Effect of Combined Zinc Oxide Nanoparticles and Ciprofloxacin on Biofilm Gene Expression in *Pseudomonas Aeruginosa*

Rasha K. Hassan[1] and Saba J. Jawad Al-Zubaidiy[2]
[1]*Scientific Research Authority, 10071 Baghdad, Iraq*
[2]*College of Education for Pure Sciences, University of Diyala, Al-Rahma Str., 32001 Baqubah, Iraq*
rasha.k.hassan@src.edu.iq, saba.jasim@uodiyala.edu

Keywords: *Pseudomonas Aeruginosa*; Zinc Oxide Tanoparticles, Ciprofloxacin, *Pela, Lasi*.

Abstract: Zinc oxide nanoparticles are promising antibacterial agents, owing to their unique physicochemical properties and broad-spectrum antibacterial activity. A study was conducted to evaluate the antibacterial efficacy of ZnO NPs against clinical isolates of *P. aeruginosa.* In addition, the impact of ZnO NPs on the expression of genes *pel*A and *las*I, the effect of ZnO NPs and ciprofloxacin on the expression of *pelA* and *lasI* genes using quantitative real-time PCR. A total 207 clinical and environmental specimens, including burn swabs, urine, wound swabs, sputum, ear swabs, endotracheal tube aspirates, bronchial washings, and operation room samples, were screened for *P. aeruginosa*. The isolates were identified and confirmed using the VITEK 2 System. Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method. Gene *pelA* and gene *lasI* were detected by PCR.The antibacterial activities of ZnO-NPs, and ciprofloxacin was measured, RNA was extracted from treated bacterial, followed by cDNA synthesis to determine the effects of ZnO-NPs and ciprofloxacin on gene expression. The cytotoxicity of ZnO-NPs was assessed using the MTT assay. From all specimens, 47 *P. aeruginosa* isolates were identified, and 20 of these isolates exhibited multidrug resistant. Molecular analysis revealed that all MDR isolates possessed both the *pelA,* and *lasI* genes. Isolates P10 and P16 were selected for further analysis because they exhibited strong biofilm formation and possessed both genes. MIC of the ZnO-NPs against *P. aeruginosa* was 1300 µg/ml. Treatment with sub-MIC of ZnO and sub-MIC of ciprofloxacin significantly reduced the expression of *pelA* and *lasI* genes, with relative expression levels of 0.163 - 0.534, respectively ($P< 0.05$). Furthermore, the combination of ZnO-NPs and ciprofloxacin demonstrated a synergistic effect, resulting in greater suppression of biofilm-related gene expression. These findings suggest for that ZnO-NPs, combination with ciprofloxacin, may represent a promising strategy for controlling MDR P. *aeruginosa* and inhibiting biofilm formation.

1 INTRODUCTION

P. aeruginosa is an opportunistic pathogen that causes a wide range of human infections, many of which can be life-threatening [1]. This bacterium possesses numerous factors, including biofilm formation, which plays a crucial role in its pathogenicity [2]. Biofilms consist of bacterial communities embedded with in a self-produced extracellular polysaccharide made by *P. aeruginosa* that made the structure of the biofilm [2], [3]. Biofilm formation contributes significantly to the persistence of *P. aeruginosa* infections, particularly those associated with implanted medical devices such as a pacemaker, peritoneal dialysis catheter, etc. [4]. Moreover, bacteria residing within biofilms can exhibit antibiotic resistance levels up to1000- fold greater than those of their planktonic counterparts [3]. Previous studies have demonstrated that quorum sensing, a bacterial cell-to-cell communication system, plays a central role in regulating biofilm formation as well as the expression of numerous virulence factors in *P. aeruginosa* [5]. Quorum sensing is responsible for bacterial species. Numerous antibiotics have been used to treat *P. aeruginosa* infections, among which ciprofloxacin, a second-generation fluoroquinolone, is widely employed due to its broad-spectrum activity against P. *aeruginosa* [4]. It exerts its antibacterial effect by inhibiting bacterial DNA gyrase and topoisomerase IV, thereby effecting by inhibiting bacterial DNA replication and ultimately leading to bacterial cell death. On the other hand, the increasing prevalence of multidrug-resistant (MDR) bacteria has encouraged

