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# Prevalence of Carbapenem resistant *Pseudomonas aeruginosa* at hospital in Baghdad, Iraq

**[1] Hadeel Abdul Muttalib Kazem, [2] Samer Abdel-Sahib Muhammad**

[1, 2] Department of Pathological Analysis, Faculty of Science, University of Kufa, Iraq

Corresponding Author: **Hadeel Abdul Muttalib Kazem**

**Abstract**

Worldwide, Infections related to healthcare are frequently caused by multidrug resistant *Pseudomonas aeruginosa*, Gram-negative bacteria, such as *Pseudomonas aeruginosa*, that are resistant to many drugs produce infections that can be treated with carbapenems, although its use is threatened by the emergence of carbapenemase-producing strains. The aim of this study was to determine the prevalence of carbapenem resistant *Pseudomonas aeruginosa* In Baghdad hospitals, by conducting susceptibility test and identification of carpenemase genes harbored by the isolates**.** 125 samples were collected from various clinical sources, and 68 isolates of *Pseudomonas aeruginosa* showed. Susceptibility tests showed that 35 isolates were carbapenem-resistant. The carbapenemase genes blaOXA-48, blaKPC and blaVIM were found in 5.7%, 2.9% and 22.9%, respectively, and the blaOXA-48 and blaVIM genes were coexpressed in one isolate, blaIMP was not observed in the isolates. For the efficient treatment of carbapenem-resistant *Pseudomonas aeruginosa* infections, epidemiological and geographic evaluation of carbapenemase-producing *Pseudomonas aeruginosa* should be taken into consideration.

**Keywords:** BlaOXA-48, BlaKPC, BlaVIM *Pseudomonas Aeruginosa*

## Introduction

Pseudomonas aeruginosa is an opportunistic pathogen, an aerobic, Gram-negative bacillus that is able to survive in moist environments, it One of the agents responsible for hospital-acquired infections, particularly in patients with burns, It is caused by its high prevalence and severity as well as inherent and acquired resistance to antibiotics (Adachi *et al* 2009) [1] Carbapenems is the drug of choice for treatment of serious infections caused by *P. aeruginosa*, it is class from antibiotics has a broad spectrum of activity, classified as β-lactam It includes (imipenem, meropenem, doripenem and ertapenem) These antibiotics are relatively stable against the hydrolysis caused by beta-lactamase enzymes (Bradley *et al* 1999; Khosravi *et al* 2008) [7, 10] Of the four classes of beta-lactamases carbapenemases belong to three of them, Based on the hydrolytic mechanisms at their active sites Ambler classes A, B, and D can be distinguished from one another (Manenzhe *et al*, 2015; Queenan *et al* 2007) [11, 12]. Serine (an amino acid) is present at the active site of Class A and Class D carbapenemases making them serine carbapenemases (serine-dependent), Class B carbapenemases on the other hand are zinc-dependent and are known as metallo-lactamases (Tsakris *et al* 2006) [14] Ambler class A carbapenemases can be plasmid encoded or chromosomal and are inhibited by clavulanic acid, a β-lactamase inhibitor; SME, IMI, NMC, GES, and KPC families are the most frequently identified class A carbapenemases mostly in *Klebsiella pneumonia*. The most prevalent enzymes belong to the VIM, IMP, SPM, GIM, SIM, and NDM families, which are plasmid-encoded (and occasionally chromosomal) in class B metallo-lactamases, Metallo-β-lactamases have been detected primarily in *P. aeruginosa* but they are also increasingly being detected in Acinetobacter species (Tsakris *et al* 2006) [14], Furthermore, class B enzymes are able to hydrolyze β-lactams except aztreonam (a monobactam) and their hydrolytic activity is inhibited by EDTA (ethylene ediammine tetra acetic acid), but not clavulanic acid. class D enzymes, also referred to as the OXA-type carbapenemases, are subdivided into five families; OXA-23, OXA-24/40, OXA-48 and OXA-58 families that are mainly plasmid-encoded (Queenan *et al* 2007) [12] Class D enzymes are not inhibited by clavulanic acid or EDTA (Manenzhe *et al* 2015) [11].

## Materials and Methods

### Bacterial Isolates

This short-term descriptive cross-sectional study in the Department of Pathological analyzes, College of Science, University of Kufa, During the period from November 2022 to March 2023, 152 isolates were collected from microbiology laboratories in teaching hospitals (Yarmouk Teaching Hospital, Medical City/Burn Center) in Baghdad. Pseudomonas aeruginosa was identified using previously described phenotypic tests (Winn *et al* 2006) [15] and verified by PCR.

