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Molecular Detection by blaIMPgene in *Pseudomonas aeruginosa* from Clinical Specimens and Relationship with Multidrug Resistance (MDR)

Marwa A. Hassan[1], Muhanad Mahdi Mohammed[2]*, Safaa Salman Dawood [3]

[1] Department of Biology, College of Science, University of Misan, Amarah, Maysan, Iraq.

[2] College of Dentistry, University of Misan, Amarah, Maysan, Iraq.

[3] Al-Manara College for Medical Sciences, Maysan, Amarah, Iraq.

***Corresponding author:** Muhanad Mahdi Mohammed,

Email: muhannad.m.m@uomisan.edu.iq

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Abstract

Due to its numerous resistance mechanisms, *Pseudomonas aeruginosa* is a significant cause of infection in hospitalized patients, leading to morbidity and mortality. As a therapy choices grows limited, the search for a new agent grows more pressing. So P. aeruginosa is an incredibly flexible Gram-negative bacterium able to flourish in a wide range of conditions, which presents major issues for doctors and nurses. One hundred specimens were gathered from clinical sources at Al-Fayhaa Hospital and Al Basrah Teaching Hospital, comprising (50) swabs from burn unit patients' exudate wounds and (50) urine specimens from patients with urinary tract infections. All of these items were cultivated in different media (89), and *P. aeruginosa* bacterial isolates were determined through microscopic examination and biochemical tests. A molecular diagnosis can be identified by using a traditional PCR approach to identify the specified amplification of gene product of the blaIMP gene, which was 77.55% in swabs from exudate wounds than the urine of patients with infections of the urinary tract, compared to 75% for *P. aeruginosa*. Isolates of bacteria had great resistance to antibiotics, and the results revealed a high degree of resistance to gatifloxacin, ciprofloxacin (88.23% each), and gentamycin (77.94%), whereas colistin (70.14%) had low resistance from bacteria. *P. aeruginosa* isolates showed multi-drug resistance (MDR). Antibiotic overuse has led to the evolution of resistant bacteria. which has resulted in inefficient treatment with antibiotics.

Keywords: *Pseudomonas aeruginosa*, blaIMP gene, Antimicrobial susceptibility, Multi-drug resistance(MDR)

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1. Introduction

Pseudomonas aeruginosa is a harmful, opportunistic bacterium that's often involved in worsening infections, especially in people who are seriously ill or have weakened immune systems. It thrives in moist environments and doesn't need many nutrients to grow [1,2]. *Pseudomonas aeruginosa* can build up over time in damp areas of healthcare settings, on medical equipment, and in hospitalized patients [3]. Sepsis, wound and soft tissue infections, urinary tract infections, ventilator-associated pneumonia, and recurrent flare-ups in cystic fibrosis patients are among the potentially fatal infections it can cause [4,5]. It also lowers the chances of successful tissue donation, can cause blood infections in burn patients, and is linked to an increased risk of death. Once *Pseudomonas aeruginosa* enters the body, it can spread to different organs and systems [6]. One

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of the most common infections it causes is endocarditis—an inflammation of the heart lining. This condition is particularly prevalent in those who have artificial heart valves or those who use intravenous drugs, as both situations give the bacteria easier access to the bloodstream. Individuals with weakened immune systems, such as those living with HIV or suffering from severe burns, are particularly at risk for developing this serious infection[7].

