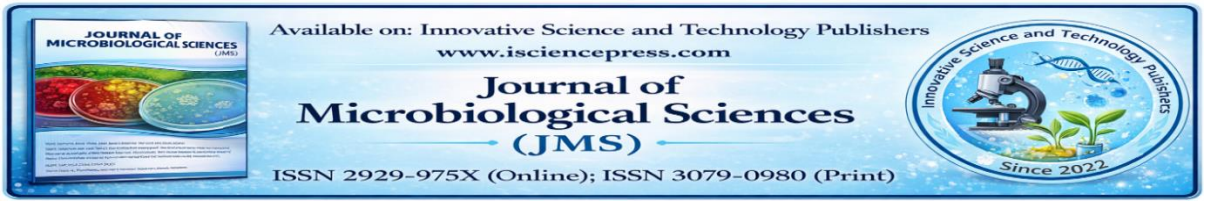
Research Article

J. Micro. Sci. Vol. 5(1), 74-83, **2026,**
https://doi.org/10.38211/jms.2026.05.210

OPEN ACCESS



Green Synthesis of Nickel Oxide Nanoparticles using pea peel extract for Attenuation of Quorum Sensing and Biofilm Formation in *Pseudomonas aeruginosa* QS107

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Article Received 16-01-2026, Article Revised 01-02-2026, Article Accepted-20-03-2026

ABSTRACT

Quorum sensing (QS) provides a means of communication between bacteria, including pathogenic bacteria. QS plays a crucial role in regulation of virulence factors, allowing bacterial communities to arrive at the required infection levels. The increase of multi-drug resistant (MDR) biofilm forming *Pseudomonas aeruginosa* strains necessitates the development of alternative therapeutic strategies that target bacterial virulence rather than cell viability. QS and biofilm formation are the key mediators of pathogenicity among MDR *P. aeruginosa*. The aim of this study was to investigate anti-quorum sensing and antibiofilm activity of nickel oxide nanoparticles (NiO-NPs) against MDR *Pseudomonas aeruginosa* QS107 (OR756454). *P. aeruginosa* strain QS107 was identified through 16S rRNA sequencing. NiO nanoparticles were synthesized via an eco-friendly method using pea peel extract (*Pisum sativum*) as reducing and stabilizing agents. The MDR profile of *P. aeruginosa* QS 107 was determined by Kirby-Bauer disc diffusion method using a standard panel of antibiotics. The production of *N*-acyl homoserine lactone (AHL) based QS signaling molecules was investigated using a bacterial biosensor *Agrobacterium tumefaciens* A136. Biofilm formation was confirmed by crystal violet ring method. The potential of NiO nanoparticles against QS and biofilm formation was carried out using well diffusion agar plate assay. The results of this study revealed that *P. aeruginosa* QS107 exhibited antibiotic resistance against more than five classes of standard antibiotics. Thus, antibiotic sensitivity testing confirmed QS107 as MDR bacterial strain. The highest antibiotic resistance was observed against Ticarcillin, Piperacillin, Imipenem, and Ciprofloxacin. Moreover, biosensor assay confirmed the robust production of AHL based QS signaling molecules by QS107 isolate. Mainly, NiO-NPs inhibited QS system and effectively disrupted biofilm formation in *P. aeruginosa* QS 107 at sub-inhibitory concentration. The green synthesized NiO-NPs showed potent quorum-quenching and antibiofilm activity against a clinical isolate of MDR *P. aeruginosa* QS107. These results suggest that pea peel derived NiO-NPs could serve as potential therapeutic agent to overcome virulence of *P. aeruginosa* infections where traditional antibiotics fail.

Key words: *P. aeruginosa*; Quorum sensing (QS); Biofilm formation; Nanoparticles; Nickel oxide

INTRODUCTION

Pseudomonas aeruginosa is a prominent Gram-negative bacterium of *Pseudomonadaceae* family characterized by its ability to thrive in diverse environmental niches (Patrick *et al*., 2025). It is widely recognized as a significant opportunistic pathogen, frequently implicated in hospital -acquired infections and ventilator-associated pneumonia (Soomro *et al*., 2024). The global escalation of multi-drug resistance (MDR) pathogens has positioned *P. aeruginosa* as the highest risk in clinical settings (Xie *et al*., 2025). *P. aeruginosa* is a leading cause of chronic infections, particularly in cystic fibrosis patients and those with compromised immune system (Borisova *et al*., 2025). The remarkable survival of *P. aeruginosa* is greatly attributed due to its ability to form biofilm, a complex multicellular aggregate encased in a self-produced matrix of extracellular polymeric substances, including polysaccharides, proteins and nucleic acids (Xie *et al*., 2025). The biofilms function as a physical barrier that restricts antibiotic penetration and protects bacterial cells making infections more difficult to treat. Current studies highlight an alarming increase in *P. aeruginosa* resistance mediated by horizontal gene transfer and upregulation of efflux pumps like Mex-AB-OprM (Tripathi *et al*., 2023). The global escalation of MDR

P. aeruginosa phenotypes has rendered most conventional antibiotics, including carbapenems and polymyxins, increasingly ineffective (Piotrowski *et al*., 2024). Further, the ability of this pathogen to transition into a biofilm-mediated state increases antibiotic resistance (Ramesh *et al*., 2025).

