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SYNERGISTIC EFFECT OF GENTAMICIN AND IRON OXIDE NANOPARTICLES ON *phzM* GENE OF *PSEUDOMONAS AERUGINOSA*

The increased use of iron-containing nanoparticles and green synthesis nanoparticles is beneficial and less harmful to the environment and human health. Working antimicrobial peptide functions by inhibiting the virulence factor of Pseudomonas aeruginosa, which is the essential mechanism of drug resistance by possessing an efflux pump important virulent factor. The main aim of the research was to study the effects of synergistic action of gentamicin (GNT) and iron nanoparticles (IONps) to solve the problem of multidrug-resistant P. aeruginosa. ***Methods.*** *The isolation and identification of multidrug-resistant P. aeruginosa from burns and wound infections were carried out using the VITEK 2 system, and the phzM genes were detected in all the strains. The effectiveness of IONps and their role in reducing phzM gene expression were investigated.* ***Results.*** *The characterization of IONps biologically manufactured using a wavelength spectrum UV-vis spectrophotometer (maximum peak at 600 nm) and tests of atomic force microscopy showed that their diameter reached 34 nm. An electron microscope examination revealed that the produced particles were spherical, indicating good homogeneity and quality of the IONps. The results showed significant down-regulation changes in phzM expression after treatment with GNT and IONps. The result of qRT-PCR in this study revealed that the fold change in the expression of the phzM gene was downregulated in response to the IONps (8μg/mL) + GNT (16μg/mL) combination.* ***Conclusions.*** *One of the justifications for using P. aeruginosa in preparing IONps is that this bacteria needs iron as a growth promoter, which helps in the formation of IONps. The inhibitory effect on the efflux pump gene expression may represent a potential strategy for controlling P. aeruginosa infections.*

Keywords: *P. aeruginosa, phzM gene, gentamicin, CT gene.*

Using extremely small material particles to build new large-scale materials is known as nanotechnology. It is the process of creating materials and devices by manipulating matter at the levels of atoms, molecules, and supramolecular (nanoscale) structures (Majeed et al., 2022). Approximately 100,000 tons of antibiotics are produced annually worldwide to treat infectious diseases.

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Overuse of antibiotics has led to multidrug resistance in pathogenic strains, especially in bacteria (Remschmidt et al., 2018). The unique properties of iron oxide nanoparticles (NPs), including their large surface area, superparamagnetism, surface-to-volume ratio, and ease of separation, have attracted a lot of attention (Ahmed et al., 2018). *P. aeruginosa* is a common environmental bacterium that can colonize a range of habitats (Danafar et al., 2018) including ones that have been contaminated by humans, like sewage, oil spills, swimming pools, and water contaminated by biocide water (Ahmed, 2018; Dwivedi, 2018). Efflux pumps have evolved as a way for bacteria to interact with the environment (Sanz-García, 2022). Iron oxide nanoparticles (IONps) have many beneficial applications in many medical fields, where the effectiveness of IONps against fungi was found, and the result was identical to the effectiveness of anti-fungal product bactrocin from *Bacillus* spp. (Minnan et al., 2019). Additional findings from a comparative analysis of antimicrobial agents, including nisin A and vancomycin antibiotics, as well as research on the impact of methicillin-resistant *Staphylococcus aureus* isolated from food sources on biofilm formation, were presented in (Ahmed, 2020). For the treatment of pathogenic bacteria, particularly Gram-positive ones, another nanotechnology uses metallic nanoparticles like argentum nanoparticles (AgNPs). Levofloxacin antibiotic-sensitive *S. epidermidis* improved the antimicrobial assay. **Nisin-Silver nanoparticles synergetics**: the study aimed to replace the majority of *P. aeruginosa* with a synergistic effect with IONps and gentamicin (GNT) as alternatives to antibiotic-resistant bacteria to reduce the most important virulence factors such as pigments (Ahmed & Kadhim, 2020).

Synthesis of NPs on multidrug-resistant *P. aeruginosa* revealed the development of diverse nanostructures with distinct antimicrobial characteristics. NPs block flux pumps and prevent the formation of biofilms, so they may be a practical substitute for treating *P. aeruginosa* microbial resistance (Shakib et al., 2024).

Materials and Methods. Sixty-two isolates of *P. aeruginosa* were obtained from 150 injury swab samples. All of them were collected from September 2022 to February 2023 from patients who were referred to Baghdad Medical City and Al-Yarmouk Teaching Hospitals. The samples were collected using sterile cotton swabs, then kept in a cool place until transported to the laboratory, then cultured on Cetrimide and MacConkey agar and incubated for 24 hr at 37 °C (Ahmed et al., 2021).

Synthesis of Iron Oxide Nanoparticles (IONps). IONps were synthesized from anhydrous Iron (III) chloride ($FeCl_3$) and iron sulfate heptahydrate ($FeSO_4{\cdot}7H_2O$) at room temperature. An aqueous solution of 0.1 mmol iron (III) chloride ($FeCl_3$) anhydrous was prepared by dissolving in 250 mL of deionized water, while 0.05 mmol of $FeSO_4{\cdot}7H_2O$ was prepared by dissolving in 250 mL of deionized water to maintain the molar ratio 2:1 (Malhotra et al., 2020). IONps were synthesized by precipitation method; 20 g of $FeSO_4{\cdot}7H_2O$ was added to 200 mL of *P. aeruginosa* named (P1) cell-free extract at 37 °C. After incubation for one day, the mixture was then centrifuged (Fig. 1). The extracellular pathway, where metal ions are reduced by the action of bacterial reducing enzymes, demonstrates how the nanoparticles were biologically generated (Noor & Mais, 2023).

