Synthesis and antibacterial activity of ZnO nanoparticles on *blaOXA* and *blaTEM* gene expression in Pseudomonas aeruginosa

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Abstract

Background: The rising percentage of multidrug resistant (MDR) *Pseudomonas aeruginosa* has emerged as a major clinical challenge in part because of the abundant transfer of β-lactamase-associated resistance genes, especially blaOXA and blaTEM. Recently, zinc oxide nanoparticles (ZnO-NPs) have been recognized as potential antimicrobial and resistance-modifying agents.

Methods: In this study we assessed the effects of biosynthesized ZnO nanoparticles on blaOXA and blaTEM gene expression in clinical *P. aeruginosa* isolates by quantitative real-time PCR (qRT-PCR).Methods: Twenty-nine clinical isolates of P. aeruginosa were obtained from different clinical specimens. The isolates carrying blaOXA and blaTEM genes were identified by molecular screening. For gene expression analysis, three typical isolates that harbored both genes were chosen. Biosynthesized ZnO nanoparticles were injected at a minimum inhibitory concentration (MIC) of 1.25 mg/ml for 18–24 h. Relative gene expression was quantified by qRT-PCR and 16S rRNA was used as reference gene for gene expression and analysed by 2^-ΔΔCt method. The results indicated that the blaOXA expression was greatly decreased in the treated isolates after treatment with ZnO nanoparticle (mean fold change ranged from 1.12 ± 0.37 in untreated isolates to 0.32 ± 0.03 in treated isolates, which is ∼71% downregulation). On the other hand, blaTEM downregulation from 1.01 ± 0.02 to 0.78 ± 0.10 represented approximately 23% downregulation. Not significant for a statistically non-significant reduction in ($P > 0.05$) but the expression level of treated isolates was always lower than the untreated controls.

Results: Biosynthesized ZnO nanoparticles inhibited β-lactamase resistance genes in *P. aeruginosa*, in particular the genes for blaOXA. These results indicate that ZnO nanoparticles can be used as resistance-modifying agents and may form an important target to control the future strategies for the prevention of antimicrobial resistance.

Keywords: *Pseudomonas aeruginosa*; Zinc oxide nanoparticles; blaOXA; blaTEM; gene expression; qRT-PCR

1. Introduction

Pseudomonas aeruginosa is one of the leading opportunistic pathogens responsible for nosocomial infections causing multiple types of clinical conditions including wound infections, burn infections, respiratory tract infections, urinary tract infections, and septicemia. The clinical control of *P. aeruginosa* infections is increasingly challenging due to its remarkable ability of establishing multi-class antimicrobial resistance via intrinsic and acquired mechanisms. One of the main mechanisms of acquired resistance present in *P. aeruginosa* is the production of β-lactamase enzymes, which are encoded by the resistance genes blaOXA and blaTEM. These enzymes break down β-lactam antibiotics and diminish their therapeutic effectiveness. The dissemination of genes encoding β-lactamase across clinical isolates has also led to the emergence of multidrug-resistant strains and has limited available treatment options as well as increased healthcare burdens worldwide. Nanotechnology has emerged as a novel modality in addressing antimicrobial resistance over the past years. Zinc oxide nanoparticles (ZnO-NPs) have obtained great research interest due to their antibacterial properties, biocompatibility, and capacity to produce reactive oxygen species (ROS). Previous reports have shown that ZnO NPs can damage bacterial cell membranes, act on metabolic pathways, and alter bacterial gene regulation. Yet details on their effect on the transcriptional expression of β-lactamase resistance genes in clinical *P. aeruginosa* isolates are scarce. Thus in this study, we aimed to determine the influence of biosynthesized ZnO nanoparticles on the expression of blaOXA and blaTEM genes in clinical *P. aeruginosa* isolates via quantitative real-time PCR (qRT-PCR).

2. Materials and Methods

2.1 Bacterial Isolates

Twenty-nine clinical isolates of Pseudomonas aeruginosa were isolated from different clinical specimens acquired from patients attending Al-Hussein Teaching Hospital, Al-Muthanna Province, Iraq, from

December 2025 to January 2026. The isolate was identified by initial bacteriological methods such as colony morphology, microscopic screening and biochemical characterization. Confirmation was carried out by using the VITEK® 2 Compact system, which is widely used for rapid and accurate identification of clinically important Gram-negative bacteria [16].

