Immunopathologia Persa
http www.immunopathol.com
DOI:10.15171/ipp.2018.26

Antibiotic resistance and presence of bla_{PER-1}, bla_{VEB-1} and bla_{PSE-1} beta-lacamases among clinical isolates of *Pseudomonas aeruginosa* from ICU settings

Abdolmajid Ghasemian[1,2], Seyyed Khalil Shokouhi Mostafavi[2], Majid Eslami[2], Mahtab Vafaei[2], Farshad Nojoomi[2*], Fatemeh Hasanvand[2]

[1]Department of Microbiology, Fasa University of Medical Sciences, Fasa, Iran
[2]Microbiology Department, Faculty of Medicine, AJA University of Medical Sciences, Tehran, Iran

***Correspondence to**
Farshad Nojoomi, Email: fnojoomi2@gmail.com

Received 14 January 2018
Accepted 10 April 2018
Published online 29 April 2018

Keywords: *Pseudomonas aeruginosa*, Intensive care unit, ESBLs, Carbapenems

Abstract

Introduction: *Pseudomonas aeruginosa* isolates are among the most common pathogens causing nosocomial infections. They are intrinsically resistant to most of antibiotics such as novel β-lactams and therefore, can develop resistance during treatment, culminating in failure in remedy.
Objectives: The aim of this study was to detect the genes encoding class A extended-spectrum beta-lactamases (ESBLs) such as *PER-1, VEB-1* and *PSE-1* among *P. aeruginosa* isolates from intensive care unit (ICU) patients.
Materials and Methods: A total of 65 isolates were collected from ICU in three hospitals of Tehran in 2016. The antibiotic susceptibility test was conducted according to Clinical and Laboratory Standards Institute (CLSI) guideline. MIC of ceftazidime was done with agar dilution method. The combine disk test was performed for detection of isolates producing ESBLs. Polymerase chain reaction (PCR) was performed to detect the *PER-1, VEB-1* and *PSE-1* genes using specific primers.
Results: Fifty-four percent (n=38) of patients were male and 46% (n=27) were female. The majority of ICU isolates were resistant to augmentin (93.8%, n=61) and cefpodoxime (84.8%, n=56). Fifty (77%) isolates were ESBL positive, among which 94% (n=47) harbored *PER-1* gene followed by 52% (n=26) *VEB-1* and 16% (n=8) *PSE-1* genes.
Conclusion: Concomitance presence of bla_{PER1} and bla_{VEB1} was observed among 10 isolates, and 7 amplified all these three genes. A high number of ICU *P. aeruginosa* isolates were ESBL producers. The frequency of bla_{VEB1} and bla_{PER1} were relatively high, while bla_{PSE1} was detected among a low number of isolates. Moreover, resistance to carbapenems was low. It is necessary to follow up ICU centers because of drug-resistant *P. aeruginosa* isolates.

Citation: Ghasemian A, Shokouhi Mostafavi SK, Eslami M, Vafaei M, Nojoomi F, Hasanvand F. Antibiotic resistance and presence of bla_{PER-1}, bla_{VEB-1} and bla_{PSE-1} beta-lacamases among clinical isolates of *Pseudomonas aeruginosa* from ICU settings. Immunopathol Persa. 2018;4(2):e26. DOI:10.15171/ipp.2018.26.

Introduction

Pseudomonas aeruginosa isolates are among the most common pathogens causing nosocomial infections. These strains are intrinsically resistant to most of drugs such as novel β-lactam antibiotics and therefore, cause failure during treatment, culminating in higher mortality (1-3). Antimicrobial resistance of *P. aeruginosa* strains and the rate of multidrug resistance are increasing and outbreaks caused by carbapenem- and multidrug-resistant isolates have been described by several surveys (4,5). The most common resistance mechanisms have been developed through down-regulation of the porin OprD, and via increased activity of multi-drug efflux pumps, primarily MexAB-OprM (6,7). The novel beta-lactamases, including AmpC beta-lactamases, extended-spectrum beta-lactamases (ESBLs) and metallo-beta-lactamases (MBLs), have emerged worldwide as a source of antimicrobial resistance in gram-negative isolates (8). The only effective β-lactam antibiotic against co-AmpC and ESBL producers are carbapenems; however, resistance to carbapenems has recently occurred increasingly with a profound rate.

