

Molecular Detection of Carbapenemase-Producing *Pseudomonas aeruginosa* Isolated from Intensive Care Units of Surgical Specialty Hospital in Erbil City

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Abstract

Background: The emergence and spread of carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) is a serious cause of nosocomial infections in critically ill patients. **Objectives:** The aim of this study was to determine the prevalence of CRPA and carriage of class B Metallo-β-lactamase resistant genes in intensive care units (ICUs) from patients with surgical-site infection or ventilator-associated pneumonia at a Surgical Specialty Hospital in Erbil city. **Materials and Methods:** During 6 months' study, a total of 80 clinical samples were collected from ICUs. The identification of *P. aeruginosa* in clinical specimens was confirmed by polymerase chain reaction (PCR) amplification of a molecular marker-*oprL* gene. Antimicrobial susceptibility was performed using Vitek-identification system. Screening of carbapenemase-producer isolates was confirmed using Rapidec Carba NP test. CRPA isolates were tested for the presence of metallo-β-lactamase (MBL)-encoding genes, including: *bla*_{VIM}, *bla*_{IMP} and *bla*_{NDM} by using PCR. **Results:** A total of 50 (62.5%) *P. aeruginosa* isolates were identified with antibiotic resistance profile (4% pan drug resistant [PDR], 20% extensively drug resistant [XDR] and 76% multidrug resistant). Twelve (24%) isolates were CRPA positive, in which the most prevalent MBL-encoding gene was *bla*_{VIM} (58.3%), *bla*_{NDM} (41.7%), and *bla*_{IMP} (33.3%). **Conclusions:** Alarming, high prevalence of CRPA with predominance of MBL-encoding genes was detected. The XDR and PDR resistance phenotypes have become highly prevalent for this nosocomial pathogen in ICU patients that may cause a therapeutic impasse. The MBL-encoding genes were predominant among clinical isolates of *P. aeruginosa*. These findings emphasize on adherence to infection prevention and control standard precautions, early detection of CRPA isolates and development of to effectively reduce the burden of carbapenem resistance.

Keywords: Carbapenemase genes, intensive care unit, *Pseudomonas aeruginosa*

INTRODUCTION

Patients in the medical-surgical intensive care unit (ICU) are about 2–5 times at greater risk of death not only from their critical illness but also from developing hospital-acquired infection complications.^[1] For instance, ventilator-associated pneumonia (VAP) is the most frequent ICU-associated infection, affecting 31% of critically ill patients on mechanical ventilation or with endotracheal intubation.^[2] Furthermore, surgical-site infection is another important cause of nosocomial infections affecting 20% surgical patients in the intensive-care unit.^[3]

It has been observed that ICUs are potential source of nosocomial pathogens causing different infectious diseases. *Pseudomonas aeruginosa* is the major predominant pathogen that frequently causes VAP which can be difficult to treat due

to intrinsic multidrug resistance development.^[4] It has been demonstrated that multidrug-resistant *P. aeruginosa* isolates exceeding >30% of ICU-associated infections. Thus, this poses a major threat to health-care system and attributed to high mortality rate.^[5]

Worldwide, increasing resistance in *P. aeruginosa* has warranted distinct attention in hospitals and associated with

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early onset of VAP, increased length of stay in the hospitals, higher case fatality rates, and complicated the selection of adequate empirical treatment in severe infection. Thus, limiting the spread of multidrug-resistant (MDR) strains of *P. aeruginosa* is considered to be infection prevention and control priority.^[6]

P. aeruginosa can be classified into different phenotypes depending on the antibiotic resistance profile, as follows: (a) MDR phenotype includes isolates that are resistant to more than one antimicrobial agent in three or more antimicrobial classes. (b) Extensively drug-resistant (XDR) phenotype includes isolates that are resistant to more than one antimicrobial agent in all antimicrobial classes, except in two or less. (c) Pan drug-resistant (PDR) phenotype includes isolates that are resistant to all antimicrobial agents in all antimicrobial classes.^[7] These antimicrobial phenotypes in *P. aeruginosa* isolate presents inactivation of β -lactamase enzymes that make β -lactams ineffective. Carbapenems are broad spectrum β -lactam antibiotics and have reliable activity against *P. aeruginosa* which was used as one of the few antibiotics for treating severe infections. However, in recent years, this bacterium has gained resistance to carbapenem antibiotics.^[8]