2.2 Molecular Detection of blaOXA and blaTEM Genes

The isolated bacterial isolates utilized with the Presto™ Mini gDNA Bacteria Kit (Geneaid, Taiwan) were used to detect their genomic DNA according to product specifications. DNA concentration and purity were measured by NanoDrop spectrophotometer, and DNA integrity confirmed on 1% agarose gel. Using specific primers, conventional PCR assays performed were used to detect blaOXA and blaTEM resistance genes. The PCR products were separated by agarose gel electrophoresis, and were shown using ultraviolet illumination. Amplicon sizes were anticipated to be 277 bp for blaOXA and 93 bp for blaTEM [17].

2.3 Selection of Representative Isolates

All the newly isolated isolates were screened for the presence of blaOXA and blaTEM genes. Multiple isolates harbored blaOXA or blaTEM separately, while only three multidrug resistant isolates harbored both resistance genes simultaneously. As a result, these isolates were selected for downstream gene expression analysis as they encompassed the target resistance genotype tested in this study [18].

2.4 Biosynthesis of Zinc Oxide Nanoparticles

Zinc oxide nanoparticles (ZnO-NPs) were biosynthesized using an aqueous extract of Matricaria chamomilla as a biological reducing and stabilizing agent. Zinc acetate dihydrate was used as the precursor salt, while sodium hydroxide solution was added to adjust the reaction pH to 9.0. The reaction mixture was continuously stirred at 70–80 °C until a white precipitate was formed, indicating nanoparticle formation. The precipitate obtained was collected, washed several times with distilled water, dried, and stored for its analyses. Green synthesis with plant extracts is both eco-friendlier but also cheap to create nanoparticles [19].

2.5 Characterization of ZnO Nanoparticles

The synthesized ZnO nanoparticles were characterized by various analytical methods. Optical properties of the nanoparticles were evaluated by UV–Visible Spectroscopy to confirm nanoparticle formation. Functional groups involved with nanoparticle stabilization were characterized using Fourier Transform Infrared Spectroscopy (FTIR). Surface morphology and particle distribution were analyzed using Atomic Force Microscopy (AFM) and Scanning Electron Microscopy (SEM), while crystallinity and phase purity were estimated by X-ray Diffraction (XRD) [20].

2.6 Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration (MIC) for biosynthesized ZnO nanoparticles against selective *P. aeruginosa* isolates was determined according to Clinical and Laboratory Standards Institute (CLSI) guidelines, using the broth microdilution procedure. The MIC was defined as the lowest concentration capable of completely inhibiting visible bacterial growth after incubation. The MIC of the present work was 1.56 mg/ml [21].

2.7 Exposure to Sub-Inhibitory Concentration of ZnO Nanoparticles

Three such *P. aeruginosa* isolates carrying blaOXA and blaTEM genes were exposed to a sub-MIC concentration (1.56 mg/ml) of biosynthesized ZnO nanoparticles for 18–24 h. untreated cultures served as controls. After incubation, bacterial cells were harvested for RNA isolation and gene expression testing.

2.8 RNA Extraction and Quantitative Real-Time PCR (qRT-PCR)

Total RNA was obtained from the treated and untreated bacterial cultures using TRIzol Reagent and was washed with 75% ethanol in the presence of chloroform phase separation and precipitated with isopropanol. The purified RNA was resuspended and the quality evaluated in nuclease free water before analysis [22]. The quantitative real-time PCR (qRT-PCR) was accomplished with a qTOWER iris Real-Time Thermal Cycler (Analytik Jena, Germany) method and the reaction was applied for 1-step RT-PCR assay. blaOXA and blaTEM gene expression levels were normalized with the housekeeping gene 16S rRNA. Relative gene expression levels were quantified by comparative Ct (2^-ΔΔCt) method. Amplification specificity was confirmed by melt curve analysis [23].

3. Results

3.1 Quantitative Real-Time PCR Analysis of blaOXA Gene Expression

Expression of the blaOXA gene was assessed in three different Pseudomonas aeruginosa isolates at a sub-MIC concentration (625 mg/ml) of biosynthesized ZnO nanoparticles, prior to and following the exposure period (18–24 hours). Relative gene expression was calculated using the 2^-ΔΔCt method with 16S rRNA as the reference gene.