Characterization of IONps. After IONps were manufactured, they were characterized using several tests such as atomic force microscopy (AFM), EDS, field emission scanning electron microscopy study (FESEM), X-ray diffraction (XRD), scanning electron microscopy (SEM), transmission electron microscopy (TEM), and zeta potential analysis (ZP) (Faiq & Ahmed, 2024). The peculiarities of synthesis and characterization methods greatly influenced the features of the nanoparticles obtained (Mohammed & Ahmed, 2020; Zahraa et al., 2022).

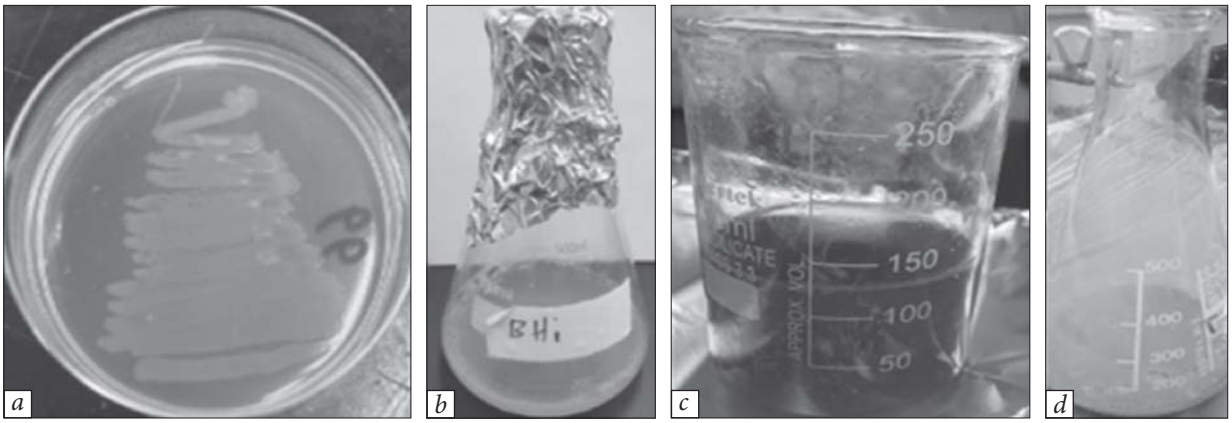


Fig. 1. Synthesis of IONps by the biological method: *a — P. aeruginosa, b —* cell free supernatant of *P. aeruginosa*, *c —* mixture of $FeCl_3$ and cell supernatant *d —* after incubation for three days

Antibiotic. In these studies, the powdered GNT antibiotic, 80 mg (Manufacture Company Merseyside, UK) was chosen (CLSI, 2023; Lafta et al., 2023).

Determination of Minimum Inhibition Concentration (MIC) of GNT and IONps. The GNT and IONps antibacterial activity were evaluated for different species of Gram-positive and Gram-negative bacteria (*S. epidermidis, S. aureus,* and *Klebsiella* spp.) isolated from patients suffering from injury. Gram-negative and positive isolates were taken from wounds and obtained from the biology laboratories at the University of Baghdad. Different concentrations (64, 32, 16, 8, and 4 μg/mL) were prepared. Bacterial suspension without any addition was used as a positive control. According to the CLSI, 2023 guidelines, the MIC of GNT for each isolate was determined (Jaffar et al., 2016) in an antibacterial activity assay using the disk diffusion method (Kadima et al., 2023).

Detection of *phzM* gene. The sequencing technique was taken from (Tang et al., 2022). Amplifications were carried out in 20μL volume containing 10μL Master Mix (2X), 1μL of primer (10 pmol), 6μL of water, and 2μL of template DNA. The PCR cycling was conducted with a PCR Express (Thermal Cycler, Thermo Fisher Scientific, USA), following this temperature program: 4-minute initial denaturation at 94 °C, followed by 30 cycles of denaturation at 94 °C for 30 sec, annealing at 55, 58, 60, 63, or 65 °C for 30 sec, and extension at 72 °C for 30 sec. The final extension step was performed at 72 °C for 7 min, followed by 10 min at 4 °C. The primer of *phzM* used is shown in Tables 1 and 2.

