Jundishapur J Microbiol. 2014 January; 7(1): e8888. DOI: 10.5812/jjm.8888
Published online 2014 January 1. **Research Article**

Antimicrobial Resistance Patterns and Prevalence of *blaPER-1* and *blaVEB-1* Genes Among ESBL-producing *Pseudomonas aeruginosa Isolates* in West of Iran

Mohammad Yousef Alikhani [1]; Zahra Karimi Tabar [1]; Fatemeh Mihani [1]; Enayat Kalantar [2]; Pegman Karami [1]; Mahnaz Sadeghi [3]; Shiva Ahdi Khosroshahi [4]; Safar Farajnia [5,*]

[1]Department of Microbiology, Faculty of Medicine, Hamadan University of Medical Sciences, Hamadan, IR Iran
[2]Department of Microbiology, School of Medicine, Alborz University of Medical Sciences, Alborz, IR Iran
[3]Tuberculosis and Lung Research Center, Tabriz University of Medical Sciences, Tabriz, IR Iran
[4]Biotechnology Research Center, Tabriz University of Medical Sciences, Tabriz, IR Iran
[5]Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, IR Iran

*Corresponding author: Safar Farajnia, Drug Applied Research Center, Tabriz University of Medical Sciences, Daneshgah Ave., Postal Code: 51656-65811, Tabriz, IR Iran. Tel: +98-9143018589, Fax: +98-4113363231, E-mail: farajnias@tbzmed.ac.ir

Received: November 15, 2012; **Revised:** January 23, 2013; **Accepted:** March 14, 2013

Background: *Pseudomonas aeruginosa* is a leading cause of nosocomial infections worldwide. Resistance of *P. aeruginosa* strains to the broad-spectrum cephalosporins may be caused by extended-spectrum β-lactamases (ESBLs).
Objectives: The aim of this study was to determine the antimicrobial resistance patterns and prevalence of PER-1 and VEB-1 type genes among ESBL producing strains of *P. aeruginosa*.
Material and Methods: A total of 106 *P. aeruginosa* isolates were collected from two university hospitals in Hamadan, Iran, during a7-month study (2009). The antimicrobial susceptibility of isolates was determined by disc diffusion method and interpreted according to the clinical and laboratory standards institute (CLSI) recommendations. Production of ESBL was determined by combined disk test and presence of PER-1 and VEB-1 type ESBL genes was identified by PCR.
Results: The resistance against broad-spectrum cephalosporins and monobactames were: cefepime (97%), cefotaxime (92.5%) ceftazidime (51%), and aztreonam (27%). Ciprofloxacin (91.5%), imipenem (84.9%) and meropenem (82.1%) were the most effective anti-pseudomonas agents in this study. The results revealed that 88.7% of the isolates were multidrug resistant, 58.25% of those were ESBL positive. Sixteen (26.6%), 9 (15%) and 3 (5%) strains among ESBL-producing strains contained *blaPER-1*, *blaVEB* and *blaPER-1-blaVEB*, respectively.
Conclusions: This study highlighted the need to establish antimicrobial resistance surveillance networks for *P. aeruginosa* to determine the appropriate empirical treatment regimens. The high prevalence of multidrug resistance and production of ESBLs in *P. aeruginosa* isolates confirms the necessity of protocols considering these issues in the hospitals.

Keywords: Pseudomonas aeruginosa; Antimicrobial Drug Resistance; Beta-lactamase

1. Background

Pseudomonas aeruginosa is an important cause of nosocomial infections, including pneumonia, burn infection, urinary tract infections, meningitis and bacteremia. The infections can be particularly develop to a severe form in immune deficient patients (1). Antibiotics have been used successfully for several decades, but resistance genes have emerged and disseminated particularly in the last few years (2).

Extended-spectrum β-lactamases (ESBLs) mediate resistance to various broad-spectrum cephalosporins, including cefotaxime, ceftriaxone, ceftazidime, and aztreonam (3). These enzymes originally collected from *Klebsiella pneumoniae* and *Escherichia coli* and recently from *P. aeruginosa* (4-7). Most of the methods for detection of ESBLs are used in bacterial species such as *Klebsiella* and *E. coli* lacking chromosomal β-lactamase activity (8, 9), However, the detection of ESBL production in *P. aeruginosa* has some difficulties, because this bacterium not only has an inducible *AmpC* enzyme but also has an efflux-mediated resistance and a higher degree of impermeability than *Enterobacteriaceae* (10, 11). The PER-1 and VEB-1 type ESBLs belong to class A of β- lactamases and is associated with high level of resistance to cephems, monobactams and ceftazidime (12, 13).