Several surveillance studies have emphasized the alarming increase of resistance to carbapenems in *P. aeruginosa* isolates. In the United States, the National Nosocomial Infections Surveillance System reported that isolates of *P. aeruginosa* was the major cause of 18% of pneumonia, 16% urinary tract infections, and 3% of blood stream infections.^[9] While consistent with the European Antimicrobial Resistance Surveillance Network (EARS-Net), an alarming increase in resistance toward antipseudomonal therapy and carbapenem resistance had exceeded 10%.^[10] Furthermore, the World Health Organization has reported *P. aeruginosa* as one of the ESKAPE pathogens in the foremost list of antimicrobial resistant isolates. As a consequence, the burden of emergence of carbapenem-resistant *P. aeruginosa* (CRPA) has also impacted hospitals with economic costs.^[11,12] Resistance against carbapenems by *P. aeruginosa* is either mediated through mutation of the outer membrane porin OprD protein, enhanced expression of MexA-MexB-OprM efflux-pump resulting in reduced level of antibiotic accumulation, upregulation of AmpC β -lactamases, and penicillin-binding protein alterations.^[13,14] Moreover, *P. aeruginosa* may acquire genes encoding carbapenemase (especially metallo- β -lactamase-MBL) through transfer of integrons, transposons, or plasmids. Combination of these mechanisms has led to widespread of carbapenem-resistant genes in *P. aeruginosa*, mainly of *bla*_{VIM}, *bla*_{IMP} and *bla*_{NDM}-family carbapenemases in different regions.^[15,16]

Although the emergence of carbapenem resistance in clinical isolates is serious public health challenge, thus far limited information is available on carbapenem-resistant genes in *P. aeruginosa* isolates in ICU of hospitals in Iraq. The major aims of this study were to determine the prevalence of carbapenemase-producing *P. aeruginosa* and to detect

carbapenem-resistant genes, including *bla*_{VIM}, *bla*_{IMP} and *bla*_{NDM}.

MATERIALS AND METHODS

Bacterial isolates

During 6 months' period from June 2019 to December 2019, a total of 80 clinical samples, including: Wound swabs, sputum, and endotracheal discharge samples were received by microbiology laboratory from ICUs of surgical specialty hospital, in Erbil city, Iraq. Isolation and identification of *P. aeruginosa* were carried out according to standard microbiological techniques, as follows: Patients samples were cultured on blood agar (Oxoid Ltd., Basingstoke, UK), MacConkey agar (Oxoid Ltd., Basingstoke, UK), and incubated at 37°C under aerobic conditions for 18–24 h. Following overnight incubation, *P. aeruginosa* isolates was identified according to culture characteristics such as Gram-negative character of the bacilli, colony characteristics, grape-like odor of culture colonies, pigment production, oxidase positivity, motility, ability to decarboxylate arginine and to grow at 42°C.^[17] Moreover, the identification of *P. aeruginosa* isolates was confirmed using an automated bacterial identification system VITEK® 2 compact system (BioMérieux). This system is a new fluorescence-based technology for the detection of bacterium and antimicrobial susceptibility testing in compliance with the Clinical and Laboratory Standard Institute (CLSI) guidelines 2014.^[18] In addition, polymerase chain reaction (PCR) using selective *oprL* gene primers was used for molecular identification of *P. aeruginosa* at species level, as described in Table 1. The size of PCR product that amplifies the open reading frame of the *oprL* gene was 504 bp.^[18]

Antimicrobial susceptibility testing

All *P. aeruginosa* isolates were tested for susceptibilities to a total 14 antimicrobial agents by using VITEK® 2 system using AST-GN30, according to manufacturer instructions. The antimicrobial agents used in this study were piperacillin, Piperacillin-Tazobactam, Ticarcillin, Ticarcillin-clavunate, ceftazidime, cefepime, imipenem, meropenem, amikacin, gentamicin, ciprofloxacin, levofloxacin, aztreonam, and colistin. Results of Vitek susceptibility testing were obtained as MIC values and shown as susceptible, intermediate, or resistant according to the updated CLSI, MIC breakpoints.^[19]

Phenotypic carbapenemase detection

Phenotypic detection of carbapenemase-producing *P. aeruginosa* isolates was carried out using Rapidec Carba NP Test (bioMérieux SA, Marcy-l'Étoile, France) according to the manufacturer's instructions. This test relies on a identifying the hydrolysis of the β -lactam ring of carbapenem antibiotics.^[20] In brief, isolated colony cultures of *P. aeruginosa* were grown onto Mueller-Hinton agar plates at 37°C for 18–24 h. An inoculum corresponding to a one calibrated full loop (10 μ L) of colonies of tested strain is taken from a culture plate and mixed into the cell. Visual reading of the plate is

