Immunological and Molecular Analysis of Virulence and Resistance of *Pseudomonas aeruginosa* in Urinary Tract Infections

Zainab Hayder Jaber Alkufaishi 1, Lamees A. Abdul-Lateef 2 *, Anwar Kadhim Hussein AL-saffar 3, Jwan Ahmed Ali Alhamawandi 4

**Abstract**

Background: *Pseudomonas aeruginosa* is an opportunistic pathogen notorious for its role in antibiotic resistance and nosocomial infections. This study investigates immunological markers in UTI with *P. aeruginosa*. Method: Between June 2022 and January 2023, 150 patients with suspected UTIs and 40 healthy controls were enrolled at AL-Hilla Teaching Hospital. Molecular tests, including PCR for MexA and pareE genes, and ELISA tests for TNF-α, IL-12, and IL-17, were conducted. Results: Out of 150 samples, 20 (13.3%) were culture-negative, while 90 harbored different bacterial species. *Pseudomonas aeruginosa* was isolated in 26.6% of samples, with 40% positive for MexA and 70% for pareE genes via PCR. UTI patients with *P. aeruginosa* showed significantly higher TNF-α (620.53 ± 18.455 ng/ml) and TNF-β levels (630.32 ± 25.150 ng/ml) compared to controls (P <0.01). Conclusion: Elevated levels of IL-12 and IL-17 were observed in *P. aeruginosa*-infected UTI patients compared to TNF-α levels, highlighting a distinct cytokine profile associated with *Pseudomonas aeruginosa* UTIs. Key words: *Pseudomonas aeruginosa*, TNF, IL-12, IL-17, pareE and MexA.

**Introduction**

*Pseudomonas aeruginosa* is a rod-shaped, opportunistic, Gram-negative bacterium that causes significant infections and nosocomial outbreaks worldwide. *P. aeruginosa* can acquire resistance genes on plasmids or undergo mutational processes that alter the expression and/or functionality of chromosomally encoded mechanisms to develop antibiotic resistance. The options for treating serious infections can be significantly limited by these drug resistance development techniques (Pelegrin et al., 2021).

Urinary tract infections (UTIs) are among the most common bacterial infections globally, occurring both as nosocomial (inpatient) and outpatient (community-acquired) infections (Dash et al., 2018). Traditionally, UTIs are classified based on clinical symptoms, laboratory data, and microbiological findings. *P. aeruginosa* represents an exceptional case of antimicrobial resistance among pathogens, as it exhibits practically all known mechanisms of resistance. These mechanisms include decreased outer membrane permeability, penicillin-binding protein modification, increased expression of efflux pump systems, alginate production, and enzymatic inactivation of antibiotics (Heidenreich et al., 2022). Two distinct methods contribute to reducing drug susceptibility: layer impermeability and efflux pump systems.
alginate production, and enzymatic inactivation of antibiotics (Heidenreich et al., 2022). Two distinct methods contribute to reducing drug susceptibility: layer impermeability and efflux pump systems (Heidenreich et al., 2022).

The overexpression of efflux pumps contributes to multidrug resistance in *Pseudomonas aeruginosa*. Most Gram-negative bacteria possess efflux pumps playing a significant regulatory role. When MexA forms a complex and some strains of *P. aeruginosa* (Ranaivomanana et al., 2023). MexA enhances resistance against β-lactams, fluoroquinolones, macrolides, chloramphenicol, cephalosporins, penicillins, and other antibiotics. The MexA-OprJ efflux system provides resistance to fluoroquinolones, macrolides, and some β-lactams, such as cefepime (George & Mallory, 2019).

*Pseudomonas aeruginosa*, a Gram-negative bacterium, is known for causing a variety of infections, particularly in immunocompromised individuals or those with underlying health conditions. The MexA protein is a key component of the MexAB-OprM efflux pump, which is a proton-driven efflux pump that expels a wide range of antibiotics and toxic compounds from the bacterial cell. This efflux pump is a critical factor in the multidrug resistance of *Pseudomonas aeruginosa* (Li et al., 2022). Persistent antibiotic-resistant *Pseudomonas aeruginosa* can be found in the lungs of cystic fibrosis patients and those with gut infections. MexA is integral to the MexAB-OprM efflux pump, playing a significant regulatory role. When MexA forms a complex with MexB, it binds to OprM, creating an effective MexAB-OprM efflux pump. This pump protects P. aeruginosa from various antibiotics, including tetracyclines, fluoroquinolones, beta-lactams, and even tannic acid (Roy et al., 2012).

