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ORIGINAL ARTICLE

Evaluated gene expressions of Metallo beta lactamase genes GIM and , VIM, SPM in *Pseudomonas aeruginosa* clinical isolates

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Abstract

Pseudomonas aeruginosa is considered as one of the human health care problems, *P. aeruginosa's* carbapenem resistance emerges by several different mechanisms, some of which include carbapenems genes. *P. aeruginosa's* carbapenem resistance is a significant health concern, So this study aims to evaluate MBL gene expressions. The study was conducted at the Department of Microbiology, AL-Mahmoodia Hospital, over one year from January to December 2022. The samples were collected from patients with different clinical sources (Burn, Urine, Wound, Sputum, Ear, and Blood), from different ages while. Samples were collected from three hospitals in Baghdad including Al-Yarmouk Teaching Hospital, AL-Mahmmodiya Hospital, and Child's Central Teaching Hospital. A study analyzed 55 *P. aeruginosa* strains from various clinical sources, the study utilizes the chemical characterization, VITEK 2 system, *16s rRNA*, antibiogram sensitivity tests, antibiotic susceptibility using eight antibiotics, including Amikacin, Ciprofloxacin, Levofloxacin, Imipenem Meropenem, Piperacillin, Cefepim and Aztreonam. The test of bacterial susceptibility revealed that each isolate was highly resistant to piperacillin, which are 96.36%, and lower resistance to Ciprofloxacin, which are 32%. Phenotypic screening carbapenem resistance methods combined the disk synergy test and conventional PCR that were used to detect isolates by using *16 S rRNA*. This proves that the bacteria is *P. aeruginosa* and computed by measuring gene expression of the target genes (*GIM, VIM, SPM)* by using the real-time PCR, which is employed for twenty-five isolates. The result indicates that the expression level of the *VIM* gene is highly regulated in carbapenem-resistance isolates compared to control isolates that is 1.00. While the expression level of gene *GIM* and *SPM* is downregulated in carbapenem-resistance isolates compared to control isolates that is 6. The carbapenem *VIM* and *GIM*, SPM (class B) genes are essential for resistance in *P. aeruginosa* induced by chromosomal changes that modify membrane permeability efflux pump overexpression for genes. As a result, many studies require for discovering new strategies to reduce the threat to public health through preventing the spread of these isolates via tight infections, control measures, and the reduction of the danger to public health.

Keywords Carbapenem resistance · *VIM* gene · *Pseudomonas aeruginosa* · *GIM SPM* gene · Gene · Real-time PC

Abbreviations

MBL	Metallo beta lactamase
DNA	Deoxyribonucleic acid
cDNA	Complementary DNA
RNA	Ribonucleic acid
rRNA	Ribosomal RNA
EDTA	Ethylene diamine tetra acetic acid
CDST	Combined disc synergy test
MDR	Multidrug resistant
MDROS	Multidrug-resistant organisms
CP-PA	Carbapenemase producing-pseudomonas aeruginosa
ISCRS	Insertion sequences with a common region
VIM	Verona integron-encoded metallo-β-lactamase
SPM	Sao Paulo metallo-β-lactamase
GIM	Germany imipenemase
OPr	Outer membrance porin
Bla	Beta lactamas
CT	Cross Threshold
MHA	Muller Hinton Agar

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MEM	Meropenem
IMP	Imipenem
CIP	Ciprofloxacin
PLR	Piperacillin
LEVO	Levofloxacin
FEP	Cefepim
AK	Amikacin
ATM	Aztreonam

Introduction

Pseudomonas aeruginosa is an important reason for severe healthcare-associated infections targeting immunocompromised patients [1]. *P. aeruginosa* emerges as a crafty human pathogen capable of starting a variety of fatal diseases. It is the most dangerous nosocomial pathogen for hospitalized patients, with a high rate of morbidity and fatality. Its ability to adapt, survive and develop resistance to numerous types of antibiotics that makes it as a disease, which poses a serious threat to human life [1, 2]. Due to the high level of inherent and acquired antibiotic resistance of *P. aeruginosa* and the advent of multi-drug resistant strains, these statistics have not changed in decades. Resistance to various antibiotics, including a group of aminoglycosides, quinolones and β-lactams, has been observed in clinical and epidemiological *P. aeruginosa* isolates [3, 4].