Table 1: List of oligonucleotide used in this study

Target gene	Primer	Oligonucleotide sequence (5'→3')	Annealing temperature (°C)	Elongation time (s)	Expected PCR product size (bp)	Reference
<i>oprL</i>	<i>oprL</i> -F-SB	GCCGAATTCAGCATTTCCAT	57	50	507	18
	<i>oprL</i> -R-SB	CTTCTTCAGCTCGACGCGACG				
<i>bla_{VIM}</i>	<i>bla_{VIM}</i> -F-SB	GATGGTGTGGTTCGCATATCGC	53	30	390	This study
	<i>bla_{VIM}</i> -R-SB	CTATCCTGGTGCTGCGCATTTCG				
<i>bla_{IMP}</i>	<i>bla_{IMP}</i> -F-SB	GAAGGYGTTTATGTTTCATACTTCG	52	30	226	This study
	<i>bla_{IMP}</i> -R-SB	GCCACTCTATTCCGCCCGTGC				
<i>Bla_{NDM}</i>	<i>bla_{NDM}</i> -F-SB	GGTTTGGCGATCTGGTTTTTC	50	30	356	This study
	<i>bla_{NDM}</i> -R-SB	CGCAACACAGCCTGACTTTC				

PCR: Polymerase chain reaction

made after 30 min of incubation at 37°C and then after 2 h, if necessary. A positive test result is indicated by a red to yellow or red to orange color changes.

Preparation of DNA template for polymerase chain reaction analysis

DNA manipulations were carried out according to standard techniques.^[21] For PCR analysis, the genomic DNA of *P. aeruginosa* was prepared using two different techniques, as follows: The first method involved genomic DNA extraction using the DNeasy tissue kit (Qiagen, Germany), according to the manufacturer's instructions.

The second method employed was the quick Chelex resin-based DNA extraction technique.^[22] Briefly, few colonies of overnight culture of *P. aeruginosa* isolates resuspended in 5 ml of Luria Bertani broth and incubated for 16 h at 37°C. Then, 1 ml of *P. aeruginosa* inoculum was harvested by centrifugation at maximum speed for 5 min at 4°. The cell pellet was then re-suspended in 300 µl of 5% (w/v) Chelex (Sigma) in sterile water, vortexed and boiled at 100° in thermal block for 10 min to release genomic DNA. Then the cell suspension was centrifuged at 10,000 ×g for 10 min at 4°C and the supernatant containing purified *P. aeruginosa* genomic DNA was transferred into a new tube and stored at -20°C. An aliquot of 2 µl of the supernatant was used as the DNA template for PCR analysis.

Carbapenemase-resistant gene identification

PCR amplification was performed using Taq polymerase (Promega, Madison, Wisconsin, USA) in accordance with the manufacturers' protocols. Carbapenem-resistant isolates of *P. aeruginosa* were screened by standard PCR for the presence of the following carbapenemase-resistant genes: *bla_{NDM}*, *bla_{VIM}* and *bla_{IMP}* using specific oligonucleotides.

PCR amplification was carried out as follows: Initial denaturation at 95°C for 5 min, denaturation at 95°C for 30 s, annealing at 50°C–57°C for 30 s., followed by elongation at 72°C for 30–50 s., and final elongation step at 72°C for 5 min to complete the extension of the primers. Finally, the PCR product was kept at 4°C until analysis. The annealing temperature was set up depending on the melting temperature of the screening primer pair (at an approximate of 5°C less

than the T_m of primers). The elongation time was determined on the basis of the length of the expected amplified DNA fragment (1 min per 1 kilobase). The list of oligonucleotide sequences used in this study and the PCR amplification conditions, particularly annealing temperature and elongation time are shown in Table 1.

The PCR reaction was prepared in a total volume of 50 µL containing 10 µM of each of upstream and downstream primers (Sigma, UK), 1.5 mM of MgCl₂, 10 µM of dNTPs mixture, 5X GoTaq® Green buffer, Taq DNA polymerase, and the reaction mixture was filled up to final volume with Nuclease-free water. Following amplification, PCR products were separated by electrophoresis with 1% (w/v) agarose gel in TAE buffer (40 mM Tris, 1 mM EDTA and 0.1% (v/v) glacial acetic acid). The agarose gel was stained with ethidium bromide (10 µg/ml), and PCR products were visualized on the ultra-violet transilluminator.