Furthermore, PaeE is an antitoxin protein that is part of a toxin-antitoxin system in *Pseudomonas aeruginosa* (Qadri et al., 2023). Antimicrobial resistance in *P. aeruginosa* is particularly concerning in clinical settings. The bacterium’s ability to form biofilms on surfaces such as catheters and ventilators makes infections harder to treat (Stewart & Costerton, 2001). Biofilm formation is facilitated by the production of alginate, a polysaccharide that protects bacteria from both the immune system and antibiotics (Hentzer et al., 2001). This biofilm-associated resistance is often seen in chronic infections, such as those found in the lungs of cystic fibrosis patients (Singh et al., 2000).

The adaptability of *P. aeruginosa* also extends to its genetic repertoire. Horizontal gene transfer is a crucial mechanism that *P. aeruginosa* uses to acquire new resistance genes. This can occur through transformation, transduction, or conjugation, allowing for rapid adaptation to new antibiotics (Ochman et al., 2000). Plasmids play a vital role in this process, often carrying multiple resistance genes and spreading them among bacterial populations (Carattoli, 2013).

Efflux pumps are not the only mechanism by which *P. aeruginosa* exhibits resistance. The modification of penicillin-binding proteins (PBPs) reduces the efficacy of β-lactam antibiotics. PBPs are essential for cell wall synthesis, and alterations can prevent β-lactam antibiotics from binding effectively, thus negating their antibacterial action (Moya et al., 2009). Additionally, the production of β-lactamases, enzymes that hydrolyze β-lactam antibiotics, further contributes to resistance (Bush, 2018).

The regulation of resistance mechanisms in *P. aeruginosa* is complex and involves multiple genetic and environmental factors. The expression of resistance genes can be induced by the presence of antibiotics, a phenomenon known as adaptive resistance (Breidenstein et al., 2011). This inducible resistance allows *P. aeruginosa* to survive in hostile environments, such as those with high antibiotic concentrations. The global rise of multidrug-resistant (MDR) *P. aeruginosa* strains poses a significant challenge for healthcare systems. Infections caused by MDR *P. aeruginosa* are associated with higher morbidity and mortality rates, longer hospital stays, and increased healthcare costs (Bassetti et al., 2018). The World Health Organization has listed *P. aeruginosa* as a critical priority pathogen for the research and development of new antibiotics (WHO, 2017). Combating *P. aeruginosa* infections requires a multifaceted approach. This includes the development of new antibiotics, the use of combination therapies to prevent the emergence of resistance, and the implementation of stringent infection control measures to prevent the spread of resistant strains (Zhao et al., 2022). Additionally, novel strategies such as bacteriophage therapy and the use of anti-virulence agents are being explored as potential treatments for *P. aeruginosa* infections (Kutter et al., 2010; Kalia & Purohit, 2011).

*Pseudomonas aeruginosa* remains a formidable pathogen due to its versatility in developing resistance mechanisms and its ability to cause severe infections. Ongoing research and innovative therapeutic approaches are crucial to managing and treating infections caused by this resilient bacterium.

**Materials and Methods**

**Study Design**

Patients at AL-Hilla Teaching Hospital's Lab unit who were suspected of having urinary tract infections (UTIs) were enrolled in the study between July 2022 and March 2023. The total sample size included 150 infected patients, 40 healthy control patients, 90 other bacterial species, and 20 patients with negative cultures. The ages of the patients ranged from twenty-five to seventy years. Data on the severity of urine color, examination for urine, and body temperature were also collected.
Urine specimens were obtained before the initiation of antibiotic treatment, with two replicates collected over two days for all patients. Morning urine was collected and placed in a sterile container (Mitchella & Nippins, 2019). Ethical consent was obtained from the hospital’s ethical review board, patients, and their guardians. All participants were verbally informed about the study, and consents for testing and publication of results were obtained before sample collection.

**Diagnosing Pseudomonas aeruginosa Infection in the Urinary Tract**

Diagnosing a *Pseudomonas aeruginosa* infection in the urinary tract typically involves a urine culture. A clean-catch or midstream urine sample is usually collected from the patient, who is instructed to clean the genital area and collect the urine in a sterile container to minimize contamination. The collected urine sample should be promptly transported to the laboratory to avoid processing delays.