the development of alternative therapeutic approaches, including nanotechnology [6]. Zinc oxide (ZnO) has attracted considerable attention because of its stability in a variety of environmental conditions, low production cost, ease of synthesis, biocompatibility, and relatively low toxicity [5], [6]. Recently, zinc oxide nanoparticles (ZnO-NPs) have been seen to be activity lethal to the pathogenic factor as *P. aeruginosa*. ZnO-NPs were found to own significant biological effects and low toxicity [6]. They may help in the functions of used antibiotics [6], [7]. ZnO-NPs can select the cell wall without going into it. As a reason, most of resistant bacteria′s antibiotic defense mechanisms are rendered ineffective [7]. These nanoparticles may substitute the tasks of frequently utilized drugs.

2 MATERIALS AND METHODS

2.1 Bacterial Isolates and Identification

Two hundred seven specimens from wounds (12.65%; n 26), burns (29.11%; n 61), ear swabs (12.65%; n 26), urine (17.72%; n 37), sputum (8.86%; n 18), endotracheal tubes (1.26%; n 4), bronchial washes (2.53%; n 6), and operation rooms (13.92%; n 29) were included in the study. These specimens were collected from patients who attended Nurse Private Hospital and General Surgery Hospital in Baghdad during the period from Oct/2024 to Jun/2025 by means of a sterile swab with media and were cultured on MacConkey agar. *P. aeruginosa* was identified depending on the morphological and biochemical tests, which consist of Gram stain, oxidase, catalase, hemolysis, fermentation of lactose, pigment formation, and growth at 42ºC. All colonies from primary cultures were then cultured on cetrimide agar for final approval and isolated by the Vitek 2 compact system. Forty-seven of *P. aeruginosa* were tested for their resistance against the following (12) antibiotics by the Kirby-Bauer method on MHA according to criteria recommended by the Clinical and Laboratory Standards Institute/2023.

The range of resistance and sensitivity in millimeters of each antibiotic: Ampicillin (AM; R:10 mm, S:17mm), Cefepime (FEP; R:14 mm, S:18 mm), Piperacillin (PRL; R: 22mm, S:17mm) Gentamicin (CN; R: 16mm, S:15mm), Polymycin B (BMD; R:15mm, S:19mm), Colistin (CT; R:15mm, S:19 mm), Ofloxacin (OFX; R:15 mm, S:19mm), Norfloxacin (NOR; R:12 mm, S:17 mm), Imipenem (IMP; R:15mm, S:19 mm), Meropenem (MNM; R:15mm, S:19mm), Ceftazidime (CAZ; R:14 mm, S:18mm), and Ciprofloxacin (CIP; R:18mm, S:25mm) [8].

2.2 Determination of Minimum Inhibitory Concentration (MIC)

2.2.1 Ciprofloxacin

Ciprofloxacin serial dilutions ranging from 0.5 to 1024 µg/ml were prepared to determine the minimum inhibitory concentration (MIC). Three control tubes were included: a sterility control containing only sterile broth, a growth control containing broth without antibiotic, and a control containing antibiotic in broth without bacterial inoculation. All tubes were incubated at 37 for 18 - 24 hours. After incubation, tubes were examined for visible bacterial growth. The MICs was defined as the lowest concentration of ciprofloxacin at which no visible growth was observed [9].

2.2.2 ZnO NPs

Zinc oxide nanoparticle material was supplied by Hongwu/China. The MIC and SUB-MIC values were determined using the conventional broth microdilution method. A series of two-fold serial dilutions of the ZnO NP stock solution was prepared in nutrient broth, yielding final concentrations of 5200, 2600, 1300, 650, 325, 162.5, 1.25, 40.6, 20.3, and 10.15 µg/ml. After vortexing, each tube was inoculated with a standardized bacterial suspension with McFarland turbidity standard. A positive growth and negative controls were included. All tubes were incubated at 35ºC for 24 hours [10].

2.3 Genotyping Detection

DNA template preparation was performed using the boiling method. Five well- isolated bacterial colonies from an overnight culture were suspended in 2 ml of D.W. The suspension was then boiled in water bath for 10 min to lyse the cells and release genomic DNA. After boiling, the samples were centrifuged, and resulting supernatant was collected. This supernatant was used as the crude DNA template for the PCR after centrifugation [11].

Table 1: Primer sequences were used in study and procedure annealing times to each gene.

