

Molecular Typing of *Klebsiella pneumoniae* Isolates by Enterobacterial Repetitive Intergenic Consensus (ERIC)-PCR

Vahideh Parsaie Mehr¹, Leili Shokoohzadeh^{2*}, Mohsen Mirzaee³, Mohammad Savari⁴

¹ Department of Microbiology, Dezful branch, Islamic Azad University, Dezful, IR Iran

² Department of Microbiology, Faculty of Medicine, Hamadan University of Medical Sciences, Hamadan, IR Iran

³ Department of Medical Laboratory Sciences, Borujerd Branch, Islamic Azad University, Borujerd, IR Iran

⁴ Department of Medical Laboratory Sciences, Faculty of Para Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, IR Iran

*Corresponding author: Leili Shokoohzadeh, Department of Microbiology, Faculty of Medicine, Hamadan University of Medical Sciences, Hamadan, IR Iran, E-mail: L.shokoohzadeh@umsha.ac.ir, Tel: +98 8138276295, Fax: +98 8138276299

Submitted: June 13, 2017; Revised: September 19, 2017; Accepted: September 25, 2017

Abstract

Background: *Klebsiella pneumoniae* is a Gram-negative bacterium and as a part of the natural microflora in human body gastrointestinal tract. *K. pneumoniae* has been known as one of the most common causes of nosocomial infections and multi-drug resistance pathogens. The aim of this study was to examine antimicrobial susceptibility and genetic relatedness among *K. pneumoniae* strains isolated from hospitals in Borujerd city in western Iran using Enterobacterial Repetitive Intergenic Consensus (ERIC)-PCR technique.

Materials and Methods: A total of 100 *K. pneumoniae* isolates were collected from Borujerd hospitals during April to September 2015. After detection and confirmation of *K. pneumoniae* isolates by conventional laboratory methods and differential tests, antibiotic susceptibility testing was performed using disk diffusion method. Also, genetic relatedness of 35 selected MDR *K. pneumoniae* isolates was examined by ERIC-PCR technique.

Results: Antibiotic susceptibility testing showed that among *K. pneumoniae* isolates, the highest antibiotic resistance was to ampicillin (91%), and the highest susceptibility was to imipenem (5%). More than 45% of the isolates showed multi resistant phenotypes. Based on ERIC-PCR results and data analysis, 32 different ERIC types.

Conclusion: The results of this study indicate the increase in multi resistant *K. pneumoniae* in hospitals under study. The results of ERIC PCR showed the high genetic diversity among *K. pneumoniae* strains, indicating the polyclonal distribution of *K. pneumoniae* isolates in Borujerd hospitals.

Keywords: *Klebsiella pneumoniae*, Antibiotic resistance, ERIC – PCR

1. Background

Klebsiella is a non-motile, Gram-negative, rod-shaped bacterium with a polysaccharide capsule. The capsule covers the entire cells surface and provides resistance against a lot of host defense mechanisms. As an opportunistic pathogen, *K. pneumoniae* is clinically very important, and 3 to 8% of hospital infections are caused by this species (1-2).

K. pneumoniae is a prevalent agent in nosocomial infections and causes pneumonia, sepsis, and urinary tract infections, particularly in patients with immunodeficiency. Infections caused by this bacterium are mainly treated with beta-lactams and fluoroquinolones (3-4).

Given the importance of infectious diseases threatening public health, especially nosocomial infections in hospitalized patients, understanding the status, distribution, and sources of *K. pneumoniae* infections can be an important issue; for this purpose, it is proved that molecular typing methods would be helpful. For the epidemiological studies purpose, bacterial molecular typing methods such as ribotyping, pulsed-field electrophoresis (PFGE) and PCR based methods are available (5-8).

Rep-PCR technique can be noted as an alternative technique to directly produce fingerprints without the use of endonuclease enzymes. In this method, oligonucleotide primers are designed based on sequences of repetitive short repeat nucleotides which are distributed in prokaryotes. This technique is rapid and reproducible and has a high discriminatory power. In this technique, three types of primer sequence are used, which are complementary with three types of repetitive elements including ERIC, BOX, and REP. ERICs are repetitive intergenic sequences of *Enterobacteriaceae* family with 126 base pairs length (9-10).

ERIC-PCR fingerprinting methods are widely used for genetic typing. According to recent information about the presence of ERIC sequences in the genome of *Escherichia coli* k-12, which is fully sequenced and available in Gen Bank, there are about 20 ERIC sequences in its entire genome.

