*Osong Public Health Res Perspect* 2015 6(3), 201–204
http://dx.doi.org/10.1016/j.phrp.2015.02.001
pISSN 2210-9099 eISSN 2233-6052

ORIGINAL ARTICLE

# High Prevalence of AmpC β-Lactamases in Clinical Isolates of *Escherichia coli* in Ilam, Iran

Abbas Maleki [a], Afra Khosravi [a], Sobhan Ghafourian [a], Iraj Pakzad [a], Shiva Hosseini [a], Rashid Ramazanzadeh [b], Nourkhoda Sadeghifard [a,*]

[a]*Clinical Microbiology, Clinical Microbiology Research Center, Ilam University of Medical Science, Ilam, Iran.*
[b]*Cellular & Molecular Research Center and Microbiology Department, Faculty of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran.*

Received: November 1, 2014
Revised: December 23, 2014
Accepted: February 2, 2015

**KEYWORDS:**
AmpC β–lactamases,
*E. coli*,
ESBLs

**Abstract**
**Objectives:** Widespread use of β-lactam antibiotics could cause resistance to this group of antibiotics in pathogenic bacteria through the production of the enzyme β-lactamases. The aim of this study is to determine the molecular detection of AmpC β-lactamases among clinical *Escherichia coli* isolated from Ilam hospitals in Ilam, Iran.
**Methods:** One hundred and twelve clinical isolates of *E. coli* were collected from hospitalized patients and were identified by biochemical tests. They were evaluated for extended spectrum beta-lactamases (ESBLs) production, and the positive strains were subjected to AmpC enzymes; for detection of AmpC cluster genes, multiplex polymerase chain reaction was applied.
**Results:** The analysis showed 62.5% of isolates were ESBLs positive and that five strains revealed the AmpC cluster genes. This is the first report of *FOXM* cluster genes in *E. coli* in Iran.
**Conclusion:** Based on our results, the prevalence of AmpC β-lactamases is increasing in Iran, which caused failure in antibiotic therapy. So, the current study recommended the revision of antibiotic policy in Iranian hospitals.

## 1. Introduction

Production of β-lactamases are the main mechanism of resistance to β-lactam antibiotics in bacteria. These enzymes hydrolyze the β-lactam ring, which leads to the inactivation of β-lactam antibiotics. In recent years, new types of β-lactamase enzymes including extended spectrum beta-lactamases (ESBLs), AmpC β-lactamases, and metallo β-lactamases have emerged. [1–3]. These enzymes are able to hydrolyze broad-spectrum cephalosporins including ceftazidime, ceftriaxone, cefepime, and monobactams (aztreonam and cephamycin). AmpC β-lactamases are resistant to 7-alfa methoxy cephalosporin and monobactams. In the late 1980s, the plasmid-borne AmpC β-lactamases were found in bacteria such as *Escherichia coli* and

*Corresponding author.
E-mail: sadeghifard@gmail.com

*Klebsiella* spp [4,5]. Currently, resistance to β-lactam antibiotics via AmpC β-lactamases in *E. coli* strains is a clinical concern [6,7]. It seems necessary to identify the AmpC β-lactamases producing bacteria in clinical isolates. For this propose we aim to identify the prevalence of AmpC β-lactamases genes in clinical isolates of *E. coli*.

## 2. Materials and methods

### 2.1. Bacterial isolates

One hundred and twelve clinical isolates of *E. coli* were collected during the period February to July 2012 from hospitalized patients in Ilam hospitals (Ilam, Iran). All the isolates were identified by biochemical tests.

### 2.2. Determination of antibiotic susceptibility

The antibiotic susceptibility assay was performed according to Clinical and Laboratory Standards Institute (CLSI) guidelines. An inoculum containing $1.5 \times 10^8$ CFU/mL of bacteria were placed on Mueller-Hinton agar. The antibiotics were cefotaxime (30 μg), ceftazidime (30 μg), and cefteriaxone (30 μg). In addition, cefoxitin as a marker for AmpC production was used. AmpC β-lactamase production was evaluated by cefoxitin minimum inhibitory concentrations (MICs) using the microdilution broth method according to CLSI guidelines [8].