Statistical analysis

The data were statistically analyzed by SPSS version 15 (SPSS Inc., Chicago, IL, USA) to compare the frequencies, percentage ± standard deviations of antibiotic resistance. The Chi-square test was provided to compare categorical data. *P* < 0.05 was considered statistically significant.

Ethical consideration

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients verbal and analytical approval before sample was taken. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee.

RESULTS

Isolation and identification of *Pseudomonas aeruginosa* isolates

A total of 50 (62.5%) nonrepetitive *P. aeruginosa* isolates were identified from 80 samples screened in this study. The most frequent source of isolation of *P. aeruginosa* from ICU patients was detected in 22 surgical-site wound infection (44%), followed by 12 endotracheal secretions (32%) and 16 sputum (24%). Genotypically, all *P. aeruginosa* isolates were

confirmed by PCR amplification for the presence housekeeping gene *oprL*, as described in Figure 1.

Antimicrobial resistance profile of *Pseudomonas aeruginosa* isolates

The pattern of antimicrobial resistance of 50 *P. aeruginosa* isolates collected from patients in ICU against 14 agents from 7 antimicrobial classes is described in Table 2. The highest resistance was seen against antipseudomonal fluoroquinolones particularly to ciprofloxacin (72.0%), amikacin (66.0%), ticarcillin (64.0%), and ceftazidime (62.0%). The most effective antibiotic against *P. aeruginosa* isolates was colistin (94.0%). In addition, this study determined the prevalence of *P. aeruginosa* phenotypes based on the antimicrobial resistance pattern, is shown in Figure 2. Results of this study demonstrated that two isolates were resistant to all antimicrobial agents of 7 classes, which was characterized as PDR *P. aeruginosa* (4%). Nonsusceptibility to six or more antimicrobial classes was observed in 12 isolates (20.0%), which was characterized as XDR *P. aeruginosa*.

Moreover, nonsusceptibility to two, three, four and five antimicrobial classes were seen in 1 (2%), 16 (32.0%) 19 (38%), and 2 (4%), respectively. Therefore, nonsusceptibility to three or more antimicrobial classes was observed in 38 isolates (76%), which characterized as MDR *P. aeruginosa*. The antimicrobial

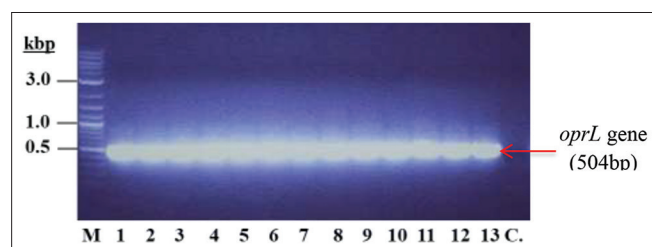


Figure 1: A presentation of polymerase chain reaction screening analysis of *oprL* gene (504 bp) detection in *Pseudomonas aeruginosa* isolates. Lane M, DNA ladder (10 kbp), lanes 1-13 represent *Pseudomonas aeruginosa* isolates

susceptibility profile of *P. aeruginosa* isolates that fit MDR, XDR, and PDR definitions is described in Figure 3; isolate no. 5 and 7 are PDR; isolate no. 1, 2, 3, 4, 6, 8, 9, 10, 11, 12 are XDR and isolate no. 13–50 are MDR.

The prevalence of carbapenem resistance in *Pseudomonas aeruginosa* isolates

Of 50 *P. aeruginosa* isolates, 12 (24%) were phenotypically confirmed as carbapenemase producers and 38 (76%) as non-carbapenemase producers using Rapidec Carba NP Test, as shown in Figure 4. Of which, 10 carbapenemase-producing *P. aeruginosa* isolates were XDR (83.3%) and 2 were PDR (16.7%) phenotypes. Of noteworthy, colistin in the polymyxins class was the most effective antimicrobial agent against CRPA isolates with a susceptibility rate of 75%. Whereas, the highest resistance was shown to carbapenem class (100%), followed by of >90% resistance in all other antimicrobial classes.

