Journal of Communicable Diseases
Volume 57, Issue 4 - 2025, Pg. No. 80-85
Peer Reviewed & Open Access Journal

Research Article

Prevalence of *Pseudomonas aeruginosa* and the effect of iron on its virulence gene in Wound Infections: A Cross-Sectional Study

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DOI: https://doi.org/10.24321/0019.5138.2026100

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How to cite this article:
Abduljabbar U A, Aburesha R A. Prevalence of *Pseudomonas aeruginosa* and the effect of iron on its virulence gene in Wound Infections: A Cross-Sectional Study. J Commun Dis. 2025;57(4):80-85.

Date of Submission: 2025-05-17
Date of Acceptance: 2025-12-28

A B S T R A C T

Background: Communicable wound infections are a significant cause of morbidity in healthcare settings. *Pseudomonas aeruginosa* is frequently implicated in hospital-acquired wound and burn infections due to its environmental persistence and virulence potential.

Objectives: To determine the prevalence of *Pseudomonas aeruginosa* in communicable wound infections and to assess the effect of iron availability on selected virulence gene expression in clinical isolates.

Materials and Methods: This cross-sectional study included 40 bacterial isolates from infected wounds and burn injuries. Phenotypic identification was followed by molecular confirmation using PCR targeting the 16S rRNA gene. Confirmed isolates were exposed to sub-minimum inhibitory concentration iron (100 µM). Expression of *fur, exoS*, and *exoA* genes was evaluated using real-time PCR, and relative expression was calculated by the $2^{-}\Delta\Delta Ct$ method.

Results: Of the 40 isolates, 30 (75%) were confirmed as *Pseudomonas aeruginosa*. Iron exposure resulted in variable regulation of the fur gene, consistent downregulation of *exoS,* and uniform upregulation of *exoA.* These findings demonstrated differential iron-mediated regulation of virulence genes.

Conclusion: Pseudomonas aeruginosa was commonly associated with communicable wound infections. Iron availability significantly influenced virulence gene expression, potentially enhancing persistence and pathogenicity. Molecular monitoring of virulence determinants may support improved infection-control strategies in healthcare settings.

Keywords: *Pseudomonas aeruginosa*, Communicable wound infection, Hospital-acquired infection, Virulence genes, Iron regulation, Gene expression

Introduction

Wound infections continue to represent a major public health concern due to their association with prolonged morbidity, extended hospital stay, increased healthcare costs, and the growing challenge of antimicrobial resistance worldwide.[1] These infections are particularly significant in hospital environments, where breakdown of skin barriers,

invasive procedures, and frequent patient contact facilitate the spread of communicable pathogens.[2] In burn and surgical wounds, the risk of infection is further amplified due to tissue necrosis, impaired local immunity, and repeated exposure to contaminated surfaces and medical equipment.[3]

Among the various bacterial pathogens implicated in wound infections, *Pseudomonas aeruginosa* remains one of the most frequently isolated organisms, especially in healthcare-associated settings.[4] It is a Gram-negative, non-fermenting bacillus with remarkable environmental adaptability, enabling it to survive in moist hospital reservoirs such as sinks, disinfectants, dressings, and medical devices.[5] The organism is easily transmitted through improper wound handling, contaminated fomites, and inadequate infection-control practices, making it an important cause of communicable wound infections.[6]

The pathogenic success of *P. aeruginosa* is largely attributed to its extensive repertoire of virulence factors, including exotoxin A, elastases, proteases, biofilm formation, and type III secretion system–associated effector proteins.[7] These factors enable the organism to invade host tissues, evade immune defenses, and establish persistent infections, particularly in vulnerable patient populations such as burn victims and those with chronic wounds.[8] In many hospital-based studies, *P. aeruginosa* has been associated with increased severity of infection, delayed wound healing, and poor clinical outcomes.[9]

In addition to its virulence potential, *P. aeruginosa* exhibits complex regulatory mechanisms that allow it to adapt to varying environmental conditions within the host. Iron availability is a critical factor influencing bacterial growth and virulence, as iron is essential for numerous metabolic processes.[10] The ferric uptake regulator (Fur) plays a central role in iron homeostasis and regulates the expression of several virulence-associated genes in response to iron levels.[11] Alterations in iron concentration within wound environments may therefore influence the expression of key virulence determinants, contributing to the persistence and transmissibility of infection.[12]

