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Prevalence of Metallo-β-lactamase–Producing Pseudomonas Aeruginosa Isolated from Clinical Samples in Iraq

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ABSTRACT

Objective: The objective of this study was to identify antibiotic susceptibility, phenotypic detection of producing P. aeruginosa in Iraq

Results: Twenty four percent and 20% of the twenty-five carbapenem-resistant P. aeruginosa (CRPA) isolates tested positive for the IMP1gene and IMP-2 genes

Respectively

Keywords: *Pseudomonas aeruginosa, β-lactams, penicillin, β-lactamase and bacterial resistance*

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1. INTRODUCTION

Metallo-β-lactamase-producing Pseudomonas aeruginosa (MPPA) is a common important source of nosocomial infection[1]. Pseudomonas aeruginosa is an opportunistic pathogen that affects the individuals have health problems such as severely burn patients and those that underwent major surgeries or immunocompromised individuals such as those with diabetes, cancer, cystic fibrosis, advanced HIV infections (acquired immunodeficiency syndrome, AIDS)[2]. This species shows resistance to a wide range of β-lactam antibiotics among Gram-negative bacteria including β-lactams, carbapenems, aminoglycosides, fluoroquinolones, and polymyxins [3] . P. aeruginosa exhibits a high degree of innate and acquired resistance to several antibiotics, which makes therapy difficult and restricts available alternatives [4]. the mechanisms of bacterial resistance to β-lactams of P aeruginosa include production of β-lactamase, change permeability, active efflux, and alter in the target[5]. Antibiotic resistance is frequently considered as an acquired trait of bacterial populations, which has become prominent very recently (in evolutionary terms) as the consequence of the introduction of antibiotics for the treatment of infectious diseases. Since resistance can be achieved either as the consequence of mutation [6] , or due to the horizontal acquisition of resistance genes. It has been largely assumed that the origin of such resistance genes are the microorganisms producing antibiotics, since they need to carry resistance elements to avoid the inhibitory action of the antibiotics they produce. Possibly due to these views regarding the origins of resistance, and the forces that shape its evolution, intrinsic resistance has not been analyzed in full detail until recently. It is of critical importance in opportunistic pathogens that present a characteristic low natural susceptibility to antibiotics. Intrinsic resistance has traditionally been

attributed to a reduced permeability of the cell envelope due to decreased uptake. This is a dependent mechanism in the sense that restricted permeability is hard-wired into the cell and slows down rather than prevents the uptake of antibiotic [7]

2. MATERIALS AND METHODS

Collection of clinical samples

A total of 148 clinical samples including burn, ear ,urine, sputum and wound were collected from patients attends Al Kindi General Hospital, Al-Karkh General Hospital and Imamain Ali Hospital and Al-Imamin Al-Kazemin Medical Cit in Baghdad governorate , from September/ 2023 to December /2023.

Identification

A total of 103 gram negative bacterial isolates from various clinical samples of admitted patients were included in the study. The isolates were identified by morphology , Gram stain, biochemical tests [8] and VITEK-2 system.

Antimicrobial susceptibility testing

1- Susceptibility testing was performed by Kirby-Bauer disc diffusion method and zones of inhibition were measured and compared with the break points of Clinical Laboratory Standards Institute CLSI (2023)[9].

2- **Metallo-β-lactamase screening:** Phenotypic detection of metallo-β-lactamases was performed by combined disc test [10].

3- **Imipenem–EDTA combined disc synergy test**

Two imipenem (10g) discs were spaced 20 mm apart atop a lawn culture of the test organism. One of the discs was then covered with 4 µL of EDTA and the plate was then incubated at 37°C for 24 h. Following incubation, the inhibition zones of the discs with imipenem alone and imipenem–EDTA combined were measured. MBL positivity was defined as a 7 mm in diameter [11].

3. MOLECULAR ANALYSIS

Extraction of genomic DNA

Genomic DNA was extracted using Wizard® Genomic DNA Purification Kit from *P. aeruginosa* isolates

Molecular detection of Metallo β-Lactamase genes

Conventional PCR was used for detection of Metallo β-Lactamase genes (IMP , IMP-1 , IMP-2 , SPM , SPM-1 , VIM , VIM-1 , VIM-2 , SIM , NDM-1 , GIM-1 , OXA-2) in *Pseudomonas aeruginosa* isolates were subjected to polymerase chain reaction (PCR) for the detection of MBL genes including IMP , IMP-1 , IMP-2 , SPM , SPM-1 , VIM , VIM-1 , VIM-2 , SIM , NDM-1 , GIM-1 , OXA-2 as described by Azimi et al. [12]. The PCR reaction mixture contained 12.5 µL of ReadyMix™ Taq PCR Reaction Mix (Sigma-Aldrich, St. Louis, MO, USA), 2.5 µL of the DNA (20 pg), and 0.5 µM of each primer and nuclease-free water made up to 25 µL. The PCR cycling conditions were presented in Table 1.

