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VIM, NDM, IMP, GES, SPM, GIM, SIM Metallobetalactamases in Carbapenem-Resistant *Pseudomonas aeruginosa* Isolates from a Turkish University Hospital

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Abstract

Background: *Pseudomonas aeruginosa* is an important opportunistic pathogen, and carbapenem resistance is an emerging problem. The determination of resistance genes is vital for epidemiological purposes, and the early determination of carbapenemase production methods is also recommended.

Objectives: The present study aimed to investigate the presence of VIM, NDM, IMP, GES, SPM, GIM, and SIM genes in *P. aeruginosa* isolates.

Methods: In this study, 200 carbapenem-resistant *P. aeruginosa* isolates were included. The DNA extraction of the carbapenem-resistant isolates was performed using the boiling method. Following the DNA extraction, optimization was conducted using the original primers. After optimization, the VIM, NDM, IMP, GES, SPM, GIM, and SIM genes were examined using the polymerase chain reaction (PCR) method.

Results: The isolates were mainly identified from the tracheal aspirate cultures (34.5%). The PCR method revealed the presence of VIM in one of the *P. aeruginosa* isolates, and the NDM gene in one isolate using. None of the isolates was positive in terms of the IMP, GES, SPM, GIM, and SIM genes.

Conclusions: In our study, two carbapenemase genes (VIM and NDM) were detected in the *P. aeruginosa* isolates.

Keywords: Carbapenem Resistance, *Pseudomonas aeruginosa*, Multiplex PCR

1. Background

Pseudomonas aeruginosa is one of the opportunistic pathogens causing hospital-acquired infections, especially in some groups of patients such as immunocompromised, burns, and cystic fibrosis (1). Infections causing multidrug-resistant *P. aeruginosa* isolates increase morbidity and mortality, enhance treatment costs, and prolong hospitalization period (2).

Carbapenems are the drug of choice in treating multidrug *P. aeruginosa* infections. The prevalence of carbapenem resistance among *P. aeruginosa* has increased worldwide (3). Because of some intrinsic resistance to antibiotics, the treatment of pseudomonas infections is a challenge. The main mechanisms for the high rates of antibiotic resistance are decreased drug accumulation because of the low permeability of cell wall and efflux pumps, chromosomal mutations, and the transfer of resistance genes by plasmids, transposones, and bacteriophage (4).

Among the carbapenemase types in *Pseudomonas* spp, according to the molecular classification of Ambler, there are serine β-lactamases such as KPC (*Klebsiella pneumoniae* carbapenemases) and GES (Guiana extended-spectrum) in Class A and OXA-198 (Oxacillinases-198) in Class D (5, 6). New metallo-beta-lactamase (MBL) enzymes, which may be responsible for the growing carbapenem resistance of non-fermentative Gram-negative (NFGN) bacilli, have been identified in recent years, which are spread worldwide (7). Many carbapenemases have been identified in *Pseudomonas* species and encompass metallo-β-lactamases (MBL) in Class B, including imipenemase (IMP), Verona integron-mediated metallo-β-lactamase (VIM), Sao Paulo MBL (SPM), Seul imipenemase (SIM), Australian imipenemase (AIM), German imipenemase (GIM), Dutch imipenemase (DIM), and new Delhi metallo β-lactamase (NDM) (8, 9).

2. Objectives

This study aimed to investigate the presence of the VIM, NDM, IMP, GES, SPM, GIM, and SIM genes accounting for the increase of resistance to carbapenem antibiotics in *P. aeruginosa* isolates.

3. Methods

In this study, 200 carbapenem-resistant *P. aeruginosa* isolates that isolated from different clinical samples were tested. Conventional methods (Gram staining, oxidase test) and the Vitek-MS (Biomeirux, France) automated system were used to identify the isolates. The antibiotic susceptibility was also tested using the Vitek 2 Compact (Biomeirux, France) automatization system. The *P. aeruginosa* isolates were stored at -20°C for further molecular studies. The presence of IMP, VIM, NDM, GES, SPM, GIM, and SIM genes was investigated by the multiplex PCR. Table 1 presents the primers used in the study (10-12). The boiling method was also used for the DNA extraction.

