ORIGINAL ARTICLE

Oman Medical Journal [2018], Vol. 33, No. 1: 37-42

Investigation of Class I, II, and III Integrons Among *Acinetobacter Baumannii* Isolates from Hospitalized Patients in Isfahan, Iran

Mehrdad Halaji, Aliakbar Rezaei, Mehrdad Zalipoor and Jamshid Faghri*

Department of Microbiology, School of medicine, Isfahan University of Medical Sciences, Isfahan, Iran

ARTICLE INFO

Article history:
Received: 10 January 2017
Accepted: 9 October 2017

Online:
DOI 10.5001/omj.2018.07

Keywords:
Acinetobacter baumannii; Drug Resistance; Integrons.

ABSTRACT

Objectives: This study aimed to determine the prevalence of class I, II, and III integrons among clinical *Acinetobacter baumannii* isolates collected from hospitalized patients. ***Methods***: This cross-sectional study was conducted at two teaching hospitals in Isfahan, Iran, from October 2015 to October 2016. A total of 147 non-duplicate *A. baumannii* isolates were collected from clinical specimens and identified as *A. baumannii* using standard microbiological methods and confirmed by genotyping. Antimicrobial susceptibility was determined using disc diffusion method, and the presence of integron genes was performed using the polymerase chain reaction. ***Results:*** Out of 147 confirmed *A. baumannii* isolates, 97.3% of isolates were extensive drug-resistant (XDR) and 2.7% were multidrug-resistant (MDR). Class I and II integrons were detected in 63.9% and 78.2% of the *A. baumannii*, respectively. Class III integron was not detected in any of the isolates. ***Conclusion:*** Our results show a high prevalence of classes I and II integrons which may play a key role in the acquisition of MDR and XDR phenotype among *A. baumannii* isolates in our region. Therefore, use of appropriate infection control in clinical settings and implementation of treatment strategies is necessary for our hospitals.

Acinetobacter is a gram-negative, non-motile, obligate aerobic, oxidase-negative, and non-fermenting rod belonging to the Moraxellaceae family. This bacterium is an important opportunist, responsible for nosocomial infections, especially in intensive care units (ICUs), which can produce various types of infection such as ventilator-associated pneumonia, bacteremia, surgical-site infections, secondary meningitis, and urinary tract infections.[1,2] Acinetobacter spp. are predominantly found in soil, water, food, animals, and humans, while its existence on surfaces of healthcare environments has been difficult to control.[3] The recent emergence of multidrug-resistant (MDR) *Acinetobacter baumannii* has become a serious problem in clinical settings and these MDR strains are spreading rapidly among hospitalized patients.[4] Several exchangeable genetic elements such as plasmids, transposons, and integrons are the most important genetic elements responsible for the transmission of antibiotic resistance genes in different gram-negative bacteria.[5] Various studies showed that integrons are significantly in relation to the presence of MDR in *A. baumannii* isolates.[3,4] These elements play a major role in the dissemination and rearrangement of resistance determinants called mobile genetic cassettes.[6] So far, several classes of integrons have been described in gram-negative bacteria.[7] All integrons have a 5'conserved segment, consist of the integrase gene, and the cassette integration site (attI), but have a distinct 3'conserved segment.[8] The most prevalent integrons belong to class Iand play an important role in the development of antimicrobial resistance and the emergence of MDR profiles in gram-negative bacteria. As for the class I integrons, the 3'conserved sequence area (3'CS) includes three open reading frames: qaEΔ1 gene which confers resistance to antiseptic compounds, a sul1 gene which confers resistance to sulfonamides and ORF5, of unknown function.[3,9] Integrons of class II are commonly found associated with the Tn7 transposon family and its 3'conserved segment containing five tns genes, which are responsible for the mobility of transposons. Class II integrons have been described most often in isolates within

*Corresponding author: faghri@med.mui.ac.ir

the Enterobacteriaceae family. Integrons of class III have also been reported but its 3'conserved segment has not been characterized.[9,10] The prevalence of different classes of integrons and their relationship with antibiotic resistance in clinical isolates of *A. baumannii* is not clear in Iran and different parts of the world. Due to the emergence of a high prevalence of multiple and extensive drug resistance (XDR) *A. baumannii* isolates in our region, the objectives of this study were to investigate the prevalence of class I–III integrons among *A. baumannii* isolates collected from hospitalized patients.

