



Journal of Medical Bacteriology



Prevalence of Integrons as the Carrier of Multidrug Resistance Genes among Clinical Isolates of *Klebsiella*

Roya Mohammadi, Azam Haddadi^{*}, Naser Harzandi

Department of Microbiology, Karaj Branch, Islamic Azad University, Karaj, Iran.

ARTICLE INFO

Article type:

Research Article

Article history:

Received: 23 Feb 2019

Revised: 14 Apr 2019

Accepted: 21 Apr 2019

Published: 13 Jun 2019

Keywords:

Integron, Integrase genes, *Klebsiella*, Multidrug Resistance genes.

ABSTRACT

Background: Multidrug resistance is a serious problem in the treatment of urinary tract infections. Integrons are ancient structures that contain determinants of a site-specific recombination system to capture genes encoding antimicrobial resistance. The aim of this study was to determine the prevalence of multidrug resistant *Klebsiella* isolates in clinical specimens. In addition, the existence of integrons in resistant isolates was assessed by amplification of integrase genes.

Methods: Susceptibility of 100 *Klebsiella* isolates was determined to 19 antibiotics by the Kirby-Bauer disc diffusion method and integron classes were detected. Then the multi-drug resistance association with the existence of the integrase gene was calculated by chi-square and Fisher exact tests.

Results: According to antibiogram results, 54 isolates were multidrug resistant. By amplification of integrase gene it was revealed that 27% of the isolates harbor integrons. Also by PCR-RFLP it was revealed that all of them were class 1. The existence of integrons was confirmed in 25 of our multidrug resistant isolates, indicating the frequency rate is high and integrons may be partly responsible for multidrug resistance.

Conclusion: Multidrug resistance suggests that strategy for treatment of patients with *Klebsiella* infections needs to be revised. The possibility of transmission of resistance genes by integrons would be decreased by treatment of patients with the appropriate antibiotics.

- **Please cite this paper as:** Mohammadi R, Haddadi A, Harzandi N. Prevalence of Integrons as the Carrier of Multidrug Resistance Genes among Clinical Isolates of *Klebsiella*. *J Med Bacteriol.* 2019; **8** (3, 4): pp.23-30.

Introduction

Urinary tract infections (UTIs) are among the most common bacterial infectious diseases encountered in clinical practice and account for significant morbidity and high medical costs and *Klebsiella pneumoniae*, an opportunistic pathogen, is responsible for about 6-17 percent of UTIs (1,2).

Dispersion of multidrug resistant *K. pneumoniae*, specifically the extended spectrum beta-lactamase (ESBL) producing strains, resulted in the failure of antibiotic treatment of patients with urinary tract infections (3). As multidrug resistance among *Enterobacteriaceae* in the hospital setting is an increasing problem, the development of control strategies is of major importance (4).

Several studies on bacterial pathogens achieved that multiple antibiotic resistance is a consequence of horizontal gene transfer. Besides, in many bacteria, exchangeable genetic elements such as plasmids, transposons and integrons are responsible for the dissemination of antibiotic resistance (5).

Integrons are conserved DNA sequences that provide an efficient means for capturing and spreading of antimicrobial resistance genes and are carried on episomal genetics structures(6). Early attempts to describe integrons suggested that they consisted of two conserved regions flanking a variable region containing one resistance gene or more (7). A more detailed description of their structure showed that these genetic elements composed of a gene encoding an integrase, gene cassettes and an integration site for the gene cassettes (att). The integrase excises and integrates the gene cassettes from and into the integron, but integrons themselves are not mobile (8). They can locate within transposons or conjugative plasmids and contribute to the gene traffic leading to the acquisition of new genes in bacteria (9).

Class 1 integrons are the most widespread and have been frequently found in ESBL producing clinical isolates of *Enterobacteriaceae* including *K. pneumoniae*. Class 2 integrons occur less frequently in ESBL producing *E. coli* and *K. pneumoniae* and finally, class 3 integrons are rarely found in ESBL producing *K. pneumoniae* (10).

