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Research Article

Investigation of *CTX-M-15* Gene Frequency in *Klebsiella pneumoniae* Strains Isolated from Urinary Tract Infections in Zanjan Hospitals, Iran

Karimi F[1], Dabir S[1], Mohammadi J[1], Nori K[1], Mahmoudi S[1], Pournajafi A[1]* and Alizadeh A[1]

1Department of Biology, Central Tehran Branch, Islamic Azad University, Tehran, Iran

***Corresponding author:** Amir Pournajafi, Department of Biology, Central Tehran Branch, Islamic Azad University, Tehran, Iran

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Abstract

Background and Aim: *Klebsiella pneumoniae* is a gram-negative pathogenic bacterium that is a common cause of nosocomial infections. Therefore, the aim of this study was to identify the molecular identification of *CTX-M-15* genes in the *Klebsiella pneumoniae* strains isolated from urinary tract infections in Zanjan hospitals.

Materials and Methods: In this descriptive-analytical study of the study of 289 cases of urinary tract infection in Zanjan medical centers in 2019, 100 isolates of *K. pneumoniae* were identified by standard bacteriological methods. Antibiotic susceptibility of the isolates was determined by disk diffusion method and ESBL-producing isolates were identified by combined disk method. The bacterial DNA was then extracted and studied by PCR using specific gene primers.

Results: The most resistant to ampicillin (73%) and tetracycline (49%) were the most sensitive to amikacin (90%) and nitrofurantoin (89%), respectively. A total of 40 samples were identified as the final ESBL producer. 10 specimens of Pseudomonas bacteria had the *CTX-M-15* gene.

Conclusion: Given the high percentage of resistance to third generation cephalosporins, careful antibiograms and avoidance of overuse of antibiotics in infections caused by ESBL-producing organisms is an inevitable necessity.

Keywords: Extended-Spectrum Beta-Lactamases; *K. pneumonia*; Urinary Tract Infection; Antibiotic Resistance; *CTX-M-15* gene

Introduction

Since sulfanamides and penicillins have come into the field, a new opportunity has emerged in the treatment of diseases. In the early days of the use of these drugs, numerous epidemics subsided. However, infections caused by infectious organisms remain a serious problem [1]. There are two important mechanisms through which increased resistance to antibiotics and other drugs. The former is due to spontaneous mutation, in the sense that the mutation occurs at a frequency of about 10 to 5%, altering the susceptibility to the drug, and the drug acts only as a selective agent and promotes the survival of resistant organisms among organisms [2]. The second mechanism of genetic exchange resistance is the genetic information that controls the drug resistance of the bacterium to both chromosomal DNA and extra-chromosomal DNA, i.e plasmids, through the transformation, conjugation, and transduction of a (resistant) cell. Transferred to another (sensitive) cell. Hospitalized patients are exposed to nosocomial infections, especially with multidrug-resistant organisms, and are one of the most important contributors to nosocomial infections and as a result mortality from Gram-negative bacilli infection. Since antibiotics, especially in ICU wards, are usually empirically due to the rush of treatment [3-4] ESBLs, with the power to hydrolyze the wide range of beta-lactam antibiotics used in clinics, pose a serious problem in medicine. Bacteria producing ESBLs with class C cephalosporinases encoded by the AmpC chromosomal gene have been the most common mechanism of resistance to Gram-negative bacilli against this antibiotic [5-6]. Since the second half of the 1980s, with the reporting of variants of ESBLs and the wide geographical distribution of these enzymes, their release has been discussed as an epidemiological phenomenon [7]. The most important ESBLs examined are *TEM* and *CTX*. *CTX* was first identified in Germany in 1989 and is divided into five groups, *CTX M1, CTX M2, CTXM8, CTXM9* and *CTXM15*, based on changes in the amino acid sequence. Generally, family members hydrolyze *CTX-M*, cefotaxime, and ceftriaxone better than ceftazidime. They are more inhibited by tazobactam than clavulanic acid [8-9]. Urinary tract infections are one of the most common human-acquired infections. In the United States, urinary tract infections are the second most common cause of upper respiratory tract infections, and many men and women are infected throughout their lives. Different factors such as age, sex and immune system influence the prevalence of UTI [10-13]. *K. pneumoniae* is one of the gram-negative bacilli of the Enterobacteriaceae family that is distributed in nature and is one of the normal flora bacteria in humans [14-16]. This opportunistic pathogen is responsible for a wide range of infections, especially in hospitalized patients, including septicemia, pneumonia, and urinary tract infections. Colonization of this bacterium is more frequent in hospitalized patients than in outpatients [17-18]. Common antibiotics to treat Klebsiella infection

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are mainly beta-lactam drugs. But over-use of these drugs has led to antibiotic resistance to this group of antibiotics in *K. pneumoniae* [19-20]. The aim of this study was to investigate the *CTX-M-15* gene in the *K. pneumoniae* strains isolated from urinary tract infections in Zanjan.