Name primers	Sequence 5-3		Annealing Tem. ºC	Product size (PB)	Ref.
LasI	F	CACATCTGGGAACTCAGC	40 cycles at 95 C for 30 s, 56 C for 30 s, and 72 C for 30 s	160	[12]
	R	ACGGATCATCATCTTCTCC			
pelA	F	CCTTCAGCCATCCGTTCTTCT	35 cycles at 94C for 30 sec, 52C for 40 sec, 72C for 50 sec	118	[13]
	R	TCGCGTACGAAGTCGACCTT			

2.4 PCR Primers to Detection of Biofilm Genes

The PCR was used to determine the presence of biofilm-related genes; the primer sequences are listed in Table 1. The reaction was carried out using Tap Master Mix, which includes Taq polymerase, dNTPs, $MgCl_2$, and the proper buffer, was used to conduct the PCR. 25 µl of reaction mixture, consisting of 12.5 µl of master mix, 1 µl of each reverse and forward primer solution, one µL of DNA, and the completed volume of nuclease-free water, was contained in each Eppendorf tube. The PCR was conducted under the subsequent condition: initial denaturation was performed at 94 ºC for 3 minutes. Then folded to thirty cycles of denaturation at 94ºC for thirty seconds each. Annealing temperature at thirty seconds, extension at 72ºC for 3.5 minutes. Table 1. The amplified DNA was subjected to separation using 1% agarose gel electrophoresis [9].

2.5 Synergistic Treatments of Inhibition *P. aeruginosa* Isolates

P. aeruginosa isolates were cultured in BHI broth, then incubated at 35º for 24 H. Then 0.1 ml of bacterial isolates were added to nutrient broth. Following the determination of the SUB-MIC for ZnO NPs and ciprofloxacin, each was prepared and added to NB (4.5 ml). The tubes were divided as follows: double tubes of each bacterium for each concentration of SUB-MIC in ZnO, a combination of ciprofloxacin and ZnO, ZnO NPs alone, and ciprofloxacin alone. After shaking each tube by vortex, they were all incubated throughout the whole night at 35ºC. Then centrifuged at 8000 rpm for 10 min; poured off the supernatant. 800 µl TRIzol was added to the clot and then RNA was extracted [7].

2.6 Detection of Gene Expression by Quantitative Real Time PCR of *pelA* and *lasI* Genes.

To determine and choose the isolates with the greatest and lowest resistance levels using gene expression, which is established through comparison of the mean CT values of validated normalized by folding = 2Aact, where they are determined by quantitative real-time PCR for the reference gene (Eub358f TCCTACGGGTATC TA AT TATCTAATCCTG) [14]· *lasI*, and *PelA* genes in the same sequence used in detection because the length of the gene was less than 200. 5 µL of the qPCR master mix. 0.25 µl of each RT mix and $MgCl_2$, 0.5 µL of each forward and reverse primer, and 2.5 µL of molecular biology grade water and, then 1 µL of RNA were used for the RT-PCR. The following guidelines were followed for doing the RT-PCR: One cycle of RT enzyme activation at 37ºC for 15 m. The first denaturation (95ºC, 10 min) and the 40th cycle (95ºC, 20 s). Temperature for annealing: extensional 72ºC for 20 seconds. Utilize the formula to determine gene expression. The equation (1) was used to detriment the difference in expression between the treatments and the reference gene [15].

$$\text{Folding} = 2^{\wedge(-\Delta\Delta Ct)}. \quad (1)$$

ΔΔCt: Cycle threshold.

Folding: Fold change.

The (2) used to give real change from the processing [16].

$$\Delta\Delta CT = CT\ \text{treated} - ACT\ \text{control}. \quad (2)$$

In (3), the ΔCT value was determined by normalizing the treatment gene expression to the reference gene.

$$\Delta CT = CT\ \text{treated} - CT\ \text{reference gene}. \quad (3)$$

2.7 Cytotoxicity Assay

The MTT test was used to measure metabolic processes in cells in order to assess cellular and cytotoxicity testing for lethality, as well as proliferation. This method was accomplished as described by Mohamed *et al.* (2025) in Alexandria, Egypt [11].

2.8 Statistical Analysis

The statistical examination detects the effect of the factors of variation on the study criteria, and the LSD was used for the marked comparison between the means (ANOVA/One-way) in this study [13].