The resulting results are shown in Table 1 and Figures 1 and 2. As measured before treatment, the mean relative expression value of blaOXA was 1.12 ± 0.37. After treatment with ZnO nanoparticles, the mean expression level was 0.32 ± 0.03. The fold-expression of treated isolates was invariably lower than untreated controls. A noticeable increase in Ct values was also noted following treatment with nanoparticles. The Ct values of blaOXA increased from 24.27–26.46 in untreated isolates to 27.85–29.94 in treated isolates, supporting a decrease in transcript abundance after exposure to ZnO nanoparticles. Although the blaOXA expression reduction was not significant (P = 0.171), the decrease in blaOXA gene expression of the intervention group was distinctly positive.

Table 1. Relative expression of the blaOXA gene in *Pseudomonas aeruginosa* isolates before and after treatment with biosynthesized ZnO nanoparticles.

Sample	blaOXA Ct	16S rRNA Ct	ΔCt	ΔΔCt	Fold Change
Before Treatment					
C 1	24.27	17.22	7.05	0.015	0.990
C 2	25.36	19.18	6.18	-0.855	1.809
C 3	26.46	18.57	7.89	0.855	0.553
Mean ± SE					**1.12 ± 0.37**
After Treatment					
C 1	29.30	20.33	8.97	1.935	0.262
C 2	29.94	21.21	8.73	1.695	0.309
C 3	27.85	19.40	8.45	1.415	0.375
Mean ± SE					**0.32 ± 0.03**
Probability (P-value)					**0.171 NS**

NS: Not significant (P > 0.05).

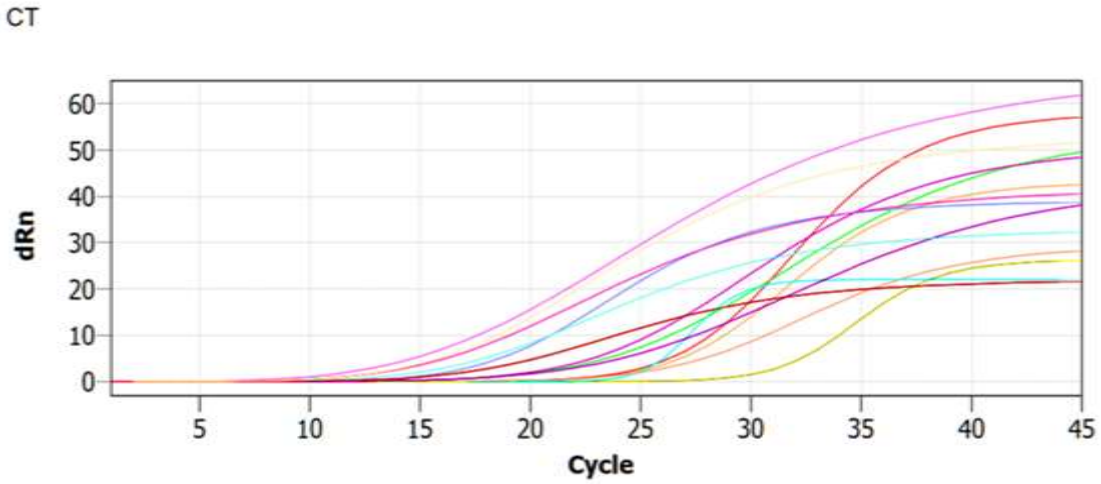

Figure 1: qRT-PCR-generated amplification plot of the blaOXA gene.

