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Original Article

Detection of the *rhlR* Gene in Clinical Isolates of *Pseudomonas aeruginosa* Using Polymerase Chain Reaction (PCR)

Nova Dwi Sulistyawati [1], Didik Wahyudi [2]

[1]Diploma III Medical Laboratory Technology Study Program, Sekolah Tinggi Ilmu Kesehatan Nasional, Surakarta City, Indonesia
[2]Department of Molecular Biology, Sekolah Tinggi Ilmu Kesehatan Nasional, Surakarta City, Indonesia.

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Corresponding author:
Didik Wahyudi
Surakarta City
didik.wahyudi@stikesnas.ac.id

Doi:

ABSTRACT

Pseudomonas aeruginosa is an opportunistic bacterium capable of forming biofilms through quorum sensing mechanisms regulated by the *rhlR* gene. The presence of this gene contributes to bacterial virulence and enhanced antibiotic resistance. This study aimed to detect the *rhlR* gene in clinical isolates of *P. aeruginosa* obtained from sputum, urine, and pus samples using the Polymerase Chain Reaction (PCR) method. This descriptive laboratory-based study analyzed six clinical isolates of *Pseudomonas aeruginosa*. Identification of isolates was performed through Gram staining, culture on MacConkey agar, and biochemical tests. DNA isolation was carried out using the Presto™ Mini gDNA Bacteria Kit, followed by qualitative and quantitative DNA analysis. Amplification of the *rhlR* gene was performed using specific primers targeting a 133 bp amplicon. The results showed that all isolates produced adequate DNA concentrations ranging from 147–155 ng/µL with purity ratios ranging from 1,1–1,9. PCR amplification demonstrated the presence of positive bands at 133 bp in all samples, indicating the presence of the *rhlR* gene. In conclusion, the *rhlR* gene was successfully detected in all clinical isolates of *Pseudomonas aeruginosa*. These findings suggest that the isolates possess quorum sensing-related virulence potential associated with biofilm formation.

Introduction

Pseudomonas aeruginosa is a Gram-negative opportunistic pathogen frequently associated with hospital-acquired infections, particularly in immunocompromised patients (Sathe et al., 2023). This bacterium can cause urinary tract infections, respiratory tract infections, wound infections, and sepsis (Wood, Kuzel, & Shafikhani, 2023). Clinical isolates of *Pseudomonas aeruginosa* are commonly obtained from sputum, urine, and pus specimens (Tuon et al., 2022). The pathogenicity of this bacterium is closely associated with the production of various virulence factors, including exotoxins, proteases, pigments, and biofilm-forming compounds (Sholihah, 2021; Wahyudi et al., 2019; Kambuno et al., 2021).

One of the important virulence mechanisms of *Pseudomonas aeruginosa* is the production of rhamnolipid, a biosurfactant regulated by the quorum sensing system (Cruz et al., 2025; Groleau et al., 2020). Rhamnolipid contributes to bacterial adhesion, biofilm maturation, and structural stability of biofilms (Cruz et al., 2020). Biofilm formation enables bacteria to survive under unfavorable environmental conditions and increases resistance to antimicrobial agents (Idrees et al., 2021; Lawal et al., 2021; Kadhim et al., 2026).

The quorum sensing system in *Pseudomonas aeruginosa* is regulated by several genes (Mukherjee et al., 2017). The *rhlR* gene functions as a transcriptional regulator that controls the expression of genes related to

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rhamnolipid production (Mukherjee et al., 2018). Activation of *rhlR* occurs through signaling molecules such as N-butyryl homoserine lactone (C4-HSL) (Jensen et al., 2006). Previous studies reported that the *rhlR* gene is highly prevalent among clinical isolates of *Pseudomonas aeruginosa*, indicating its significant role in bacterial pathogenicity and biofilm formation (Zalianti et al., 2025; Malgaonkar & Nair, 2019; Kadhim et al., 2026). However, local epidemiological data regarding the distribution of this virulence gene across diverse clinical specimens—such as sputum, urine, and pus—remains limited. Understanding this distribution is important for characterizing the virulence potential of local clinical strains.