The pathogenicity of *P. aeruginosa* is coordinated by quorum-sensing (QS) system, a density dependent cell to cell communication system. The communication system is primarily regulated by *Las* and *rhl* systems, utilizing signaling molecules called *N*-acyl homoserine lactones (AHLs) (Patrick *et al*., 2025; Jatt, 2023). QS is critical mechanism for pathogenic microorganisms, as it coordinates the expression of virulence factors only when the bacterial population reaches a threshold density. The synchronized behaviour enables the pathogen to overwhelm host immune defences, thereby enhancing its survival and colonization success. Understanding the genetic and molecular basis of biofilm formation in *P. aeruginosa* is of utmost importance due to its association with increased antibiotic resistance and treatment failure. Through QS system, bacteria coordinate the release of harmful toxins, enzymes and maturation of biofilms, allowing the bacterial colony to attack the host's immune system, as a single powerful unit (Xie *et al*., 2025). This collective QS based behaviour creates a physical barrier that traditional antibiotics cannot penetrate, often leading to treatment failure. Consequently, this imposes selective pressure that drives a further increase in antibiotic resistance. In this regard, there is a critical need of quorum-quenching (QQ), a strategy that inhibits QS system and disarms pathogens without killing them.

Among QS inhibitor agents, nanoparticles (NPs) have emerged as a revolutionary tool due to their unique physicochemical properties (Touati *et al*., 2025). Mainly, NiO-NPs have gained significant attention for their effective antimicrobial and antibiofilm activities (Irum *et al*., 2021). Recent studies suggest that NPs can attenuate the growth and biofilm formation of *P. aeruginosa* by disrupting cell membrane integrity and interfering with molecular mechanism of QS (Irum *et al*., 2021; Maruthupandy *et al*., 2020). Unlike bulk substances, these NPs can penetrate the dense EPS matrix of a biofilm to interact directly with the bacterial cells (Xie *et al*., 2025). Despite extensive research on various metallic NPs, yet the specific potential of NiO-NPs in inhibition of QS pathways remains largely unexplored.

This study was carried out to evaluate the efficacy of NiO-NPs in attenuating QS and inhibiting biofilm formation in the clinical isolate of *P. aeruginosa* QS107. The current research investigates the impact of NiO-NPs on QS regulated virulence traits. This study seeks to provide a strategy to develop NiO-NPs based inhibitors that can control bacterial pathogenicity and enhance the performance of existing antibiotic therapy.

MATERIALS AND METHODS

Bacterial strains and culture conditions: The clinical isolate of *Pseudomonas aeruginosa* strain QS107 (OR756454) was recovered from a pus sample using standard microbiological sampling protocol. The study was approved by the advanced studies and research board (ASRB), University of Sindh, Jamshoro, Pakistan, vide letter No. DRGS/1905-2024. Preliminary identification was performed through biochemical characterization including oxidase and cetrimide tests (Soomro *et al*., 2024). The QS107 isolate was further identified based on 16S rRNA gene sequence analysis (Patrick *et al*., 2025). The resulting gene sequence was blasted at NCBI database (www.ncbi.nlm.nih.gov./genbank/).The biosensor strain *Agrobacterium tumefaciens* A136 used for quorum sensing assays was utilized and maintained on Luria-Bertani (LB) medium. All bacterial strains were preserved in 20% glycerol and stored at -80 °C. The bacterial strains were sub-cultured in Luria - Bertani (LB) broth at 37 °C for 2hr prior to experimental work.

Green synthesis of Nickel oxide nanoparticles (NiO NPs): NiO-NPs were synthesized using eco-friendly approach utilizing pea peel (*Pisum Sativum*), as reducing and stabilizing agents as per method described by Ahmad & Kalra, (2020), with a slight modification. Briefly, cleaned and dried pea peels were boiled in deionized water to obtain an aqueous extract. Briefly, cleaned and dried pea peels were boiled in deionized water to obtain an aqueous extract. A 20 mL of the obtained extract was added dropwise to 80 mL of 0.1M nickel nitrate solution (1:4 v/v) with constant stirring. The resulting solution was dried at 100 °C and calcined at 400 °C for 3h to obtain crystalline NiO-NPs. The nanoparticles were characterized using Ultraviolet-Visible **(**UV-Vis) spectroscopy, Fourier transform infrared (FTIR) spectroscopy, and X-ray diffraction **(**XRD), and Scanning Electron Microscopy (SEM) analysis to confirm their size and morphology (Alarjani *et al*., 2022).

