

Research Article

Molecular Identification of *Pseudomonas aeruginosa* and Analysis of *phzA* and *phzM* Genes in Clinical Wound and Burn Isolates

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A B S T R A C T

Background: *Pseudomonas aeruginosa* is considered to be one of the most virulent opportunistic pathogens involved in wound and burn infections, due to its arsenal of virulence factors and its capacity to establish itself within damaged tissues.

Aim: The present study was designed to isolate and identify *P. aeruginosa* from cases of wound and burn infections and screen the confirmed isolates for the presence of *phzA* and *phzM* virulence genes.

Methodology: A total of 48 clinical specimens were received from patients with wound and burn infections. All samples were subjected to standard bacteriological culture methods on appropriate media and then incubated at 37°C for 24 hours. After the incubation period, bacterial growth was detected in 38 samples, and 10 samples showed no bacterial growth. Of the positive cultures, 18 isolates were initially identified as *Pseudomonas* spp., while 20 belonged to other bacterial species. Confirmation of the presumptive *Pseudomonas* isolates at the molecular level was carried out by amplifying the 16S ribosomal RNA (rRNA) gene.

Results: Ten isolates were identified as *Pseudomonas aeruginosa*. Genomic DNA was then extracted from all confirmed isolates and used for polymerase chain reaction (PCR) analysis to detect the *phzA* and *phzM* genes.

Conclusion: Molecular analysis revealed that the *phzA* and *phzM* genes were present in all ten confirmed *P. aeruginosa* isolates, showing a prevalence of 100%. The results showed a high distribution of these virulence-associated genes among the recovered isolates and indicate their potential role in the pathogenicity of *P. aeruginosa* associated with wound and burn infections.

Keywords: *Pseudomonas aeruginosa*, *phzM* gene, *phzA* gene, Pyocyanin biosynthesis, Burn and wound infections

Introduction

Burn and wound infections continue to be a major public health problem worldwide because they are associated with delayed healing, long hospitalization, high cost of health care, and high mortality rates.^{1,2} The skin barrier, when damaged after a burn or any other trauma, provides an ideal environment for microbes to stay and cause infection.^{3,4} Among the different types of microbes that cause these infections, *Pseudomonas aeruginosa* is one of the most common and important ones because it possesses processes to fight against drugs and mechanisms that make it easy for this pathogen to cause disease.^{5,6}

Pseudomonas aeruginosa is a Gram-negative, aerobic, non-spore-forming bacterium, commonly found in hospitals and often harvested from burn units and surgical wards. The organism has an outstanding capability to stay alive in harsh environmental conditions and establish chronic infections through biofilm formation, quorum sensing systems, and production of extracellular enzymes and toxins.^{7,8} As a result, *P. aeruginosa* infections are usually hard to treat and have poor clinical outcomes.⁹

The pathogenic potential of *P. aeruginosa* primarily relates to its capacity to produce a wide array of virulence determinants, which include elastase, protease, exotoxin A, phospholipases, rhamnolipids, and phenazine compounds.¹⁰ Among these factors, pyocyanin is considered one of the most important phenazine-derived virulence molecules. Pyocyanin is a blue-green redox-active pigment that can produce reactive oxygen species; it disrupts cellular respiration, impairs host immune response, and promotes tissue damage.^{11,12} Pyocyanin has been reported to play a significant role in bacterial persistence and disease progression in burn and wound infections by several investigators.

Pyocyanin biosynthesis is carried out by a group of phenazine biosynthetic genes, and one of the most important ones in the group is *phzA*. The phenazine operon, which is responsible for phenazine-1-carboxylic acid synthesis, also contains the *phzA* gene. This gene has been directly linked with the virulence potential of *P. aeruginosa* isolates, and it may help them in the colonization of host tissues as well as in escaping immune defenses. Therefore, molecular detection of *phzA* has become a very useful tool in the assessment of pathogenicity in clinical isolates.^{13,14}

The *phzA* and *phzM* genes are among the most important virulence-associated genes in *P. aeruginosa*, which participate in the phenazine biosynthetic pathway. Phenazine is responsible for bacterial pathogenicity by inducing oxidative stress and promoting biofilm development.¹⁵

Recent reports have underlined the increasing prevalence of virulence-associated genes in multidrug-resistant *P. aeruginosa* isolates recovered from burn and wound infections.¹⁶ Knowledge about the distribution of the *phzA* gene in clinical isolates is vital for understanding the molecular mechanisms underlying bacterial pathogenicity and developing new therapeutic and preventive strategies. Furthermore, virulence-related genetic markers could be useful in enhancing epidemiological surveillance and implementation of effective infection-control measures. The present study was designed to determine the prevalence of the *phzA* and *phzM* genes in *Pseudomonas aeruginosa* isolates obtained from burn and wound infections as virulence-associated genetic determinants.