METHODS

We performed a cross-sectional study from October 2015 to October 2016 at two teaching hospitals affiliated to Isfahan University of Medical Sciences, Isfahan, Iran. The non-duplicated *A. baumannii* isolates were obtained from various clinical specimens such as blood, wounds, and urine, and were transported to the microbiology laboratory for further analysis. The specimens were cultured on blood agar and MacConkey's agar (Merck, Germany) and incubated overnight at 37 °C. Bacterial isolates were identified as *A. baumannii* using standard microbiological methods, including Gram staining, oxidative or fermentative metabolism, catalase, oxidase, motility, and production of acid from different sugars and genotypic method (the presence of the *bla*OXA-51 gene).[11] The confirmed isolates were stored at -80 °C in brain heart infusion broth containing 20% glycerol.

The antibiotic susceptibility pattern was determined based on disk diffusion method on Mueller–Hinton agar (Himedia, India) according to the Clinical and Laboratory Standards Institute (CLSI) recommendation for imipenem (10 µg), meropenem (10 µg), ertapenem (10 µg), cefepime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), piperacillin-tazobactam (100/10 µg), gentamicin (10 µg), amikacin (30 µg), ciprofloxacin (5 µg), co-trimoxazole (25 µg), and tetracycline (30 µg) disks (MAST, Merseyside, UK).

The CLSI interpretive criteria for *Pseudomonas aeruginosa* was applied to determine the susceptibility to polymyxin B and colistin. *P. aeruginosa* ATCC 27853 were used as standard quality controls.[12] MDR and XDR were estimated according to previously described definitions.[13]

A phenol-chloroform method was used to extract genomic DNA as described previously.[14] The extracted DNA were dissolved in 100 µl sterile distilled water and stored at -20 °C. The polymerase chain reaction (PCR) was performed to detect *bla*OXA-51 and the presence of class I, II, and III integrase genes using the specific primers.[15]

The conditions for PCR amplification were: initial denaturation at 94 °C for 5 minutes, followed by 35 cycles of denaturation at 94 °C for 45 seconds, primer annealing at 54 °C for *intI1* and *intI2* and 52 °C for *intI3* and extension at 72 °C for 45 seconds, and a final extension at 72 °C for 7 minutes.

Amplification products were analyzed using 1.5% agarose gel with KBC power load dye (CinnaGen Co. Iran). Positive results were confirmed by direct sequencing of the PCR products.

Statistical analysis was performed using SPSS Statistics (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.). The results are presented using descriptive statistics in terms of relative frequency. Fisher's exact test was used to determine any statistical association. Statistical significance was regarded as p-values < 0.050.

RESULTS

A total of 147 confirmed *A. baumannii* isolates were collected from different clinical samples of studied

Table 1: Distribution of integron-positive *Acinetobacter baumannii* isolates based on clinical specimens and different wards.

Isolates	IF 1 positive (n = 94), n (%)	IF 2 positive (n = 115), n (%)
Wards		
ICU	58 (61.7)	79 (68.7)
Emergency	12 (12.8)	9 (7.8)
Surgery	13 (13.8)	14 (12.2)
Internal medicine	11 (11.7)	13 (11.3)
Clinical sample		
Trachea	48 (51.1)	64 (55.7)
Urine	4 (4.3)	5 (4.3)
CSF	10 (10.6)	9 (7.8)
Wound	11 (11.7)	13 (11.3)
Sputum	13 (13.8)	14 (12.2)
Blood	3 (3.2)	4 (3.5)
Others	5 (5.3)	6 (5.2)

ICU: intensive care unit; CSF: cerebrospinal fluid.

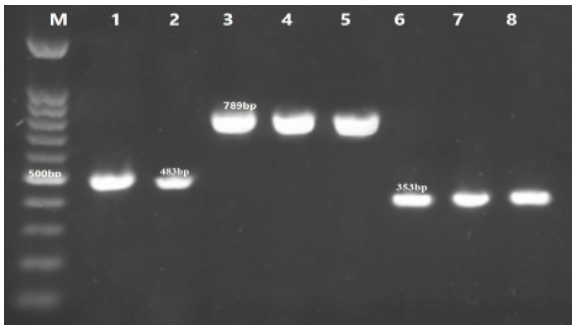

Figure 1: Electrophoresis of the polymerase chain reaction amplification products of *intI1, intI2,* and *oxa51* gene. Lane M: DNA Marker; 1–3: *intI1* gene; 4,5: *intI2* gene; 6–8: *bla*OXA-51 gene.

