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Original Paper

Molecular Detection of *pilA* Gene and Antimicrobial Resistance Pattern of *Pseudomonas aeruginosa* Isolated from Burn Patients

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ABSTRACT

Background: *Pseudomonas aeruginosa* is a highly infective and highly antibiotic-resistant pathogen and is considered as one of the most important opportunistic bacteria associated with burn wound infections.

Aim: The aim of the present study was to evaluate patterns of antibiotic resistance of *P. aeruginosa* isolates acquired from burn patients at Al-Diwaniyah, Iraq along with the frequency for *pilA* virulence gene.

Material & Methods: Burn wound swabs were collected in January and April 2025 (n=100). Isolation and identification of *P. aeruginosa* was done by conventional microbiological and biochemical techniques including growth on cetrimide agar. According to The Clinical & Laboratory Standards Institute (CLSI), antimicrobial susceptibility testing was carried out using the disc diffusion method and molecular detection of *pilA* gene was performed by polymerase chain reaction (PCR). 42 (42.0%) of the 100 clinical samples yielded *P. aeruginosa*.

Results: The antimicrobial susceptibility data demonstrated high rates of resistance to several commonly used antibiotics, including ceftazidime and gentamicin (85.7%); cefepime (71.4%); aztreonam (61.9%); tobramycin (59.5%) and piperacillin/tazobactam (57.1%). Moderate resistance rates were determined for meropenem and ciprofloxacin as well as colistin (50.0%). Molecular analysis revealed the presence of *pilA* gene in 38 (90.5%) of the isolates indicating a strong association of the gene with bacterial pathogenic activity, adhesion, movement and biofilm formation.

Conclusions: The results of the study indicated the critical contribution of a virulence gene *pilA* in the pathogenesis of burn infection and the elevated rate of multi-drug-resistant *P. aeruginosa* among the patients with burns. The findings emphasize the need for ongoing surveillance, judicious use of antibiotics and proper implementation of infection control procedures to curtail the high prevalence of resistant and highly virulent strains of *P. aeruginosa* in the health care settings.

KEYWORDS: *P. aeruginosa, Burn infection, pilA gene, Antimicrobial susceptibility*

1. INTRODUCTION

Pseudomonas aeruginosa is a gram-negative aerobic bacterium isolated from many environments, including soil, plants, and human tissue. Major binding components such as flagella, pili and biofilms allow *P. aeruginosa* to thrive on a variety of surfaces including medical equipment. It has been recognized as a major contributor to hospital acquired infection and antibiotic resistance (1). *P. aeruginosa* forms a leading cause of bacterial infection in humans. Numerous conditions, including bacteremia, dermatitis, respiratory system infections, burn wound infections, or soft tissue infections, are associated with it, especially in hospitalized patients as well as immunocompromised individuals (2). Further, patients with burns were especially at risk to this bacterial infection during the period of their hospitalization, resulting in high morbidity and mortality rates as well. Several studies have associated an elevated death rate in *P. aeruginosa* infections to the microbe's potential for quick adaptability to the environment, swift growth of antibiotic resistance, and the formation of numerous virulence factors (3). Further study showed that antipseudomonal drugs are less able to penetrate the pathogen's cell walls and have an increased genetic potential to develop treatment resistance rapidly. The multidrug resistant isolates of these bacteria are a serious threat for infection control as they can cause fatal and often incurable diseases (4).

