Subtyping of *Escherichia coli* Clinical Specimens Resistant to Beta-Lactam Antibiotics Using Pulsed-Field Gel Electrophoresis

Hamid LavaKhamseh¹, Parviz Mohajeri², Abbas Farahani³, Samaneh Rouhi¹⁴, Pegah Shakib¹⁴, Rashid Ramazanzadeh¹⁴∗

¹Student Research Committee, Kurdistan University of Medical Sciences, Sanandaj, Iran; ²Department of Microbiology, Kermanshah University of Medical Sciences, Kermanshah, Iran; ³Department of Microbiology, Jundishapur University of Medical Sciences, Ahvaz, Iran; ⁴Cellular and Molecular Research Center, Kurdistan University of Medical Sciences, Sanandaj, Iran; ⁵Department of Microbiology, Kurdistan University of Medical Sciences, Sanandaj, Iran

Received Jul 14, 2017; Accepted Oct 11, 2017

**Introduction:** Pulsed-Field Gel Electrophoresis (PFGE) is used for molecular fingerprinting of *Escherichia coli* (E. coli) isolates and determining their origin. The present study aimed to determine the subtypes of E. coli isolated from clinical specimens resistant to beta-lactam antibiotics. **Methods:** 120 E. coli isolates were collected from outpatients and inpatients admitted to Toohid and Besat hospitals in Sanandaj city, Iran. Antimicrobial susceptibility test was performed using the disk diffusion method. The E. coli isolates resistant to beta-lactam antibiotics, were analyzed by PFGE and the generated images interpreted by molecular BioNumerics software V7.1. **Results:** The resistance ratios of isolates to antibiotics were as follows: cefixime (67.5%), ampicillin (53.33%), cefotaxime (41.66%), ceftriaxone (36.66%), ceftazidime (24.16%), cefitoxime (20%), cephalothin (15%) and imipenem (0.83%). The 21 E. coli strains that were resistant to beta-lactam antibiotics were clustered by visual analyses. DNA fingerprinting of E. coli strains by PFGE revealed five patterns, pulsortypes I, II, III, IV, and V. A high similarity (75-80%) was observed among isolates obtained from outpatients and inpatients. **Conclusion:** Resistance to beta-lactam antibiotics was observed among the E. coli isolates. These isolates had rates of resistance ranging from 0.8% for imipenem as the lowest, to 68.2% for cefixime as the highest. PFGE detected a high rate of similarity between resistant isolates to beta-lactam antibiotics. This similarity suggests that the strains isolated from different patients have a common ancestor. Our findings necessitate implementing appropriate control measures for antibiotic prescription. *J Med Microbiol Infect Dis, 2017, 5 (1-2): 5 pages.*

**Keywords:** *Escherichia coli*, Beta-Lactam Antibiotics, Pulsed-Field Gel Electrophoresis, Genetic relationship.

INTRODUCTION

*Escherichia coli* (E. coli) bacteria are normal inhabitants of intestines of most animals. Some stains can cause a wide range of intestinal and extraintestinal diseases, such as diarrhea, urinary tract infections (UTI), septicemia and neonatal meningitis [1]. *Escherichia coli* is frequently isolated from patients with urinary or gastrointestinal infections at hospitals and other medical centers [2]. Beta-lactam antibiotics constitute a significant class of chemotherapeutic agents currently employed in the treatment of diseases caused by E. coli. Easy access, low toxicity, high efficiency, and effectiveness are the reasons for widespread use of beta-lactam antibiotics in medicine [3, 4].

In recent years, antibiotic resistance, especially to beta-lactam combinations has become a significant problem for the empirical treatment of patients. Antimicrobial resistance in E. coli has created concern, particularly E. coli strains with resistance to beta-lactams, i.e., those that are resistant to ampicillin and cephalosporins [5-8]. The rate of resistance to antibiotics in bacteria may differ due to irregular prescription, lack of proper policies for using antibiotics, gene mutations, and transfer of resistant genes by transportation tools such as plasmids and bacteriophages from one bacterium to another that can result in resistance in other bacteria [9, 10].

On the other hand, molecular fingerprinting of bacterial isolates for identifying their clonal distribution and the predominant type of isolates that are resistant to antibiotics is an approach for detecting the origin of these isolates and preventing and controlling the dispersion of resistant strains as well [11, 12]. Pulsed-Field Gel Electrophoresis (PFGE) method is the most successful fingerprinting technique for molecular epidemiology purposes and applies to a vast range of pathogens and bacterial species such as *E. coli* isolates [13-15].

