

Research Article

Investigation of *bla*_{IMP-1}, *bla*_{VIM-1}, *bla*_{OXA-48} and *bla*_{NDM-1} carbapenemase encoding genes among MBL-producing *Pseudomonas aeruginosa*

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Abstract

Pseudomonas aeruginosa (*P. aeruginosa*) places among major opportunistic nosocomial pathogen which has developed extensive drug resistance. Due to uncontrolled consumption of antibiotics, multidrug-resistant (MDR) *P. aeruginosa* species are increasingly isolated from various settings of hospitals globally. This research aimed to determine the genes reproducing *bla*_{OXA-48}, *bla*_{IMP-1}, *bla*_{VIM-1} and *bla*_{NDM-1} metallo beta lactamase (MBL) genes from MDR *P. aeruginosa* isolates. Herein, the isolates of 200 *P. aeruginosa* have been obtained from microbiology laboratory of burn ward. The antibiotic susceptibility profile was using Disc Diffusion Method (DDM) and in compliance with CLSI (Clinical and Laboratory Standards Institute) advice. Study of MBL-bearing strains and the existence of encoding genes was specified by means of polymerase chain reaction. In addition, pulsed field gel electrophoresis (PFGE) is performed for typing. In this study, 124 (62%) were extended spectrum β -lactamase producer and 51 (25.5%) were MBL producers. Moreover, 148 (74%) isolates were MDR-*P. aeruginosa*. Additionally, 42 (21%), 21 (10%), 10 (5%) and 2 (1%) isolates carried the *bla*_{IMP-1}, *bla*_{OXA-48}, *bla*_{VIM-1} and *bla*_{NDM-1} genes, respectively. The PFGE showed no genetic relationships among isolates. The study observed high rate of MDR *P. aeruginosa* in hospital settings, though not being outbreak, which nearly half of them carried carbapenemase enzymes. Therefore, the proper control of related infections and appropriate prescription and consumption of antibiotics is essential.

Keywords: Antibiotic resistance, Carbapenemase, Metallo-beta-lactamase, *P. aeruginosa*

INTRODUCTION

Pseudomonas aeruginosa can be defined as one of the major causes of severe infections like systemic pneumonia, burns, cystic fibrosis, skin inflammation and some infections related to respiratory tract, urinary tract, soft tissue bone and joint, gastrointestinal, blood and cornea, bacteremia, septicemia infections especially in patients with neutropenia and transplantation (Barry *et al.*, 2021; Hasset *et al.*, 2021). The species need narrow nutrition requirements and withstand in harsh environmental conditions. In addition, inherent and acquired drug resistance mechanisms which have

caused failure in their eradication is a concern (Hu *et al.*, 2021; Zheng *et al.*, 2022). Carbapenem resistance among Gram-negative species has been induced for several years being induced by producing carbapenemases such as metallo-beta-lactamase (MBL) enzymes which have the ability to resist against nearly all common β -lactams. MBLs have a wide substrate spectrum and are hydrolyzing all beta-lactam excluding the monobactams (Shamkhali and Shahriari, 2021; Tchakal-Mesbahi *et al.*, 2021). Besides, the MBL encoding genes on class 1 integron are based on a plasmid that can transmit the elements of transmissible genes such as plasmids and transposons, mostly found

among *P. aeruginosa* strains (Khademi et al., 2021; Tunyapanit et al., 2021). The *bla*_{IMP-1} MBL was firstly found in *Serratia marcescens* in 1991. In recent decade, several MBLs have been spread such as *bla*_{VIM}, *bla*_{SPM} and *bla*_{GIM} the enzymes require bivalent cations such as Zn²⁺ for their activity (Odoi et al., 2021; Olaniran et al., 2022). The important issues and menace for healthcare settings regarding MBLs include their wide range of substrate for function and their genetic location in genetic elements which promote their facilitating transmission to other pathogens (Yoon and Jeong, 2021). In this work, the major goal was to determine carbapenemase genes such as MBLs among MDR-*P. aeruginosa* clinical isolates.