Polymerase Chain Reaction (PCR) is a molecular technique widely used for rapid and specific amplification of DNA fragments (Zalianti et al., 2025; Jen, 2016). PCR provides high sensitivity and specificity for detecting virulence-associated genes in bacteria (Xiao et al., 2026; Ali et al., 2022). Therefore, this study aimed to detect the presence of the *rhlR* gene in clinical isolates of *Pseudomonas aeruginosa* obtained from different clinical specimens using the PCR method.

Methods

This study used a descriptive laboratory-based design to detect the *rhlR* gene in clinical isolates of *Pseudomonas aeruginosa*. The study was conducted at the Bacteriology Laboratory, Molecular Biology Laboratory, and Quantitative Analytical Chemistry Laboratory of Sekolah Tinggi Ilmu Kesehatan Nasional Surakarta from July 2025 to March 2026. A total of six clinical isolates of *Pseudomonas aeruginosa* were included in this study, consisting of two sputum isolates, two urine isolates, and two pus isolates obtained from RSUD Dr. Moewardi Surakarta.

Bacterial isolates were characterized through Gram staining, culture on MacConkey agar, and biochemical tests including TSIA, SIM, citrate, urea, methyl red, Voges-Proskauer, and PAD tests. DNA isolation was performed using the Presto™ Mini gDNA Bacteria Kit according to the manufacturer's protocol. The procedure included cell lysis, DNA binding, washing, and elution steps (Zalianti et al., 2025; Kristi et al., 2025; Wahyudi & Soetarto, 2021; Jensen et al., 2006).

Qualitative analysis of DNA was carried out using 1,5% agarose gel electrophoresis and visualization under UV transillumination (Zalianti et al., 2025). Quantitative analysis was performed using a UV-Vis spectrophotometer at wavelengths of 260 nm and 280 nm to determine DNA concentration and purity (Cruz et al., 2025). PCR amplification was performed using forward primer 5'-TGCATTTTATCGATCAGGGC-3' and reverse primer 5'-CACTTCCTTTTCCAGGACG-3' targeting an amplicon size of 133 bp (Hemmati et al., 2024). The PCR reaction was prepared in a total volume of 25 µL, consisting of 12 µL of 2 × PCR master mix, 2 µL of forward primer (10 µM), 2 µL of reverse primer (10 µM), 4 µL of nuclease-free water, and 5 µL of DNA template (Mukherjee et al., 2018). The amplification protocol consisted of an initial denaturation at 96 °C for 3 minutes, followed by 35 cycles of denaturation at 96 °C for 30 seconds, annealing at 59,2 °C for 30 seconds, and extension at 72 °C for 60 seconds, with a final extension step at 72 °C for 5 minutes (Jensen et al., 2006; Zalianti et al., 2025). Data were analyzed descriptively based on visualization of amplification bands on agarose gel electrophoresis. Positive detection of the *rhlR* gene was indicated by the presence of a 133 bp DNA band.

Results

Microscopic examination using Gram staining demonstrated that all isolates appeared as Gram-negative rod-shaped bacteria. Growth on MacConkey agar revealed circular colonies with yellowish-green pigmentation characteristic of *Pseudomonas aeruginosa*. Biochemical tests confirmed the identity of all isolates as *Pseudomonas aeruginosa* (Figure 1).

