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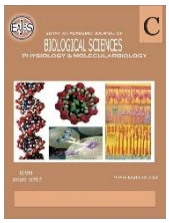

Molecular Detection of Metallo-Beta Lactamases Among Carbapenem-Resistant *Pseudomonas aeruginosa* Isolated From Patients With Burn in Baghdad City, Iraq

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ABSTRACT

Metallo-lactamases (MBL) have the ability to hydrolyze, carbapenems, which are crucial medications used to treat *Pseudomonas aeruginosa* infections, To effectively treat infections brought on by this bacterium, quick detection of the MBL-producing *Pseudomonas aeruginosa* is required. In this study, Polymerase chain reaction (PCR) analysis was used to examine the prevalence of MBL-producing *Pseudomonas aeruginosa* in burn and non-burn patients, 68 *Pseudomonas aeruginosa* isolates were collected from different clinical sources from two hospitals in Baghdad. Antibiotic susceptibility tests were PCR performed on carbapenem-resistant isolates, Among the *Pseudomonas aeruginosa* isolates, 50% were meropenem and 39% were imipenem, Among all *Pseudomonas aeruginosa* isolates, 63.23% were multidrug-resistant and 51.5% were resistant to carbapenems (meropenem and imipenem), It has been determined blaVIM, blaSPM and blaNDM in 22.9%, 2.9% and 5.7% respectively blaIMP were not observed. For the efficient treatment of carbapenem-resistant *P. aeruginosa* infections, epidemiological and geographic evaluation of MBL-producing *P. aeruginosa* should be taken into consideration.

INTRODUCTION

Pseudomonas aeruginosa is an opportunistic pathogen, an aerobic, Gram-negative bacillus that is able to survive in moist environments, it is 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) 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 (Khosravi *et al.*,2008) 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). 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). All beta-lactams with the exception of monobactames, can be hydrolyzed effectively by metallo-beta-lactamases (MBL) which belong to class B of the structural categorization of beta-lactamases (Gutierrez *et al.*, 2007; Palzkill *et al.*, 2013).

Some bacteria's bacterial chromosomes have genes that encode MBLs while other bacteria have heterologous genes that they acquired through the transfer of mobile genetic elements, As a result, many bacterial strains, including *P. aeruginosa*, can disseminate acquired MBL, There are around nine distinct categories of acquired MBL. The IMP, VIM, SPM, and NDM types of enzymes are the most significant types that contribute to epidemiological and clinical investigations (Cornaglia *et al.*, 2011). To effectively treat infections brought on by this bacteria, as well as to stop the transmission of the organism within and across hospitals, it is crucial to identify MBL-producing *P. aeruginosa* as soon as possible, Several approaches, including phenotypic and molecular techniques, have been proposed for the screening of MBL-producing *P. aeruginosa*(Walsh *et al.*,2005). One of the most important appearance methods is the combined disk, MBL detection is more sensitive and precise using molecular techniques including PCR, DNA probes, cloning, and sequencing, However, they are technically challenging and rarely readily available in clinical laboratories (Walsh *et al.*,2005; Wirth *et al.*,2009).

MATERIALS AND METHODS

Bacterial Isolates:

This brief descriptive cross-sectional study was conducted from November 2022 to March 2023 in the Department of Pathological Analyses, College of Science, University of Kufa. 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) 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 agents (i.e.penicillins/ cephalosporins, carbapenems, aminoglycosides and fluoroquinolones), that isolate was considered as multidrug resistant (MDR).

Detection of MBLs by PCR:

PCR analysis was performed for blaIMP, blaVIM, blaSPM and blaNDM genes For genes blaIMP, blaVIM The primers used were described by (Dallenn et al 2010) (IMP-F TTGAACACTCCATTTACDG, IMP-R GATYGAGAATTAAGCCACYCT with Product size of 139bp and annealing temperature at55 ºC) (VIM-F GATGGTGTTTGGTCGCATA, VIM-R GA TTTGCTCCGTGGCCGAAA with Product size of 390bp and annealing temperature at55 ºC) And use the primer for the gene NDM described by (Poirel et al 2010) (NDM-F GGTTTGGCGATCTGGTTTTC, NDM-R CGGAATGGCTCATCACGATC with Product size of 621bp and annealing temperature at 63.4 ºC) And use the primer for the gene SPM described by (Lee *et al.*,2005). SPM- FCCTACAATCTAACGGCGACC, SPM-R TCGCCGTGTCCAFFTATAAC with a Product size of 650bp and annealing temperature at 59 ºC) DNA was extracted using the boiling method. Briefly, frozen bacteria were subcultured 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, and 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%) was multi-resistant, and antibiotics 7 (20%) and 28 (80%) isolates were from burn and non-burn patients respectively. PCR analysis was performed for the *P. aeruginosa* isolates Resistance to carbapenem. Genes have been discovered blaVIM, 4 (11.4%) isolates from burn patients, 4 (11.4%) isolates from non-burn patients, blaSPM and blaNDM 1(2.9%), 2(5.7%) respectively. Figures 1,2 and 3 do not detect blaIMP.

