Identifying CTX-M Resistance Gene Beta-lactamase in the Separated Escherichia Coli from Urine Samples by Polymerase Chain Reaction (PCR) in Khoramabad, Iran

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Abstract

The most important factor in resistance to cephalosporin is \textit{Escherichia coli} bacteria producing Extended Spectrum Beta Lactamases (ESBLs). Over the past decade, CTX-M enzymes have been the most common type of broad-spectrum beta-lactamases in Europe, Canada and Asia. Therefore, the purpose of the research was to study the prevalence of \textit{E. coli} bacteria producing ESBL and CTX-M-I group with molecular method. There were totally gathered 257 urine samples from 12 medical laboratories in Khoramabad. After culturing them on EMB agar environment at 37°C for 24 h and biochemical testing for confirmation, there were isolated 100 samples of 257 samples of \textit{Escherichia coli}. Presence of CTX-M gene was studied by PCR method on the separated isolates in diagnostic tests of Diffusion agar disk and combined disk. There were 31 (31\%) strains of ESBL producing out of 100 examined strains. PCR process to detect CTX-M genes showed that 16 out of 31 strains of ESBL (51.161\%) contained the considered gene. The results of this study show high percentage of beta-lactamases resistant among \textit{E. coli} strains. This indicates a serious public danger that all measures must be taken to avoid this danger.

Key Words: Extended Spectrum Beta- Lactamases (ESBL), CTX-M-I, Polymerase chain reaction, Urinary infection, Escherichia coli

Introduction

\textit{Escherichia coli} (\textit{E. coli}) is one of the most common bacterial factors that is isolated from human infections and causes urinary infection, gastrointestinal and meningitis in newborns. The bacteria are one of hospital opportunistic pathogens that have been resistant because of acquisition of plasmids encoding ESBL to beta-lactam antibiotics such as cephalosporin. As a result, infections caused by the bacteria are treated difficultly. The beta-lactam antibiotics include penicillin, cephalosporin and menobaktam etc. They connect bender proteins to penicillin (PBPs) in cell walls of bacteria control transpeptidase and inhibit transglutaminyl; as a result, there were

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destroyed cell wall and bacteria. Producing beta-lactamase is main mechanism of bacterial resistance to beta-lactam antibiotics. The enzymes hydrolyze and inactivate beta-lactam antibiotics before they reach PBPs in the cytoplasm membrane. Beta-lactamases are classified two categories: molecular (Ambler, 1980) and functional (Bush- Jacoby-Medeiros, 1989). Ambler (1980) proposed molecular classification, when there were only known four sequences of beta-lactamase amino acids. Based on the basic structure, beta-lactamase is divided to four classes of molecule, A to D. Classes D, C and A are lactamase gluteal and class B includes types of containing zinc. Class A includes chromosomal penicillinase negative gram bacteria including Extended Spectrum Beta- Lactamases (ESBLs). Class C includes type AmpC that hydrolyzes cephalosporin and it is resistant to beta-lactam inhibitors. Class D includes oxacilinase (OXA) with plasmid origin that cannot be handled by clavnic acid. Class B includes metallobeta-lactamases that they are beta-lactamase containing zinc and are found in pseudomonas aeroginosa and bacteroides. Beta-lactamase classification in terms of performance began when cephalosporin were distinct from penicillinase and divided into four functional groups. The first group includes cephalosporinase that cannot properly be handled by clavulanic acid and they belong molecular class C. The second group consists penicillinase and cephalosporinase or both that are generally handled by beta-lactamase inhibitors and they belong to molecular class A or D. Beta-lactamases in the group (TEM, SHV, CTX-M) are always increased, and they are divided into several subgroups according to their substrate. The third group included metallobeta-lactamases that use zinc ions to destroy beta-lactam rings. These enzymes can hydrolyze penicillin, cephalosporin and carbapenem. Carbapenem are also inhibited by subgroup 2f. The group members belong to molecular class B, the fourth group includes penicillinase that are not inhibited by clavulanic acid. This group does not belong to any class of molecules. Recent studies divide beta-lactamase into four groups: 1) Extended SpectrumBeta Lactamases; 2) beta-lactamase derivatives of class A resistant inhibitors; 3) AmpC beta-lactamase related with plasmid; and 4) hydrolysis beta-lactamase of carbapenem. Extended SpectrumBeta Lactamases (ESBLs) are a class of beta-lactamase enzymes that are important in antimicrobial therapy. These enzymes can hydrolyze oxy-minobeta-lactam completely in structure of the third generation of cephalosporin. These enzymes were first diagnosed in 1980s, which they were mostly TEM, SHV type; as a
result, there have been created point mutations of main enzymes without extended spectrum activity. CTX-M family Of ESBLs was firstly reported in 1989 on Germany, and then it was spread around the world. This enzyme has been often reported in E. coli, clebsiella, but it has been observed in other enterobacteriaceae. The family of ESBLs is divided into five groups: 1) group CTX-M 1,3 includes CTX-M 1,3,10,12,15,22, 23,28; 2) group CTX-M 2 includes CTX-M 2,4,5,6,7,20; 3) group, CTX-M 8 includes CTX-M 8; 4) group CTX-M 9 includes CTX-M 9,13,14,16,17,19,21,24,27; and 5) group CTX-M 25 includes CTX-M 25. The beta-lactamases have genetic correlation with members TEM and SHV beta-lactamase. Instead, there is high similarity between chromosomal AmpC enzyme (especially KLU-1 and KLU-2) with CTX-M enzymes, as which there have been proposed theories suggesting that these enzymes are derived from similar species. New kinetic studies show that CTX-M enzymes hydrolyze cephalothin and cephaloridine better than benzyl penicillin. They also hydrolyze cefotaxime and ceftazidime more than ceftazidime. Ceftazidime hydrolysis is not enough to cause clinical resistance to bacteria against the antibiotics. The presence of an amino acid serine at position 237 that is available in all CTX-M enzymes plays a critical role in a wide spectrum of beta-lactamase activity of these enzymes.

**Methodology**

In this study, there were collected 257 urine samples during 6 months from 12 medical laboratory in Khoramabad from people with urine infection and then they were isolated after culturing on EMB agar environment and biochemical differentiation tests such as TSI, Simon citrate, urease, MR/VP, SIM using standard tables of E. coli isolates. Antibiotic resistance was determined by antibiogram test through disk diffusion method and antibiotic discs that were prepared by MAST company including cefotaxime (30 µg), ceftazidime (30 µg). To study Extended Spectrum Beta-Lactamases (ESBL), bacteria resistant to cephalosporin were studied using discs of ceftazidime (30 µg), ceftazidime + clavulanic acid (10-30 µg) and cefotaxime (30 µg), cefotaxime + clavulanic acid (10-30 µg) produced by Mast company. ESBL production was determined by increasing diameter to 5mm or more around in disc ceftazidime-clavulanic acid and cefotaxime-clavulanic acid. Then for polymerase chain reaction (PCR), there was extracted DNA after culturing on Mueller Hinton agar environment,