3 RESULTS

3.1 Characteristic of Isolates

Twenty MDR *P. aeruginosa* isolates from forty-seven clinical specimens. All (47) isolates appeared Gram-negative, they were positive for oxidase and catalase, and non- fermenting lactose. When *P. aeruginosa* cultured on cetrimide agar, they were greenish-yellow and mucoid, and the diagnosis was confirmed by the Vitek 2 compact system.

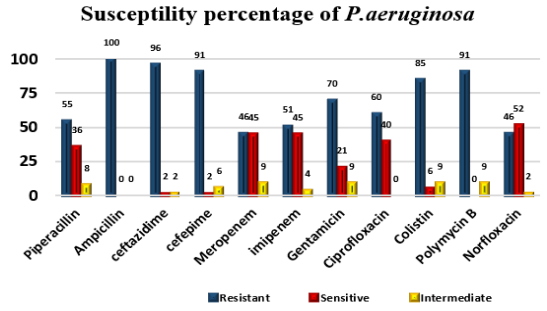

Figure 1: Antiprogram of *P. aeruginosa* isolates and the susceptibility percentage against different antibiotics.

Figure 2: Antibiotic susceptibility test for *P. aeruginosa* using 12antibiotics.

3.2 Antibiotic Susceptibility of *P. aeruginosa*

Using the Kirby-Bauer disc methods, 47 *P. aeruginosa* isolates from various clinical sources were tested for antibiotic susceptibility to 12 antibiotics from distinct groups. Results are indicated in Figure 1 and 2. was found that most of the bacterial isolates was resistant showing results. Results also showed that the isolate *P. aeruginosa* ten were resistant to all antibiotics (100%), while the four were resistant to 11 antibiotics (91.6%); then the one isolate was resistant to ten antibiotics (83.3%), four isolates were resistant to nine antibiotics (75%), seven isolates were resistant to eight antibiotics (66.6%), four isolates were resistant to seven antibiotics (58%), six isolates were resistant to six antibiotics (50%), the eight isolates were resistant to five antibiotics (41%), and finally, two isolates were resistant to four antibiotics (33%). The antimicrobial resistance pattern found in this investigation caused worries because the isolates were very resistant to the typical antibiotic that the patients were taking. This result was agreed with in a study conducted by Yosif at the collage of Science, University of Baghdad (2023), [14].

3.3 Molecular Detection of Pel polysaccharide *pelA* gene

In this study, the prevalence of *pelA* in MDR and hypervirulent *P. aeruginosa* isolates was determined by amplification of the gene using specific primers. Showed each of the twenty isolates has the *pelA* gene in their genomic DNA, which was located at 118 bp. This result appears in Figure 3. That agreement with a study done by Omran *et al*. (2026) in Baghdad showed that *pelA* genes were part of the essential genome of *P. aeruginosa* [15]. Additionally, *Pel* was the main element of the biofilm matrix and has been linked with a variety of biological roles, particularly in relation to the defense of the bacterial cell against the immune system and antibiotics [16].

3.4 Molecular Detection of the *LasI* Gene (*lasI*)

Results of gel electrophoresis for amplification products of *lasI* showed that twenty of these isolates harbor lasI in their genomic DNA. This result was illustrated in Figure 4. Furthermore, these results were in conformity with a study done by Kadhim *et al*. (2025) inside Iran, which found all the specimens of *P. aeruginosa* have *lasI/lasR* [12]. So, this study agreement with a study done in the USA, at Oregon State University, Corvallis, treated integrated experiments with mathematical modeling to quantitatively analyze the *LasI/LasR* quorum sensing pathway in the opportunistic pathogen [17]. The results showed that *P. aeruginosa* isolates had a remarkably high incidence of treatment resistance. Additionally, the quorum-sensing system was significantly correlated with drug resistance and

biofilm formation, suggesting that it played a crucial role in the development of nosocomial infections carried by *P. aeruginosa* [16].