hospitals. Overall, 95 (62.5%) *A. baumannii* isolates were obtained from male and 57 (37.5%) from female samples. Table 1 shows the distribution of integron-positive isolates based on clinical specimens and different wards. Accordingly, most of the integron-positive isolates were obtained from the trachea followed by sputum. Of the 147 isolates, 97.3% (143/147) of *A. baumannii* isolates were identified as XDR and 2.7% (4/147) of isolates were MDR.

PCR amplification of the three classes of integron genes revealed that 94 isolates (63.9%) carried class I integrons, 115 isolates (78.2%) harbored class II integrons, and 73 isolates (49.6%) had both classes of integron genes. Class III integron was not found in any isolates [Figure 1]. The results also showed that all integron-positive isolates were resistant to imipenem, meropenem, and ceftriaxone and the most effective antibiotics against integron-positive isolates were colistin.

The antimicrobial resistance patterns of integron-positive and integron-negative isolates are shown in Tables 2 and 3. We observed a significant correlation between the presence of class I and class II integron with higher rate of resistance related to some antibiotics such as imipenem, meropenem, and ceftazidime (p-value < 0.001).

DISCUSSION

The spread of clinical multidrug resistance *A. baumannii* isolates with high resistance to different classes of antibiotics has become a serious problem.[16] Resistance to antimicrobial agents is often associated with the horizontal transmission of antimicrobial resistance genes via mobile elements, such as plasmids and transposons.[17] Therefore, the emergence of the antimicrobial resistance genes through integrons in MDR *A. baumannii* isolates has become a major concern in the treatment of infections caused by these bacteria.

In recent years, this is the first study addressing the emergence of XDR *A. baumannii* isolates based on a standard definition for these isolates in our region, and there are few studies on the frequency of XDR and MDR isolates. Our finding revealed a high rate of XDR *A. baumannii* isolates (97.3%; 143/147),

Table 2: Antibiotic susceptibility pattern of class I integron-positive and integron-negative of *Acinetobacter baumannii* strains.

Class	Antibiotics	Integron-positive 1 n = 94		Integron-negative n = 53		*p*-value
		Resistant n (%)	Sensitive n (%)	Resistant n (%)	Sensitive n (%)	
Carbapenems	Imipenem	94 (100)	-	52 (98.1)	1 (1.8)	< 0.001
	Meropenem	94 (100)	-	51 (96.2)	2 (3.7)	< 0.001
	Ertapenem	94 (100)	-	53 (100)	-	1.000
Cephalosporins	Cefepime	93 (98.9)	1 (1.0)	53 (100)	-	1.000
	Ceftriaxone	94 (100)	-	53 (100)	-	1.000
	Ceftazidime	90 (95.7)	4 (4.2)	52 (98.1)	1 (1.8)	< 0.001
β-lactam/β-lactamase inhibitor	Piperacillin/ tazobactam	92 (97.8)	2 (2.1)	53 (100)	-	< 0.001
Tetracyclines	Tetracycline	66 (70.2)	28 (29.7)	50 (94.3)	3 (5.7)	0.001
Fluoroquinolones	Ciprofloxacin	94 (100)	-	52 (98.1)	1 (1.8)	< 0.001
Aminoglycosides	Amikacin	85 (90.4)	9 (9.5)	47 (88.6)	6 (11.3)	< 0.001
	Gentamycin	89 (94.6)	5 (5.3)	50 (94.3)	3 (5.7)	1.000
Sulfonamide	Co-trimoxazole	92 (97.8)	2 (2.1)	52 (98.1)	1 (1.8)	1.000
Polymyxins	Colistin	-	94 (100)	-	53 (100)	1.000

Table 3: Antibiotic susceptibility pattern of class II integron-positive and integron-negative of *Acinetobacter baumannii* strains.

