

Molecular Characterization of AmpC β -Lactamases among *Klebsiella pneumoniae* Isolated from Ilam and Tehran Hospitals, from Iran

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(Received: 27 July 2013; accepted: 19 September 2013)

The common use of beta lactam antibiotics for treatment of bacteria infections leads to increase the world wide microbial resistance by producing beta lactamase enzyme among clinical isolates . In recent years, the production of broad-spectrum beta-lactamase enzymes in clinical isolates , especially *E.coli* and *Klebsiella* bacteria are common. Typical Ampc enzymes (class C- ESBLs) confirm resistance to most oxyimino cephalosporins. The aim of this study is to determine the prevalence of AmpC type extended spectrum beta lactamases genes in clinical isolates of *Klebsiella pneumoniae*. 108 clinical sample of *K.pneumoniae*, isolated from hospitalized patients procured from two hospitals in Ilam and Tehran. To identify Ampc genes, PCR method was used. 95/3 percent of isolates were resistant to Cefoxitin , 49 isolates were positive for FOXM cluster genes , 35 were positive for DHAM cluster genes and 6 were positive for CITM cluster genes. Our results showed that among of clinical isolates of *Klebsiella pneumoniae*, prevalence broad-spectrum beta-lactamase enzymes and Ampc genes are relatively high.

Key words: *Klebsiella pneumoniae*, Beta-lactamase broad-spectrum, Ampc genes.

β -lactamases are the Bactrian enzymes that are responsible for their resistance to β -lactam antibiotics as like penicillins , cephamycins, and carbapenems¹ . The lactamase enzyme breaks the $\hat{\alpha}$ -lactam ring and deactivating the molecule's antibacterial properties. $\hat{\alpha}$ -lactamases are found as a mechanism to induction of resistance to many β -lactam antibiotics. Resistant to antimicrobial agents are increased in the world wide² AmpC β -lactamases first appeared in the late 1970's as an inducible resistance in organisms that would

overproduce their chromosomal Ampc gene, probably due to the use of cephamycins and the introduction of $\hat{\alpha}$ -lactamase inhibitor combinations. The first plasmid-mediated AmpC β -lactamase was isolated in 1988 from a *Klebsiella pneumoniae*^{3,4}. AmpC type β -lactamases are commonly isolated from extended-spectrum cephalosporin-resistant Gram-negative bacteria⁵ .The increasing widespread of plasmid-mediated AmpC β -lactamases in members of the Enterobacteriaceae is a subject of great concern worldwide. Prevalent plasmid-mediated Ampc $\hat{\alpha}$ -lactamase detection methods include an insusceptibility screen using cephamycin (usually

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cefoxitin) disc^{6,7}. plasmid-mediated Ampc β -lactamase resistance in *E.coli* and *Kelebsiella* is an emerging problem worldwide. Phenotypic detection of microorganisms containing the Ampc is difficult. visible of the expanding reports plasmid-mediated Ampc β -lactamase producing strains of *Klebsiella spp* and scarceness of molecular studies in our country, the aim of this study carried out for detection of AmpC β -lactamases genes in clinical *Klebsiella pneumoniae* strains isolated (which all of them are ESBLs positive) from two large scale hospital in Iran.

MATERIALS AND METHODS

Bacterial isolates

In this study, the number of 108 clinical sample of *K.pneumoniae*, isolated from hospitalized patients got from two hospitals in Ilam and Tehran cities. All the isolates were identified by biochemical and antibiogram test.

Determination of antibiotic susceptibility

The isolates were tested by the disk diffusion method with different types of antibiotics according to CLSI guidelines⁸. An inoculum containing 10⁶ CFU was placed on Mueller-Hinton agar. One disk containing, cefotaxime (30 μ g), ceftazidime (30 μ g) and ceftetriaxone (30 μ g), was thereafter placed on the agar plates. The plates were then incubated at 37 °C for 24h. *Klebsiella pneumoniae* ATCC 700603 were used as quality controls in each susceptibility determination.

Also the use of cefoxitin resistance as a screening marker for AmpC production is quite reliable. According to NCCLS protocol the cefoxitin MICs with microdilution method was used to indicate Ampc β -lactamase production().

DNA Extraction

K.pneumoniae strains were cultured in LB broth at 37°C overnight, and then DNA was extracted by using the DNA extraction kit (bionner company).

PCR Assay

PCR was used to identify the most common plasmid mediated AmpC cluster genes ACC, FOX, MOX, DHA, CIT and EBC. The PCR system (25 μ l) was composed of 1X PCR buffer, 2 mM MgCl₂, 0.2 mM dNTP, 10 pmol of primers, 1U Taq DNA polymerase (Ferments, UK) and A total of 1 μ l of DNA extract was used for each reaction. The sequences of primers used for detection of FOXM, CITM, MOXM, DHAM, ACCM and EBCM are shown in Table 1. PCR conditions included 35 cycles of amplification under the following conditions: denaturation at 95 C for 30 s, annealing for 1 min at primer set specific temperatures (Table 1), and extension at 72 C for 1 min. Cycling was followed by a final extension at 72 C for 10 min. PCR product (10 μ l) was analyzed by gel electrophoresis with 1.5 percent agarose. Gels were stained with DNA Safe Stain and visualized by gel document. A 100-bp DNA ladder was used as a molecular marker.

