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Prevalence of *CTX-M* Genes in Bacterial Strain Isolated from Patients Hospitalized in ICU Units in the City of Qom, Iran

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ABSTRACT

***Background*:** Pathogenic bacteria because of beta-lactam antibiotic resistance genes are dangerous to society. This resistance due to ESBL genes, plasmids and transposons that by receiving or mutation occurs. The most important factor beta-lactam antibiotic resistance is beta lactamase enzymes. The purpose of this study was to evaluate the frequency of hospital opportunistic pathogenic bacteria producing ESBL and *CTX-M* genes identified are molecular methods.

***Methods*:** In this study, 500 isolates from patients in the ICU of hospitals in the city of Qom was diagnosed by standard biochemical tests. Combined disk test for isolated resistant strains of ESBL was performed in order to identify. Then, strains producing ESBL, DNA extraction and *CTX-M* genes were detected by PCR.

***Results*:** A total of 500 strains isolated, 20 strains (51.28%) of *P. aeruginosa* strains, 40 strains (62.5%) of *E. coli* strains, 38 strains (48.1%) of *K. pneumoniae* strains, 8 strains (33.33%) *A. baumannii* bacteria strains and 4 strains (23.52%) of the strains of *Enterobacter* were carrying *CTX-M* genes.

***Conclusion*:** This study represents a high percentage of beta-lactamase resistance among hospital opportunistic pathogens bacteria. Due to the high prevalence of antibiotic resistance carried out detailed antibiogram tests in infections caused by ESBL-producing organisms is necessary.

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Introduction

Indiscriminate use of antibiotics is causing to resistance mechanisms in bacteria. This mechanism can be received resistance genes via plasmids, transposons, or the mutation. Beta-lactam antibiotics are widely used group of antibiotics in the country. The enzyme by hydrolysis of the core beta-lactam antibiotics will be disabled (1). In themid-1980s, a new group of enzymes with a wide range of broad-spectrum (B-lactamase), capable of destroying cephalosporins were presented such as cefotaxime, ceftriaxone, and ceftazidime that called ESBL. These enzymes are inhibited by beta-lactamase inhibitors such as clavulanic acid, sulbactam and tazobactam (2). ESBLs have been including a number of mutations Enzymes that to give Hydrolysis broad-spectrum beta-lactam antibiotics. For the first time in 1983 in Germany, the enzymes isolated of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (3). ESBL in the division by Bush, Jacob and Medeiros took place, were classified into four main groups A to D (4). One of the enzyme beta-lactamase, CTX-M enzymes, respectively. This enzyme is belongs to the class A molecular Bush classification. These enzymes are not associated with TEM or SHV. Only about 14% identical to the two-lactamase (5). CTX-M enzymes are divided into 5 groups: Group 1consisted of all kinds; CTX-M-1, 3, 10, 12, 15, 22, 23, 28; Group2, including CTX-M-2, 4, 5, 6, 7, 20; Group 8, including CTX-M-8; group 9, including CTX-M9, 13, 14, 16, 17, 19, 21, 24, 27; And Group 25, including CTX-M-25 (6, 7, 8). These enzymes due to their higher activity against cefotaxime than other beta-lactams substrate by oxy imino b-lactamadministration (ceftazidime, ceftriaxone or cephepim) have been named after this. Also, Hydrolysis of benzyl penicillin better than cephalothin or cephaloridine (9-11).

So far, the family CTX-M, 53 enzymes have been identified (12). Over the past decade type of CTX-M beta-lactamase developed in much of the world. The first of these types of beta-lactamase were diagnosed in the isolated organisms such as epidemic or sporadic in Germany, Argentina and France in the early 1990s (13). The prevalence of CTX-M on samples of intestinal bacteria in Columbia 47.9% and 25% were reported in France (14, 15). In Australia, 33% of strains of ESBL producing Enterobacteriaceae with *CTX-M* genes were identified (16). This enzyme with multiple resistance genes could communicate with other antibiotics. The development and release of multiple resistance genes causing the increase in the incidence of diseases and related costs of therapy. Considering the prevalence of this type of enzymes and Antibiotic Resistance Pattern control, prevention and treatment of infections caused by these bacteria is important. Therefore, the study of CTX-M gene in isolates of *Enterobacter*, *Acinetobacter*, *Klebsiella*, *Escherichia coli* and *Pseudomonas aeruginosa* ESBL-producing isolates from ICU of hospitals in Qom was investigated.