Antibiotic sensitivity Testing: The antibiotic resistance profile of *Pseudomonas aeruginosa* QS107 was investigated using the Kirby-Bauer disc diffusion method, following the Clinical Laboratory Standards Institute (CLSI) guidelines M100-S35 (2025). Antibiotic susceptibility testing was performed on Muller Hinton Agar (MHA), prepared according to manufacturer's instructions. QS107 culture suspension was inoculated on MHA agar medium followed by placing the selected antibiotics. The medium plate was incubated at 37 ºC for 24 hrs. A panel of antibiotics (listed in Table 2) awas evaluated against the clinical isolate of QS 107.

Table 1. List of antibiotics, corresponding potencies, and source of antibiotic discs used for antibiotic susceptibility testing.

Antibiotic Name	Potency (Concentration)	Manufacturer
Colistin	10 µg	Oxoid Ltd., UK
Ofloxacin	5 µg	Oxoid Ltd., UK
Levofloxacin	5 µg	Oxoid Ltd., UK
Ciprofloxacin	5 µg	Oxoid Ltd., UK
Amikacin	30 µg	Oxoid Ltd., UK
Gentamicin	10 µg	Oxoid Ltd., UK
Imipenem	10 µg	Oxoid Ltd., UK
Ceftazidime	30 µg	Oxoid Ltd., UK
Cefepime	30 µg	Oxoid Ltd., UK
Ticarcillin/Clavulanic acid	10 µg	Oxoid Ltd., UK

Detection of AHL based QS signaling molecules: The clinical isolate of *P. aeruginosa* QS107 was screened qualitatively for the production of *N*-acyl homoserine lactone (AHL) based QS signaling molecules using *A. tumefaciens* A136 biosensor bacterium by agar plate cross-feeding bioassay (Patrick *et al*., 2025; Jatt *et al.,* 2023). Briefly, the test strain was co-cultured with the reporter strain *Agrobacterium tumefaciens* A136 on LB agar supplemented with X-gal (40 µg/mL) as a chromogenic substance. An AHL deficient bacterial strain served as a negative control to validate the specificity of the assay. The plates were incubated at 35 °C for 24hrs. The production of AHLs was confirmed by the production of blue color pigmentation in A136 reporter strain (Patrick *et al*., 2025).

Anti-quorum sensing activity of NiO-NPs: The ability of NiO-NPs to inhibit the QS system was evaluated using well diffusion agar plate assay. Briefly, the cell-free supernatant was separated by centrifugation at 10,000 rpm for 15 minutes. The obtained supernatant was then tested against the biosensor strain *A. tumefaciens* A136. Wells (approximately 6 mm in diameter) were punched into LB-agar plate supplemented with X-gal (40 µg/mL), and 60 µL of each supernatant was added to respective wells (Jatt *et al*., 2015). Inhibition of QS signaling molecules was confirmed by the absence of blue pigmentation (Touati *et al*., 2025).

Biofilm formation and Antibiofilm activity of NiO-NPs: The biofilm forming ability of *P. aeruginosa* QS107 strain was determined qualitatively by tube method and quantified through crystal violet staining, following the protocol of Eladawy *et al*. (2020). Briefly, a fresh bacterial culture was inoculated into 10 mL of a tryptic soya broth (TSB) tube supplemented with 1% glucose and incubated statically at 37°C for 24hrs. Sterile TSB without bacterial inoculation served as a negative control. After incubation, culture was aspirated, and the adhered biofilm was washed with phosphate-buffered saline (PBS, pH 7.4). After air drying, tubes were kept in inverted position for 30-45 minutes for complete dryness. Subsequently, tubes were stained with 1% crystal violet for 15 minutes. Tubes were washed with distilled water to remove excess stains. The formation of a clear ring at the air-liquid surface qualitatively indicated biofilm formation.

The antibiofilm activity of NiO-NPs was evaluated at concentrations of 30, 60, and 100 µg/mL, which were confirmed as sub-inhibitory doses (Sub-MIC). To quantify the percentages of biofilm inhibition and disruption, the bound dye was solubilized with 30% glacial acetic acid. The optical density (OD) of the resulting solution was measured at 570 nm using a spectrophotometer. The percentage of inhibition was calculated based on the reduction in OD compared to the untreated control (Khan *et al*., 2023; Salim *et al*., 2025).