Key point

Concomitance presence of blaPER1 and blaVEB1 was observed among *P. aeruginosa* isolates. A high number of ICU *P. aeruginosa* isolates were ESBL producers. The frequency of bla_{VEB1} and bla_{PER1} were relatively high, while bla_{PSE1} was detected among a low number of isolates. The frequency of carbapenem resistance was low.

However, the increasing use of these compounds has culminated in the emergence of carbapenem-resistant *P. aeruginosa* clinical isolates, limiting treatment choices. Detection of isolates producing ESBLs in the clinical laboratory is an important issue for guidance of proper therapeutic schemes and the implementation of the infection control measures. The combined or synergistic methods are phenotypic routes of ESBLs detection (9,10). In *P. aeruginosa* several classes of enzymes such as class A ESBLs have been identified including bla_{PER} (mostly from Turkey), bla_{VEB} (from South-East Asia, France and Bulgaria), $bla_{GES/IBC}$ (France, Greece and South Africa) and other less common types. These six carbapenemase types have low similarity at the genetic level, and yet they have identical hydrolysis profiles. The bla_{PER} occurs less frequently, but has clinical importance by conferring resistance to oxy-imino-beta-lactams (11,12). Unfortunately, several antibiotic resistance mechanisms often have been occurred simultaneously, thereby conferring multidrug-resistant phenotypes.

Objectives

The aim of this study was to detect the genes encoding class A ESBLs of PER-1, VEB-1 and PSE-1 among clinical isolates of *P. aeruginosa* in ICU patients.

Materials and Methods

Bacterial isolates

A total of 65 isolates of *P. aeruginosa* were collected from intensive care unit (ICU) patients in several hospitals of Tehran between the years 2015 and 2016. Tests and culture media for identification of these isolates included: catalase and oxidase tests, hydrogen sulfide (H2S), indol, motility (SIM medium), triple sugar iron agar (TSI), and methyl red (MR), Voges-Proskauer (VP), Simon citrate, urease, oxidative/fermentative (OF), Macconkey agar and cetrimide agar.

Antibiotic susceptibility test

The antibiotic susceptibility test was conducted according to the CLSI guidelines. The antibiotic disks used included: aztreonam (30 µg), augmentin (30 µg), amikacin (30 µg), piperacillin (100 µg), ofloxacin (5 µg), ceftriaxone (30 µg), cefoperazone (75 µg), cefotaxime (30 µg), cefpodoxime (10 µg), ciprofloxacin (5 µg), carbenicillin (100 µg), levofloxacin (5 µg), meropenem (10 µg), netilmicin (30 µg), piperacillin-tazobactam (110 µg), imipenem (10 µg), tobramycin (10 µg), ticarcillin (75 µg), gentamicin (120 µg), ceftazidime (30 µg) and cefepime (30 µg).

Phenotypic detection of ESBL producers

The combine disk test was performed to detect the isolates producing ESBLs. The ceftazidime and cefotaxime disks with or without clavulanic acid were used in this test. A difference of more than 5 mm between these disks along with clavulanic acid was considered as positive.

DNA extraction

One colony of each isolate was suspended in 10 mL Lauria Bertani (LB) broth and incubated an overnight at 37℃. The tubes were centrifuged at 4000 rpm for 10 minutes and the precipitate was used for extraction. DNA was isolated by a boiling method and DNA Extraction kit (DIAtom DNA Prep 100).

Polymerase chain reaction

Polymerase chain reaction (PCR) was performed to detect the *PER-1, VEB-1* and *PSE-1* encoding genes with specific primers (Table 1).

