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

Yeliz Tanriverdi Cayci 1, *, Ilknur Biyik 1 and Asuman Birinci 1

1Department of Medical Microbiology, Faculty of Medicine, Ondokuz Mayis University, Samsun, Turkey

*Corresponding author: Department of Medical Microbiology, Faculty of Medicine, Ondokuz Mayis University, Samsun, Turkey. Email: yeliztanriverdi@gmail.com

**Received** 2021 August 26; **Accepted** 2022 April 27.

**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 pozitif 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   | VIM-F GTT TGG TCG CAT ATC GCA AC | 389 | (10) |
|       | 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   | IMP-F GGA ATA GAG TGG CTT AAT TCT C | 188 | (12) |
|       | IMP-R CCA AHC CAC TAC GTT ATC T |   |   |
| GES   | GES-F ATG GGC TTC ATT CAC GCA C | 863 | (10) |
|       | GES-R CTA TTT GTC GGT GCT CAG GA |   |   |
| SPM   | SPM-F AAA ATC TGG GTA CGC AAA GG | 271 | (12) |
|       | SPM-R ACA TTA TCC GCT GGA ACA GG |   |   |
| GIM   | GIM-F TGG ACA CAC CIT GGT CIG AA | 477 | (12) |
|       | GIM-R AAC TIC CAA CIT TGC CAT GC |   |   |
| SIM   | SIM-F TAC AAG GGA TIC GGC ATC G | 570 | (12) |
|       | SIM-R TAA TGG CCT GTT CCC AFG 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) |

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-lactamas (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.

Funding/Support: This study was funded by no organization.

References

1. Er H, Altindis M, Asik G, Demir C. [Molecular epidemiology of beta-lactamases in ceftazidime-resistant Pseudomonas aeruginosa isolates]. Mikrobiyol Bul. 2015;49(2):56-65. Turkish. [PubMed ID: 2667816]. https://doi.org/10.5787/mb.8901.

2. Rossi Goncalves I, Dantas RCC, Ferreira MI, Batista D, Gontijo-Filho PP, Ribas RM. Carbapenem-resistant Pseudomonas aeruginosa: association with virulence genes and biofilm formation. Braz J Microbiol. 2017;48(2):211-7. [PubMed ID: 28034598]. [PubMed Central ID: PMC5710441]. https://doi.org/10.1016/j.bjm.2016.11.004.

3. Guççu AU, Kilic A, Bedir O, Yılmaz S, Günsel M. [Investigation of metallo-beta-lactamase production in multi-drug resistant Pseudomonas aeruginosa isolates showing carbapenemase activity isolated from intensive care units]. Gülhane Tip Derg. 2013;55:276-80. Turkish.

4. Lambert PA. Mechanisms of antibiotic resistance in Pseudomonas aeruginosa. J R Soc Med. 2002;95(Suppl 4):22-6.

5. Villegas MV, Loians K, Correa A, Kattan JN, Lopez JA, Quin P, et al. First identification of Pseudomonas aeruginosa isolates producing a KPC-type carbapenem-hydrolyzing beta-lactamase. Antimicrob Agents Chemother. 2007;51(4):1553-5. [PubMed ID: 17266212]. [PubMed Central ID: PMC1855450]. https://doi.org/10.1128/AAC.0405-06.

6. El Garch F, Bogaerts P, Bebrone C, Galleni M, Glupczynski Y. OXA-198, an acquired carbapenem-hydrolyzing class D beta-lactamase from Pseudomonas aeruginosa. Antimicrob Agents Chemother. 2011;55(10):4828-33. [PubMed ID: 21788471]. [PubMed Central ID: PMC3186976]. https://doi.org/10.1128/AAC.00522-11.

7. Çağlar H, [The detection of metallo-beta-laktamaz (mbl) enzyme in gram negative non fermentative bacteria by different methods and the comparison of these methods]. Elazig, Turkey: First University; 2012.

8. Cornaglia G, Giamarello H, Rossolini GM. Metallo-beta-lactamases: a last frontier for beta-lactams? Lancet Infect Dis. 2011;11(5):381-93. [PubMed ID: 21503894]. https://doi.org/10.1016/S1473-3099(11)70056-4.

9. Jovic C, Lebsanovic Z, Suliagie V, Rackov G, Begovic J, Topisirovic I, et al. Emergence of NDM-1 metallo-beta-lactamase in Pseudomonas aeruginosa clinical isolates from Serbia. Antimicrob Agents Chemother. 2011;55(4):3929-31. [PubMed ID: 21646490]. [PubMed Central ID: PMC3147624]. https://doi.org/10.1128/AAC.00226-11.

