Clinical research

Detection of IMP and VIM genes in *Pseudomonas aeruginosa* isolated from Egyptian patients

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Abstract

Introduction: Metallo-β-lactamase production among *Pseudomonas aeruginosa* is a major health problem worldwide. *Pseudomonas aeruginosa* acquire several mechanisms of resistance towards carbapenems through the production of metallo-β-lactamases, especially VIM and IMP. The problem of multi-drug-resistant *Pseudomonas aeruginosa* is increasing all over the world, reaching dangerous levels. The aim of this study was to detect the metallo-β-lactamases bla_{VIM} and bla_{IMP} genes in *Pseudomonas aeruginosa* strains in Suez Canal University Hospital in Ismailia, Egypt.
Material and methods: A cross-sectional descriptive study was conducted on 65 *Pseudomonas aeruginosa* strains. Genotypic detection of bla_{VIM} and bla_{IMP} was reached by using polymerase chain reaction.
Results: Out of 65 *Pseudomonas aeruginosa* strains , bla_{VIM} gene was present in four females and one male, with an age of 42.9 ±18.1; two cases were isolated from the Oncology Department, and one case each was present in the Burn Unit, Surgery Ward, and Intensive Care Unit. The bla_{VIM} gene was expressed in four stains, while the bla_{IMP} gene was not expressed in any strain.
Conclusions: The carbapenem resistance in our patients can be referred to as metallo-β-lactamases bla_{VIM} type. The problem of metallo-β-lactamases and carbapenem resistance requires ongoing surveillance, strong preventive measures, and implementation of infection control policies and procedures. Also, routine diagnostic laboratory methods should be performed, and synthesis of antimicrobial products with new effecting mechanism should be implemented in hospitals.

Key words: metallo-β-lactamases, bla_{VIM}, bla_{IMP}, Egypt.

Introduction

One of the most prevalent opportunistic organisms is *Pseudomonas aeruginosa*, which has started to grow worldwide [1]. It is considered as one of the most extensive organisms, resistant to all anti-pseudomonal antibiotics (carbapenems and aminoglycosides) [2]. For this reason, the best choice of treatment is carbapenems, in spite of the incidence of resistance to these antibiotics [3].

Pseudomonas aeruginosa acquires several mechanisms of resistance towards carbapenems through the production of metallo-β-lactamases (MβLs), especially VIM (Verona integron-encoded metallo β-lactamase) and IMP (imipenem active metallo-β-lactamase) [4, 5], alteration and/or loss of OprD (outer membrane porin protein), hyperproduction of AmpC β-lactamase [6], and increased expression of efflux pump [5].

Carbapenemase resistance belongs to Ambler classes A, B, and D [7, 8], but MβL VIM and IMP belong to Ambler B. MβLs are inhibited by ethylenediaminetetraacetic acid (EDTA) and sodium mercaptoacetic acid (SMA), but they are not affected by β-lactase inhibitors such as clavulanic acid, sulbactam, and tazobactam [9].

The problem of multi-drug-resistant (MDR) *Pseudomonas aeruginosa* has augmented all over the world, thus reaching a dangerous level [10]. It is noteworthy that several studies that have been conducted all over the world reveal that the problem of MβL in *Pseudomonas aeruginosa* is increasing in different hospitals in various parts of the world [11–13]. It has thus been necessary to identify these MβLs and treat them [13].

The aim of the study was to detect the MβL bla_{VIM} and bla_{IMP} genes in *Pseudomonas aeruginosa* strains in patients hospitalised in Suez Canal University Hospital, Ismailia, Egypt.

Material and methods

A cross-sectional, descriptive study was conducted on 65 *Pseudomonas aeruginosa* strains obtained from clinical specimens from hospitalised patients admitted to the Suez Canal University Hospital in Ismailia during the period from January 2017 to September 2017, because the MβL enzymes had previously been detected phenotypically in the strains [14] by using the modified Hodge test (MHT) and double disk synergy test (DDST) [15, 16]. Our inclusion criteria were patients admitted to the hospital 48 h after infection and who had been suffering from it regardless of their age or sex. We excluded those who showed signs or symptoms of infection 48 h after admission to the hospital and/or those receiving antibiotics 48 h before specimen collection.

DNA extraction

DNA was extracted from the bacterial cells using GeneJET genomic DNA purification kit (Cat#K0721, Thermo Fisher Scientific, USA) and following the manufacturer's protocol. Up to 2×10^9 bacterial cells were harvested in 1.5-ml micro-centrifuge tubes by centrifugal for 10 min at 5000 × g. Then, the resulting pellets were suspended in 180 µl of digestion solution and 20 µl of proteinase K and incubated at 56°C in a thermo-mixer until complete lysis occurred (~30 min).

RNase A solution (20 µl) was added and incubated for 10 min in order to obtain RNA-free samples. After 200 µl of lysis solution and 400 µl of ethanol (50%) had been added and mixed thoroughly to the samples, the prepared lysate was transferred to the specified columns and centrifuged for 1 min at 6000 × g. The DNA absorbed on the silica membrane was washed twice in 500 µl wash buffer I and II, respectively.

