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Original Research Article

Molecular Detection of Beta-Lactamase Genes and Phenotypic Resistance Patterns in *Pseudomonas aeruginosa* Isolates from Diwaniyah Hospitals, Iraq

Dania Ali Azeiz Al-Saadi[1*], Hind Fieq Abdalameer Alhachim[1], Sarah Nair Abdul ameer Aljumaily[2]

[1]Department of Pathological Analysis, College of Science, University of Al-Qadisiyah, Iraq
[2]Directorate of Education in Al-Diwaniyah, Al-Namariq Secondary School for Girls, Iraq

***Corresponding Author:** Dania Ali Azeiz Al-Saadi
Department of Pathological Analysis, College of Science, University of Al-Qadisiyah, Iraq

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Abstract: ***Background*:** *Pseudomonas aeruginosa* is an opportunistic bacterial pathogen responsible for a significant proportion of hospital infections, especially in intensive care units (ICUs). The bacterium's ability to resist multiple drugs and survive in hospitals makes it more likely to stick around and cause disease. In Diwaniyah Governorate, repeated studies have explored the phenotypic and molecular characteristics of *Pseudomonas aeruginosa,* particularly its beta-lactamase production and antibiotic resistance. The identification of genetic markers linked to drug resistance is essential for designing antimicrobial guided strategies. Even though previous studies have evaluated Pseudomonas aeruginosa in several hospital settings, little is known about its molecular characterization and resistance profile on this setting. Knowing the prevalence of resistant genes such as GES, blaOXA-48 and SPM may help in establishing measures for infection control. ***Objectives*** **:**The objective of this study was to use molecular techniques to isolate and identify *Pseudomonas aeruginosa* from clinical samples, find out what kind of antibiotics they are resistant to, and look for genes that make beta-lactamase. ***Materials*** **:**The study involved the different culture media were used, and identification relied on several biochemical reagents, such as Catalase, Oxidase, IMVC, and TSI tests, in addition to the Vitek2 Compact system. To detect β-lactamase activity, Penicillin G solution, starch solution, and iodine solution were applied in the rapid iodine method. For molecular work, genomic DNA was extracted using the Genomic DNA Mini Kit (Geneaid, USA). Preparation of electrophoresis gels required agarose powder (1.5%), TBE buffer (1X), and ethidium bromide as a DNA stain. The amplification of target genes was carried out using Wizpure PCR FDmix, along with specific primers for *16S rRNA, blaSPM, blaGES,* and *blaOXA-48* genes, in addition to PCR-grade water. ***Results*:** *Pseudomonas aeruginosa* was confirmed in 15 out of 40 clinical samples (37.5%). The highest isolation rate was found in burns (60%), then wounds (26.6%), and finally urine (13.4%). The rapid iodine test and the Vitek 2 system showed that 80% of the isolates made beta-lactamase, which means they were resistant to most antibiotics except for tigecycline. Polymerase chain reaction (PCR) analysis indicated that all isolates possessed the 16S rRNA gene, 75% contained the OXA-48 gene, 40% harboured the bla GES gene, and 20% possessed the SPM gene. This study is one of the first to show what *Pseudomonas aeruginosa* is made of in the Diwaniyah Governorate. It also shows how resistant it is and what genes are responsible for making beta-lactamase. ***Conclusion*:** The results show how important it is to keep an eye on things all the time, follow strict infection control rules, and have antimicrobial stewardship programs in place to stop the spread of drug-resistant Pseudomonas aeruginosa in healthcare settings.

Keywords: *Pseudomonas Aeruginosa*, Beta-Lactamase Production, Antibiotic Resistance, Molecular Characterization, PCR Detection, Nosocomial Infections.

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1. INTRODUCTION

Pseudomonas aeruginosa is a common and dangerous opportunistic pathogen that can cause serious hospital-acquired infections in people with weak immune systems. Its amazing ability to fight off many antibiotics, including beta-lactams, is becoming harder and harder to find in clinics. The production of beta-lactamases, especially ESBLs and MBLs, is a major reason why these bacteria are less likely to respond to beta-lactams. This has made treatment options limited and increased hospital and overall mortality [1].