In low- and middle-income countries, including regions of South Asia and the Middle East, *P. aeruginosa* accounts for a substantial proportion of wound and burn infections and is frequently linked with hospital outbreaks.[13] Despite its clinical importance, limited data are available on the molecular behavior of virulence genes among clinical isolates in relation to environmental factors such as iron availability. Understanding both the prevalence and pathogenic potential of *P. aeruginosa* in communicable wound infections is essential for improving diagnostic accuracy, guiding infection-control strategies, and reducing hospital-acquired transmission.[14]

Therefore, the present cross-sectional study was undertaken to determine the prevalence and association of *Pseudomonas aeruginosa* in communicable wound infections and to evaluate selected virulence-related gene expression patterns in clinical isolates, thereby contributing to a better understanding of its role in hospital-acquired wound infections.

Materials And Methods

Study Design

This clinical laboratory–based cross-sectional study was conducted to evaluate the prevalence and association of *Pseudomonas aeruginosa* in communicable wound infections. The study was carried out in the Department of Microbiology of a tertiary care teaching hospital over a defined study period after obtaining institutional approval.

A total of 40 clinical isolates were included in the study. These isolates were obtained from patients presenting with infected wounds and burn injuries who were clinically suspected to have bacterial wound infections. Only non-duplicate isolates from individual patients were included to avoid repetition.

Inclusion and Exclusion Criteria

Inclusion criteria consisted of bacterial isolates obtained from clinically evident wound and burn infections showing features of infection such as discharge, inflammation, or delayed healing.

Exclusion criteria included isolates obtained from non-infected wounds, repeat isolates from the same patient, and samples showing mixed bacterial growth where *Pseudomonas aeruginosa* could not be clearly identified.

Sample Collection and Processing

Wound swabs or pus samples were collected aseptically from infected wound sites using sterile cotton swabs. The specimens were immediately transported to the microbiology laboratory and processed without delay.

Phenotypic Identification of Isolates

All samples were cultured on appropriate selective and differential media. The isolates were initially identified as *Pseudomonas aeruginosa* based on colony morphology, pigment production, oxidase positivity, and routine biochemical tests. Presumptive identification was made prior to molecular confirmation.

Molecular Confirmation of *Pseudomonas aeruginosa*

Genomic DNA was extracted from all presumptive isolates using standard extraction procedures. Molecular confirmation was performed by conventional polymerase chain reaction (PCR) targeting the 16S rRNA gene specific for *Pseudomonas aeruginosa.* The amplified PCR products

ISSN: 0019-5138
DOI: https://doiorg/10.24321/0019.5138.2026100

were analyzed by agarose gel electrophoresis, and isolates showing the expected amplicon size were considered molecularly confirmed.

Iron Exposure at Sub-Minimum Inhibitory Concentration

Molecularly confirmed isolates were exposed to a sub-minimum inhibitory concentration (sub-MIC) of iron (100 µM) to evaluate its effect on virulence gene expression. Treated and untreated bacterial cultures were incubated under identical conditions for a fixed duration prior to RNA extraction.

RNA Extraction and cDNA Synthesis

Total RNA was extracted from treated and untreated bacterial cultures using TRIzol™ reagent following the manufacturer's instructions. The quality and quantity of RNA were assessed, and complementary DNA (cDNA) was synthesized using a reverse transcription kit for downstream gene expression analysis.

Quantitative Real-Time PCR for Virulence Gene Expression

Real-time quantitative PCR (RT-qPCR) was performed to assess the expression of selected virulence-related genes (fur, exoS, and exoA). The 16S rRNA gene was used as the internal housekeeping control. Amplification reactions were carried out using gene-specific primers under standardized cycling conditions.

Analysis of Relative Gene Expression

Relative gene expression levels were calculated using the $2^{-}\Delta\Delta Ct$ method by comparing treated and untreated samples. Changes in gene expression were expressed as fold changes to determine upregulation or downregulation of virulence genes following iron exposure.

Statistical Analysis

The data obtained were compiled and analyzed descriptively. Using SPSS version 25. Gene expression results were presented as fold change values, and prevalence was expressed as percentages. No inferential statistical tests were applied, as the primary objective of the study was descriptive molecular analysis.

Results

Prevalence of *Pseudomonas aeruginosa* in Communicable Wound Infections

A total of 40 clinical isolates obtained from patients with communicable wound and burn infections were included in the study. All isolates were initially identified as *Pseudomonas aeruginosa* based on phenotypic and biochemical characteristics. Molecular confirmation using conventional PCR targeting the 16S rRNA gene confirmed 30 isolates, yielding an overall prevalence of 75%. The remaining 10 isolates (25%) did not show amplification of the target gene and were excluded from further molecular analysis.