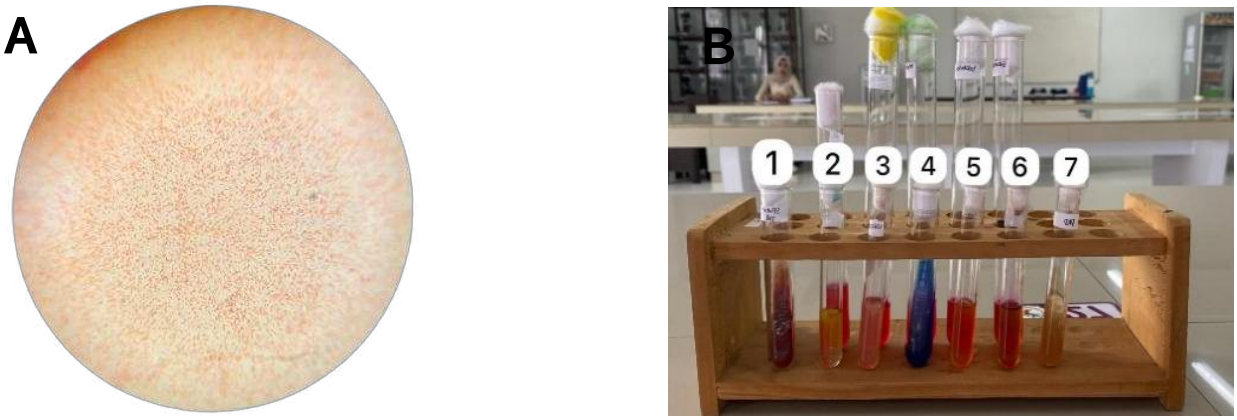

Figure 1. Microscopic Image of *Pseudomonas aeruginosa* Sample (A) Biochemical Test Results of *Pseudomonas aeruginosa* Isolates From Clinical Samples (B) (1. Triple Sugar Iron Agar (TSIA), 2. Sulfide Indole Motility (SIM), 3. Urea, 4. Citrate, 5. Methyl Red, 6. Voges Proskauer, 7. Potato Dextrose Agar)

DNA isolation successfully produced genomic DNA from all isolates. Agarose gel electrophoresis showed clear and thick DNA bands without significant smearing, indicating intact DNA (Figure 2).

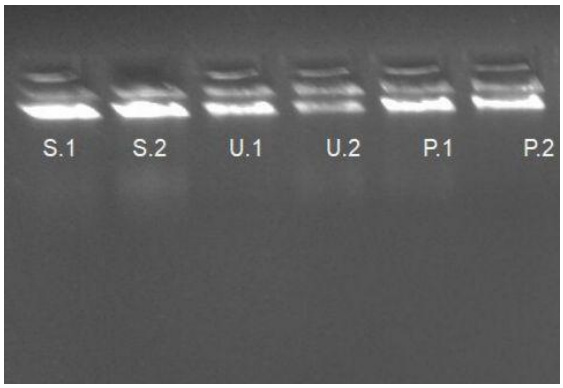

Figure 2. Qualitative Test Results of *Pseudomonas aeruginosa* DNA Isolation by Electrophoresis

The highest DNA purity value was observed in sample S1 with a ratio of 1,9, while the lowest purity values were found in samples U2, P1, and P2 with ratios of 1,1. DNA concentrations ranged from 147–155 ng/µL (Table 1). Optimization of annealing temperature was performed using gradient PCR at temperatures ranging from 50 °C to 60 °C. The optimal annealing temperature was determined to be 59,2 °C because it produced a clear amplification band at the expected target size without smearing (Figure 3).

Table 1. Quantitative Analysis of DNA Isolates

Sample Code	A260	A280	DNA Purity	DNA Concentration (ng/µL)
S1	0,062	0,057	1,9	155
S2	0,060	0,056	1,7	150
U1	0,060	0,056	1,7	150
U2	0,059	0,055	1,1	147
P1	0,060	0,054	1,1	150
P2	0,059	0,054	1,1	147

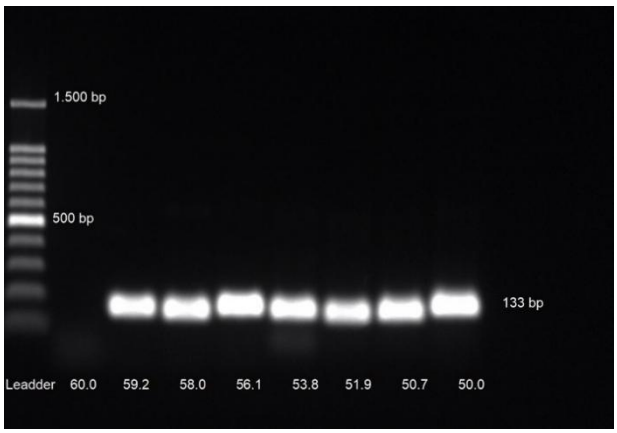

Figure 3. Visualization of Annealing Temperature Optimization (50–60 °C) for PCR Amplification of *Pseudomonas aeruginosa* DNA Samples

