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**Original article**

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

Vahideh Parsaie Mehr[1], Leili Shokoohizadeh[2]*, Mohsen Mirzaee[3], Mohammad Savari[4]

[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 Shokoohizadeh, Department of Microbiology, Faculty of Medicine, Hamadan University of Medical Sciences, Hamadan, IR Iran, E-mail: L.shokoohizadeh@umsha.ac.ir, Tel: +98 8138276295, Fax: +98 8138276299

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**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. pneumonia* isolates were collected from Borujerd hospitals during April to September 2015. After detection and confirmation of *K. pneumonia* 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. pneumonia* isolates was examined by ERIC - PCR technique.
**Results**: Antibiotic susceptibility testing showed that among *K. pneumonia* 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 geniuses; 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. pneumonia* 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. pneumonia* susceptibility to 13 antibiotics including ampicillin, gentamicin, chloramphenicol, tetracycline, ceftriaxone, nitrofurantoin, imipenem, azithromycin, ciprofloxacin, amoxicillin-clavulanic acid,





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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 94ºC for 3 min, followed by 35 cycles of denaturation at 94ºC for 1 min, annealing at 48ºC for 1 min, extension at 72 ºC for 2 min, and a final cycle of amplification at 72ºC 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.

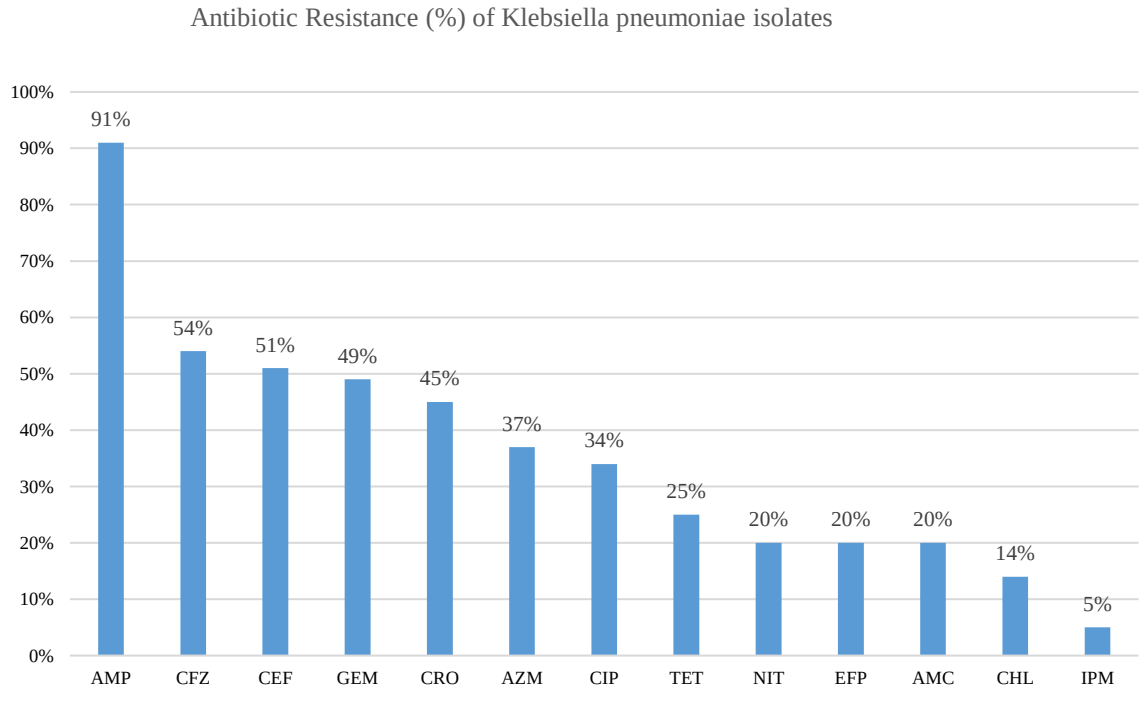


**Figure 1. Prevalence (%) of antibiotic resistance of *Klebsiella pneumoniae* isolates in Borujerd hospitals.**
**AMP; ampicillin, CFZ; ceftazidime, CEF; cephalothin, GEM; gentamicin, CRO: ceftriaxone, AZM; azithromycin, CIP; ciprofloxacin, TET; tetracycline, NIT; nitrofurantoin, EFP; cefepime, AMC; ampicillin-clavulanic acid, CHL; chloramphenicol, IMI; imipenem**