The increasing prevalence of multidrug-resistant (MDR) *P. aeruginosa* in Iraq has emerged as a significant public health concern. However, documentation regarding the molecular characterisation of beta-lactamase genes and their association with phenotypic resistance patterns in *P. aeruginosa* isolates from Diwaniyah hospitals is limited. Understanding the genetic basis of resistance is essential for implementing effective infection control measures and directing appropriate antibiotic therapy [2].

Nosocomial infections, also known as hospital-acquired infections (HAIs), are those that develop 48 hours or more after being admitted into a hospital and involve great clinical and economic costs. The development and dissemination of antimicrobial resistance (AMR) in HAI pathogens compromise effectiveness of standard empiric regimens, prolong length of stay, morbidity, and mortality, as well as raise healthcare costs [3].

Antibiotic resistance in *P. aeruginosa* can be acquired or intrinsically caused by transferable genetic elements such as integrons and plasmids. Numerous mechanisms contribute to this resistance, such as altered target sites in the bacterial cell wall, increased drug delivery through efflux pump overactivity, decreased antibiotic uptake due to decreased permeability of the outer cell membrane, and the production of carbapenemases, which break down all beta-lactam antibiotics, including carbapenems [5, 4].

2. MATERIALS AND METHODS

2.1. Sample Collection

The study included various clinical samples taken from different parts of the body for hospitalized patients of all ages and both sexes, such as wounds, burns, and urine. For the period of August 2024 to February 2025, 40 samples were collected from hospitals and health centers in the Diwaniyah Governorate, specifically the Women's and Children's Hospital, Diwaniyah General Teaching Hospital, and Public Health Laboratory.

2.2 Isolation and Identification

Swabs and samples were taken directly to the lab to look for certain cultural, microscopic, and biochemical traits. They were then grown for 24 hours at 37°C in an aerobic environment. To identify the bacterial species, culture media were used, and a series of biochemical tests (catalase, oxidase, IMVC, and TSI tests) as well as the morphological and microscopic characteristics of the isolates growing on the plate were used. The Vitek2 Compact and the previously published [6] validated the diagnosis of *P. aeruginosa* isolates.

2.3 Rapid Standard Iodine Method for Detecting the Ability of Bacteria to Produce Beta-Lactamase Enzymes

a. Using sterile wooden sticks, several colonies that had just formed and were between 18 and 24 hours old were moved to a microdilution plate with 100 microlitres of penicillin G solution in each hole. The colonies had been grown on the heart-brain infusion medium. The colonies were mixed well and then put in an incubator for 30 minutes at 37 °C.
b. To guarantee uniformity of the contents, add 50 microliters of starch solution and 20 microliters of iodine solution, both of which should be thoroughly mixed with wooden sticks. Iodine and starch react to generate the blue color, and it is a good sign when the color changes quickly from blue to white in about five minutes.

Table 1: Primers used in this study with their nucleotide sequence and amplification size

Gene name	Oligo sequence (5'-3') (primer)	Product Size (bp)	Reference
16 srRNA	F TCAACCTGGGAACTGCATCC	688 bp	[7]
	R CAGACTGCGATCCGGACTAC		
bla SPM	F AAAATCTGGGTACGCAAA CG	271 bp	[8]
	R ACATTATCCGCTGGAACAGG		
bla GES	F ATGCGCTTCATTCACGCAC	860 bp	[16]
	R CTATTTGTCCGTGCTAAGG		
bla OXA-48	F TTGGTGGCATCGATTATCGG	744 bp	[10]
	R GAGCACTTCTTTTGTGATGGC		

2.4 Genomic DNA Extraction

Nucleic Acid was extracted from *Pseudomonas aeruginosa* using Geneaid USA's pre-made Genomic DNA Mini Kit in accordance with their instructions.