Casting of the horizontal gel

Agarose gel solution was poured into the gel tray after sealing of both edges with cellophane tape, then agarose was allowed to solidify at room temperature for 30 min. The comb was carefully removed and the gel was placed in the gel tray, then tray was filled with 1X TAE-electrophoresis buffer until the buffer reached 3-5 mm over the surface of the gel.Table (1): Gene-specific primer sequence.

Primer Name	Sequence (5` 3`) ⟶	Tm (°C)	Product Size(bp)	Reference
IMP	F: CCCACGTATGCATCTGAATTAACAA	64	256	This Study
	R: CGGACTTTGGCCAAGCTTCTA			
SPM	F: GGGTACGCAAACGCTTATGG	64	229	This Study
	R: CCTTCGCTTCAGATCCTCGT			

VIM	F: GAAGGACTCTCATCGAGCGG R: AGACGTGCGTGACAACTCAT	65	156	This Study
VIM-1	F: AGTGGTGAGTATCCGACAG R: ATGAAAGTGCGTGGAGAC	55	261	Ghae *et al.*, 2018
VIM-2	F: GGTGTTTGGTCGCATATC R: AGACGTGCGTGACAACTC	56	468	Saeed *et al* ., 2019
IMP-1	F: TCCATTTACGGCTAAAGATACTG R: CAGGCAGCCAAACCACTAC	57	302	Rajab *et al.*, 2018
IMP-2	F: CGAGAAGCTTGAAGAAGGTG R: AGCCTGTTCCCATGTACG	57	613	Pour *et al.*, 2018
SPM-1	F: GCGTTTTGTTTGTTGCTC R: TTGGGGATGTGAGACTAC	54	803	Azii *et al.*, 2018
SIM	F: TACAAGGGATTCGGCATC R: TAATGGCCTGTTCCCATG	55	571	Peymi *etal.*, 2018
NDM-1	F: GGCGGAATGGCTCATCAC R: CGCAACACAGCCTGACTTTC	60	287	Joji *et al.*, 2018
GIM	F: TCGACACACCTTGGTCTG R: AACTTCCAACTTTGCCATG	55	477	Abaza *et al.*, 2018
Oxa2	F: CAAGCCAAAGGCACGATAG R: GAGTTGACTGCCGGGTTG	58	695	Bennet *et al.*, 2018

Statistical analysis

The data were analyzed using SPSS version 20 and Graph Pad Prism version 6.0. Positive and negative predictive values were also computed.

4. RESULTS AND DISCUSSION

Bacterial strains, antibiotic susceptibility and MBL phenotypic test.

A total of One hundred and forty-eight samples were enrolled in this study which include burn swabs (n=69, 46.62%), wound swab (n=45, 30.40%), sputum from patients with lower respiratory tract infection (n=14, 9.45%) and urine samples

of patients who admitted with symptoms and signs of urinary tract infection (n=20, 13.51%) figure (1) . the majority of samples were collected from inpatients (103 , 69.59%), while (45, 30.40%) samples were collected from outpatient.