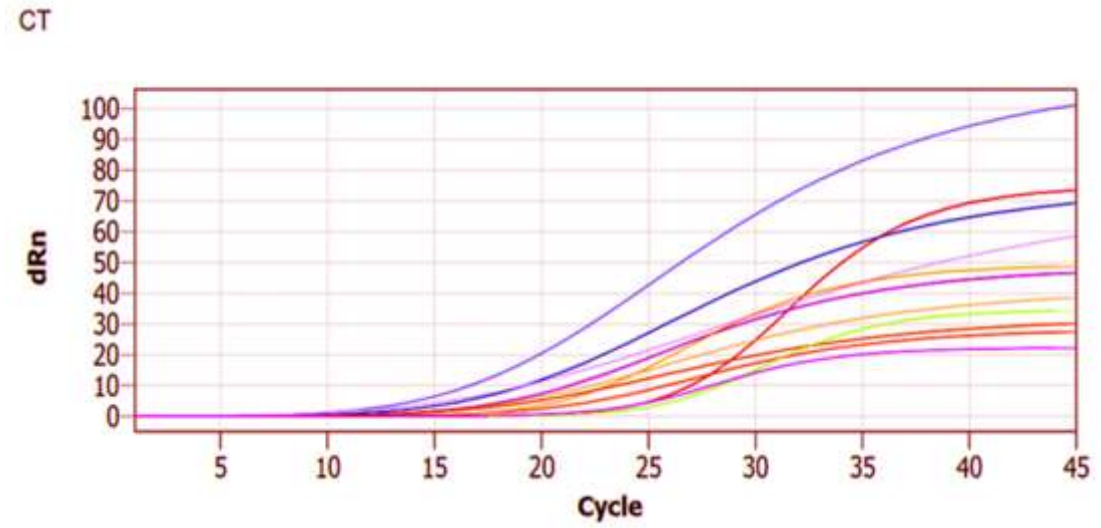

Figure 2: Relative expression profile of the blaOXA gene before and after ZnO nanoparticle treatment.

3.2 Quantitative Real-Time PCR Analysis of blaTEM Gene Expression

The effect of biosynthesized ZnO nanoparticles on blaTEM gene expression in the three Pseudomonas aeruginosa isolates was also evaluated using qRT-PCR analysis. Relative expression levels were normalized to the housekeeping gene 16S rRNA and calculated by the comparative Ct method.

The results shown in Table 2 and Figures 3 and 4 indicated that blaTEM expression decreased after treatment with ZnO nanoparticles. The average fold-expression decreased from 1.01 ± 0.02 in the untreated isolates to 0.78 ± 0.10 after treatment. The Ct values of blaTEM varied between 24.83–27.32 pre-treatment and 25.57–28.16 post-treatment. In general, treated isolates exhibited higher Ct values than untreated controls, suggesting lower transcript levels. The reduction was not statistically significant (P = 0.154). However, the expression levels of all treated isolates were lower than those of untreated control isolates.

Table 2: Relative expression of the blaTEM gene in Pseudomonas aeruginosa isolates prior to and after treatment with biosynthesized ZnO nanoparticles.

Sample	blaTEM Ct	16S rRNA Ct	ΔCt	ΔΔCt	Fold Change
Before Treatment					
C1	26.40	17.80	8.60	-0.020	1.014
C2	27.32	18.76	8.56	-0.060	1.042
C3	24.83	16.15	8.68	0.060	0.959
Mean ± SE					**1.01 ± 0.02**
After Treatment					
C1	25.57	16.45	9.12	0.500	0.707
C2	25.80	16.64	9.16	0.540	0.688
C3	28.16	19.44	8.72	0.100	0.933
Mean ± SE					**0.78 ± 0.10**
Probability (P-value)					**0.154 NS**

NS: Not significant (P > 0.05).

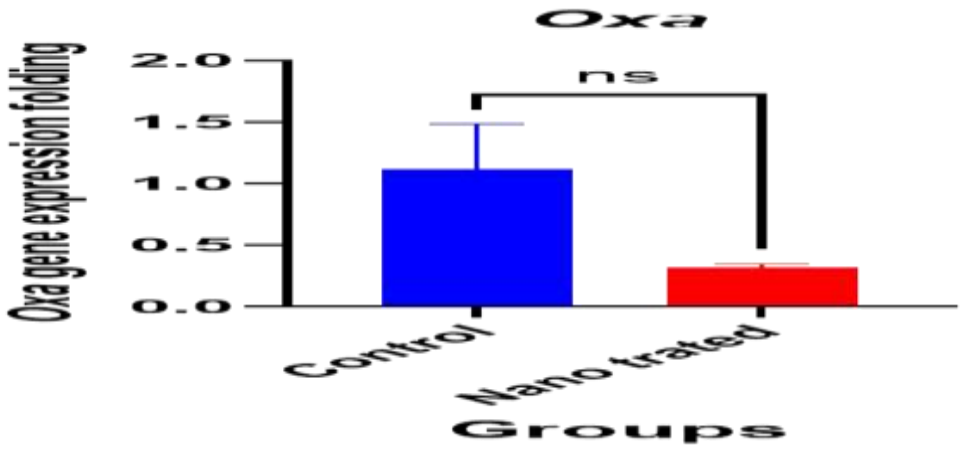

Figure 3: qRT-PCR-derived amplification plot of the blaTEM gene.