Material and method

Identification of bacteria

In this study of 500 clinical samples sent to the laboratory, were examined. These samples include urine, blood, wounds, and other respiratory secretions, purulent discharge and sterile fluids from the Intensive Care Unit (ICU) of the hospitals of Qom. The sample based on the location of isolation on blood agar-agar, Chocolate agar, Eosin methylene blue agar, Taiwan glycol agar-agar and Macconkey agar were cultured on medium.

Then using Gram staining to determine the form of microscopic organisms, catalase and oxidase tests based on differential tests such as TSI, Simmons Citrate Agar, urease, lysine, SIM and MR/VP and decarboxylase agar using standard diagnostic methods and biochemical were identified (17) (Figure 1).

Table 1. Primers were designed and used in this study.

No.	Primer Name	3 'Oligo sequences5'-	No of Bases
1	CTXF	atgtgcagyaccagtaargt	20
2	CTXR	tgggtraartargtsaccaga	21

Table 2. Temperature cycle-time PCR.

Step	Temperature(°c)	Time	Cycle
Initial Denaturation	94	4(min)	
Denaturation	94	45(SC)	**35**
Annealing	57	40(SC)	
Extension	72	60(SC)	
Final extension	72	5(min)	**1**

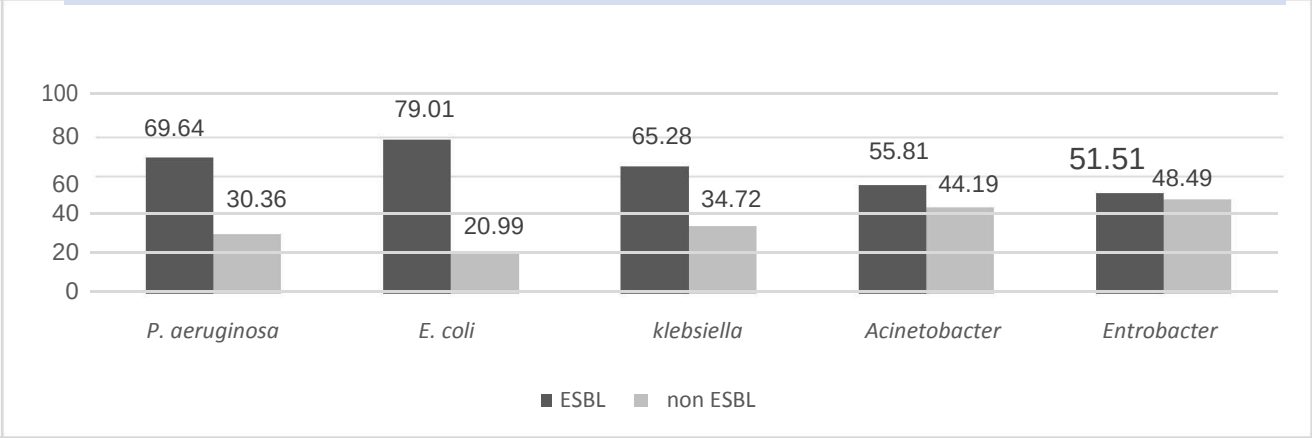

Figure 1. Distribution of bacteria in clinical samples.

Antibiotic resistance pattern

To identify beta-lactamase (ESBL) producing bacteria first screening test using the disk diffusion method (Kirby Bauer), on Mueller Hinton agar medium was performed according to CLSI guidelines. The Disc antibiotic cefotaxime, ceftazidime (company MAST) was used (18) (Figure 4).

Phenotypic identification beta-lactamase (ESBL) producing strains

The beta-lactamase (ESBL) by Combined disk method using Ceftazidimediscs (30 g), ceftazidime + clavulanic acid (10-30 g) and cefotaxime (30 g), cefotaxime + clavulanic acid (10-30 g) prepared from MAST company was studied. ESBL producing bacteria by increasing the size of the zone of growth inhibition around the disk combinations 5 mm or more were determined (19) (Figure 3 and Figure 4).

The polymerase chain reaction (PCR) Extraction of bacterial genomic

Then for polymerase chain reaction (PCR) the ESBL phenotype positive bacteria in broth LB (Lur-Bertani)temperature 37 °C for 24 hours overnight were cultured and then bacterial strains were isolated using by DNA Extraction Kit (DNP TM) –SinaClon.

