



Detection of the *pqsA* Gene and Histological Changes Induced by *Pseudomonas aeruginosa*

Zahraa Hasan Khaleefah^{1*}  , and Enas Abdalhadi Hussain²  

^{1,2}Department of Biology, College of Education Pure Science (Ibn Al-Haitham), University of Baghdad, Baghdad, Iraq.

*Corresponding Author.

Received:18/January/2026

Accepted:17/May/2026

Published: 20/October/2026

<https://doi.org/10.30526/39.4.4421>



© 2026. The Author(s). Published by College of Education for Pure Science (Ibn Al-Haitham), University of Baghdad. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International License](https://creativecommons.org/licenses/by/4.0/)

Abstract

Pseudomonas aeruginosa uses quorum-sensing systems to coordinate activities regulated by specific genes, such as metabolic and environmental responses. The *pqsA* gene is a key component of the *pqs* pathway (quinolone signaling in *Pseudomonas aeruginosa*). This study focused on applying a mouse model to some local isolates of *P. aeruginosa* and identifying those carrying the *pqsA* gene. The study included isolating 150 samples for analysis, which resulted in confirmation of the presence of 58 isolates of *P. aeruginosa*. The specimens including wound swabs, burn samples, urine samples, middle ear swabs, and sputum were collected from different hospitals. The presence of the *pqsA* gene was confirmed molecularly using polymerase chain reaction (PCR) on 19 selected isolates, all of which showed positive amplification (448 bp). The experimental mouse model consisted of Swiss albino mice, divided into a control group and two treatment groups with low (1×10^4 CFU) and high (1×10^8 CFU) concentration of bacteria. We evaluated body weight before and after exposure and also performed histological examinations of the liver and lung tissue. The results showed that increased bacterial concentrations led to increased weight loss and extensive histological changes. In contrast, low concentrations produced only minor tissue changes. Statistical analysis (results are presented as means $\pm$ SE calculated using one-way ANOVA) confirmed these findings as statistically significant ($p \leq 0.05$). Based on the above information, it can be concluded that the *pqsA* gene is highly conserved in local isolates of *P. aeruginosa* and that the presence of this gene leads to significant effects in host tissue in controlled *in vivo* studies. Therefore, this research contributes to understanding of the bacterial quorum sensing mechanism, the pathogenicity of bacterial species and their ability to interact with host bacteria in *in vivo* studies.

Keywords: *Pseudomonas aeruginosa*; *pqsA* gene; Quorum sensing; Molecular detection; Animal model; Histological alterations.

1. Introduction

Microorganisms possess sophisticated regulatory mechanisms by which they can respond appropriately to a variety of global, environmental, biological, and chemical stimuli¹. The most important bacterial communication system is quorum sensing - the means by which bacteria coordinate their collective actions through gene regulation in response to the density of their population². In the case of *Pseudomonas aeruginosa*, quorum sensing regulates a broad range of metabolic processes, biofilm formation, and the synthesis of many secondary metabolites, ultimately affecting the complete biological behaviour of the organism^{3,4}.

PQS (*Pseudomonas* quinolone signal) gene *pqsA* is a key factor in the quorum-sensing process of *Pseudomonas aeruginosa*. The *pqsA* gene is viewed as a pivotal regulatory factor that aids in coordinating bacterial actions and allowing bacteria to respond to the environment⁵. Through

molecular methods for identifying *pqsA* and other genes of quorum sensing, you gain knowledge regarding the genetic potential of isolated bacteria and their ability to regulate their growth based on laboratory conditions⁶.

The biological impacts associated with systems that control bacteria must also be evaluated to gain a thorough understanding of the impact bacteria may have on a large scale in addition to assessing the genetics of the bacteria⁷. The use of animal models provides a controlled means of quantifying generalised effects to various tissues due to exposure to a bacteria or group of bacteria⁸. By looking at the histological structure of a tissue, one can identify changes caused by bacteria and the response from the host's biological systems to those changes, without any implication of clinical or pathological results⁹. The response of the host's biological system to bacterium regulates a dynamic interaction between the host and the microbe¹⁰.

Past studies show that using *Pseudomonas aeruginosa* in animal studies creates verifiable changes within tissues based on how much bacteria were used and how long the body has encountered them^{11,12}. Typically these changes demonstrate either an increase in the number of immune cells along with decreased cellular integrity (amount of damage the tissue sustains) and other vascular effects as a result of the activation of the bacterial regulatory systems¹³. Understanding the relationship between these biological changes and their genetic determinants sheds light on the role that the various bacterial communication systems play in controlling their host environment¹⁴.

This research aimed to understand how the *pqsA* gene, which is responsible for quorum sensing, affects the ability of *Pseudomonas aeruginosa* bacteria to communicate with other cells. This gene was detected molecularly using polymerase chain; additionally, histological studies were conducted on mouse tissue from a model used to investigate the biological effects of the *pqsA* gene. Using both methods, the researchers were able to evaluate how the *pqsA* gene affects the function and health of surrounding tissues. Ultimately, this study will provide a better understanding of how bacteria communicate with each other and the impact of this communication on the host.