2.5 Preparation of Agarose Gel

It was prepared according to the method of Sambrook and his group as follows:

a. Using a magnetic hot plate stirrer, dissolve 1.5 g of Agarose gel in 100 ml of TBE buffer

solution at a concentration of (1X) for 15 minutes.

b. Three microliters of the radioactive DNA dye ethidium bromide were added to the gel and thoroughly mixed with it after it had cooled to 50°C.
c. After pouring the acro gel into the migration mold (the tray holding the comb), the gel was allowed to solidify for fifteen minutes at room temperature. The comb was then carefully taken out of the gel to create and define the holes (wells) in the gel that were required for injecting the amplified samples.

2.6 PCR Master Mix Preparation

1. As directed by the business that provided the Wizpure PCR FDmix and as indicated in Table 2 below, prepare this mixture in PCR tubes:

Table 2: Components of the PCR master mix

Mixture Ingredients	Size
PCR FD mix	1Tube
10mM Forward Primer	1.5 µL
10mM Reverse Primer	1.5 µL
Template DNA	5 µL
PCR water	12 µL
Total	**20 µL**

2. Once the mixture tubes were ready, they were put in the Vortex machine for five seconds. The tubes were then put in the PCR thermocycler machine to carry out the DNA amplification procedure under the best possible thermal cycle conditions.
3. The program that uses heat cycles to amplify DNA Based on the reaction, the thermocycler PCR apparatus was utilized and set up for the genes being investigated.

3. RESULTS AND DISCUSSION

3.1 Numbers and Percentages of Pseudomonas Aeruginosa Isolation

Insgesamt wurden 15 Isolate identifiziert (Isolation rate: 37.5 %). *Pseudomonas aeruginosa* exhibited the highest isolation rate from burns (60%) and the second highest from wound infections (26.6%). In contrast, urine samples showed the lowest isolation rate (13.4%). The results align with those of the study by [11], which found that *P. aeruginosa* was the most frequently isolated organism among burn patients, representing 59.3% of all isolates. This corroborates the idea that burn wounds, which are moist and rich in protein, offer an ideal breeding ground for bacteria. Likewise, the present results correspond with those of [12], who noted that *P. aeruginosa* isolation rates from wound infections ranged from 20% to 30%, closely aligning with the 26.6% observed in this study. In contrast, the results of this study are different from those of [13], who found that the rate of isolating *P. aeruginosa* from urine samples was much higher (38%). This was associated with the frequent use of urinary catheters, particularly in intensive care units. In short, the results of this study show how important the clinical environment is for finding the sources of *P. aeruginosa* infections. They also stress how important it is to follow strict infection control rules, especially in burn and surgical units, to stop the spread of resistant *P. aeruginosa* strains.

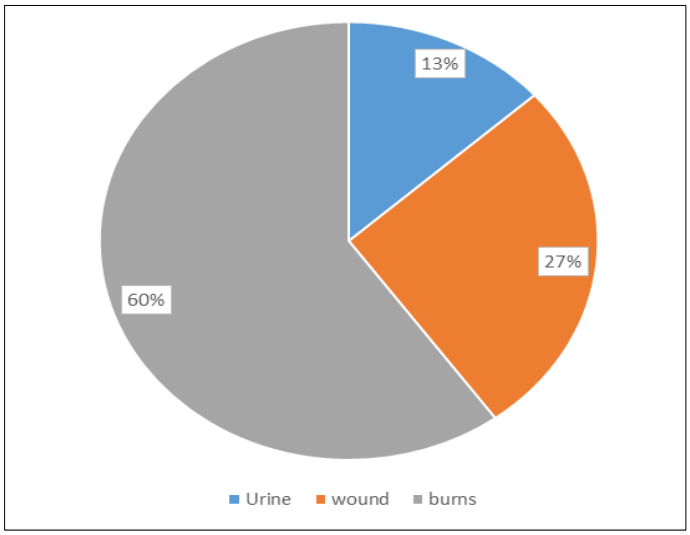

Figure 1: Distribution of Pseudomonas aeruginosa isolates according to sample source

The results showed that 12 out of 15 isolates, representing 80%, had the ability to produce beta-lactamase enzymes, and that the resistance shown by these bacteria to some antibiotics, such as penicillins and cephalosporins that contain a beta-lactam ring in their structure, is consistent with what was previously mentioned [14]. Based on the results of the Vitek device, the majority of the isolates showed resistance to the antibiotics under study with a resistance rate of 100%, with the exception of the antibiotic tigecycline, which demonstrated its effectiveness and inhibition of bacteria, as the percentage of sensitivity of bacteria to this antibiotic ranged between (0.3-1).