3.5 Determinations of Minimum Inhibition Concentration MIC

Two *P. aeruginosa* isolates, (P10) from sputum and (P16) from urine, were selected for this study. Both isolates were characterized by strong biofilm forming ability and the presence of *lasI* and *pelA genes in their genomes*. In addition, both isolates exhibited XDR profiles against all antibiotics tested in this study. The aim of this experiment was to determine the minimum inhibitory concentration sub-MIC values. For ciprofloxacin (256-512 µg/ml). This result was consistent with the findings of Hameed (2023), who reported similar MIC values for *P. aeruginosa* isolates collected from patients in Baghdad [18]. Additionally, the result of MIC and sub-MIC to zinc oxide nanoparticles to the same bacterial isolates was at the level of (1300-650µg/ml) respectively. A study by Ahmed et al. (2023), a finding of the present study, demonstrated the effectiveness of ZnO-NPS in inhibiting the growth of *P. aeruginosa* [19]. Results are indicated in Table 2.

Table 2: Minimum inhibition concentration to ZnO- NPs with *P. aeruginosa* isolates and sub-MIC and MBC.

P. aeruginosa NO.	Sub-MIC µg/ml	MIC µg/ml	MBC µg/ml
p10	650	1300	2600
P16	650	1300	2600

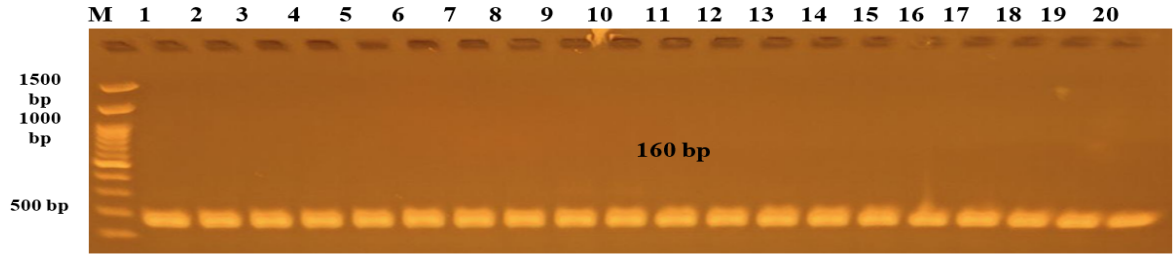

Figure 3: Amplification products of *P. aeruginosa pelA* after electrophoresis on 1.5% agarose gel for 60 min at 7v/cm2. (M): DNA ladder marker; Lanes (1-20): Bacterial isolates number.

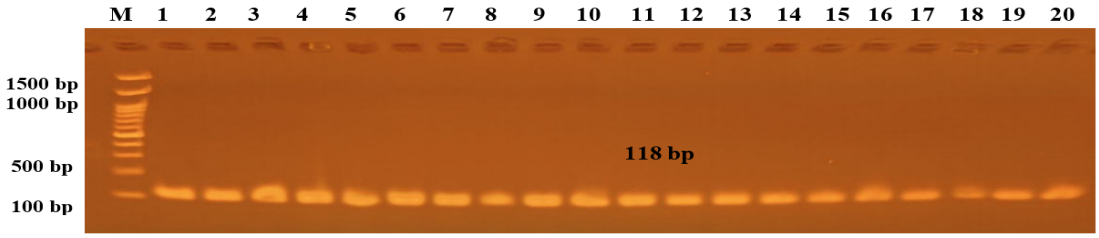

Figure 4: Amplification products of *P. aeruginosa lasI* gene after electrophoresis on 1.5% agarose gel 60 min at 70 volt/cm.(M): DNA ladder marker; Lanes (1-20): Bacterial isolate number.

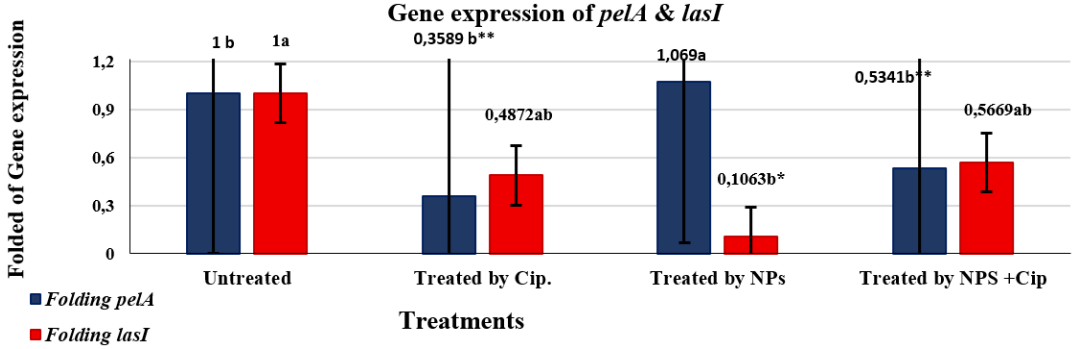

Figure 5: Expression pelA and lasI gene of P. aeruginosa isolates after 24 hours from incubation at 37ºC.