In comparison, carbapenem-sensitive *P. aeruginosa* (CSPA) isolates were significantly more susceptible to polymyxin and carbapenem antimicrobial classes (100% vs. 75%) and 100%

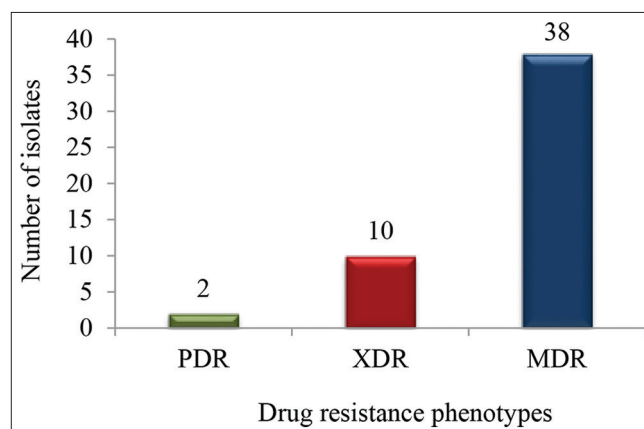


Figure 2: Prevalence of antimicrobial resistance phenotypes of *Pseudomonas aeruginosa* isolates

Table 2: Prevalence of antimicrobial resistant isolates against antimicrobial agents in seven antimicrobial classes

Antimicrobial classes	Antimicrobial agents	Resistant isolates, n (%)	Sensitive isolates, n (%)
Antipseudomonal penicillins + β -lactamase inhibitors	Ticarcillin	32 (64.0)	18 (36.0)
	Ticarcillin - Clavulanate	29 (58.0)	21 (42.0)
	Piperacillin	24 (48.0)	26 (52.0)
	Piperacillin - Tazobactam	29 (58.0)	21 (42.0)
Antipseudomonal cephalosporins	Ceftazidim	31 (62.0)	19 (38.0)
	Cefepim	27 (54.0)	23 (46.0)
Antipseudomonal carbapenems	Imipenem	12 (24.0)	38 (76.0)
	Meropenem	12 (24.0)	38 (76.0)
Aminoglycosides	Amikacin	33 (66.0)	17 (34.0)
	Gentamicin	30 (60.0)	20 (40.0)
Antipseudomonal fluoroquinolones	Ciprofloxacin	36 (72.0)	14 (28.0)
	Levofloxacin	30 (60.0)	20 (40.0)
Monobactams	Aztreonam	26 (52.0)	24 (48.0)
Polymyxins	Colistin	3 (6.0)	47 (94.0)

Isolate no.	Antimicrobial categories							
	Antipseudomonal penicillins + β -lactamase inhibitors	Antipseudomonal cephalosporins	Antipseudomonal carbapenems	Aminoglycosides	Antipseudomonal fluoroquinolones	Monobactams	Polymyxins	
1								XDR
2								XDR
3								XDR
4								XDR
5								PDR
6								XDR
7								PDR
8								XDR
9								XDR
10								XDR
11								XDR
12								XDR
13								MDR
14								MDR
15								MDR
16								MDR
17								MDR
18								MDR
19								MDR
20								MDR
21								MDR
22								MDR
23								MDR
24								MDR
25								MDR
26								MDR
27								MDR
28								MDR
29								MDR
30								MDR
31								MDR
32								MDR
33								MDR
34								MDR
35								MDR
36								MDR
37								MDR
38								MDR
39								MDR
40								MDR
41								MDR
42								MDR
43								MDR
44								MDR
45								MDR
46								MDR
47								MDR
48								MDR
49								MDR
50								MDR

Figure 3: Antimicrobial susceptibility profile of *Pseudomonas aeruginosa* isolates that fit multidrug resistant, extensively drug resistant and pan drug resistant definitions; isolate no. 5 and 7 are pan drug resistant; isolate no. 1, 2, 3, 4, 6, 8, 9, 10, 11, 12 are extensively drug resistant and isolate no. 13 to 50 are multidrug resistant

vs. none, respectively), followed by monobactams class (60.5% vs. 8.3%), piperacillin (63.2% vs. 16.7%), cefipime (57.9% vs. 83.3%), each of gentamicin and levofloxacin (47.4% vs. 16.7%).

These findings indicate that the rate of antibiotic resistance for all antimicrobial agents tested were significantly ($P < 0.05$) higher in the CRPA isolates as compared with CSPA isolates, as described in Table 3.

Furthermore, all phenotypically confirmed 12 carbapenemase-producing isolates were further investigated for the presence of carbapenem-resistant genes. PCR screening

analysis confirmed that 7 CRPA isolates harbored *bla*_{VIM} gene (58.3%) [Figure 5a], 4 CRPA isolates harbored *bla*_{IMP} gene (33.3%) [Figure 5b], and 5 CRPA isolates were detected having *bla*_{NDM} gene (41.7%) [Figure 5c].