As there is not much published data available on the detection of integrons in MDR isolates of *Klebsiella* in the immigration friendly city of Karaj, Iran, the aim of this study was to survey the frequency of MDR and class 1 and 2 integrons by restriction fragment length polymorphism analysis of PCR products (PCR-RFLP) of clinical isolates of *Klebsiella* and to investigate associations between resistance to some antibiotics and existence of integrons.

Materials and Methods

Bacterial isolates

Between June and December 2017, 147 non-duplicate *Klebsiella* isolates from urine samples were collected from four hospitals and two private clinical laboratories of Alborz province, Karaj city. One hundred isolates were identified as *Klebsiella* based on API 20E kit.

Antimicrobial susceptibility test

The susceptibilities of all isolates to different antibiotics were determined by Kirby- Bauer disc diffusion method, as suggested by the CLSI (11). The zone of inhibition of each isolate was tested on Muller–Hinton agar medium with commercial antimicrobial discs (Padtan Teb Co., Tehran, Iran). The antibiotic discs used in this study were gentamicin (10 µg), amikacin (30 µg), amoxicillin (10 µg), ceftazidime (30 µg), cephalothin (30 µg), cefalexin (30 µg), cefotaxime (30 µg), ceftizoxime (30 µg), ceftriaxon (30 µg), imipenem (10 µg), nalidixic acid (30 µg),

ciprofloxacin (5 µg), norfloxacin (10 µg), trimethoprim-sulfamethoxazole (25 µg), tetracycline (30 µg), chloramphenicol (30 µg), ofloxacin (5 µg), amoxicillin/clavulanic acid (20/10 µg) and nitrofurantoin (300 µg). *E. coli* ATCC 25922 was used as the reference strain for antibiotic susceptibility tests.

Screening for ESBL producing isolates

ESBL producers were detected by phenotypic confirmatory tests (PCT) (CLSI). The isolates showing resistance to one or more 3rd generation cephalosporins such as ceftazidime (30 µg), cefotaxime (30 µg) and ceftriaxone (30 µg) were tested for ESBL production by double disc-diffusion test (DDDT) using four discs cefazidime (CAZ: 30 µg), ceftazidime-clavulanic acid (30/10 µg), and cefotaxime (CTX: 30 µg), cefotaxime-clavulanic acid (30/10 µg). The inocula and incubation conditions were same as for standard disc diffusion recommendations. A >5 mm increase in zone diameter for either antimicrobial agent tested in combination with clavulanic acid versus its zone when tested alone was considered as ESBL positive (12). *K. pneumoniae* ATCC 700603 and *Escherichia coli* ATCC 25922 were used as positive control and negative control respectively.

Detection of integrons and variable regions by PCR-RFLP

Detection of integrons was performed by PCR using genomic DNA extracted by boiling method (13). PCR amplification of integrase genes was performed in 25 µl reaction mixtures containing 2 µl of template DNA, 2.5 µl of 10X PCR buffer, 0.5 µl of 10 mM dNTPs, 150 µM MgCl₂, 0.2 µl of 5u/µl Taq DNA polymerase (CinnaGen, Tehran, Iran) and 0.7 µl of each primers (20 pmol), (Faza Biotech, Tehran, Iran) as follows: hep35 (5'-TGCGGGTYAARGATBTKGATTT-3') and hep36 (5'-CARCACATGCGTRTARAT-

3'), where B = C or G or T, K = G or T, R = A or G and Y = C or T. Amplifications were performed in a Applied Biosystem - Veriti Thermal Cycler using the following condition: early denaturation at 94°C for 5 minutes followed by 40 cycles of 94 °C for 30 seconds, 64°C for 50 seconds and 72 °C for 100 seconds, with a final elongation step of 10 minutes at 72 °C. The length of the expected amplified fragment was about 491 bp. The amplified PCR products were resolved by electrophoresis in 1% agarose gel and visualized by DNA safe stain.

Clinical isolate of *E. coli* harboring integrons class 1 and 2 genes (kindly provided from, research center for gastroenterology and liver diseases, Iran) was used as positive control. Also, a tube includes PCR reaction with no DNA template was used as negative control for all PCRs.