Statistical analysis: All experiments were performed in triplicate, and the results are expressed as mean ± standard deviation (SD). Statistical significance was evaluated using one-way analysis of variance (ANOVA). p-value <0.05 was considered as statistically significant (Khan *et al*., 2019).

RESULTS

Bacterial strains and culture conditions: The clinical isolate obtained from a pus sample, designated as QS107 exhibited characteristics growth on cetrimide agar, appearing greenish-blue colonies. Phenotypic characteristic was confirmed through biochemical testing, where QS107 bacterial strain showed positive reactions for oxidase test, consistent with the profile of *Pseudomonas aeruginosa* (Figure.1). Further, molecular identification via 16S rRNA gene sequencing confirmed the QS107 as *P. aeruginosa*, with accession number OR756454. NCBI blast tool revealed 99.9% similarity with the existing *P. aeruginosa* type strain in the GenBank database.

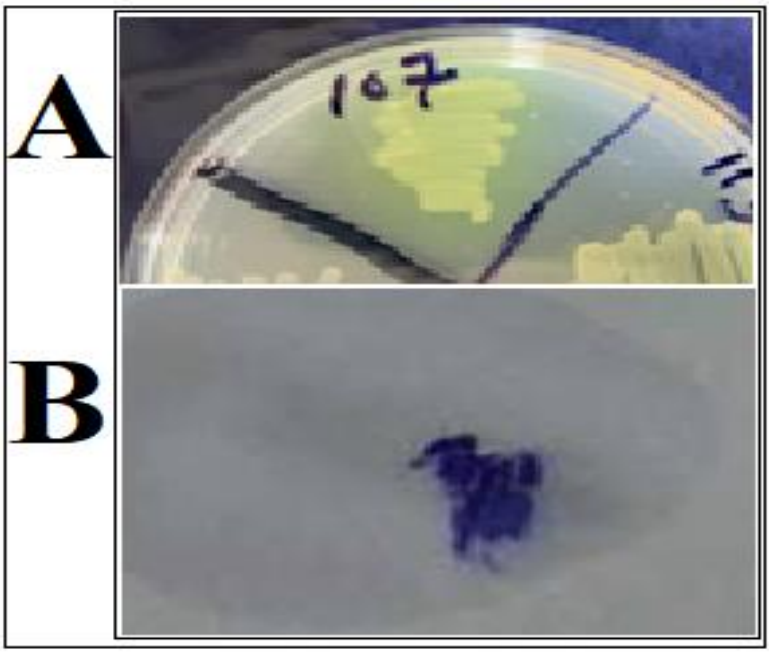


Figure 1. Selective growth of *P. aeruginosa* QS107 on Cetrimide agar (A), biochemical validation via Oxidase test showing a positive result (B).

Green synthesis of nickel oxide nanoparticles (NiO-NPs): Primarily, the green synthesis of NiO-NPs was determined by a clear change in color of the reaction mixture. Spectrophotometric analysis via UV-Vis Spectroscopy confirmed a sharp absorption peak at the wavelength of 360 nm. The disappearance of absorption peaks for characteristic Ni (II) absorptions occurring at wavelengths of 315, 349, 365, and 700 nm following the bioreduction process suggests that the reduction of nickel to NiO-NPs has taken place. Characterization via XRD analysis further confirmed the identity of the NiO-NPs. The diffraction pattern exhibited prominent peaks at 2θ values of 37.2º, 43. 26º, 44.4º, 62.9º, which correspond to the face-cantered cubic (FCC) crystal structure of NiO with the approximate size of 30 nm. The results obtained consistent with our XRD pattern are presented for the synthesis of NiO-NPs, where the XRD pattern indicated a presence of distinct diffraction peaks at 2θ = 37.28°, 43.29°, 62.9°, and 75.44° corresponding to the crystal planes of (111), (200), (220), and (311), respectively. These peaks are consistent with the face-centered cubic structure of NiO, thus demonstrating that nickel oxide nanoparticles have been successfully synthesized. Furthermore, SEM analysis confirmed the formation of NiO-NPs, revealing their surface morphology and distribution. The FTIR spectrum of the aqueous pea peel extract showed various peaks due to plant-derived biomolecules that were involved in reducing and stabilizing the nanoparticles during their preparation. The broad peak observed at about 3333 cm^{-1} corresponds to O-H stretching vibration of phenols, alcohol, and polysaccharide molecules, showing strong hydrogen bonding among them. Another important peak at 2924 cm^{-1} indicates the presence of lipids and carbohydrates through the stretching vibration of the C-H bond in aliphatic structures. Moreover, the prominent peak at about 1615 cm^{-1} suggests the involvement of proteins and phenolic structures through the vibrations of C=O (amide I) and/or aromatic C=C bonds. Additional peaks around 1516 and 1400 cm^{-1} indicate aromatic and carboxylate group vibration, respectively, whereas the very intense peak around 1014 –1030 cm^{-1} suggests the presence of C-O stretch vibrations in alcohol and polysaccharide molecule (Figure . 2).