MBLs are bacterial enzymes that give resistance to most β -lactam antibiotics, including carbapenems, which have the largest spectrum of action. This resistance mechanism endangers public health by substantially reducing treatment choices for serious bacterial infections. Carbapenems in *P. aeruginosa* are classified into three classes: class A, B and C. class B, blaVIM, blaGIM, and blaSPM Synthesis of carbapenemases, which give resistance to large amounts of commercially available β-lactams, is the most problematic acquired resistance of *P. aeruginosa* [1–3]. The group B carbapenemases, such as Verona integron-encoded metallolactamases (VIM), are the most prevalent MBL of the bacterium *P. aeruginosa* and the source of many infections24 out of 46 VIM variations, involves VIM-43, were discovered in the United States. Integrons, which also include various antibiotic resistance genes, contain the genes for VIM, promoting their worldwide proliferation. Saao Pauulo metallo-β-lacctamaase(SPM) in South America at the moment has one of the highest ratios of resistance to carbapenem in the global. The type of gene blaSPM can be found on either chromosomes or plasmids [4–6]. Germany imipenemasee (GIM)In 2002, GIM, was discovered in the isolates of *P. aeruginosa* from Germany. GIM-1 has less than 45% identification of the amino acid sequence with important clinical MBL genes. Infections due to carbapenem-resistant bacteria, such as *P. aeruginosa* (CR-PA), are often contributed to healthcare across the world. In the case of long-term intensive care and wards in hospitals, the most widespread *P. aeruginosa* contributes to ventilator-associated pneumonia (VAP), and it is the second-leading source in critical care hospitals. Additionally, it is the third most common source of catheter-related urinary tract infections [5]. The main reasons for the resistance of carbapenems in *P. aeruginosa* are various. The Efflux pump MexAB-OprM is overexpressed in the first mechanism, which causes the medication to be effluxed [6]. With the possible exception of imipenem, this results in the resistance to the majority of beta-lactam drugs. The deactivation of the OprD outer membrane protein and increased AmpC beta-lactamase synthesis are the second mechanism [7]. The most antipseudomonal beta-lactams are susceptible to resistance obtained by the combination of mechanisms. The creation of carbapenemases is less common than others, but it is the possible emerging route of carbapenem resistance in *P. aeruginosa* isolates [8]. The carbapenem resistance mechanism is essential for that since it considerably decreases the effectiveness of widely used antipseudomonal drugs. *P. aeruginosa* frequently carries carbapenem resistance that determinants on plasmids of the IncP type, class I integrins that carry the *blaVIM* gene, and other mobile genetic elements that are linked to sequences of insertion with common regions (ISCRs). This improves the organism's capacity to the spread resistance among various species [9, 10]. Treatment failure is probable as a result of the pathogenic bacteria *P. aeruginosa* (CP-PA) strains that produce carbapenemase frequent resistance to all of these treatments. Infection control measures have been considered since CP-PA has been connected to nosocomial transmission [11]. The problem of antibiotic resistance in Gram-negative organisms continues to grow greatly. Broad-spectrum-lactam antibiotics such as carbapenems are used as a last resort against multidrug-resistant organisms (MDROs). The requirement of identification and address MDROs that are also carbapenem-resistant that is growing as a result of increased resistance to these last-resort medications. The three classes of carbapenems are found in bacteria *P. aeruginosa* they are the following: class A(bla KPC), class B included (blaIMP, blaVIM, blaNDM, blaSIM, blaGIM, and blaSPM) and class D(bla OXA) [7].

Materials and methods

Ethical statements for human

I confirm that all experiments were performed in accordance with relevant guidelines and regulations. The study proposal was accepted by biology department, college of science of Mustansiriyah University, Baghdad, Iraq in September 2021.

Isolation and Identification of *P. aeruginosa*

The study was conducted at the Department of Microbiology, AL-Mahmoodia Hospital, for one year from January to December 2022. The samples were collected from patients with different clinical sources (Burn, Urine, Wound, Sputum, Ear, and Blood), from different ages. Additionally, samples were collected from three hospitals in Baghdad including Al-Yarmouk Teaching Hospital, AL-Mahmmodiya Hospital, and Child's Central Teaching Hospital. Blood, MacConkey and Cetrimide agars were used. According to phenotypic traits, biochemical testing, and the VITEK 2 compact system, fifty-five isolates of *P. aeruginosa* were identified. The EasyPure® Bacteria Genomic DNA Kit (Transgene biotech /China) was used to extract the DNA. Cell harvesting involves the bacterial culture into 1.5 µl microcentrifuge tubes, centrifuging them at 14–16 000 × g for 1 min, and then discarding the supernatant DNA extract by using the manufacturing strategy. 16 S rRNA primers was used to confirm identification. The total 25 µl volume used for the PCR amplification, which is contained 3 µl of DNA and 20 µl of Accu power premix, which was used. 1 ml from each primer (10 pmol/1µl), as well as up to 25 µl with nucleases free water. After that, 5 min start of denaturation of that at 94 °C, 25 cycles of 30 s at 94 °C, 30 s at 63 °C at the optimum temperature for annealing, and 30 s at 72 °C were carried out. Then, a procedure of 10 further minutes at 72 °C was conducted. Using a thermal cycler, this program required approximately 45 min to finish the target DNA amplification. The gel electrophoresis technique with EtBr. stain was used to detect PCR products [12].