The widespread use of broad-spectrum antibiotics in these settings has resulted in the continuous emergence of MDR and XDR *P. aeruginosa* (5). The bacterium's capacity to mutate rapidly and to develop resistance to antibiotics complicates treatment. Reports indicate a death rate of 77% in the last 25 years in burn victims infected with *P. aeruginosa* (6). The

European Centre for Disease Prevention and Control (ECDC) reports that transmission rates of MDR *P. aeruginosa* are variable by zones and require adapted treatment programs by zone (7). The wide distribution of MDR strains highlighted by ECDC data underlines the necessity of continuous surveillance and adaptive strategies to mitigate resistance emergence (8). The risk of infection by XDR *P. aeruginosa* is elevated in the intensive care unit (ICU), especially in critically ill patients with invasive devices and prolonged antibiotic use. A recent study from a Portuguese third-level hospital reported an incidence of XDR isolation of around 3.7% among *P. aeruginosa* patients, with an associated mortality of 47% for respiratory tract infections and 44% for bacteremia, which indicates its clinical severity (9). The incidence of XDR is still high in the world, particularly in ICU-acquired infections, where biofilm formation on medical devices and selective antibiotic pressure maintain the persistence of resistance (10).

The pathogenic activity of *Pseudomonas aeruginosa* relies on the synthesis of various compounds associated with cells and biofilms, including flagella, pili, lipopolysaccharide, and alginate capsules. Also, it can secrete proteins, in particular extracellular virulence elements (toxins and enzymes) that could contribute to *P. aeruginosa* pathogenicity (11). These elements are located on plasmids or chromosomes and play a role in bacterial adherence and colonization, host immunological suppression, as well as immune escape that creates an antibiotic resistance barrier (12). The *pilA* gene of *P. aeruginosa* is required for the synthesis and assembly of type four pili and mutations within these genes may affect the ability of the bacteria to attach to surfaces, produce biofilm and spread infection (13). This study examined the distribution of the (*pilA*) gene among *P. aeruginosa* that was isolated from burn patients due to the significance of *P. aeruginosa* genes for virulence and the possibility of antibiotic resistance.

2. MATERIALS AND METHODS

Isolation and Identification of Pseudomonas aeruginosa

Between January and April of 2025, 100 clinical isolates were collected from burn patients at the Center of the Burn in Al-Diwaniyah, Iraq. All study participants were informed that their data would be used for research purposes. Cetrimide agar is a medium used to isolate *Pseudomonas aeruginosa*. Cetrimide is a quaternary ammonium molecule that is specific for Pseudomonas species and inhibits the growth of most other bacteria. Furthermore, the cetrimide agar contains malachite green, which helps to identify *Pseudomonas aeruginosa* as it gives a characteristic blue-green color.

Antimicrobial susceptibility experimentation

Susceptibility testing was done by disc diffusion method on Mueller-Hinton agar according to CLSI standards. 100 µg piperacillin 100/10 µg piperacillin-tazobactam 30 µg cefepime 30 µg ceftazidime 10 µg imipenem 10 µg meropenem 30 µg aztreonam 5 µg ciprofloxacin 5 µg gentamicin 10 µg tobramycin 30 µg amikacin. Diameters of inhibition zones were evaluated according to CLSI recommendations (14). MDR phenotypes are defined as resistance to three or more classes of antimicrobial drugs, including at least one antibiotic. The XDR isolates were the ones that were resistant to at least one antibiotic in each class of antibiotics used except one or two types (15). A positive reaction is indicated by the presence of a zone of inhibition around the antibiotic-inhibitor disk, while the antibiotic disk shows no such zone.

DNA Extraction Protocol

After centrifuging 200 microliters of the overnight growth for 30 seconds at 13,000 rpm, 1.5ml was isolated from the supernatant in a 2ml microcentrifuge tube. To create a homogeneous suspension, the pellet was dissolved in 200µl TL buffer, then removed, and thoroughly mixed with a 20ul proteinase K solution. After 10 minutes of incubation at 56°C in the water bath, the sample was fully lysed. The specimens were treated by 200 microliters of GB buffer, vortexed to create a homogenous mixture for about 15 seconds, then incubated at 56°C for 10 minutes. Following that, 200µl of pure ethanol is then added and vortexed or pipetted. A fresh 2ml tube containing the GeNet Bio genomic DNA purification column was placed after the lysate was carefully transferred, without wetting the rim, into the spin column reservoir for 1 minute at 10,000 rpm. The column then centrifuged the collecting tube and released it containing the flow-through solution. Following adding 500 microliters of GW1 buffer and centrifuging for one minute at 10,000 rpm, the flow-through was taken out of and a purification column was returned into the collecting tube. Next, 500 microliters of GW2 were added to the GeNet Bio genomic DNA purification column and centrifuged for one minute at 10,000 rpm. After centrifuging the tube, take out the flow-through and put the spin column and its collecting tube back together. Centrifuge at 12,000 rpm for 12 minutes to fully extract the ethanol and make sure the droplet is not stuck to the tube's bottom. To perform the elution, 1.5ml of the spin column was then transferred to a fresh tube. The