Gene expression of *phzM* genes of *P. aeruginosa*. The influence of IONps with GNT suspension on the investigated *phzM* gene expression was studied for 4 isolates of *P. aeruginosa*. Measurements were made of the genes' expression

Table 1. **Primer sequencing used in this study**

Primer Name	Sequence 5`-3`	Annealing Temp. (°C)	Product size (bp)	Source
phzM	F: ACGGCTGTGGCGGTTTA R :CCGTGACCGTCGCATT	60	~180	Tang *et al.,* 2022
House keeping gene (*fbp*)	F: CCTACCTGTTGGTCTTCGACCCG R: GCTGATGTTGTCGTGGGTGAGG	55	53	Ahmed & Al-Awadi, 2024

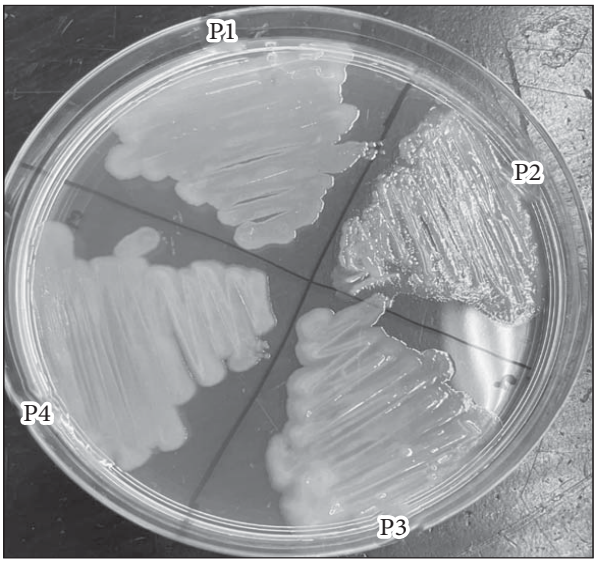


Fig. 2. *P. aeruginosa* culture media on Cetrimide agar after 24 h of incubation at 37 °C

levels after receiving sub-MIC treatment with nanoparticles synthesis.

RNA purification. RNA was isolated from the sample by the protocol of TRIzol™ Reagent according to Sabharwal et al. (Sabharwal et al., 2014).

Quantitative real-time polymerase chain reaction (qRT-PCR). The RT-PCR reaction should be carried out in a nuclease-free environment. The preparation of the RNA sample, the assembling of a reaction mixture, PCR, and analysis of the reaction should be performed in separate areas. The thermal cycler was set up so that the synthesis of cDNA could be followed immediately by qPCR amplification (Table 3) (Livak et al., 2001). The expression of the *fbp* gene, serving as a housekeeping gene, was used to normalize the results as demonstrated below:

Folding $=2^{-\Delta\Delta CT}$

ΔCT = CT gene — CT House Keeping gene

$\Delta\Delta CT = \Delta CT$ Treated or Control — ΔCT Control

Results. In this study, *P. aeruginosa* isolates were identified according to colony shapes using selective media. On Cetrimide agar, *P. aeruginosa* isolates appear as mucoid, smooth colonies with flat edges and elevated centers. Cetrimide agar is a selective/differential medium that contains 0.3 g of Cetrimide, which inhibits the growth of microorganisms other than *P. aeruginosa.*

Confirmation of identification by the VITEK 2 system. The identification of *Pseudomonas* isolates was confirmed using the VITEK 2 system, and 4 isolates showed multidrug resistance (MDR). They were named P1, P2, P3, and P4 (Fig. 2).

Ultraviolet-visible spectroscopy. Ultraviolet-visible (UV-visible) spectroscopy absorbance is used to characterize NPs, including their sizes. According to its outcomes, IONps showed a maximum peak at 600 nm (Fig. 3).

X-ray diffraction (XRD). The XRD spectrum of the IONps powdered sample is shown in Fig. 4, Confirmed formation of IONps by revealing three prominent peaks at 31.9450, 35.8371, and 45.6745.

AFM analysis. AFM was used to determine the particle size and surface morphology of IONps. 2D and 3D topography images of IONps are shown in Fig. 5.

TEM analysis. TEM analysis is a useful method for the determination of sizes and shapes of synthesized NPs (Fig. 6). These images confirm that IONps has an average size within 25—

Table 2. **Program PCR amplification of *P. aeruginosa* genes**

Steps	°C	m:s	Cycle
Initial Denaturation	95	05:00	1
Denaturation	95	00:30	30
Annealing	60 or 63	00:30	
Extension	72	00:30	
Final extension	72	07:00	1
Hold	10	10:00	

Table 3. **Real-Time PCR Program**

Steps	°C	Min: sec	Cycle
RT. Enzyme Activation	37	15:00	1
Initial Denaturation	95	05:00	
Denaturation	95	00:20	40

30 nm, spherical form, and good homogeneity, which demonstrates a good quality of the IONps.

Field Emission SEM Study. The majority of the produced nanoparticles were spherical, IONps that were produced biologically had an average size of 19—30 nm (Fig. 7) and were aggregated in a variety of places.

Antimicrobial activity of different concentrations of nanoparticles. MIC determination. The prepared by biogenic method IONps at different concentrations (Figs. 8, *a* and 8, *b*) were assessed for their antibacterial activity against Gram-positive bacteria. The inhibition zone was between 3—16 mm; this study was performed using the disk-diffusion assay, and growth inhibition zones were measured after incubation at 37 °C for 24 h. The results showed the effectiveness of IONps against different types of Gram-positive and Gram-negative bacteria that are resistant to antibiotics.