Antimicrobial susceptibility test

The Kirby-Bauer method was used to determine the antibiogram activity: The Kirby-Bauer method was used for all the bacterial isolates to find out the antibiotic susceptibility test for 8 different antimicrobial agents, and antimicrobial susceptibility was tested according to the CLSI (2022) criteria. To prepare the bacterial suspension, a few bacterial colonies from the overnight culture were transferred to 5 ml of normal saline and adjusted to 0.5 McFarland turbidity(1.5×10^8 CFU/ml). Impenem (Imp10 mg), Levofloxacin (LEV 5 mg), Ciprofloxacin (CIP10 mg), Piperacillin (PRL 100 mg), Aztreonam (ATM 30 mg), Amikacin (AK 10 mg), Cefepime (FEP 10 mg), and Meropenem (MEM 10 mg).

Phenotypic detection of carbapenemase

According to Yong et al., imipenem-resistant strains were utilized to detect MBL strains phenotypically [13]. The combination of the IMP-EDTA disc test is referred to as the CDST (combined disc synergy test). The CLSI recommended that test organisms be placed on Mueller Hinton agar plates. A 0.5 M EDTA solution was made by dissolving 18.61 g in 100 ml of filtered water and adjusting the pH to 8.0 with NaOH. The mixture was disinfected by autoclaving. To reach the right concentrations (750 g), one of the two 10 ml imipenem discs (Becton Dickinson) was placed on the plate, and 10 ml of the EDTA-containing solution was added in the required proportions. During 16–24 h of incubation in the air at 37 degrees Celsius, the inhibition zones of the Imipenem and Imipenem-EDTA discs were compared [14, 15].

Extraction of genomic DNA

By using Easy Pure® Bacteria Genomic DNA kit (TRANS Gen Biotech/China), genomic DNA was extracted to detect 16 S rRNA by conventional PCR using primers as shown in Table 1.

Carbapememase gene quantitative expression using real-time PCR

To quantify gene expression for carbapenem resistance and normalize the results with the housekeeping gene 16 s rRNA, 25 isolates of *P. aeruginosa* underwent quantitative Real-time PCR. The procedure consists of the following steps:

Table 1 PCR primers that were used for genotypic detection of bacterial isolates

Primers		Primer Sequence (5′-3′)	Size bp	References
16s rRNA (detection)	(Foreword)	5′- TGCCTGGTAGTGGGGGATAA-3′	505	[16]
	(Reverse)	5′- GGATGCAGTTCCCAGGTTGA-3′		
16s-rRNA expression	(Foreword)	GAGCGGATAACAATTTCACACAGG	96	[17]
	(Reverse)	CGCCAGGGTTTTCCCAGTCACGAC		
SPM-1 A	(Foreword)	AAAATCTGGGTACGCAAACG	271	[18]
	(Reverse)	ACATTATCCGCTGGAACAGG		
GIM-1	(Foreword)	TCGACACACCTTGGTCTGAA	477	[18]
	(Reverse)	AACTTCCAACTTTGCCATGC		
VIM family	(Foreword)	GATGGTGTTTGGTCGCATA	390	[18]
	(Reverse)	CGAATGCGCAGCACCAG		

RNA extraction

Twenty-five *P. aeruginosa* isolates (out of 55) were grown on Brain Heart Infusion Broth at 37 °C up to (OD600:0.8–1.0), and the bacterial cell was then collected using a centrifuge at 12,000 g/min to extract the RNA.It was extracted from bacteria isolates in accordance with the manufacturer's instructions. RNA was isolated from bacteria isolates using a kit from (Transgene Biotech/China) cat. No. (ER501) according to the manufacturer's instructions. Total RNA was mixed with DNase enzyme and quickly kept at (70 °C) to prevent contamination. A nanodrop was used to test the purity and concentration of RNA.