The reaction mixture for these genes was: 10XPCR buffer = 2.5 µL, MgCl2 (50 mM)= 1.5 µL, dNTP (10 Mm)= 0.75 µL, forward primer (100 µM)= 2.5 µl, reverse primer (100 µM)=2.5 µL, Taq DNA polymerase (5 U/µl)= 0.2 µL, Template (DNA)= 1 µL and nuclease-free H_2O= 14.05 µL.

Ethical issues

The research followed the tenets of the Declaration of Helsinki. This study was approved by AJA University of Medical Sciences, Tehran, Iran (Grant # 9504).

Data analysis

Data were analyzed using GraphPad Prism 6 and SPSS 20. The Student's t-test was applied for analysis, and *P* value of < 0.05 was considered as the significance of differences.

Results

Of the total 65 isolates, 46.1% (n=30) were collected from urine, followed by 15.9% (n=11) in pneumonia, 10.8% in blood and other clinical sites. Fifty-four percent (n=38) of patients were male and 46% (n=27) were female.

Antibiotic susceptibility test

The majority of the isolates were resistant to augmentin (93.8%, n=61) and cefpodoxime (84.8%, n=56). The frequency of resistance to other antibiotics were as follows: CAZ (60%, n=39), CPM (47.6%, n=31), PRL (44.6, n=29),

Table 1. The specific primers used in this study

Primer	Sequence 3' to 5'	Product size	Reference
bla*PER-*1	F: ATG AAT GTC ATT ATA AAA GCT R: TTA ATT TGG GCT TAG GG	927	1,12
bla*PSE-*1	F: AATGGCAATCAGCGCTTC R: GCGCGACTGTGATGTATA	699	9,12
bla*VEB-*1	F: CGA CTT CCA TTT CCC GAT GC R: GGA CTC TGC AAC AAA TAC GC	624	9,12

CRO (64.6%, n=42), PY (53.8%, n=35), ATM (57%, n=37), CPZ (46.1%, n=30), CTX (58.4%, n=38), TC (55.3%, n=36), IMI (18.4%, n=12), MEM (20%, n=13), CIP (43.07%, n=28), OFX (26.1%, n=17), LEV (49.2%, n=32), NET (38.4%, n=25), GEN (36.9%, n=24), TN (46.1%, n=30) and AK (41.5%, n=27).

Phenotypic detection of ESBL producers

Fifty (77%) isolates of *P. aeruginosa* were ESBL positive (Table 2). The majority of the ESBL positive isolates belonged to urine infection (61%, n=40), followed by wound (23%, n=15) and sputum (15.3%, n=10).

Detection of VEB-1, PER-1 and PSE-1 encoding genes

Fifty (77%) isolates were ESBL positive, among which 94% (n=47) harbored bla_{PER-1} gene followed by 52% (n=26) bla_{VEB-1} and 16% (n=8) bla_{PSE-1} genes (Figures 1 and 2). Concomitance presence of bla_{PER-1} and bla_{VEB1} was observed among 10 isolates, and 7 amplified all these three genes (Table 2). There was a relationship between the presence of these genes and resistance to ceftazidime, ceftriaxone, cefpodoxime and cefotaxime.

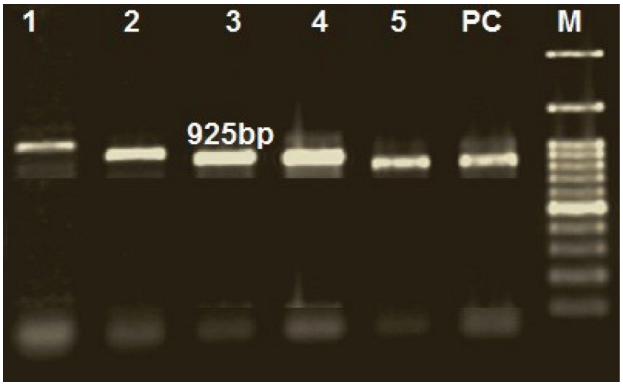

Figure 1. The bla_{PSE1} gene with 925bp product size. Columns 1-5: positive samples; M: marker; column PC: positive control