2. Objectives

The aim of this study was to determine the antibacterial resistance patterns and prevalence of *PER-1* and VEB type ESBLs among *P. aeruginosa* isolated from patients in west of Iran.

3. Materials and Methods

3.1. Bacterial Isolates

A total of 106 isolates of *P. aeruginosa* recovered from

Implication for health policy/practice/research/medical education:
This study highlights the need for an antimicrobial resistance surveillance network for *P. aeruginosa* to monitor the trends and new types of resistance mechanism emerging.

various clinical specimens (Table 1) in Beasat Teaching Hospital at the Hamadan University of Medical Sciences during a 7-month study in 2009. The identification were carried out by colonial morphology, positive oxidase test, pigment formation; growth test at 42°C on nutrient agar, Gram staining and motility test.

Table 1. Distribution of *P. seudomonas aeruginosa* by Site of Isolation

Source	Isolates, No. (%)
Burn wounds	55 (51.9)
Trashes	20 (18.9)
Urine	12 (11.3)
Blood	7 (6.6)
Feces	6 (5.7)
Sputum	5 (4.7)
CSF[a]	1 (0.9)
Total	106 (100)

[a] Abbreviation: CFS, Cerebral spinal fluid

3.2. Antibiotic Susceptibility Test

Antibiotic susceptibility of the isolates was determined by standard disk diffusion method (14). The following antibiotics were used: gentamicin (30 μg), aztreonam (30 μg), meropenem (10 μg), imipenem (10 μg), amikacin (30 μg), tobramycin (30 μg), piperacillin (30 μg), ceftazidime (30 μg), ciprofloxacin (5 μg), ofloxacin (5 μg), cefepime (30 μg) and cefotaxime (30 μg) (Himedia, India). *P. aeruginosa* ATCC 27853 was used as control. The results were interpreted according to the clinical and laboratory standards institute (CLSI) (15).

3.3. Phenotypic Detection of Beta-Lactamase

The isolates were tested for the ESBLs production by using combine disk test (CDT) as CLSI recommendations. CDT were performed on ceftazidime, cefotaxime, cefepime and aztreonam resistant strains by placing disks of ceftazidime, and cefotaxime (30 μg each) at a 20 mm distance from a disk containing ceftazidime-clavulanic acid (30/10 μg), cefotaxime-clavulanic acid (30/10μg) and cefepime-clavulanic acid (30/10 μg) (16). ESBL production was inferred when the cephalosporin inhibitory zones were expanded by the clavulanate.

3.4. PCR Amplification

PCR amplifications were done using specific primers for the β-lactamases PER-1, and VEB-1 genes, as described previously (17). PCR was performed for all ESBL-producers which their resistance to cephalosporins and phenotypic was identified by confirmatory tests. DNA was extracted by the boiling method as previously described (17). The DNA amplification program consisted of an initial denaturation (94 °C, 5 minutes) followed by 35 cycles of denaturation (94 °C, 60 seconds), annealing (50 °C for PER-1 and 55 °C for VEB-1, 60 seconds), extension (72 °C, 45 seconds) and a single final extension for 5 minutes at 72 °C. Reaction mixtures for PCR contained 1.5mM $MgCl_2$, 0.5 mM of each primer, 0.2 mM of dNTPs, 1 U of Taq polymerase, 1X PCR buffer and 2 μL of DNA. Primers PER-F (5-AATTTGGGCTTAGGGCAGAA-3') and PER-R (5'-ATGAATGTCATTATAAAAGC-3') were used for blaPER-1; and VEB-F (5'-CGACTTCCATTTCCCGATGC-3') and VEB-R (5'-GGACTCTGCAACAAAT AC GC-3') were used for blaVEB-1 amplification.