Synthesis of cDNA

To evaluate the cDNA synthesis, the Easy Script® One-Step gDNA removal and cDNA Synthesis Super Mix (TRANS/China) kit's instructions, genomic DNA was successfully removed. Reaction mix, random primer, anchored oligo dT, genomic DNA remover, RNase-free water, E-mix reverse transcriptase, and the final quantity of total RNA were added as necessary for cDNA synthesis. There are three stages in a thermal cycler: the first is at 25 °C for 10 min, the second is at 42 °C for 15 min, and the last stage is to inactivate the enzyme at 85 ċ for 5 s.

Real-time PCR reaction

The Trans Start® Top Green qPCR super mix (TRANS Gene biotech/China) cat. no. (AQ131-01) was used to initiate the RT-PCR reaction. The reaction mixture included 10 µl of master mix, 1 µl of each primer listed in Table 1 3 µl of template DNA, and 20 µl of nuclease-free water for the final volume. The RT-PCR conditions included a temperature profile of 94 °C for 1 min, 40 cycles of 94 °C for 10 s for the initial denaturation, annealing at 54, 56, and 58 °C for 15 s, and finally, 72 °C for 20 s for the phase of extension. The variations between the reference gene's (CT) mean threshold cycle and the gene's primary (CT), for both the test specimens and controls, were then computed. The variations between the two tests and two control samples were used to determine the CT index. For the amplification by RT-PCR, the real time PCR- assays for targets were run on an instrument G-STORM/UK. RTPCR, which permits to detect and measure the aggregation of amplified products to be as the reaction progresses "real time". In the PCR plot, it was appeared the fluorescence from amplification reaction and cycle numbers on the X-axis, while the Y-axis was appeared proportional the amount of amplified product in the reaction tube [26].

Statistical analysis

The Statistical Analysis System- SAS (2018) program was used to detect the effect of different factors on study parameters. Least Significant Difference- LDS was used to significantly compare between means. The chi-square test was used to significantly compare between percentages (0.05 and 0.01 probability). Estimate of Odd Ratio and CI in this study.

Results

Bacterial isolates and antimicrobial sensitivity testing

The current study showed that the identification of fifty-five clinical isolates of *P. aeruginosa* from different clinical samples., including sputum from (11)20.00%, blood from (2) 3.64%, wounds from (6) 10.91%, burns from (19) 34.54%, ears from (5) 9.09%, and urine from (12) 21.82% for patients who were hospitalized in different hospitals in Baghdad between September and December 2022. In this study, all 55 isolates were confirmed by *16s rRNA* with 505 bp band size as shown in Fig. 1. According to Table 2, the antibiogram test of the fifty- five isolates of *P.aeruginosa* showed resistance rates to Imipenem at 40.00% and Meropenem at 34.55%. In the current study, all isolates showed widespread antibiotic resistance. Also, it showed the highest resist rates to Amikacin 96.36% and Piperacillin 96.36% respectively.

PCR result for16s-rRNA

The traditional PCR was carried out satisfactorily. In this investigation, 505 bp bands were amplified as 16 S, and then 25 isolates verified as *P. aeruginosa* by molecular detection utilizing *16s rRNA* were employed in PCR, as shown in Fig. 1

Table 2 Antimicrobial susceptibility testing

Antibiotics	S	R	I	P-value
MEM	(28) 50.91%	(19) 34.55%	(8) 14.55%	0.0004**
IMP	(31) 56.36%	(22) 40.00%	(2) 3.64%	0.0001**
PRL	(0) 0.00%	(53) 96.36%	(2) 3.64%	0.0001**
CIP	(31) 56.36%	(18) 32.72%	(6) 10.91%	0.0001**
LEVO	(28) 50.91%	(20) 36.36%	(7) 12.73%	0.0001**
FEP	(7) 12.72	(45) 81.82%	(3) 5.45%	0.0001**
AK	(0) 0.00%	(53) 96.36%	(2) 3.64%	0.0001**
ATM	(18) 32.73%	(22) 40.00%	(15) 27.27%	0.237 NS
P-value	0.0001**	0.0001**	0.0082**	

**P ≤ 0.01

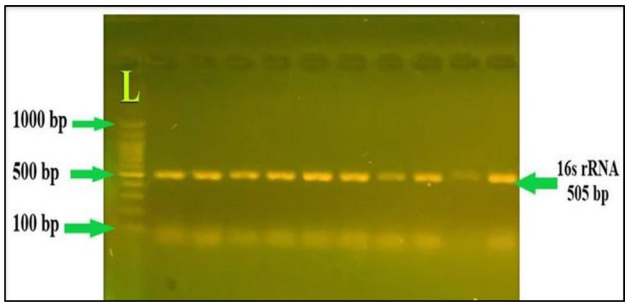

Fig. 1 Gel electrophoresis for *16s rRNA* 505 bp, (Agarose 1%, At 100 Volts, 60 min.) Visualized under UV light after staining with ethidium bromide

