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ORIGINAL ARTICLE

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

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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

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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 1.5×10^8 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*,
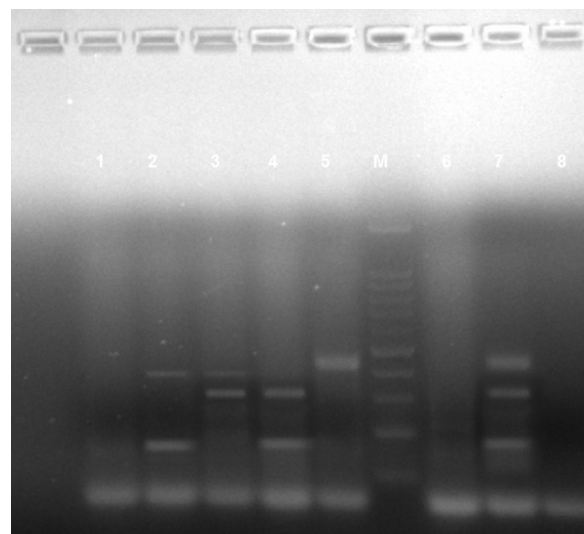

Figure 1. 1 and 8 = negative control; 2 = FOXM (190 bp) and DHAM (405 bp); 3 = EBCM (302 bp) and DHAM (405 bp); 4 = EBCM (302 bp) and FOXM (190 bp); 5 = CITM (462 bp); M = size marker 100 bp; 6 = FOXM (190 bp); 7 = FOXM (190bp), EBCM (302 bp) and CITM (462 bp).

FOX, *MOX*, *DHA*, *CIT*, and *EBC*. The PCR system (25 μL) was composed of 1× PCR buffer, 2 mM MgCl₂, 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.

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		
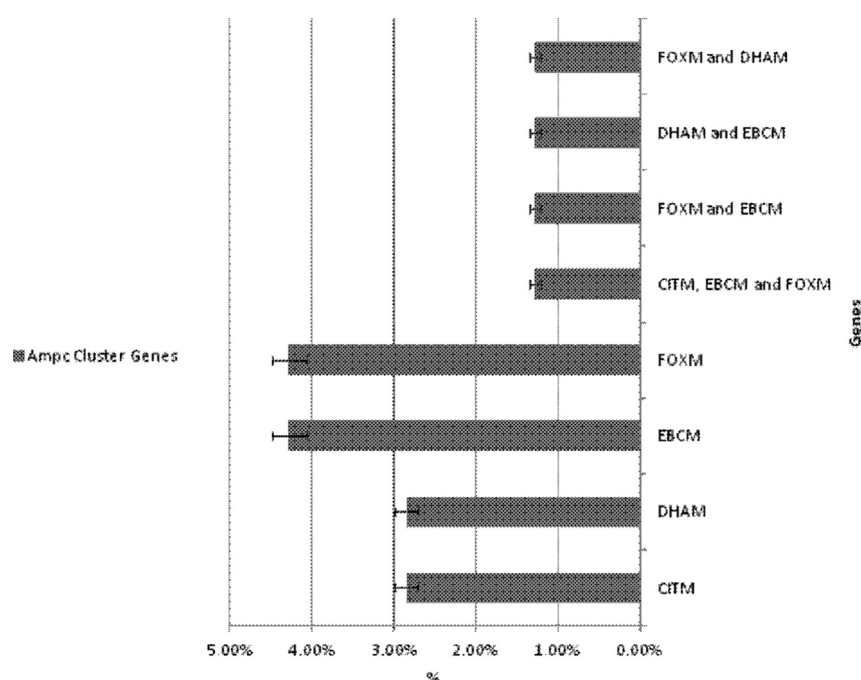
Figure 2. Prevalence of different AmpC cluster genes in ESBL positive *E. coli* strains.

3. Results

3.1. ESBLs were presented in large scale of isolates

The analysis revealed 62.5% of isolates ($n = 70$) were ESBLs positive and that antibiotic susceptibility was resistant to ceftazidime, cefotaxime, and cefteriaxone. MICs microdilution broth test for identification of cefoxitin resistance isolates showed that among the 70 ESBL-positive strains, 40% ($n = 28$) of isolates exhibited resistance to cefoxitin (MIC $\geq$ 32 mg/L).

3.2. Multiplex PCR showed AmpC cluster genes in ESBL-positive strains

The multiplex PCR results of 70 ESBL-positive strains revealed that two isolates (85%) were positive for *CITM* cluster gene, two isolates (85%) were positive for *DHAM* cluster gene, three isolates (28%) were positive for *EBCM* cluster gene, and three isolates (28%) were positive for *FOXM* cluster gene. One strain presented *CITM*, *EBCM*, and *FOXM* cluster genes simultaneously. One strain had *FOXM* and *EBCM* cluster genes, one strain showed *DHAM* and *EBCM* cluster genes, and finally, in one strain *FOXM* and *DHAM* cluster genes were observed (Figures 1 and 2). *ACCM* and *MOXM* cluster genes were not found in this study.

4. Discussion

β-Lactamases are the main defensive system against β-lactam antibiotics. As long as β-lactam antibiotics have been used in clinical treatment, β-lactamases have had a main role in treatment failure. Approximately 30 years ago, researchers identified the plasmids responsible for antibiotics resistance genes in *E. coli* and other Enterobacteriaceae [9]. The chromosomal AmpC was found in a small scale in *E. coli*, whereas most strains showed the AmpC enzyme in plasmids in *E. coli* [4]. In this study, among 112 isolates of *E. coli*, 70 isolates indicated as ESBL positive phenotypically showed that 28 of them had the AmpC phenotype. These 28 isolates were resistant to cefoxitin (MIC $\geq$ 32 mg/L). According to the PCR assay results, we found four types of cluster genes in five isolates (14%) indicating the prevalence of AmpC β-lactamases in Iran. Many important factors, such as the indiscriminate use and availability of antibiotics and their ease of preparation from pharmacies, could be noted. The study by Mansouri et al [10] indicated that among 88 clinical isolates of *E. coli*, five (7%) had AmpC genes that belong to *DHAM*, *CITM*, and *EBCM* cluster genes. Japoni-Nejad et al [11] showed that in Iran among 100 isolates of *Klebsiella pneumonia*, 19 isolates had AmpC genes that belong to *DHAM*, *CITM*, *EBCM*, and *MOXM* cluster genes. The study by Dallai et al [12] showed that among 128 clinical isolates, five were positive for *DHA* cluster gene. According to our investigations in this study we reported the first case of *FOXM* cluster gene in *E. coli* in Iran. Based on the aforementioned studies, the prevalence of AmpC β-lactamases is increasing in Iran.

Conflicts of interest

The authors declare no conflicts of interest.

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