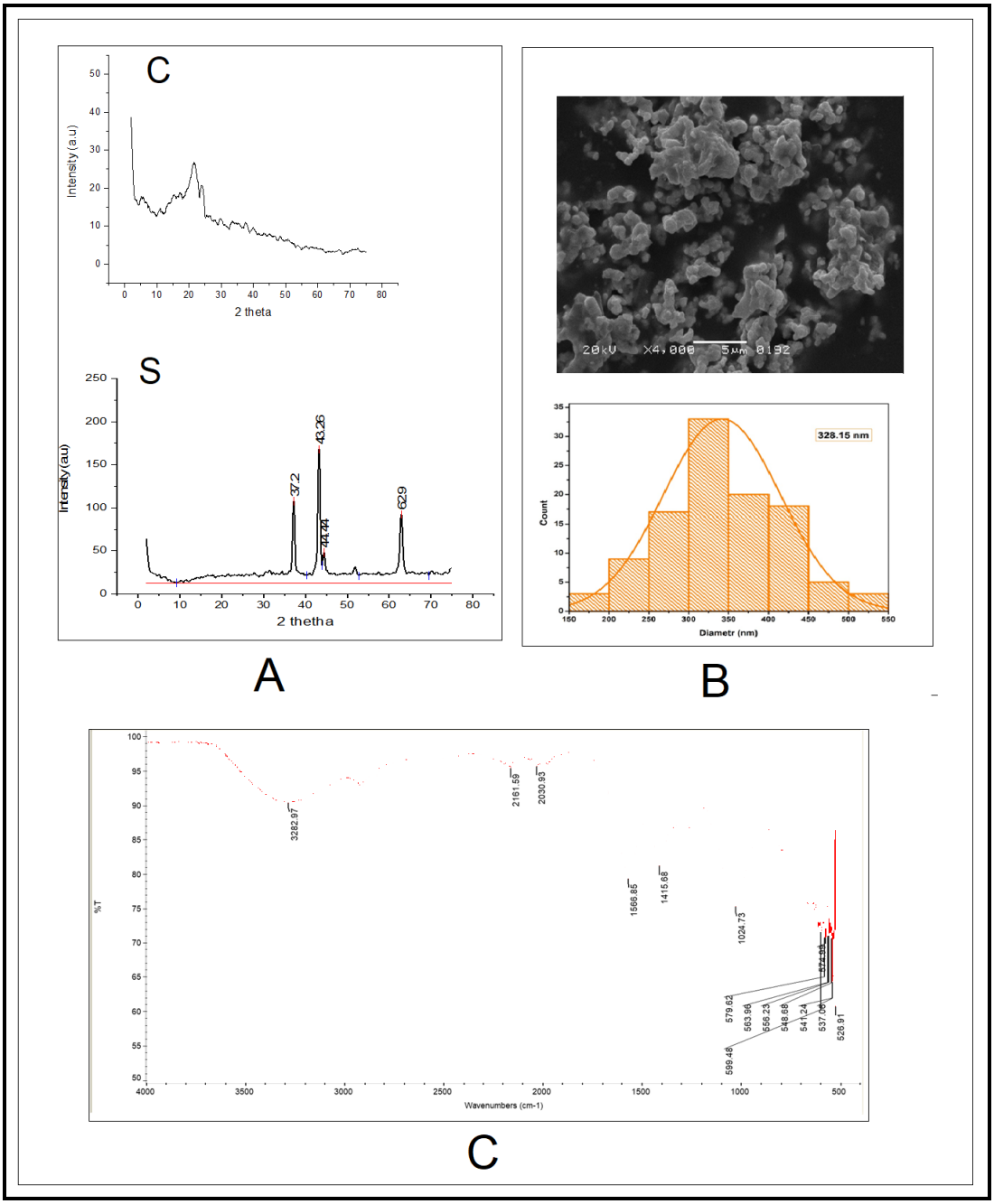


Figure 2. Structural and morphological characterization of green synthesized NiO-NPs. (A) XRD patterns of control (C) and the synthesized nanoparticles sample (S). (B) SEM micrograph displaying surface morphology. (C) FTIR spectra illustrating the functional groups involved in stabilization.