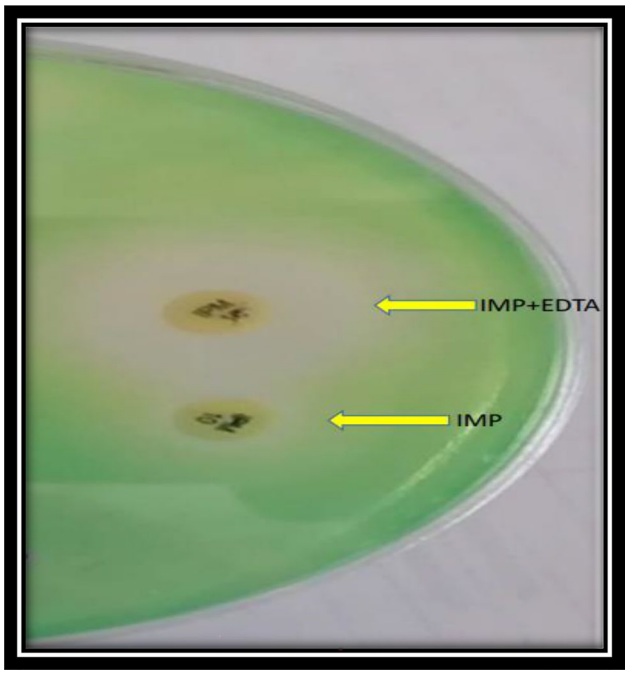

Fig. 2 Isolate showing positive carbapenemase-producing *P.aeruginosa* test with ≥7 mm increase in the zone of inhibition of imipenem + ethylenediaminetetraacetic acid as compared to imipenem alone

Phenotypic detection of carbapenemase

The Combined Disc Synergy Test showed (20/55) isolates that could produce carbapenemase enzymes. (17/20)85% of isolates were positive for the CDST test, while most imipenem-resistant bacteria tested positive for metallo-beta-lactamase. The imipenem-EDTA combination disc test was performed to determine whether imipenem resistance is induced by MβL production or by other mechanisms in 20 imipenem-resistant bacteria in Fig. 2 is an example of an imipenem-EDTA combination disc test.

Evaluation of gene expression of *VIM, SPM,* and *GIM*

The quantitative Real-Time PCR technique with *16s rRNA* housekeeping gene was then used to normalize the data. To evaluate *VIM*, *SPM*, and *GIM* gene expressions in the bacteria *P. aeruginosa* isolates.

Housekeeping gene expression

The results of *16s rRNA* Ct results ranged from 23.32 to 21.85 with an average of 22.58 as shown in (Fig. 3A & B).

VIM gene expression

As shown in Fig. 4C & D, the results of *VIM* Ct values varied from 17.7 to 24.8 with an average of 21.25 showing the expression compared to the 16s rRNA (Ct = 23.32) reference gene. VIM gene is highly regulated in carbapenem-resistance isolates compared to 16s rRNA. These result show high significantly, **($P \leq 0.01$).

SPM gene expression

As shown in Fig. 5E & F, the results of the *SPM* Ct values varied from 24.87 to 26.1 with an average of 25.48, showing the expression relative to the 16s rRNA (Ct = 23.32) reference gene. expression level of *SPM* genes is downregulated in carbapenem-resistance isolates compared to 16s rRNA. these result show high significantly, **($P \leq 0.01$).

Results of *GIM* gene expression

The expression is shown in Fig. 6G & H by *GIM* Ct values, which varied from 32.2 to 33.55 with an average of 32.875. expression level of *GIM* genes is downregulated in carbapenem-resistance isolates compared to 16s rRNA, these result show non-significantly.

Discussion

The pathogen *P. aeruginosa* is an aggressive bacterium that especially in immunocompromised and burn patients, can cause a variety of diseases. [19, 20]. In the previous few decades, it was observed that there is an increase in the carbapenem resistance, which is now a serious health risk. Therefore, it is essential to early determine MβL producers in order to treat infections effectively, which will reduce the resistance rate and nosocomial transmission [21]. Table 2 provides an overview of the antibacterial effectiveness of specific drugs against Pseudomonas. The current study showed an astounding increase in *P. aeruginosa* resistance to the medicines utilized in this investigation, The study's