MATERIAL AND METHODS

Bacterial strains

A total of 200 *P. aeruginosa* isolates were obtained from the Microbiology Laboratory of Burn Ward and identified using biochemical tests including growth in Cetrimide Agar culture (Merck, Germany), motility, oxidase and catalase tests, Indole production in SIM Agar, TSI Agar (Merck, Germany) pattern, and growth in 42° C. The isolates were kept at a temperature of -70 Celsius for further study.

Antibiotic susceptibility test

The antibiotic susceptibility profile was implemented using ampicillin, ceftazidime, tetracycline, erythromycin, ciprofloxacin and imipenem disks as advised via CLSI (Clinical and Laboratory Standards Institute) version 2018. Extended spectrum β-lactamase (ESBL) producing isolates were indicated using synergy test between co-amoxiclav and ceftazidime disks onto the Mueller Hinton agar (MHA) (Merk, Germany) medium.

Metallo-beta-lactamase detection using phenotypic method

The combine disk method including imipenem (IMP) and IMP-EDTA disks was performed using disk diffusion onto the MHA medium based on CLSI standards (Clinical and Laboratory Standards Institute, 2018) and metallo-beta-lactamase producing strains were identified according to susceptibility of halo zone difference

>5mm. for this method, firstly a bacterial suspension that is equal to the half McFarland standard is prepared and lawn onto the MHA (Merck, Germany) medium and then, the IMP and IMP-EDTA disks (Rosco, Denmark) were placed with 2 cm distance onto the medium and incubated for a period between (18 and 24) hrs, a temperature of 37 Celsius. The inhibition zone is measured to be more than 5mm difference between the disks as a positive result (MBL- producing strain). Herein, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC25923 and *E. coli* ATCC25922 were utilized as control of all tests.

PCR detection of carbapenemases and MBLs

The total genomic DNA was extracted with the use of boiling technique (Neumann et al, 1992). After that, PCR was done to detect the MBLs involving *bla*_{VIM-1}, *bla*_{IMP-1}, *bla*_{NDM-1} *bla*_{OXA-48} genes by means of primers. The thermal conditions included initial denaturation for 3mins at a temperature of 94°C, denaturation for 1 min at a temperature of 94 Celsius, annealing for 45 seconds, at 61-65°C, 72°C for 1 min and final extension at 10min in a total of 35 cycles (Table 1).

To do PCR, 1.5 μL of MgCl₂, 0.24 μL of dNTP_s, and one unit of Taq polymerase enzyme 5 Picomole of each primer and 1μL of template DNA were added to PCR master mix (Fermentase, Lithuania) in 25 μL as a final volume. To observe and identify banding sizes of products, in a 2% agarose gel prepared with 1X TBE, 100 bp DNA marker (Fermentase, Lithuania) was used.

Pulsed field gel electrophoresis (PFGE)

The isolates' molecular typing is done with the use of PFGE approach as previously described. A similarity of >90% was considered for being in a common type.

RESULTS

Antibiotic resistance profile

Among 200 *P. aeruginosa* isolates, resistance to Ceftazidime (86%), Ciprofloxacin (68%) Piperacillin-Tazobactam (36%), Imipenem (34%), Meropenem (34%), Amikacin (33%), and Gentamycin (33%) and was observed. Notably, 66 (33%) isolates were MDR which exhibited resistance to all 7 tested antibiotics (Table 2). In the phenotypic tests, 124 (62%) and 51

Table 1. MBL genes PCR information

Primer	Sequence 5' to 3'	Annealing (°C)	Product(bp)
<i>bla</i> _{IMP-1}	F: GGGTGGGGCGTTGTTCCCTA R: TCTATTCCGCCCGTGCTGTC	62	198
<i>bla</i> _{VIM}	F: CATTGTCCGTGATGGTGATGAGT R: GCGTGTCCGACGGTGATGC	61	250
<i>bla</i> _{OXA-48}	F: CGCCCGCGTCGACGTTCAAGAT R: TCGGCCAGCAGCGGATAGGACAC	65	484
<i>Bla</i> _{NDM1}	F: CGCACCTCATGTTTGAATTCGCC R: GTCGCAAAGCCCAGCTTCGC	61	1015