Antibiotic Sensitivity Testing: Antibiotic sensitivity testing results revealed *P. aeruginosa* QS107 as a multi-drug resistant (MDR) pathogen. QS107 showed resistance against various antibiotics, including Ticarcillin, Piperacillin, Imipenem, and Ciprofloxacin, Gentamicin, and Ceftazidime (Fig. 3). *P. aeruginosa* QS107 was found susceptible to Levofloxacin, Amikacin, Colistin and Piperacillin/Tazobactam, Ticarcillin / Clavulanic acid and Cefepime. *P. aeruginosa* QS107 was confirmed as multi-drug resistant (MDR) bacterium, because it exhibited resistance to various antibiotics used in this study.



Figure 3. Antibiotic sensitivity of *P. aeruginosa* QS107

Detection of AHL based QS signaling molecules: The production of AHL based QS signaling molecules was confirmed by cross feeding bioassay using A136 reporter strain. The results revealed a clear change of color into blue color, thus confirmed the production of AHL signaling molecules by the test strain QS107 (Figure. 4).

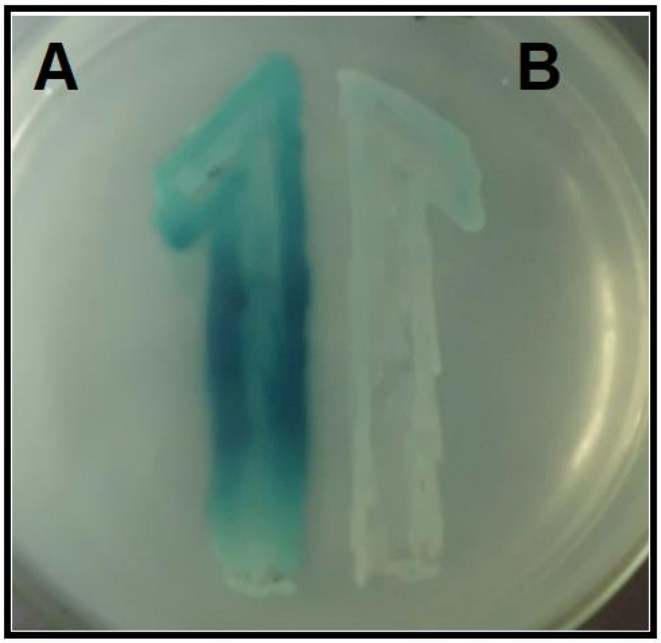


Figure 4. AHL production in presence of reporter strain *A. tumefaciens* A136 (A), and a test strain *P. aeruginosa* QS107 (B).

Anti-Quorum Sensing activity of NiO-NPs: The potential of NiO-NPs to disrupt bacterial intercellular communication system was evaluated by inhibition of the production of AHL based QS signaling molecules. Anti-quorum sensing assay using well-diffusion bioassay in presence of reporter strain clearly reduced the production of AHL signaling molecules. Moreover, no significant effect on bacterial growth kinetics was observed ($P>0.05$) at sub-MIC levels of NiO-NPs (30, 60, and 100 µg/mL) as summarized in Table 2

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Table 2. Bacterial growth (OD_{600}) in the presence of sub-MIC NiO-NPs.

Time (h)	**Control** (Untreated)	**NiO-NPs** (30 µg/mL)	**NiO-NPs** (60 µg/mL)	**NiO-NPs** (100 µg/mL)
0	0.03±0.01	0.03±0.01	0.03±0.01	0.03±0.01
12 (Mid-log)	0.87±0.05	0.85±0.04	0.82±0.04	0.81±0.05

Values are presented as Mean ±SD(n+3), P>0.05 vs. control

Biofilm formation and Antibiofilm activity of NiO-NPs: Biofilm formation assay revealed *P. aeruginosa* QS107 as a strong biofilm forming bacterial pathogen, A clear ring formation was observed by crystal violet assay after 48h of incubation. The results of antibiofilm activity of NiO-NPs demonstrated potent antibiofilm efficacy after treating with NiO-NPs (Fig. 5). Further, quantitative elution of the crystal violet stain confirmed the suppression of initial biofilm at sub-MIC levels of NiO-NPs with a potent disruptive effect on pre-formed biofilm.