GeNet Bio genomic DNA purification kit column membrane was filled with 200 microliters of the elution buffer. The genomic DNA elution was centrifuged for one minute at 10,0000 rpm after being left at room temperature for one minute. After that, the purified DNA was taken out right away and kept for further use at -20°C (PrimePrep Genomic DNA extraction kit, GeNet Bio, Korea).

Protocol of PCR Technique

Each gene was amplified by PCR in a reaction volume of 25µl. The Master Mix tube contains 2.5µl, 1µl of forward and reverse primers, 1µl of DNA template and 9.5µl of sterile (D. W.) deionized water (16). Amplified PCR products were identified using agarose gel electrophoresis. Depending on the size of the amplified product, a DNA marker (Neogen/USA) was applied to each gel. The PCR condition was applied to the reaction.

Statistical analysis

The data was analyzed by IBM Corp.'s Statistical Package for the Social Sciences (SPSS) version 25.0 (Armonk, New York, USA). Proportions were compared using a Chi-squared test for correlations. A $P<0.050$ was considered statistically significant.

Ethics Approval

The Ethics Committee of the Al-Diwaniyah Health Directorate in Iraq gave its approval to this study. Prior to being enrolled in the study, each participant filled out a signed informed consent form.

3. RESULTS

Based on microscopy, traditional biochemical testing and identification using cetrimide agar medium, 42 (42.0%) of the 100 swab samples taken from burn patients were found to be *P. aeruginosa*.

Antibiotics susceptibility testing

The test was used to calculate the potential resistance of isolates of P. to eleven antibiotics (table 1). The disc diffusion technique was used to test all 42 isolates for antibiotic susceptibility according to CLSI-2022 guidelines. According to the findings, the tested isolates displayed patterns of sensitivity and high levels of resistance to these antibiotics. 36 isolates (85.7%) had complete resistance to gentamicin and ceftazidime. 30 (71.4%) for Cefepime, 26 (61.9%) for Aztreonam, 25 (59.5%) for Tobramycin, 24 (57.1%) for Piperacillin and Tazobactam, and 21 (50.0%) for Meropenem, Ciprofloxacin, and Colistin came next.

Table 1: Patterns of antibiotic susceptibility in the isolates of *P. aeruginosa*

Antimicrobial agents	Interpretation		
	Sensitive	Intermediate	Resistance
Piperacillin\ Tazobactam	18 (42.9%)	0	24 (57.1%)
Ceftazidime	6 (14.3%)	0	36 (85.7%)
Aztreonam	16 (38.1%)	0	26 (61.9%)
Tobramycin	16 (38.1%)	1 (2.4%)	25 (59.5%)
Cefepime	9 (21.4%)	3 (7.2%)	30 (71.4%)
Imipenem	20 (47.6%)	6 (14.3%)	16 (38.1%)
Meropenem	20 (47.6%)	1 (2.4%)	21 (50.0%)
Amikacin	20 (47.6%)	3 (7.2%)	19 (45.2%)
Gentimicin	4 (9.5%)	2 (4.8%)	36 (85.7%)
Ciprofloxacin	19 (45.2%)	2 (4.8%)	21 (50.0%)
Colistin	21 (50.0%)	0	21 (50.0%)