Class	Antibiotics	Integron-positive 2 n = 115		Integron-negative n = 32		*p*-value
		Resistant n (%)	Sensitive n (%)	Resistant n (%)	Sensitive n (%)	
Carbapenems	Imipenem	115 (100)	-	31 (96.8)	1 (3.1)	< 0.001
	Meropenem	115 (100)	-	31 (96.8)	1 (3.1)	< 0.001
	Ertapenem	114 (99.1)	1 (0.8)	32 (100)	-	1.000
Cephalosporins	Cefepime	115 (100)	-	31 (96.8)	1 (3.1)	< 0.001
	Ceftriaxone	115 (100)	-	32 (100)	-	< 0.001
	Ceftazidime	112 (97.3)	3 (2.6)	30 (93.7)	2 (6.2)	< 0.001
β-lactam/β-lactamase inhibitor	Piperacillin/ tazobactam	114 (99.1)	1 (0.8)	32 (100)	-	1.000
Tetracyclines	Tetracycline	109 (94.7)	6 (5.2)	28 (87.5)	4 (12.5)	< 0.001
Fluoroquinolones	Ciprofloxacin	114 (99.1)	1 (0.8)	32 (100)	-	1.000
Aminoglycosides	Amikacin	101 (87.8)	14 (12.1)	31 (96.8)	1 (3.1)	< 0.001
	Gentamycin	107 (93.0)	8 (6.9)	31 (96.8)	1 (3.1)	< 0.001
Sulfonamide	Co-trimoxazole	113 (98.2)	2 (1.7)	31 (96.8)	1 (3.1)	< 0.001
Polymyxins	Colistin	-	115 (100)	-	32 (100)	1.000

which were resistant to several antimicrobial agents used for the treatment of *A. baumannii* infections.

The rate of XDR isolates in our region is close to the study conducted by Jasemi et al,[18] in which the rate of XDR isolates was detected in 91.3% from Iranian healthcare settings. However, the frequency of XDR isolates described by Jasemi et al (60%),[18] Maspi et al (71.2%),[19] and Fazeli et al (62.8%)[20] are slightly lower than our finding. However, the high incidence of XDR isolates may be related to misdiagnosis and subsequent useless antibiotic prescriptions in our healthcare setting.

Due to high rate of antibiotic resistance in our isolates, there were no correlation between distribution of antibiotic resistance and different clinical samples. However, the most antibiotic resistance were among respiratory samples where *A. baumannii* are a major cause of respiratory infections.

Our data indicated 63.9% and 78.2% of *A. baumannii* isolates carried *intI1* and *intI2* integron-integrase genes, respectively. Meanwhile, the *intI3* integron-integrase gene was not detected in any isolates.

Numerous studies have been investigating the presence of class I and II integrons in clinical *A. baumannii* isolates from Iran and other parts of the world that it is important to note that in most of these studies, the higher prevalence of class I integron was reported, however, our study indicates otherwise.[21–24]

In contrast to previous data, our finding may explain the emergence of the high prevalence of *intI2* for the first time in our region, which indicates a concern in the future and increase the antibiotic resistance associated with the presence of class II integron. Closest to our finding, in a study conducted by Mirnejad et al,[3] the prevalence of class I and class II integrons was reported in 42% (21/50) and 82% (41/50) of isolates. Ramírez et al,[16] reported the high prevalence of the *intI2* gene (68%) in clinical samples isolated from Buenos Aires City, Argentina. Kamalbeik et al,[25] also reported 65% of the clinical isolates *A. baumannii* were classified as class II integrons. The previous study indicates the rate of incidence of class I in *A. baumannii* isolates is variable due to geographical distribution and origin of infections. Goudarzi et al,[26] showed that 74.1% and 12.5% of *A. baumannii* isolates harboring class I and II integrons, respectively. Another Iranian study showed 96.7% and 43.3% of XDR *A. baumannii* isolates were positive for class I and class II integrons, respectively.[27]

According to antibiotic susceptibility pattern, the presence of class I and class II integron showed a significant correlation with higher rates of antibiotic resistance with most antibiotics used for in vitro compared to integron-negative isolates.

In addition, we compared the antimicrobial resistance pattern. The most effective antibiotic against integron-negative and integron-positive isolates were polymixins with 100% susceptibility and

followed by tetracyclines for class I integron-positive and amikacin for class II integron-positive isolates.