2. Materials and Methods

2.1. Bacterial Isolates

A total of 150 clinical specimens were collected, including wound swabs, burn samples, urine samples, middle ear swabs, and sputum. These samples included seven different locations Ghazi al-Hariri Hospital (Medical City), Child Protection Hospital, Burns Hospital, Al-Kindi Hospital, Imam Ali Hospital, Al-Sadr Hospital, and Central Children's Hospital. The types of specimens included: wound, burn, urinary tract samples, middle ear swab, and sputum.

The specimens were cultured and processed to determine the presence of *Pseudomonas aeruginosa*. After identifying the specific strain, 58 of the 150 samples were found to contain *Pseudomonas aeruginosa*; these 58 samples were subsequently used for further analysis.

2.2. Phenotypic and Biochemical Identification

Bacterial isolation was performed using standard microbiological techniques, including culturing on MacConkey agar, Cetrimide agar, and *Pseudomonas* agar, followed by incubation at 37°C for 24 hours¹⁵.

Bacterial isolates underwent biochemical testing for oxidase, catalase, indole, methyl red, Voges–Proskauer, and citrate (IMViC) tests, and final confirmation was performed using the automated Vitek-2 system for the identification *Pseudomonas aeruginosa* isolates¹⁶.

2.3. Experimental Animals

Swiss albino BALB/c mice, free from any diseases aged between 6 and 8 weeks, with a body and weighting between 22 and 30 grams were selected for this study. The mice were kept in the same environment described above, with all experimental access to food and irrigation available from their environmental source.

2.4. Experimental Design

Fifteen mice were randomly assigned to three treatment groups (5 mice/group). The treatment groups were: Control group (injected with sterile solution only), Group I (injected with 1×10^4 CFU of *Pseudomonas aeruginosa*), and Group II (injected with 1×10^8 CFU of *Pseudomonas aeruginosa*). Bacterial cells were suspended in sterile normal saline (0.85% NaCl), which was also used for the control group injections to ensure consistency. Injections were administered intraperitoneally using sterile syringes. All injections were administered at a dose of 0.1 mL over the course of a one-week trial. The weights of all mice were measured before injection and at the end of the trial week.

2.5. Molecular Detection of the *pqsA* Gene

Genomic DNA was extracted from *Pseudomonas aeruginosa* isolates using a commercial genomic DNA extraction kit (Geneaid Genomic DNA Mini Kit, Taiwan) according to the manufacturer's instructions. The purity and concentration of the extracted DNA were assessed using a NanoDrop spectrophotometer, and samples were stored at -20°C until further use. The presence of the *pqsA* gene was detected by polymerase chain reaction (PCR) in 19 representative isolates. Amplification was performed using specific primers targeting the *pqsA* gene as follows: Forward primer: 5'-GACCCCGAACACCTCGAC-3'

Reverse primer: 5'-CGGTTCAGCAGGATGCC-3' (expected amplicon size: 448 bp).

PCR reactions were carried out in a total volume of 25 μL containing 12.5 μL of 2 $\times$ Master Mix, 1 μL of each primer (10 pmol), 5 μL of DNA template, and nuclease-free water to complete the volume. The amplification conditions included an initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 30 sec, annealing at 58°C for 30 sec, and extension at 72°C for 45 sec, with a final extension at 72°C for 5 min. The PCR products were separated by electrophoresis on 1.5% agarose gel stained with ethidium bromide, and visualized under UV illumination. A 100 bp DNA ladder was used as a molecular size marker. The presence of the *pqsA* gene was confirmed by the appearance of a distinct band at 448 bp, as previously described¹⁷.

2.6. Histological Examination

After exposing the mice to the bacteria for a week, the researchers euthanized them and collected lung and liver tissue samples. After excising these tissues, they were fixed in dilute neutral formalin solution, and then processed using conventional histologic techniques, including paraffin embedding, microtome sectioning, and hematoxylin and eosin H&E) staining¹⁸.

The tissue sections were examined under a light microscopy to identify changes in tissue morphology and pathological responses to bacteria¹⁹.

2.7. Statistical Analysis

To express the mean ($\pm$ standard error of (SE)) of the quantitative data gathered during the study, the body weights of mice within each group were determined (mouse/mice weight before and after the exposure period). One-way analysis of variance (ANOVA) was completed to statistically compare the control, 1×10^4 and 1×10^8 Bacterial Groups, in order to determine whether there was a statistically significant difference between the groups. In all cases where a statistically significant difference was detected between the groups, the relevant post hoc multiple comparison tests were performed to identify the differences between specific groups. A probability of $p \leq 0.05$ was regarded as statistically significant²⁰. Analyses were performed through the use of IBM Corp.'s SPSS statistics software version 26 (IBM Corp.) to conduct all statistical analyses.

3. Results

3.1. Isolation and Identification of *Pseudomonas aeruginosa*

The isolates were obtained from infection-related samples (wound, burn, urinary tract, and middle ear) and clinical samples (sputum). Among 150 specimens collected, 58 bacterial isolates were identified as *Pseudomonas aeruginosa* using colony morphology, Gram staining and

biochemical assays (oxidase, catalase, indole, methyl red, Voges-Proskauer, and citrate (IMViC)). The bacterial identity was definitively confirmed using the automated Vitek-2 system. The source of these isolates was infectious samples specifically wound, burn, urinary tract and middle ear infections and Clinical samples specifically sputum.