Synergistic antibacterial effect of GNT and IONps assay. Taking into account that the studied bacteria have an increased resistance to GNT antibiotics, there was studied the synergistic effect with IONps. The antibacterial assay of biological IONps and their mechanism of action on bacterial species cellular levels have been reviewed and discussed. Nanostructured materials may

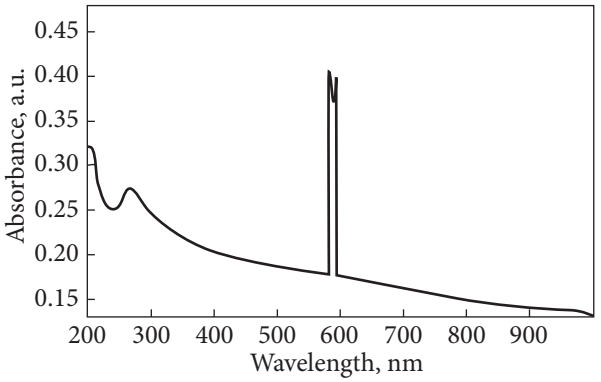


Fig. 3. UV-visible spectrophotometry of IONps

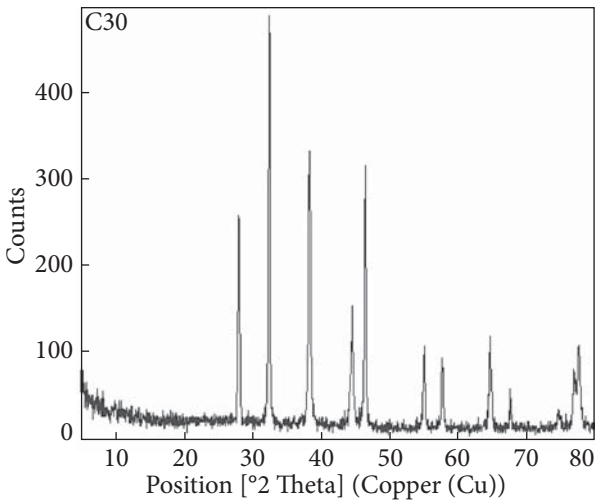
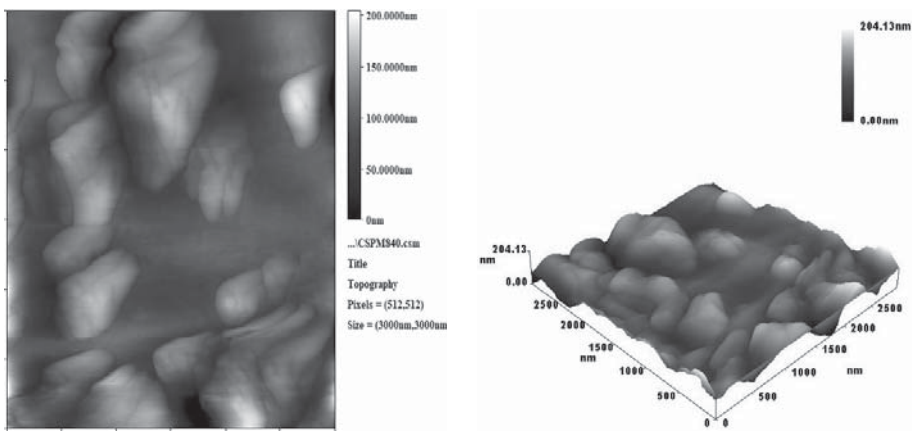


Fig. 4. XRD analysis of synthesized IONps



a *b*

Fig. 5. The synthesized IONps: *a* — 2D AFM of IONps, *b* — 3D AFM of IONps, and a chart of granularity distribution of IONps