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%
Colistin	11	16.2%	57	83.8%

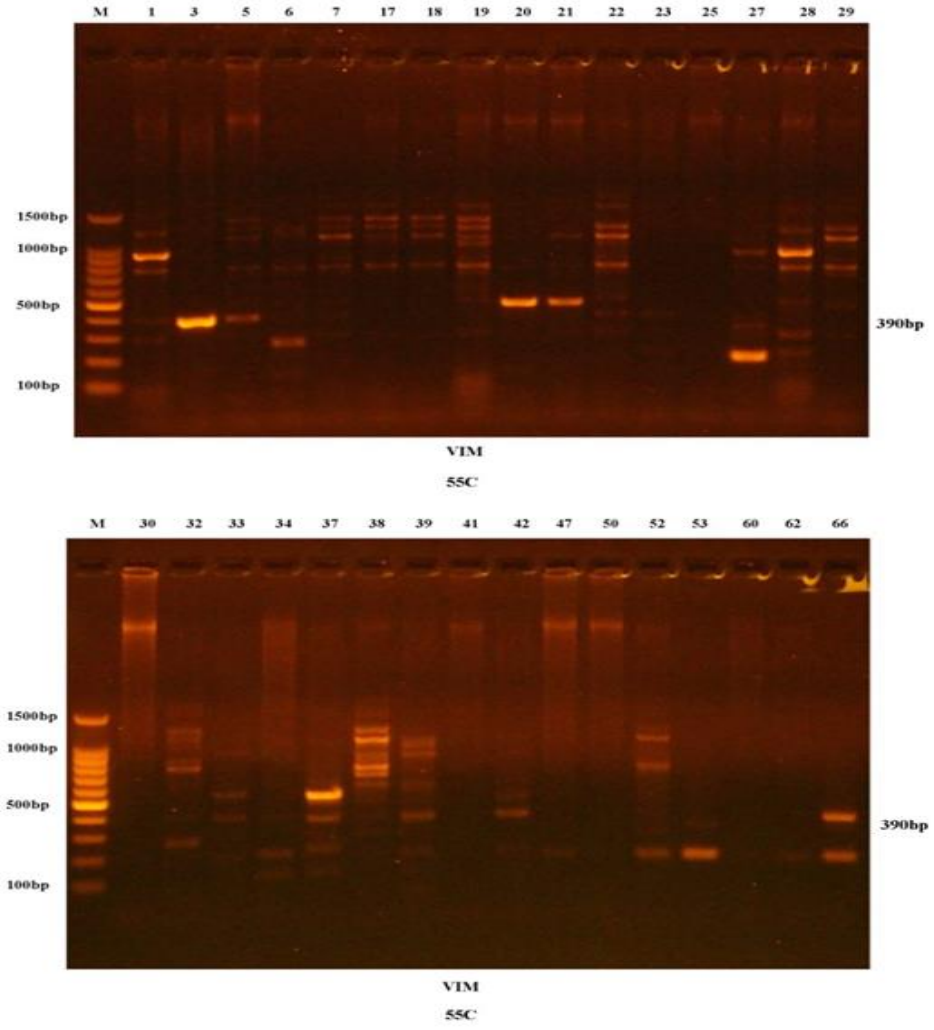

Fig.1: Results of the amplification of VIM gene of bacterial species were fractionated on 2% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 1-29resemble 390bp PCR products