3.2. Molecular Detection of the *pqsA* Gene

The presence of the *pqsA* gene was confirmed by PCR analyses of 19 representative strains of *Pseudomonas aeruginosa*. All isolates showed a positive PCR result of 448 base pairs (bp) compared to the ladder marker of 100 bp (**Figure 1**). This result indicates that the *pqsA* gene is conserved in all tested isolates.

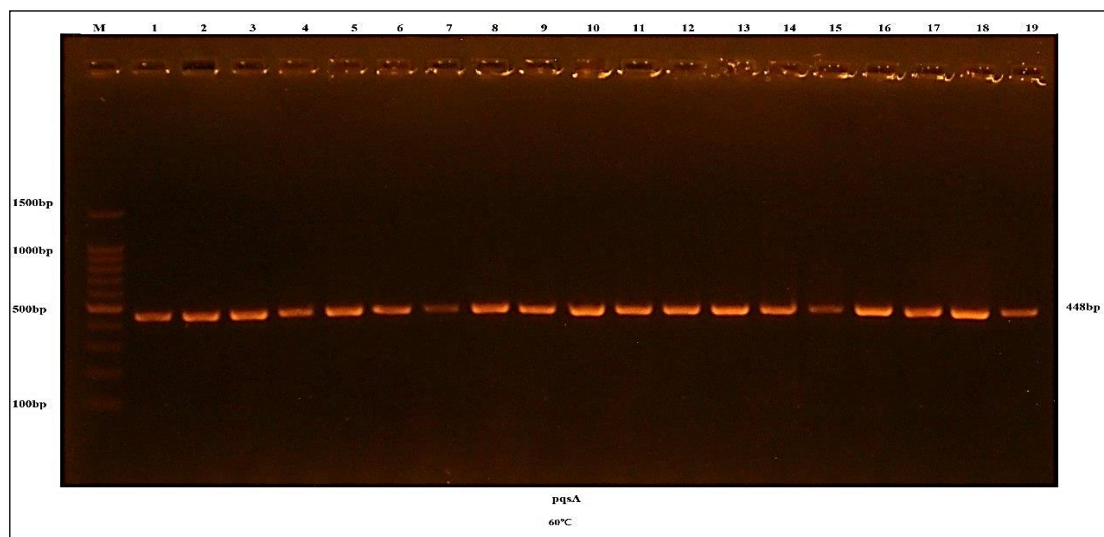


Figure 1. PCR amplification of *pqsA* genes in representative *Pseudomonas aeruginosa* isolates (19 samples) was performed using *pqsA*-specific primers. PCR products were separated using agarose gel (1.5%) and then stained with ethidium bromide for clarity. A 100-base-pair DNA ladder was used as a molecular size (M) marker. Each sample produced a single amplification product of 448 bp delete and put comm., indicating the presence of the *pqsA* gene in each sample.

3.3. Body weight measurements

The mean body weights of the mice in the control and experimental groups before and after the one-week of exposure are summarized in **Table 1**.

Table 1. Body weight of mice before and after one-week exposure to *Pseudomonas aeruginosa* at different concentrations

Group	Before (Mean $\pm$ SE, g)	After (Mean $\pm$ SE, g)	Significance (After)
Control	26.52 $\pm$ 1.26	28.04 $\pm$ 1.11	b
Group I (1×10^4)	25.28 $\pm$ 1.41	24.88 $\pm$ 1.37	b
Group II (1×10^8)	27.02 $\pm$ 0.67	21.82 $\pm$ 0.56	a
p-value	0.001*		

* Significant differences at $p \leq 0.05$, the different letter indicated significant differences, the same letter indicated non-significant differences.

Group means (Mean $\pm$ SE) were calculated for each group with the following assumptions: Group A has the largest value; Group B has the second largest; Group C has the least. After one week, data for each group were evaluated using one-way analysis of variance. Groups that have different letters (a,b) represent statistically significant differences at $p \leq 0.05$, whereas groups with identical letters do not have statistically significant differences.

- Control: The average change in bodyweight for the control group was 26.52 $\pm$ 1.26 grams to a new mean of 28.04 $\pm$ 1.11 grams.
- Group I (1×10^4 CFU): The average change in bodyweight for group I was 25.28 $\pm$ 1.41 grams to a new mean of 24.88 $\pm$ 1.37 grams.

- Group II (1×10^8 CFU): The average change in bodyweight for group II was 27.02 ± 0.67 grams to a new mean of 21.82 ± 0.56 grams.

The statistical analysis demonstrated that the decrease in bodyweight for the high concentration group (1×10^8 CFU) was statistically different from the control and low concentration groups ($p \leq 0.05$) while there was no substantial difference in the decrease in bodyweight between control and low concentration groups

3.4. Histological Examination

The histopathological examination of hepatic tissue revealed clear concentration-dependent alterations in tissue architecture among the experimental groups.

In the control group, liver sections demonstrated a preserved hepatic lobular organization with intact hepatocytes arranged in normal hepatic cords radiating from the central vein. Hepatocytes exhibited normal cytoplasmic and nuclear morphology without signs of degeneration or inflammatory infiltration (**Figure 2**).