Our results are in agreement with previous reports conducted by Mirnejad et al,[3] Japoni-Nejad et al,[28] and Chen et al,[29] which have shown the significant differences with an increased risk of antibiotic resistance in relation to the presence of a different class of integrons.[30]

These associations demonstrating the important role of integrons in antibiotic resistance and thereby in the epidemic behavior of *A. baumannii*. It should be noted that the resistance of integron-negative *A. baumannii* isolates is probably due to other mechanisms of antibiotics resistance.

CONCLUSION

Our findings highlight the emergence of class II integrons. Moreover, results of our study contribute to the understanding of the distribution of class I and II integrons in *A. baumannii* and its association with MDR and XDR isolates in our region. The use of appropriate infection control measured in clinical settings and implementation of treatment strategies is very necessary for our health care setting.

Disclosure
The authors declared no conflict of interest. No funding was received for this study.

REFERENCE

1. Moradi J, Hashemi FB, Bahador A. Antibiotic Resistance of Acinetobacter baumannii in Iran: A Systemic Review of the Published Literature. Osong Public Health Res Perspect 2015 Apr;6(2):79-86.
2. Hwa WE, Subramaniam G, Navaratnam P, Sekaran SD. Detection and characterization of class 1 integrons among carbapenem-resistant isolates of Acinetobacter spp. in Malaysia. J Microbiol Immunol Infect 2009 Feb;42(1):54-62.
3. Mirnejad R, Mostofi S, Masjedian F. Antibiotic resistance and carriage class 1 and 2 integrons in clinical isolates of Acinetobacter baumannii from Tehran, Iran. Asian Pac J Trop Biomed 2013 Feb;3(2):140-145.
4. Aryanezhad M, Shakibaie MR, Karmostaji A, Shakibaie S. Prevalence of class 1, 2, and 3 integrons and biofilm formation in pseudomonas aeruginosa and Acinetobacter baumannii among ICU and non-ICU patients. Infection. Epidemiology and Microbiology 2016;2:1-7.
5. Bennett PM. Plasmid encoded antibiotic resistance: acquisition and transfer of antibiotic resistance genes in bacteria. Br J Pharmacol 2008 Mar;153(Suppl 1):S347-S357.
6. Koczura R, Przyszlakowska B, Mokracka J, Kaznowski A. Class 1 integrons and antibiotic resistance of clinical Acinetobacter calcoaceticus-baumannii complex in Poznań, Poland. Curr Microbiol 2014 Sep;69(3):258-262.
7. Gillings MR. Integrons: past, present, and future. Microbiol Mol Biol Rev 2014 Jun;78(2):257-277.
8. Deng Y, Bao X, Ji L, Chen L, Liu J, Miao J, et al. Resistance integrons: class 1, 2 and 3 integrons. Ann Clin Microbiol Antimicrob 2015 Oct;14:45.
9. Japoni S, Japoni A, Farshad S, Ali AA, Jamalidoust M. Association between existence of integrons and multi-drug resistance in Acinetobacter isolated from patients in southern Iran. Pol J Microbiol 2011;60(2):163-168.
10. Khosravi Y, Tay ST, Vadivelu J. Analysis of integrons and associated gene cassettes of metallo-β-lactamase-positive Pseudomonas aeruginosa in Malaysia. J Med Microbiol 2011 Jul;60(Pt 7):988-994.
11. Turton JF, Woodford N, Glover J, Yarde S, Kaufmann ME, Pitt TL. Identification of Acinetobacter baumannii by detection of the blaOXA-51-like carbapenemase gene intrinsic to this species. J Clin Microbiol 2006 Aug;44(8):2974-2976.
12. Wayne P. CLSI. Performance standards for antimicrobial susceptibility testing; Twenty-second informational supplement. CLSI document M100-S22. Clinical and Laboratory Standards Institute. 2015.
13. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 2012 Mar;18(3):268-281.
14. Shamsizadeh Z, Nikaeen M, Esfahani BN, Mirhoseini SH, Hatamzadeh M, Hassanzadeh A. Detection of antibiotic resistant Acinetobacter baumannii in various hospital environments: potential sources for transmission of Acinetobacter infections. Environ Health Prev Med 2017;22:44.
15. Machado E, Cantón R, Baquero F, Galán JC, Rollán A, Peixe L, et al. Integron content of extended-spectrum-beta-lactamase-producing Escherichia coli strains over 12 years in a single hospital in Madrid, Spain. Antimicrob Agents Chemother 2005 May;49(5):1823-1829.
16. Ramírez MS, Morales A, Vilacoba E, Márquez C, Centrón D. Class 2 integrons dissemination among multidrug resistance (MDR) clones of Acinetobacter baumannii. Curr Microbiol 2012 Mar;64(3):290-293.
17. van Hoek AH, Mevius D, Guerra B, Mullany P, Roberts AP, Aarts HJ. Acquired antibiotic resistance genes: an overview. Front Microbiol 2011 Sep;2:203.
18. Jasemi S, Douraghi M, Adibhesami H, Zeraati H, Rahbar M, Boroumand MA, et al. Trend of extensively drug-resistant Acinetobacter baumannii and the remaining therapeutic options: a multicenter study in Tehran, Iran over a 3-year period. Lett Appl Microbiol 2016 Dec;63(6):466-472.
19. Maspi H, Mahmoodzadeh Hosseini H, Amin M, Imani Fooladi AA. High prevalence of extensively drug-resistant and metallo beta-lactamase-producing clinical Acinetobacter baumannii in Iran. Microb Pathog 2016 Sep;98:155-159.
20. Fazeli H, Taraghian A, Kamali R, Poursina F, Esfahani BN, Moghim S. Molecular identification and antimicrobial resistance profile of Acinetobacter baumannii isolated from nosocomial infections of a teaching hospital in Isfahan, Iran. Avicenna Journal of Clinical Microbiology and Infection 2014;1(3):e21489.
21. Sung JY, Koo SH, Kim S, Kwon KC. Epidemiological characterizations of class 1 integrons from multidrug-resistant acinetobacter isolates in Daejeon, Korea. Ann Lab Med 2014 Jul;34(4):293-299.
22. Martins N, Picão RC, Adams-Sapper S, Riley LW, Moreira BM. Association of class 1 and 2 integrons with multidrug-resistant Acinetobacter baumannii international clones and Acinetobacter nosocomialis isolates. Antimicrob Agents Chemother 2015 Jan;59(1):698-701.
23. Çıçek AC, Düzgün AO, Saral A, Kayman T, Çızmecı Z, Balcı PO, et al. Detection of class 1 integron in Acinetobacter baumannii isolates collected from nine hospitals in Turkey. Asian Pac J Trop Biomed 2013 Sep;3(9):743-747.
24. Taherikalani M, Maleki A, Sadeghifard N, Mohammadzadeh