The ability of *P. aeruginosa* to cause disease is partly due to its virulence factors and genetic adaptability, which help it survive in many environments. Some of these traits help the bacteria attach and colonize, while others enable them to invade tissues, release harmful enzymes, and cause damage[8]. Inflammation and poisoning are caused by this infection in system parts such as the urinary system, lungs, kidneys, thin tissues, and skin injury. It has been determined that roughly 6% of those who receive burns die as a result of infection with this bacterium [9]. The collection and examination of cerebrospinal fluid, blood, pus, blood, and vestibular secretions are required for laboratory diagnosis [10]. These bacteria are causing significant infections that are difficult to treat and eradicate due to their widespread antibiotic resistance. It is the most common cause of burns and wound damage [11,12]. These microbes cause wound contamination, which results in tissue collapse and blood plasma ejection outside the skin, providing an ideal environment for bacterial development [13]. In recent times, the frequency of multidrug-resistant *P. aeruginosa* (MDR) has increased dramatically, posing a danger to global general health and leading to significant mortality and morbidity. This is due to this microorganism's propensity to develop resistance to the most potent antibiotics, and the issues caused by multidrug-resistant *P. aeruginosa* have caused widespread public concern. High incidences of metallo-lactamase (MBL)-producing *P. aeruginosa* in patients in hospitals have been recorded in the last decade. Several studies investigated MDR strain risk variables and their connection with death. MDR stains were linked with an increase in mortality (OR 4.89) as compared to susceptible infections. The World Health Organization (WHO) has identified three bacterial species that require treatment with alternative medicines, including carbapenem-resistant P. aeruginosa [14]. Antimicrobial resistance is one of today's most important public health issues, and it is growing in poor countries. [15] *P. aeruginosa* is resistant to a variety of antimicrobial treatments. It is typically resistant to aminoglycosides, semisynthetic penicillins such as ticarcillin and piperacillin, third- and fourth-generation cephalosporins (cefepime and ceftazidime, respectively), fluoroquinolones (except ertapenem), and carbapenems [16]. In nosocomial medical care, resistance to antibiotics in bacteria is reaching near-crisis proportions, with many bacterial isolates now multi-resistant due to the inclusion of extra DNA components. A recent study has shown that resistance gene markers exist on plasmids and can be transmitted, with many studies confirming this solely through plasmid curing experiments [17]. Infections caused by *Pseudomonas aeruginosa* can develop independently and are well known for their ability to resist many commonly used antibiotics. This resistance makes treatment more difficult—especially as more strains emerge that are resistant to multiple types of drugs. Research has shown that these bacteria are considered multidrug-resistant when they can withstand at least three different classes of antibiotics, such as carbapenems, aminoglycosides, antipseudomonal penicillins, cephalosporins, and quinolones [18]. This study aims to determine how often the bla-IMP gene appears in clinical samples and to isolate and analyze these multidrug-resistant *P. aeruginosa* strains.

2. Materials and methods

2.1. Collection of specimens

Clinical samples were collected (100), including (50) samples of swabs from exudate wounds of the burn unit patients and (50) urine specimens from patients with urinary tract infections collected from Al-Fayhaa Hospital and Al Basrah Teaching Hospital.

2.2 Isolation and Identification of *P. aeruginosa*

The clinical specimens are grown on nutrient agar, MacConkey agar, Blood agar, and Cetrimide agar for 24 hours at 37 °C. These isolates could represent P. aeruginosa growing on Cetrimide agar with P. aeruginosa characterization, such as a blue-greenish hue, mucous membrane colony, smooth form, and a fruity odor. Colonies appeared as light greenish and lactose non-fermenter colonies on another MacConkey agar plate; however, on blood agar, colonies appeared as enormous, opaque, irregular colonies with butyrous texture (shiny), emitting fruity odor and B-hemolytic colonies. On Nutrient agar, it appeared yellowish-green, smooth, had a flat surface, and an elevated center. Additional identifying procedures include biochemical tests such as an oxidase test and an oxidation reaction, which detect morphological characteristics [19].

2.3 Molecular detection of isolates

2.4 Genomic DNA extraction

The DNA from the genome of *P. aeruginosa* isolates was obtained employing a DNA kit (Geneaid, USA) and the manufacturer's protocol.

2.5 P. aeruginosa genotypic detection

The presence of the blaIMP gene was detected using PCR, with the primer sequences listed in Table 1. The PCR reaction mixture had a total volume of 25 µl and included: 2 µl of extracted DNA, 1 µl of each primer, 12.5 µl of 2X Master Mix, and 8.5 µl of nuclease-free water. The PCR conditions included a 5-minute initial denaturation at 95°C, 30 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 30 seconds. The last extension phase was performed for seven minutes at 72°C. A Gel Doc system was used to visualize the results after the PCR products were separated using 1.5% agarose gel electrophoresis (Bio-Rad, USA) [20].