PCR amplification results demonstrated that all six clinical isolates produced DNA bands at 133 bp, indicating positive detection of the *rhlR* gene. Optimization of annealing temperature was performed using gradient PCR at temperatures ranging from 50 °C to 60 °C. The optimal annealing temperature was determined to be 59,2 °C because it produced a clear amplification band at the expected target size without smearing (Figure 3).

PCR amplification results demonstrated that all six clinical isolates produced DNA bands at 133 bp, indicating positive detection of the *rhlR* gene (Figure 4).

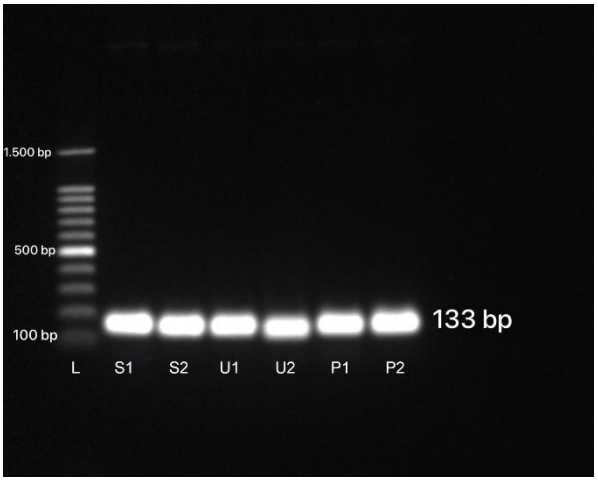

Figure 4. Visualization Results of the rhlR Gene in *Pseudomonas aeruginosa* Isolates From Several Clinical Samples Using PCR (S = Isolate from Sputum Sample; U = Isolate from Urine Sample; P = Isolate from Pus Sample)

Discussion

The present study successfully detected the *rhlR* gene in all clinical isolates of *Pseudomonas aeruginosa* obtained from sputum, urine, and pus specimens. The findings indicate that the isolates possess quorum sensing-associated virulence mechanisms that may contribute to biofilm formation and bacterial pathogenicity. The presence of the *rhlR* gene in all isolates supports the hypothesis that quorum sensing systems are highly conserved among clinical strains of *Pseudomonas aeruginosa* and play an important role in infection persistence.

The microscopic and biochemical characterization confirmed that all isolates exhibited the typical characteristics of *Pseudomonas aeruginosa*, including Gram-negative rod morphology, pigment production on MacConkey agar, and biochemical reactions consistent with previous reports by Wahyudi & Soetarto (2021), who stated that *Pseudomonas aeruginosa* commonly demonstrates oxidative metabolism, citrate positivity, and pigment production associated with pyocyanin synthesis. Similar findings were also reported by Wahyudi et al. (2019), who observed that clinical isolates from respiratory and wound infections predominantly showed identical phenotypic characteristics.

The DNA isolation process in this study produced genomic DNA with concentrations ranging from 147–155 ng/µL, indicating adequate DNA yield for PCR amplification. Although several samples demonstrated purity ratios below the ideal value of 1,8–2,0, amplification was still successfully achieved. According to Hemmati et al.

(2024), DNA purity values below the optimal range may indicate contamination by proteins, salts, or residual ethanol during extraction procedures. However, Hemmati et al. (2024) explained that PCR amplification may still occur effectively when sufficient DNA concentration is available despite suboptimal purity values. This condition was also observed in the present study, where all isolates produced clear amplification bands despite varying purity levels.