In the low bacterial concentration group (1×10^4 CFU), mild histopathological changes were observed. These included focal hepatocellular necrosis accompanied by early degenerative changes in hepatocyte morphology. Additionally, mild lymphocytic infiltration was detected within the hepatic parenchyma, suggesting an early inflammatory response to bacterial exposure (**Figure 3a**). Despite these changes, the overall lobular architecture remained largely preserved.

In the high bacterial concentration group (1×10^8 CFU), severe hepatic injury was evident. Extensive hepatocellular necrosis was observed, characterized by pronounced nuclear alterations including karyolysis and cytoplasmic disintegration. Marked inflammatory cell infiltration was present throughout the hepatic tissue, along with significant vascular congestion. These findings indicate severe disruption of hepatic architecture and functional impairment in a dose-dependent manner (**Figures 3b and 3c**).

The histological analysis of pulmonary tissues also revealed progressive structural damage correlated with increasing bacterial concentration.

In the control group, lung sections showed normal histoarchitecture with intact alveolar sacs, thin alveolar septa, and patent bronchioles lined by continuous epithelial cells. No evidence of edema, inflammation, or vascular congestion was observed (**Figure 4**).

In the low concentration group (1×10^4 CFU), mild pathological alterations were detected. These included slight alveolar edema characterized by minimal fluid accumulation within alveolar spaces, as well as partial disruption of bronchiolar epithelial lining. Mild inflammatory changes were also observed, although overall alveolar structure remained relatively preserved (**Figure 5a**).

In the high concentration group (1×10^8 CFU), severe pulmonary damage was evident. Extensive alveolar edema was observed, leading to marked impairment of gas-exchange surfaces. There was also pronounced disruption and loss of continuity in the bronchiolar epithelium. In addition, significant vascular congestion was present, indicating severe inflammatory and circulatory disturbances within lung tissue (**Figures 5b and 5c**).

These findings demonstrate a clear dose-dependent pattern of pulmonary injury characterized by progressive edema, epithelial destruction, and vascular alterations.

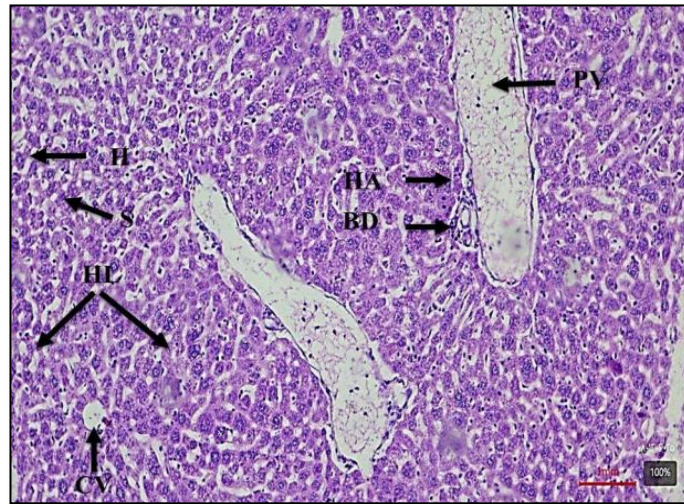


Figure 2. Cross section of the mice liver (Control group) showing: Central vein (CV), Hepatocytes (H), sinusoid (S), portal vein branch (PV), hepatic artery branch (HA), bile duct branch (BD), and hepatic lobule (HL).(H&E, 10x).

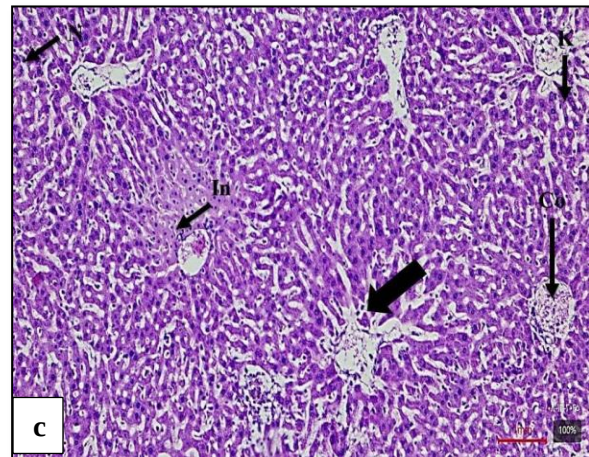
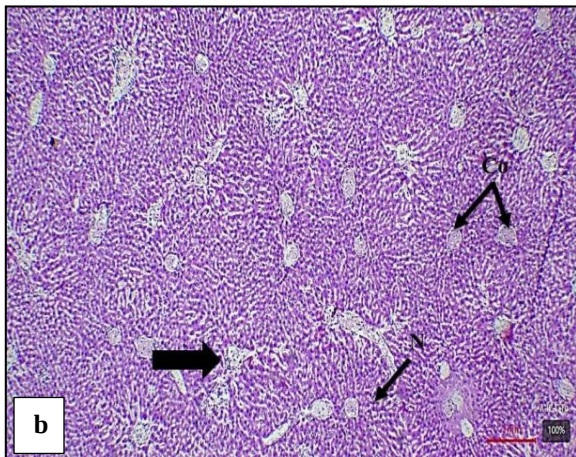


Figure 3. Cross sections of mouse liver from bacteria-treated groups showing concentration-dependent histopathological alterations. The 10^4 CFU group (Figure 3a) demonstrates hepatocyte necrosis (N) and mild lymphocytic infiltration within hepatic sinusoids and around the central vein ($\rightarrow$). The 10^8 CFU group shows more severe lesions, where Figure 3b illustrates extensive hepatocyte necrosis (N), karyolysis (K), inflammatory cell infiltration within hepatic sinusoids ($\rightarrow$), and vascular congestion (Co), while Figure 3c presents higher magnification of the same group highlighting marked hepatocyte necrosis (N), karyolysis (K), prominent inflammatory infiltration, and vascular congestion (Co). H&E, a, 10x; b, 4x; c, 10x.