Table (1): The sequence of blaIMP gene primers for amplification The genes.

Gene	Sequence		Amplico Size, bp	Refe.
blaIMP	F	5"GGAATAGAGGCTTAAYTCTC"3	232	[20]
	R	5"GGTTTAAYAAAACAACCACC"3		

3. Antimicrobial susceptibility testing

Antibiotic susceptibility was determined using the Kirby-Bauer diffusion technique with discs [21]. The antimicrobial discs have been bought from Oxoid (Hampshire, England) and include, piperacillin-tazobactam (TPZ, 110µg), piperacillin (PRL, 100µg), ceftazidime (CAZ, 30µg), cefoperazone (CEP, 75µg), cefepime (FEP, 30µg), colistin sulfate (CT, 10µg), ciprofloxacin (CIP, 5µg), gentamycin (CN, 10µg), , aztreonam (ATM, 30µg), meropenem (Mem,10 µg), aztreonam (ATM, 30µg), tobramycin(TOB, 10µg), amikacin (AK, 30µg) and gatifloxacin (GAT, 5µg). The suspension of bacteria was produced using overnight cultures on Muller-Hinton agar (Oxoid, Hampshire, England). Soluble density has been modified to the 0.5 McFarland standard, which equates to about (1.5108 CFU/mL). Using sterile cotton swabs, Suspensions were inoculated on the surface of the Muller-Hinton agar plate. The plates are dried and incubated overnight at 37o °C before applying the antibiotic discs. The diameter of the inhibitory zones around the discs is measured. The Clinical Laboratory Standards Institute is used to analyze the findings [22].

3.1 Results

Clinical specimens were analyzed for the presence of *Pseudomonas aeruginosa* in one hundred samples. Bacterial isolation and identification confirmed the presence of (89) *P. aeruginosa* isolates, including 40/50 (80%) from the urine of patients with urinary tract infections and 49/50 (98%) from swabs from exudate wounds. Some biochemical experiments are conducted to provide further validation. Some biochemical tests find that 89 *P. aeruginosa* isolates are positive for the catalase, oxidase, motility, and B-hemolysis tests; negative for Gram's stain; and able to develop into blue-greenish colonies on cetrimide agar (at 37 °C for 24 hours).

3.2 Molecular Detection of *P. aeruginosa*

Genomic DNA was isolated from *P. aeruginosa* isolates using a genomic DNA purification kit (Geneaid, USA). Extraction of genomic DNA from 89 isolates verified by microscopy. A Nanodrop spectrophotometer was used to assess DNA concentration and purity; all of the isolates had DNA concentrations ranging from 50 to 100 ng/l, as well as the purity of the isolated DNA. PCR was used to identify *P. aeruginosa* isolates containing the blaIMP genes. The results of the present investigation shows that a few *P. aeruginosa* isolates with blaIMP gene, blaIMP gene molecular weight 232bp, showed 30 (75%) of the 40 isolates urine of patients with urinary tract infections, swabs from exudate wounds, while 38 (77.55%) of the 49 wound swab samples were positive, in significant variations (p. value = <0.05) show table (2) and figure (1).

Table (2): *P. aeruginosa* identification using genotypic methods

Type of specimen	No. of *P. aeruginosa* isolates	blaIMP gene	
		Positive	Negative
swabs from exudate wounds	49	38(77.55%)	11(22. 44%)
urine of patients with urinary tract infection	40	30(75%)	10 (25%)
Total	89	68(76.40%)	21(23.59%)