Then, the columns were centrifuged at full speed for 1 min, in order to ensure elimination of all impurities. Finally, the columns were transferred to new 1.5-ml collection tubes and 200 µl of elusion buffer was added; the tubes were incubated at room temperature for 2 min and then centrifuged at 8000 × g for 1 min to elute the DNA. The extracted DNA concentration and relating purity were assessed using NanoDrop ND-1000 (Nanodrop, USA).

Real-time PCR

The MβL-encoding bla_{IMP}- and bla_{VIM}-type genes were detected via SYBR green multiplex real-time PCR analysis. The following primers (Invitrogen, CA) were used for IMP: forward: 5′- GAATAG(A/G)(A/G) TGGCTTAA(C/T)TCTC-3'; reverse: 5′- CCAAAC(C/T) ACTA(G/C)GTTATC-3', for VIM: forward: 5'- GTTTG-GTCGCA TATCGCAAC-3'; reverse: 5'-AATGCGCAG-CACCAGGATAG-3', and for 16S *rRNA* as internal control: forward: 5'-AGAGTTTGATCCTGGCTCAG-3'; reverse: 5'-ACGGCTACCTTGTTACGACTT-3' at a final concentration 1.0 µM, 0.1 µM, and 0.04 µM for bla_{IMP}, bla_{VIM}, and 16S rRNA, respectively [17]. Amplification was performed in a 48-µl mixture containing three pairs of primers at their respective concentrations, 2 µl of the template by using the DNA at concentration 100 pg/reaction, 25 µl of Platinum SYBR green qPCR SuperMix (Platinum Taq DNA polymerase, SYBR green I dye, Tris-HCl, KCl, 6 mM $MgCl_2$, 400 µM dGTP, 400 µM dATP, 400 µM dCTP, 800 µM dUTP, uracil DNA glycosylase, and stabilisers- Cat. No. 11733-038, Invitrogen, CA).

Rox reference dye was included in each reaction to normalise the fluorescent reporter signal. The PCR was performed by StepOne™ Real-Time PCR System manufactured by Biosystems, using the following conditions: initial denaturation at 94°C for 5 min; 35 cycles of 94°C for 20 s, 53°C for 45 s, and 72°C for 30 s. A melt curve step was prepared (from 68°C gradually increasing by 0.5°C/s to 95°C, with acquisition data every 1 s).

Melt curves were then converted to melting peaks by plotting the negative derivative of fluorescence versus temperature (–dF2/dT vs. T and –dF3/dT vs. T). Post-PCR agarose gel (1.5%) containing 0.5 µg/ml ethidium bromide was performed; the predicted lengths of the amplicons were 188,

Table I. Geographic distribution of the patients studied according to the personal data of the cases (n = 65) [14]

Variable		Results
Gender	Male	90 (43.1%)
	Female	120 (56.9%)
Age		42.9 ±18.1

382, and 1499 base pairs for bla_{IMP}, bla_{VIM}, and 16S rRNA, respectively [17].

Ethical considerations

The study obtained approval from the Ethics Committee of the Faculty of Medicine, Suez Canal University (FOMSCU), Ismailia, Egypt.

Statistical analysis

Data was analysed by Texasoft WINKS, 4.651 software (Texasoft, Texas, USA). The numerical data were expressed as means and standard deviations. The qualitative data were expressed in frequencies and percentages. Student's *t*-test was applied in order to compare between two independent groups. Frequencies of categorical variables were compared using the χ^2 test and Fishers' exact test when appropriate.

For quantitative data, the comparison between the three groups was performed by an analysis of variance (ANOVA) followed by post-hoc Newman-Keuls multiple comparison tests. A two-tailed $p < 0.05$ was considered significant.

Results

Geographic distribution of the patients studied according to the personal data of the cases presented in Table I.

Out of 65 *Pseudomonas aeruginosa* strains, the bla_{VIM} gene was present in 4 females and 1 male, both with age 42.9 ±18.1. Two cases were isolated from the Oncology Department, and one case each was from the Burn Unit, Surgery ward, and Intensive Care Unit. The most common specimen was pus and blood (Table II).

The bla_{VIM} gene is remarkably present among female patients (10.8%) in the following departments: Intensive Care Unit, Oncology, Surgery, and Burn Unit at a percentage of 10, 9.5, 9, and 9, respectively. For the type of specimen, it is present at the highest level in blood specimens and was not present in urine.

The relationship between the bla_{VIM} gene and the antibiotic sensitivity according to the disc diffusion method is presented in Table III. It is worth mentioning that this gene is present in different antibiotic groups such as ampicillin, piperacillin, ciprofloxacin, cefepime, and imipenem in the following percentages: 7.7, 8.2, 12.9, 12.2, and 16.0, respectively.

The bla_{VIM} gene is expressed in four MDR strains by using MHT and EDTA-IMP DDST phenotypic confirmatory test (Table IV).