In the laboratory, the urine sample is streaked onto a culture medium, such as blood agar or MacConkey agar plates. These culture plates are then incubated at an appropriate temperature (usually around 35-37°C) for 24 to 48 hours, as *Pseudomonas aeruginosa* can grow at various temperatures, including body temperature. After incubation, the microbiologist examines the culture plates for bacterial growth. *Pseudomonas aeruginosa* colonies typically exhibit a distinctive greenish color and characteristic odor, aiding in identification. If Pseudomonas-like colonies are observed, a subculture may be performed to obtain pure isolates, followed by further biochemical tests or molecular methods, such as PCR, to confirm the presence of *Pseudomonas aeruginosa*. Once identification is confirmed, antibiotic susceptibility testing is conducted to determine which antibiotics are effective against the isolated Pseudomonas strain, guiding appropriate antibiotic therapy. It’s important to note that *Pseudomonas aeruginosa* is an opportunistic pathogen often associated with healthcare-associated infections. Therefore, a diagnosis of Pseudomonas infection in the urinary tract may indicate an underlying medical condition, catheter-associated infection, or other factors. The choice of antibiotics for treatment should be based on the results of antibiotic susceptibility testing to ensure effective treatment. The specific procedures and culture media used in a clinical laboratory may vary, so it’s essential to follow the laboratory’s protocols and guidelines for urine culture and bacterial identification. Additionally, the use of more advanced diagnostic techniques, such as polymerase chain reaction (PCR) and genetic testing, can provide faster and more specific results in some cases.

**DNA Extraction**

This procedure utilized components from a genomic DNA purification kit, provided by the manufacturer Genomic (USA).

__Reference__

Mitchella, R. S., & Nippins, C. L. (2019). Ethical consent was obtained from the hospital’s ethical review board, patients, and their guardians. All participants were verbally informed about the study, and consents for testing and publication of results were obtained before sample collection.
Table 1: Detection of MexA, ParE genes in *Pseudomonas aeruginosa*

<table>
<thead>
<tr>
<th>Genes</th>
<th>Primer sequence (5´-3´)</th>
<th>Size BP</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>parE</td>
<td>F5´-CGGCGTTCGTCTCGGGGCGTGGTGAAGGA-3´&lt;br&gt;R5´-TCGAGGGGCTAGTAGATCTCCTGCGC-3´</td>
<td>580 bp</td>
<td>Feng et al., 2019</td>
</tr>
<tr>
<td>MexA</td>
<td>GTTCCCCAAACCGAACAACG-3´&lt;br&gt;ACCTTGTTCTGCACGTTCAC-3´</td>
<td>150 bp</td>
<td>Feng et al., 2019</td>
</tr>
</tbody>
</table>

Figure 1. Positive and negative culture were detected in this study.

Table 2. Initial isolation and identification of *Pseudomonas aeruginosa* by culturing, biochemical tests and culturing test.

<table>
<thead>
<tr>
<th>Total No. of negative culture</th>
<th>Positive <em>Pseudomonas aeruginosa</em></th>
<th>Other types of MO</th>
</tr>
</thead>
<tbody>
<tr>
<td>20(13.33%)</td>
<td>40(26.6%)</td>
<td>90(73.3%)</td>
</tr>
</tbody>
</table>

Figure 2. MexA gene at (150 bp) of *Pseudomonas aeruginosa* amplified by PCR was electrophoresed on agarose gel (1.5%) for 55 minutes at 5 volts per centimeter. For 1:30 hours, use one TBE buffer. L: A 100–500 bp DNA ladder.

Figure 3. *Pseudomonas aeruginosa*’s parE gene at 580 bp was amplified by PCR and subjected to Agarose gel electrophoresis (1.5%) at 5 volts per cent for 55 minutes. For 1:30 hours, use one TBE buffer. L: A 100–700 bp DNA ladder.