ERIC PCR is often used to analyze the genetic diversity among *Enterobacteriaceae* family such as *Klebsiella pneumoniae*. ERIC patterns and numbers are different in bacterial genotypes; therefore, they can be used as genetic markers for genetic diversity among bacteria (11).

2. Objective

Given the importance of *K. pneumoniae* in nosocomial infections and no report on molecular epidemiology of *K. pneumoniae* strains in Borujerd hospitals, the aim of this study was to examine the antibiotic resistance and genetic relatedness of clinical *K. pneumoniae* isolates in Borujerd hospitals by ERIC-PCR technique.

3. Materials and Methods

3.1. Bacterial strains

A total of 100 isolates of *K. pneumoniae* were collected during April to September 2015 from Borujerd hospitals. The isolates were identified as *K. pneumoniae* using conventional microbiological tests (12).

3.2. Antibiotics susceptibility testing

Disk diffusion method was used to determine *K. pneumoniae* susceptibility to 13 antibiotics including ampicillin, gentamicin, chloramphenicol, tetracycline, ceftriaxone, nitrofurantoin, imipenem, azithromycin, ciprofloxacin, amoxicillin-clavulanic acid,

cefazolin, cefepime, and cephalothin based on CLSI 2015 (13) criteria. The antibiotic disks were supplied by Rosco Company, Denmark.

3.3. DNA extraction

Genomic DNAs were extracted from isolates overnight cultures at 37°C using the commercial genomic DNA Purification Kit (SinaClon, Iran) according to the manufacturer's instructions.

3.4. ERIC PCR

Thirty-five isolates were selected for analysis by ERIC-PCR based on clinical samples type (urine, trachea, wound, and blood), MDR isolates antibiotic resistance profile, sampling location, hospitals (three major hospitals), and hospital wards (internal, ICU, pediatric, emergency wards and outpatients or OP). Primer sequence used in this study was: ERIC (F): 5' - ATG TAA GCT CCT GGG GAT TCA C-3, ERIC (R): 5' - AAG TAA GTG ACT GGG GTG AGC G-3' (14). Amplification was performed in PCR buffer (New England Biolabs) containing each deoxynucleoside triphosphate (dNTP) at 200 mM with 10 pmol of each primer, approximately 10–50 ng of template DNA and 1U of Taq DNA polymerase (New England Biolabs) in a volume of 25 µL PCR reaction. Thermal cycling conditions consisted of an initial denaturation cycle of amplification at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 48°C for 1 min, extension at 72 °C for 2 min, and a final cycle of amplification at 72°C for 5 min. Genes fragments were separated by electrophoresis on 1.2% (w/v) agarose gels, stained with ethidium bromide (5 mg.L⁻¹), visualized with UV, and imaged using a Gel Doc TM XR image analysis station (Bio-Rad, Hercules, CA,USA). Products sizes were estimated using 100 bp and 1 kb DNA ladders (New England Biolabs) as molecular size markers.

3.5. ERIC PCR profiles Analysis

The strains similarity was detected based on the analysis of the profiles (Banding) by Total Lab software using Dice method for comparison and UPGMA method for clustering.

4. Results

Overall, 70% of *K. pneumoniae* strains were collected from urine samples, 14% from trachea, and 3% from wounds and blood samples. About 48% of *K. pneumoniae* strains were isolated from female and 52% from male patients.

4.1. Frequency of antibiotic resistance

Among 100 *K. pneumoniae* isolates, the highest antibiotic resistance was observed to ampicillin (91%), and the highest susceptibility was observed to imipenem (5%). The antibiotic resistance rates were as follows: 54% to cefazolin; 51% to cephalothin; 20% to nitrofurantoin, cefepime, and amoxicillin-clavulanic acid. The prevalence rates of resistance to antibiotics used in this study are shown in Figure 1.

Based on the results of antibiotic susceptibility testing, a high level of diversity in antibiotic resistance was observed among *K. pneumoniae* isolates. Resistance to at least one agent of three or more antimicrobial categories were detected in more than 45% of the isolates and designated as MDR isolates (15). The most prevalent simultaneous resistance pattern (51%) among these strains was resistance to ampicillin, cefazolin, and cephalothin. It should be noted, simultaneous resistance was observed to 11 antibiotics including ampicillin, chloramphenicol, cefazolin, tetracycline, gentamicin, cefepime, aztreonam, ceftriaxone, nitrofurantoin, amoxicillin - clavulanic acid, and ciprofloxacin in 8% of the isolates.