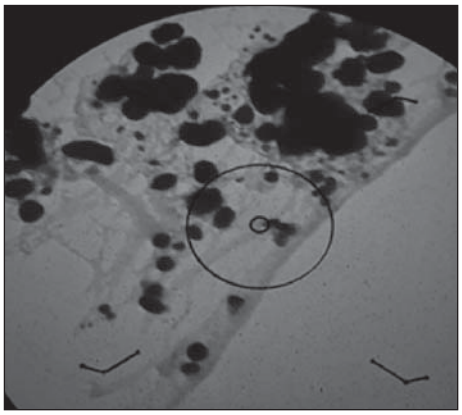


Fig. 6. TEM images of the IONps

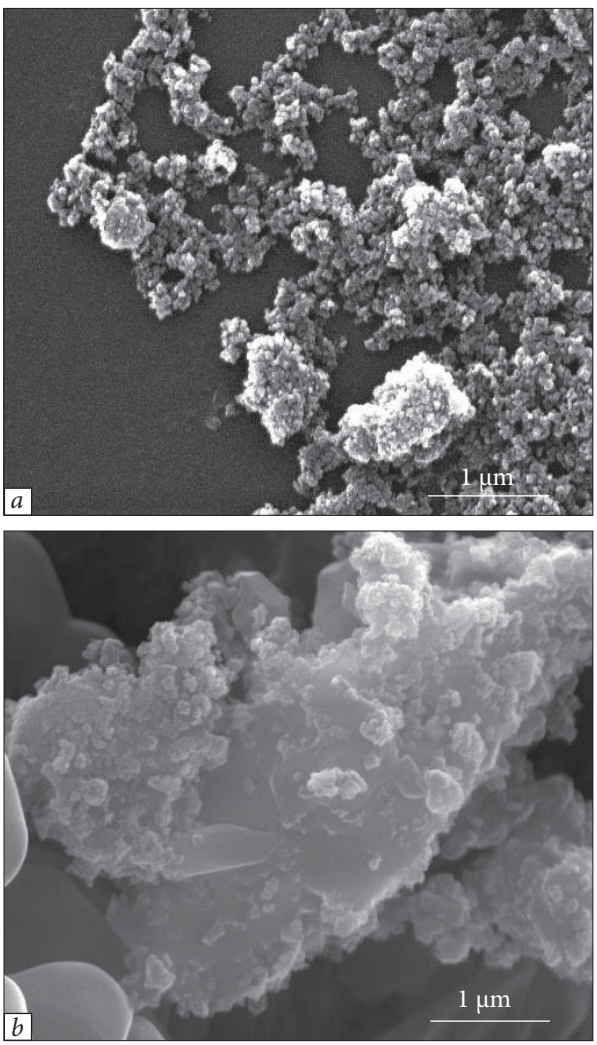


Fig. 7. SEM and size distribution of biosynthesized IONps: *a* — Spherical, *b* — Aggregated

also be applied in combination with antibiotics to increase their antibacterial effects. The MIC of GNT and IONps for the MDR bacteria isolates was determined. We noticed the synergistic effect against both Gram-negative and Gram-positive bacteria (Table 4). The statistical analysis emphasized a significant difference ($p < 0.05$) in the dissemination. The MIC of IONps was chosen (8 μg/mL), which showed the highest resistance against bacteria. The mean zone of bacterial inhibition after treatment with IONps (8 μg/mL) + GNT (16 μg/mL) showed large and significant differences regarding Gram-negative bacteria.

We note a synergistic effect of GNT with IONps against different species of bacteria, where the diameter of the growth inhibition zone ranged from 12 to 25 mm. However, it is less effective against Gram-positive bacteria *S. aureus* where the inhibition diameter reached 12 mm compared to more effective action against *P. aeruginosa* (15 mm).

The purpose of this study was to evaluate GNT's anti-virulence efficacy against *P. aeruginosa in vitro* using IONps to create novel, possibly anti-infective medications. These results imply that the combination of IONps and antibiotics has a synergistic effect that could make it

Table 4. **Mean ± SD zone (in mm) of growth inhibition of bacteria treated with IONps (8 μg/mL) and IONps (8 μg/mL) + GNT (16 μg/mL)**

Bacterial isolate	Mean ± SD zone of bacteria inhibition, mm		*p* Value
	IONps (8 μg/mL)	IONps (8 μg/mL) + GNT (16 μg/mL)	
S. aureus	12.2 ± 0.2	15.3 ± 0.3	<0.0001 **
S. epidermidis	18.3 ± 0.4	19.23 ± 0.5	0.0037 **
P. aeruginosa	15.4 ± 0.4	25.5 ± 0.4	<0.0001 **
Klebsiella spp.	17.16 ± 0.21	21.6 ± 0.25	<0.0001 **