Table 2. Antibiotic resistance pattern of *P. aeruginosa* isolates

Disk/Resistance (N=200)	Resistance N (%)	Intermediate N (%)	Sensitive N (%)
Ceftazidime	172 (86)	9 (4.5)	19 (9.5)
Ciprofloxacin	136 (68)	4 (2)	60 (30)
Piperacillin-Tazobactam	72 (36)	3 (1.5)	125 (62.5)
Imipenem	68 (34)	2 (1)	130 (65)
Meropenem	68 (34)	3 (1.5)	129 (64.5)
Amikacin	66 (33)	4 (2)	130 (65)
Gentamycin	66 (33)	3 (1.5)	131 (65.5)

Table 3. Rate of ESBL and MBL enzymes

β -lactamase	No (%)
ESBL*	124 (62)
MBL**	51 (25.5)

*extended-spectrum β -lactamase, **metallo- β -lactamase

(25.5%) of them were ESBL and MBL producers, respectively (Table 3).

PCR results

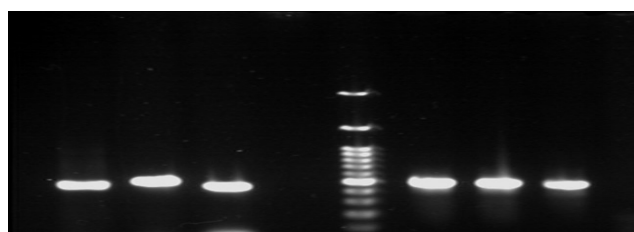
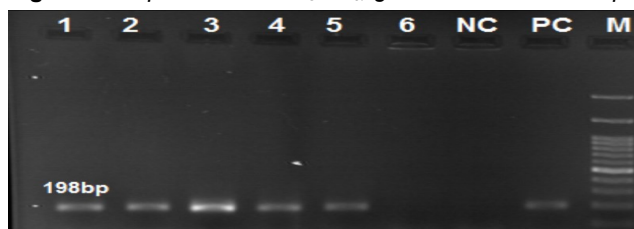
The present study observed that 42 (21%), 21 (10%), 10 (5%) and 2 (1%) isolates carried the *bla*_{IMP-1}, *bla*_{OXA-48}, *bla*_{VIM-1} and *bla*_{NDM-1} genes, respectively (Fig. 1-4). One MDR- and MBL-producing isolate carried all these genes. These isolates were obtained from a male patient with age of 72 years, with previous hospitalization and history of consumption of cephalosporins and carbapenems.

Genetic typing

The PFGE showed no genetic relation among isolates considering 90% similarity index (Fig. 5).

DISCUSSION

MBLs are critical due to resistance against carbapenems and majority of β -lactams as an effective antibiotic used against infections caused by *P. aeruginosa*. Due to high resistance rate of bacteria producing these enzymes and failure in antibiotic treatment and extreme infection such as septicemia and pneumonia, the death risk due to the presence of these strains is higher compared to other bacteria resistant to imipenem in hospital infections (Mathlouthi *et al.*, 2015; Al-Dawodeyah *et al.*, 2018). Owing to the fact that genes encoding these enzymes are located on the genetic transmittable elements like plasmids as well as the transmission of mentioned genes to other strains. Quick examination and recognition of strains resistant to carbapenem, especially imipenem in terms of epidemiology and helping therapeutic factors in selecting adequate antibiotic for successfully treating patients

**Fig. 1.** PCR products of *bla*_{OXA-48} gene with size of 484bp**Fig. 2.** PCR products of *bla*_{IMP-1} gene with size of 198bp**Fig. 3.** PCR products of *bla*_{VIM-1} gene with size of 250bp**Fig. 4.** PCR products of *bla*_{NDM} gene with size of 1015bp