Effect of Iron Exposure on fur Gene Expression

Following exposure to sub-minimum inhibitory concentration (sub-MIC) iron (100 µM), differential expression of the fur gene was observed among the confirmed isolates. Upregulation of fur was detected in four isolates, with fold-change values reaching up to 6.06, indicating activation of iron-responsive regulation. In contrast, two isolates showed downregulation, with fold-change values ranging between 0.57 and 0.80. This variability suggested isolate-dependent regulatory responses to iron availability.

Expression Pattern of *exoS* Gene After Iron Exposure

The *exoS* gene demonstrated a consistent downregulation in all tested isolates following iron exposure. Fold-change values ranged from 0.25 to 0.90, indicating partial to marked repression of exoS expression under iron-replete conditions. This uniform trend suggested strong iron-associated suppression of this virulence gene.

Expression Pattern of *exoA* Gene After Iron Exposure

In contrast to exoS, the *exoA* gene showed upregulation in all tested isolates after exposure to sub-MIC iron. The degree of upregulation varied across isolates, with fold-change values ranging from 1.40 to 21.10. This consistent increase indicated that *exoA* expression was positively influenced by iron availability and may be regulated through mechanisms beyond classical iron-mediated repression.

Table 1.Molecular Confirmation of *Pseudomonas* aeruginosa Isolates

(n = 40)

Identification Method	Number of Isolates	Percentage (%)
Phenotypic identification	40	100
PCR-confirmed (16S rRNA)	30	75
PCR-negative	10	25

ISSN: 0019-5138
DOI: https://doiorg/10.24321/0019.5138.2026100

Table 2.Expression Pattern of Virulence Genes Following Iron Exposure

(n = 6 isolates)

Gene	Upregulated, n (%)	Downregulated, n (%)	Fold Change Range
fur	4 (66.7%)	2 (33.3%)	0.57 – 6.06
exoS	0 (0%)	6 (100%)	0.25 – 0.90
exoA	6 (100%)	0 (0%)	1.40 – 21.10

