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ISSN 2411-3514 ONLINE
ISSN 1997-2490PRINTED

Al-Qadisiyah Journal Of Pure Science (QJPS)

Vol. 24, No. 2, p.p 6-11, Year2019

Molecular detection of metallo-β-lactamase genes in carbapenem-resistant isolates of *Pseudomonas aeruginosa* recovered from patients in Al-Diwaniyah province, Iraq

Received : 22/7/2019 **Accepted :19/8/2019**

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ABSTRACT:

Introduction: The World Health Organization(WHO) has rated Carbapenems Resistant *Pseudomonas aeruginosa*from top priority pathogens to research and develop new antibiotics.
Objectives: The current work intended to detect the presence of the metallo-β-lactamase genes in *Pseudomonasaeruginosa* isolates cultivated from some healthcare centers in Al-Diwaniyah City, Iraq.
Materials and methods: After induction of specific traditional cultivation and identification methods for 630 samples (244 burn swabs, 163 sputa, 115 urine samples, and 108 ear swabs), a monoplex polymerase chain reaction (MPCR) method aiming at 8 metallo-β-lactamases (MBLs) genes was followed. Partial-gene sequencing of the MBLs was also performed to understand some of the evolution history of those isolates.
Results: The *P. aeruginosa* was identified in 100 isolates. MBL-related MPCR results showed the presence of bla_{VIM}, bla_{SPM}, bla_{GIM}, bla_{NDM}, bla_{AIM},bla_{SIM}, and bla_{DIM} genes in 16 (66.6%), and 14 (14.3%), 13 (54.1%), 1 (36.5%), 9 (37.5%), 8 (33.3%), 2 (8.3%), respectively, of the isolates. No detection of the bla_{IMP} was observed. The phylogenic study demonstrated high matching rates, 99%, with global strains from the NCBI-related database.
Conclusion: To the best of our awareness, the current work is the first in Iraq identifying metallo-β-lactamase genes in *Pseudomonasaeruginosa* isolates.

KEYWORDS: metallo-β-lactamase genes, phylogeny, *Pseudomonasaeruginosa.*

1. INTRODUCTION

Pseudomonas aeruginosa is among the most important pathogenic organisms for nosocomial infections in patients taking chemical therapy or having cystic fibrosis and serious injuries with huge rates of morbidity and mortality*(Alikhani et al*., 2017; Maroui *et al*., 2017; Schaber *et al*., 2007). Multi-drug resistance (MDR) isolates of *P. aeruginosa* emergence is increasingly reported in the world because the presence of major alterations facing the genetic materials related to the resistance against a wide range of antibiotics (Bassetti *et al*., 2018). resulting in a significant global public health issue (Mohamed *et al*., 2018). The Clinical & Laboratory Standards Institute: CLSI Guidelines (CLSI) has been followed to identify groups of antibiotics challenging those bacteria such as β-lactams; aminoclycosides, carbapenem, and lipopeptides, quinolones (Institute–CLSI, 2017). This antibiotic resistance induced by bacteria is acquired by different mechanisms; however, it can be performed by certain bacterial enzymes such as Class B (β-lactamase); metallo-β-lactamases that needs Zn^{++} for their action to destroy the β-lactam ring (Bonomo, 2017; Jean *et al*., 2015; Khan *et al*., 2017). The current work intended to detect the presence of the metallo-β-lactamase genes in *Pseudomonasaeruginosa* isolates cultivated from some healthcare centers in Al-Diwaniyah City, Iraq.

2. Materials and methods

2.1 Patients and sample collection

In the present study, 630 samples were collected as (244 burn swabs, 163 sputa, 115 urine samples, and 108 ear swabs) from patients in the period of July to December, 2018, for which various healthcare centers in Al-Diwaniyah province, Iraq, were employed to collect these samples. Technical-aseptic conditions were utilized when collecting and handling the samples and tools. These samples were then transported using an ice-box to a laboratory in the University of Al-Qadisiyah, Diwaniyah City, Iraq, for required test induction.