### 2.3. DNA extraction

*E. coli* strains were cultured in lysogeny broth at 37°C overnight, and then DNA was extracted using the DNA extraction kit (Bionner Company, Daejeon, Korea).

### 2.4. Multiplex polymerase chain reaction assay

Multiplex polymerase chain reaction (PCR) assay was performed for identification of the most common plasmid mediated AmpC cluster genes including *ACC*, *FOX*, *MOX*, *DHA*, *CIT*, and *EBC*. The PCR system (25 μL) was composed of 1× PCR buffer, 2 mM $MgCl_2$, 0.2 mM dNTP, 10 pmol of primers, 1U Taq DNA polymerase (Ferments, UK), and a total of 1 μL of DNA extract was used for each reaction. The sequences of primers are presented in Table 1. PCR conditions were 35 cycles of amplification under the following conditions: denaturation at 95°C for 30 seconds, annealing at 64°C for 1 minute, and extension at 72°C for 1 minute. Cycling was followed by a final extension at 72°C for 10 minutes. PCR product (10 μL) was analyzed using gel electrophoresis with 1.5% agarose. Gels were stained with DNA Safe Stain and visualized using gel documentation. A 100-bp DNA ladder was used as a molecular marker.

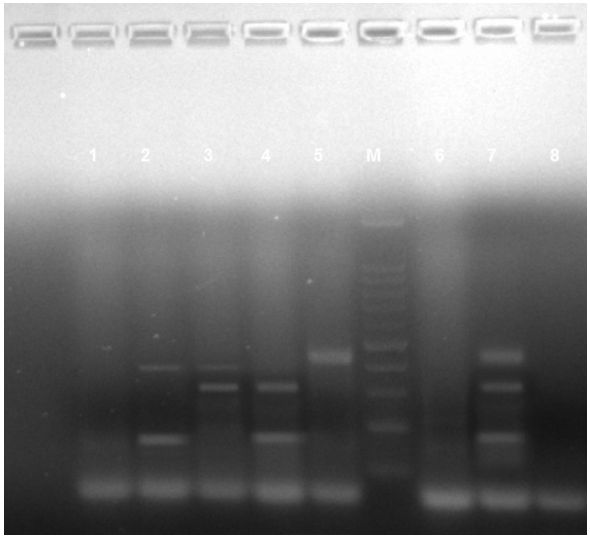


**Figure 1.** 1 and 8 = negative control; 2 = FOXM (190 bp) and DHAM (405 bp); 3 = EBCM (302 bp) and DHAM (405 bp); 4 = EBCM (302 bp) and FOXM (190 bp); 5 = CITM (462 bp); M = size marker 100 bp; 6 = FOXM (190 bp); 7 = FOXM (190bp), EBCM (302 bp) and CITM (462 bp).

**Table 1.** Sequences of primers.

| Primer | Sequence (5′ to 3′) | Expected amplicon size (bp) | Annealing |
|---|---|---|---|
| MOXM-F | GCT GCT CAA GGA GCA CAG GAT | 520 | 64 |
| MOXM-R | CAC ATT GAC ATA GGT GTG GTG G | | |
| CITM-F | TGG CCA GAA CTG ACA GGC AAA | 462 | 64 |
| CITM-R | TTT CTC CTG AAC GTG GCT GGC | | |
| DHAM-F | AAC TTT CAC AGG TGT GCT GGG T | 405 | 64 |
| DHAM-R | CCG TAC GCA TAC TGG CTT TGC | | |
| ACCM-F | AAC AGC CTC AGC AGC CGG TTA | 346 | 64 |
| ACCM-R | TTC GCC GCA ATC ATC CCT AGC | | |
| EBCM-F | TCG GTA AAG CCG ATG TTG CGG | 302 | 64 |
| EBCM-R | CTT CCA CTG CGG CTG CCA GTT | | |
| FOXM-F | AAC ATG GGG TAT CAG GGA GAT G | 190 | 64 |
| FOXM-R | CAA AGC GCG TAA CCG GAT TGG | | |