### Antimicrobial Susceptibility Testing

All isolates were tested by the Kirby-Bauer method as recommended by the Clinical and Laboratory Standards Institute (CLSI; 2022) E.coli strain ATCC 25922 was used as a quality control for the antibiotics tested. The following were tested: imipenem (10 µg), Meropenem (10) Ceftazidime (30 µg), Ceftriaxone (10 µg), Cefepime (30 µg), Piperacillin (100 µg), Ticarcillin (75) Piperacillin/tazobactam (10/100) µg), gentamicin (10 µg), amikacin (30 µg), tobramycin (10 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), aztreonam (30 µg), colistin (10 µg) (Bioanalyse) When the isolate was resistant to three or more classes of antip seudomonal agent (i.e.penicillins /cephalosporins, carbapenems, aminoglycosides and fluoroquinolones), that isolate was considered as multidrug resistant (MDR).

### Detection of by PCR

PCR analysis was performed for blaIMP, blaVIM,blaKPC, blaOXA-48, For genes blaIMP, blaVIM The primers used were described by (Dallenn *et al* 2010) [8] (IMP-F TTGAACACTCCATTTACDG, IMP-R GATYGAGAATTAAGCCACYCT with Product size of 139bp and annealing temperature at55 ºC) (VIM-F GATGGTGTTTGGTCGCATA,VIM-R GATTTGCTCCGTGGCCGAAA with Product size of 390bp and annealing temperature at 55 ºC) And use the primer for the gene blaKPC described by (Bardford *et al* 2004) [6] KPC-FATG TCA GTC TAT CGC CT, KPC-RTTT TCA GAG CCT TGC CC with Product size of 893bp and annealing temperature at 62 ºC) And use the primer for the gene blaOXA-48 described by (Dallenn *et al* 2010) [8] OXA-48-FGCTTGATCGCCCTCGATT, OXA-48-R GATTTGCTCCGTGGCCGAAA with Product size of 281bp and annealing temperature at 50 ºC) DNA was extracted using the boiling method. Briefly, frozen bacteria were sub cultured onto Mueller–Hinton's agar (Merck, Germany) before DNA extraction. One to five bacterial colonies were suspended in 500 µL of 1X TrisEDTA buffer and heated at 95°C for 10 minutes and placed at room temperature for 5 minutes. The suspension was then placed at -20°C for 10 minutes and after centrifugation at 14,000 rpm for 10 minutes at 4°C, 2 µL of supernatant was used as the template for a 50 µL PCR reaction. The master mixture for detection of all genes consisted of: 5 µL of 10X reaction buffer, 2 µL of 50 mM MgCl2, 1 µL of 2.5 mM dNTPs, 2 µL of each 20 pmol/µL primer, 0.4 µL Taq polymerase 5 U/µL and 35.6 µL distilled water. DNA was amplified in a Master cycler Eppendorf (Eppendorf, Germany) under the following conditions: initial denaturation for 5 minutes at 95°C followed by 30 cycles at 95°C for 30 seconds, at specific annealing temperature for 30 seconds, then at 72°C for 30 seconds. A final extension was performed for 7 minutes at 72°C and PCR products were kept at 4°C. Amplicons were electrophoresed on a 2 % agarose gel with 0.5 µg/mL ethidium bromide in 1X Tris Borate EDTA buffer. Gels were visualized and photographed under ultraviolet illumination (primers were obtained from TAG Copenhagen A/S, Denmark and all chemical materials from Cinna Gen, Iran).

### Statistical Analysis

Data were coded and entered using the statistical package for (SPSS) version 26 (IBM Corp., Armonk, NY, USA). Data were summarized using minimum and maximum in quantitative data and using frequency (count) and relative frequency (percentage) for categorical data.

## Results

Among the 152 isolates, 68 *Pseudomonas aeruginosa* isolates were recorded, 35 isolates were resistant to carbapenem. Table 1 shows the clinical sources of *Pseudomonas aeruginosa* isolates. 34 (50%) were meropenem-resistant, 27 (39%) were imipenem -resistant, and susceptibility tests to other antibiotics are summarized in Table 2. Among the isolates *Pseudomonas aeruginosa* Resistance to carbapenem 12 (34.3%) were multi- resistant, antibiotics. PCR analysis was performed for the *P. aeruginosa* isolates Resistance to carbapenem. Genes have been discovered blaVIM, 8 (22.9%) isolates, and were not detected blaIMP. blaKPC and blaOXA-84 1(2.9%), 2(5.7%) respectively. Figure 1, 2, and 3.