Table 3 shows the percentage of beta-lactamase production in *Pseudomonas aeruginosa* isolates from different sources. 80% of the isolates were beta-lactamase producers and 20% were non-producers. One of these isolates was from burns and two from urine did not produce beta-lactamases.

Table 3: Ability of P. aeruginosa isolates to produce beta-lactamase enzymes according source isolated

Ability to produce beta-lactamase enzymes	Burn	Wound	Urine	Percentage %
Producing isolate	8	4	0	80%
Non-producing isolate	1	0	2	20%

As shown in Figure 2, white represents a positive value in the rapid standard iodine method, while dark represents a negative value. The distinct color differences observed in the microplate assay reflect the specific enzyme activity of the tested samples, which is used to assess bacterial starch hydrolysis or other biochemical properties.

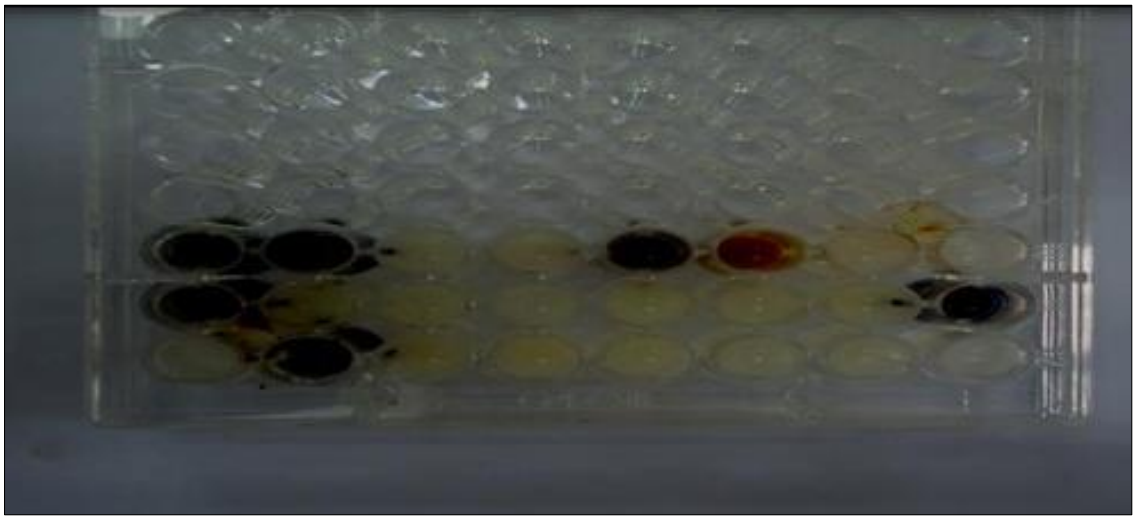

Figure 2: Shows the rapid standard iodine method (White is positive, Dark is negative)

3.2. Molecular Diagnosis of P. Aeruginosa through Polymerase Chain Reaction

The 16srRNA gene was tested in all 15 bacterial isolates studied and was found to be present in 100% of them. Virulence genes such as bla *SPM* were found in 20% of the isolates; bla *GES* in 40%; and bla *OXA-48* in 75%. The results of amplification by PCR for these genes are displayed in Figures (3, 4, 5, and 6).