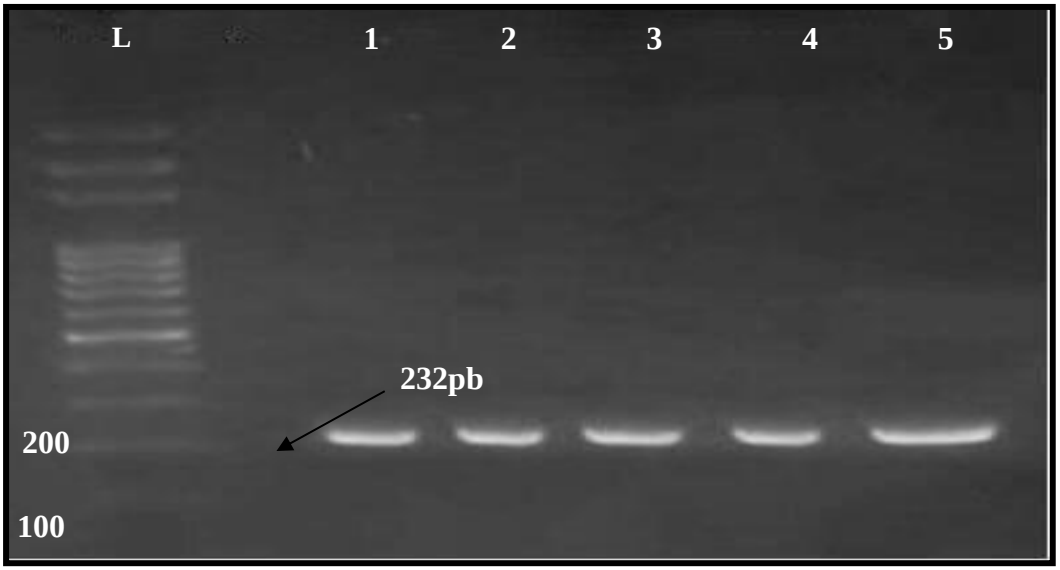

Figure 1: blaIMP genes gel electrophoresis amplified products of PCR from *P. aeruginosa* isolated by the PCR technique. 1.5% agarose gel l: DNA ladder (100-3000bp).

3.3. Antimicrobial Susceptibility Testing of *P. aeruginosa*

P. aeruginosa antibiotic resistance demonstrated diverse patterns of resistance with various drugs (Figure 1). Bacteria resistance to the antibiotics ciprofloxacin and gatifloxacin has been determined to be high (88.23% each), as was resistance to gentamycin (77.94%) and meropenem (72.05%). Bacterial resistance to cefoperazone, cefepime, piperacillin, and tobramycin is found to be intermediate (66.17%), amikacin (51.47%), piperacillin-tazobactam, ceftazidime, and aztreonam (48.52%, 41.17%, and 35.29%, respectively). Only colistin (14.70%) showed low bacterial resistance. Twenty isolates are sensitive to all antibiotics tested (29.41%). Only three isolates show pan-drug resistance to all antibiotics. Testing for antibiotic susceptibility revealed that 35 of the 68 P. aeruginosa isolates in this study are MDR (non-susceptible to more than a single antibiotic in three or more antibacterial classes), as shown in Table 3 and Figure 2.