Sequence selection and design of specific primers for PCR Reaction

After the primer design and study on the characteristics of gene *CTX-M*, to assess the specificity of the primers, the primer with the National Center for Biotechnology Information at the site of application Primer BLAST (National Center for biotechnology information) NCBI, evaluated (Table 1).

Polymerase chain reaction

PCR reaction was used to examine each of the genes with Condition 25 microliter in a standard size. The reaction volume of 1.5 micro molar of Mg^{2+}, 0.2 micro molar of dNTPs, 100 nano gram of DNA extracted from the bacteria, forward and Reverse primer concentration of 0.5 micro molar and Taq DNA Polymerase enzyme 1 unit of enzyme were used. The PCR process according to sources has been set by thermal cycler device (20-22) (Table 2).Then was mixed the 7 microliter of the PCR product with 1 microliter of 6X Loading dye Buffer (Sinaclon Co.) and electrophorese was performed by 1% agarose gel containing Ethidium Bromide at a 100 volts of voltage for 40 minutes (Figure 5 and Figure 6).

Result

Distribution of bacteria in clinical samples

Of the 500 clinical samples isolated from the ICU of the hospitals of Qom, 182 strains (36.4%) *Klebsiella*, 119 strains (23.8%), *Escherichia coli*, 87 strains (17.4%), *Pseudomonas aeruginosa*, 64 isolates (12.8%) and 48 *Acinetobacter* strains (9.6%) were *Enterobacter*.

Antibiotic resistance patterns

Antibiotic resistance rates among 500 strains isolated are shown in Figure 4.

Phenotype-ESBL test results

According to the results of the disc combines 39 strains (69.64%) of the strains of *Pseudomonas aeruginosa* isolates, 64 strains (79.01 %) of strains of *Escherichia coli* isolates, 79 strains (65.28%) of strains of *Klebsiella* isolates, 24strains (55.81%) of the isolates *Acinetobacter* isolate and 17strains (51.51%) of *Enterobacter* strains were ESBL producing isolates.

Table 3. Antibiotic resistance patterns of isolates (percent).

Strains / **Antibiotices**	***Pseudomonas***		***Escherichia coli***		***Klebsiella***		***Acinetobacter***		***Entrobacter***	
	R	S	R	S	R	S	R	S	R	**S**
CAZ	64/36	35/64	68/06	31/94	66/48	33/52	67/18	32/82	68/75	**31/25**
CTX	51/72	48/28	52/94	47/06	58/24	41/76	65/62	34/38	64/58	**35/42**

**CTX : Cefotaxime

* CAZ : Ceftazidime

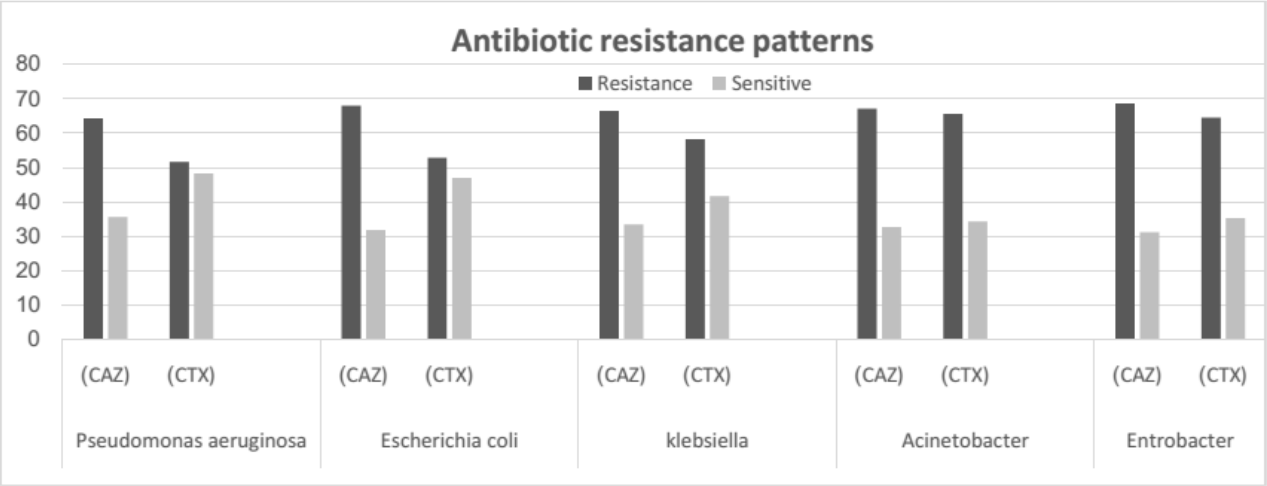

Figure 2. Chart of antibiotic resistance strains isolated.