Optimization of annealing temperature is a critical step in PCR because inappropriate temperatures can reduce amplification specificity and generate nonspecific bands. In this study, the optimal annealing temperature was determined to be 59,2 °C, producing clear bands at the expected amplicon size of 133 bp. Zalianti et al. (2025) reported that annealing temperatures approaching the primer melting temperature improve primer specificity and reduce nonspecific amplification. Similar findings were reported by Cruz et al. (2020), who demonstrated that optimization of PCR conditions significantly improves amplification accuracy in bacterial virulence gene detection.

The detection of the *rhlR* gene in all isolates indicates that these bacteria possess quorum sensing regulatory systems associated with rhamnolipid production and biofilm formation. The *rhlR* gene functions as a transcriptional regulator activated by C4-HSL signaling molecules and controls the expression of genes involved in virulence factor production. According to Mukherjee et al. (2017) and Groleau et al. (2020) the quorum sensing system in *Pseudomonas aeruginosa* regulates bacterial communication and coordinates the expression of virulence-associated genes in response to population density. The activation of *rhlR* contributes to enhanced biofilm maturation and bacterial adaptation under environmental stress conditions.

The findings of this study are consistent with previous research conducted by Asfahl et al. (2022), who reported a high prevalence of the *rhlR* gene among clinical isolates of *Pseudomonas aeruginosa* collected from intensive care unit patients. Their study demonstrated that isolates carrying the *rhlR* gene exhibited stronger biofilm-forming ability and increased resistance to multiple antibiotics. Similarly, Hemmati et al. (2024) found that more than 90% of clinical isolates possessed quorum sensing genes, including *rhlR*, indicating the importance of these genes in bacterial virulence regulation.

Biofilm formation is considered one of the major pathogenic mechanisms of *Pseudomonas aeruginosa*. Biofilms provide structural protection against host immune responses and reduce antibiotic penetration. Ali et al. (2021) explained that rhamnolipid production contributes to biofilm architecture and facilitates bacterial dissemination within infected tissues. In addition, Wahyudi et al. (2019) reported that biofilm-producing isolates are more frequently associated with chronic and recurrent infections due to their increased tolerance to antimicrobial therapy.

The presence of the *rhlR* gene in isolates derived from sputum, urine, and pus specimens also indicates the adaptability of *Pseudomonas aeruginosa* to different clinical environments. Respiratory isolates are commonly associated with chronic lung infections, particularly in immunocompromised patients, while urine and pus isolates are frequently involved in catheter-associated urinary tract infections and wound infections. Elnegery et al. (2021) and Groleau et al. (2020) emphasized that quorum sensing systems enhance bacterial survival across diverse ecological niches by regulating virulence and metabolic adaptation.

Overall, the results of this study suggest that the *rhlR* gene may serve as an important molecular marker for identifying virulent strains of *Pseudomonas aeruginosa*. Molecular detection of quorum sensing genes could provide additional information regarding bacterial pathogenic potential and may contribute to the development of targeted therapeutic strategies, including quorum sensing inhibition approaches aimed at reducing biofilm formation and antimicrobial resistance.

Nevertheless, this study has several limitations, particularly the relatively small number of clinical isolates analyzed. Therefore, the findings of this study cannot yet be generalized to represent the overall distribution of the *rhlR* gene in *Pseudomonas aeruginosa* clinical isolates. Further studies involving larger sample sizes and broader geographical coverage are needed to obtain more comprehensive epidemiological data and strengthen the generalizability of the findings.

Conclusions

The *rhlR* gene was successfully detected in all clinical isolates of *Pseudomonas aeruginosa* obtained from sputum, urine, and pus specimens using the PCR method. The presence of this gene suggests the involvement of quorum sensing mechanisms associated with virulence and biofilm formation in the analyzed isolates.

Author contributions

NDS and DW conducted biochemical identification tests on bacterial isolates, including DNA extraction, primer determination, and PCR programming. DW verified and validated PCR and electrophoresis products, and wrote articles for publication.

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Ethical approval statement

This research has obtained ethical approval from the Ethics Committee of the Universitas Sebelas Maret Hospital with certificate number No.009.I/UN27.46/TA.04.19/KEP/EC/2026.

Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Supplementary materials

No supplementary material available.

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