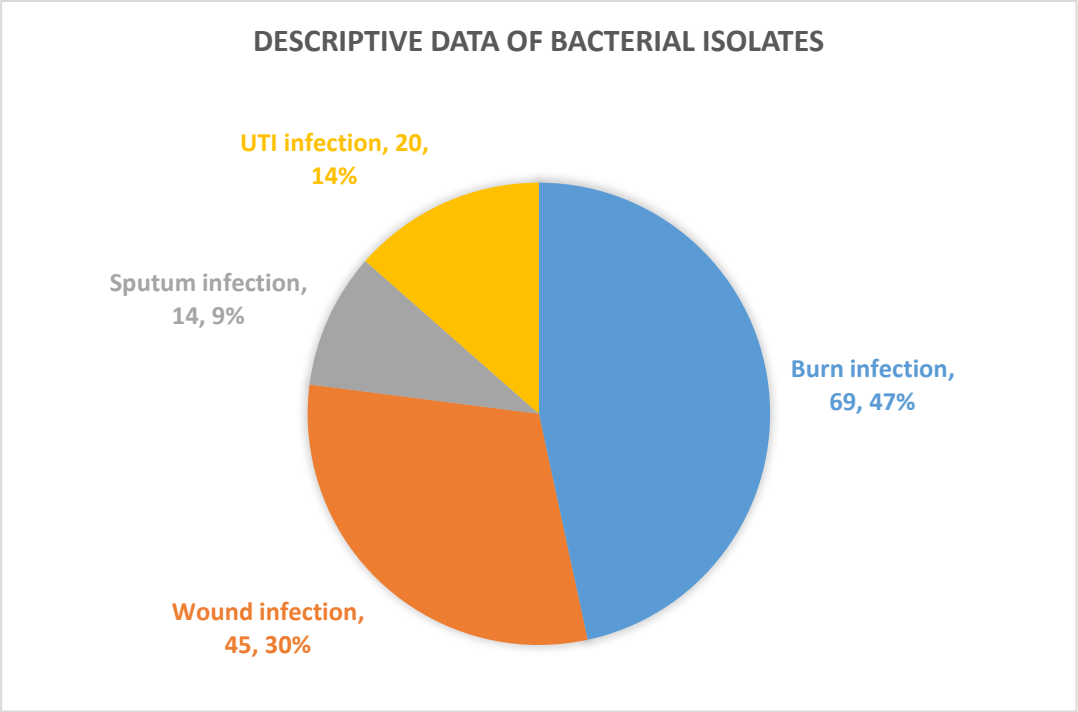


Figure (1): Distribution of clinical samples bacteria

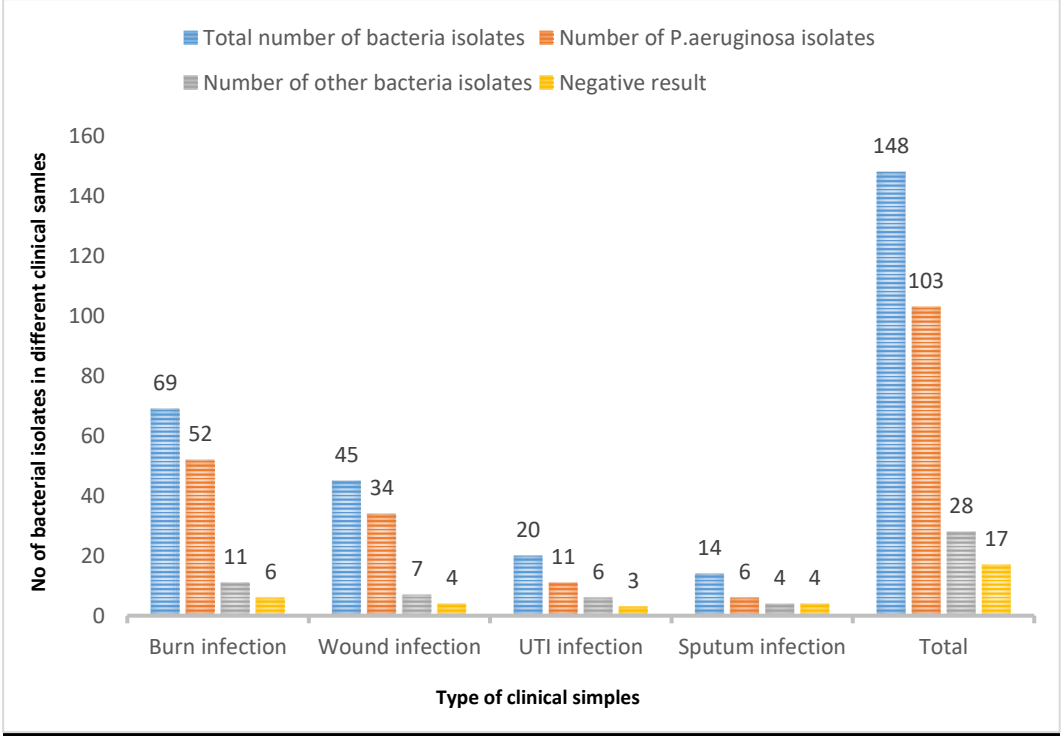


Figure (2): The distribution of bacterial isolates among different clinical samples

In this local study, the diagnosis showed that the highest percentage of Pseudomonas aeruginosa bacteria was 20% compared to other bacteria, and the most affected sites were burns and wounds. These findings are compatible with previous

study conducted in Wasit by Zeyad et al., 2018 but theses results incompatible with previous study in Baghdad by Al-Kadhmi in 2016 (22,23), who reported that percentage of S. aureus was higher than P. aeruginosa this difference in results can be attributed to sample differences. The most frequent cause of carbapenem resistance in isolates of P. aeruginosa and Enterobacteriaceae is the development of MBLs. The results also shown that one hundred and three isolates had high resistance to imipenem , and isolate No. B-16 showed the highest percentage of resistance. All of these might have a significant impact on the rising trend of antibiotic resistance in the nation overall and in the research area under consideration specifically.