Table 1. Primers Used For Per Assay

primer	Sequence (5' to 3')	Expected amplicon size (bp)	Annealing	Refrence
MOXM-F	GCT GCT CAA GGA GCA CAG GATC	520	64	17
MOXM-R	AC ATT GAC ATA GGT GTG GTG G			
CITM-F	TGG CCA GAA CTG ACA GGC AAA	462	64	17
CITM-R	TTT CTC CTG AAC GTG GCT GGC			
DHAM-F	AAC TTT CAC AGG TGT GCT GGG	405	52	17
DHAM-R	TCCG TAC GCA TAC TGG CTT TGC			
ACCM-F	AAC AGC CTC AGC AGC CGG TTAT	346	64	17
ACCM-R	TC GCC GCA ATC ATC CCT AGC			
EBCM-F	TCG GTA AAG CCG ATG TTG CGG	302	64	17
EBCM-R	CTT CCA CTG CGG CTG CCA GTT			
FOXM-F	AAC ATG GGG TAT CAG GGA GAT	190	64	17
FOXM-R	GCAA AGC GCG TAA CCG GAT TGG			

RESULTS AND DISCUSSION

MICs microdilution broth test showed that among the 108 *Klebsiella pneumoniae* isolates, 103 (95/3 percent of strains) were cefoxitin resistant. Disc diffusion method showed that all isolates (100%) were resistant to ceftazidime, ceftriaxone and cefotaxime.

PCR results showed that among the 108 isolates of *Klebsiella pneumoniae*, 49 isolates (45/3 %) were positive for FOXM cluster gene (Fig.1), 35 (32/4 %) were positive for DHAM cluster gene (Fig.2) and 6 (5/5 %) were positive for CITM cluster gene (Fig.3). All isolates were lacking the ACCM, EBCM and MOXM genes. Our results showed that among the 108 strains 12 isolate are possess the DHAM and FOXM genes and 3 isolated were CITM-DHAM genes positive simultaneously.

Plasmid-mediated AmpC β -lactamase producing *K. pneumoniae* are being increasingly found in many parts of the world. ESBLs producing pathogens and plasmid-mediated AmpC β -lactamases pose a serious threat to patient treatment. The prevalence of AmpC β -lactamase in Iran is not known, due to the limited number of surveillance studies seeking clinical strains producing AmpC β -lactamases and inaccurate clinical laboratories for detecting resistance mechanism. Reducing the spread of plasmid-mediated AmpC resistance in hospitals requires the identification of the resistant genes involved in bacterial infections.

In this study, laboratory approved ESBLs, screened for AmpC production by cefoxitin resistance marker.

The rate of resistance to antibiotics among strains isolated from the Ilam hospital was higher of the strains isolated from Milad hospital in Tehran.

To prevent mortality from undiagnosed AmpC β -lactamase dependent infections, it is recommended that β -lactamase strain rings that are not cleaved by clavulanic acid and are resistant to 3rd generation cephalosporins and carbapenem be screened by molecular methods for identification of resistance for the purpose of control of spread of AmpC β -lactamase. Though β -lactamase plasmid positive bacteria separation would be reduced. This can prevent prevalence of AmpC

β -lactamase that transfer easily from one bacterium to other through their plasmid and show high treatment risk is useful. In order to prevent prevalence of microbial antibiotic resistance to AmpC β -lactamase, it is suggested that physicians should pay attention to antibiogram before antibiotic prescription and drugstores should not deliver any antibiotic without doctor prescribed medicine.

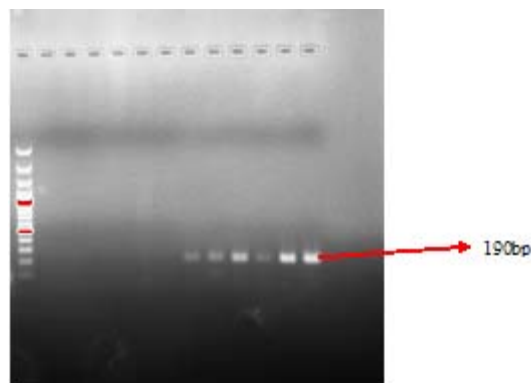


Fig.1. FOXM gene with 190 bp molecular weight

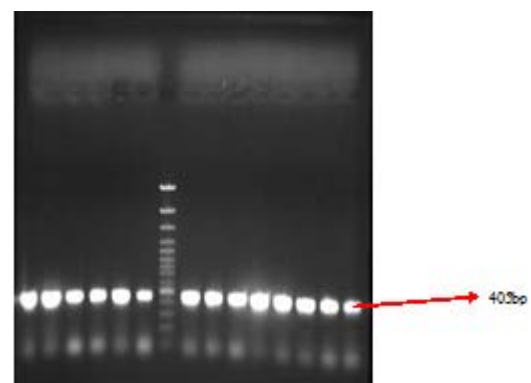


Fig.2. DHAM gene with 405 bp molecular weight

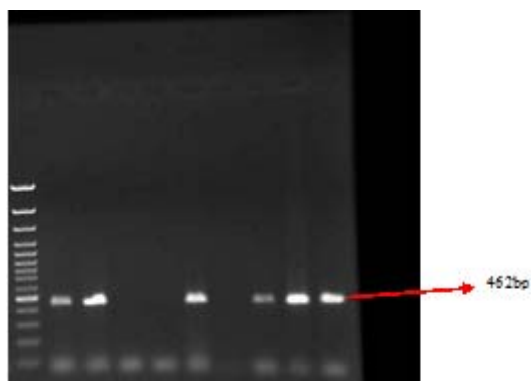


Fig.3. CITM gene with 462 bp molecular weight

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