2.2 Molecular detection

2.2.1 Genomic DNA extraction

A specific kit of genomic DNA extraction was used manufactured by (Geneaid, USA). The protocol provided with this kit was followed. A NanoDrop was utilized to estimate the quantity and the quality of the DNA obtained from the process of extraction. The DNA was kept frozen (-20°C) until the next tests were performed.

2.2.2 Monoplex polymerase chain reaction

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A monoplex polymerase chain reaction (MPCR) method aiming at metallo-β-lactamase; *bla*$_{VIM}$,*bla*$_{SPM}$, *bla*$_{GIM}$, *bla*$_{NDM}$, *bla*$_{AIM}$,*bla*$_{SIM}$,*bla*$_{IMP}$, and *bla*$_{DIM}$ coding genes was used. Table (1) demonstrates the primers employed for the current work.

Table 1: the primers of the PCR

Primer		Sequence (5→3)	Product size	Reference
NDM	F	ACC GCC TGG ACC GAT GAC CA	264	(Zarfel *et al.*, 2011)
	R	GCC AAA GTT GGG CGC GGT TG		
IMP	F	GAA TAGGGT GGC TTA ACT CTC	188	(Ellington et al., 2007)
	R	CCA AAC CAC TAG GTT ATC		
SIM	F	TAC AAG GGA TTC GGC ATC G	570	(Lee *et al.*, 2005)
	R	TAA TGG CCT GTT CCC ATG TG		
VIM	F	GTT TGGT CGC ATA TCG CAA C	382	(Pitout *et al.*, 2005)
	R	AAT GCG CAG CAC CAG GAT AG		
SPM	F	AAA ATC TGG GTA CGC AAA CG	271	(Ellington *et al.*, 2007)
	R	ACA TTA TCC GCT GGA ACA GG		
GIM	F	TCG ACA CAC CTT GGT CTG AA	477	(Ellington *et al.*, 2007)
	R	AAC TTC CAA CTT TGC CAT GC		
DIM	F	TAA CGA CGA GGT ACC TGA GC	410	(Murugan*et al.*,2018)
	R	ACC ACA CCA CTA CGT TGT CT		
AIM	F	CTG AAG GTG TAC GGA AA CAC	322	(Poirel*et al.*,2011)
	R	GTT CGG CCA CCT CGA ATT G		

The Accupower PCR Pre Mix (Bioneer Company, Korea) was recruited to prepare the PCR solutions (master mix). Here, DNA at 5µl, each primer (F or R) at 2µl, and water (PCR type) at 11µl to complete add-up to the total volume, 20µl. The conditions of the thermocycler are demonstrated in table (2).

Table 2: The reaction conditions of the thermocycler

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Gene	Time and temperature (°C)					No. of cycles
	Initial denaturation	Cycling condition			Final extension	
		Denaturation	Annealing	Extension		
bla$_{SPM}$	95/2 min	95/30 sec	56/30 sec	72/110 sce	72/5 min	30
bla$_{VIM}$	95/2 min	95/30 sec	58/30 sec	72/110 sce	72/5 min	30
bla$_{NDM}$	94/5 min	95/30 sec	63.5/30 sec	72/30 sec	72/10 min	35
bla$_{SIM}$	94/5 min	94/30 sec	57/30 sec	72/30 sec	72/5 min	36
bla$_{GIM}$	94/5 min	94/30 sec	56/30 sec	72/30 sec	72/5 min	36
bla$_{AIM}$	94/10 min	94/30 sec	58/30 sec	72/50 min	72/5 min	36

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*bla*DIM	94/5 min	94/30 sec	58.3/30 sec	72/1.5 min	72/7 min	35
bla IMP	95/5 min	95/5 min	52/30 sec	72/50 min	72/5 min	35

An electrophoresed-1%-agarose gel pre-exposed to ethidium bromide was used. Then, the products of the PCR were screened utilizing a UV imager.

