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

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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. 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 (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 GenBank, 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. 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,
Molecular Typing of Clinical Isolates of Klebsiella pneumoniae

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

3.3. DNA extraction
Genomic DNA was 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 cefepime; 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; cefazidine, CEF; cephalothin, GEM; gentamicin, CRO; ceftriaxone, AZM; azithromycin, CIP; ciprofloxacin, TET; tetracycline, NT; nitrofurantoin, EFP; cefepime, AMC; amoxicillin-clavulanic acid, CHL; chloramphenicol, IMI; imipenem](image-url)


113
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>
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<th>Source</th>
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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

Figure 2. Dendrogram of ERIC-PCR results for 35 K. pneumoniae strains isolated from Borujerd hospitals.
diversity of \textit{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 \textit{K. pneumoniae} strains. Our results showed a high level of diversity in antibiotic resistance and ERIC profiles among \textit{K. pneumoniae} strains isolated from Boroujerdi hospitals. This diversity causes problems for the treatment of infections due to \textit{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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