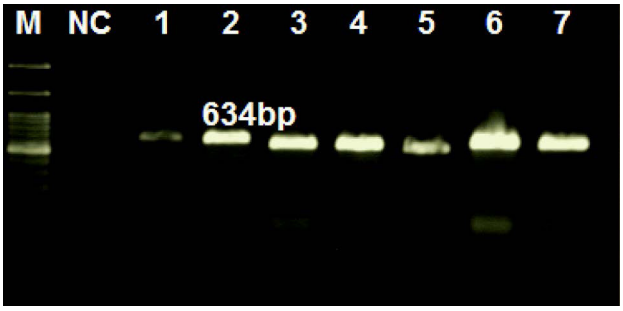

Figure 2. PCR products of bla_{VEB1} gene with 634 bp size. Columns 1-6: positive samples; column 7: positive control; M: marker; NC: negative control.

Discussion

Pseudomonas aeruginosa is a prevalent nosocomial pathogen that causes a wide spectrum of opportunistic and nosocomial infections. Isolates of *P. aeruginosa* are resistant to a variety of antibiotics with mechanisms including intrinsic capabilities and acquired (chromosome or plasmid-mediated) resistance. In the present study, most of isolates were collected from urinary tract infections. Several other previous studies confirm our results (12,13). The urine is the main route for antibiotic excretion from the body and thus, most of resistant isolates were collected from this site. In this study, the majority of the isolates were resistant to augmentin/co-amoxiclav and cefpodoxime. Moreover, most of them were resistant to third generation cephalosporins (aztreonam, ceftriaxone and cefotaxime). These antibiotics are important in the treatment of gram-negative rods, and thus the increasing resistance is a great concern. About 0.3% of the total genes in *P. aeruginosa* are responsible for antibiotic resistance (14). Approximately 77% of ICU isolates were ESBL producers which is comparable with previous studies (15,16). Susceptibility to clavulanic acid is a diagnostic method. Resistance to clavulanic acid indicates the probable presence of AmpC and other enzymes that are not inhibited by this inhibitor (17). On the other hand, de-repression of the chromosomal AmpC β-lactamases can culminate in the decreased susceptibility to β-lactam antibiotics.

Table 2. The relationships of third generation cephalosporins resistance, MIC and the concomitance presence of beta- lactamase genes among ESBL positive isolates

Isolate	Sex	MIC	Third generation cephalosporins resistance	Concomitance genes
1	M	4	CTX, CAZ, CRO, CPM	Bla_{PER-1}, bla_{VEB1}
2	M	4	CTX, CAZ, CRO	Bla_{PER-1}, bla_{VEB1}
3	F	8	CTX, CAZ, CPM	Bla_{PER-1}, bla_{VEB1}, bla_{PSE1}
4	F	8	CTX, CAZ, CRO, CPM	Bla_{PER-1}, bla_{VEB1}
5	M	8	CTX, CAZ, CRO, CPM	Bla_{PER-1}, bla_{VEB1}, bla_{PSE1}
6	F	16	CTX, CRO, CPM	Bla_{PER-1}, bla_{VEB1}, bla_{PSE1}
7	F	16	CTX, CAZ, CRO, CPM	Bla_{PER-1}, bla_{VEB1}, bla_{PSE1}
8	F	32	CTX, CRO, CPM	Bla_{PER-1}, bla_{VEB1}, bla_{PSE1}
9	M	32	CTX, CAZ, CRO, CPM	Bla_{PER-1}, bla_{VEB1}, bla_{PSE1}
10	F	32	CTX, CAZ, CRO, CPM	Bla_{PER-1}, bla_{VEB1}, bla_{PSE1}

F: female, M: male, CTX: cefotaxime, CAZ: ceftazidime, CRO: ceftriaxone, CPM: cefpodoxime.