Interestingly, PCR amplification showed that 4 CRPA isolates (33.3%) harbored two genes, as follows: *bla*_{VIM} and *bla*_{IMP} in 2 isolates (16.67%), *bla*_{VIM} and *bla*_{NDM} genes were found to co-exist in one isolate (8.33%), and *bla*_{IMP} and *bla*_{NDM} genes were found to co-exist in one isolate (8.33%). Multiple gene carriage is shown in Table 4.

DISCUSSION

P. aeruginosa represents a serious therapeutic challenge for dissemination of hospital-acquired infections with each passing year as a result of the rising resistance to cephalosporin antimicrobials and acquiring resistant determinants. Carbapenems are considered one of the most effective drugs used for treating severe infections caused by MDR Gram-negative bacteria including *P. aeruginosa* isolates; however, the enormous capacity these superbugs to emerge and spread resistance to carbapenems causing a growing threat to health-care settings of global dimensions.^[23]

More recently, hospitals in various areas of Iraq showed a dramatic change in the resistance pattern of *P. aeruginosa*

clinical isolates toward carbapenem class of antibiotics. The increased over-use of carbapenem antibiotics – as the



Figure 4: Representative confirmation of carbapenemase production in *Pseudomonas aeruginosa* isolates by using Rapidec Carba NP Test. The positivity of the reaction of tested isolate is observed in well (e) compared to the control well (d). (a) Negative result for Carbapenemase production corresponded to same color (red) as in control well, and (b) Positive result for Carbapenemase production corresponded to a color change from red to yellow

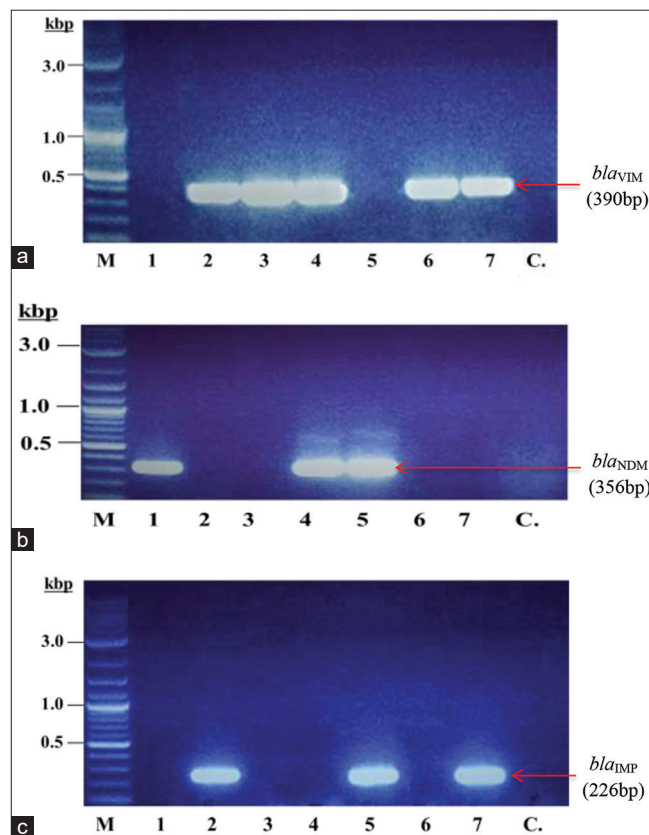


Figure 5: A representation of Polymerase Chain Reaction (PCR) screenings of Carbapenemase-resistant genes in XDR *P. aeruginosa* isolates. M, molecular marker 10 kbp ladder; Lanes 1 to 7, *P. aeruginosa* isolates; C, indicates negative control (RNase free water). a) *P. aeruginosa* isolates of 2, 3, 4, 6 and 7 showed amplified *bla_{VIM}* target gene with 390bp PCR size product, b) *P. aeruginosa* isolates of 1, 4, and 5 showed amplified *bla_{NDM}* target gene with 356bp PCR size product, and c) *P. aeruginosa* isolates of 2, 5, and 7 showed amplified *bla_{IMP}* target gene with 226bp PCR size product

Table 3: Comparison of resistance pattern between carbapenem-resistant *Pseudomonas aeruginosa* and carbapenem-sensitive *Pseudomonas aeruginosa* isolates in this study