(Muddassir *et al.*, 2021; Fang *et al.*, 2022). Additionally, controlling the strains resisting drugs and preventing from dissipation of such infections in health care centers is considered necessary (Eslami *et al.*, 2018; Ghasemian *et al.*, 2018). In this investigation, EDTA as an inhibitor of MBLs was employed for diagnosis through DDST (double-disk synergy test). The use of this method was simple, it was easily interpretable and without the problems of other MBL inhibitors such as toxicity of 2-Mercapto Propionic Acid and variability of

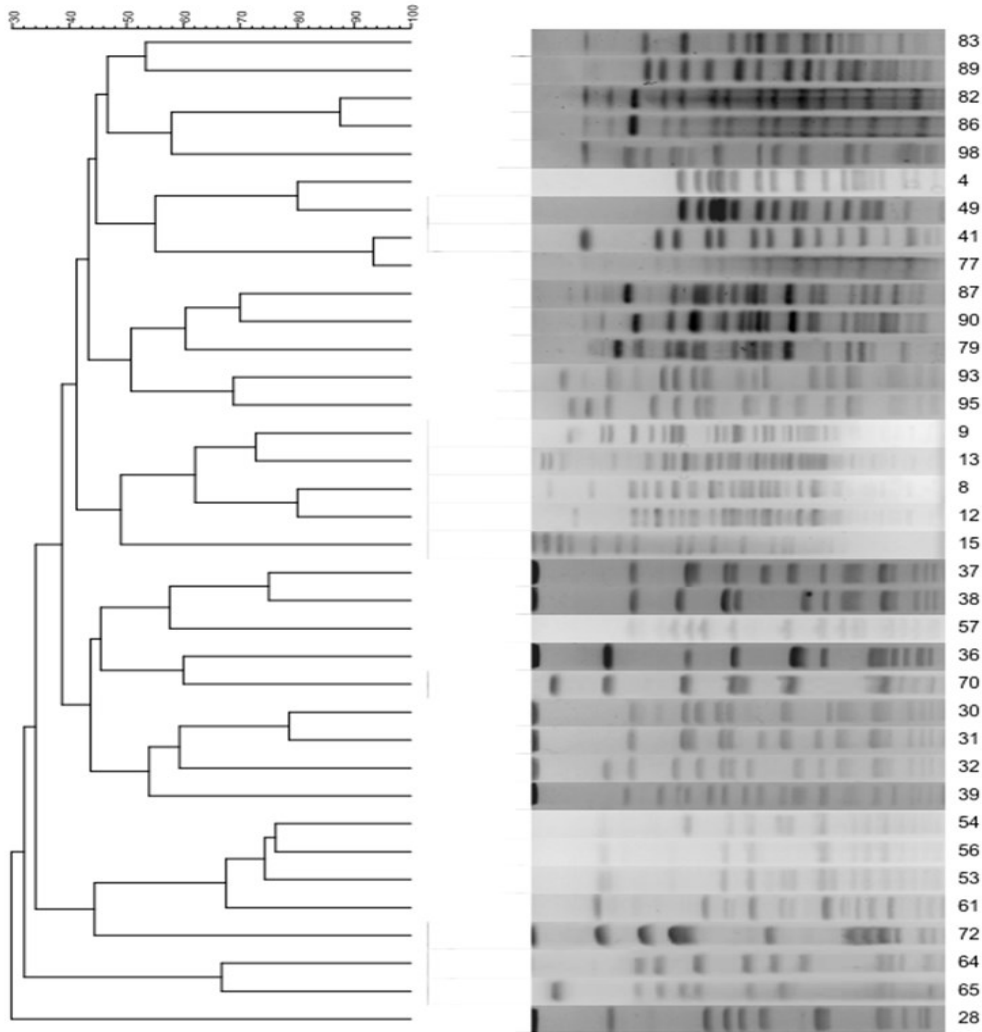


Fig. 5. PFGE pattern of carbapenemase-producing strains of *P. aeruginosa*; the UPGMA analysis using gel compare exhibited no genetic relation among the isolates