Class 1, 2, and 3 integrons were analyzed by RFLP using RsaI and HinfI restriction enzymes (Cinagene, Iran). The products of each distinct RFLP were analyzed by electrophoresis in 1.3% agarose gel (14). For the class 1 integron carrying isolates, PCR amplification of variable regions was performed in 20 µl reaction mixtures containing 30 ng DNA template, 20 µM of each dNTP, 150 µM MgCl₂, 0.2 U Super Taq DNA polymerase and 1 pM of forward primer 5'-GGCATCCAAGCAGCAAG- 3' which anneals at positions 1206–1190 of the intI1 gene, and reverse primer 5'-AAGCAGACTTGACCTGA- 3') which anneals at positions 1342–1326 (15). Amplification of the integron variable regions was carried out with initial denaturation of 4 min at 94 °C followed by 35 cycles of 1 min at 94 °C, 1 min at 51 °C and 1 min at 72 °C with a final extension of 10 min at 72 °C. The amplified PCR products were resolved by electrophoresis in 1% agarose gel and visualized by DNA safe stain.

Statistical analysis

Chi-square and Fisher exact test were used to calculate the association between antibiotic resistance and presence of integron. P value of less than 0.05 was considered statistically significant.

Results

A total of 147 clinical isolates of *Klebsiella* were isolated from three hospital and two private clinical laboratories of Karaj city, 100 isolates were confirmed as *Klebsiella* by API-20E strips. Of the 100 *Klebsiella* isolates, 86 and 14 isolates were identified as *Klebsiella pneumoniae* and *Klebsiella oxytoca* respectively. Seventy-nine isolates were obtained from women and 21 from men. Total isolates were subjected to antibiotic susceptibility test by Kirby-Bauer disc diffusion method as suggested by the Clinical and Laboratory Standards Institute (11). The antimicrobial agents were chosen to cover different classes of antibiotics. According to antibiogram results, the rate of resistance to antibiotics were in the following order: amoxicillin (99%), nitrofurantoin (61%), amoxicillin-clavulanic acid (46%), cephalexin (29%), cephalotin (29%), tetracycline (27%), cefotaxime (24%), ceftazidime (24%), trimethoprim-sulfamethoxazole (24%), ceftriaxone (22%), ceftizoxime (18%), gentamicin (13%), amikacin (12%), nalidixic acid (12%), chloramphenicol (12%), imipenem (12%), ciprofloxacin (11%), norfloxacin (11%), ofloxacin (11%). Eighty three isolates (54%) were multidrug resistant (resistant to at least three unrelated antibiotics). Resistant to four, five and six or more antibiotics were 36%, 33% and 29% respectively.

Phenotypic identification of ESBLs

ESBLs screening of strains by phenotypic confirmatory disc test showed that out of 100 isolates, 77 (87.5%) *Klebsiella pneumoniae* and 11(12.5%) *Klebsiella oxytoca* were chosen as ESBLs producing candidates. The double disc-diffusion test confirmed that 23(92%) *Klebsiella pneumoniae* and 2(8%) *Klebsiella oxytoca* produce ESBLs.

As mentioned above resistance to fluoroquinolones, amikacin and imipenem was about 11-12% but resistance to these antibiotics among ESBL producing *Klebsiella* isolates in this study was higher (almost 32-48%).

Frequency of integrons

By amplification of the integrase gene, it was found that 27 isolates (27%) harbor antibiotic resistance integrons. By PCRRFLP, only class 1 integron was found in all integron harboring isolates (Figure 1).

Class 2 and 3 integrons were not detected among our isolates. Results showed that 13 (48%) of the ESBL producing *Klebsiella* isolates harbored class 1 integron. Comparison of MDR phenotypes in the two groups of isolates showed that integron-positive strains had much higher rate of MDR (100%) compared with integron-negative strains (56%).

The integron harboring isolates were significantly more resistant to all used antibiotics but only there was found a significant correlation between the presence of integrons and resistance to nalidixic acid, ($p \leq 0.01$), ofloxacin and norfloxacin ($p \leq 0.03$), ceftizoxim ($p \leq 0.003$) and finally ciprofloxacin ($p \leq 0.004$) (Table 1).