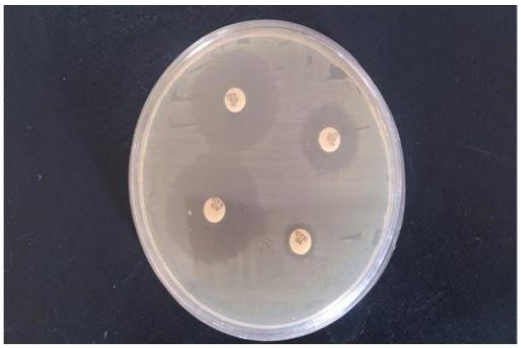

Figure 3. Inhibition in combination disk method with screening.

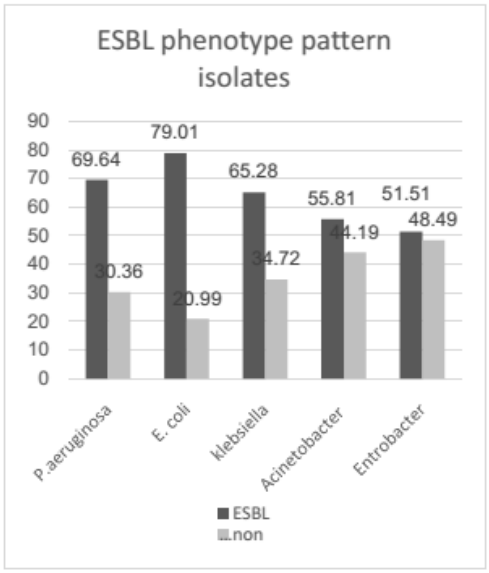

Figure 4. ESBL phenotypepatternisolates.

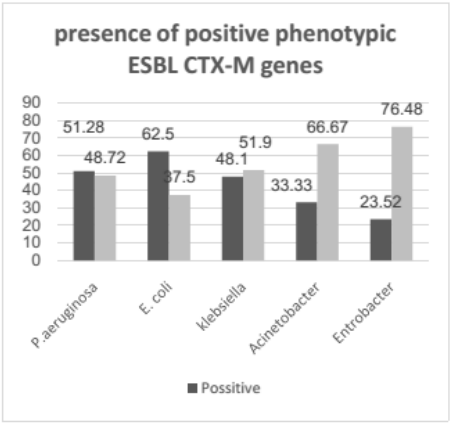

Figure 5. Chart of presence of positive phenotypic ESBL CTX-M genes.

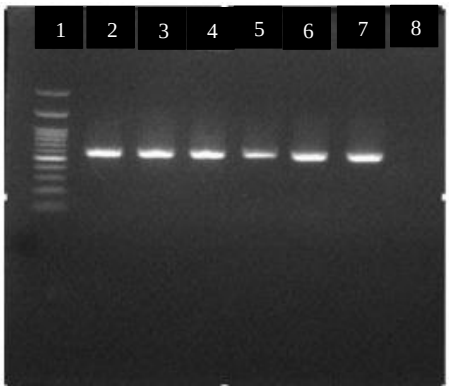

Figure 6. Agarose gel electrophoresis of PCR products of strains CTX-M genes producing ESBL, Row1: marker (100bp DNA ladder), Row 2: gene amplification products CTX-M (bp600) in the standard strain, Row 3: amplified product *Acinetobacter* samples, Row 4: amplified product *Klebsiella* samples, Row 5: amplified product from E. coli samples, Row 6: amplified product from *Pseudomonas* samples, Row7: amplified product from *Entrobacter* samples, Row 8: amplified products without CTX-M genes isolated from clinical samples..

CTX-M Gene PCR results

The PCR assay of 500 strains isolated, 20 strains (51.28%) of *P. aeruginosa* strains, 40 strains (62.5%) of *E. coli* strains, 38 strains (48.1%) of *K. pneumoniae* strains, 8 strains (33.33%) *A. bomani* bacteria strains and 4 strains (23.52%) of the strains of *Enterobacter* were carrying *CTX-M* genes.