**Table 1:** Source of *P.aeruginosa* Clinical Isolates (No= 68)

| Source of isolation | *p. aeruginosa* Isolates No | % |
|---|---|---|
| Wound | 15 | 55.5 |
| Urine | 12 | 38.7 |
| Burn | 10 | 62.5 |
| Sputum | 8 | 32 |
| Ear | 14 | 38.3 |
| CSF | 6 | 37.5 |
| blood | 3 | 37.5 |
| Total | 68 | 44.7 |

**Table 2:** Antimicrobial Susceptibility in *P. aeruginosa* Clinical Isolates

| Antibiotic | Resistant | % | Sensitive | % |
|---|---|---|---|---|
| Meropenem | 34 | 50% | 34 | 50% |
| Imipenem | 27 | 39.7% | 41 | 60.3% |
| Amikacin | 60 | 88.2% | 8 | 11.8% |
| Gentamicin | 44 | 64.7% | 24 | 35.3% |
| Tobramycin | 36 | 52.9% | 32 | 47.1 % |
| Ceflazidime | 62 | 91.2% | 6 | 8.8% |
| Ceftriaxone | 60 | 88.2% | 8 | 11.8 % |
| Cefepime | 63 | 92.6 % | 5 | 7.4% |
| Ciprofloxacin | 26 | 38.2% | 42 | 61.8% |
| Levofloxacin | 28 | 41.2 % | 40 | 58.8 % |
| Piperacillin | 41 | 60.3 % | 27 | 39.7 % |
| Ticarcillin | 65 | 95.6% | 3 | 4.4% |
| piperacillin/ tazobactam | 21 | 30.9% | 47 | 69.1% |
| Azteronam | 28 | 41.2% | 40 | 58.8% |

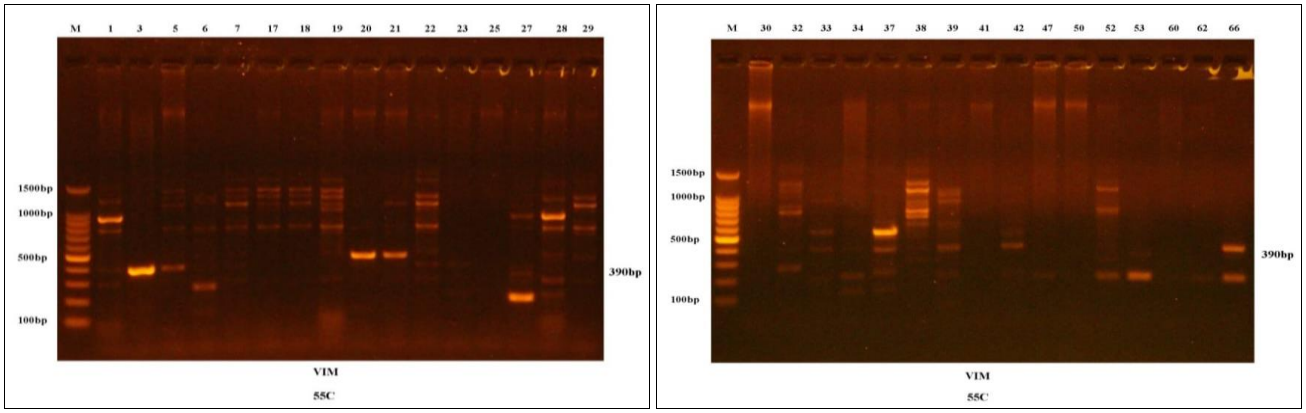


**Fig 1:** Results of the amplification of VIM gene of bacterial species were fractionated on 2% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 1-29resemble 390bp PCR products