Detection of phenotypic Metallo-β –lactamase

As shown in figure 3, detection of metallo β-lactamases (MBL) were done by Meropenem-EDTA double disks test. Some carbapenem resistance *Pseudomonas aeruginosa* isolates were MBL producers. One hundred and three isolates had high resistance to imipenem , and isolate No. B-16 showed the highest percentage of resistance. *Pseudomonas aeruginosa* tested exhibits an inhibition diameter 2.5 cm around of imipenem EDTA disc compared to this imipenem disc alone. EDTA is a chelating agent that prevents the expression of metallo-beta-lactamase activity.

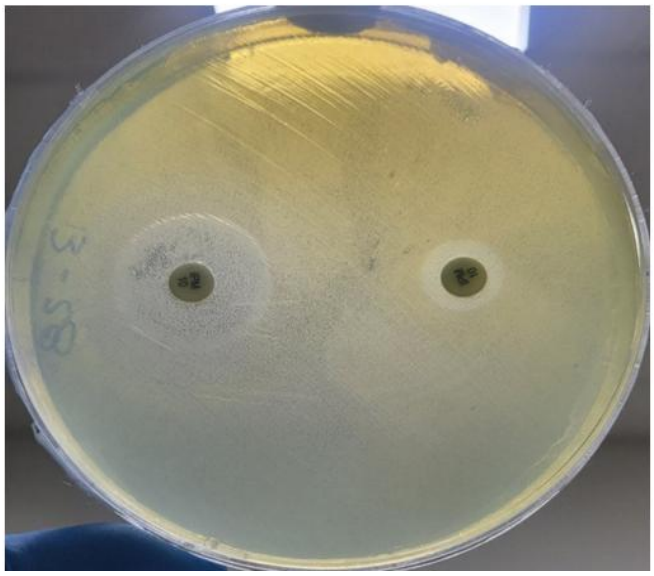


Figure (3): Detection of Metallo-beta-lactamase producer by *Pseudomonas aeruginosa* in B-16 sample

Genotypic detection

PCR was carried out on the DNA samples of 12 carbapenem resistance *P,aeruginosa* isolates for Metallo β-Lactamase genes (IMP , IMP-1 , IMP-2 , SPM , SPM-1 , VIM , VIM-1 , VIM-2 , SIM , NDM-1 , GIM-1 , OXA-2), using specific primer for each one. The results in figure (4) showed that 6 out of the 25 isolates (24%) have IMP1gene (imipenem metallo-β-lactamase). The IMP-F and IMP-R primers are used in detection of IMP gene. The fragment size was determined to be 256 base pairs.