Antimicrobial classes	Antimicrobial agents	CRPA, n (%)	CSPA, n (%)
Antipseudomonal penicillins + β -lactamase inhibitors	Ticarcillin	12 (100.0)	20 (52.6)
	Ticarcillin - Clavulanate	11 (91.7)	18 (47.4)
	Piperacillin	10 (83.3)	14 (36.8)
	Piperacillin - Tazobactam	11 (91.7)	18 (47.4)
Antipseudomonal cephalosporins	Ceftazidim	11 (91.7)	20 (52.6)
	Cefepim	11 (91.7)	16 (42.1)
Antipseudomonal carbapenems	Imipenem	12 (100.0)	0 (0.0)
	Meropenem	12 (100.0)	0 (0.0)
Aminoglycosides	Amikacin	10 (83.3)	23 (60.5)
	Gentamicin	10 (83.3)	20 (52.6)
Antipseudomonal fluoroquinolones	Ciprofloxacin	12 (100.0)	24 (63.2)
	Levofloxacin	10 (83.3)	20 (52.6)
Monobactams	Aztreonam	11 (91.7)	15 (39.5)
Polymyxins	Colistin	3 (25.0)	0 (0.0)

P. aeruginosa: *Pseudomonas aeruginosa*, CRPA: CSPA: Carbapenem-sensitive *P. aeruginosa*, CRSA: Carbapenem-resistant *P. aeruginosa*

Table 4: Distribution of carbapenemase-resistant genes among *Pseudomonas aeruginosa* isolates, specimen source and resistance phenotypes

Isolate number	Resistance phenotype	Specimen type	Carbapenemase resistance genes		
			<i>bla</i> _{VIM}	<i>bla</i> _{NDM}	<i>bla</i> _{IMP}
1	XDR	Wound infection	—	+	—
2	XDR	Endotracheal secretions	+	—	+
3	XDR	Wound infection	+	—	—
4	XDR	Endotracheal secretions	+	+	—
5	PDR	Wound infection	—	+	+
6	XDR	Endotracheal secretions	+	—	—
7	PDR	Wound infection	+	—	+
8	XDR	Sputum	—	+	—
9	XDR	Sputum	+	—	—
10	XDR	Endotracheal secretions	+	—	—
11	XDR	Endotracheal secretions	—	—	+
12	XDR	Endotracheal secretions	—	+	—

XDR: Extensively drug resistant, (—): negative for carbapenemase-resistant gene, (+): positive for carbapenemase-resistant gene

last resource of drug for treatment of infections has derived selection pressure and permitted development of more resistant strains which have a potential for rapid dissemination. In most countries around the continents, the prevalence of CRPA was ranged between 3.3% and 75.3%.^[24]

In the present study, the prevalence of resistance against antipseudomonal antimicrobial classes was investigated showing a high resistance rate among antimicrobial agents in which 76.0%, 20.0%, and 4.0% of *P. aeruginosa* isolates displayed an MDR, XDR, and PDR resistance phenotypes. This high resistance pattern is worrisome. The prevalence rate of carbapenem resistance was 24.0%. This rate reflects a caution for treatment options of severe infections in hospitals.

This result was in agreement with similar findings described by researchers in a studied hospital in Wasit.^[25] A recent study by Al-abedi and Al-Mayahi in 2019 showed higher rate of resistance of *P. aeruginosa* toward carbapenem class antibiotics (34.95%) in Al-Diwaniyah hospitals.^[26] Another study showed the occurrence of 40% of CRPA isolates identified in a studied hospital at Babylon, Iraq.^[27] Furthermore, Shilba *et al.*, in 2015, concluded an increased resistance of *P. aeruginosa* against carbapenems (68.34%) in studied hospitals in Karbala province.^[28] However, earlier studies reported lower prevalence of CRPA that were isolated in studied hospitals of Baghdad (8%),^[29] Najaf (12.4%),^[30] and Duhok (12.7%).^[31] Among the neighboring countries, such as Turkey,^[32] Gulf regions,^[33] and Iran,^[34] high prevalence of CRPA was reported often and it ranged between 63% and 70% from pooled data. Thus, far collectively, this implies that there has been an increasing trend in the dissemination of CRPA rate among Iraqi hospitals over years from 8% to 68.34% during the 10 years according to the fore mentioned pooled earlier investigations. In these cases, the use of antimicrobial regimens for treatment of infections becomes limited to use of polymyxins (polymyxin B and colistin) to treat infected patients by these CRPA isolates.