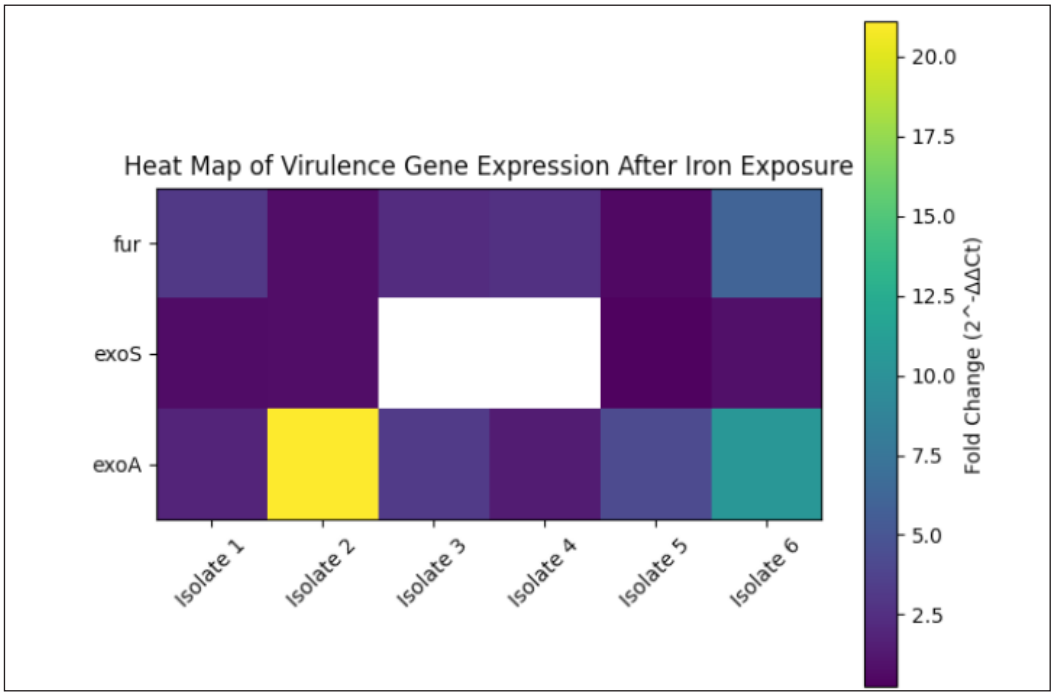

Figure 1.Heat Map of Virulence Gene Expression After Iron Exposure

Result Tables

Discussion

In the present cross-sectional study, *Pseudomonas aeruginosa* was found to be frequently associated with communicable wound infections, with molecular confirmation achieved in 75% of phenotypically identified isolates. This finding reinforced the well-documented role of *P. aeruginosa* as an important healthcare-associated pathogen capable of persisting in hospital environments and spreading through contaminated surfaces, medical devices, and improper wound handling.[4,5] The discrepancy observed between phenotypic identification and PCR confirmation highlighted the limitations of conventional biochemical methods and emphasized the need for molecular techniques for accurate diagnosis, as also reported by Tawfeeq et al. and Altaai et al.[22,23]

Iron exposure at sub-minimum inhibitory concentration resulted in variable expression of the fur gene among the isolates. Upregulation of fur in the majority of isolates indicated activation of iron-responsive regulatory pathways, whereas downregulation in a few isolates suggested strain-specific regulatory variability. Similar heterogeneity in Fur-mediated regulation has been described in previous studies, which noted that bacterial iron homeostasis mechanisms differ among clinical isolates due to genetic diversity and environmental adaptation.[11,13] Chandrangsu et al. further demonstrated that iron-dependent regulators do not respond uniformly across bacterial populations, supporting the variability observed in the present study.[10]

A key finding of this study was the consistent downregulation of the *exoS* gene in all tested isolates following iron exposure. Exoenzyme S is a major type III secretion system–associated virulence factor involved in host cell invasion and acute infection. Its suppression under iron-replete conditions strongly suggested iron-dependent repression, likely mediated through Fur activation. This observation was in agreement with earlier reports that demonstrated iron-mediated suppression of T3SS effector genes in *P. aeruginosa*.[11,15] Khodayary et al. also reported that exoS expression is highly sensitive to environmental cues and nutrient availability, particularly in burn isolates.[16] The uniform downregulation of *exoS* across isolates in the present study indicated that iron availability may reduce

ISSN: 0019-5138
DOI: https://doiorg/10.24321/0019.5138.2026100

invasive virulence while promoting adaptation to a more persistent infection state.

In contrast, the exoA gene exhibited consistent upregulation in all isolates following iron exposure. Exotoxin A is a potent tissue-damaging toxin associated with chronic infection and delayed wound healing. The persistent upregulation of exoA, despite iron abundance, suggested that its regulation is not solely dependent on Fur-mediated pathways. Similar findings have been reported by Reinhart and Oglesby-Sherrouse, who demonstrated that exotoxin A expression may be influenced by alternative regulatory systems depending on the iron source and environmental conditions.[11] Davinic et al. further showed that the virulence factor regulator (Vfr) can override classical Fur repression and promote exotoxin A production under specific physiological states.[25] These mechanisms likely explained the divergent regulatory behavior of exoA observed in the present study.

The contrasting expression patterns of exoS and exoA underscored the complexity of virulence regulation in *P. aeruginosa.* While iron availability appeared to suppress invasion-associated factors such as exoS, it simultaneously enhanced expression of tissue-damaging toxins such as exoA, potentially favoring chronic infection and increased tissue destruction. This regulatory divergence has been highlighted in previous literature, which emphasized that Fur does not uniformly control all virulence genes and that certain toxins escape iron-mediated repression.[13,15] Such adaptive behavior may provide a survival advantage to P. aeruginosa in wound environments rich in iron released from damaged tissues.

From a clinical perspective, these findings were particularly relevant in the context of hospital-acquired wound infections. Studies from low- and middle-income countries have reported a high burden of *P. aeruginosa* infections in burn and surgical wounds, often associated with poor outcomes and prolonged hospital stays.[8,9] The present study suggested that iron availability within wound exudates may contribute to enhanced exotoxin-mediated pathogenicity, thereby facilitating disease persistence and transmission within healthcare settings. This observation highlighted the importance of molecular surveillance of virulence genes alongside routine microbiological diagnostics.

Conclusion

This clinical cross-sectional study demonstrated a significant association of *Pseudomonas aeruginosa* with communicable wound infections, confirming its frequent involvement in hospital-acquired wound and burn infections. Molecular identification using PCR proved to be a reliable method for confirming *P. aeruginosa* among clinically suspected isolates, emphasizing the importance of incorporating molecular diagnostics into routine laboratory practice.