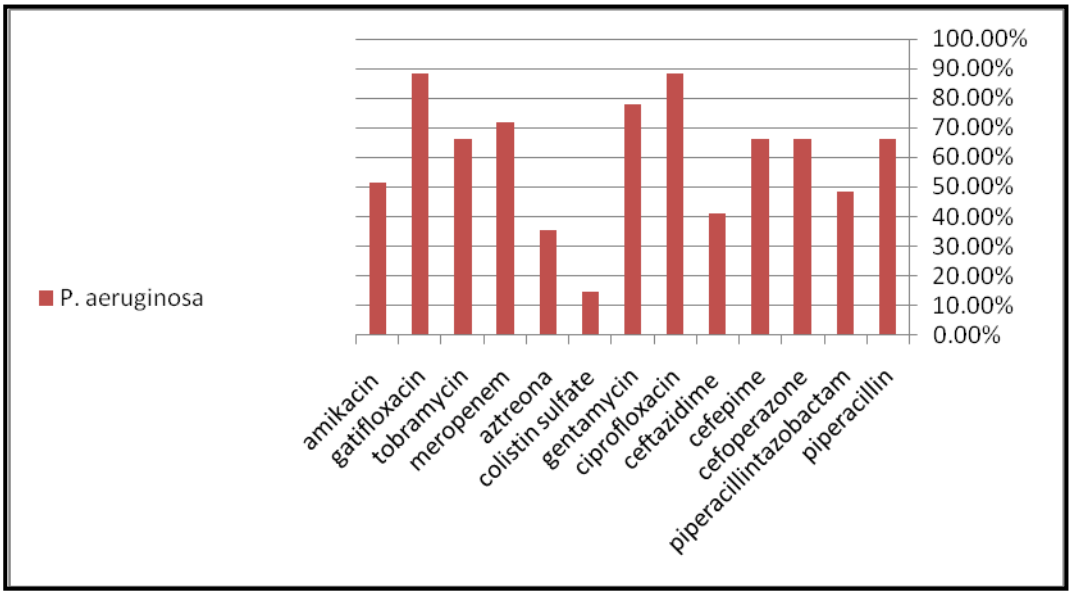

Figure 2: The percentage of *P. aeruginosa* that are resistant to antibiotics that have been examined

Table (3): The prevalence of multidrug-resistant *P. aeruginosa* isolates

Number of resistant isolates	Number of Antibiotics classes	Antibiotic classifications
6	4	B-lactams, fluoroquinolones, aminoglycosides and lipopeptide
25	3	fluoroquinolones, β-lactams, and aminoglycosides.
4		B lactams, lipopeptide and fluoroquinolones

4. Discussion

Bacterial contamination remains a major health concern in hospitals, as it can lead to serious infections in patients. Infections resulting from contaminated wounds and burns are becoming increasingly common (1). This study aimed to investigate the types of bacteria present in samples collected from patients in the burn unit—specifically, wound exudate swabs—and from individuals with urinary tract infections, using urine samples. These samples were obtained from Al-Fayhaa Hospital and Al-Basrah Teaching Hospital. Most of the bacterial isolates showed beta-hemolysis when grown on blood agar, although a few showed no hemolysis at all. Since they are unable to break down lactose, all isolates grew well on MacConkey agar but formed pale colonies. Further testing shows that every isolate is positive for both oxidase and catalase enzymes(5). Our study also finds that *Pseudomonas aeruginosa* is highly prevalent, with 89 out of 100 samples testing positive, indicating significant contamination among patients and within the hospitals examined.

A very high percentage (98%) of the collected cases showed wound infections, strongly indicating bacterial contamination. These results are in line with previous findings (23). Infections caused by *Pseudomonas aeruginosa* often occur during hospital-related outbreaks, likely because this bacterium is an opportunistic pathogen. It takes advantage of disruptions—whether widespread or localized—in the body's natural defenses. This allows it to invade and bypass both general and specialized immune barriers.

Finding this bacterium in surgical wounds suggests poor hygiene practices—not only from the patient and their clothing, but also possibly from hospital staff. It also points to contamination in the hospital environment, including medical equipment, beds, and other surfaces (24). The high level of contamination by this and other bacteria may be due to two main factors. First, doctors often rely on a limited range of antibiotics, which may not always be effective. Second, the overuse and misuse of antibiotics by the general public—often without medical advice—has led to the development of drug-resistant bacterial strains. Additionally, some bacteria have become resistant to the disinfectants and cleaning chemicals commonly used in hospitals for sterilization [25]. *Pseudomonas aeruginosa* is often found on the beds of patients with infected wounds or burns and can easily spread to the hands of healthcare workers who come into contact with them. It can also travel through the air, spreading from sources like water, disinfectants, or contaminated surgical tools to other areas of the hospital. This makes the hospital environment itself a significant factor in increasing patients' risk of infection from this bacterium [26].

4.1 Molecular Detection of *P. aeruginosa*

Although our phenotypic methods did not produce similar results, our findings were convergent and can be utilized in conjunction with molecular methods [27]. PCR investigation revealed that the blaIMP gene is present in 68 (76.40%) of the positive isolates of *P. aeruginosa*.

The overall incidence of MBL in the present investigation was roughly comparable to the rates in various studies. For example, hospitals in Baghdad have isolated 37.5% of MBL producers in various clinical specimens. Another study discovered 3.95% of MBL producers in patients with burn infections in Sulaimani City and 12.7% of MBL-producing P. aeruginosa in wound samples at Duhok Hospital in Iraq [28].