This is attributed to the production of beta-lactamase enzymes by these bacteria and their resistance to antibiotics in this class through modifications to their outer membrane proteins and efflux pumps [15, 16]. *Pseudomonas aeruginosa* is regarded as a multidrug-resistant bacterium, possessing various mechanisms that confer resistance to these medications. These include the production of beta-lactamase enzymes that deactivate antibiotics, the use of efflux pumps to remove antibiotics from the cell, and the presence of certain genes on its chromosomes or plasmids that facilitate antibiotic resistance, as illustrated in Figure (7). This is among the key factors contributing to the studied bacteria's resistance to antibiotics [17-19].

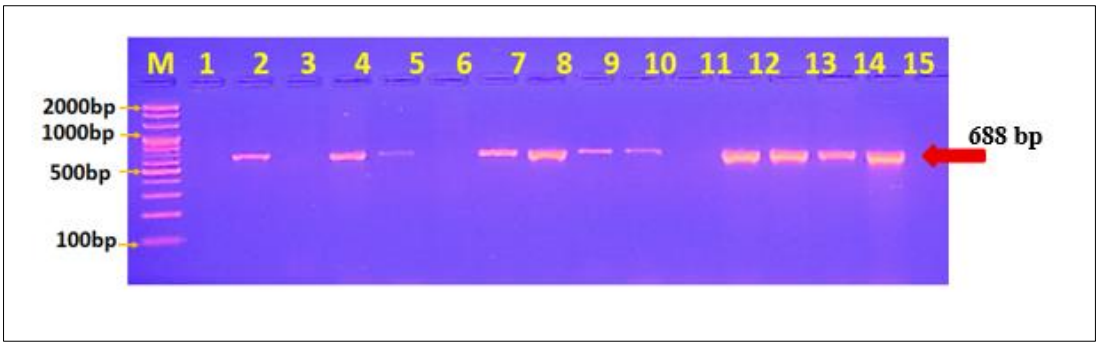

Figure 3: PCR product of 16srRNA gene (688 bp)
Line (M): DNA ladder 2000 base-pair
Lines (2, 4, 5, 7-14): Positive result of 16srRNA gene

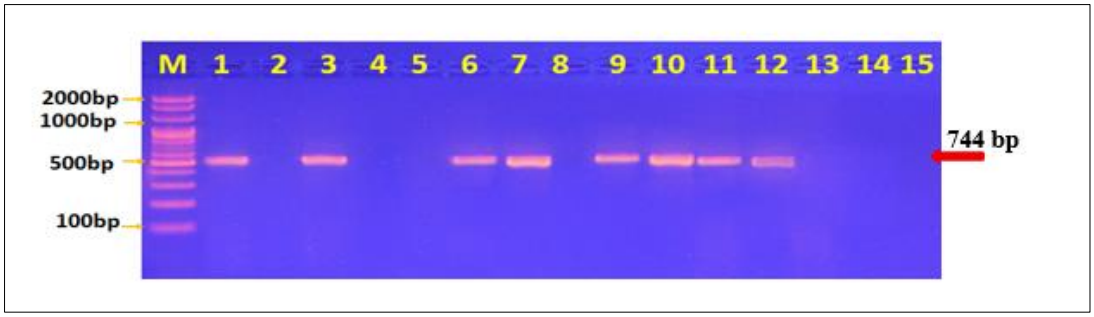

Figure 4: PCR product of beta-lactamase gene (*bla* OXA-48, 744bp)
Line (M): DNA ladder 2000 base-pair.
Lines (1,3,6,7,9-12): Positive result of *bla* OXA-48 gene