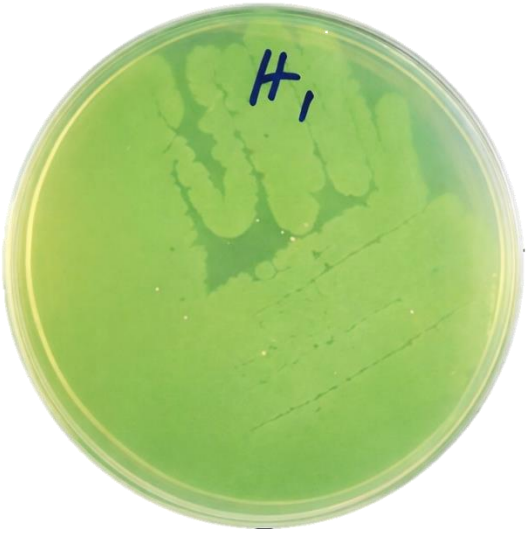

Figure 1: Growth of *Pseudomonas arogenosia* on cetrimide agar

Detection of virulence factor gene

The virulence gene (*pilA* gene) in each of the 42 *P. aeruginosa* burn isolates was identified via molecular analysis. According to the results, 38 (90.5%) of the *P. aeruginosa* isolates produced unique and positive *pilA* gene bands.

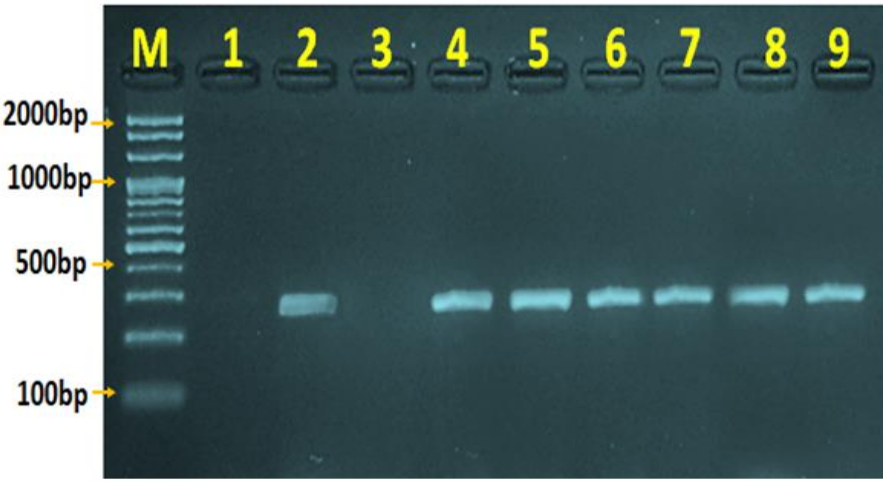

Figure 2: Agarose gel electrophoresis image displaying PCR product analysis for *Pseudomonas aeruginosa* virulence factor *pilA* gene discovery

4. DISCUSSION

A common cause of burn inflammation is *Pseudomonas aeruginosa*. It is present in soil, water, small amounts in the intestines, and the moist areas of hospitals. They are mainly responsible for burn infections (17). In this study, 42(42.0%) of the total *P. aeruginosa* isolates were recovered from burn swabs. Immunosuppressive effects of burn injuries and the local wound environment promote pathogen colonization and growth. The most common isolated organism from burns is *Pseudomonas aeruginosa* and it is often the predominant pathogen in established burn infections (18). *P. aeruginosa* impacts the blood metabolome through the increase in the size of infected burns and the ensuing sepsis, resulting from alterations in certain metabolites that may be employed as biomarkers for the early diagnosis of sepsis caused by *P. aeruginosa* (19).