Discussion

Pathogenic and opportunistic bacteria in different part of hospitals, particularly in ICU, to acquisition of plasmids encoding lactamase (ESBL), a broad-spectrum beta-lactam antibiotics such as Cephalosporins were resistant (23). The treatment of bacteria in particular of multidrug-resistant strains, because of their widespread resistance to antimicrobial drugs is difficult (24). Beta-lactamase hydrolysis by beta-lactam drugs are the most common mechanism of resistance in Gram-negative bacteria of medical science. The worldwide emergence of ESBL is an important issue. The presence and identify of this enzyme plays an important role in treatment. This study of CTX-M gene in isolates of *Enterobacter*, *Acinetobacter*, *Klebsiella*, *Escherichia coli* and *Pseudomonas aeruginosa* ESBL-producing isolates from ICU of hospitals in Qom was investigated. The most frequent isolates of *K. pneumoniae* strains isolated 182 (36.4%) strains of *E. coli* and 119 isolates (23.8%) and *P. aeruginosa* isolates, 87 (17.4%), respectively. According to the results of the disc combines 39 strains (69.64%) of the strains of *Pseudomonas aeruginosa* isolates, 64 strains (79.01 %) of strains of *Escherichia coli* isolates, 79 strains (65.28%) of strains of *Klebsiella* isolates, 24 strains (55.81%) of the isolates *Acinetobacter* isolate and 17 strains (51.51%) of *Enterobacter* strains were ESBL producing isolates. The frequency of CTXM gene showed through of 500 strains isolated, 20 strains (51.28%) of *P. aeruginosa* strains, 40 strains (62.5%) of *E. coli* strains, 38 strains (48.1%) of *K. pneumoniae* strains, 8 strains (33.33%) *A. bomani* bacteria strains and 4 strains (23.52%) of the strains of *Enterobacter* were carrying *CTX-M* genes. In a study conducted in Korea and North India 27 and 26.6% of isolates of Enterobacteriaceaeas a productive factor beta-lactamase (ESBL) enzymes were reported (25, 26). Tavajjohi and colleagues in 2011 ESBL genes in Pseudomonas aeruginosa isolated from hospitals studied and showed that 8.1% of the total bacteria in the hospitals are ESBL genes (27).

Acinetobacter baumannii, an opportunistic pathogen with high virulence and one of the causes of nosocomial infections during the past 30 years. Research in 2006 showed that beta-lactamase enzymes in *Acinetobacter baumannii* associated with a broad spectrum of plasmids and chromosomes and resistant strains can set a family of genes encoding antibiotic resistance, including resistance to simultaneously carry and transmit to each other (28). Most strains to ceftazidime, cefotaxime, were resistant. In a study conducted Nasehi L and colleagues (2010), the prevalence of CTX-M 1.2% reported. (29). In this study antibiotic resistance 23.52% reported that this difference could be due to the uncontrolled use of antibiotics has led to the emergence of resistant strains in hospitals.

Klebsiella pneumoniae is one of the most important infectious agents, particularly in patients who are admitted to the hospital. Recently, drug resistance, it is considered. This resistance is such that the bacterial virulence factors are considered. In the study of Behzadian Nejadand colleagues in 2009, the prevalence of *CTX-M* 87.5% was reported (30).In this study, the prevalence of *CTX-M* in *Klebsiella pneumoniae* was 48.1 percent.

Escherichia coli is the most common bacterial agents that cause urinary tract infections, gastrointestinal and meningitis in humans. Soltan Dallal, M and colleagues in 2010 showed that 64% of the 200 isolates of ESBL-positive isolates and 57.8% were carrying *CTX-M* genes (31). Mayer and his colleagues in 2008 showed

that the frequency of ESBL producing *E. coli* in the ICU in Germany from 14 percent in 2001 to 52 percent in 2007 reached (32).

Conclusion

This study represents a high percentage of beta-lactamase resistance among hospital opportunistic pathogens bacteria. Due to the indiscriminate use of antibiotics, the prevalence of resistance among pathogens in hospital can be a wakeup call for the community. In addition, the detailed tests antibiogram pattern of infections caused by ESBL-producing organisms is necessary. To control the enzymes needed to identify ESBL by laboratories and suitable beta-lactam drugs.

Conflict of interest

None declared conflicts of interest.

Financial disclosure

There is no financial disclosure.

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