2.2 Phylogenetic analysis

The partial-gene DNA sequencing was done on the successive PCR metallo-β-lactamase genes. Using the products of the PCR, sequencing by Macrogen Company, South Korea, was performed. Data from the process were processed via the NCBI-based BLAST website plus the MEGA v6.0 to generate the phylogenetic tree following the Unweighted Pair Group Methods with Arithmetic Mean (UPGMA tree) method.

2.3 Statistical analysis

The Statistical Package for Social Science (SPSS), was utilized in the current study. Chi-square test was employed. Probability ≤0.05 ($p \leq 0.05$) was used(Petrie and Watson, 2006).

3. Results

The *P. aeruginosa* was identified in 100 isolates. MBL-related MPCR results showed the presence of *bla*VIM, *bla*SPM, *bla*GIM, *bla*NDM, *bla*AIM,*bla*SIM, and *bla*DIM genes in 16 (66.6%), and 14 (14.3%), 13 (54.1%), 1 (36.5%), 9 (37.5%), 8 (33.3%), 2 (8.3%), respectively, of the isolates, figure 1. No detection of the *bla*IMP was observed.

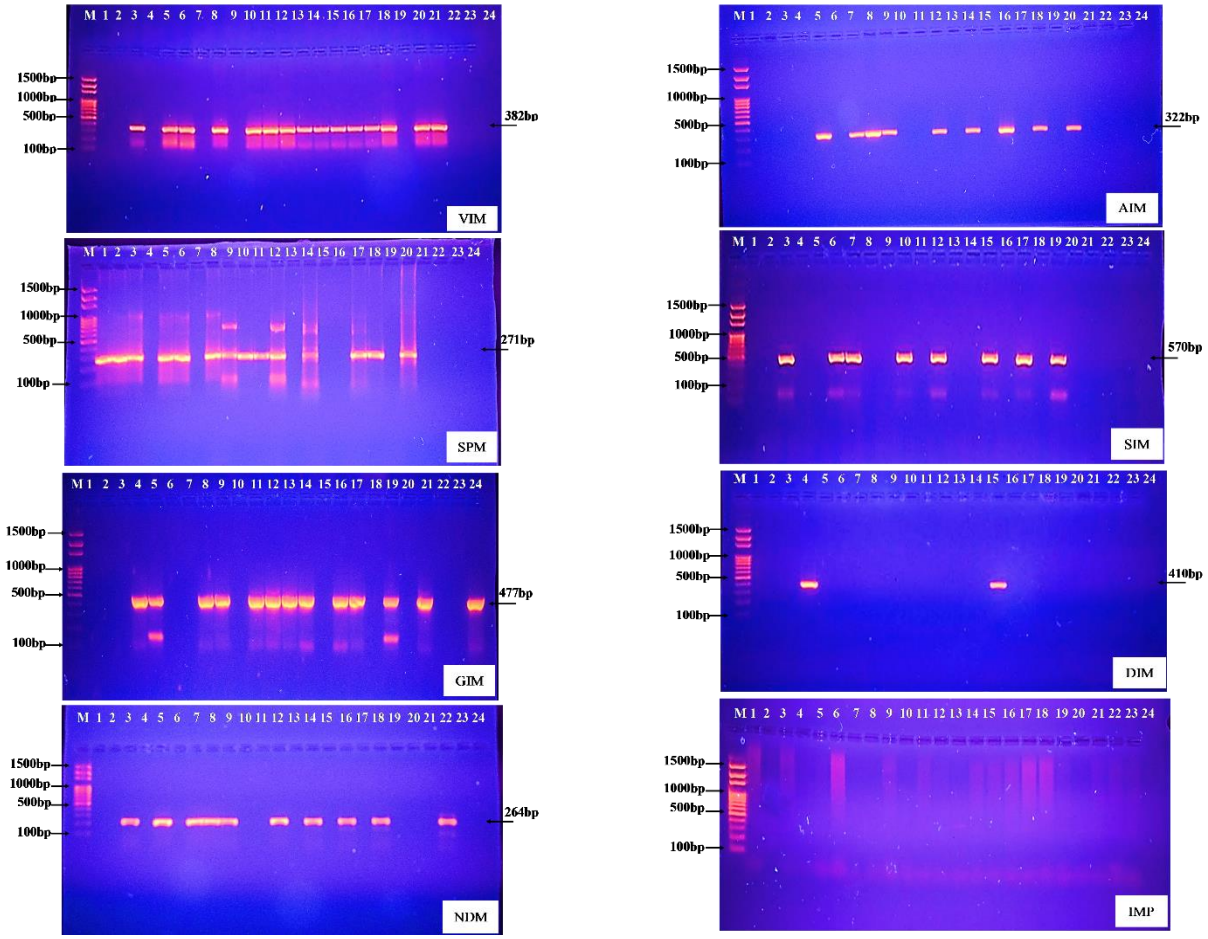

Figure 1: Agarose gel electrophoresis of the metallo-β-lactamase genes from isolates of *P. aeruginosa*. M: ladder, 1500-100bp. No IMP gene was detected.

Available online at http://qu.edu.iq/journalsc/index.php/JOPS

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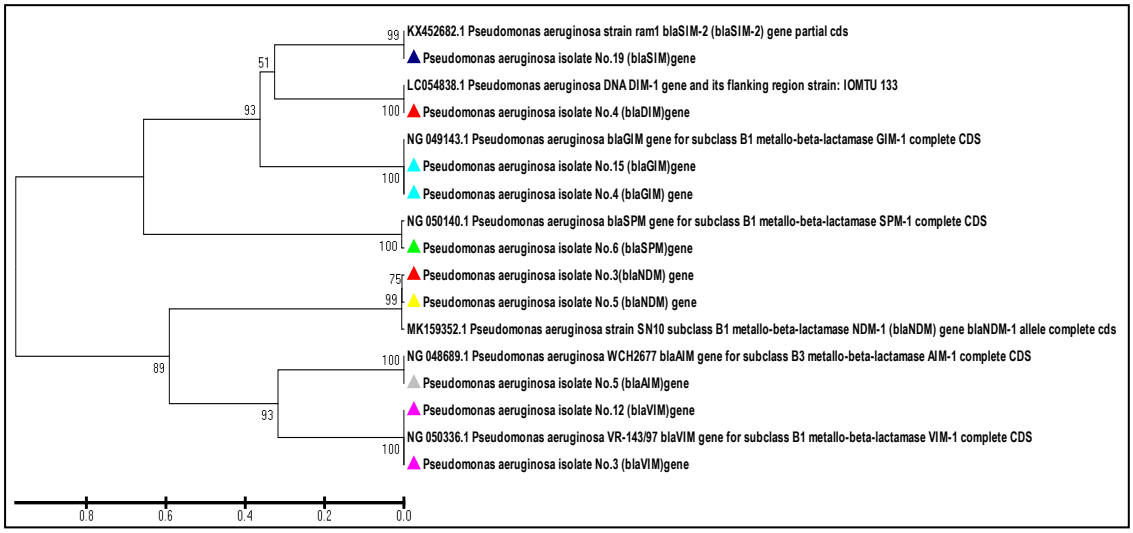

Figure 2: The phylogenetic tree via the sequencing of the metallo-β-lactamase genes in the isolates of *P. aeruginosa*

Table 3: The Homology Sequence identity for the current study isolates of the *P. aeruginosa* with NCBI-BLAST world strains