4. Results

4.1. Antimicrobial Susceptibility Test

Table 2 shows the antimicrobial susceptibility pattern of *P. aeruginosa* strains. Ciprofloxacin (91.5%), imipenem

Table 2. Antimicrobial Susceptibility Pattern of *P. aeruginosa* Strains

Antimicrobials	Resistance, No. (%)	Sensitive, No. (%)	Intermediate, No. (%)
Amikacin	32 (30.2)	70 (66)	4 (3.8)
Ceftazidime	54 (51)	40 (37.7)	12(11.3)
Aztreonam	29 (27.4)	70 (66)	7 (6.6)
Cefepime	103 (97.2)	1 (0.9)	2 (1.9)
cefotaxime	53 (50)	10 (9.4)	43 (40.6)
Ciprofloxacin	5 (4.7)	97 (91.5)	4 (3.8)
Gentamicin	39 (36.8)	55 (51.9)	12 (11.3)
Imipenem	8 (7.5)	90 (84.9)	8 (7.5)
Meropenem	14 (13.2)	87 (82.1)	5 (4.7)
Ofloxacin	31 (29.2)	72 (67.9)	3 (2.8)
Piperacillin	99 (93.4)	7 (6.6)	0
Tobramycin	39 (36.8)	67 (63.2)	0

(84.9%) and meropenem (82.1%) were the most active antimicrobial agents followed by ofloxacin (67.9% susceptibility). Aztreonam, a monobactam, and amikacin showed antibiotic activity against 66% of the strains. Susceptibility to the cephalosporins was reduced to 37.7% for ceftazidime, followed by cefotaxime (9.4%) and Cefepime (0.9%).

ESBL Production: Among 106 isolates, 94 (88.7%) were multidrug resistant and 60(58.3%) were putative ESBL producers using phenotypic confirmatory tests (Table 3). Sixteen (26.6%), 9 (15%) and 3 (5%) strains among 60 ESBL-producing strains had *blaPER-1* (Figure 1), *blaVEB-1* (Figure 2), and *blaPER-1- blaVEB* related genes, respectively (Table 4).

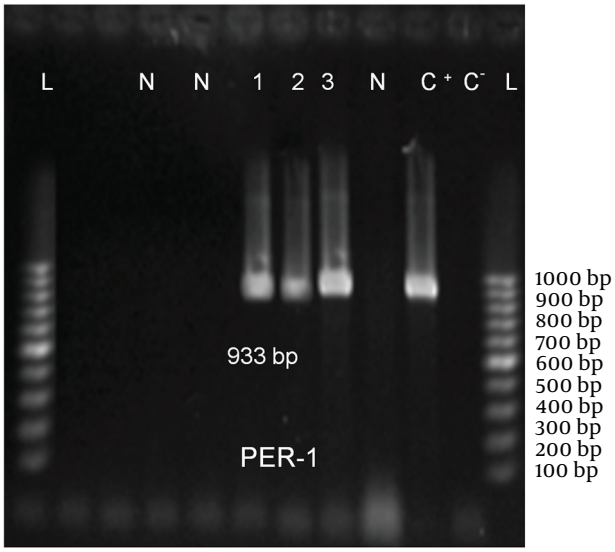
Table 3. Susceptibility Pattern to Antimicrobial Agents in ESBLs and non ESBLs-producing *P. aeruginosa* Strains

	ESBL Positive (n=60)			ESBL Negative (n=46)		
Antimicrobials	**Resistant, No. (%)**	**Susceptible, No. (%)**	**Intermediate, No. (%)**	**Resistant, No. (%)**	**Susceptible, No. (%)**	**Intermediate, No. (%)**
Amikacin	29 (48.3)	30 (50)	1 (1.7)	4 (9.3)	37 (86)	2 (4.7)
Ceftazidime	40 (66.7)	18 (30)	2 (3.3)	13 (28.3)	24 (55.2)	6 (13)
Aztreonam	25 (41.7)	32 (53.3)	3 (5)	3 (6.97)	39 (90.69)	1 (2.32)
Cefepime	58 (96.7)	0	2 (3.3)	42 (97.67)	1 (2.32)	0
cefotaxime	41 (68.3)	6 (10)	13 (21.7)	12 (27.90)	3 (6.97)	28 (65.11)
Ciprofloxacin	5 (8.3)	50 (83.3)	5 (8.3)	0	42 (97.67)	1 (2.32)
Gentamicin	32 (53.3)	28 (46.7)	0	7 (16.27)	36 (83.72)	0
Imipenem	7 (11.7)	45 (75)	8 (13.3)	1 (2.32)	41 (95.34)	1 (2.32)
Meropenem	13 (21.7)	44 (73.3)	3 (5)	2 (4.65)	39 (90.69)	2 (4.65)
Ofloxacin	30 (50)	29 (48.3)	1 (1.7)	4 (9.30)	37 (86.04)	2 (4.65)
Piperacillin	54 (90)	6 (10)	0	41 (95.34)	2 (4.65)	0
Tobramycin	31 (51.6)	28 (46.7)	1 (1.7)	4 (9.30)	39 (90.69)	0