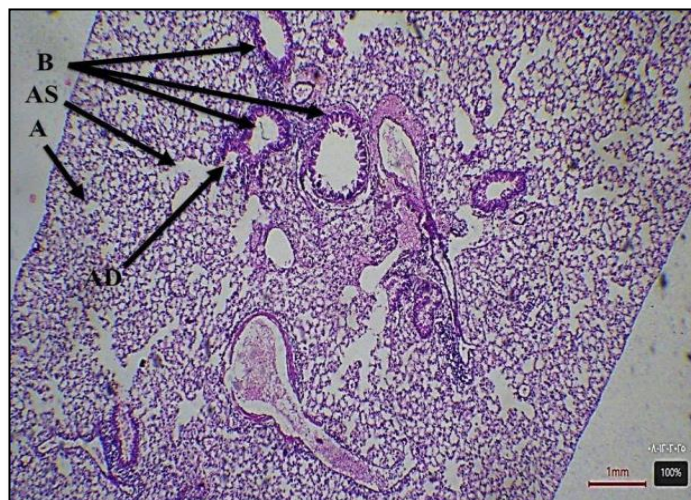


Figure 4. Cross section of the mice lung (Control group) showing: Alveolus (A), alveolar duct (AD), alveolar sac (AS), bronchioles (B). (H&E, 4x).

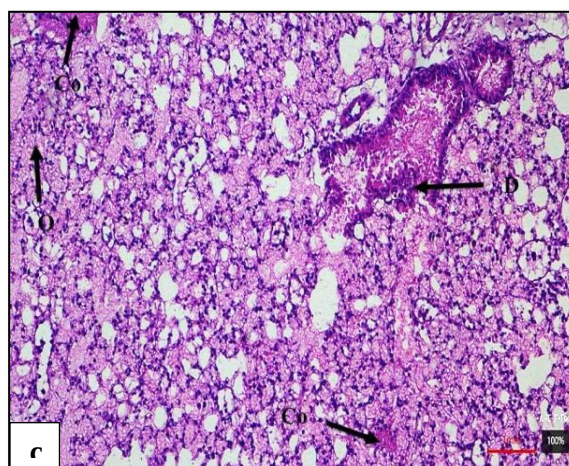
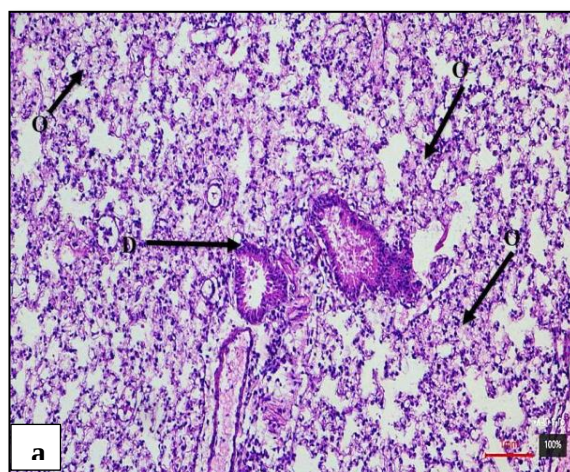


Figure 5. Cross sections of mouse lung from bacteria-treated groups showing concentration-dependent histopathological alterations. The 10⁴ CFU group (Figure 5a) demonstrates pneumonia-like changes characterized by mild alveolar oedema (O) and destruction of bronchiolar epithelium (D). The 10⁸ CFU group shows more severe lesions, where Figure 5b illustrates marked pneumonia-like changes with alveolar oedema (O), destruction of bronchiolar epithelium (D), and vascular congestion (Co), while Figure 5c presents higher magnification of the same group highlighting pronounced alveolar damage, epithelial disruption, and vascular congestion (Co). H&E. b, 10x; c, 4x; d, 10x.

4. Discussion

The *pqsA* gene encodes anthranilate-CoA ligase, a key enzyme in the biosynthesis of Pseudomonas quinolone signal (PQS), which plays a central role in quorum sensing regulation. This system controls virulence factors, biofilm formation, and host tissue damage⁵. In this study we investigated the molecular and biological properties of *Pseudomonas aeruginosa* isolates obtained from a clinical specimen and their impact on a genetically engineered animal model (mouse). The analyses show that all 19 of the isolates we tested contained a functional copy of the *pqsA* gene. This indicates that this type of quorum-sensing regulatory gene is very well conserved within the local population of these bacteria²¹. The fact that these bacteria have the *pqsA* gene means that they are capable of having a coordinated response to their environment through the regulation of many different metabolic and physiological activities²². Therefore, this type of bacteria may result in an observable change in biological activity, which should be evident within the tissue of the infected animal²³.