3.6 Gene Expression of *pelA* and *lasI* Genes of Iofilm

To investigate the expression of biofilm-related genes in two *P. aeruginosa* isolates, P10 and P16, ciprofloxacin and zinc oxide nanoparticles, and their combination for 24 hours. Total RNA was subsequently extracted from the treated isolates. The RNA concentrations ranged 34.364 to 96.103 ng/µL, while the purity (A260/A280) ranged from 1.0–2.119. The extracted RNA was then used for gene expression analysis. Ciprofloxacin and zinc oxide nanoparticles were applied at sub-MIC concentrations, either individually or in combination were used at a 1:1 ratio. The expression level of the biofilm-associated gene *pelA* was subsequently determined using quantitative real-time PCR.showed Figure 5.

These finding suggest that ciprofloxacin, either alone or in combination with ZnO-NPs, effectively downregulated the expression of the biofilm-associated *PelA* gene, whereas ZnO-NPs alone exhibited only a modest suppressive effect on pelA expression (relative expression 0.624 relative to the untreated control) under the tested conditions, considerably weaker than that produced by ciprofloxacin or the combination treatment. The results were illustrated in Figure 4. Treated with ZnO-NPs, resulted in a significant reduction ($P<0.05$) in the expression of the *lasI* gene, with a relative expression value of (0.163) Ciprofloxacin treatment also decreased *lasI* gene expression, yielding a value of (0.4872), Likewise, the combination that ZnO-NPs and ciprofloxacin reduced *lasI* expression to (0.5669) [7]. These findings indicate that ZnO-NPs exerted a stronger inhibitory effect on *lasI* gene expression than on the *pelA* gene expression.

Note: the specific relative-expression values quoted above for pelA and lasI under each treatment should be cross-checked against the source qRT-PCR data underlying Figure 5, as the numeric values currently reported in the text for the ZnO, ciprofloxacin, and combination groups do not fully align with those plotted in Figure 5 for the two genes.

3.7 Cytotoxicity of an ZnO-NPs in HDF Cells

The results of the cytotoxicity analysis demonstrated that zinc oxide NPs exerted a dose-dependent effect on the Human Dermal Fibroblast HDF cell line after 72 hrs. The cells were treated with different concentrations of ZnO NPs (120, 60, 30, 15, 7.5, 3.75, 1.87, and 0 µg/mL). The results indicated that cell viability varied according to the concentrations of ZnO-NPs used. As shown in Figure 6. the biological activity of ZnO NPs against the results demonstrated that the HDF cells decreased with increasing concentrations of ZnO-NPs indicating a concentration-dependent cytotoxic effect. The highest cell viability was observed in the untreated control group (0 µg/mL) and at the lowest concentration tested, where cell viability remained between (100-99%). The results appearance in Figure 6.

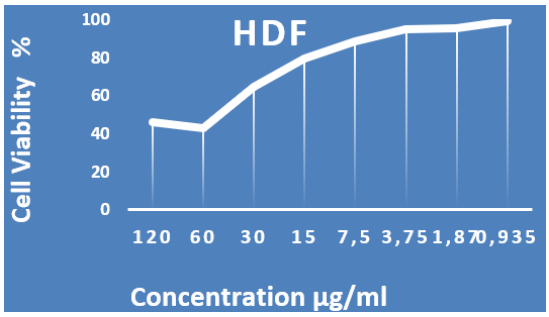

Figure 6: Cytotoxicity of ZnO NPs towards normal cells.