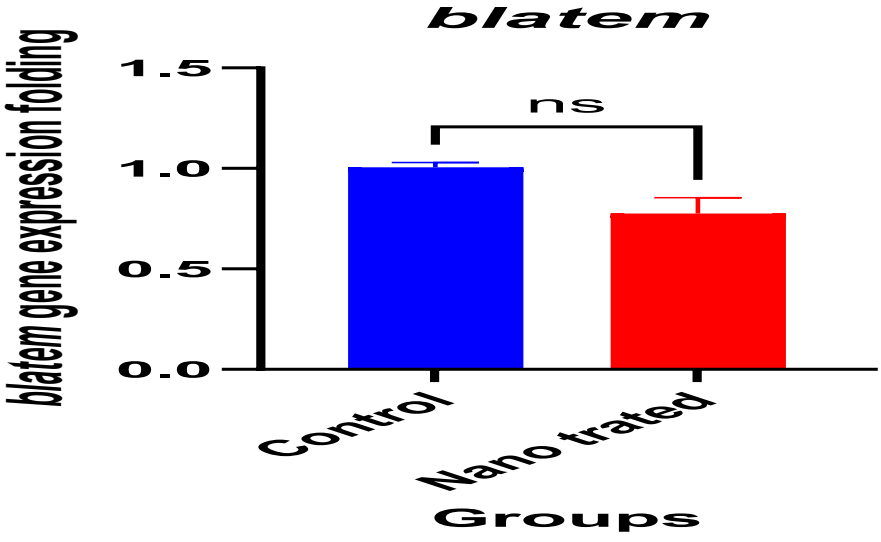

Figure 4: Relative expression profile of the blaTEM gene before and after ZnO nanoparticle treatment.

3.3 Comparative Response of blaOXA and blaTEM Genes to ZnO Nanoparticle Treatment

Results were evaluated, and the expression profiles of the two β-lactamase genes were compared. It was observed that blaOXA showed a greater decrease in expression after exposure to ZnO nanoparticles than blaTEM. Mean blaOXA expression decreased from 1.12 to 0.32, while blaTEM decreased from 1.01 to 0.78. The expression of blaOXA was approximately 71% reduced by fold-change, and blaTEM was approximately 23%. Neither reduction achieved statistical significance; nevertheless, the results indicate that ZnO nanoparticle treatment enhanced the response of blaOXA under the experimental conditions in this research.