Materials and Methods

In this descriptive study, 289 urine samples were collected from outpatients and inpatients of Zanjan hospitals during three months from November to December of 2019 and were cultured on EMB (Merck Company, Germany). Then routine biochemical tests were performed on the colonies. Also, standard strain of *K. pneumoniae* ATCC700603 was used as quality control. Combined disk test was used to evaluate ESBL producing strains. This experiment was performed using ceftazidime (30µg), cefotaxime (30µg), ceftazidime/clavulanic acid (30µg/10µg) and Cefotaxime/clavulanic acid (30µg/10µg). For this test, the isolates under study were suspended in physiological saline and their turbidity was adjusted to 0.5 McFarland standard. Then, cotton swabs were cultured in Muller Hinton Agar medium in three directions and after 24 h incubation at 37°C, the growth zone diameter was recorded around the discs. Then, cotton swabs were cultured in Muller Hinton Agar medium in three directions and after 24 h incubation at 37°C, the growth zone diameter was recorded around the discs. Increase in diameter of more than 5 mm in diameter growth zone around ceftazidime/clavulanic acid (30µg/10µg) and cefotaxime/clavulanic acid (30µg/10µg) discs compared to ceftazidime (30µg) and cefotaxime (30µg) discs) Indicates ESBL positive of sample and recorded as positive result. In this experiment *E. coli* ATCC 25922 was used as negative control and *E. coli* ATCC 35218 as positive control. After confirmation of the presence of *K. pneumoniae*, the antibiogram for the samples was recommended by the Clinical and Laboratory Standards Institute. Antibiotic discs used were tetracycline (30µg), nitrofurantoin (300µg), ceftazidime (30µg), ampicillin sulbactam (10µg), amoxicillin (25µg), amoxicillin-clavulanic (25µg), nalidixic acid (30µg), amikacin (30µg), tobramycin (10µg), imipenem (10µg), ciprofloxacin (5µg) and gentamicin (10µg), (Media Companies). After 24-hour incubation at 37°C using a ruler, the growth zone around the discs was measured and compared to the CLSI standards. According to the manufacturer's instructions, the results were based on sensitivity (S) and resistance (R) was reported and semi-susceptible halos were recorded as (I).

After determining the phenotypically positive isolates, the DNA of the identified samples was extracted using kits Oiagen, Hilden (Germany). The PCR reaction was performed with a final volume of 25µl, including 1µl of each primer, Mr. Mix 12.5µl, DNA pattern 3.5µl and 7µl of distilled water (all consumables were manufactured by Sinagen Iran). Thermal Cycler device program contains 35 cycles with 4-minute temperature conditions and initial return at 94°C, connection at 60°C for 45 seconds, lengthening at 72°C for 1 minute and finally lengthening. The final was done at 72°C for 10 min. The PCR product was then evaluated on 1% agarose gel with electrophoresis and the gel containing PCR products was placed in a tank containing ethidium bromide for 15 to 20 minutes after the end of the electrophoresis period. Printed. The *K. pneumoniae* ATCC 700603 strain with the *CTXM15* gene were used as positive control. In order to statistically analyze the data, the twentieth version of SPSS software and Chi-square test were used. A significant boundary was set at $p < 0.05$ (Table 1).

Table 1: Primers used in this study.

van A, B	Amplicon size(bp)
CTXM15 F: 5' CACACGTGGAATTTAGGGACT3'	996
CTXM15 R: 5' GCCGTCTAAGGCCATAAACA3'	

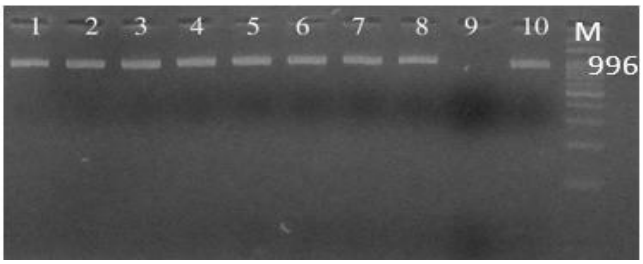

Figure 1: Display of *CTX-M-15* gene isolates (M: marker, NC: negative control, PC: positive control, 1 to 9: *CTX-M-15* gene isolates).

Results

In this study, 289 urine samples were collected from 100 (34.60%) *K. pneumoniae*. 60 specimens were isolated from the inpatients ward and 40 samples from the out patients ward. Based on the results of the combined disk test, 40 samples were identified as final ESBL producers. Of the 40 strains of ESBL producing *K. pneumoniae*, 10 samples had *CTX-M-15* genes (Figure 1). The results of the sensitivity test against the 12 selected antibiotics are shown in Table 2.

