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Insights into the Role of *pslA* and *pslB* Genes in Biofilm-Mediated Multidrug Resistance of *Pseudomonas aeruginosa*

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1. Dear Editor,

We read with great interest the article by Amini et al., titled *"Prevalence of pslA and pslB Biofilm-Related Genes in Pseudomonas (P.) aeruginosa Isolates from ICU Patients: A Cross-Sectional PCR-Based Study."* published in Iran J Med Microbiol. 2025;19(3):150-8. The authors' investigation on biofilm-associated genes among multidrug-resistant (*MDR*) *P. aeruginosa* isolates from intensive care unit (ICU) patients provides valuable insight into the selected molecular factors involved in biofilm formation and antibiotic resistance in clinical settings **(1)**.

The reported prevalence of *pslA* (98.9%) and *pslB* (100%) among biofilm-forming *P. aeruginosa* isolates is striking and highlights the widespread distribution of these biofilm-associated genes among ICU-associated isolates **(1)**. These findings are particularly significant given the global rise in carbapenem-resistant *P. aeruginosa* and the increasing burden of device-associated infections **(2)**. These findings suggest a possible relationship between *pslA/pslB* gene presence and MDR phenotypes in *P. aeruginosa*, supporting the concept that biofilm formation may contribute to antimicrobial tolerance and persistence.

We commend the authors for employing a comprehensive methodological approach that combined phenotypic biofilm assays with genetic screening. The identification of a *pslA*-negative yet weak biofilm-producing isolate is particularly intriguing and may be compensatory involvement of alternative pathways, such as *pel* or *alg* operons. This observation supports the view that biofilm formation in *P. aeruginosa* is a multifactorial process regulated by a complex network of genetic and environmental signals rather than by single operon **(3)**.

Nevertheless, we wish to highlight several considerations that could enhance the translational relevance of such studies. First, although the crystal

violet assay is widely used for biofilm quantification, integrating viability-based methods like resazurin reduction, and imaging-based approaches such as confocal microscopy, could provide detailed information on the physiological state of biofilm cells and their antimicrobial tolerance. Second, quantitative gene expression analysis using RT-qPCR could determine whether *pslA* and *pslB* are differentially regulated in response to antibiotic stress or host-derived cues; such information could guide the design of biofilm-targeted therapeutics.

Moreover, the near-universal detection of *psl* genes among ICU patient isolates underscores the urgent need to develop anti-biofilm strategies for nosocomial infection control. Enzyme-based biofilm dispersal agents, quorum-sensing inhibitors, and emerging nanomaterial-based systems have shown potential in disrupting Psl-dependent matrices. Combining these approaches with conventional antibiotics may improve antibiofilm activity and treatment efficacy, although clinical benefits require further validation in ICU-acquired *P. aeruginosa* infections (data from Amini et al) are summarized in Table 1. Table 2 summarizes the findings of the cited study and the methodological additions highlighted in this letter.

Table 1. Reported prevalence of biofilm-associated genes in *p. aeruginosa* isolates in Amini et al **(1)**.

Gene	Reported prevalence	Study Context" and cite Amini et al[1] in the caption	Interpretation/remark
pslA	98.9%	Biofilm-forming P. aeruginosa isolates from ICU patients	Very high prevalence; supports the relevance of Psl in ICU-associated biofilm formation
pslB	100%	Biofilm-forming P. aeruginosa isolates from ICU patients	Universally detected in the cited study; underscores its potential as a biomarker/therapeutic target

Table 2. Cited study findings and methodological additions highlighted in this letter.

Domain	In the cited study (Amini et al., 2025)	Added value emphasized in this Letter to the Editor
Biofilm assessment	Crystal violet assay (phenotypic quantification)	Consider adding viability-based approaches (e.g., resazurin reduction, confocal microscopy) to better assess tolerance and physiology within biofilms.
Genetic assessment	PCR-based screening of pslA/pslB	Add RT-qPCR to assess gene expression (expression under antibiotic stress/host cues)
Interpretation of exceptions	Notes a pslA-negative isolate with weak biofilm	Highlights multifactorial biofilm formation, possible compensation via pel/alg operons.
Clinical and infection prevention and control implication	High prevalence in ICU setting	Supports the need for anti-biofilm strategies in infection prevention/control and therapy optimization
Biofilm assessment	Crystal violet assay (phenotypic quantification)	Consider adding viability-based approaches (e.g., resazurin reduction, confocal microscopy) to better assess tolerance and physiology within biofilms.

In conclusion, Amini et al.'s study contributes meaningfully to our understanding of biofilm genetics in multidrug-resistant *P. aeruginosa*. The reported association of *pslA* and *pslB* with robust biofilm formation suggests that these genes may have potential as molecular biomarkers and therapeutic targets. Future investigations expanding to transcriptomic and proteomic profiling, alongside clinical outcome correlation, may be valuable for developing precision anti-biofilm interventions and improving patient management in high-risk hospital environments.

2. Declarations

2.1 Acknowledgment

Nil.

2.2 Ethical Considerations

None declared.

2.3 Authors' Contributions

All authors have read and approved the final manuscript.

2.4 Conflict of Interests

The authors declare no conflict of interest.

2.5 Financial Support and Sponsorship

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2.6 Using Artificial Intelligence Tools (AI Tools)

The authors did not used A.I tools. All content, interpretations, and conclusions are solely the work of the authors.

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