*Correspondence:* Rashid Ramazanzadeh
Cellular and Molecular Research Center and Department of Microbiology, Faculty of Medicine, Kurdistan University of Medical Sciences, Pasdaran St., Sanandaj, Iran, 6617713446
Email: atrop_t51@yahoo.com
Tel/Fax: +98 (87) 33664654

http://jommid.pasteur.ac.ir
According to different studies that deployed PFGE, genetic profiles of *E. coli* isolates can be different according to their place of origin and their way of spread in the environment. By genetic analysis of the bacteria strains using a different method such as PFGE specific information can be obtained related to antibiotic resistance, origin and clonal similarity of bacteria [16]. This study aimed to identify source, origin, relationship, and subtyping of *E. coli* isolates obtained from clinical specimens resistant to beta-lactam antibiotics, using PFGE in the city of Sanandaj, Kurdistan province, Iran.

**MATERIAL AND METHODS**

**Bacterial Isolates.** We collected 120 isolates of *E. coli* from various specimens of hospitalized patients and outpatient during November 2013 to April 2014 from two teaching hospitals of Toohid and Besat in the city of Sanandaj, Kurdistan province, Iran. The specimens included: urine (107; 89.16%), stool (2; 1.66%), blood (10; 8.33%) and cerebrospinal fluid (CSF) (1; 0.83%). The bacteria were isolated and identified by the standard bacteriological procedures and biochemical tests. For detection of Enterobacteriaceae, the isolates were first cultured on MacConkey and Eosin-methylene blue (EMB) agar (Merck, Germany). Then Gram-stain, oxidase and catalase, indole, methyl red (MR), Voges-Proskauer (VP) and Simmons’ citrate (IMViC) and Sulfur-Indole-Motility (SIM) (motility test) tests (Merck, Germany) were applied to identify the *E. coli* species [17]. Final confirmation of the isolates was done using a standard Enterobacteriaceae detection system; Analytical Profile Index (API) 20E Kit (Biomerieux, Franc), a biochemical panel for identification and differentiation of members of the Enterobacteriaceae family. This kit included twenty mini-test chambers in plastic strip containing dehydrated media, which were used for each test [18].

**Antibiotic Susceptibility.** The Kirby-Bauer disk diffusion method was used for identification of isolates resistant to beta-lactam antibiotics according to clinical and laboratory standards institute (CLSI) [19]. First, a McFarland concentration of $10^9$ CFU/ml was prepared and inoculated into Mueller-Hinton agar (MHA) (Difco, Detroit, Mich., USA). The tested antibiotics were ampicillin (Conventional), cefixime, cefalothin, ceftazidime, ceftriaxone, and imipenem (Cephalosporins). (Mast Co., UK). The strain *E. coli* ATCC 25922 was used as positive control [20].

**Pulsed-Field Gel Electrophoresis.** In this part, 21 isolates of *E. coli* that were resistant to beta-lactam antibiotics were selected for PFGE analysis. The strain *E. coli* ATCC 25922 was included in the assay as the external reference. Genotyping of all organisms was performed by the digestion enzyme *XbaI* (Fermentas, USA). Electrophoresis in a pulsed-field electrophoresis system (Chef Mapper; Bio-Rad Laboratories, Hercules, CA, USA) was conducted according to the following program: temperature 14°C; voltage 6V/cm; reorientation angle, 120°; switch ramp 2.2-54. 2 s for 19 h. The gels were stained with ethidium bromide, and the developed patterns were photographed with a UV gel Doc (BIO-RAD, USA). The Lambda Ladder PFGE Marker (NEB, US) was used as the standard/reference strain. Analysis of banding patterns was done with the bioNumerics software V7.1[21].

**RESULTS**

Out of the 120 *E. coli* isolates, 81 (67.50%) were resistant to beta-lactam antibiotics. From 107 isolates originated from urine samples, 75 (92.59%) showed resistant to different beta-lactams antibiotics. In stool, all 2 (100%) isolates were resistant. Also, 4 (40%) of 10 *E. coli* isolates from blood were resistant to beta-lactams antibiotics in this research. The resistant rate to antibiotics were as follows: CEF (67.5%), AMP (53.3%), CTX (41.66%), CRO (36.66%), CAZ (24.16%), ZOX (20%), CEF (15%) and IPM (0.83%). The highest rate of resistance was against CEF and the lowest to IPM (Table 1). The PFGE analysis clustered 21 beta-lactam-resistant *E. coli* isolates into five pulsortypes, I, II, III, IV, and V. In cluster I, 2 samples had genetic similarity of about 77% and in cluster II, in 3 samples genetic similarity of about 77% was observed. Also in each of clusters III, IV, and V, 2 samples had genetic similarity of about 75%, 80% and 75%, respectively. The pulsortypes I and IV were dominant in outpatients and hospitalized patients, respectively. The isolates in pulsortype IV shared the highest similarity (about 80%) compared to other clusters. Most of the resistant isolates belonged to the cluster II and IV (Fig. 1) (Table 2).

**DISCUSSION**

Resistance to beta-lactam antibiotics is a serious problem for patients and its prevalence among clinical isolates is on the increase [1]. A study showed that among 100 *E. coli* isolates collected from different clinical specimens, 56% were ESBL-producing; also, 95% of ESBL-producing isolates were resistant to CRO and CTX, 93% to CAZ, 86% to azithromycin (AZM), 79% to cefazolin (CFZ), and 43% to IPM [9].