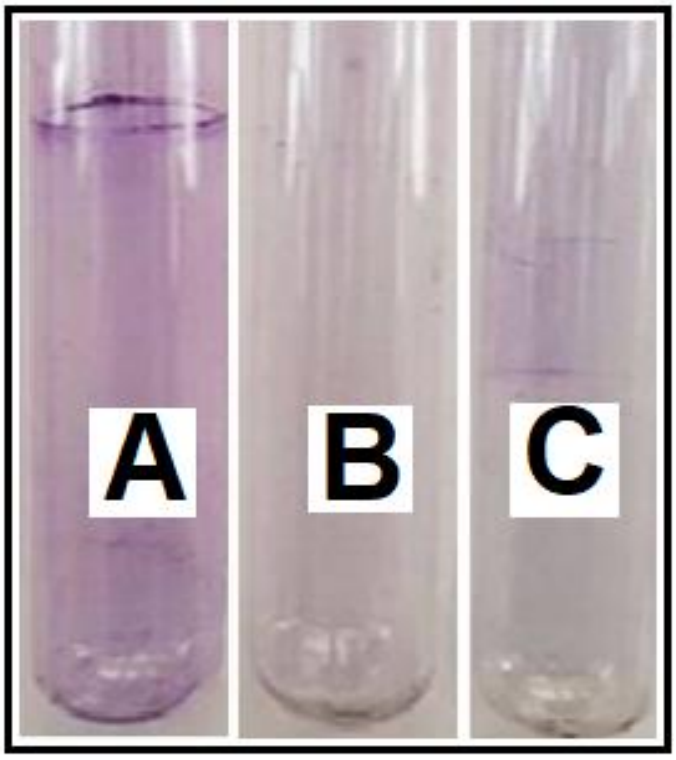


Figure 5. Biofilm formation by QS107 (A), Treating with NiO-NPs (B), Negative control (C)

DISCUSSION

Quorum sensing (QS) is the signal transduction network that serves as a master regulator of bacterial pathogenesis. *P. aeruginosa* has been recognized as a critical priority pathogen due to its complex QS network, which plays a crucial role in regulation of virulence factors and biofilm formation (Ho *et al*., 2025). The emergence of multi-drug resistant (MDR) *P. aeruginosa* strains necessitates the consideration of non-traditional therapeutic strategies, specifically the application of nanotechnology (Hetta *et al*., 2023). The green synthesis of nanoparticles is of great advantage due to their ecofriendly nature and the potential capping of nanoparticles with bioactive phytochemicals, which may enhance their biological activity (Mahdi Salih *et al*., 2025). This study reveals that the green synthesized nickel oxide nanoparticles (NiO-NPs) act as potent quorum sensing (QS) inhibitors against a clinical isolate of *P. aeruginosa* QS107. NiO-NPs synthesized in this study exhibited higher anti-quorum sensing and antibiofilm activity at lower concentrations. A previous study reported biofilm inhibition of *P. aeruginosa* using NiO-NPs at concentrations of 100-500 µg/mL (Al-Shabib *et al*., 2018). However, in this study NiO-NPs achieved a comparable reduction at sub-MIC levels of 30-100 µg/mL. This enhanced bioactivity can be attributed to the unique synergistic effect of the phenolic compounds from *P. sativum* peel, which act as efficient capping agents, potentially increasing the surface to volume ratio and bioavailability of the nanoparticles compared to those synthesized via traditional chemical routes (Priya *et al*., 2023).

This study revealed the identification of a clinical isolate as a *P. aeruginosa* QS107 (OR756454), based on biochemical characterization followed by molecular identification using 16S rRNA gene analysis. Proper identification of the pathogenic

bacteria is a very crucial in adopting proper antibiotic therapy, ant-quorum sensing and antibiofilm assays. The use of 16S rRNA gene sequencing remains the widely accepted and standardized method offering unmatched reliability and accuracy in bacterial classification (Patrick *et al*., 2025). Initial screening of the test strain QS107 confirmed the robust production of AHL signaling molecules. When streaked together with A136 reporter strain, a distinct blue coloration was observed (Fig. 4). The production of blue coloration indicates the high levels of TraR-mediated beta galactosidase activity (Jatt *et al*., 2023). Antibiotics sensitivity testing results revealed that strain QS107 exhibits a multi-drug resistance (MDR) phenotype, showing resistance to several classes of antibiotics including Ticarcillin, Piperacillin, Imipenem, and Ciprofloxacin, Gentamicin, and Ceftazidime. Previously, *P. aeruginosa* has also showed the highest resistance to various antibiotics including Ticarcilin and Ofloxacin (Davarzani *et al*., 2021; Flores-Velazquez and Perez, 2021). The findings of this study also categorize this bacterium as an MDR pathogen, consistent with global trends of escalating resistance in *P. aeruginosa* clinical isolates.