Class 1 integron harboring isolates possessed one to four DNA sequences manifested as seven different sized amplicons in the range of 0.1 to 2.5 kbp and isolates with similar profiles for class 1 integron variable regions had the same antibiotic resistance phenotypes (Table 2).

Table 1. Association between antibiotic resistance and presence of integrins in *Klebsiella* isolates.

Antibiotic	No. of integron positive isolates	No. of integron negative isolates	PV	Antibiotic	No. of integron positive isolates	No. of integron negative isolates	PV
Cephalexin	20	9	0	Ceftriaxone	15	7	0
Gentamicin	11	2	0	Cefotaxime	16	8	0
Norfloxacin	6	5	0.03	Ceftizoxime	10	8	0.003
Ofloxacin	6	5	0.03	Nitrofurantoin	15	46	0.499
Nalidixic acid	7	5	0.01	Tetracycline	19	8	0
Nalidixic acid	7	5	0.01	Co-trimoxazole	20	4	0
Cefalotin	20	9	0	Amoxicillin	27	72	0.543
Ciprofloxacin	7	4	0.004	Amikacin	4	8	0.6
Ceftazidime	17	7	0	Imipenem	9	3	0
amoxicillin/clavulanate	24	22	0	Chloramphenicol	7	5	0.01

Table 2. Distribution of variable region fragments sizes among class I integron carrying *K. pneumoniae* clinical isolates.

No. of isolates	No. of bands	Band size (bp)	Antibiotic resistance profiles
1	3	170, 270, 650	CN, GM, NA, NOR, CF, CP, CAZ, CRO, AMC, CTX, CT, FM, TE, AMX, OFX, SXT, AN, IPM
1	2	400, 1000	CN, GM, CF, CAZ, CRO, AMC, CTX, AMX, SXT, AN, IPM
1	4	400, 600, 700	FM, TE, AMX, SXT, C
1	2	100, 250, 600	CN, GM, CF, CAZ, CRO, CTX, AMC, AMX, SXT
4	3	100, 300, 600	CN, NA, NOR, CF, CP, CAZ, CRO, AMC, CTX, CT, FM, TE, AMX, OFX, IPM, C
16	3	400, 600, 900	CN, NA, NOR, CF, CP, CAZ, CRO, AMC, CTX, CT, FM, TE, AMX, OFX, IPM, C
1	2	400, 500	CN, CP, CT, TE, AMX, AMC