In this study, among ESBL producers, 94% and 52% amplified bla_{PER1} and bla_{VEB1} respectively, suggesting the presence of other enzymes such as Amp-C and MBLs or possibility of efflux pumps interference. In the study by Shacheraghi et al in ESBL positive isolates, the prevalence of bla_{VEB-1} and bla_{PER-1} was 100% and 68.3%, respectively (18), but a low number (5.8%) of imipenem-resistant isolates contained bla_{PER-1} in another study. In a study by Davodian et al, among wound isolates the 90% and 88% were resistant to augmentin and cefpodoxime, 40% of them being bla_{VEB1} positive (15).

Besides this, in our study, resistance to imipenem and meropenem was low (18.4 and 20%, respectively), suggesting that the low presence of carbapenem-hydrolyzing enzymes in addition to class A enzymes.

However, continuous and prolonged antibiotic periods are likely to be a critical factor in the selection of highly antibiotic-resistant *P. aeruginosa* strains. Therefore, combination therapy (usually with a β-lactam and an aminoglycoside) is now important to eradicate infections due to *P. aeruginosa*. The broad-spectrum carbapenems are efficient drugs for the eradication of infections caused by *P. aeruginosa*; however their efficacy is increasingly compromised due to the emergence and the worldwide dissemination of carbapenem- resistant strains. Fortunately, in our study the resistance to carbapenems was low.

A high number isolates of *P. aeruginosa* from ICU were ESBL producers. The frequency of bla_{VEB1} and bla_{PER1} were relatively high, while bla_{PSE1} was detected among a low number of isolates. ICU wards are of main sources for infections with drug-resistant strains. Continuous and prolonged antibiotic periods, hospitalization and misuse are pivotal factors in the selection of highly resistant strains. Combination therapy (usually with a β-lactam and an aminoglycoside) is important to treat Pseudomonas infections.

Authors' contribution

AG, SKSM and ME performed the study. FN supervised the work. MV and FH participated in data analysis.

Conflicts of interest

There is no conflict of interest regarding the publication of this paper.

Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors.

Funding/Support

This work was financially supported by the AJA University of Medical Sciences, Tehran, Iran.