NiO-NPs were successfully synthesized via an eco-friendly approach pea peel extract. The NiO nanoparticles in XRD analysis showed peaks at 2θ values of 37.2º, 43. 26º, 44.4º, 62.9º corresponds to NiO crystal structure, similar results for NiO-NPs were obtained previously (Ali *et al*., 2022). On the other hand, the FTIR analysis of NiO nanoparticles exhibited significant shifts and changes in intensities of these functional groups, thus proving their contribution to the synthesis process. It was observed that the O-H absorption band has shifted to approximately 3282 cm-1 with decreased intensity, meaning that it participated in the reduction process and stabilization. The band at approximately 1615 cm-1 has shifted to 1566 cm-1, proving the contribution of carbonyl and aromatic functional groups to complexation of nickel ions. Moreover, there is also a band at approximately 1415 cm-1, thus implying that carboxylate functional groups participated in metal complexation. Finally, the C-O stretching vibration band has shifted to 1024 cm-1, proving that polysaccharides participated in the capping of nanoparticles. Most importantly, there have been detected distinct new absorption bands within the range of ~599-526 cm^{-1} representing Ni-O bonds. Similar results for green synthesis NiO-NPs are reported, a broad band appeared at around ~3400 cm^{-1} due to the N-H stretching mode, which suggests the presence of organic materials such as proteins and metabolites that functioned as capping and stabilizing agents during the green synthesis process. The appearance of peaks in the fingerprint region indicates the contribution of organic functional groups associated with the surfaces of nanoparticles. Furthermore, the observation of weak peaks at ~468, ~522, ~630, ~719, and ~833 cm^{-1} is attributed to the Ni-O stretching vibration, belonging to the range of the metal-oxygen vibration (~1000-400 cm^{-1}) (Ahmed *et al*., 2022; Olajire and Mohammed, 2020).

The resulting NiO-NPs demonstrated potent anti-quorum sensing activities in *P. aeruginosa* QS107. This is highly important because QS system in *P. aeruginosa* is a command center for virulence. The mechanism by which NPs interfere with the QS system in bacteria involves both physical and chemical interactions with the cell envelop and signaling proteins (Hayat *et al*., 2019). Unlike traditional antibiotics that target essential metabolic pathways leading to high selective pressure, NPs disrupt the signaling pathway, which may potentially disrupt the communication required for biofilm formation. These findings underscore the critical role of nanotechnology in developing effective strategies to combat MDR bacteria. The anti-biofilm assay also showed the promising results. In general, biofilms are responsible for 80% of the persistent human bacterial infections due to EPS matrix which works as a physical barrier to antibiotics (Singh *et al*., 2021). The current results of antibiofilm assay suggest that NiO-NPs either inhibit initial attachment of bacterial cells or interfere with the EPS production regulated by QS system. Targeting the QS-mediated biofilms in *P. aeruginosa* represents a primary strategy for addressing the challenges of multidimensional antibiotic resistance. The novelty of this study lies in the eco-friendly green synthesis of NiO-NPs using pea peel (*Pisum sativum*) extract as a sustainable reducing and capping agent. While a recent study reported the synthesis of NiO-NPs for environmental remediation via photocatalysis (Haq *et al*., 2025), to our knowledge this is the first report where NiO-NPs synthesized from pea peels are used as anti-quorum sensing and anti-biofilm activity against MDR *P. aeruginosa.* Our "waste-to-wealth" approach provides a cost-effective fabrication method and identifies a novel, non-lethal strategy for combating persistent bacterial infections. Future research should focus on in vivo validation of NiO-NPs using animal models to confirm their safety and therapeutic efficiency in living system. Additionally, molecular docking of *P. sativum*-capped NiO-NPs with QS receptor proteins could provide deeper mechanistic insights into the binding affinity and signal disruption observed in this study.

CONCLUSION

This study successfully demonstrates the eco-friendly synthesis of Nickel Oxide nanoparticles (NiO-NPs) using pea peel extract as a natural reducing and stabilizing agent. This eco-friendly synthesis approach not only minimizes the uses of hazardous chemicals but also provides a cost-effective and sustainable pathway for NP production. NiO-NPs possess potent anti-quorum

sensing and antibiofilm properties, effectively disrupting communication signaling in pathogenic bacteria. These findings are highly significant in the context of the emergence of MDR bacteria, against which traditional antibiotics often fail. By targeting the virulence factors rather than just cell viability, NPs may offer promising alternative approach for controlling persistent infections while reducing the selective pressure for resistance.

CONFLICT OF INTEREST:

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

Conceptualization: A.N.M, ASQ, F.M.A. Methodology: F.M.A, B.K, QAS. Analysis: A.N.M, A.S.Q, Q.A.S, A.S.D. Writing, Review, Editing: F.M.A, A.N.M, A.S.Q, A.P, A.S.D, B.K. The manuscript has been reviewed and endorsed by all the authors.

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