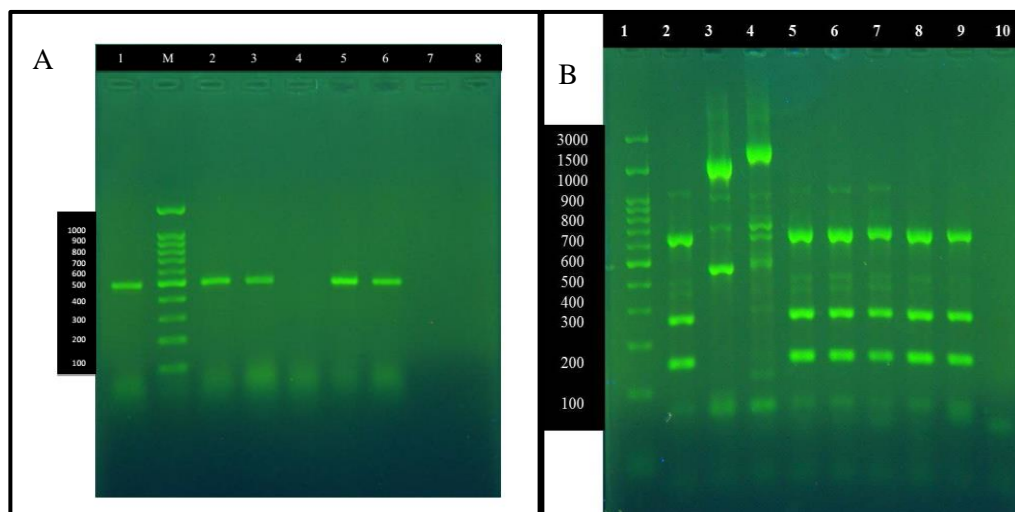


Figure 1. Figure 1: A. PCR analysis and restriction fragment length polymorphism analysis of PCR products of *Klebsiella* antibiotic resistance integrons. M: 100 bp ladder, Lane 1: positive control (491 bp), Lane 2, 3: Class 1 integron (PCR product, a 491 bp fragment), Lane 4: Negative control, Lane 5: Class 1 integron (PCR product treated by RsaI, a 491 bp fragment), Lane 6: Class 1 integron (PCR product treated by HinfI, a 491 bp fragment). B. PCR amplification of class 1 integron variable regions in *Klebsiella* isolates (Lanes 2-9). Lane 1, DNA molecular weight marker.

Cefalexin (CN), Gentamicin (GM), Nalidixic acid (NA), cephalothin (CF), ciprofloxacin (CP), ceftriaxone (CRO), ceftizoxime (CT), nitrofurantoin (FM), tetracycline (TE), amoxicillin (AMX), ofloxacin (OFX), trimethoprim-sulfamethoxazole (SXT), amikacin (AN), chloramphenicol (C), Imipenem (IPM), amoxicillin/clavulanic acid (AMC), Cefotaxime (CTX), Ceftazidime (CAZ), Norfloxacin (NOR).

Discussion

Due to exposure to various antibiotics, many pathogenic and commensal organisms show multidrug resistant phenotype. Often, this antimicrobial resistance is encoded by integrons that may be found on plasmids or that are integrated into the bacterial chromosome (16). It has been suggested that integrons are as reservoirs of antimicrobial resistance genes within microbial populations (17). Class I integrons play a

particularly important role in the development of multidrug resistance in clinical bacterial isolates (10) and appear to be prevalent in nature (16).

Similar with the results of Xuan et al. (17) and Mobarak- Qamsari et al. (10) our results showed that integron-positive strains had higher rate of MDR (100%) compared with integron-negative strains (56%), indicating the correlation of integron carriage with multidrug resistance.

It has been reported that compared to non-ESBL strains, ESBL producing *K. pneumoniae* harbor higher rates of class 1 integrons (10, 19). In the current study, 48% of the ESBL positive isolates carried class 1 integrons. This is similar to the results of Mobarak-Qamsarei et al. who found that 44% of ESBL positive isolates carried class 1 integrons (10).

In our study, a significant correlation was found between the presence of integrons and resistance to fluoroquinolones (norfloxacin, ciprofloxacin, ofloxacin, nalidixic acid), chloramphenicol and

ceftizoxim. This is comparable to those of Japoni et al. (20) who have reported a significant association between integron carriage and resistance to certain antibiotics, including gentamicin, amikacin, cephalotin, ciprofloxacin, nalidixic acid, norfloxacin and co-trimoxazole, but the findings of Mobarak-qamsari et al. have indicated a significant association between integron carriage and higher rates of resistance to β -lactams and aminoglycosides (10). Nevertheless, to confirm this association genetically, integron cassette regions should be sequenced.

The bacterial isolates in this study were sensitive to ofloxacin (88%), norfloxacin, (84%) and ciprofloxacin (75%). The double disc-diffusion test confirmed that out of 100 *Klebsiella* isolates 25 isolates produce ESBLs. There are several studies have done in Iran that reports the prevalence of ESBL as high as 33-69% (21, 22, 23). Also Arbabi et al (24) explained that the prevalence of extended spectrum beta-lactamase (ESBL) producing *Klebsiella pneumoniae* strains isolated from urinary tract infections in Miald Hospital in Tehran was 30%. It was obtained during a large study of 1610 *Escherichia coli* and 785 *K. pneumoniae* isolates from 31 centers in 10 European countries from 1997 to 2000, that the prevalence of ESBL in these organisms ranged from as low as 1.5% in Germany to as high as 39-47% in Russia, Poland, and Turkey (25). Mac Danel et al. performed a systematic literature review and found that the incidence of ESBL-*E. coli* and ESBL-*Klebsiella* infections in the United States has increased, with slightly higher rates of ESBL-*Klebsiella* infections (26).