References

1. Basak S, Khodke M, Bose S, Mallick SK. Inducible AmpC Beta-Lactamase producing *Pseudomonas aeruginosa* isolated in a rural hospital of central India. J Clin Diagn Res. 2009;3:1921-7.
2. Ghasemian A, Salimian Rizi K, Rajabi Vardanjani H, Nojoomi F. Prevalence of Clinically Isolated Metallo-beta-lactamase-producing *Pseudomonas aeruginosa*, Coding Genes, and Possible Risk Factors in Iran. Iran J Pathol. 2018;13:1-9.
3. Nojoomi F, Ghasemian A. Effect of overgrowth or decrease in gut microbiota on health and disease. Arch Pediatr Infect Dis. 2016;4:e34558.
4. Kohlenberg A, Weitzel-Kage D, Van der Linden P, Sohr D, Vögeler S, Kola A, et al. Outbreak of carbapenem-resistant *Pseudomonas aeruginosa* infection in a surgical intensive care unit. J Hosp Infect. 2010;74:350-7. doi: 10.1016/j.jhin.2009.10.024.
5. Fernandes R, Amador P, Prudêncio C. β-Lactams: chemical structure, mode of action and mechanisms of resistance. Rev Med Microbiol. 2013;24:7–17.
6. Saderi H, Karimi Z, Owlia P, Bahar MA, Akhavi Rad SMB. Phenotypic detection of Metallo-beta-Lactamase producing *Pseudomonas aeruginosa* strains isolated from burned patients. Iran J Pathol. 2008;3:20-4.
7. Salehi M, Ghasemian A, Shokouhi Mostafavi SK, Nojoomi F, Ashiani D, Rajabi Vardanjani H. The epidemiology of *Candida* species isolated from urinary tract infections. Arch Clin Infect Dis. 2016;11:e37743. doi: 10.5812/archcid.37743.
8. Gupta V, Garg R, Garg S, Chander J, Attri AK. Coexistence of extended spectrum beta-lactamases, AmpC beta-lactamases and metallo-beta-lactamases in Acinetobacter baumannii from burns patients: a report from a tertiary care centre of India. Ann burns fire disast. 2013;26:189-92.
9. Tavajjohi Z, Moniri R. Detection of ESBLs and MDR in *Pseudomonas aeruginosa* in a tertiary-care teaching hospital. Iranian J Clin Infect Dis. 2011;6:18-23.
10. Hosseini-Mazinani SM, Eftekhar F, Milani M, Ghandili S. Characterization of β-Lactamases from Urinary Isolates of Escherichia coli in Tehran. Iran Biomed J. 2007;11:95-9.
11. Szabó D, Szentandrássy J, Juhász Z, Katona K, Nagy K, Rókusz L. Imported PER-1 producing *Pseudomonas aeruginosa*, PER-1 producing Acinetobacter baumanii and VIM-2-producing *Pseudomonas aeruginosa* strains in Hungary. Ann clin microbiol antimicrob. 2008;7:12. doi: 10.1186/1476-0711-7-12.
12. Spagnolo AM, Orlando P, Panatto D, Perdelli F, Cristina ML. An overview of carbapenem-resistant Klebsiella pneumoniae: epidemiology and control measures. Rev Med Microbiol. 2014;25:7-14. doi: 10.1097/MRM.0b013e328365c51e.
13. Fooladi AAI, Ch MH, Amin M, Amani J. Applications and modifications of aptamers: potential tool for medical microbiology. Rev Med Microbiol. 2016;27:107-20. doi: 10.1097/MRM.0000000000000064.
14. Yusuf E, Van Herendael B, Verbrugghe W, Ieven M, Goovaerts E, Bergs K, et al. Emergence of antimicrobial resistance to *Pseudomonas aeruginosa* in the intensive care unit: association with the duration of antibiotic exposure and mode of administration. Ann Intens Care. 2017;7:72. doi: 10.1186/s13613-017-0296-z.
15. Davodian E, Sadeghifard N, Ghasemian A, Noorbakhsh S. Molecular Detection of bla_{VEB-1} Beta-Lactamase Encoding Gene Among Extended Spectrum B-Lactamase Positive Wound Isolates of *Pseudomonas aeruginosa*. Arch Pediatr Infect Dis. 2015;3:e26362. doi: 10.5812/pedinfect.26362.
16. Davodian E, Sadeghifard N, Ghasemian A, Noorbakhsh S. Presence of bla_{PER-1} and bla_{VEB-1} beta-lactamase genes among isolates of *Pseudomonas aeruginosa* from South West of Iran. J Epidemiol Glob Health. 2016;6:211-3. doi: 10.1016/j.jegh.2016.02.002.
17. Müller A, Hächler H, Stephan R, Lehner A. Presence of AmpC beta-lactamases, CSA-1, CSA-2, CMA-1, and CMA-2 conferring an unusual resistance phenotype in cronobacter sakazakii and Cronobacter malonaticus. Microb Drug Resist. 2014;20:275-80. doi: 10.1089/mdr.2013.0188.
18. Shacheraghi F, Shakibaie MR, Noveiri H. Molecular identification of ESBL Genes bla_{GES-1}, bla_{VEB-1}, bla_{CTX-M}, bla_{OXA-1}, bla_{OXA-4}, bla_{OXA-10} and bla_{PER-1} in *Pseudomonas aeruginosa* strains isolated from burn patients by PCR, RFLP and sequencing techniques. Int J Biol life Sci. 2010;3:138-42.