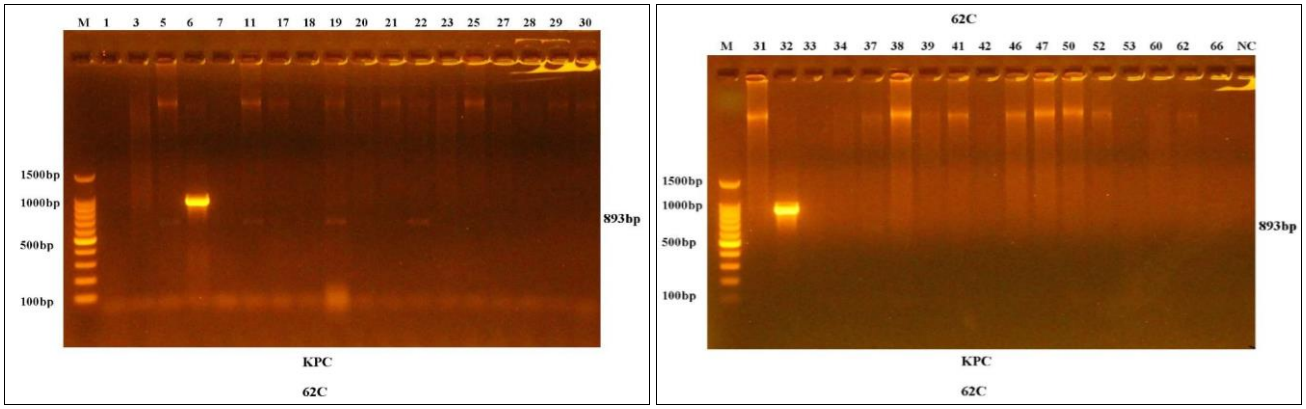


**Fig 2:** Results of the amplification of KPC gene of bacterial species were fractionated on 2% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 31-66resemble 893bp PCR products

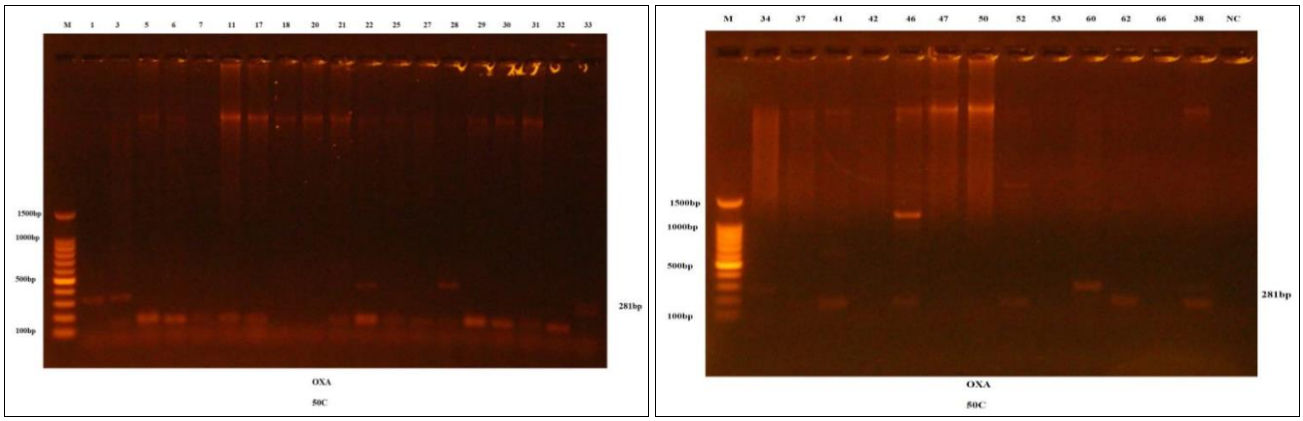


**Fig 3:** Results of the amplification of OXA gene of bacterial species were fractionated on 2% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 1-33 resemble 281bp PCR products

## Discussion

One of the main issues in treating hospitalized patients is antibiotic resistance among gram-negative bacteria like *P. aeruginosa*., Multiple mechanisms, including the synthesis of MBL, contribute to β-lactam resistance in *P. aeruginosa*., MBL enzymes are capable of hydrolyzing carbapenems, Nosocomial infections are commonly treated with, meropenem and imipenem although the efficiency of these antibiotics has been hampered by rising resistance to them. This study showed a high rate of carbapenem resistance in Baghdad hospitals. Meropenem and imipenem were tested, and resistance to meropenem was 50% and to imipenem 39%. Many studies showed higher resistance to meropenem compared to imipenem (Shatti 2021; Al-kazrage 2021) [13, 5] Interestingly, blaVIM (22.9%) is the most common carbapenemase identified in carbapenem-resistant *P. aeruginosa* isolates, and higher rates have been reported in Iran at 40% (Dogonch 2018) [9] and it was not recorded in Baghdad according to (Algebra 2018) [3]. In this study, one isolate (2.9%) containing blaKPC was identified among the 35 carbapenem-resistant *P. aeruginosa* isolates. It was reported (Al-Abedi and Al-Mayahi 2019) [2] reported 11 isolates containing blaKPC. In this study, two isolates (5.7%) containing blaOXA-48 were identified among carbapenem-resistant *P. aeruginosa* isolates, and this is in line with (Al-Janahi 2020) [4] who reported two isolates out of 34 carbapenem-resistant *P. aeruginosa* isolates. According to the results of this study, one isolate (2.9%)

was identified that contains blaVIM in addition to its coexistence with blaOXA-48. (Al-Janahi 2020) [4] reported the co-occurrence of these two genes among carbapenem-resistant *P. aeruginosa* isolates.