Table II. Association between demographic characteristics and *blaVIM* gene carriage among *Pseudomonas aeruginosa* isolates

Parameter	*N*	bla_{VIM} in study samples (n = 65)				*P*-value
		Present	%	Absent	%	
Gender:						0.380
Male	28	1	3.6	27	96.4	
Female	37	4	10.8	33	89.1	
Department:						0.941
Burn unit	11	1	9	10	91	
Surgery	11	1	9	10	91	
Oncology	21	2	9.5	19	90.5	
Intensive care unit	10	1	10	9	90	
Urology	5	0	0	5	100	
Neonatal intensive care unit	7	0	0	7	100	
Specimen:						0.771
Urine	8	0	0	8	100	
Pus	24	2	8.3	22	91.7	
Blood	17	2	11.8	15	88.2	
Sputum	16	1	6.3	15	93.7	

Table III. Association between bla_{VIM} gene carriage and antibiotic sensitivity among *Pseudomonas aeruginosa* isolates

Variable	N	bla_{VIM} in study samples (n = 65)				P-value
		Present	%	Absent	%	
Ampicillin:						< 0.001*
Resistant	65	5	7.7	60	92.3	
Piperacillin:						0.720
Resistant	61	5	8.2	56	91.8	
Intermediate	4	0	0	4	100	
Ciprofloxacin:						0.319
Sensitive	32	1	3.1	31	96.9	
Resistant	31	4	12.9	27	87.1	
Intermediate	2	0	0	2	100	
Cefepime:						0.206
Sensitive	20	0	0	20	100	
Resistant	41	5	12.2	36	87.8	
Intermediate	4	0	0	4	100	
Aztreonam:						0.684
Sensitive	31	2	6.5	29	93.5	
Resistant	28	2	7.1	26	92.9	
Intermediate	6	1	16.7	5	83.3	
Gentamicin:						0.639
Sensitive	45	3	6.7	42	93.3	
Resistant	20	2	10	18	90	
Meropenem:						0.401
Sensitive	41	2	4.9	39	95.1	
Resistant	12	1	8.3	11	91.7	
Intermediate	12	2	16.7	10	83.3	
Imipenem:						0.129
Sensitive	29	1	3.4	28	96.6	
Resistant	25	4	16.0	21	84.0	
Intermediate	11	0	0	11	100	

**P-value < 0.05 considered significant.*

For the bla_{IMP} gene, it was not expressed in any strain in any of the 65 samples.

Discussion

Drug-resistant *Pseudomonas aeruginosa* is considered a major problem, and several antibiotics such as β-lactamases, quinolones, and aminoglycosides are used to solve this problem [18]. The antibiotic resistance pattern fluctuates, especially in the case of imipenem [19]. Carbapenems are often used to treat MDR *Pseudomonas aeruginosa* [20]. The production of MβLs enzymes (IMP and VIM) is considered to be the cause of antibiotic resistance to imipenem [21, 22].

Literature reports that there are several phenotypic methods used to detect MβLs, but their role is limited because not all MβLs carry the same enzyme [23, 24].

The MβLs producing *Pseudomonas aeruginosa* in Egypt vary according to the infection control policies and procedures applied in hospitals. The sample size of the population studied in one study [25] was estimated at 82%, while in another it was very low, reaching just 32.3% [26].

In the present study, bla_{VIM} is the gene detected in all isolates. This copes with several studies recognising that this is dominant worldwide and associated with hospital-acquired outbreaks [27,

Table IV. Association between bla_{VIM} gene carriage and the phenotypic confirmatory methods

Parameter	*N*	bla_{VIM} in study samples (*n* = 65)				*P*-value
		Present	%	Absent	%	
Multi-drug resistance:						0.174
Present	33	4	12.1	29	87.9	
Absent	32	1	3.1	31	96.9	
MHT:						0.222
Total isolated *P. aeruginosa*	35	4	11.4	31	88.6	
MDR strains	20	1	3.3	29	96.7	
DDST:						0.013
Present	20	4	20.0	16	80.0	
Absent	45	1	2.2	44	97.8	

28]. MβLs producing isolates is a nationwide concern in Egypt, and many efforts are being made to confront it in order to decrease morbidity and mortality.

The bla_{IMP} gene was not expressed in any strain among the population studied. This is in agreement with the study by Shahcheraghi *et al.* conducted in Imam Khomeini Hospital in Tehran on 243 *Pseudomonas. aeruginosa* strains because the study did not observe the bla_{IMP} gene among the isolates [29].

Fazeli *et al.* conducted their study of *Pseudomonas aeruginosa* MβLs (sensitive to both imipenem and meropenem) by sing PCR technique. However, no bla_{IMP} gene was expressed among all the samples [30].

The study results were limited to a sample of 65 isolates. The reason behind this was due to insufficient financial support and the short duration of the study period. However, we anticipate the expansion of the work on a large sample size over a longer period.

In conclusion, the problem of MβLs and carbapenem resistance requires ongoing surveillance, strong preventive measures, and implementation of infection-control policies and procedures. Also, routine diagnostic laboratory methods should be performed as well as synthesis of antimicrobial products, and new effective mechanisms should be introduced.

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Conflict of interest

The authors declare no conflict of interest

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