4. Results

This results indicated that tracheal aspirate cultures were the most common samples (34.5%) of the carbapenem-resistant *P. aeruginosa* isolates (Table 2). The VIM and NDM genes were noticed in two of the *P. aeruginosa* (P32, P190) isolates (Figure 1).

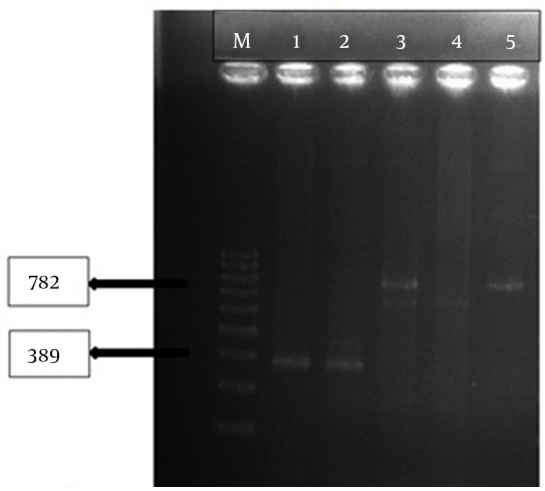

Figure 1. PCR gel image with VIM and NDM positive strain (M: Marker, 1: VIM pozitif strain, 2: VIM positive control, 3: NDM pozitif strain, 4: Negative strain, 5: NDM pozitif control).

5. Discussion

Carbapenems are among the best options for treating infections caused by multidrug-resistant Gram-negative bacteria; however, their inappropriate usage has increased resistance. Due to the increased prevalence of the MDR strains, the treatment of infections caused by *P. aeruginosa* is challenging (13, 14). The "Carbapenem Antimicrobials Pseudomonas Isolate Testing at regional Locations (CAPITAL) surveillance programme" in 2010 indicated that the overall rates of carbapenem-resistant *P. aeruginosa* ranged from 7.4 to 35.4%. Moreover, the rates of carbapenem resistance in a meta-analysis study in 2015 ranged from 8.7 to 50.4% among the *P. aeruginosa* isolates (15, 16). In this regard, the upregulation of efflux pumps, decreased outer membrane permeability, and acquired metallo-beta-lactamases (MBL) are introduced as the main factors leading to carbapenem resistance (17). The *P. aeruginosa* MBLs were detected in the early 1990s, the first representatives of which are IMP and VIM type enzymes (18, 19).

In Brazil, the SPM-1 gene, the most frequent metallo-lactamase gene, was first introduced in 2002 and then reported in different sites (20, 21). The SPM-1 gene region was detected in 33 out of 129 carbapenem-resistant *P. aeruginosa* isolates isolated from the hospitalized patients during 1998 - 2012, in four and three of which the VIM-2 and GES-3 genes were detected, respectively. Furthermore, the SPM-1 and KPC-2 links were found in the nine strains, and the SPM-1, VIM-2, and KPC-2 link was observed in one strain (22).

Carbapenem resistance genes (VIM, PER, IMP, GES, KPC, OXA) were examined in the ceftazidime-resistant *P. aeruginosa* strains (n = 195) isolated from the hospitalized patients using the PCR test. In this study, the OXA-10 (n = 5), OXA-14 (n = 4), VIM-2 (n = 4), IMP-1 (n = 2), and GES-1 (n = 26) determinants were detected; however, all isolates were negative for the PER and KPC genes (1).

Amoureux et al. isolated IMP-19 producing *P. aeruginosa* from seven patients with nosocomial infections linked to contaminated sinks in France (23). Their findings showed that the prevalence of MBL producers among imipenem-resistant *P. aeruginosa* was lower in France than the other countries, and that the VIM producers were mainly determined during the outbreaks (8, 24-26). Various MBLs, including IMP, VIM, NDM, SPM, GIM, and SIM were identified worldwide; however, NDM, VIM, and IMP genes are frequently observed in *P. aeruginosa* in India (27). The IMP and VIM-producing *Pseudomonas* isolates have been reported in different geographical regions (28).