## Conclusions

Carbapenem-resistance prevalence is comparatively high in isolates *Pseudomonas aeruginosa* In Baghdad hospitals, the emergence of carbapenemase-producing strains (VIM, KPC, OXA-48), It is alarming that infections are spreading in hospitals among patients and health care workers.

## References


1. Adachi JA, Perego C, Graviss L, Dvorak T, Hachem R, Chemaly RF, *et al*. The role of interventional molecular epidemiology in controlling clonal clusters of multidrug resistant Pseudomonas aeruginosa in critically ill cancer patients. Am J Infect Control.2009; 37(6):442-446.
2. Al-Abedi KJ, Al-Mayahi FS. Molecular detection of serine carbapenemase genes in carbapenem-resistant isolates of Pseudomonas aeruginosa recovered from patients in Al- Diwaniyah province, Iraq. J. Pure Appl. Microbiol. 2019; 13(3):1775-1782.
3. Algebra ML. Molecular study of ampenem-resistant Pseudomonas aeruginosa isolated from different clinical samples. A Thesis Submitted to the Council of College Education for Pure Sciences/Ibn Al-Haytham-University of Baghdad, 2018.
4. Al-Janahi HCL. Occurrence and Molecular Characterization of Metallo-β-Lactamase (MBLs)-Producing Pseudomonas aeruginosa in Najaf Hospitals A Thesis Submitted to the Council of the Faculty of Medicine, University of Kufa, 2020.
5. Al-kazrage HAD. Inhibition of Virulence Factors in Pseudomonas aeruginosa Isolated from Clinical Samples Using Galardin Loaded AgPEG Nano composite. A Dissertation Submitted to the Council of the College of Biotechnology, University of Al-Nahrain, 2021.
6. Bradford PA, Bratu S, Urban C, Visalli M, Mariano N. Emergence of Carbapenem-resistant Klebsiella Species Possessing the class A Carbapenem Hydrolyzing KPC-2 and Inhibitor-Resistant TEM-30 β-lactamases in New York City. Clin Infect Dis. 2004; 39:55-60.
7. Bradley JS, Garau J, Lode H, Rolston KV, Wilson SE, Quinn JP. Carbapenems in clinical practice: A guide to their use in serious infection. Int J Antimicrob Agents. 1999; 11(2):93-100.
8. Dallenne C, Da Costa A, Decré D, Favier C, Arlet G. Development of a set of multiplex PCR assays for the detection of genes encoding important β-lactamases in Enterobacteriaceae. Journal of Antimicrobial Chemotherapy. 2010; 65(3):490-495.
9. Dogonchi AA, Ghaemi EA, Ardebili A, Yazdansetad S, Pournajaf A. Metallo-β-lactamase-mediated resistance among clinical carbapenem-resistant Pseudomonas aeruginosa isolates in northern Iran: apotential threat to clinical therapeutics. Tzu-Chi Medical Journal. 2018; 30(2):90-96.
10. Khosravi AD, Mihani F. Detection of metallo-beta-lactamase-producing Pseudomonas aeruginosa strains isolated from burn patients in Ahwaz, Iran. Diagn Microbiol Infect Dis. 2008; 60(1):125-128.
11. Manenzhe RI, Zar HJ, Nicol MP, Kaba M. The spread of carbapenemase producing bacteria in Africa: A systematic review. J Antimicrob Chemother. 2015; 70(1):23-40.
12. Queenan AM, Bush K. Carbapenemases: the versatile beta-lactamases. Clin Microbiol Rev. 2007; 20(3):440-458
13. Shatti. Study the association among biofilm formation and quorum sensing genes in locally isolated multidrug resistant pseudomonas aeruginosa. A thesisSubmitted to the College of Medicine, University of AL-Mustansiriyah, 2021.
14. Tsakris A, Ikonomidis A, Pournaras S, Tzouvelekis LS, Sofianou D, Legakis NJ, *et al*. VIM-1 metallo-beta-lactamase in Acinetobacter baumannii. Emerg Infect Dis. 2006; 12(6):981-983.
15. Winn W, Allen S, Janda W, Koneman E, Procop G, Schreckenberger P. Koneman's Color Atlas and Textbook of Diagnostic microbiology. 6 edPhiladelphia: Lippincott Williams & Wilkins, 2006.