The selected bacterial concentrations (1×10^4 and 1×10^8 CFU) were chosen to represent low and high infection doses based on previous experimental infection models, allowing evaluation of dose-dependent effects on host tissues²⁴.

The body weight of the mice exposed varying amounts of bacteria is a useful way to measure how their bodies react to these amounts²⁵. Mice exposed to the highest concentration of bacteria (1×10^8 CFU) lost significantly more weight than the control group ($p \leq 0.05$), while the first group with the lowest concentration (1×10^4 CFU) lost only a small amount, a difference that was not statistically significant. Thus, it appears that the amount of bacteria in the mice's bodies had a significant effect on how the mice responded to the bacteria, even though they did not exhibit any symptoms of being ill²⁶. Additionally, this should enhance researchers' confidence when using body weight as an indicator of how accurately their studies represent what happens to humans when exposed to the same amount of bacteria²⁷.

A histological analysis of liver and lung tissues was performed to confirm these quantitative observations. In the high concentration group, liver tissue showed significant necrosis of hepatocytes, karyolysis, infiltrating inflammatory cells, and vascular congestion. In lung tissue, there was evidence of alveolar edema and bronchiolar epithelial destruction, as well as vascular congestion. The histological results for the low concentration group were limited to slight changes in the morphology of the tissue; thus, it would appear that there existed a direct correlation between the type of bacteria and the amount to which it affected the host's tissue²⁸. Histological findings consistent with these observations have been reported by others studies and appear to be mediated through the action of the *pqsA* gene, a regulatory gene of *Pseudomonas aeruginosa*, on the tissue response of the host²⁹⁻³¹.

Using the statistical analysis of mean $\pm$ SE and one-way ANOVA confirms these results. A significant difference has been found between control and high concentration groups, although no significant difference has been found between the control and low concentration groups, for body weight change and tissue change. In addition to using gene detection data to represent both frequency and percentage, the data illustrates the prevalence of the *pqsA* gene among the isolates tested. Based on these results, it can be concluded that the biological effects seen here are significant and represent repeatable biological findings in controlled laboratory environments³².

These collective results provide evidence for a strong correlation between the *pqsA* quorum-sensing gene and quantifiable responses from the host, such as physiological (body mass index) and histopathological changes⁶. While these changes do not correlate with any clinical diseases, they provide a glimpse into the wider range of biological functions related to the regulatory systems of bacteria³³. Thus, the combination of molecular, quantitative, and histological approaches provides a complete understanding of how genes encoding quorum-sensing, like *pqsA*, change the impact of bacteria on the tissues of the host³⁴. Additionally, they offer further insight into the importance of using experimental models for studying microorganisms³⁵.

The *pqsA* gene plays a critical role in PQS-dependent quorum sensing, which regulates biofilm

formation and enhances bacterial survival. Additionally, PQS signaling contributes to the production of virulence factors that promote tissue damage, including proteases and toxins. This explains the severe histological alterations observed in the high concentration group²⁹.

It is important to note that the observed histopathological changes cannot be attributed solely to the *pqsA* gene, as *P.aeruginosa* possesses multiple virulence factors. However, the presence of the *pqsA* gene suggests a potential role in quorum sensing regulation, which may contribute to the observed effects. Further studies involving gene expression analysis or mutant strains are required to confirm its direct role³⁴.

Ultimately, these results highlight that the presence of genetic factors in bacteria play an important role in determining how the host responds, even if the study was performed in a nonmedical laboratory set up. By combining study from both areas, we can gain further insight into the behaviour of and pathways taken by bacteria and how this affects the host; therefore, adding to our knowledge about both basic research in microbiology as well as the field of biology³⁶.

5. Conclusion

The *pqsA* gene is conserved across all isolates of *Pseudomonas aeruginosa* obtained from mice that were exposed to varying concentrations of the same region. The results indicated a dose-dependant effect when performing controlled-dose exposures on infected animals as illustrated by a marked decrease in mouse body weight with increased infection levels, major structural damage to the animal's liver and lungs, and minimal structural injury to the animal's liver and/or lung tissue for lower inoculation concentrations. These results illustrate the relationship of bacterial regulatory gene activity with host biological response in a controlled environment, providing fundamental comprehension of the role of quorum-sensing bacteria's activity of all experimental models used.

Acknowledgment

The authors would like to express their immense gratitude to the Development of Biology, College of Education for Pure Science (Ibn Al-Haitham), University of Baghdad, Baghdad, Iraq, for their invaluable support during the practical parts of this paper.

Conflict of Interest

The authors declare that they have no conflicts of interest.

Funding

No founding.

Ethical Clearance

The Ethical and Scientific Committee of the College of Education for Pure Science (Ibn Al-Haitham), University of Baghdad granted the study its approval (Approval No. EC-92 dated 18/1/2026).