Sensitivity to ofloxacin, norfloxacin and ciprofloxacin among ESBL producing *Klebsiella* isolates were 64%, 48% and 28% respectively. Apparently it appears that, ESBL positive isolates are less sensitive to quinolones especially ciprofloxacin compared to non- ESBL producing isolates. In this study, ESBL producing isolates were completely resistant to amoxicillin,

cephalexin, cephalotin (100%) and resistance rate to ceftazidim and cefotaxim were 96%. As it seems resistance to cephalosporins is high among ESBL producing isolates and ofloxacin, norfloxacin and imipenem, showed better in vitro activity this results is comparable to the results of Azimi et al. (27) showed that imipenem and ceftriaxone are more effective against *Klebsiella* isolates. Yao et al. (19) reported similar findings, according to their results imipenem and the fourth-generation cephalosporin, cefepime, showed better in vitro activity than third-generation cephalosporin, such as cefotaxime, ceftazidime and ceftriaxone against ESBL producing *K. pneumoniae*.

Conclusion

The production of ESBLs and integrons will continue to threaten the usefulness of antibiotics as therapeutic agents. In conclusion convenient estimates of ESBL infections when combined with other mechanisms of resistance will allow for the appropriate targeting of resources toward research, drug discovery, antimicrobial protection, and infection prevention.

Acknowledgment

This study was supported by Islamic Azad University Karaj Branch.

Conflict of interest

Roya Mohammadi, Azam Haddadi, Naser Harzandi declare that they have no conflicts of interest.

References

1. Saraylu J, Fallah Mehrabadi J, Imani Fooladi AA, et al. Prevalence and evaluation of toxin genes among Uropathogenic *Escherichia coli* clinical isolates by duplex PCR. *J Med*

- Bacteriol* 2012; **1**:17-22.
2. Babypadmini S, Appalaraju B. Extended Spectrum β -lactamases in urinary isolates of *Escherichia coli* and *Klebsiella pneumoniae* prevalence and susceptibility pattern in a tertiary care hospitals, Indian. *J Med Microbiol* 2004; **22**:172-74.
 3. Paterson DL, Bonomo RA. Extended-spectrum beta-lactamases: a clinical update. *Clin Microbiol Rev* 2005; **18**:657-86.
 4. Leverstein-van Hall MA, Paauw A, Box ATA, et al. Presence of integron-associated resistance in the community is widespread and contributes to multidrug resistance in the hospital. *J clin Microbiol* 2002; **40**:3038-40.
 5. Zarei-Yazdeli M, Eslami G, Zandi H, et al. Prevalence of class 1, 2 and 3 integrons among multidrug-resistant *Pseudomonas aeruginosa* in Yazd, Iran. *Iran J Microbiol* 2018; **10**:300-6.
 6. Haddadi A, Mikaili Ghezeljeh S, shavandi M. Prevalence of class 1 and 2 integrons among the multidrug resistant uropathogenic strains of *Escherichia coli*. *Asian Biomed* 2015; **9**:49-54.
 7. Peter M. Bennett. Integrons and gene cassettes: a genetic construction kit for bacteria. *J Antimicrob Chemother* 1999; **43**:1-4.
 8. Fluit AC, Schmitz FJ. Resistance. integrons and super-integrons. *Clin Microbiol Infect* 2004; **10**:272-88.
 9. Sepp E, Stsepetova J, Lõivukene K, et al. The occurrence of antimicrobial resistance and class 1 integrons among commensal *Escherichia coli* isolates from infants and elderly persons. *Ann Clin Microbiol Antimicrob* 2009; **8**:1-6.
 10. Mobarak-Qamsari M, Ashayeri-Panah M, Eftekhari F, et al. Integron mediated multidrug resistance in extended spectrum beta-lactamase-producing clinical isolates of *Klebsiella pneumoniae*. *Braz J Microbiol* 2014; **44**:849-54.
 11. Cockerill FA. Performance standards for antimicrobial disc susceptibility testing. Twenty-first informational supplement: Clinical Laboratory Standards Institute (CLSI); 2011.
 12. Bauer AW, Kirby MM, Sherris JC, et al. Antibiotic susceptibility testing by a standardized single disc method. *AM J Clin Pathol* 1966; **36**:493-96.
 13. Queipo-Ortuno MI, Colmenero JD, Macias M, et al. Preparation of bacterial DNA template by boiling and effect of immunoglobulin G as an inhibitor in real-time PCR for serum samples from patients with brucellosis. *Clin Vaccine Immunol* 2008; **15**:93-96.
 14. Ahangarzadeh Rezaee M, Sheikhalizadeh V, Hasani A. Detection of integrons among multi-drug resistant (MDR) *Escherichia coli* strains isolated from clinical specimens in northern west of Iran. *Braz J Microbiol* 2011; **42**:1308-13.
 15. Levesque C, Piche L, Larose C, et al. PCR mapping of integrons reveals several novel combinations of resistance genes. *Antimicrob Agents Chemother* 1999; **39**:185-91.
 16. Goldstein C, Lee MD, Sanchez S, et al. Incidence of class 1 and 2 integrons in clinical and commensal bacteria from livestock, companion animals, and exotics. *Antimicrob Agents Chemother* 2001; **45**:723-26.
 17. Xuan X, Xuan L, Mei L, et al. Molecular characterisations of integrons in clinical isolates of *Klebsiella pneumoniae* in a Chinese tertiary hospital. *Microb Pathog* 2017; **104**:164-70.
 18. Najjar NG, Farah MJ, Hashwa FA, et al. Antibiotic resistance patterns and sequencing of class I integron from uropathogenic *Escherichia coli* in Lebanon. *Lett Appl Microbiol* 2010; **51**:456-61.
 19. Yao F, Quian Y, Chen S, et al. Incidence of