**Significant differences at $p < 0.05$ between groups in a row

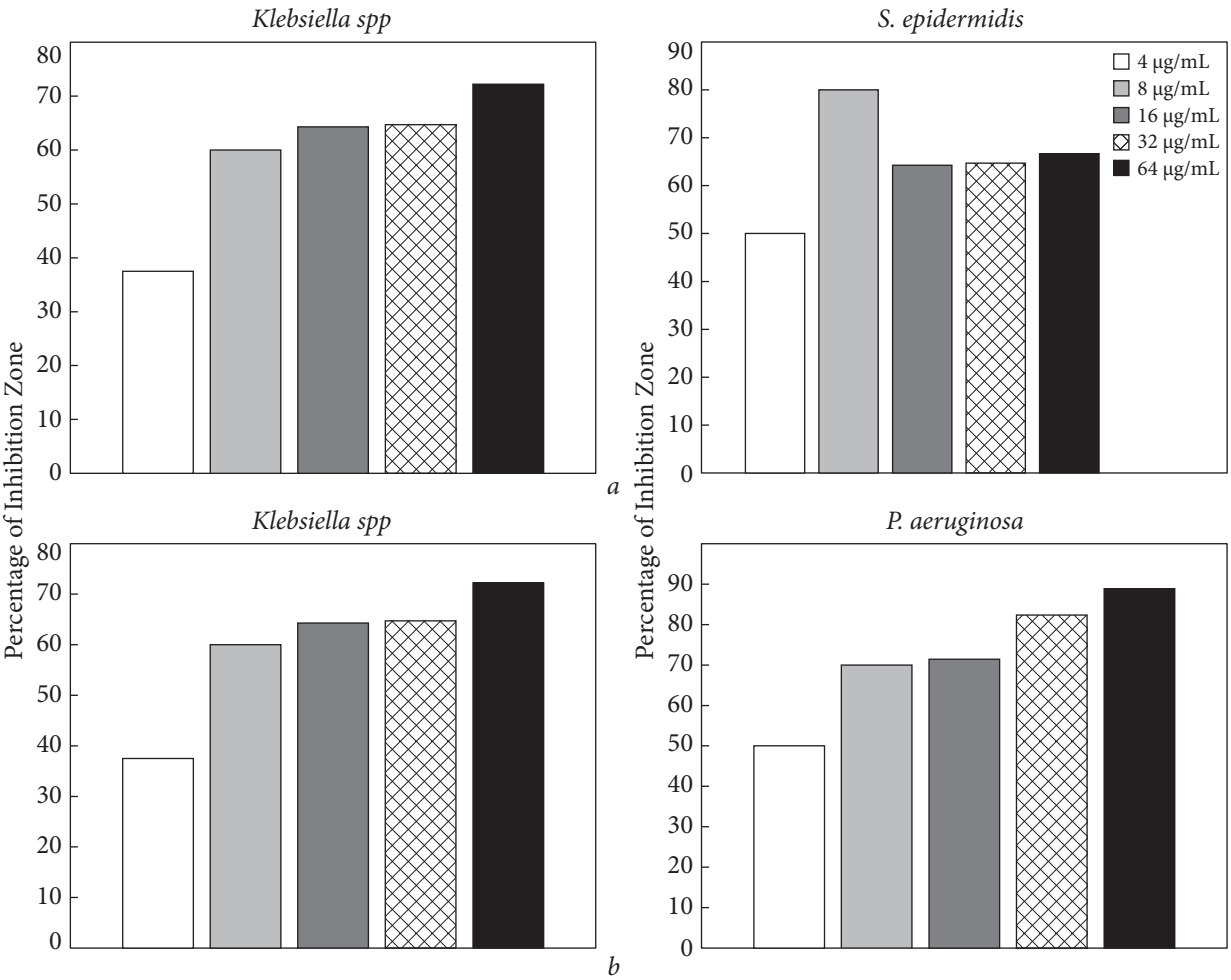


Fig. 8. The zone of growth inhibition (in mm) of bacteria treated with different concentrations (in μg/mL) of IONps *a* — against *S. aureus* and *S. epidermidis; b* — against *Klebsiella* spp. and *P. aeruginosa*

an efficient antimicrobial agent against *P. aeruginosa* infections that show resistance to several medications.

Detection of *phzM* gene. The *phzM* gene is known to be involved in the synthesis of pyocyanin, a virulence factor produced by *Pseudomonas*. Decreased *phzM* expression could potentially reduce the production of pyocyanin, which in turn may impact the pathogenicity and host interactions of *Pseudomonas.* The PCR results identified nine isolates of *P. aeruginosa* with the *phzM* gene, selected based on their MDR. The positive gene result was subsequently confirmed through electrophoresis at 75 volts for 50 min and with an ultraviolet (UV) transilluminator. The present study revealed the presence of a sharp, singular, and non-dispersed 180 bp *phzM* gene band, which was clearly distinguished from the DNA ladder, as demonstrated in Fig. 9.

Effect of IONps on *phzM* gene expression. RT-PCR reveals downregulation in *phzM* expression after treatment with IONps compared to housekeeping gene expression in bacteria. The result of qRT-PCR in this study revealed that the Fold change in expression of the *phzM* gene was downregulated in response to IONps

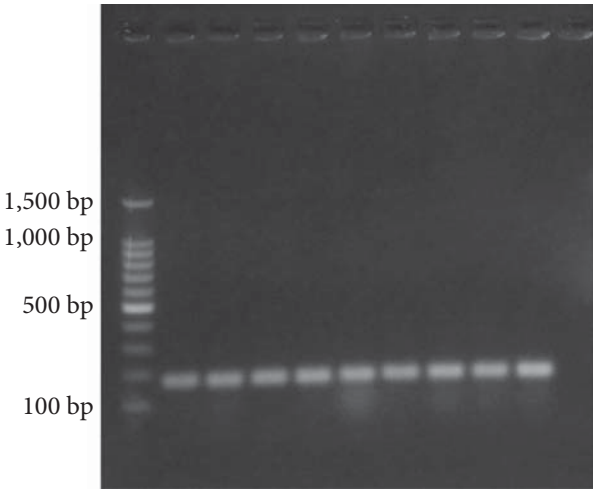


Fig. 9. Results of the amplification of the *phzM* gene of *P. aeruginosa* bacterial samples were fractionated on 1.5% agarose gel electrophoresis stained with Eth. Br. M: 100bp ladder marker. Lanes Z1-Z9 resemble 180bp PCR products

(8 μg/mL) + GNT (16 μg/mL). Table 5 shows that the expression of the *M* genes was significantly (p <0.0001) downregulated (fold of change: 0.68 ± 0.04, 0.49 ± 0.03, and 0.61 ± 0.09, respectively) after treatment with IONps + GNT compared with control (no treatment). The P2 isolate from wounds after treatment with IONps showed that there was no significant difference for each of the *phzM* genes, while significant differences were shown for P1, P3, and P4 strains after treatment, and down-regulation of *phzM* gene was observed.