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*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 | |



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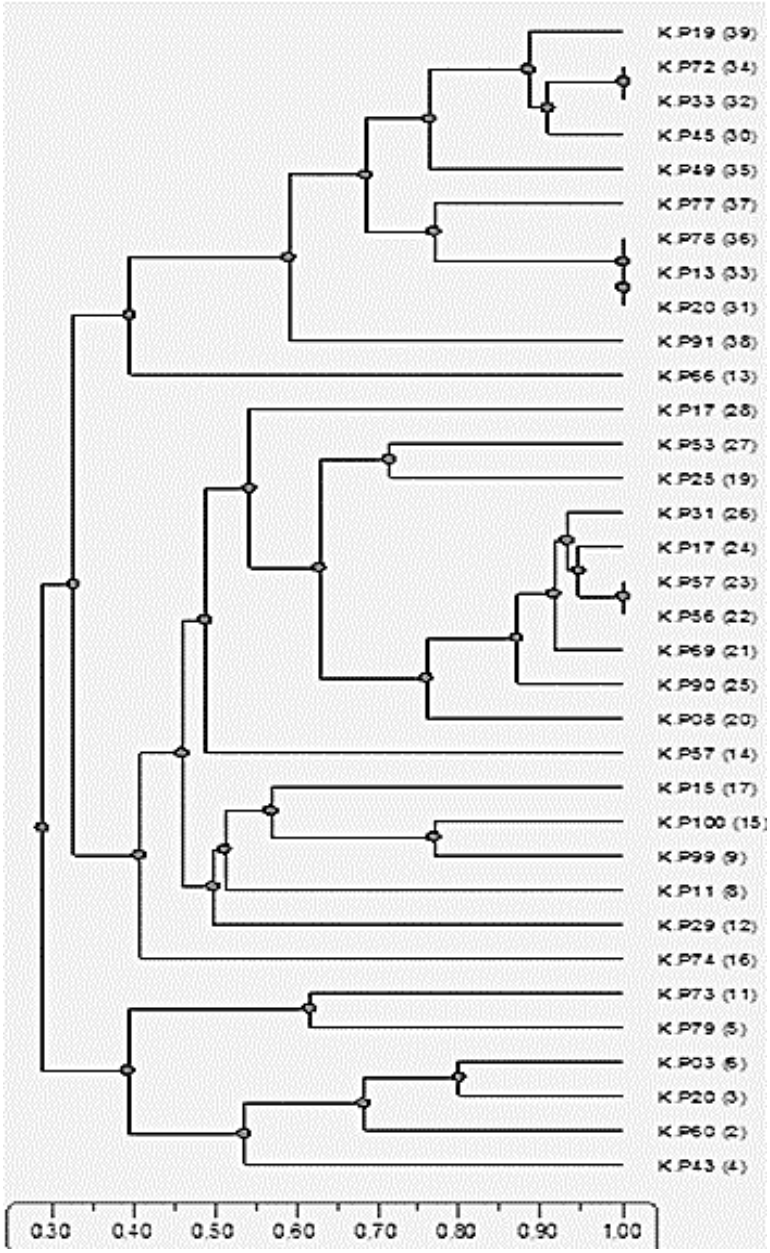


**Figure 2. Dendrogram of ERIC-PCR results for 35 *K. pneumoniae* strains isolated from Borujerd hospitals.**

## 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





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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 whiles 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.

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## Authors' Contribution

All authors contributed equally to this study.

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