- extended spectrum-lactamases and characterization of integrons in extended-spectrum β -lactamase producing *Klebsiella pneumoniae* isolated in Shantou, China. *Acta Biochim Biophys Sin* 2007; **39**:527-32.
20. Japoni A, Gudarzi M, Farshad SH, et al. Assay for integrons and pattern of antibiotic resistance in clinical *Escherichia coli* strains by PCR-RFLP in southern Iran. *Jpn J Infect Dis* 2008; **61**:85-8.
 21. Nasehi L, Shahcheraghi F, Nikbin VS, et al. *PER*, *CTX-M*, *TEM* and *SHV* Beta-lactamases in clinical isolates of *Klebsiella pneumoniae* isolated from Tehran, Iran. *Iran J Basic Med Sci* 2010; **13**:111-18.
 22. Feizabadi MM, Delfani S, Raji N, et al. Distribution of blaTEM, blaSHV, blaCTX-M genes among clinical isolates of *Klebsiella pneumoniae* at Labbafinejad Hospital, Tehran, Iran. *Microb Drug Resist* 2010; **16**:49-53.
 23. Shahcheraghi F, Moezi H, Feizabadi MM. Distribution of TEM and SHV β -lactamase genes among *Klebsiella pneumoniae* strains isolated from patients in Tehran. *Med Sci Monit* 2007; **13**:247-50.
 24. Arbabi L, Rahbar M, Jabbari M. Extended-spectrum β -lactamase-producing *E. coli* and *Klebsiella pneumoniae* isolated from urinary tract infections in Milad Hospital, Tehran, Iran. *Health MED* 2012; **6**:2818-22.
 25. Goossens H. MYSTIC program: summary of European data from 1997 to 2000. *Diagn Microb Infect Dis* 2001; **41**:183-89.
 26. McDanel J, Schweizer M, Crabb V, et al. Incidence of Extended-spectrum β -lactamase (ESBL)-Producing *Escherichia coli* and *Klebsiella* infections in the United States: A systematic literature review. *Infect Control Hosp Epidemiol* 2017; **38**:1209-15.
 27. Azimi L, Erajyian GH, Talebi M. Phenotypic and molecular characterization of plasmid mediated AmpC among clinical isolates of *Klebsiella pneumoniae* isolated from different hospitals in Tehran. *J Clin Diagn Res* 2015; **9**:1-3.