The production of MBL enzymes, particularly IMP, VIM, and

NDM have been identified as the most predominant determinants of carbapenem resistance and are becoming highly widely distributed in various countries globally.^[35] A recent survey implied an alarming increasing trend in the dissemination of “high risk” CRPA isolates harboring MBL-resistant genes across Eastern Mediterranean countries.^[36]

According to results of this study, 58.3% and 33.3% of CRPA isolates were *bla*_{VIM} and *bla*_{IMP} genes positive, respectively. These findings were in conformity with a study by El-Domany *et al.* in Egypt which demonstrated the presence of *bla*_{VIM} and *bla*_{IMP} genes in 8 (57%) and 5 (35%) isolates out of 14 IMP-resistant *P. aeruginosa* clinical isolates.^[37] On the other hand, this rate was higher than findings of a study conducted in Sulaimaniya which reported that 10.73% and 18.64% of CRPA isolates harbored *bla*_{VIM} and *bla*_{IMP} genes, respectively.^[38] In another study conducted in Bahrain, Joji *et al.* reported 47.5% isolates positive for the *bla*_{VIM} gene.^[39] On the contrary, a previous study showed a considerably higher incidence of *bla*_{VIM} (85%) and *bla*_{IMP} (57%) genes in MBL-producing *P. aeruginosa* that were isolated from different clinical samples in Erbil city.^[40] Furthermore, a study by Al-Charrakh *et al.*^[29] revealed that in a studied hospital of Baghdad the *bla*_{VIM} gene was not detected in any of the tested MBL-producing *P. aeruginosa* isolates, whilst 50% these isolates were *bla*_{IMP} gene positive. A possible explanation for variations in incidence rate of MBL enzyme types of genes are likely due to different geographical regions, a widespread indiscriminate use of antimicrobial agents, specimen type and size, and genotypic variations. These factors facilitate dissemination of these resistant isolates.^[41]

The emergence of the most clinically significant carbapenemase NDM determinant has received much attention recently as a growing threat affecting humans worldwide. The major sources of dissemination of this type carbapenemase are India and Middle Eastern countries.^[42] High prevalence of *bla*_{NDM} gene (41.7%) in the CRPA isolates was detected in

this study. This finding was in conformity with two recent studies conducted in the Iraqi hospitals which observed high incidence of *P. aeruginosa* clinical isolates carrying *bla*_{NDM} gene variants.^[30,43] Lower rate of *bla*_{NDM} gene (1.12%) positive isolates was found in a studied hospital in Sulaimaniya.^[43] On the contrary, several previous studies did not detect *bla*_{NDM} gene positive *P. aeruginosa* isolates.^[38]

Interestingly, it was observed that interestingly clinical isolates of CRPA carried multiple carbapenemase-resistant genes. One isolate carried both *bla*_{VIM} and *bla*_{NDM} genes. Similar cases were shown in studied hospitals in Najaf city,^[30] and in neighboring countries such as Bahrain^[39] and Saudi Arabia.^[44] Moreover, one CRPA isolate harbored both *bla*_{VIM} and *bla*_{IMP} genes. Higher rate of co-existence of these later MBL genes were also observed in India.^[45] Surprisingly, one CRPA isolate harbored both *bla*_{NDM} and *bla*_{IMP} genes in this study.

Taken together, findings of this study indicate that the prevalence of MBL producing-*P. aeruginosa* isolates harboring carbapenem resistant genes are increasing in Iraq. Furthermore, the strength of agreement between the phenotypic detection of carbapenemase-production and PCR screening is strong since the sensitivity and specificity of phenotypic carbapenem detection using Rapidec Carba NP Test in relation to detection of these MBL-resistant genes by PCR is 100% and 94.4%, respectively, according to previous studies.^[46,47]

CONCLUSIONS

In the light of these findings, this study concludes that the prevalence of CRPA and MBL resistant genes reached at an alarming rate and the XDR and PDR resistance phenotypes are becoming highly prevalent for this nosocomial pathogen in ICU patients and which may cause a therapeutic impasse. This study emphasizes on development of antimicrobial stewardship to prevent indiscriminate use of carbapenem antibiotics and early detection of carbapenem-resistant isolates is vital to prevent the risk of cross-transmission of these isolates among critically-ill patients. Hence, the implementation of active surveillance and strict adherence to infection prevention and control measures could be effective strategies to reduce the carbapenemase resistance trend.

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Conflicts of interest

There are no conflicts of interest.

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