References

1. Lee JW. Bacterial regulatory mechanisms for the control of cellular processes: simple organisms' complex regulation. J Microbiol. 2023;61(3):273–276. <https://doi.org/10.1007/s12275-023-00036-6>.
2. Lafta IJ, Sadeq Z. *Pseudomonas aeruginosa* is an effective indicator for screening of quorum sensing inhibition by plant extracts. Iraqi J Vet Med. 2024;48(1):54–62. <https://doi.org/10.30539/nzsc0y49>.
3. Hameed HQ, Hasan AA, Abdullah RM. Effect of *Olea europaea* L extraction and TiO₂ nanoparticles against *Pseudomonas aeruginosa*. Indian J Public Health Res Dev. 2019;10(6):1218–1223. <https://doi.org/10.5958/0976-5506.2019.01459.1>.
4. Zhang X, Li S, Zhang D, Zheng S, Zhou DI, Hou Q, Han H. A comprehensive review of the

- pathogenic mechanisms of *Pseudomonas aeruginosa*: synergistic effects of virulence factors, quorum sensing, and biofilm formation. *Front Microbiol.* 2025;16:1619626. <https://doi.org/10.3389/fmicb.2025.1619626>.
5. García-Reyes S, Soberón-Chávez G, Cocotl-Yanez M. The third quorum-sensing system of *Pseudomonas aeruginosa*: *Pseudomonas* quinolone signal and the enigmatic PqsE protein. *J Med Microbiol.* 2020;69(1):25–34. <https://doi.org/10.1099/jmm.0.001116>.
 6. Montagut EJ, Marco MP. Biological and clinical significance of quorum sensing alkylquinolones: current analytical and bioanalytical methods for their quantification. *Anal Bioanal Chem.* 2021;413(18):4599–4618. <https://doi.org/10.1007/s00216-021-03356-x>.
 7. Bervoets I, Charlier D. Diversity, versatility and complexity of bacterial gene regulation mechanisms: opportunities and drawbacks for applications in synthetic biology. *FEMS Microbiol Rev.* 2019;43(3):304–339. <https://doi.org/10.1093/femsre/fuz001>.
 8. Douglas AE. 2019. Simple animal models for microbiome research. *Nature Reviews Microbiology*, 17(12), 764-775. <https://doi.org/10.1038/s41579-019-0242-1>.
 9. Velez-Hoyos A, Jimenez-Tobon GA. Highlights of infectious agents in tissue. *Pathology.* 2022;54(2):217–224. <https://doi.org/10.1016/j.pathol.2021.10.004>.
 10. Proal AD, Lindseth IA, Marshall TG. Microbe–microbe and host–microbe interactions drive microbiome dysbiosis and inflammatory processes. *Discov Med.* 2017;23(124):51–60.
 11. Bobrov AG, Getnet D, Swierczewski B, Jacobs A, Medina-Rojas M, Tyner S, Antonic V. Evaluation of *Pseudomonas aeruginosa* pathogenesis and therapeutics in military-relevant animal infection models. *APMIS.* 2022;130(7):436–457. <https://doi.org/10.1111/apm.13119>.
 12. Wood SJ, Kuzel TM, Shafikhani SH. *Pseudomonas aeruginosa*: infections, animal modeling, and therapeutics. *Cells.* 2023;12(1):199. <https://doi.org/10.3390/cells12010199>.
 13. Chegini Z, Noei M, Hemmati J, Arabestani MR, Shariati A. The destruction of mucosal barriers, epithelial remodeling, and impaired mucociliary clearance: possible pathogenic mechanisms of *Pseudomonas aeruginosa* and *Staphylococcus aureus* in chronic rhinosinusitis. *Cell Commun Signal.* 2023;21(1):306. <https://doi.org/10.1186/s12964-023-01347-2>.
 14. Lichtenberg M, Jakobsen TH, Kühl M, Kolpen M, Jensen PØ, Bjarnsholt T. The structure–function relationship of *Pseudomonas aeruginosa* in infections and its influence on the microenvironment. *FEMS Microbiol Rev.* 2022;46(5):fuac018. <https://doi.org/10.1093/femsre/fuac018>.
 15. Tinrat S, Jiraprasertwong O. Probiotic potential and phytase-producing capacity of yeast isolated from Thai traditional fermentation starter (Look-pang). *Folia Microbiol.* 2025;70:1–17. <https://doi.org/10.1007/s12223-025-01314-z>.
 16. Al-Rubaye MRS, Mohammed TK, Abdullah HN. Isolation and diagnosis of multidrug resistance *Pseudomonas aeruginosa* from wound and burn patients in Baghdad City. *Indian J Forensic Med Toxicol.* 2020;14(3):2431–2437. <https://doi.org/10.37506/ijfmt.v14i3.10801>.
 17. Abdelrahim SS, Hassuna NA, Waly NG, Kotb DN, Abdelhamid H, Zaki S. Coexistence of plasmid-mediated quinolone resistance (PMQR) and extended-spectrum beta-lactamase (ESBL) genes among clinical *Pseudomonas aeruginosa* isolates in Egypt. *BMC Microbiol.* 2024;24(1):175. <https://doi.org/10.1186/s12866-024-03319-z>.
 18. Bayram EN, Al-Bakri NA, Al-Shmgani HS. Zinc chloride can mitigate the alterations in metallothionein and some apoptotic proteins induced by cadmium chloride in mice hepatocytes: a histological and immunohistochemical study. *J Toxicol.* 2023;2023(1):2200539. <https://doi.org/10.1155/2023/2200539>.
 19. Bancroft JD, Gamble M. Theory and practice of histological techniques. 7th ed. Philadelphia: Churchill Livingstone Elsevier; 2013. p. 172–186.
 20. Çanga D, Efe C. Using contrasts in one-way analysis of variance with control groups and an application. *J Agric Sci Technol A.* 2017;7:474–478. <https://doi.org/10.17265/2161-6256/2017.07.003>.
 21. Prescott RD, Decho AW. Flexibility and adaptability of quorum sensing in nature. *Trends Microbiol.* 2020;28(6):436–444. <https://doi.org/10.1016/j.tim.2019.12.004>.
 22. Wang Y, Zhang SP, Zhang MY, Kempfer ML, Guo DD, Han JT, He YX. The antitoxin MqsA homologue in *Pseudomonas fluorescens* 2P24 has a rewired regulatory circuit through evolution. *Environ Microbiol.* 2019;21(5):1740–1756. <https://doi.org/10.1111/1462-2920.14538>.
 23. MacInnes JI, Van Immerseel F, Boyce JD, Rycroft AN, Vázquez-Boland JA. Pathogenesis of bacterial