Table 4. Association of Antimicrobial Resistance Pattern and ESBL Genotypes

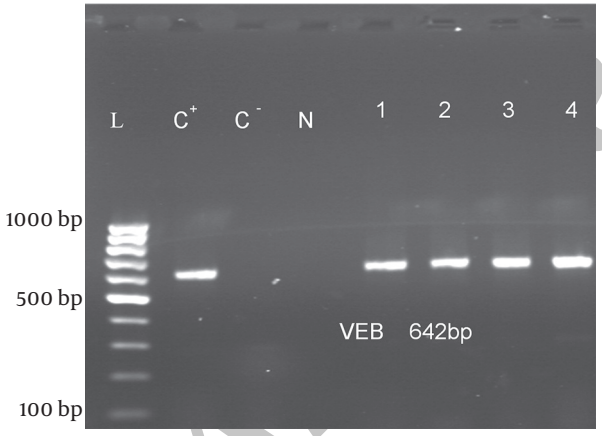
	PER-1:16 (26.6%)			VEB: 9 (15%)		
Antimicrobials	**Resistant, No. (%)**	**Susceptible, No. (%)**	**Intermediate, No. (%)**	**Resistant, No. (%)**	**Susceptible, No. (%)**	**Intermediate, No. (%)**
Amikacin	10 (62.5)	5 (31.25)	1(6.25)	5 (55.5)	2 (22.2)	2 (22.2)
Ceftazidime	12 (75)	4 (25)	0	9 (100)	0	0
Aztreonam	10 (62.5)	4 (25)	2 (12.5)	9 (100)	0	0
Cefepime	16 (100)	0	0	9 (100)	0	0
cefotaxime	87.5 (14)	0	2 (12.5)	9 (100)	0	0
Ciprofloxacin	2 (12.5)	13 (81.25)	1 (6.25)	2 (22.2)	6 (66.6)	1 (11.11)
Gentamicin	10(62.5)	6 (37.5)	0	6 (66.6)	3 (33.3)	0
Imipenem	5 (31.25)	5 (31.25)	6 (37.5)	1 (11.11)	6 (66.6)	2 (22.2)
Meropenem	7 (43.75)	8 (50)	1 (6.25)	6 (66.6)	2 (22.2)	1 (11.11)
Ofloxacin	11 (68.8)	5 (31.25)	0	8 (88.8)	1 (11.11)	0
Piperacillin	16 (100)	0	0	9 (100)	0	0
Tobramycin	10 (62.5)	6 (37.5)	0	6 (66.6)	3 (33.3)	0

Figure 1. PCR Amplification of *blaPER-1* Gene



L: 100 bp DNA ladder, C^-: negative control, C^+: positive control, N: PER-1 negative strains, 1-3: PCR products from PER-1positive strains.

Figure 2. PCR Amplification of *blaVEB-1* Gene



L: 100 bp DNA ladder, C^-: negative control, C^+: positive control, N: negative strain, 1-4: PCR products fromVEB-1 positive strains.

5. Discussion

P. aeruginosa has a high resistance to antibiotics and is a common cause of morbidity and mortality in hospitalized and immunocompromised patients (18). Treatment of *P. aeruginosa* infections is complicated by the inhered and acquired resistance to the most of commonly used antimicrobial agents (19). The results from this study showed the high resistance of *P. aeruginosa* to most of used antimicrobial agents. It was also demonstrated that the prevalence of antibiotic resistance of the isolates was very high in comparison to other studies and most of *P. aeruginosa* isolates (88.7%) were multi-drug resistant (resistant to ≥ 3 different antibiotic classes) (20-25).

The prevalence of ESBL- producing *P. aeruginosa* isolates in this study was also higher than other investigations (5, 20, 26, 27). Among 60 ESBL-positive strains, 16 (26.6%), 9 (15%) and 3 (5%) contained *PER-1*, *VEB-1* and *PER-1-VEB-1* genes, respectively. These results indicated that the prevalence of *VEB-1* gene in our area, is higher than Turkey and Korea, but the prevalence of *VEB-1* and *PER-1* genes, is lower than in Thailand (94.44% *blaVEB-1*) and Italy (34.61% *blaPER-1*) (5, 13, 26). Data on the prevalence of ESBL- producing *P. aeruginosa* strains in our area is limited. In the study performed by Shahcheraghi and colleges on *P. aeruginosa* isolates in Tehran, the rate of *blaVEB* and *blaPER* ESBLs were reported 24% and 17%, respectively, that was similar to our results (28). The high prevalence of *PER-1* and *VEB-1* indicated the high resistance to penicillins, ceftazidime and cefotaxime, as reported by other studies (26, 29).