4.2 Antimicrobial Susceptibility Testing of *P. aeruginosa*

This study focuses on analyzing *Pseudomonas aeruginosa* strains obtained from clinical settings that exhibit antibiotic resistance. Multidrug-resistant (MDR) bacteria have emerged as a result of antibiotic abuse, which are especially difficult to treat due to their resistance to multiple medications. Antimicrobial resistance is a growing global

concern, particularly in developing countries[29]. To help prevent the spread of resistant bacteria, it's crucial to accurately and promptly identify *P. aeruginosa* and determine its antibiotic sensitivity, which can guide appropriate treatment and reduce unnecessary antibiotic use (13)**.**

This study investigated the antimicrobial resistance of *Pseudomonas aeruginosa* from various sources. Among the antibiotics tested, gentamicin was the most commonly used aminoglycoside, showing a resistance rate of 77.94%, followed closely by carbapenems such as meropenem, at 72.05%. We observed a ciprofloxacin resistance rate of 88.23%, which aligns with a 61.53% resistance rate reported by [30]. In contrast, another study by [31] reports a significantly lower resistance rate of just 25%. The level of resistance to ciprofloxacin may be influenced by how often fluoroquinolones are prescribed and the easy availability of oral formulations. Aminoglycosides are essential broad-spectrum antibiotics that work primarily by disrupting bacterial cell membranes and inhibiting protein synthesis [32]. In our study, resistance to amikacin is observed at 51.47%, which is notably lower than the 77.94% resistance rate seen with gentamicin. These findings are in line with previous research by (33), where gentamicin resistance (57.8%) was also higher than that of amikacin (35%). Another study [34] reports amikacin as the most effective aminoglycoside, with only 10% resistance. The variability in aminoglycoside resistance among clinical isolates is influenced by several factors, including the specific bacterial strain, resistance mechanisms, geographical location, and the particular antibiotic used (35). Carbapenems are often considered the most effective antibiotics for treating multidrug-resistant (*MDR*) *Pseudomonas aeruginosa*. However, rising resistance to these drugs is making it increasingly difficult for healthcare professionals to manage infections, leaving limited treatment options. It's crucial to take proper precautions to prevent the spread and amplification of genes responsible for producing carbapenemase enzymes [32]. This study also shows that bacteria are becoming increasingly resistant to β-lactam antibiotics. In particular, resistance in hospital-acquired *P. aeruginosa* infections has become a serious concern, especially against third- and fourth-generation cephalosporins.

Bacteria can become resistant to antibiotics through several molecular processes. These include producing extended-spectrum beta-lactamases (ESBLs), carrying *bla* genes within integrons, failing to properly express porin genes, or changing the structure of antibiotic target sites. In our study, 35 out of 68 samples (about 51.5%) were found to have multidrug-resistant (*MDR*) strains. This result is very similar to another study that reported an MDR rate of 52% [31]. It is also clear that 66.17% of the *P. aeruginosa* isolates are resistant to piperacillin. A recent study (36) reports a similar resistance rate of 56.5%, while another study [37] finds a lower rate of just 28%. In our research, the isolates also showed resistance to the piperacillin-tazobactam combination at a rate of 48.52%. This is slightly lower than the rate reported by study [33], but significantly higher than the 4.9% resistance observed in study [38].

4.3 Conclusion

The current investigation shows a high prevalence of P. aeruginosa in exudate wound swabs from burn unit patients. Resistance is the most prevalent to aminoglycosides (Amikacin and Gentamicin) and fluoroquinolones (Ciprofloxacin), with resistance to Colistin being the least common. The rise and spread of MDR P. aeruginosa is undeniable. Antibacterial research is inadequate to address the clinical issues posed by MDR bacterial crises. Antibiotic treatment failure could result from pharmaceutical misuse and abuse, as well as antibiotic prescriptions without susceptibility testing and extensive usage of broad-spectrum antibiotics.

Ethical approval: The study's ethics approval was obtained from Al-Fayhaa Hospital and Al Basrah

Teaching Hospital. (and participants before enrollment, all participants provided written informed consent.

Conflict of interest:

The authors declare that they have no conflict of interest.

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