- infections in animals. Wiley-Blackwell; 2022.
24. Sender R, Fuchs S, Milo R. Revised estimates for the number of human and bacteria cells in the body. *PLoS Biol.* 2016;14(8):e1002533. <https://doi.org/10.1101/036103>
 25. Zhang X, Zhang Z, Shu Q, Xu C, Zheng Q, Guo Z, Lu C. Copper clusters: an effective antibacterial for eradicating multidrug-resistant bacterial infection *in vitro* and *in vivo*. *Adv Funct Mater.* 2021;31(14):2008720. <https://doi.org/10.1002/adfm.202008720>.
 26. Pletzer D, Mansour SC, Wuerth K, Rahanjam N, Hancock RE. New mouse model for chronic infections by gram-negative bacteria enabling the study of anti-infective efficacy and host–microbe interactions. *mBio.* 2017;8(1):e01128-16. <https://doi.org/10.1128/mbio.00140-17>.
 27. Ma Z, Zuo T, Frey N, Rangrez AY. A systematic framework for understanding the microbiome in human health and disease: from basic principles to clinical translation. *Signal Transduct Target Ther.* 2024;9(1):237. <https://doi.org/10.1038/s41392-024-01946-6>.
 28. Jakobsen TH, Kirkegaard JB, Lichtenberg M, Kvich LA, Gottlieb H, McNally M, Bjarnsholt T. Detection limitations of bacteria in tissue samples. *Bone Joint Res.* 2025;14(6):560–567. <https://doi.org/10.1302/2046-3758.146.BJR-2024-0410.R1>
 29. Bala A, Chhibber S, Harjai K. Pseudomonas quinolone signalling system: a component of quorum sensing cascade is a crucial player in the acute urinary tract infection caused by *Pseudomonas aeruginosa*. *Int J Med Microbiol.* 2014;304(8):1199–1208. <https://doi.org/10.1016/j.ijmm.2014.08.013>.
 30. Chen J, Lu Y, Ye F, Zhang H, Zhou Y, Li J, Wang H. A small-molecule inhibitor of the anthranilyl-CoA synthetase PqsA for the treatment of multidrug-resistant *Pseudomonas aeruginosa*. *Microbiol Spectr.* 2022;10(4):e02764-21. <https://doi.org/10.1128/spectrum.02764-21>.
 31. Dehbashi S, Tahmasebi H, Alikhani MY, Vidal JE, Seifalian A, Arabestani MR. The healing effect of Pseudomonas quinolone signal (PQS) with co-infection of *Staphylococcus aureus* and *Pseudomonas aeruginosa*: a preclinical animal co-infection model. *J Infect Public Health.* 2024;17(2):329–338. <https://doi.org/10.1016/j.jiph.2023.12.016>.
 32. Voelkl B, Altman NS, Forsman A, Forstmeier W, Gurevitch J, Jaric I, Würbel H. Reproducibility of animal research in light of biological variation. *Nat Rev Neurosci.* 2020;21(7):384–393. <https://doi.org/10.1038/s41583-020-0313-3>.
 33. Schulze A, Mitterer F, Pombo JP, Schild S. Biofilms by bacterial human pathogens: clinical relevance, development, composition and regulation, therapeutical strategies. *Microbial Cell.* 2021;8(2):28. <https://doi.org/10.15698/mic2021.02.741>.
 34. Xiao Y, Zou H, Li J, Song T, Lv W, Wang W, Tao S. Impact of quorum sensing signaling molecules in gram-negative bacteria on host cells: current understanding and future perspectives. *Gut Microbes.* 2022;14(1):2039048. <https://doi.org/10.1080/19490976.2022.2039048>.
 35. Qian Y, Lan F, Venturelli OS. Towards a deeper understanding of microbial communities: integrating experimental data with dynamic models. *Curr Opin Microbiol.* 2021;62:84–92. <https://doi.org/10.1016/j.mib.2021.05.003>.
 36. Lopatkin AJ, Collins JJ. Predictive biology: modelling, understanding and harnessing microbial complexity. *Nat Rev Microbiol.* 2020;18(9):507–520. <https://doi.org/10.1038/s41579-020-0372-5>.