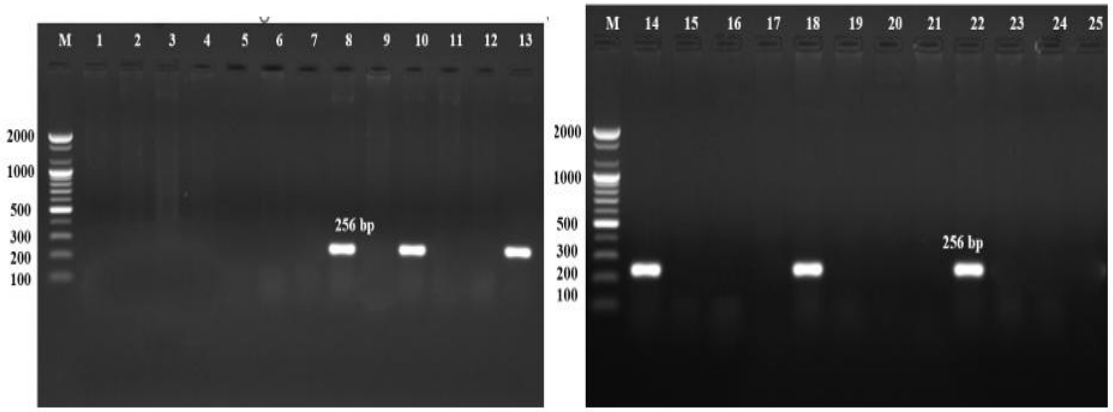
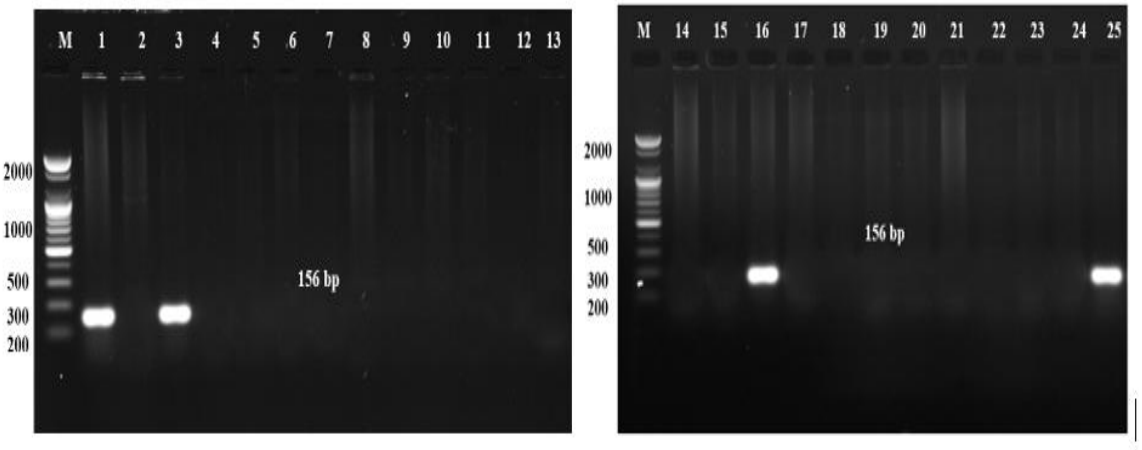


Figure (4): Electrophoresis of PCR products for amplification of IMP gene in *P. aeruginosa* , isolates on agarose gel (1%) at 5 V/cm for 60 min.

The emergence of P. aeruginosa-acquired metallo-β-lactamase (MBL) is most worrisome and poses a serious threat during treatment and infection control (13). The incidence of carbapenem resistance among clinical isolates of *P. aeruginosa* has been raised, predominantly due to production of carbapenemases, including the imipenem metallo-β-lactamase (IMP), VIM (Verona imipenemase), SPM (São Paolo metallo-β- lactamase), and GIM (German imipenemase) (14). The results showed that no one of the 25 isolates have IMP1 while 5 (20%) have IMP-2 genes (imipenem metallo-β-lactamase). The fragment size was determined to be 302 and 613 base pairs. The results in figure (5) showed that 4 out of the 25 isolates (16%) have VIM gene (Verona integron-encoded metallo-β-lactamases). The VIM-F and VIM-R primers are used in detection of VIM gene. The fragment size was determined to be 156 base pairs.



Figure(5):Electrophoresis of PCR products for amplification of *VIM* gene

The result of study carried in Baghdad city indicates that the expression level of the VIM gene is highly regulated in carbapenem-resistance isolates compared to control isolates. The carbapenem VIM gene are essential for resistance in *P. aeruginosa* induced by chromosomal changes that modify membrane permeability efflux pump overexpression for genes (15). Carbapenem-resistant *P. aeruginosa* clinical isolates obtained from hospitalised patients were positive for blaVIM gene in about 1.3% in the study of Hishinuma *et al.* (16) .The class B carbapenemases, such as Verona integron-encoded metallo-β-lactamases (VIM) and Imipenem metallo-β-lactamases (IMP), are the most frequent.The genes encoding IMP and VIM are located on integrons, which also carry other antibiotics resistance genes favoring their worldwide dissemination (17).The results showed that no one out of the 25 isolates have VIM-1 or VIM-2, SPM and SPMI gene . The results in figure (6) showed that no gene detected out of the 25 isolates have SIM-1 gene (Seul imipenemase). The fragment size was determined to be 571 base pairs.