E-test. It also benefits from high sensibility (Aksoy and Tuğrul, 2020; Radhika *et al.*, 2022). Yet, concerning the probability of occurrence of false results, performing molecular tests such as PCR seems to be necessary. The present study observed that resistance to resistance to Ceftazidime (86%), Ciprofloxacin (68%) Piperacillin-Tazobactam (36%), Imipenem (34%), Meropenem (34%), Amikacin (33%), and Gentamycin (33%) and was observed. Notably, 66 (33%) isolates were MDR which exhibited resistance to all antibiotics. In the phenotypic tests, 124 (62%) and 51 (25.5%) of them were ESBL and MBL producers, respectively. The results of present study were quite similar to those documented by Mahfoud *et al.* (2015) from Syria; Al-Agamy *et al.* (2016) from Saudi Arabia; Zorgani *et al.* (2015) from Libya for imipenem and meropenem (43.9%, 40.9%), (38.2%, 52.5%), (36%, 46%) respectively while the results much less than those acknowledged by Joji *et al.*, (2019) from Bahrain were (88%, 90%); Sid Ahmed *et al.*, (2020) from Qatar were (90%,

90.2%); Ramadan *et al.*, (2018) from Egypt were (78%, 78%) and Mathlouthi *et al.*, (2015) from Libya were (87%, 79%) for imipenem and meropenem respectively. Generally, the results of these studies indicate the increasing resistance against imipenem during a decade. Concerning the similarity of mentioned studies in terms of using the disk diffusion method, the variations in results could be due to differences in resistance in various areas, sample type or the type of used disks. Different studies shown that patients suffering from infections because of the MBL-producing *P. aeruginosa* strains received various antibiotics as therapy and the death (mortality rate) due to infections by this type of bacteria were 20 % in study conducted in Iran (Vala *et al.*, 2014), 29-year-old female death case from Italy (Carugati *et al.*, 2020).

The present study show that, 42 (21%), 21 (10%), 10 (5%) and 2 (1%) isolates carried the *bla*_{IMP-1}, *bla*_{OXA-48}, *bla*_{VIM-1} and *bla*_{NDM-1} genes, respectively. One MDR- and MBL-producing isolate carried all these genes. The fre-

quency of genes were very low when compared with Salimi and Eftekhari (2014) from Iran, who found *bla*_{IMP} and *bla*_{VIM} present in 56.25% and 46.8% of *P. aeruginosa* isolates, respectively. Same thing for *bla*_{OXA-48} and *bla*_{NDM} were present in 18% and 32% respectively (Khosravi et al., 2019). *bla*_{IMP-1} and *bla*_{VIM-1} were present among *P. aeruginosa* in different frequencies as documented by Aghamiri et al., (2014) (9%, 33%); Azimi et al., (2018) (15.6%, 17.5%) and Alkhudhairy and Al-Shammari, (2020) (25%, 33.3) for *bla*_{IMP-1} and *bla*_{VIM-1} respectively. Same results were also highlighted in an Iranian study, which found the percentage of *bla*_{IMP-1} was (5.77%) and *bla*_{VIM-1} was (17.31%) (Jabalamelis et al., 2018). MBL producing *P. aeruginosa* (especially those carrying *bla*_{IMP-1}) can confer resistance to other antibiotics like aminoglycosides, fluoroquinolones and cephalosporins (Ejikeugwu et al., 2018).

Conclusion

MBL-producing *P. aeruginosa* has evolved worldwide with a high mortality rate. This study reports MDR-*P. aeruginosa* producing MBL conferring cross-resistance to cephalosporin and aminoglycosides. Therefore, the proper control of related infections and appropriate prescription and consumption of antibiotics is essential.

Conflict of interest

The authors declare that they have no conflict of interest.

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