Hakemi Vala et al. evaluated the presence of classes A, B, and D (IMP, VIM, SPM, KPC, GIM, DIM, BIC, OXA-48, CTX-M15, and NDM genes) β-lactamases among *P. aeruginosa* isolates

Table 1. Sequence of Primers in the Study

Genes	Primers Sequence	Expected Amplicon Size (bp)	References
VIM		389	(10)
VIM-F	GTT TGG TCG CAT ATC GCA AC		
VIM-R	AAT GCG CAG CAC CAG GAT AG		
NDM			
NDM-F	GCA GCT TGT CGG CCA TGC GGG C	782	(10)
NDM-R	GGT CGC GAA GCT GAG CAC CGC AT		
IMP		188	(12)
IMP-F	GGA ATA GAG TGG CTT AAT TCT C		
IMP-R	CCA AAC CAC TAC GTT ATC T		
GES		863	(11)
GES-F	ATG CGC TTC ATT CAC GCA C		
GES-R	CTA TTT GTC CGT GCT CAG GA		
SPM		271	(12)
SPM-F	AAA ATC TGG GTA CGC AAA CG		
SPM-R	ACA TTA TCC GCT GGA ACA GG		
GIM		477	(12)
GIM-F	TCG ACA CAC CTT GGT CTG AA		
GIM-R	AAC TTC CAA CTT TGC CAT GC		
SIM		570	(12)
SIM-F	TAC AAG GGA TTC GGC ATC G		
SIM-R	TAA TGG CCT GTT CCC ATG TG		

Table 2. Distribution of Specimens of *Pseudomonas aeruginosa* Isolates

Specimens	No. (%)
Tracheal aspirate culture	69 (34.5)
Sputum culture	36 (18)
Urine culture	34 (17)
Exudate culture	22 (11)
Blood culture (Aerob)	15 (7.5)
Wound culture	10 (5)
Catheter	5 (2.5)
Sterile body liquid	5 (2.5)
BOS	2 (1)
Swab	1 (0.5)
Bronchoalveolar lavage (BAL) culture	1 (0.5)

(29). They reported that the frequencies of the positivity of the CTX-M15 and IMP genes among 47 P. aeruginosa isolates were four (8.5%) and one (2.1%), respectively (29). Fallah et al. showed that the *P. aeruginosa* isolates harboured the IMP gene in their study (30).

Zafer et al. examined the prevalence of metallo-β-lactamases (MBL) in *P. aeruginosa* isolates (n = 122) collected from two different hospitals in Cairo, Egypt, and found out that the VIM-2 gene was the most prevalent MBL gene (31). In a study on the presence of the IMP-6, VIM-2, KPC, GES, NDM, and OXA-48 genes, IMP-6 and VIM-2 MBLs were identified in 17 and four of the 329 *P. aeruginosa* isolates (32).

VIM-2 is the dominant MBL gene associated with the outbreaks caused by MBL producing *P. aeruginosa* worldwide (33, 34).

Resistance in *P. aeruginosa* is an emerging problem worldwide, and carbapenem resistance is one of the critical problems caused by the spread of carbapenemases. VIM is frequently isolated from carbapenem-resistant *P. aeruginosa* isolates. In the present study, we detected one isolate harbouring VIM. Moreover, NDM was isolated from the carbapenem-resistant isolates during the last decade, and one isolate was positive for NDM in our study.

Footnotes

Authors' Contribution: Study concept and design: Y. T. C.; acquisition of data: Y. T. C. and I. B.; analysis and interpretation of data: Y. T. C. and I. B.; critical revision of the manuscript for important intellectual content: Y. T. C. and A. B.; statistical analysis: Y. T. C. and I. B.; administrative, technical, and material support: Y. T. C. and I.B.; study supervision: Y. T. C. and I. B.

Conflict of Interests: There is no conflict of interests among the authors.

Data Reproducibility: The data presented in this study are openly available in one of the repositories or will be available on request from the corresponding author by this journal representative at any time during submission or after publication. Otherwise, all consequences of possible withdrawal or future retraction will be with the corresponding author.

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