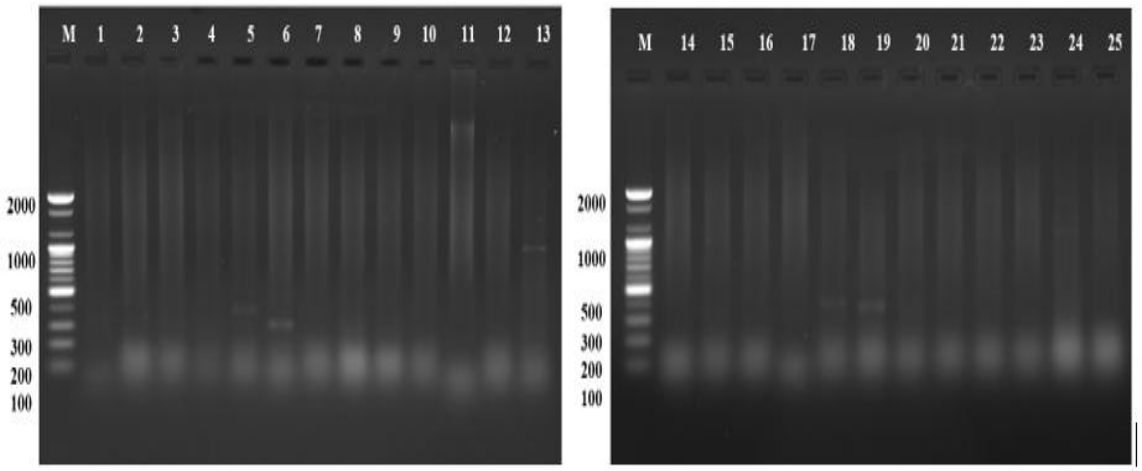


Figure (6): Electrophoresis of PCR products for amplification of *SIM1* gene in *P. aeruginosa*

Seul imipenemase (SIM) was one of Many carbapenemases that have been identified in *Pseudomonas* species and encompass metallo-β-lactamases (MBL) in Class B. Class B metallo-β-lactamases (MBLs), such as SIM-1, are capable of hydrolysing almost all β-lactams including carbapenems but not monobactams, and they can be inactivated by metal chelators such as EDTA but not by sulbactam and tazobactam that are effective against class A serine β-lactamases (18). The results showed that no gene deteced out of the 25 isolates have NDM-1 gene (new Delhi metallo β-lactamase). The fragment size was determined to be 287 base pairs. The results in figure (7) showed that 22 (88%) out of the 25 isolates have GIM-1 gene (Germany imipenemase). The fragment size was determined to be 477 base pair.

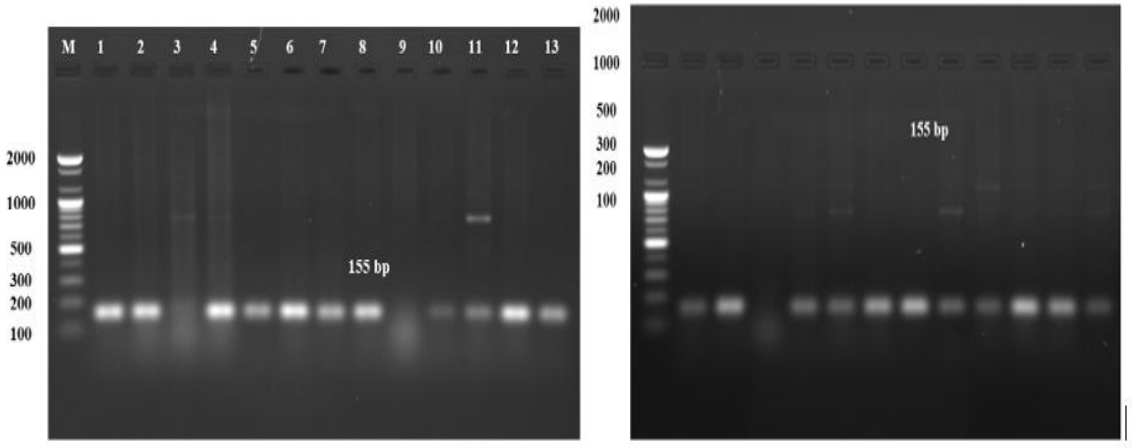


Figure (7):Electrophoresis of PCR products for amplification of *GIM-1* gene

The genes responsible for the production of MBLs are typically part of an integron structure and are carried on transferable plasmids but can also be part of the chromosome. Therefore, because of the integron-associated gene cassettes, P. aeruginosa isolates producing MBL are often resistant to different groups of antimicrobial agents which can be transferred to various types of bacteria (19). The results in figure (8) showed that 3 (12%) detected out of the 25 isolates have OXA-2 gene (Oxacillinases). The fragment size was determined to be 695 base pairs.