Materials and Methods

Sample collection

A cross-sectional study was performed from February 2026 to June 2026 at Al-Yarmouk Education Hospital in Baghdad. 48 samples were collected from patients with wound and burn infections, at ages 18-42 years, both male and female, in sterile containers with proper aseptic techniques. The samples were immediately brought to the microbiology laboratory of the hospital to maintain their integrity and avoid contamination.

Primary isolation of bacteria was carried out using standard microbiological techniques. The samples were inoculated onto suitable culture media and incubated at 37°C for 24 hours under aerobic conditions. Following incubation, the bacterial growth was assessed based on colony morphology, pigmentation, and the characteristic odor associated with *Pseudomonas* species.

Preliminary identification of *Pseudomonas* spp. was done by Gram staining and standard biochemical tests.¹⁷ Gram-negative, motile rods showing oxidase and catalase positivity were selected for further studies. Confirmation of *Pseudomonas aeruginosa* was carried out through molecular identification targeting the 16S rRNA gene.¹⁸

Bacterial genomic DNA extraction

Genomic DNA from the confirmed *P. aeruginosa* isolates was extracted using the FavorPrepTotal DNA Mini Kit from FavorGen, Korea. The purity and concentration of extracted DNA were measured by using a Nanodrop One UV-Vis spectrophotometer. Briefly, bacterial pellets were obtained by centrifugation and were lysed enzymatically and chemically with the lysis buffers provided in the kit. The lysate was put in a spin column for DNA binding and then washed with different washing buffers to remove impurities. The final step was to elute purified genomic DNA with elution buffer, and frozen at -20 °C until PCR analysis could be performed.¹⁹

PCR amplification of 16S rRNA and *phzA* and *phzM* genes

The polymerase chain reaction (PCR) was used out for molecular confirmation of *Pseudomonas aeruginosa* by amplifying the 16S rRNA gene and detecting the *phzA* and *phzM* virulence genes. The sequences of primers and the sizes of the amplicons expected are given in Table 1.

Table 1. Target genes and PCR primers used for molecular detection

Gene	Primer	5'-3' Sequence	Size (bp)
16S rRNA	F	GGGGATCTTAGGACCTCA	953bp
	R	TCCTTAGAGTGCCACCCG	
PhzA	F	GTTCACCGACAACCTGGAGT	359bp
	R	TCTCGAGTTCGAAGGAATGG	
PhzM	F	AGGATCCGAACACCTCTG	191bp
	R	TATTTGCGGAACGGGTATTC	

The PCR reaction mixture was prepared in a total volume of 25 µL, which included 12.5 µL of Hot FirePol® Master Mix (Solis BioDyne), 3 µL of template DNA (1.5 µL of each primer, forward and reverse primer), 4.5 µL of nuclease-free water and 5 µL of the purified DNA template. The thermal cycling conditions were as follows: 94°C for 5 minutes; then 35 cycles of 58°C for 45 seconds, 49°C for 45 seconds, and 72°C for 30 seconds; and finally, 72°C for 7 minutes. The same cycling conditions were used for both target genes. The program used was illustrated in Table 2.²⁰

Table 2. Program of PCR amplification of *Pseudomonas aeruginosa* genes

Steps	Temperature	Time (m:S)	Number of Cycle
Denaturation	94°C	05:00	1
Annealing 16S rRNA	58°C	00:45	35
Annealing <i>phzA</i>	49°C	00:45	
Annealing <i>phzM</i>	49°C	00:45	
Extension	72°C	00:30	
Final extension	72°C	07:00	
Hold	4°C	10:00	1

Agarose gel electrophoresis

PCR products were analyzed by agarose gel electrophoresis. Agarose gel (1.5%) was made by dissolving 1.5 g in 100 mL

of 1× TAE buffer with heating until it completely dissolved. Once the solution had cooled to around 60–70°C, 3 µL of RedSafe DNA stain was added and the gel was cast.

After solidification, 10 µL of PCR products were loaded into the wells alongside a DNA ladder. Electrophoresis was performed at 80 V for 80 minutes. The DNA bands were then visualized and captured using a gel documentation system.²¹

Results and Discussion

Forty-eight clinical samples were obtained from patients with wound and burn infections. After incubation at 37°C for 24 hours, 38 (79.1%) samples showed bacterial growth, while no growth was observed in 10 (20.8%) samples. Of the positive cultures, 18 (47.3%) isolates exhibited morphological and biochemical characteristics typical of *Pseudomonas* species. The other 20 (52.6%) isolates were different. Molecular identification by sequencing the 16S rRNA gene later confirmed that 10 of the isolates were *Pseudomonas aeruginosa* (Figure 1). This study confirmed with another study that sequencing of the 16S rRNA gene is an accurate and more reliable molecular bacterial identification method for differentiating *P. aeruginosa* from *Pseudomonas* species and it also reduces the misidentifications that may occur when using traditional biochemical methods.^{18,22}