Pseudomonas aeruginosa local isolate	beta-lactamase antibiotic resistance gene	Genbank Accession number	NCBI-BLAST Homology Sequence identity (%)			
			Identical resistance isolate	Genbank Accession number	Identity (%)	SNP
P. aeruginosa isolate No.3	*bla*VIM-1	MN182488	*Pseudomonas aeruginosa* VR-143/97	NG_050336.1	99%	C/G , C/A
P. aeruginosa isolate No.12	*bla*VIM-1	MN182489	*Pseudomonas aeruginosa* VR-143/97	NG_050336.1	99%	A/T , C/A
P. aeruginosa isolate No.6	*bla*SPM-1	MN182492	*Pseudomonas aeruginosa* subclass B1	NG_050140.1	99%	G/C
P. aeruginosa isolate No.4	*bla*GIM-1	MN182486	*Pseudomonas aeruginosa* subclass B1	NG_049143.1	99%	-
P. aeruginosa isolate No.15	*bla*GIM-1	MN182487	*Pseudomonas aeruginosa* subclass B1	NG_049143.1	100%	-
P. aeruginosa isolate No.3	*bla*NDM-1	MN182484	*Pseudomonas aeruginosa* strain SN10	MK159352.1	99%	G/T , C/G
P. aeruginosa isolate No.5	*bla*NDM-1	MN182485	*Pseudomonas aeruginosa* strain SN10	MK159352.1	99%	G/T , C/A
P. aeruginosa isolate No.5	*bla*AIM-1	MN182495	*Pseudomonas aeruginosa* WCH2677	NG_048689.1	99%	C/A
P. aeruginosa isolate No.19	*bla* SIM-2	MN182496	*Pseudomonas aeruginosa* strain ram1	KX452682.1	99%	A/T , C/T
P. aeruginosa isolate No.4	*bla*DIM-1	MN182493	*Pseudomonas aeruginosa* strain: IOMTU 133	LC054838.1	99%	T/A , A/T

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4. Discussion

The production of carbapenemases is one of the most important mechanisms for carbapenems resistance (Nordmann*et al*., 2012). and has increased significantly in the gram negative bacilli in the last ten years (Jean *et al*., 2015).

The PCR results showed that the *bla*$_{VIM}$ gene was the most common MBL gene among the studied isolates. This is the first report in the hospitals in the city of Diwaniyah. However, the first report in Iraq was from a study conducted by (Al-Shara, 2013). The *bla*$_{SPM}$ gene was identified in 14 the carbapenim-resistant isolates. *bla*$_{SPM-1}$ gene was first identified in *P. aeruginosa* isolated from a leukemia patient in Sao Paulo Hospital in Brazil (Toleman *et al*., 2002). The *bla*$_{GIM}$ gene was identified in the isolates; however, this gene was not identified in the study by (Al-Shara, 2013). The results indicated *bla*$_{NDM}$ gene in the isolates which was also reported in isolates from *P. aeruginosa* in France recovered from a patient who had previously been transferred to a hospital in Serbia (Janvier *et al*., 2013). *bla*$_{AIM}$ was identified in the isolates of the current study for the first time in Iraq, although it was recognized in *P. aeruginosa* bacteria from Australia (Yong *et al*., 2012). The *bla*$_{SIM}$ gene was found in the isolates of the current work, in addition, this gene was in *Acinetobacter pittii* from a hospital in Seoul, Korea in 2003 (Lee *et al*., 2005). *bla*$_{DIM}$ was identified in only two isolates of the present investigation, recovered from two patients in the specialized burns center in Diwaniyah. The gene was first identified in isolated *Pseudomonas stutzeri* of a Dutch patient in 2007 (Deshpande *et al*., 2014).

5.Conclusion

To the best of our awareness, the current work is the first in Iraq identifying metallo-β-lactamase genes in *Pseudomonasaeruginosa* isolates.

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Available online at http://qu.edu.iq/journalsc/index.php/JOPS

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Vol. 24, No. 2, p.p 6-11, Year2019

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