In conclusion, these findings suggest that the synergistic use of IONps + GNT can have an inhibitory effect on *phzM* gene expression, which may decrease the function of genes involved in pyocyanin biosynthesis.

This research aimed to assess the anti-virulence activity of IONps + GNT against *P. aeruginosa* infection *in vitro.* Its findings support the further development of new potential antimicrobial drugs. In particular, they suggest that *Enterococcus faecium* (E5) suspension has the potential to serve as an effective antimicrobial agent against *P. aeruginosa* MDR strains.

Discussion. One of the most significant pathogens in the world, *P. aeruginosa* is distinguished by high genetic variability. The virulence trait of *P. aeruginosa* strain is a complicated topic. The majority of virulence genes are involved in the biosynthesis of pyocyanin. One illustrative aspect of their complex drug-resistance spectrum and variable pathogenicity potential may be their specific genotype composition (Wang et al., 2023). *P. aeruginosa* isolates with varying degrees of biofilm intensity possessed *psl* and *pel.* Nevertheless, there was only a slight association found between the GNT MIC and the gene expression. There was a discernible strong positive correlation with the minimum inhibitory concentration of GNT (Al-Sheikhly et al., 2020).

According to Nowruz et al. (Nowruz et al., 2012), strains of *P. aeruginosa* that have the *phzM* or *phzS* genes are unable to produce pyocyanin. Consequently, *P. aeruginosa's* pyocyanine production is significantly influenced by these genes. When combined with silver nanoparticles, pyocyanin exhibits synergistic antimicrobial effects against various microbial strains.

As compared to the other isolates under study, the *Pseudomonas* spp. isolates we assessed had higher AMR and biofilm-forming capacities. These results emphasize how critical it is to create novel antimicrobial drugs and anti-biofilm treatments to combat infections brought on by these bacteria (Almaghrabi et al., 2024). The

Table 5. **Fold of change of *phzM* gene expression in *Pseudomonas aeruginosa* before and after treatment with IONps + GNT**

Gene Type *phz M*	Mean ± SD gene expression fold of change		Sig.	***p*-value**
	Before	After		
P1	1.0	0.68 ± 0.04	**	<0.0001
P2	1.0	0.94 ± 0.02	NS	0.3921
P3	1.0	0.49 ± 0.03	**	<0.0001
P4	1.0	0.61 ± 0.09	**	<0.0001

**Significant differences are shown for P1, P3, and P4 strains after treatment

results of the nanoparticle tests showed a color change after three days and the spectrophotometry confirmed this (Lin et al., 2014; Lakshminarayanan et al., 2021).

Antibiotic activity was discovered through assessment and analysis of the synthesized nanoparticles' synergistic effect. The antibacterial properties of the drugs (gentamycin, penicillin, tetracycline, azithromycin, and ciprofloxacin) were increased when ZnS nanoparticles were present (Ismael & Zaidan, 2023). A wide variety of particles, such as metal oxides, naturally occurring antimicrobial substrates, and carbon-based nanomaterials can be classified as nanoparticles with antimicrobial properties. Particularly, due to their distinct physicochemical characteristics and high surface-to-volume ratio, nanoparticles have antimicrobial activity. Compared to the synthesis of antibiotics, the preparation of antimicrobial nanoparticles is less complex (Al-Awsi et al., 2023). Biogenic IONps were tested against pathogenic microorganisms such as *P. aeruginosa*, *Escherichia coli*, *Shigella* sp., *Staphylococcus* sp., and *Aspergillus niger* to determine their antimicrobial properties and estimate their potential for use in the biomedical and therapeutic domains (Al-Brahim, 2023). Synergistic solutions can be prepared for future use as nanomedical therapy as well as chemotherapeutic or preventive agents against pathogens. They can also be used as advantageous antibacterial product formulations in the pharmaceutical industry (Awatif & Aseel, 2023). Biosynthesized Fe_3O_4 NPs showed maximum absorbance in the 200—300 nm region. The XRD crystallinity analysis was applied, and the purity of Fe_3O_4 nanoparticles was confirmed (Predescu et al., 2018). The nanoparticle powder's XRD spectra showed unique peaks that matched the diffraction peaks (Faisal et al., 2023). The diameters of nanoparticles prepared by the biological method and determined by the AFM method (Mais & Khadija, 2020) showed an average roughness of about 1.27 nm and an average size of about 34 nm.

The TEM images of nanoparticles at different magnifications are in close agreement with the results. Additionally, they correlate with (Seddiq et al., 2023). However, other findings demonstrated the use of magnetic nanoparticles in synthesis through minute variations in shape and a wider size diversity, the sizes of agglomerates of the IONps, which reached a diameter of 8—33 nm (Roca et al., 2019).