The IC50 value (the half-maximum inhibitory concentration) could not be determined after 48 hours of exposure because ZnO-NPs did not produce a 50% reduction in cell viability at the tested concentrations. However, after 72 h. of exposure, the IC50 was estimated at 66.85 %. These findings are consistent with those reported by Jaiyan *et al.* (2025), Who demonstrated that ZnO exhibit acceptable biocompatibility at appropriate concentrations while highlighting the importance of further investigations into long-term toxicity and the development of more efficient delivery systems to enhance their safety and activity [20]. Furthermore, this result agreement with the study conducted in Egypt by Salah *et al* (2019), which demonstrated that ZnO NPs significantly reduced the expression of the *lasI* gene, which is responsible for the synthesis of AHL 3-oxo-C12-HSl [21]. The commercial ZnO NPs were assessed for antimicrobial activity and biological compatibility in HDF-type lines. Their biological effects were strongly influenced by the method of preparation, some of them showed intact human dermal fibroblast proliferation at concentrations up to <500 µg/ml, with a noticeable decline in viability at > 250µg/ml.

4 CONCLUSIONS

According to the results of the present study that present study revealed that the majority of local clinical MDR *P. aeruginosa* clinical isolates harbored the biofilm-associated genes *pelA* and *lasI*. Molecular detection confirmed the presence of both genes in all examined isolates; biofilm plays a vital role in multidrug resistance in clinical *P. aeruginosa* isolates. The results of detecting biofilm genes demonstrated the presence of *pelA* and *lasI* genes in all local isolates of *P. aeruginosa*. And the influence of the zinc oxide nanoparticles on the expression of biofilm genes of *P. aeruginosa* was significant and exhibited variability in their inhibitory effects, attributable to their mechanisms of biofilm disruption, including penetration into the biofilm matrix, degradation of biofilm constituents, and inhibition of bacterial growth. The findings also demonstrated that ZnO-NPs significantly affected the expression of biofilm-related genes. The inhibitory effects varied according to the target gene and treatment applied. The ZnO exhibited a stronger suppressive effect on *lasI* gene expression than on *pelA*, suggesting a potential role in disrupting quorum sensing pathways in *P. aeruginosa*. This effect may be attributed to several mechanisms, including penetration of the biofilm matrix, degradation of biofilm components, interference with bacterial signaling systems, and inhibition of bacterial growth. Additionally, ciprofloxacin alone produced a marked reduction in the expression of biofilm-associated genes, while the combination of ciprofloxacin and ZnO-NPS also appearance to have a significant inhibitory effect. These findings suggest that it may enhance the efficacy of the ciprofloxacin by facilitating it′s penetration or augmenting it′s impact on the bacterial cell or inhibiting the resistance of bacteria or enhancing drug delivery. Consistent with the cytotoxicity assay performed in this study, ZnO-NPs showed a favorable short-term safety profile on normal cells, with measurable cytotoxicity emerging only after prolonged (72 h) exposure (IC50 = 66.85%).

ACKNOWLEDGEMENTS

The authors express their gratitude to the microbiology laboratory unit in a private Nursing Hospital in Baghdad to providing them with a place to work.

REFERENCES

[1] N. Sathe, P. Beech, L. Croft, C. Suphioglu, A. Kapat, and E. Athan, “Pseudomonas aeruginosa: Infections and novel approaches to treatment ‘Knowing the enemy’ the threat of Pseudomonas aeruginosa and exploring novel approaches to treatment,” Infectious Medicine, vol. 2, no. 3, pp. 178-194, 2023.

[2] A. J. Kunz Coyne, A. El Ghali, D. Holger, N. Rebold, and M. J. Rybak, “Therapeutic strategies for emerging multidrug-resistant Pseudomonas aeruginosa,” Infect. Dis. Ther., vol. 11, no. 2, pp. 661-682, 2022.

[3] Banerjee, P. Chowdhury, K. Bauri, B. Saha, and P. De, “Inhibition and eradication of bacterial biofilm using polymeric materials,” Biomater. Sci., vol. 11, no. 1, pp. 11-36, 2023.

[4] D. K. Süer, “Molecular detection of some virulence genes in Pseudomonas aeruginosa isolated from clinical sources,” Ph.D. dissertation, Near East Univ., 2021.

[5] A. K. Khajuria et al., “In vitro antioxidant and antibacterial activities of biogenic synthesized zinc oxide nanoparticles using leaf extract of Mallotus philippinensis,” Sci. Rep., vol. 15, no. 1, p. 6541, 2025.

[6] H. Al-Momani et al., “Low-dose zinc oxide nanoparticles trigger the growth and biofilm formation of Pseudomonas aeruginosa: A hormetic response,” BMC Microbiol., vol. 24, no. 1, p. 290, 2024.