Antibiotic Resistance (%) of *Klebsiella pneumoniae* isolates

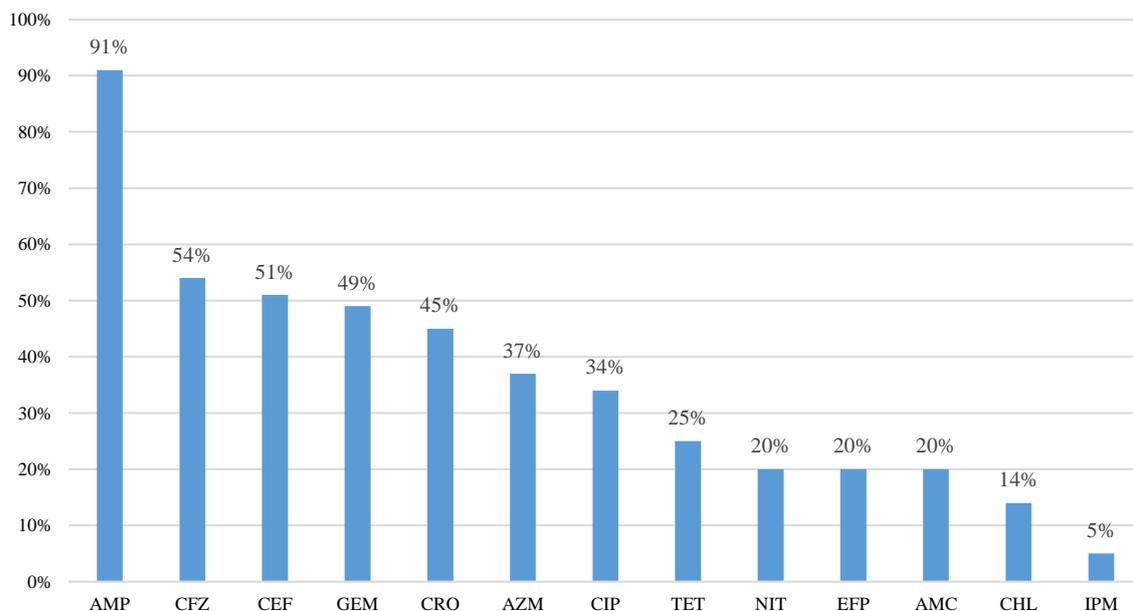


Figure 1. Prevalence (%) of antibiotic resistance of *Klebsiella pneumoniae* isolates in Borujerd hospitals.

AMP; ampicillin, CFZ; ceftazidime, CEF; cephalothin, GEM; gentamicin, CRO: ceftriaxone, AZM; azithromycin, CIP; ciprofloxacin, TET; tetracycline, NIT; nitrofurantoin, EFP; cefepime, AMC; ampicillin-clavulanic acid, CHL; chloramphenicol, IMI; imipenem

4.2. ERIC PCR

Gel electrophoresis image of ERIC-PCR products is presented in Figure 2. The number of bands was varied from 3 to 13 bands, and the size was varied from 200 bp to more than 1kb. The strains similarity among the strains was detected based on the analysis of the profiles (Banding) by Total Lab software (Newcastle, England) using Dice method for comparison and UPGMA method for clustering. Based on the electrophoresis and software analysis results, a high level of genetic diversity was observed among *K. pneumoniae* isolates (Figure 2).

A total of 32 different ERIC profiles (E-types) were observed, two common types include 2 isolates, and one common type includes 3 isolates, and other isolates showed a unique pattern. Indeed, high genetic diversity represents non-clonal distribution of *K. pneumoniae* strains in studied hospitals. All *K. pneumoniae* strains isolated from ICU patients' urine samples belonged to type B (78, 13, 20 isolate numbers) and showed similar antibiotics resistance patterns. *K. pneumoniae* strains belonging to type A (72 and 33 isolate numbers) and type C (57 and 6 isolate numbers) were isolated from different sources and wards and showed different antibiotics resistance patterns (Table 1).

Table 1. Characterization of *K. pneumonia* isolates according sex of patients, hospital, wards, source and ERIC type.