Antimicrobial resistance poses a major challenge to global efforts to control infectious diseases. *P. aeruginosa* is inherently resistant to many antimicrobials due to low outer membrane permeability, the presence of several efflux pumps that are constantly active and the synthesis of diverse antimicrobial-inactivating enzymes (20). As per Qin et al. (20), *P. aeruginosa* can form biofilm more efficiently, which leads to decreased antibiotic penetration and bacteria access. This study researched the antibiotic resistance as well as existence of metallo-beta-lactamase along with extended-spectrum beta-lactamase generating genes.

The antibiotic susceptibility test results showed complete resistance to gentamicin and ceftazidime by 36 (85.7%) isolates followed by Cefepime 30 (71.4%), Aztreonam 26 (61.9%), Tobramycin 25 (59.5%), Piperacillin and Tazobactam 24 (57.1%), Meropenem, Ciprofloxacin and Colistin 21 (50.0%). β-lactams are important for therapy of *P. aeruginosa* infection. However, a substantial proportion of *P. aeruginosa* are resistant to these antibiotics, which complicates infection treatment and leads to poor patient outcomes. Such resistances may involve alterations in the penicillin-binding protein, necessary for enhanced efflux or reduced uptake of antibiotics, degradation of β-lactams through increased expression or altered specificity of substrate of β-lactamases (AmpC), or horizontal gene transfer resulting in the acquisition of β-lactamases, as well as changes in metabolism and biofilm formation. Aminoglycoside resistance can be mediated by aminoglycoside-modifying enzymes, which catalytically alter the structure of these antibiotics to varying extents, or by RNA methyltransferases, which alter the target site of 16S rRNA. Resistance to fluoroquinolones can result from mutations in the target sites of parC and gyrA, which are components of topoisomerase IV and DNA gyrase, respectively (21). In a local study in Duhok, the percentage of resistance to piperacillin, ceftazidime, and cefepime was 100%, followed by meropenem and tobramycin with 79.2% and 64.6%, respectively (22). In a study from Baghdad, resistance rates to piperacillin, gentamicin, cefepime, imipenem, tobramycin and meropenem were 85.5%, 73.9%, 76.8%, 71%, 71% and 63.8%, respectively (23). But the other study done by Al-Wahid (24) in Al-Diwaniyah showed that the percentages of resistance to cefepime, ceftazidime, piperacillin, aztreonam, gentamicin, ciprofloxacin, imipenem, meropenem and amikacin were 39%, 35%, 31%, 29%, 23%, 21%, 18%, 16% and 13% respectively. Variations in resistance levels among research may be due to differences in geographical distribution, size of the sample of the studies and antibacterial standards across the world.

The results of the present study showed the *pilA* gene was detected in 90.5% of the 42 isolates of *P. aeruginosa*.

The *pilA* gene encodes components of type IV pili necessary for adhesion of bacteria, twith motility and biofilm formation. These pili allow the formation of microcolonies and initial attachment of bacteria to host tissues (25). Mutations in this gene have been shown to affect *P. aeruginosa*'s ability to form biofilm, adhere to surfaces and spread infection. Reduced virulence in infected mouse models and lack of biofilm formation on abiotic surfaces was reported in many studies of a mutant strain of *P. aeruginosa* that has a mutation in the *pilA* gene. (26).

CONCLUSION

The current study showed that the *P. aeruginosa* isolates are resistant to the antibiotics under test. at a considerable level. shown that *P. aeruginosa* is one of the major active bacteria in burn infections.

The prevalence of virulence gene (*pilA*) among the isolates of *P. aeruginosa* was identified in this investigation. Moreover, the high incidence of these genes greatly affects the *P. aeruginosa* pathogenicity in burn infections.

These findings may facilitate the evolution of resistant types of *P. aeruginosa* that could infect healthy people and patients within the hospital.

Thus, new strategies are recommended to control infections and to optimize the use of antibiotics to prevent the emergence of drug-resistant *P. aeruginosa*.

Conflicts Of Interest

The author asserts that there are no conflicts of interest.

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