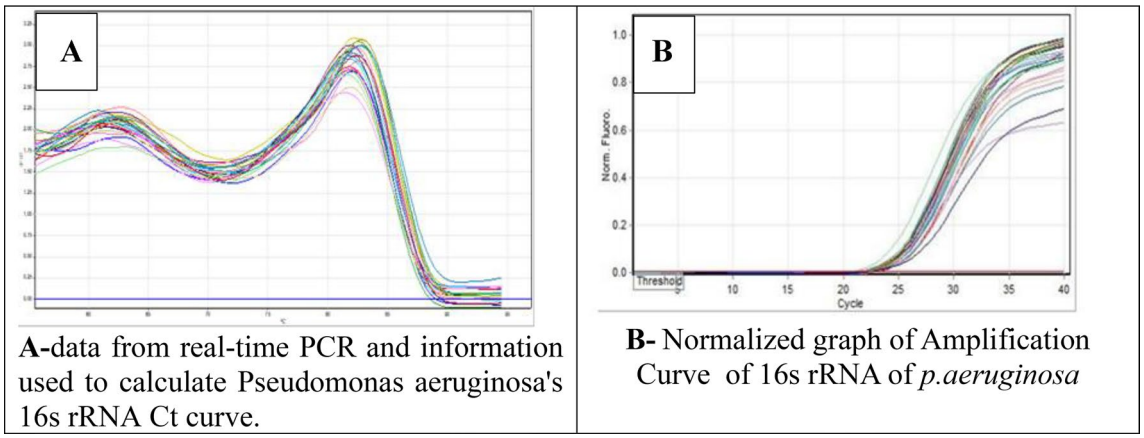

Fig. 3 Comparative quantitation and amplification curve of 16s rRNA

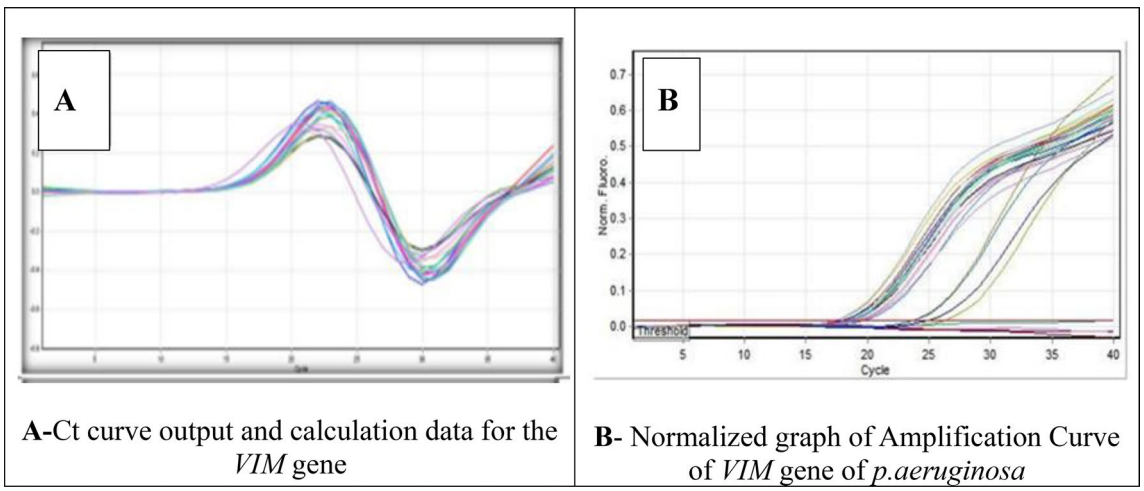

Fig. 4 Comparative quantitation and amplification curve of *VIM* genes

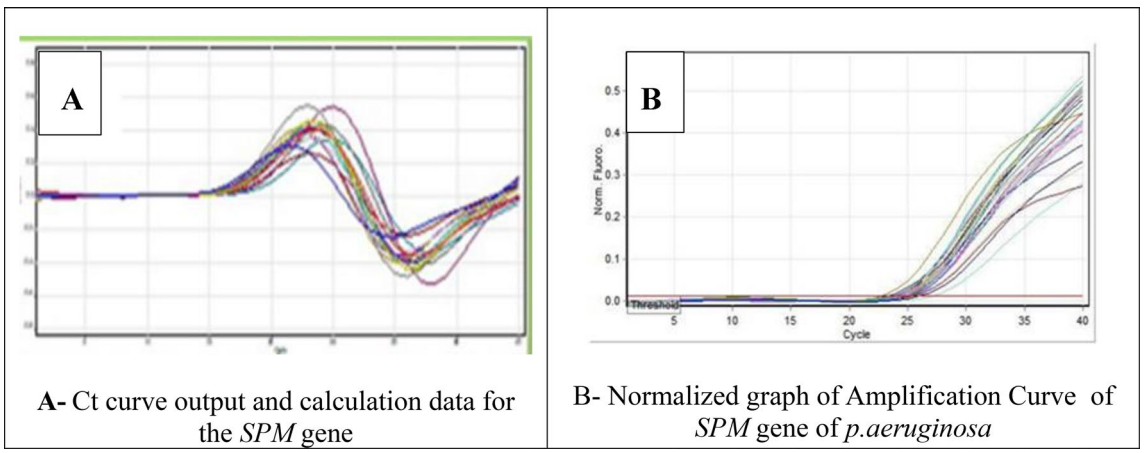

Fig. 5 Comparative quantitation and amplification curve of *SPM* genes

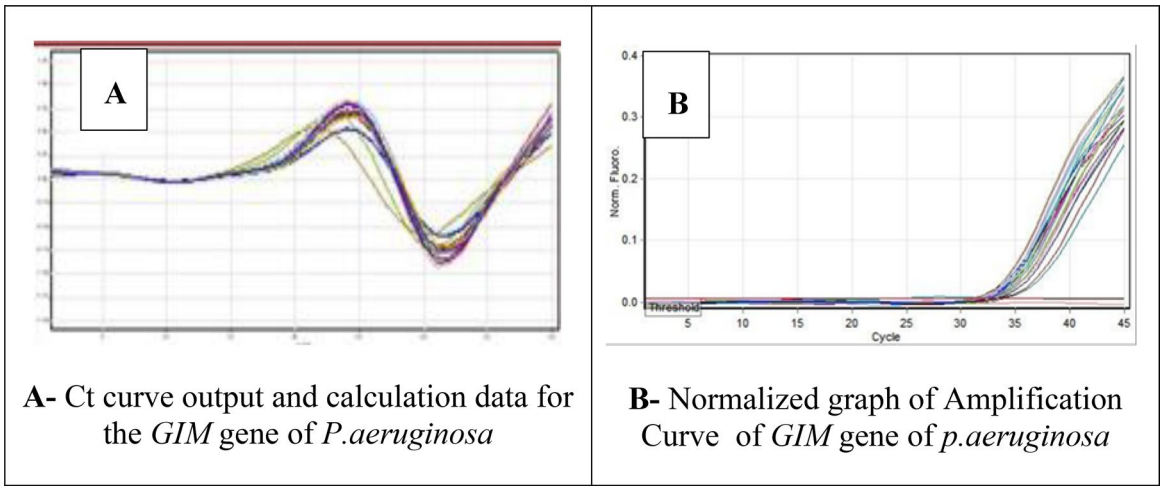

Fig. 6 Comparative quantitation and amplification curve of *GIM* genes

findings, which are reported in Table 2, demonstrate a striking increase in pseudomonal resistance to penicillins like piperacillin (96,66%), a beta-lactam antibiotic that is representative of this class of antibiotics. These findings corroborated research from. The percentage of people who are resistant to cephalosporins, including cefepime, is 81.82%. This resistance rate is quite comparable to studies. Cefepime's representation of cefepime's level of resistance to other antibiotics was *P.aeruginosa* the outer membrane, which serves as an efficient intrinsic barrier to accessing the targets within the cell wall, cytoplasmic membrane, or within the cytoplasm, may confer resistance to this antibiotic. Modifications in outer membrane permeability via changes in porin protein channels represent a component of many resistance mechanisms. In addition, the periplasmic gap allows inactivating enzymes secreted from the inner membrane to work more effectively. Reduced permeability via the outer membrane, reduced absorption through the cytoplasmic membrane, and active efflux back out across the cytoplasmic membrane are the methods by which intracellular quantities of medicines are constrained. The results of antibiotic sensitivity in the present study were lower compared to another research [22, 23]. This could belong to a difference in the sample size or population size in addition to healthcare practices in those regions. MβL-producing bacteria increased in reported nosocomial epidemics in recent years, which is highly concerning because they have few therapeutic options. It was shown there are more than 20 (36.36%) of *P. aeruginosa* isolates that were imipenem-resistant, with 17 (85%) of these isolates being MβL phenotypically productive [22, 24]. It was observed that 85% of A strain of *P. aeruginosa* that is resistant to imipenem produced MβL and that 55/20 (36.36%) of the isolates showed antibiotic resistance [25]. assessed155 the pathogenic bacteria *P. aeruginosa* strains from various clinical samples were examined, and it was shown that 40% of them were resistant to imipenem and 85% were MβL-positive [26]. The