The results were consistent with the results (Vasantharaj et al., 2019) that the inhibition zone for Fe_3O_4 NPs was 14.6 mm for *P. mirabilis* and 11.6 mm for *S. epidermidis*. The possible mechanism of the antibacterial activity of the Fe_3O_4 NPs can be affected by the magnetic IONps (positive charge) and pathogenic bacteria (negative charge) (Gudkov et al., 2021). Investigating the antibacterial effectiveness of WDA was the best method, and this method agreed with that (Abed et al., 2021) was the first to evaluate the niacin and volatile oil activity as an antimicrobial agent (assay by the WDA method). According to the detection of optical concentrations of both NPs and their synergetic use with antibiotics, compared to metal oxide NPs, the synergistic use of NPs with antibiotics increases their effectiveness against microbes compared to using the antibiotic alone (Muzammil et al., 2018). A similar result was obtained by (Patra & Baek, 2017). Simultaneous use of silver NPs with antibiotics inhibited protein synthesis or other various modes of action and chemical structures. Notably, there was no evidence of DNA degradation, as indicated by the absence of any smearing of the gene band (Jennifer et al., 2014). *P. aeruginosa* and antimicrobial peptides subjected to hematite (α-Fe_2O_3) IONps with varying diameters.

The use of conjugated or green-synthesized nanoparticles has increased over the past ten years due to enhanced antibiotic activity assay when combined with metallic NPs such as silver, iron, and zinc (Ahmed et al., 2023).

Using the microtiter plate method and an ELISA reader machine, the disruption of MDR

bacteria, such as MRSA *Staphylococcus*, suggests the use of a synergistic drug with a lower concentration of both vancomycin and bacitracin to treat the biofilm formation. This is the best option for reducing a microbial pathogen-formed biofilm, which is crucial for controlling infections linked to biofilm in patients with medical artifacts (Ahmed & Al-Shimmary, 2018). On the other hand, since pyocyanin production and antibiotic resistance are positively correlated, researchers have looked into the impact of low concentrations of ethanol on various antibiotics. Ethanol and antibiotics decreased *P. aeruginosa's* resistance to antibiotics by blocking pyocyanin (Montelongo-Martíne et al., 2023), while another result by (Jabłońska et al., 2022) shows the other hand: synthetic zinc oxide inhibited pyocyanin production by *P. aeruginosa.* The antibacterial and antibiofilm effectiveness of biosynthesized AgNps against pathogenic bacteria biofilm-forming clinically isolated were also detected (El-Sapagh et al., 2023).

Conclusions. Using IONPs as an alternative to antibiotics can have a significant impact on the use of antibiotics, especially considering the global issue of antibiotic resistance. The synthesis of these nanoparticles is safe for the environment. They have been proven to be effective in inhibiting the expression of vital virulence factors, including the expression of the efflux pump in *P. aeruginosa.* This inhibitory effect on the efflux pump gene expression may represent a potential strategy for controlling *P. aeruginosa* infections.

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Author's Contribution. Mais E. Ahmed planned the research work, performed the experiments and did the data analysis.

Zahraa A. Abdul Muhsin performed the experiments and contributed to the writing of the paper and publishing it as a correspondence author.

Conflict of Interest. The authors declare no conflict of interest.

Ethical Approval. This case report was conducted according to the Declaration of Helsinki. The collection and evaluation of data were confidential according to the health policy of the Iraqi Ministry of Health.

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СИНЕРГІЧНИЙ ВПЛИВ ГЕНТАМІЦИНУ ТА НАНОЧАСТОК ОКСИДУ ЗАЛІЗА НА ГЕН *phzM* СИНЬОГНІЙНОЇ ПАЛИЧКИ

Збільшення використання залізовмісних наночасток і наночасток, виготовлених методом зеленого синтезу, є корисним і менш шкідливим для навколишнього середовища і здоров'я людини. Дія антимікробного пептиду полягає в інгібуванні фактору вірулентності синьогнійної палички, який є основним механізмом розвитку лікарської резистентності і діє шляхом впливу на важливий вірулентний фактор — ефлюксну помпу. Основною **метою** дослідження було вивчення можливості використання гентаміцину з наночастками заліза для вирішення проблеми мультирезистентності *P. aeruginosa*. **Методи.** Виділення мультирезистентних штамів *P. aeruginosa* з опікових ран та їх ідентифікацію проводили за допомогою системи VITEK 2; гени *phzM* виявлено в усіх чотирьох виділених штамах. Далі проводили дослідження ефективності наночастинок заліза та їх ролі у зниженні експресії генів *phzM*. **Результати.** УФ спектроскопія наночастинок заліза, отриманих біологічним шляхом, показала максимальний пік при 600 нм, а атомно-силова мікроскопія визначила їхній діаметр 34 нм. Дослідження під електронним мікроскопом показали, що отримані частинки мали сферичну форму, що свідчить про хорошу гомогенність і якість наночастинок оксиду заліза. Після обробки гентаміцином і наночастинками оксиду заліза були виявлені значні зміни в експресії генів *phzM*. Результати qRT-ПЛР показали, що рівень експресії генів *phzM* був знижений у відповідь на обробку наночастинками оксиду заліза (8 мкг/мл) + гентаміцин (16 мкг/мл). **Висновки.** Використання *P. aeruginosa* для отримання наночастинок заліза є одним з обґрунтувань використання цієї бактерії, яка потребує заліза як стимулятора росту, що сприяє утворенню наночастинок заліза, а інгібуючий вплив на експресію гена ефлюксної помпи може бути потенційною стратегією боротьби з інфекціями, спричиненими *P. aeruginosa.*

Ключові слова: *P. aeruginosa, ген phzM, GNT, ген CT.*