10. Doyle D, Peirano G, Lascols C, Lloyd T, Church DL, Pitout JD. Laboratory detection of Enterobacteriaceae that produce carbapenemases. J Clin Microbiol. 2012;50(12):3877-80. [PubMed ID: 2299375]. [PubMed Central ID: PMC3503014]. https://doi.org/10.1128/JCM.02177-12.

11. Cicek AC, Saral A, Iraz M, Ceylan A, Duzgun AO, Peleg AY, et al. OXA and GES-type beta-lactamases predominate in extensively drug-resistant Acinetobacter baumannii isolates from a Turkish University Hospital. Clin Microbiol Infect. 2014;20(5):410-5. [PubMed ID: 23957892]. https://doi.org/10.1016/j.cmi.2013.09.033.

12. Aksoy MD, Cavuslu S, Turgul HM. Investigation of Metallo Beta Lactamases and OXA-class beta-lactamases in Carbapenem Resistant Acinetobacter baumannii Strains isolated from Inpatients. Balkan Med J. 2015;32(1):79-83. [PubMed ID: 25759776]. [PubMed Central ID: PMC4342442]. https://doi.org/10.5529/balkanmedj.2015.35.02.

13. Mahmoud A, Zahran W, Hindawi G, Labib A, Galal R. Prevalence of Multidrug-Resistant Pseudomonas aeruginosa in Patients with Nosocomial Infections at a University Hospital in Egypt, with Special Reference to Typing Methods. Journal of Virology & Microbiology. 2013;2013:1-11. https://doi.org/10.5171/2013.290047.

14. Mohanam I, Menon T. Coexistence of metallo-beta-lactamase-encoding genes in Pseudomonas aeruginosa. Indian J Med Res. 2017;166(Supplement 3):S46-52. [PubMed ID: 29203955]. [PubMed Central ID: PMC5735570]. https://doi.org/10.4103/ijmr.IJMR_29_16.

15. Morrow BJ, Pillar CM, Deane J, Sahn DF, Lynch AS, Flamm RK, et al. Activities of carbapenem and comparator agents against contemporary US Pseudomonas aeruginosa isolates from the CAPITAL surveillance program. Diagn Microbiol Infect Dis. 2013;75(4):412-6. [PubMed ID: 23391609]. https://doi.org/10.1016/j.diagmicrobio.2012.12.012.

16. Liu Q, Li X, Li W, Du X, He JQ, Tao C, et al. Influence of carbapenem resistance on mortality of patients with Pseudomonas aeruginosa infection: a meta-analysis. Sci Rep. 2015;5:13175. [PubMed ID: 26084747]. [PubMed Central ID: PMC479982]. https://doi.org/10.1038/srep13175.

17. Rodríguez-Martínez JM, Poirel L, Nordmann P. Molecular epidemiology and mechanisms of carbapenem resistance in Pseudomonas aeruginosa. Antimicrob Agents Chemother. 2009;53(4):4783-8. [PubMed ID: 19718025]. [PubMed Central ID: PMC2772299]. https://doi.org/10.1128/AAC.00574-09.

18. Watanabe M, Iyobe S, Inoue M, Mitsuhashi S. Transferable imipenem resistance in Pseudomonas aeruginosa. Antimicrob Agents Chemother. 1991;35(3):547-51. [PubMed ID: 1906695]. [PubMed Central ID: PMC244956]. https://doi.org/10.1128/AAC.35.3.547.

19. Giani T, Marchese A, Coppo E, Kroumova V, Rossolini GM. VIM-1-producing Pseudomonas mosselii isolates in Italy, predating known VIM-producing index strains. Antimicrob Agents Chemother. 2012;56(4):2266-7. [PubMed ID: 22390981]. [PubMed Central ID: PMC318374]. https://doi.org/10.1128/AAC.00695-11.

20. Toleman MA, Simm AM, Murphy TA, Gales AC, Biedenbach DJ, Jones RN, et al. Molecular characterization of SPM-1, a novel metallo-beta-lactamase isolated in Latin America: report from the SENTRY antimicrobial surveillance programme. J Antimicrob Chemother. 2002;50(5):673-9. [PubMed ID: 12407123]. https://doi.org/10.1093/jac/dkf210.

21. Sader HS, Castanheira M, Mendes RE, Toleman M, Walsh TR, Jones RN. Dissemination and diversity of metallo-beta-lactamasas in Latin America: report from the SENTRY Antimicrobial Surveillance Program. Int J Antimicrob Agents. 2005;25(1):57-61. [PubMed ID: 15820827]. https://doi.org/10.1016/j.ijantimicag.2004.08.013.