This is the first report about the presence of these enzymes in *P. aeruginosa* isolates from west of Iran. It has shown that ESBL production in strains of *P. aeruginosa* can greatly complicate the clinical management of infection if advanced care is not taken. However, further studies are needed to determine other ESBL- types in *P.aeruginosa* strains in this area and their role in resistance to other antibiotic classes. The results of this study emphasizes on the need for a surveillance network to monitor the trends and emerge of new resistance mechanism in *P. aeruginosa* from different geographic regions. Therefore, the improvement in antibiotic prescription policies and infection control programs are of high necessity to prevent the spread of such resistant infectious agents.

Acknowledgements

No Acknowledgments.

Authors' Contribution

All authors contributed in the research equally.

Financial disclosure

There is not any financial interest.

Funding/Support

This work was financially supported by Hamadan and Tabriz University of Medical Sciences.

References

1. Pagani L, Mantengoli E, Migliavacca R, Nucleo E, Pollini S, Spalla M, et al. Multifocal detection of multidrug-resistant Pseudomonas aeruginosa producing the PER-1 extended-spectrum beta-lactamase in Northern Italy. *J Clin Microbiol.* 2004;**42**(6):2523–9.
2. Isturiz RE, Carbon C. Antibiotic use in developing countries. *Infect Control Hosp Epidemiol.* 2000;**21**(6):394–7.
3. Livermore DM, Brown DF. Detection of beta-lactamase-mediated resistance. *J Antimicrob Chemother.* 2001;**48 Suppl 1**:59–64.
4. Ben-Mahrez Kamel, Rejiba Samia, Belhadj Cherifa, Belhadj Omrane. β-lactamase-mediated resistance to extended spectrum