Table 3. Determination of cytokines in *Pseudomonas aeruginosa*.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sample</th>
<th>N</th>
<th>Mean ± S.E</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α</td>
<td>sick</td>
<td>20</td>
<td>620.53 ± 18.455</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>20</td>
<td>280.69 ± 108.80</td>
<td></td>
</tr>
<tr>
<td>TNF-β</td>
<td>sick</td>
<td>20</td>
<td>630.32 ± 25.150</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>20</td>
<td>230.13 ± 20.818</td>
<td></td>
</tr>
<tr>
<td>IL-12</td>
<td>sick</td>
<td>20</td>
<td>690.82 ± 34.169</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>20</td>
<td>249.22 ± 6.2316</td>
<td></td>
</tr>
<tr>
<td>IL-17</td>
<td>sick</td>
<td>20</td>
<td>650.13 ± 34.460</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>20</td>
<td>220.24 ± 12.078</td>
<td></td>
</tr>
</tbody>
</table>
Discussion
In 26.6% of cases, *Pseudomonas aeruginosa* was isolated. These results are consistent with research by Li et al. (2022), which found that M. tuberculosis was isolated from 38% of patients with UTIs. Understanding the pathophysiology of *Pseudomonas aeruginosa* requires the identification of its virulence determinants that play a role in human disease (Buscan et al., 2019). Recent research suggests that one of the virulent genes of *Pseudomonas aeruginosa* may contribute to the bacteria’s ability to survive inside human macrophages (Sundararajan & Muniyan, 2021). UTIs, which affect two million people annually, are among the leading causes of chronic infections. To comprehend UTI pathogenesis, it is essential to characterize the virulence determinants of *Pseudomonas aeruginosa* that are relevant to human diseases (Kanabalan et al., 2021). According to a recent study, a virulent gene of *Pseudomonas aeruginosa* may also facilitate the bacteria's survival within human urinary tracts (Huang et al., 2022). The ability of *Pseudomonas aeruginosa* to cause disease, or virulence, depends on its capacity to invade host cells and thwart the microbicidal activities of macrophages. The *Pseudomonas aeruginosa* genome appears to encode bacterial factors that reflect a highly evolved and coordinated program of immune evasion strategies that interfere with both innate and adaptive immunity, causing disease even in fully immunocompetent hosts (Chen et al., 2023). Additionally, the inflammatory response and the outcome of bacterial infections are heavily influenced by the cytokine network (Pan et al., 2019). TNF-α plays a crucial role in protective immunity and pathogenesis against *Pseudomonas aeruginosa*. Granuloma formation is vital for containing a *Pseudomonas aeruginosa* infection, working synergistically with gamma interferon to boost nitric oxide metabolite synthesis and aid in bacterial eradication. Studies have shown that cytokine levels in urinary tract specimens from individuals with UTIs are higher than those in individuals with less severe illness (Hu et al., 2022). Cytokine analysis of urine has been recommended as a means of differentiating between individuals with active *Pseudomonas aeruginosa* infections and healthy controls. The increased reactivation of UTIs in patients treated with monoclonal anti-TNF-α antibodies underscores the importance of TNF-α in regulating bacilli in the latent stage (Pollara et al., 2021). Both IL-12 and IL-17 activities have been observed in these contexts. The presence of IL-17 causes an increase in IL-12 production, which may be an immunological mechanism for dampening Th1 responses. This interplay may block an intrinsic mechanism that typically results in severe disease (Shi et al., 2022).

Conclusion
The identification of *Pseudomonas aeruginosa* using specific primer genes proved to be more specific compared to conventional biochemical tests. Crucial virulence genes were identified, highlighting their importance in the pathogenicity of *Pseudomonas aeruginosa*. The presence of these bacteria correlates with an increase in cytokine levels, indicating a significant immune response.

Studying the immunological and molecular aspects of *Pseudomonas aeruginosa*, particularly its virulence genes, is essential for understanding its pathogenic mechanisms and developing targeted therapeutic strategies. This comprehensive approach, integrating both immunological and molecular techniques, offers valuable insights into the bacterium's virulence factors, contributing to a better understanding of its pathogenicity and identification of potential therapeutic targets. This method enhances our ability to detect and analyze the factors that contribute to *Pseudomonas aeruginosa* infections, ultimately aiding in the development of more effective treatments.

Author contributions
Z.H.J.A. conducted the experiments, L.A.A.L. performed the statistical analysis, A.K.H.A. wrote, J.A.A.A edited, and reviewed the article.

Acknowledgment
The authors were grateful to department for their support.

Competing financial interests
The authors have no conflict of interest.

References