22. Rizek C, Fu L, Dos Santos LC, Leite G, Ramos J, Rossi F, et al. Characterization of carbapenem-resistant Pseudomonas aeruginosa clinical isolates, carrying multiple genes coding for this antibiotic resistance. Ann Clin Microbiol Antimicrob. 2014;13(4):3. [PubMed ID: 2579208]. [PubMed Central ID: PMC428277]. https://doi.org/10.1186/s12941-014-0043-3.
23. Amoureux L, Riedweg K, Chapuis A, Bador J, Siebor E, Pechinot A, et al. Nosocomial Infections with IMP-19-Producing Pseudomonas aeruginosa Linked to Contaminated Sinks, France. Emerg Infect Dis. 2017;23(2):304-7. [PubMed ID: 28098548]. [PubMed Central ID: PMC5324815]. https://doi.org/10.3201/eid2302.160649.

24. Corvec S, Poirel L, Espaze E, Giraudieu C, Druegeon H, Nordmann P. Long-term evolution of a nosocomial outbreak of Pseudomonas aeruginosa producing VIM-2 metallo-enzyme. J Hosp Infect. 2008;68(1):73-82. [PubMed ID: 18079018].

25. Fournier D, Richardot C, Muller E, Robert-Nicoud M, Llanes C, Pleziat P, et al. Complexity of resistance mechanisms to imipenem in intensive care unit strains of Pseudomonas aeruginosa. J Antimicrob Chemother. 2013;68(8):1772-80. [PubMed ID: 23587654]. https://doi.org/10.1093/jac/dkt098.

26. Ambrogi V, Cavalie L, Mantion B, Ghiglia MJ, Cointault O, Dubois D, et al. Transmission of metallo-beta-lactamase-producing Pseudomonas aeruginosa in a nephrology-transplant intensive care unit with potential link to the environment. J Hosp Infect. 2016;92(1):27-9. [PubMed ID: 26597635].

27. Shanthi M, Sekar U, Kamalanathan A, Sekar B. Detection of New Delhi metallo beta lactamase-1 (NDM-1) carbapenemase in Pseudomonas aeruginosa in a single centre in southern India. Indian J Med Res. 2014;140(4):546-50. [PubMed ID: 25488450].

28. Bahar MA, Jamali S, Samadikuchaksaraei A. Imipenem-resistant Pseudomonas aeruginosa strains carry metallo-beta-lactamase gene bla(VIM) in a level I Iranian burn hospital. Burns. 2010;36(6):826-30. [PubMed ID: 20045260]. https://doi.org/10.1016/j.burns.2009.10.011.

29. Deshpande LM, Jones RN, Woosley LN, Castanheira M. Retrospective molecular analysis of DIM-1 metallo-beta-lactamase discovered in Pseudomonas stutzeri from India in 2000. Antimicrob Agents Chemother. 2014;58(1):596-8. [PubMed ID: 24145536]. [PubMed Central ID: PMC4277442].

30. Fallah F, Borhan RS, Hashemi A. Detection of bla(IMP) and bla(VIM) metallo-beta-lactamases genes among Pseudomonas aeruginosa strains. Int J Burns Trauma. 2015;2(2):122-4. [PubMed ID: 2638331]. [PubMed Central ID: PMC4616667].

31. Zafer MM, Al-Agamy MH, Al-Mahallawy HA, Amin MA, Ashour MS. Antimicrobial resistance pattern and their beta-lactamase encoding genes among Pseudomonas aeruginosa strains isolated from cancer patients. Biomed Res Int. 2014;2014:101635. [PubMed ID: 2470747].

32. Hongo JS, Kim JO, Lee H, Bae IK, Jeong SH, Lee K. Characteristics of Metallo-beta-Lactamase-Producing Pseudomonas aeruginosa in Korea. Infect Chemother. 2015;47(1):33-40. [PubMed ID: 25844261]. [PubMed Central ID: PMC4384452]. https://doi.org/10.3947/ic.2015.47.1.33.

33. Deshpande LM, Jones RN, Woosley LN, Castanheira M. Retrospective molecular analysis of DIM-1 metallo-beta-lactamase discovered in Pseudomonas stutzeri from India in 2000. Antimicrob Agents Chemother. 2014;58(1):596-8. [PubMed ID: 24145536]. [PubMed Central ID: PMC4277442].

34. Elias J, Schoen C, Heinz G, Valenza G, Gerharz E, Gerharz H, et al. Nosocomial outbreak of VIM-2 metallo-beta-lactamase-producing Pseudomonas aeruginosa associated with retrograde urography. Clin Microbiol Infect. 2010;16(9):1494-500. [PubMed ID: 20041895]. https://doi.org/10.1111/j.1469-0691.2009.03146.x.