cephalosporins among clinical isolates of Pseudomonas aeruginosa. *Res Microbiol.* 1999;**150**(6):403–406.
5. Lee S, Park YJ, Kim M, Lee HK, Han K, Kang CS, et al. Prevalence of Ambler class A and D beta-lactamases among clinical isolates of Pseudomonas aeruginosa in Korea. *J Antimicrob Chemother.* 2005;**56**(1):122–7.
6. Poirel L, Lebessi E, Castro M, Fevre C, Foustoukou M, Nordmann P. Nosocomial outbreak of extended-spectrum beta-lactamase SHV-5-producing isolates of Pseudomonas aeruginosa in Athens, Greece. *Antimicrob Agents Chemother.* 2004;**48**(6):2277–9.
7. Weldhagen GF. Rapid detection and sequence-specific differentiation of extended-spectrum beta-lactamase GES-2 from Pseudomonas aeruginosa by use of a real-time PCR assay. *Antimicrob Agents Chemother.* 2004;**48**(10):4059–62.
8. Essack SY. Laboratory detection of extended-spectrum β-lactamases (ESBLs)—The need for a reliable, reproducible method. *Diag Microbiol Infect Dis.* 2000;**37**(4):293–295.
9. Jacoby GA, Han P. Detection of extended-spectrum beta-lactamases in clinical isolates of Klebsiella pneumoniae and Escherichia coli. *J Clin Microbiol.* 1996;**34**(4):908–11.
10. Poirel L, Menuteau O, Agoli N, Cattoen C, Nordmann P. Outbreak of extended-spectrum beta-lactamase VEB-1-producing isolates of Acinetobacter baumannii in a French hospital. *J Clin Microbiol.* 2003;**41**(8):3542–7.
11. Wolska MK, Bukowski K, Jakubczak A. [Occurrence of beta-lactamase type ESBL and IBL in Pseudomonas aeruginosa rods]. *Med Dosw Mikrobiol.* 2001;**53**(1):45–51.
12. Aubert D, Poirel L, Chevalier J, Leotard S, Pages JM, Nordmann P. Oxacillinase-mediated resistance to cefepime and susceptibility to ceftazidime in Pseudomonas aeruginosa. *Antimicrob Agents Chemother.* 2001;**45**(6):1615–20.
13. Endimiani A, Luzzaro F, Pini B, Amicosante G, Rossolini GM, Toniolo AQ. Pseudomonas aeruginosa bloodstream infections: risk factors and treatment outcome related to expression of the PER-1 extended-spectrum beta-lactamase. *BMC Infect Dis.* 2006;**6**:52.
14. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol.* 1966;**45**(4):493–6.
15. Wayne PA. Performance standards for antimicrobial susceptibility testing. *Ninth informational supplement NCCLS document M100-S9. National Committee for Clinical Laboratory Standards.* 2008.
16. Yousefi S, Farajnia S, Nahaei MR, Akhi MT, Ghotaslou R, Soroush MH, et al. Detection of metallo-beta-lactamase-encoding genes among clinical isolates of Pseudomonas aeruginosa in northwest of Iran. *Diagn Microbiol Infect Dis.* 2010;**68**(3):322–5.
17. Sohrabi N, Farajnia S, Akhi MT, Nahaei MR, Naghili B, Peymani A, et al. Prevalence of OXA-type beta-lactamases among Acinetobacter baumannii isolates from Northwest of Iran. *Microb Drug Resist.* 2012;**18**(4):385–9.
18. Todar Kenneth. *Todar's online textbook of bacteriology.*: University of Wisconsin-Madison Department of Bacteriology; 2006.
19. Hauser AR, Sriram P. Severe Pseudomonas aeruginosa infections. Tackling the conundrum of drug resistance. *Postgrad Med.* 2005;**117**(1):41–8.
20. Chayakulkeeree M, Junsriwong P, Keerasuntonpong A, Tribuddharat C, Thamlikitkul V. Epidemiology of extended-spectrum beta-lactamase producing gram-negative bacilli at Siriraj Hospital, Thailand, 2003. *Southeast Asian J Trop Med Public Health.* 2005;**36**(6):1503–9.
21. Kaushik Robin, Kumar Sandeep, Sharma Rajeev, Lal Pawanindra. Bacteriology of burn wounds — the first three years in a new burn unit at the Medical College Chandigarh. *Burns.* 2001;**27**(6):595–597.
22. Mirsalehian A, Feizabadi M, Nakhjavani FA, Jabalameli F, Goli H, Kalantari N. Detection of VEB-1, OXA-10 and PER-1 genotypes in extended-spectrum beta-lactamase-producing Pseudomonas aeruginosa strains isolated from burn patients. *Burns.* 2010;**36**(1):70–4.
23. Nikbin VS, Abdi-Ali A, Feizabadi MM, Gharavi S. Pulsed field gel electrophoresis & plasmid profile of Pseudomonas aeruginosa at two hospitals in Tehran, Iran. *Indian J Med Res.* 2007;**126**(2):146–51.
24. Ozumba UC, Jiburum BC. Bacteriology of burn wounds in Enugu, Nigeria. *Burns.* 2000;**26**(2):178–180.
25. Song Wonkeun, Lee Kyu Man, Kang Hee Jung, Shin Dong Hoon, Kim Dong Kun. Microbiologic aspects of predominant bacteria isolated from the burn patients in Korea. *Burns.* 2001;**27**(2):136–139.
26. Celenza G, Pellegrini C, Caccamo M, Segatore B, Amicosante G, Perilli M. Spread of bla(CTX-M-type) and bla(PER-2) beta-lactamase genes in clinical isolates from Bolivian hospitals. *J Antimicrob Chemother.* 2006;**57**(5):975–8.
27. Chanawong A, M'Zali FH, Heritage J, Lulitanond A, Hawkey PM. SHV-12, SHV-5, SHV-2a and VEB-1 extended-spectrum beta-lactamases in Gram-negative bacteria isolated in a university hospital in Thailand. *J Antimicrob Chemother.* 2001;**48**(6):839–52.
28. Shahcheraghi F, Nikbin VS, Feizabadi MM. Prevalence of ESBLs genes among multidrug-resistant isolates of Pseudomonas aeruginosa isolated from patients in Tehran. *Microb Drug Resist.* 2009;**15**(1):37–9.
29. Tsakris A, Vatopoulos AC, Tzouvelekis LS, Legakis NJ. Diversity of resistance phenotypes and plasmid analysis in multi-resistant 0:12 Pseudomonas aeruginosa. *Eur J Epidemiol.* 1992;**8**(6):865–70.