The study further revealed that iron availability plays a crucial role in modulating virulence gene expression in *P. aeruginosa*. Exposure to sub-minimum inhibitory concentration iron resulted in consistent suppression of the invasion-associated exoS gene, while the tissue-damaging exoA gene remained persistently upregulated. These findings indicated a differential regulatory response that may favour persistence and enhanced pathogenicity of the organism in wound environments.

Conflict of interest: None

Source of funding: None

Declaration of Generative AI and AI-Assisted Technologies in the Writing Process: None

References

1. Al-Maeni MA. Detecting the Variation in the lasI Gene and Their Relation with Biofilm in Pseudomonas aeruginosa Isolates. Iraqi Journal of Science. 2024 Jan 30:79-89. [Google Scholar]
2. Kaushik MS, Singh P, Tiwari B, Mishra AK. Ferric uptake regulator (FUR) protein: properties and implications in cyanobacteria. Annals of microbiology. 2016 Mar;66(1):61-75. [Google Scholar]
3. Al-Sheikhly MA, Musleh LN, Al-Mathkhury HJ. Gene expression of pelA and pslA in Pseudomonas aeruginosa under gentamicin stress. Iraqi journal of Science. 2020 Feb 27:295-305. [Google Scholar]
4. Behzadi P, Ambrosi C, Scribano D, Zanetti S, Sarshar M, Gajdács M, Donadu MG. Current perspectives on Pseudomonas aeruginosa: epidemiology, virulence and contemporary strategies to combat multidrug-resistant (MDR) pathogens. Frontiers in Microbiology. 2022 Jul 26;13:975616. [Google Scholar]
5. Todar K. Todar's online textbook of bacteriology [Internet]. 2004 [Google Scholar]
6. Diggle SP, Whiteley M. Microbe Profile: Pseudomonas aeruginosa: opportunistic pathogen and lab rat. Microbiology. 2020 Jan;166(1):30-3. [Google Scholar]
7. Al-Khazraji SF. Study on tssC1 gene mediating biofilm antibiotics resistance of Pseudomonas aeruginosa. Iraqi Journal of Science. 2023 Sep 30:4356-65. [Google Scholar]
8. Capatina D, Feier B, Hosu O, Tertis M, Cristea C. Analytical methods for the characterization and diagnosis of infection with Pseudomonas aeruginosa: A critical review. Analytica Chimica Acta. 2022 Apr 29;1204:339696. [Google Scholar]
9. Muhammad SR, Abed IJ. Evaluating of the Synergistic Effect of three Essential Oils on the Virulence Factors (resistance gene) of Pseudomonas aeruginosa and Serratia fonticola Isolated from Wounds. Iraqi Journal of Science. 2023 Oct 30:4982-99. [Google Scholar]

10. Al NM. Role of Some Virulence Genes and Antibiotic Susceptibility of Pseudomonas aeruginosa Isolated from Different Clinical Samples. Iraqi journal of biotechnology. 2022;21(2). [Google Scholar]
11. Chandrangsu P, Rensing C, Helmann JD. Metal homeostasis and resistance in bacteria. Nature Reviews Microbiology. 2017 Jun;15(6):338-50. [Google Scholar]
12. Flayyih M, Sweedan E, Mohammed L. Study The Effect Of Some Inhibitor Factors On Production Of Some Virulence Factors Of Pseudomonas Aeruginosa And Their Ability For Adhesion To Contact Lenses. Iraqi Journal of Science. 2012;53(1):25-9. [Google Scholar]
13. Reinhart AA, Oglesby-Sherrouse AG. Regulation of Pseudomonas aeruginosa virulence by distinct iron sources. Genes. 2016 Dec 14;7(12):126. [Google Scholar]
14. Porcheron G, Dozois CM. Interplay between iron homeostasis and virulence: Fur and RyhB as major regulators of bacterial pathogenicity. Veterinary microbiology. 2015 Aug 31;179(1-2):2-14. [Google Scholar]
15. Javanmardi F, Emami A, Pirbonyeh N, Keshavarzi A, Rajaee M. A systematic review and meta-analysis on Exo-toxins prevalence in hospital acquired Pseudomonas aeruginosa isolates. Infection, Genetics and Evolution. 2019 Nov 1;75:104037. [Google Scholar]

ISSN: 0019-5138
DOI: https://doiorg/10.24321/0019.5138.2026100