Discussion

Broad-spectrum beta-lactamases are a group of beta-lactamase enzymes that are of particular importance in antimicrobial therapy. The rate of ESBL production among Enterobacteriaceae varies worldwide [21]. In the present study, from 100 *K. pneumoniae* isolates, 60 samples from the inpatient ward and 40 samples from the outpatients ward were isolated. Based on the results of the combined disk test, 40 samples were identified as final ESBL producers. The highest resistance to ampicillin (73%) and tetracycline (49%) were the most sensitive to amikacin (90%) and nitrofurantoin (89%),

Table 2: Frequency of antibiotic resistance pattern of *K. pneumoniae* strains isolated from urinary tract infections.

Antibiotics	Resistance	Intermediate	Sensitive
Tetracycline	49	10	41
Nitrofurantoin	7	4	89
Ceftazidime	29	29	42
Ampicillin Sulbactam	73	10	17
Amoxicillin	43	16	41
Amoxicillin-Clavulanic	45	0	55
Nalidixic Acid	30	18	52
Amikacin	8	0	92
Tobramycin	18	2	80
Imipenem	21	4	75
Ciprofloxacin	31	3	66
Gentamicin	10	5	85

respectively. However, Shah Cheraghi et al., reported the prevalence of *K. pneumoniae* isolated from urine specimens more than other pathogenic agents [22]. In a 2007 study, Amirmozafari and colleagues found that 61.2% of isolates of *K. pneumoniae* had drug resistance. Of these, 20.4% had 100% drug resistance to all cephalosporins (cefexime, ceftriaxone, ceftazidime, etc.) [23]. In a paper published by Taslima et al. In 2007 in Bangladesh, resistance to ceftazidime (36%), gentamicin (27%), tetracycline (27%), ciprofloxacin (45%) was reported [24]. Feiz Sarshar and Akya showed the highest and lowest resistance to ampicillin and carbapenem antibiotics, respectively, from the 60 isolates tested in 2016. 45% of the isolates were ESBL-producing enzyme. Most of the ESBL enzymes were in hospital isolates (88%) compared to outpatient samples (11%). The highest and lowest resistance were observed to ampicillin and carbapenem antibiotics, respectively [25]. Mobasher Kare Jeddi and colleagues showed that 23 (91.43%) isolates were resistant to ceftazidime and 42 (89.26%) isolates were resistant to cefotaxime. Of 47 isolates (97.87%) 46, *K. pneumoniae* isolates were ESBL positive. 100% of K.pneumoniae isolates were susceptible to imipenem [26]. Sarvazad and Darbouy showed that 60.82% of cefotaxime resistant isolates, 40.2% ceftriaxone resistant isolates, 62.88% ceftazidime resistant isolates, 3.09% isolates resistant to imipenem, 39.17% of isolates were resistant to cefepime, 64.94% isolates were resistant to cefixime, 26.8% were resistant to amikacin [27]. Rostam Zad and Padervand In 2017, out of 31 positive ESBL phenotypic isolates, only 17 had the *bla CTX-M-15* gene [28]. Soroush and Ghane showed Most of the isolates were resistant to Cotrimoxazole (72.3%), Gentamicin (67.7%) and Ampicillin (69.2%) and the highest susceptibility was seen for Ciprofloxacin (50.8%) Tetracycline (49.2%), Imipenem (46.3%) and Ceftriaxone (43.1%). Among the ESBL-producing genes, *blaCTX-M* (55.3%) was the most prevalent, followed by *blaTEM* (41.5%) and *blaSHV* (10.7%). The results showed that 1.5% of the isolates had concurrently *blaTEM/blaSHV* and *blaSHV/blaCTX-M* genes and 21.6% of isolates the *blaTEM/blaCTX-M* genes [29]. Thaghimosleh and khajehkarimaldini showed Based on the results of PCR, 43 (86%) of the samples had phenotypic ESBL enzymes, 20 (47%) had *CTX* gene, 8 (18%) had *TEM* gene, and 15 (34%) had Both the *CTX* and *TEM* genes [30]. Pourali Sheshblouki and Mardaneh in 2016, showed that 70.3% of the isolates possess the *blaCTX* gene [31]. Regional differences in different parts of the world give rise to different antibiotic responses, and even patterns of antibiotic resistance may vary from one hospital to another in one country. The origin of these differences are: genetic differences between individuals, genetic differences of strains, differences in cultural and economic backgrounds. Therefore, the treatment pattern used in different regions is different depending on the specific characteristics of a region.

Conclusion

Due to the increased antibiotic resistance among the strains, it is recommended that antibiogram testing be performed before treatment. Also, preventing bacterial strains and therapeutic failures that lead to complication of the infection can be prevented by proper use of existing medicines, completing the course of treatment and avoiding as many antibiotics as possible. Further research in this field will increase our knowledge and more effective exposure to the antibiotic resistance of emerging microorganisms.

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