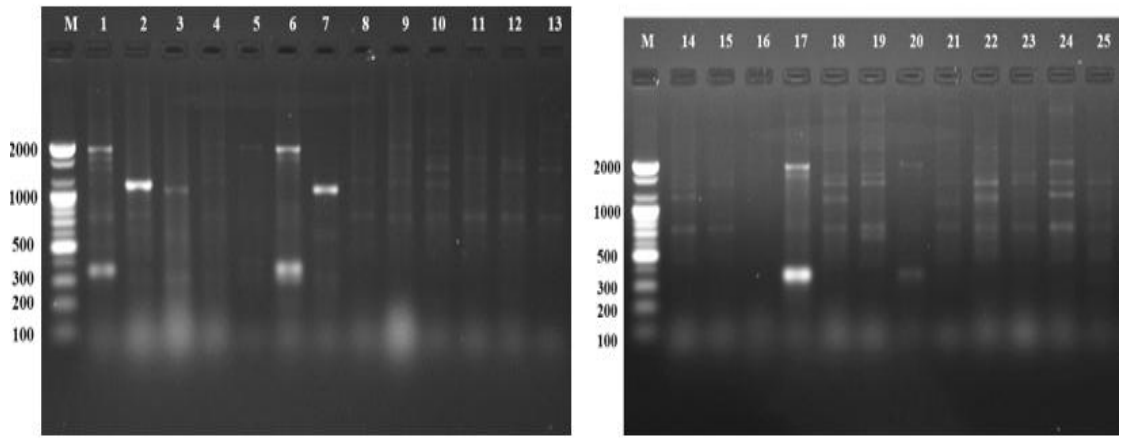


Figure (8):Electrophoresis of PCR products for amplification of *OXA2* gene

Pseudomonas aeruginosa is the documented source of oxacillinase (OXA type), a class D -lactamase that hydrolyzes Oxacillin, but it has also been found in numerous other gram-negative bacteria, including Enterobacteriaceae. In general, OXA-type enzymes are a diverse category that exhibits variation in amino acid sequences and substrate profiles (20). However, it has been shown that a number of OXA-type variations hydrolyze cephalosporins, cephems, and/or monobactams. According to a recent review, there are 27 oxacillinase enzymes described as extended-spectrum. These enzymes' substrates include third- and/or fourth-generation Cephalosporins in addition to Penicillins and early Cephalosporins. Most extended-spectrum oxacillinases derive from OXA-10 and OXA-2(21,22).

5. CONCLUSIONS

The study demonstrated how P.aeroginosa isolates with betalactam genes spread among patients with various infections, posing a greater risk to hospitalised patients in Iraq. More importantly, though, is that avoiding overuse or misuse of antibiotics can counteract the negative effects of bacteria that produce MBL and are resistant to multiple drugs. Furthermore, we anticipate that more MBL variants will be found in Iraq within the upcoming years.

Authors' contributions

Each author examined and approved the final draft critically, and they both made equal contributions to the concept and manuscript's development..

REFERENCES

[1] Zeyad K. Hussein, Haider S. Kadhim, Jabbar S. Hassan, Detection of New Delhi Metallo-Beta-Lactamase-1 (blaNDM-1) in Carbapenem-Resistant Pseudomonas aeruginosa Isolated from Clinical Samples in Wasit Hospitals, Iraqi JMS 2018; Vol. 16(3).

[2] Héctor Sambranod, Julio César Castilloa,b, Carlos W. Ramosa, Brenda de Mayorgac, Olga Chena, Ovidio Durána, Carmelo Ciniglioa, Criseida Aguilara, Osvaldo Cisternae, Magaly de Chiala, (2021): Prevalence of antibiotic resistance and virulent factors in nosocomial clinical isolates of Pseudomonas aeruginosa from Panamá, The Brazilian Journal of Infectious Diseases , Vol. 25. Issue 1. DOI: 10.1016/j.bjid.2020.11.003.

[3] Bergen PJ, Bulitta JB, Forrest A, Tsuji BT, Li J, Nation RL. Pharmacokinetic/pharmacodynamic investigation of colistin against Pseudomonas aeruginosa using an in vitro model. Antimicrob Agents Chemother. 2010;54:3783–3789.

[4] Saderi H, Owlia P. Detection of multidrug resistant (MDR) and extremely drug resistant (XDR) P aeruginosa isolated from patients in Tehran, Iran. Iranian journal of pathology. 2015;10(4):265.

