi: 10.15406/jbmoa.2016.02.00040.
6. Ahmed ME, Naser W, Hassoon HA. The study of the efficacy of bacteriocin isolated from the genus Salmonella and its role in treating Basra water pollution. Samarra J Pure App Sci. 2022; 4(3): 79-88.
7. Nasrollahzadeh M, Sajadi SM, Sajjadi M, et al. Chapter one: An introduction to nanotechnology. Interface Science and Technology; 2019; 28: 1-27. doi: https://doi.org/10.1016/B978-0-12-813586-0.00001-8
8. Sinha K, Ghosh J, Sil PC. 2- New pesticides: A cutting-edge view of contributions from nanotechnology for the development of sustainable agricultural pest control. In New Pesticides and Soil Sensors. 2017; 2017: 47-79. doi: https://doi.org/10.1016/B978-0-12-804299-1.00003-5.
9. Khan FS, Naseer M, Hayat M, et al. Transformers in vision: A survey. ACM computing surveys. ACM Computing Surveys. 2022; 54(10s): 1-41. doi: https://doi.org/10.1145/3505244.
10. Ahmed ME, Al-Awadi AQ, Abbas AF. Focus of synergistic bacteriocin-nanoparticles enhancing antimicrobial activity assay. Microbiol J. 2023; 6; 95-104. doi: https://doi.org/10.15407/microbiolj85.06.095.
11. Ahmed ME, Al-lam MQ, Abd Ali DM. Evaluation of antimicrobial activity of plants extract against bacterial pathogens isolated from urinary tract infection among males' patients. Al-Anbar Med J. 2021; 17(1): 20-4. doi: 10.33091/amj.2021.171060.
12. Samali B, Nemati S, Sharafi P, et al. Feasibility analysis of the use of rigid polyurethane foam in modular sandwich panels for rapid assembly structures. Int J GEOMATE. 2018; 15(51). 113-20. doi: https://doi.org/10.21660/2018.51.06166.
13. Ahmed ME, Ahmed ZM, Thamer A. The evolutionary effects of bacillin and S-pyocin bacteriocin and their effects on propionibacterium acnes and fungi. Biochem Cell Arch. 2022; 20(Supplement 2): 3645-9. doi: https://connectjournals.com/03896.2020.20.3645.
14. Ahmed ME, Mousa IS, Al-Halbosiy MMF, et al. The anti-Leishmaniasis activity of Purified Bacteriocin Staphylococci and Pyocin Isolated from Staphylococcus aureus and *Pseudomonas aeruginosa*. Iraqi J Sci. 2018; 59(2A): 645-53. doi: 10.24996/ijs.2018.59.2A.2.
15. Muunim HH, Al-Mossawei MT, Ahemd ME. The comparative study among the MRSAcin, nisin a and vancomycin, on biofilm formation by methicillin resistance *Staphylococcus aureus* isolated from food sources. Int J Drug Delivery Technol. 2019; 9(3): 176-81. doi: 10.25258/ijddt.9.3.31.
16. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976; 72: 248-54. doi: 10.1006/abio.1976.9999.
17. Faiq NH, Ahmed ME. Effect of biosynthesized zinc oxide nanoparticles on phenotypic and genotypic biofilm formation of *Proteus mirabilis*. Baghdad Sci J. 2024; 21(3): 0894. doi: https://doi.org/10.21123/bsj.2023.8067.
18. Mohammed LS, Saeed L. Effects of ZnO NPS on *Streptococcus pyogenes* in vivo. Ann Trop Med Public Health. 2020; 23(02): S452. doi: https://DOI:10.36295/ASRO.2020.23228
19. Kadhim ZH, Ahmed ME, Şimşek I. Biologically synthesized copper 506 nanoparticles from *S. epidermidis* on resistant *S. aureus* and cytotoxic assay. Bionatura. 2023; 8(1): 1-12. doi: http://dx.doi.org/10.21931/RB/CSS/2023.08.01.54
20. Ahmed ME, Kadhim AR. Alternative preservatives of a "Nisin A" with silver nanoparticles for bacteria isolation from the local food markets of Baghdad

City. Medico Legal Update. 2020. 20(4): 945-951. doi: https://doi.org/10.37506/mlu.v20i4.1946.