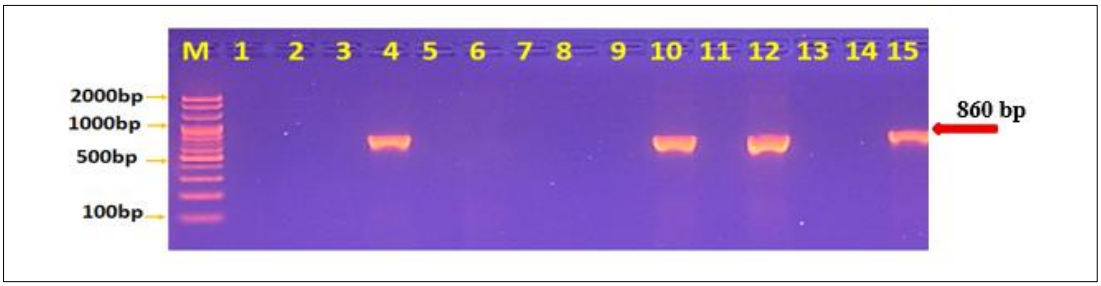

Figure 5: PCR product of beta-lactamase gene (*bla* GES, 860 bp)
Line (M): DNA ladder 2000 base-pair
Lines (4,10,12,15): Positive result of *bla* GES gene

Figure 6: PCR product of beta-lactamase gene (*bla* SPM, 271 bp)
Line (M): DNA ladder 2000 base-pair
Line (4): Positive result of *bla* SPM gene

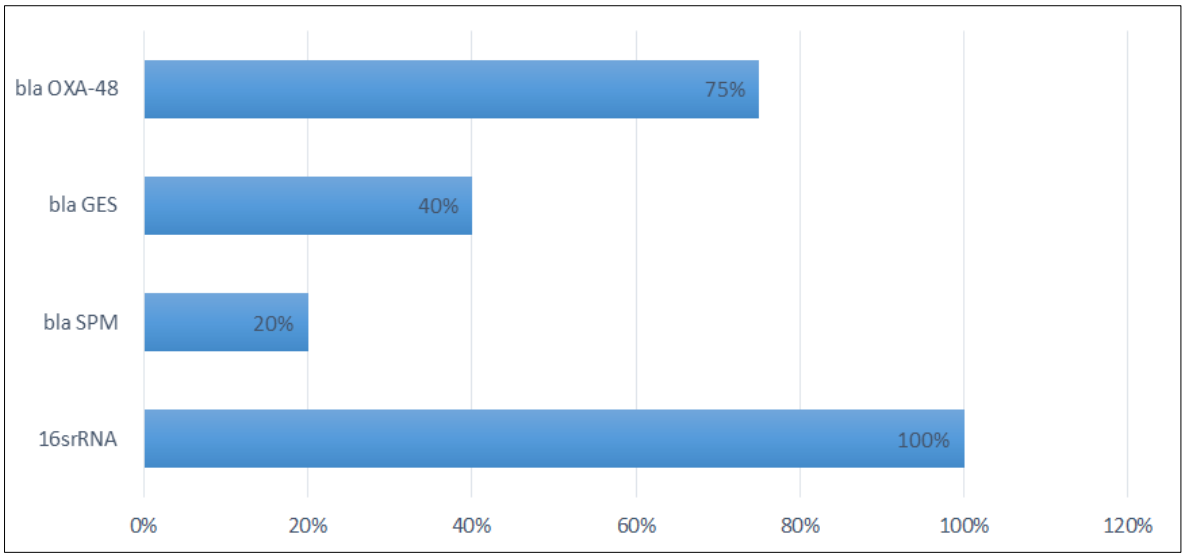

Figure 7: The percentage of gene prevalence in *Pseudomonas aeruginosa* bacteria under study.

4. CONCLUSION

This uncovers a considerable presence of *Pseudomonas aeruginosa* in clinical samples from the Diwaniyah Governorate, showing notable resistance to the majority of antibiotics while being sensitive to tigecycline. All isolates possessed the 16S rRNA gene, with 75% carrying the *bla OXA-48* gene, 40% the *bla GES* gene, and 20% the *bla SPM* gene, demonstrating a significant potential for beta-lactamase production. The findings emphasize the urgent necessity of bolstering infection control measures, enhancing antibiotic stewardship programs, and maintaining ongoing molecular surveillance of multidrug-resistant *Pseudomonas aeruginosa* prevalence in healthcare settings. It is therefore important for further studies to determine treatment options other than therapy, including approaches that focus on new antimicrobials or methods of targeted gene inhibition for dealing with this erheblicher pathogen.

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