Isolate	Male/Female	Hospital code	Ward	Source	E-Type
1	F		ICU	Urine	
72	M		Internal		A
33	F		OP	Urine	A
4	M		ICU		
5	M		Internal	Urine	
6	M		Pediatric	Urine	
78	M		ICU	Urine	B
13	M		ICU	Urine	B
20	M		ICU	Urine	B
10	F		Internal		
11	M		Internal	Urine	
12	M	1	ICU		
13	F	1	Internal	Urine	
14	F		Internal	Urine	
15	F		Internal	Urine	
16	M		ICU		
57	F		OP	Urine	C
6	F		ICU	Urine	C
19	M	1	ICU	Urine	
20	M	1	ICU		
21	M	1	ICU		
8	M		ICU	Urine	
2	M		ICU	Urine	
49	M		ICU	Urine	
5	F		ICU	Urine	
60	M		Internal	Urine	
56	F	2	Emergency	Urine	
57	M	1	Internal		
33	F		Internal	Urine	
66	M			Urine	
77	M		Internal	Urine	
29	F		ICU	Urine	
74	F		Internal	Urine	
12	F		OP	Urine	
90	F		Pediatric	Urine	

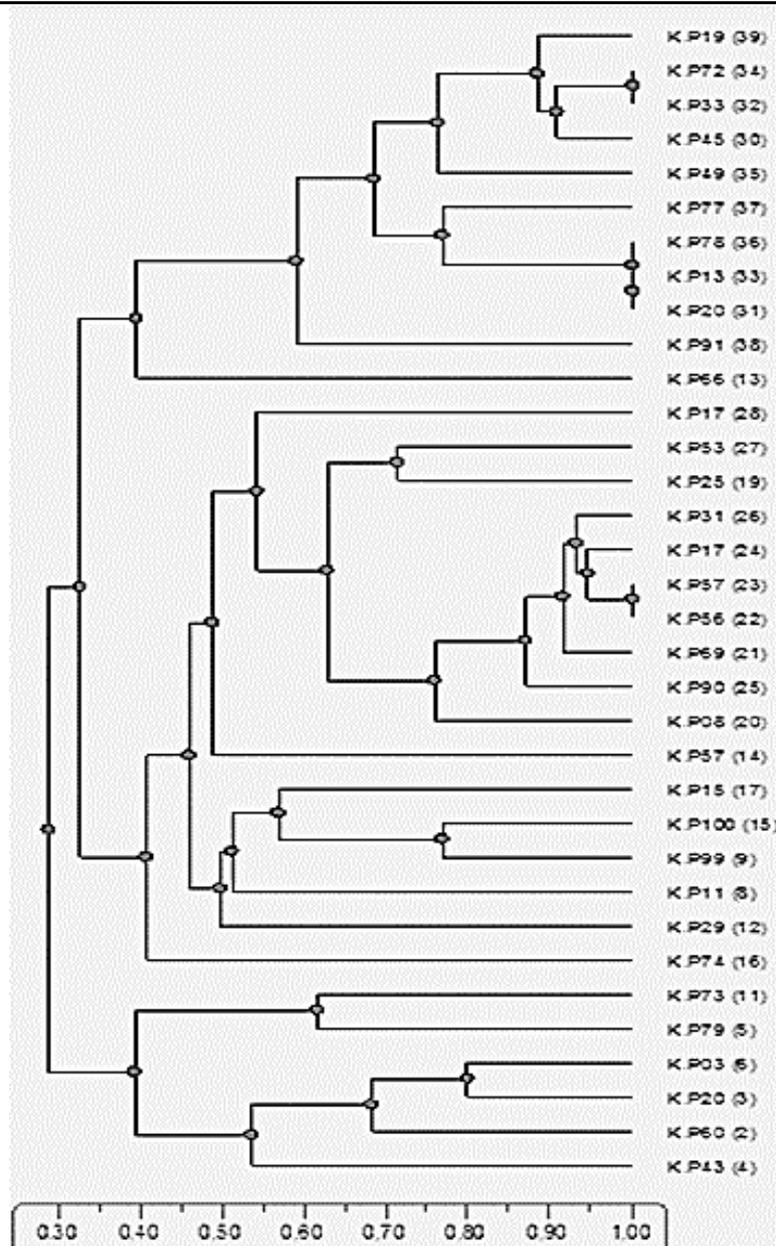


Figure 2. Dendrogram of ERIC-PCR results for 35 *K. pneumoniae* strains isolated from Borujerd hospitals.