21. Lafta FM, Mohammed RK, Alhammer AH, et al. Cytotoxic potential of neem (*Azadirachta indica A. Juss*) oil. Trop J Nat Prod Res. 2023; 7(12): 5436-40. doi: http://www.doi.org/10.26538/tjnpr/v7i12.11.

22. Pourakbari M, Seidavi A, Asadpour L, et al. Probiotic level effects on growth performance, carcass traits, blood parameters, cecal microbiota, and immune response of broilers. An Acad Bras Cienc. 2016; 88(2): 1011-21. doi: 10.1590/0001-3765201620150071.

23. Dorri K, Modaresi F, Shakibaie MR, et al. Effect of gold nanoparticles on the expression of efflux pump mexA and mexB genes of *Pseudomonas aeruginosa* strains by quantitative real-time PCR. Pharmacia. 2022 69(1): 125-33. doi: https://doi.org/10.3897/pharmacia.69.e77608

24. Dehari D, Chaudhuri A, Kumar DN, et al. A bacteriophage microgel effectively treats the multidrug-resistant *Acinetobacter baumannii* bacterial infections in burn wounds. Pharmaceuticals (Basel). 2023; 16(7): 942. doi: 10.3390/ph16070942.

25. Le MN, Nguyen TH, Trinh VM, et al. Comprehensive analysis of bacteriocins produced by the hypermucoviscous *Klebsiella pneumoniae* species complex. Microbiol Spectr. 2023; 11(3): e0086323. doi: 10.1128/spectrum.00863-23.

26. Yadav P, Pandey S, Dubey SK. Selenite bioreduction with concomitant green synthesis of selenium nanoparticles by a selenite resistant EPS and siderophore producing terrestrial bacterium. Biometals. 20233 6(5): 1027-45. doi: 10.1007/s10534-023-00503-y.

27. Hussein HG, El-Sayed ER, Younis NA, et al. Harnessing endophytic fungi for biosynthesis of selenium nanoparticles and exploring their bioactivities. AMB Express. 2022; 12(1): 68. doi: 10.1186/s13568-022-01408-8.

28. Quddus S, Liaqat Z, Azam S, et al. Identification of efflux pump mutations in *Pseudomonas aeruginosa* from clinical samples. Antibiotics (Basel). 2023; 12(3): 486. doi: 10.3390/antibiotics12030486.

29. Yousefi S, Nazari M, Ramazanzadeh R, et al. Association of carbapenem and multidrug resistance with the expression of efflux pump-encoding genes in *Pseudomonas aeruginosa* clinical isolates. Acta Microbiol Immunol Hung. 2023; 70(2): 161-6. doi: 10.1556/030.2023.02029.

30. Mokhtari H, Shafighi ST, Salehzadeh A, et al. Investigating the effect of thymol active ingredient and ZnO nanoparticle conjugated by thiosemicarbazone on the expression of efflux pump and biofilm genes in *Pseudomonas aeruginosa*. J Genet Resour, 2023; 9(1): 75-82. doi: 10.22080/jgr.2023.24604.1338.

31. Abdolhosseini M, Zamani H, Salehzadeh A. Synergistic antimicrobial potential of ciprofloxacin with silver nanoparticles conjugated to thiosemicarbazide against ciprofloxacin resistant *Pseudomonas aeruginosa* by attenuation of MexA-B efflux pump genes. Biologia 2019; 74: 1191-6. doi: https://doi.org/10.2478/s11756-019-00269-0.

32. Azizi H, Akbari N, Kheirandish F, et al. Biogenic synthesized copper oxide nanoparticles by *Bacillus subtilis*: Investigating antibacterial activity on the mexAB-oprM efflux pump genes and cytotoxic effect on MCF-7 cells. J Basic Microbiol. 2023; 63(9): 960-70. doi: 10.1002/jobm.202200718.

Correspondence to Maryam F. Salman
E-mail: maryam.f.salman@aliraqia.edu.iq
Received Jan. 16th 2024
Accepted Mar. 3rd 2024