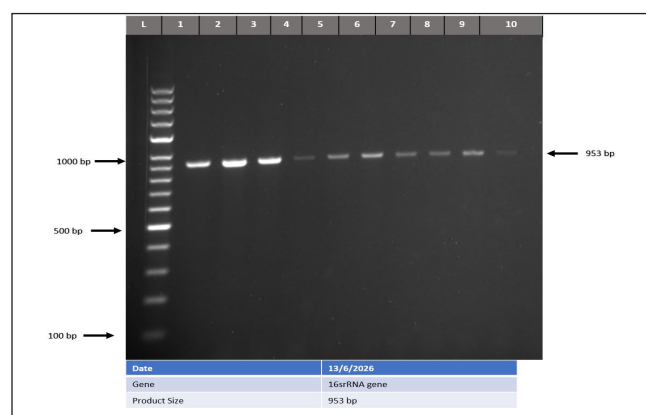


Figure 1. Agarose gel electrophoresis of 16S rRNA PCR products of *Pseudomonas aeruginosa* isolates (953 bp) under UV illumination

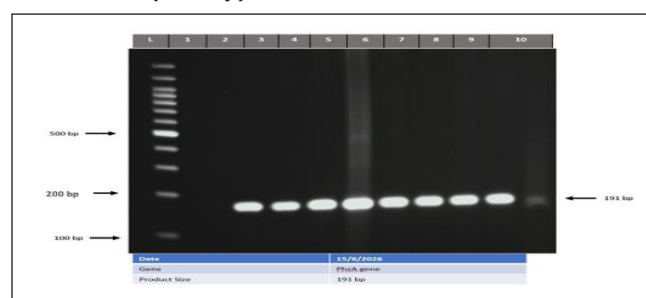


Figure 2. PCR Amplification of the *phzA* gene (191 bp) under UV illumination

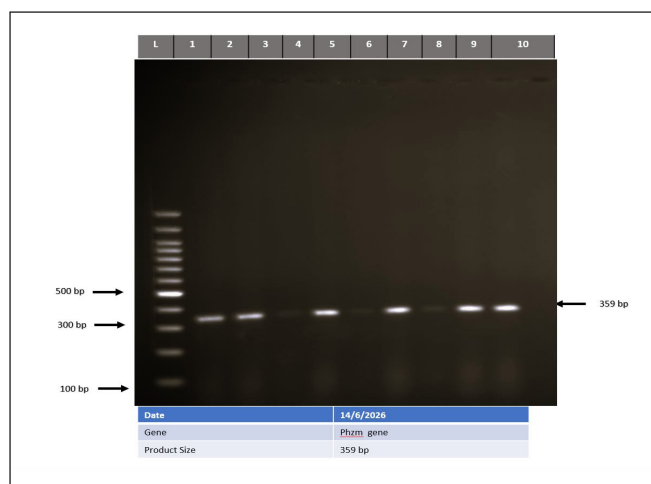


Figure 3. PCR amplification of the *phzM* gene (3595 bp) under UV illumination

The confirmed *P. aeruginosa* isolates were further screened for *phzA* and *phzM* genes by PCR analysis. All 10 (100%) isolates showed the presence of *phzA* and *phzM* genes (Figures 2, 3). This high prevalence indicates a conserved genetic distribution of phenazine biosynthetic genes among clinical isolates from wound and burn infections.

The high prevalence of *P. aeruginosa* in wound and burn infections in this study is in line with Moradali *et al.*23, who noted that the organism is one of the most common opportunistic pathogens, since it can survive under severe conditions, form biofilms, and persist in tissues. The high isolation rates of *P. aeruginosa* in burn wound infections as reported by Ghasemian *et al.*8 were associated with its high adaptability and resistance mechanisms and a similar study was reported by Al-Naji *et al.*24. The detection of the *phzA* and *phzM* genes in all isolates is consistent with the findings of Serafim *et al.*12, who demonstrated a widespread distribution of phenazine biosynthetic genes in strains of *P. aeruginosa*, and their significance in the expression of virulence. Phenazine compounds, particularly pyocyanin, were reported by Abdelaziz *et al.*11 to cause oxidative stress, tissue damage, and immunosuppression during infection.

The presence of *phzA* and *phzM* genes in all isolates indicates that these are components of phenazine biosynthesis pathway, which culminates in pyocyanin production. These results are similar to those of Schiessl *et al.*25, who demonstrated that phenazine genes were necessary for the bacteria to survive and have a competitive advantage in infected tissues. However, since this study is based only on a few confirmed isolates without analyzing gene expression, the presence of *phzA* and *phzM* genes indicates only a genetic potential for virulence. Further studies are needed to assess their level of expression and relation to disease severity and patterns of antimicrobial resistance.

Conclusion

The study demonstrates that *Pseudomonas aeruginosa* is a significant pathogen in wound and burn infections, and that the *phzA* and *phzM* genes are universally present among clinical isolates. This highlights the potential role of phenazine biosynthesis in bacterial pathogenicity and persistence.

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