D, Soroush S, Asadollahi P, et al. Dissemination of class 1, 2 and 3 integrons among different multidrug resistant isolates of Acinetobacter baumannii in Tehran hospitals, Iran. Pol J Microbiol 2011;60(2):169-174.

25. Kamalbeik S, Talaie H, Mahdavinejad A, Karimi A, Salimi A. Multidrug-resistant Acinetobacter baumannii infection in intensive care unit patients in a hospital with building construction: is there an association? Korean J Anesthesiol 2014 Apr;66(4):295-299.
26. Goudarzi H, Azad M, Seyedjavadi SS, Azimi H, Chirani AS, Omrani VF, et al. Characterization of integrons and associated gene cassettes in Acinetobacter baumannii strains isolated from intensive care unit in Tehran, Iran. J Acute Dis 2016;5(5):386-392.
27. Najar Peerayeh S, Karmostaji A. Molecular identification of resistance determinants, integrons and genetic relatedness of extensively drug resistant Acinetobacter baumannii isolated from hospitals in Tehran, Iran. Jundishapur J Microbiol 2015 Jul;8(7):e27021.
28. Japoni-Nejad A, Farshad S, van Belkum A, Ghaznavi-Rad E. Novel cassette array in a class 1 integron in clinical isolates of Acinetobacter baumannii from central Iran. Int J Med Microbiol 2013 Dec;303(8):645-650.
29. Chen J, Li H, Yang J, Zhan R, Chen A, Yan Y. Prevalence and Characterization of Integrons in Multidrug Resistant Acinetobacter baumannii in Eastern China: A Multiple-Hospital Study. Int J Environ Res Public Health 2015 Aug;12(8):10093-10105.
30. Fazeli H, Fatahi M, Faghri J, Akbari R. Molecular study of PER and VEB genes is multidrug resistant Pseudomonas aeroginosa isolated from clinical specimens in Isfahan/Iran and their antibiotic resistance patterns. J Kerman Univ Med Sci 2012;19(4):345-353.