4. Discussion

Antimicrobial resistance in *Pseudomonas aeruginosa* is among the most pressing issues in modern medical field as a result of the very high prevalence of many β-lactamase genes causing high resistance to β-lactam antibiotics in the whole population [24]. As a result, alternative strategies that may neutralize mechanisms of resistance have attracted greater interest, and metal oxide nanoparticles exhibit significant potential [25]. In the present investigation, ZnO nanoparticles were synthesized and the expression of blaOXA gene in the tested *P. aeruginosa* isolates was markedly suppressed. The average fold expression decreased from 1.12 ± 0.37 in untreated isolates to 0.32 ± 0.03 after the sub-MIC ZnO nanoparticle exposure (71% downregulation). Though not statistically significant ($P = 0.171$), all treated isolates consistently showed lower expression levels than untreated isolates. A single trend is indicative of a biologically relevant (compared to random) response from isolated cells to exposure to nanoparticle rather than a random pattern. Further confirmation of this hypothesis, the Ct values were increased in response to treatment post-treatment. Since Ct is the inverse of transcripts, change from 24.27–26.46 before to 27.85–29.94 after treatment reflects a decrease of blaOXA mRNA levels. Such results indicate that ZnO nanoparticles might suppress transcriptional function, but not the resistance gene. Interpretation of this data is consistent with the presence of the gene after post-treatment. Several mechanisms could account for the blaOXA expression suppression observed. Zinc oxide nanoparticles produce reactive oxygen species (ROS), such as hydroxyl radicals, superoxide anions, and hydrogen peroxide, which can promote oxidative stress in bacterial cells [26]. High ROS concentrations may have a role in cellular membrane, protein, and nucleic acid damage which may disturb established gene transcription regulation. Oxidative stress may also disturb transcription factors and global regulatory networks governing antibiotic resistance determinants [27], which can lead to decreased expression of β-lactamase genes. These results are consistent with earlier studies showing the possible binding of ZnO nanoparticles to regulate bacterial gene expression. Alshaibani et al. [28] reported that ZnO nanoparticles markedly modified the transcription of several antimicrobial resistance genes in Gram-negative bacteria by oxidative stress-mediated mechanisms. Similarly, Ahmed et al. [29] also showed that after nanoparticle treatment with nanomaterials, the expression of resistance-associated determinants was reduced, so a potential dual function of nanomaterials may operate not only as antibacterial agents but as resistance-modifying substances. Ibrahim et al. [30] observed further downregulation of the β-lactamase-related genes after treatment by metal nanoparticles. This suggests that a strain of drug-induced stress which was caused by nanosized ones could influence resistance gene regulation. Unlike blaOXA, the blaTEM gene showed a more moderate response to ZnO nanoparticle treatment. The mean expression level decreased from 1.01 ± 0.02 to 0.78 ± 0.10, with a 23% decrease in expression. Despite the aforementioned statistically non-significant decrease ($P = 0.154$) it may suggest that blaTEM expression was not affected as significantly as blaOXA under the same experimental conditions. These changes in the response of blaOXA and blaTEM imply that resistance-based factors are not equally responsive to manipulation by nanoparticles. The same observation was observed by Singh et al. [32], which demonstrated that different bacterial species, nanoparticle functionalities, and experimental conditions conditioned nanoparticle intervention on resistance genes. This complexity illustrates the difficulty in defining bacterial stress response, and highlights the necessity of gene-specific assessment for antimicrobial resistance modulation. A salient finding from the present study is that the presence of blaOXA and blaTEM genes before and after treatments were verified by conventional PCR, whereas qRT-PCR demonstrated inhibition of transcriptional activities for the nanoparticles. The biological implication of this distinction is that, in fact, ZnO nanoparticles did not eliminate the genetic determinant, rather they affected its expression. Meaning, mRNA levels reduction could result in less β-lactamase being synthesized as well as possibly lower resistance phenotype but that association should be confirmed by further proteomic and phenotypic studies [33]. Statistically insignificant results in the current study are also cautionary. There were only three representative isolates encoding for each resistance gene, which restricted statistical power. Nevertheless, the continuous decrease between all treated isolates, especially as it pertains to blaOXA, indicates that ZnO nanoparticles had an observable biological effect and require further investigation using larger numbers of samples and related resistance determinants. Their limitations have previously been recognized in the molecular studies of gene expression based on clinical bacterial isolates [34]. Taken together, the results show that biosynthesized ZnO nanoparticles may affect transcriptional activity of clinically relevant β-lactamase genes in P. aeruginosa. The higher suppression for blaOXA compared to blaTEM indicates selective action of the ZnO nanoparticles on specific types of resistance pathways. These findings confirm the emergence of potential nanoparticles for use as

adjunctive strategies for the challenge of antimicrobial resistance rather than as standalone antibacterial agents.

5. Conclusion

In this study, novel biosynthesized zinc oxide nanoparticles

- affect the expression of crucial β-lactamase resistance genes in
- Clinical *Pseudomonas aeruginosa* isolates.

Quantitative real-time PCR analysis demonstrated that expression levels of blaOXA and blaTEM genes decreased after a sub-MIC concentration (1.56 mg/ml) of ZnO nanoparticles was applied. BlaOXA had a much higher downregulation of 71% compared with the blaTEM, indicating moderate suppression of 23%. While the changes were not significant, all treated isolates showed lower expression levels compared with untreated controls, consistent with a biologically relevant response to nanoparticle exposure. Findings suggest that the ZnO nanoparticles may not directly target genes for killing but could instead disrupt the transcriptional activity of resistance genes. Collectively, the findings indicate a possible use of biosynthesized ZnO nanoparticles as molecular resistance modulators inhibiting the expression of clinically relevant β-lactamase genes in *P. aeruginosa*. More diverse isolates and other resistance determinants should be studied to discover the molecular mechanisms on how nanoparticle and nanomaterials regulate genes and their potential use as antimicrobial therapies adjuvant.

6. Future Perspectives

Future investigations of biosynthesized ZnO nanoparticles on more antimicrobial resistance genes, and the effect of transcriptional suppression on phenotypic antibiotic susceptibility may be required. Comprehensive transcriptomic and proteomic studies might provide more depth to elucidate the molecular elements of nanoparticle-dependent gene modulation.

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