[7] H. Valadbeigi et al., “Effect of ZnO nanoparticles on biofilm formation and gene expression of toxin-antitoxin system in clinical isolates of Pseudomonas aeruginosa,” Ann. Clin. Microbiol. Antimicrob., vol. 22, no. 1, p. 89, 2023.

[8] S. Rai, D. Dash, and N. Agarwal, “Introducing the new face of CLSI M100 in 2023: An explanatory review,” Indian J. Med. Microbiol., vol. 46, p. 100432, 2023.

[9] A. Azemin, N. Alias, and A. Kari, “Identification of Pseudomonas aeruginosa strains isolated from Dorper sheep milk with subclinical-mastitis infection by uniplex PCR using 16S rRNA, lasI/R, gyrB and ecfX genes,” Malaysian Journal of Fundamental and Applied Sciences, vol. 18, no. 1, pp. 30-42, 2022.

[10] F. HajiSadeghi, A. Sobhani Nasab, S. Rashki, H. Zandi, S. Afsharikhah, Z. S. Fatemi-Nasab, and A. Nazari-Alam, “Evaluation of antimicrobial effect ZnO nanoparticles and clove extract against *Pseudomonas aeruginosa* isolated from clinical samples,” *Microbe*, vol. 7, Art. no. 100363, 2025, doi: 10.1016/j.microb.2025.100363.

[11] A. N. Mohamed et al., “Antimicrobial, anti-inflammatory, anticancer and antiviral activity of bioactive compounds from Pseudomonas aeruginosa,” Microb. Biosyst., vol. 10, no. 1, pp. 123-134, 2025.

[12] Z. A. Kadhim, J. S. Hassan, and A. S. Rawa’a, “Correlation between quorum sensing genes (lasI and lasR) and antibiotic resistance in Pseudomonas aeruginosa,” Iraqi J. Med. Sci., vol. 23, no. 2, 2025.

[13] Z. Li et al., “Antibacterial activity and mechanism of sodium houttuyfonate against heteroresistant Pseudomonas aeruginosa,” Front. Microbiol., vol. 16, p. 1523182, 2025.

[14] M. A. Yousif and L. A. Yaaqoob, "Comparison between extracellular and intracellular extract of Lactobacillus plantarum in Fe_2O_3 nanoparticle preparation and their effect against pathogenic Pseudomonas aeruginosa," M.S. thesis, Univ. Baghdad, 2023.

[15] A. H. Omran, Z. A. A. Hosain, and H. T. Ibrahim, "Effect of chloroxylenol on gene expression of pslA and pelA in Pseudomonas aeruginosa," Iraqi J. Sci., pp. 846-854, 2026.

[16] J. C. Van Loon, F. Le Mauff, M. A. Vargas, S. Gilbert, R. Pfoh, Z. A. Morrison, et al., "Structural and functional analysis of Pseudomonas aeruginosa PelA provides insight into the modification of the Pel exopolysaccharide," Journal of Biological Chemistry, vol. 301, no. 5, p. 108432, 2025.

[17] S. Thomas, "Linking molecular mechanisms to population-level behavior using mathematical models of Pseudomonas aeruginosa quorum sensing," Ph.D. dissertation, Georgia Institute of Technology, 2023.

[18] H. G. Hameed, "Pseudomonas aeruginosa epidemiology and antibiotic resistance: A five years retrospective study in Iraq," Al-Esraa University College Journal for Medical Sciences, vol. 4, no. 6, pp. 31-54, 2023.

[19] M. Ahamad Khan, S. A. Lone, M. Shahid, M. T. Zeyad, A. Syed, A. Ehtram, et al., "Phytogenically synthesized zinc oxide nanoparticles (ZnO-NPs) potentially inhibit the bacterial pathogens: In vitro studies," Toxics, vol. 11, no. 5, p. 452, 2023.

[20] T. Jaiyan et al., "Commercial zinc oxide nanoparticles: Mechanistic investigation and biocompatibility evaluation," Appl. Nano, vol. 6, no. 4, p. 26, 2025.

[21] M. M. Saleh et al., "Zinc oxide nanoparticles inhibit quorum sensing and virulence in Pseudomonas aeruginosa," Afr. Health Sci., vol. 19, no. 2, p. 2043, 2019.