5. Discussion

Studies conducted in various countries have shown that *Enterobacteriaceae* family members are often etiologic agents of UTI, among which *K. pneumoniae* causes 16-17% of urinary tract infections, and *E. coli* is the second most common cause of urinary tract infections (16-17). In this study, 83% of *K. pneumoniae* strains were isolated from urine cultures of patients. About 45% of these urine samples were obtained from ICU. In recent years, we have been faced with the emergence of a high level of resistance to available antibiotics in *K. pneumoniae* worldwide, especially in hospitals. In this study, more than 60% of *K. pneumoniae* strains were multi-resistant. The results of this study and other studies indicated the increase in resistance to antibiotics and the emergence of MDR *K. pneumoniae* in hospitals (18-21). In Iran, studies indicated the genetic diversity in

Enterobacteriaceae family members such as *E. coli* and recently *K. pneumoniae* by ERIC-PCR (21-22). In the present study, a high level of genetic diversity among different strains of *K. pneumoniae* was observed. In a study by Ramazanzadeh et al. (2013) conducted on the genetic diversity in clinical isolates of *E. coli* isolated from hospitals of major city in western Iran, ERIC-PCR, allowed typing of the 230 isolates into 205 ERIC types which were then grouped into twenty (C01-C20) as main clusters (21). Seifi et al. (2016) also reported a high level of genetic diversity among the *K. pneumoniae* strains in a hospital in Tehran (22). Results from studies conducted on other countries also indicated the genetic diversity of *K. pneumoniae* strains (23-25). In this study, a total of 32 different ERIC profiles were observed among 35 isolates. Thus, 28 isolates of *K. pneumoniae* were not set in any cluster and formed unique profiles. In fact, the genetic

diversity of *K. pneumoniae* strains represents non-clonal distribution of this bacterium in studied hospitals. All strains in type B were isolated from the same hospital ICU ward and collected from urine and showed similar antibiotics resistance patterns. This may show an inter-ward clonal distribution in this unit while the types A and C, consisting of two isolates and belonging to different wards of hospitals, indicate the intra-ward clonal distribution.

6. Conclusion

Our findings indicate the suitability and usefulness of ERIC-PCR technique for the purpose of molecular typing and epidemiological studies of nosocomial infections and investigating the genetic diversity among hospital pathogens including *K. pneumoniae* strains. Our results showed a high level of diversity in antibiotic resistance and ERIC profiles among *K. pneumoniae* strains isolated from Borujerd hospitals. This diversity causes problems for the treatment of infections due to *K. pneumoniae* strains in hospitals.

Conflict of Interests

The authors declare they have no conflict of interest.

Acknowledgment

We would like to thank all the staff at Microbiology Laboratory of Borujerd hospitals.

Authors' Contribution

All authors contributed equally to this study.