real-time PCR was used to assess the gene expression of *GIM, VIM*, and *SPM*. SYBR green, which was used as an indicator dye. Gene expression was measured as Ct (Cycle Threshold), where higher Ct values indicate lower gene expression and lower Ct values indicate higher gene expression. In this study, the 25 isolates that were confirmed by molecular detection using *16 S rRNA* as *P. aeruginosa* were used. Reference or housekeeping genes have historically been used in Real-time PCR methods to regulate sample-to-sample variation [26, 27]. The expression level of (*GIM, VIM*, and *SPM*) genes among 25 *P. aeruginosa* isolates were conducted by using cDNA. The expression of target genes was normalized to the *16s rRNA* as a housekeeping gene. Our result indicates that the expression level of the *VIM* gene is highly regulated in carbapenem-resistance isolates compared to control isolates is 1.0. While the expression level of *GIM, SPM* genes is downregulated in carbapenem-resistance isolates compared to control isolates is 5 this result shown, these suggesting that other undiscovered factors or pathways likely contribute to the up or down-regulated of *VIM, SPM* and *GIM* that are important of resistance in *P. aeruginosa* is induced by chromosomal changes that modify membrane permeability efflux pump overexpression. Recent research has noted the rising incidence of *P. aeruginosa* which is resistant to carbapenem. Ambler class B (such as *GIM, VIM,SPM*) β-lactamases, which hydrolyze carbapenem, are responsible for this resistance [28, 29]. Gram-negative bacteria are the main cause of nosocomial infections. One of the biggest medical difficulties has been these infections. In hospitals, *Pseudomonas aeruginosa* is an opportunistic bacterium that is responsible for a wide range of nosocomial infections. We are seeing this bacterium develop a considerable resistance to antibiotics as a result of people using a wide range of drugs carelessly

[30]. The spread of multiple antibiotic-resistance genes is linked to the great antibiotic resistance of this bacterium. Several studies show that *P. aeruginosa* is resistant to most antibiotics, including quinolones and beta-lactam, and that it develops an increased resistance to aminoglycoside [31, 32]. The major reasons for the resistance to carbapenem in *P. aeruginosa* are oprD genetic changes and the production of the Metallo-β-lactamase *Vim*, *Spm*, according to parallel findings from many different nations [33]. The SPM gene was found in 27 out of 32 P. aeruginosa isolates, according to reports Hassuna et al., 2020 [34]. Khan et al. (2020) also found that only 6% of 50 isolates have the *SPM* gene [35]. Four of the sixteen isolates of *P. aeruginosa* that were carbapenem-resistant included the *VIM* gene according to Gill et al. (2020) [36]. On the other hand, according to Musila et al. (2021), the *VIM* gene shown in 50% of isolates [37]. According to findings from additional research achieved at AL-Mahmmodiya Hospital, resistance isolates had *GIM, VIM* and *SPM* genes. None of the isolates that were susceptible to the imipenem carried these genes. The percentage of MβL genes was different, but the differences were not significant. It was also observed that these resistance genes were almost widespread. Prevalence variations showed that different types of Metallo-β-Lactmase genes were distributed differently in various geographical.

Conclusion

The *VIM family* gene, *SPM-1 A* genes, and *GIM-1* genes were found in imipenem-resistant *P. aeruginosa* that isolated from a hospital in Baghdad. It also showed that the MβL strains are multi-drug resistant; as a result, effort should now be directed toward reducing the threat to the public's health by preventing the threat to public health by preventing the spread of such strains through strong infection control measures. To avoid spreading *P. aeruginosa* infection, it may be useful to identify bacteria that are resistant to the antibiotic imipenem and check for the production of the beta-lactamase enzyme.

Limitation of this study

(1) small samples size.
(2) limit time specified to carried study.
(3) Some molecular techniques weren't able to be used due to no funding for this work.

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Author contributions All authors contributed equally in writing—original draft preparation, all authors have read and agreed to the published version of the manuscript.

Funding This research received no external funding.

Data availability No Data associated in the manuscript.

Declarations

Conflict of interest The authors declare no conflict of interest.

Ethical approval The Ethics Committee of the Mustansiriyah University approved and oversaw this study.

Consent to Publish All authors agree to publish this work.

Informed consent All patients gave their written informed consents before inclusion.

Research involving human and animals' participants This study involved the clinical isolates of *P. aeruginosa* from patients who are suffering from infections of sputum, blood, wounds, burns and urine at Baghdad hospitals, Baghdad, Iraq.

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