[5] Jean-Claude Pechdre and Thilo Kohler(1999): Patterns and modes of p-lactam resistance in Pseudomonas aeruginosa, Clin Microbiol Infect 1999; 5: S15-Sl8.

[6] Sara Hernando-Amado*, Fernando Sanz-García, José Luis Martínez (2020): Rapid and robust evolution of collateral sensitivityin Pseudomonas aeruginosa antibiotic-resistant mutants, Sci. Adv. 2020; 6 : eaba5493.

[7] James Redfern, Janine Wallace, Alex van Belkum, Magali Jaillard, Elliot Whittard, Roobinidevi Ragupathy, Joanna Verran, Peter Kelly & Mark Charles Enright(2021): Biofilm associated genotypes of multiple

antibiotic resistant Pseudomonas aeruginosa, Redfern et al. BMC Genomics (2021) 22:572 https://doi.org/10.1186/s12864-021-07818-5

[8] Collee JG, Diguid JP, Fraser AG. 14th ed. Edinburgh: Churchill Livingstone; 1996. Mackie and McCartney practical Medical Microbiology.

[9] (CLSI,2023) .

[10] Ghusoon A. Abdulhasan*, Sulaiman D. Sulaiman,Curcumin as Efflux Pump Inhibitor Agent for Enhancement Treatment Against Multidrug Resistant Pseudomonas aeruginosa Isolates, Iraqi Journal of Science, 2020, Volume 61, Issue 1, Pages 59 -67

[11] Yong D, Lee K, Yum JH, Shin HB, Rossolini GM, Chong Y. Imipenem-EDTA disc method for differentiation of Metallo-beta- lactamase -producing clinical isolates of pseudomonas spp. and Acinetobacter spp. J Clin Microbiol. 2002;40(10):3798-3801.

[12] Azimi A, Peymani A, Pour PK. Phenotypic and molecular detection of metallo-beta-lactamase-producing Pseudomonas aeruginosa isolates from patients with burns in Tehran, Iran. Rev Soc Bras Med Trop 2018;51:610–5.10.1590/0037-8682-0174-2017

[13] Hateet, R. (2021). Isolation and Identification of Some Bacteria Contemn in Burn Wounds in Misan, Iraq. Archives of Razi Institute, 76(6), 1665.

[14] Karami, P., Mohajeri, P., Mashouf, R. Y., Karami, M., Yaghoobi, M. H., Dastan, D., & Alikhani, M. Y. (2019). Molecular characterization of clinical and environmental Pseudomonas aeruginosa isolated in a burn center. Saudi journal of biological sciences, 26(7), 1731-1736.

[15] Munya, A. H. B. (2022). Microbiological evaluation of natural juices in the city of Benghazi (Doctoral dissertation, Benghazi University).

[16] Ramanarayana, P., Kumari, G. D., Kumar, P. A., & Bindu, C. (2023). Cultural characterization and molecular identification of Pseudomonas aeruginosa from milk samples

[17] Deshmukh, D. G., Damle, A. S., Bajaj, J. K., Bhakre, J. B., & Patwardhan, N. S. (2011). Metallo-β-lactamase-producing clinical isolates from patients of a tertiary care hospital. Journal of laboratory physicians, 3(02), 093-097.

[18] HASAN, Sarah Ahmed; NAJATI, Ali Mohamed; ABASS, Kasim Sakran. Prevalence and antibiotic resistance of "pseudomonas aeruginosa" isolated from clinical samples in Kirkuk City, Iraq. Eurasia J Biosci, 2020, 14.1: 1821-5.

[19] Iswarya, M. (2020). Isolation and Speciation of Non-Fermenting Gramnegative Bacilli from Various Clinical Samples with Special Reference to Phenotypic Detection of Metallobetalactamase in Pseudomonas Species at a Tertiary Care Hospital (Doctoral dissertation, Rajiv Gandhi University of Health Sciences (India)).

[20] Meletis G, Bagkeri M. Pseudomonas aeruginosa: multi- drug-resistance development and treatment options. In: Basak C, editor. Infection Control. Rijeka, Croatia: IntechOpen, 2013: 33–56.

[21] Queenan AM, Bush K. Carbapenemases: the versatile beta-lactamases. Clin Microbiol Rev 2007;20: 440–458.

[22] Al-Huraishi M.A.M. Molecular detection of multidrug resistance Acinetobacter baumannii from different clinical samples. MSc Thesis. College of Medicine, AlNahrain University, Iraq; 2016..