Funding/Support

No fund was received for this research

References

- Ryan KJ, Ray CG. *Medical microbiology: An introduction to infectious diseases*. McGraw-Hill; 2004.
- Podschun R, Ullmann U. *Klebsiella* spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. *Clin Microbiol Rev*. 1998; 11(4):589-603.
- Sanchez UM, Bello TH, Dominguez YM, Mella MS, Zemelman ZR, Gonzalez RG. Transference of extended-spectrum beta-lactamases from nosocomial strains of *Klebsiella pneumoniae* to other species of Enterobacteriaceae. *Rev Med Chil*. 2006; 134(4):415-20.
- Ogawa W, Li DW, Yu P, Begum A, Mizushima T, Kuroda T, et al. Multidrug resistance in *Klebsiella pneumoniae* MGH78578 and cloning of genes responsible for the resistance. *Biol Pharm Bull*. 2005; 28(8):1505-8.
- Crawford JT. Genotyping in contact investigations: A CDC perspective. *Int J Tuberc Lung Dis*. 2003; 7(12): S453-7.
- Lessig R, Zoledziwska M, Fahr K, Kostrzewa M, Dobosz T, Kleeman WJ. Y-SNP genotyping: A new approach in forensic analysis. *Forensic Sci Int*. 2005; 154(2): 128-36.
- Kok J, Buist G, Zomer AL, Van Hijum SA, Kuipers OP. Comparative and functional genomics of lactococci. *FEMS Microbiol Rev*. 2005; 29(3): 411-33.
- Schwartz DC, Cantor CA. Separation of yeast chromosome-sized DNA by pulsed-field gel electrophoresis. *Cell*. 1984; 37(1): 67-75.
- Ranjbar R, Karami A, Farshad SH, Giammanco GM, Mammina C. Typing methods used in the molecular epidemiology of microbial pathogens: A how-to guide. *New Microbiol*. 2014; 37(1): 1-15.
- Hiatt KL, Seal BS. Use of repetitive element palindromic PCR (rep-PCR) for the epidemiologic discrimination of food borne pathogens. *Methods Mol Biol*. 2009; 551: 49-58.
- Barus T, Hanjaya I, Sadeli J, LAY BW, Suwanto A, Yulandi A. Genetic diversity of *Klebsiella* spp. isolated from tempe based on enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC-PCR). *Hayati J Biosci*. 2013; 20 (4):171-6.
- Ewing WH. *Edward and Ewing's Identification of Enterobacteriaceae*. 4th ed. New York: Elsevier Science Publishing Co.; 1986: 169-81.
- Clinical and Laboratory Standard Institute. M07-A9: 2012. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically*. Wayne Pa; 2012.
- Versalovic J, Koeuth T, Lupski JR. Distribution of repetitive DNA sequences in Eubacteria and application to fingerprinting of bacterial genomes. *Nucleic Acids Res*. 1991; 19(24): 6823-31.
- Duggan JM, Oldfield GS, Ghosh HK. Septicemia as a hospital hazard. *J Hosp Infect*. 1985; 6(4): 406-12.
- Henshke-Bar-Meir R, Yinnon AM, Rudensky B, Attias D, Schlesinger Y, Raveh D. Assessment of the clinical significance of production of extended-spectrum beta-lactamases (ESBL) by Enterobacteriaceae. *Infection*. 2006; 34(2): 66-74.
- Cabral AB, Melo RD, Maciel MA, Lopes AC. Multidrug resistance genes, including blaKPC and blaCTX-M-2, among *Klebsiella pneumoniae* isolated in Recife, Brazil. *Rev Soc Bras Med Trop*. 2012; 45 (5): 572-8.
- Wei ZQ, Chen YG, Yu YS, Lu WX, Li LJ. Nosocomial spread of multi-resistant *Klebsiella pneumoniae* containing a plasmid encoding multiple beta-lactamases. *J Med Microbiol*. 2005; 54(9): 885-8.
- Perez-Moreno MO, Centelles-Serrano MJ, Cortell-Ortola M, Fort-Gallifa I, Ruizb J, Llovet-Lombartea MI, et al. Molecular epidemiology and resistance mechanisms involved in reduced susceptibility to amoxicillin/clavulanic acid in *Klebsiella pneumoniae* isolates from a chronic care centre. *Int J Antimicrob Agents*. 2011; 37(5): 462-6.
- Peerayeh SN, Rostami E, Siadat SD, Derakhshan S. High rate of aminoglycoside resistance in CTX-M-15 producing *Klebsiella pneumoniae* isolates in Tehran, Iran. *Lab Med*. 2014; 45(3):231-7
- Ramazanzadeh R, Zamani S, Zamani S. Genetic diversity in clinical isolates of *Escherichia coli* by enterobacterial repetitive intergenic consensus (ERIC)-PCR technique in Sanandaj hospitals. *Iran J Microbiol*. 2013; 5(2):126-31.
- Seifi K, Kazemian H, Heidari H, Rezagholizadeh F, Saeed Y, Shirvani F, et al. Evaluation of biofilm formation among *Klebsiella pneumoniae* isolates and molecular characterization by ERIC-PCR. *Jundishapur J Microbiol*. 2016; 9(1): e30682.
- Yan JJ, Hsueh PR, Lu JJ, Chang FY, Shyr JM, Wan JH, et al. Extended-spectrum beta-lactamases and plasmid-mediated AmpC enzymes among clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* from seven medical centers in Taiwan. *Antimicrob Agents Chemother*. 2006; 50(5):1861-4.
- Edelstein M, Pimkin M, Palagin I, Edelstein I, Stratchounski L. Prevalence and molecular epidemiology of CTX-M extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in Russian hospitals. *Antimicrob Agents Chemother*. 2003; 47(12):3724-32.
- Lagha N, Abdelouahid DE, Hassaine H, Robin FE, Bonnet R. First characterization of CTXM-15 and DHA-1-lactamases among clinical isolates of *Klebsiella pneumoniae* in Laghouat Hospital, Algeria. *Afr J Microbiol Res*. 2014; 8(11):1221-7.

How to cite this article: ParsaieMehr V., Shokoohizadeh L., Mirzaee M., Savari M. Molecular Typing of Clinical Isolates of *Klebsiellapneumoniae* by Enterobacterial Repetitive Intergenic Consensus (ERIC)-PCR. *Infection, Epidemiology and Microbiology*. 2017; 3(4): 112-116.