### Table 1. Antimicrobial susceptibility pattern of *E. coli* isolates

<table>
<thead>
<tr>
<th>Type of Antibiotic</th>
<th>No. of Sensitive (%)</th>
<th>No. of Resistant (%)</th>
<th>No. of tested isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin (AMP)</td>
<td>56 (46.6)</td>
<td>64 (53.3)</td>
<td>120</td>
</tr>
<tr>
<td>Cefixime (CFM)</td>
<td>39 (32.5)</td>
<td>81 (67.5)</td>
<td>120</td>
</tr>
<tr>
<td>Cefotaxime (CTX)</td>
<td>70 (58.33)</td>
<td>50 (41.66)</td>
<td>120</td>
</tr>
<tr>
<td>Cefalothin (CEF)</td>
<td>102 (85)</td>
<td>18 (15)</td>
<td>120</td>
</tr>
<tr>
<td>Ceftazidime (CAZ)</td>
<td>91 (75.83)</td>
<td>29 (24.16)</td>
<td>120</td>
</tr>
<tr>
<td>Ceftriaxone (ZOX)</td>
<td>96 (80)</td>
<td>24 (20)</td>
<td>120</td>
</tr>
<tr>
<td>Imipenem (IPM)</td>
<td>76 (63.33)</td>
<td>44 (36.66)</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>119 (99.16)</td>
<td>1 (0.83)</td>
<td>120</td>
</tr>
</tbody>
</table>
Table 2. Antibiotic resistance pattern in isolates of different clusters (N, Sample Number; R, Resistant; S, Sensitive; I, Intermediate, considered as resistant in this study)

<table>
<thead>
<tr>
<th>PFGE Cluster</th>
<th>N</th>
<th>AMP</th>
<th>CFM</th>
<th>CTX</th>
<th>CEF</th>
<th>CAZ</th>
<th>ZOX</th>
<th>CRO</th>
<th>IPM</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>13</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>I</td>
<td>S</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>I</td>
<td>S</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>II</td>
<td>106</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>I</td>
<td>S</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>69</td>
<td>I</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>III</td>
<td>68</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>23</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>IV</td>
<td>102</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>27</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>V</td>
<td>39</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>I</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>82</td>
<td>I</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
</tbody>
</table>

Fig. 1. PFGE dendrogram of E. coli isolates resistant to beta-lactam antibiotics. The vertical black line shows the 70% cut-off (inp, hospitalized patients; outp, outpatient; U, urine specimens; S, stool specimens; B, blood specimens; B, Besat hospitals; T, Toohid hospitals)

The results in our study showed that 67.5% of E. coli isolates were resistant to beta-lactam antibiotics, which is a lower rate compared to the previous study. In previous studies from Iran on 148 E. coli isolates from patients, the highest resistance rate was observed to CFM (57.9%) [22] and the lowest rate to IMP (5%) [23]. In our study, the highest and lowest rates of resistance were to CFM (68.2%) and IPM (0.8%), respectively. In our results, the high similarity (75-80%) between isolates from outpatients and inpatients indicates the prevalence of these isolates among the community and hospitals as well. Different studies have shown subtyping of bacteria using PFGE. This method showed the genetic diversity between 155 E. coli strains that were resistant to antibiotics [12]. In another study different pulsortypes were observed in 29 out of 40 carbapenem enzymes-producing Enterobacteriaceae isolates using PFGE [13]. A study showed that among the isolates, 80-90% of similarity was observed with 4 clusters [16].
survey on 200 uropathogenic *E. coli* (UPEC) isolates showed that 24.5% of them were positive for ESBL by the disk diffusion and PCR methods, and PFGE analysis exhibited 10 different genotypes among the isolates [24]. In another research PCR detected blaOXA-51-like, blaOXA-23-like and blaOXA-24-like/40-like genes in 48 *Acinetobacter baumannii* strains, and PFGE detected 11 unique clones [25]. In our study, PFGE analysis clusters the *E. coli* isolates in pulsotypes I, II, III, IV, and V. We had some problems in our study, e.g., the *E. coli* isolates from stool samples did not grow and we had to work only on two sample. Also due to the lack of financial resources, only 21 from 81 resistant isolates were used for PFGE analysis. However, in this study, heterogeneity was observed in these clusters, and no particular source can be cited for the spread of these isolates. In the current study, a high level of resistant to beta-lactam antibiotics (0.8% to 68.2%) was observed among *E. coli* isolates obtained from hospitalized patients and outpatients and this was an indication of a widespread occurrence of beta-lactam antibiotics’ resistance among the isolates. Also, the pulsotype analysis showed a high similarity between resistance isolates. So an efficient and sustained control measures and proper antibiotic policies should be observed in the hospitals.

ACKNOWLEDGEMENT

This is part of a MSc student thesis. The authors wish to extend their gratitude to the Research Deputy of the Kurdistan University of Medical Sciences for their financial support.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

REFERENCES