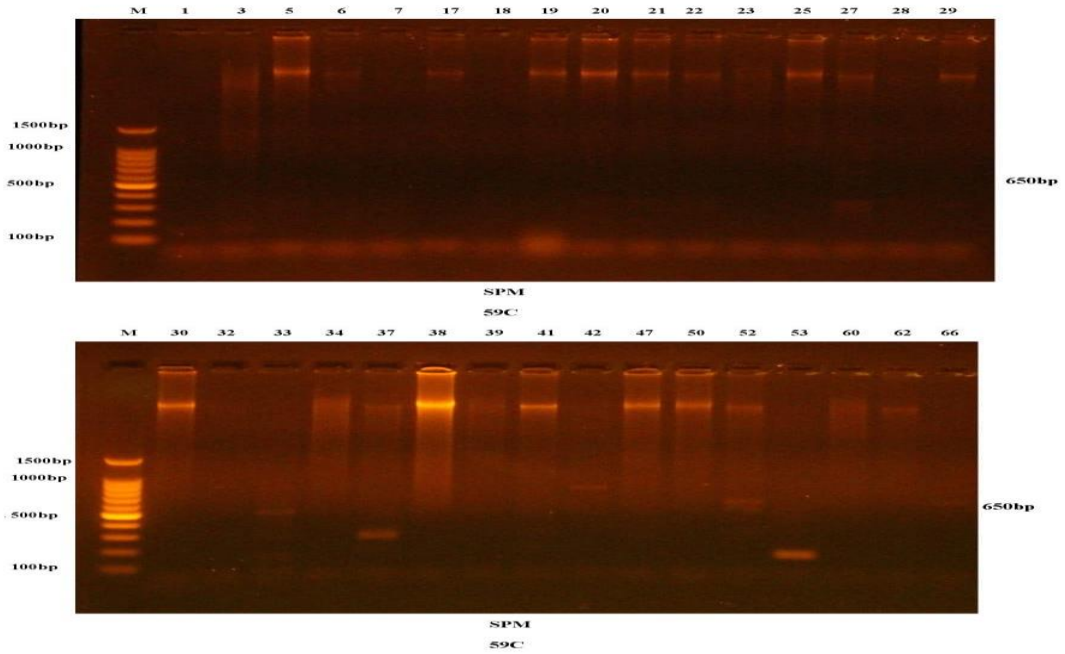

Fig.2: Results of the amplification of SPM gene of bacterial species were fractionated on 2% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 30-66resemble 650bp PCR products

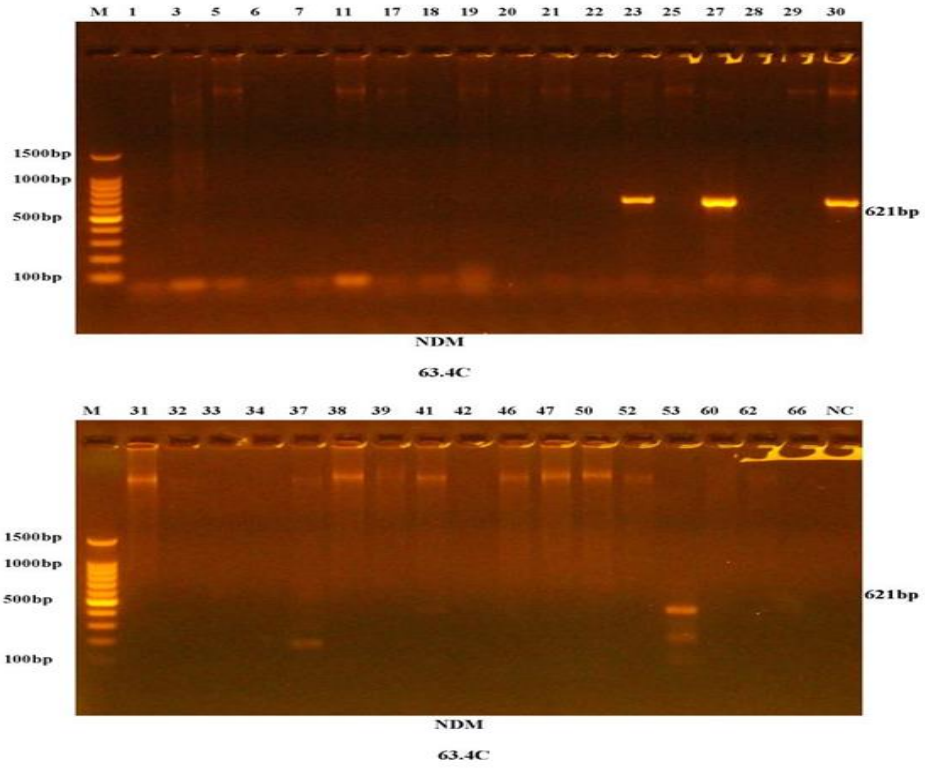

Fig.3: Results of the amplification of NDM gene of bacterial species were fractionated on 2% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 31-66resemble 621bp PCR products.

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). This study showed that 4 (11.4%) isolates were positive for blaVIM genes from burn patients that were resistant to meropenem and imipenem. Another study conducted by (Khosravi and MiHani 2008) performed on burn patients in Ahvaz, Shows a higher rate of 8 (19.51%). As for blaSPM it was identified in one isolate (2.9%) and it is the least common in this study among carbapenem-resistant *P. aeruginosa* isolates, (Al-Janahi 2020) reported two positive SPM isolates in Najaf. As for blaNDM, it was identified in two (5.7%) carbapenem-resistant *P. aeruginosa* isolates, and (Alsaady 2022) reported 5 isolates containing blaNDM.

Conclusions

Knowledge of the prevalence and processes underlying carbapenem resistance in *P.aeruginosa* allows us to develop effective treatment plans to combat nosocomial infections.

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