Examination of the prevalence of Escherichia coli containing the CTX-M-1 gene in urinary tract infection samples collected from Zanjan hospitals, Iran

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Abstract:

Background and Aim: The increasing use of beta-lactam antimicrobials in the treatment of bacterial infections has increased resistance to them. One of the current problems in the treatment of nosocomial infections is the resistance of enzymes to broad-spectrum beta-lactamases (ESBL) among clinical isolates, especially Escherichia coli. Therefore, the aim of this study was to identify the molecular identification of CTX-M-1 genes in the E. coli 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 E. coli 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 50 samples were identified as the final ESBL producer. 39 specimens of E. coli bacteria had the CTX-M-1 gene.

Conclusion: The genes studied in this study were all located on the chromosome of E. coli bacteria. Therefore, further investigation of ESBL genes such as CTX-M-1 gene seems necessary to control this bacterium.

Keywords: CTX-M-1 gene, Extended-Spectrum Beta-Lactamases, E. coli, Urinary Tract Infection, Antibiotic Resistance

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, ie 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). E. coli is one of the natural inhabitants of the human and animal intestines. It goes, but it is also found in water, soil and even plants (14). E. coli is one of the most common bacterial causes of infections. The pharmacological resistance of this bacterium is of great importance, especially in patients admitted to hospitals (15). This bacterium is one of the most common microbial causes of urinary tract infections and is a causative agent (16). Many nosocomial infections, such as sepsis, ulcerative colitis, gastroenteritis, and neonatal meningitis (17-18). Escherichia coli is one of the hospital’s opportunistic pathogens and is due to acquisition Plasmids that encode broad-spectrum beta-lactamases have become resistant to beta-lactam antibiotics. For this reason, treatment of infections caused by E. coli is difficult. Antimicrobial resistance has been reported worldwide in E. coli, and the rapid increase in resistance to the bacterium has caused great concern in developing and developed countries (19-20). The aim of this study was to investigate the CTX-M-1 gene in the E. coli 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. 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 E. coli, the antibiogram for the samples was recommended by the Clinical and Laboratory Standards Institute. Antibiotic discs used were tetracycline (30 µg), nitrofurantoin (300 µg), ceftazidime (30 µg), ampicillin sulbactam (10 µg), amoxicillin (25 µg), amoxicillin-clavulanic (25 µg), nalidixic acid (30 µg), amikacin (30 µg), tobramycin (10 µg), imipenem (10 µg), ciprofloxacin (5 µg) and gentamicin (10 µg). (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 phenotype of isolates, the DNA of the identified samples was extracted using kits Oiagen, Hilden (Germany). The PCR reaction was performed with a final volume of 25 µl, including 1 µl of each primer, Mr. Mix 12.5 µl, DNA pattern 3.5 µl and 7 µl 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. The E.coli ATCC 13911 strain with the CTX-M-1 gene were used as positive control. Primers used in this study: CTXM1 F:5’ CGTGGCGATGAATAAGCTG 3’ and R: 5’ GTGTGGATATTGCCTTTCATCC 3’. 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.

Results & Discussions
In this study, 289 urine samples were collected from 100 (34.60%) E.coli. 60 specimens were isolated from the inpatients ward and 40 samples from the outpatients ward. Based on the results of the combined disk test, 50 samples were identified as final ESBL producers. Of the 50 strains of ESBL producing E.coli, 39 samples had CTX-M-1 genes. The results of the sensitivity test against the 12 selected antibiotics are shown in Table 1.
Table 1. Frequency of antibiotic resistance pattern of E. coli strains isolated from urinary tract infections

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

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 E.coli 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, 50 samples were identified as final ESBL producers. 39 specimens of E.coli bacteria had the CTX-M1 gene. The highest resistance to ampicillin (73%) and tetracycline (49%) were the most sensitive to amikacin (90%) and nitrofurantoin (89%), respectively. Wesam et al. (2009) have examined the resistance pattern of Pseudomonas to the antibiotics and the results showed that this bacterium has the highest resistance to the antibiotics of nalidixic acid and tetracycline (22). In another study in Arak in 2013, the resistance of this bacterium to the following antibiotics has been reported: ceftazidime(33.3%), imipenem(22.2%), amikacin(20.3%), ciprofloxacin (15.7%) and gentamicin(19.4%) (23). In the study by Ahadi et al., the resistance of it to imipenem(55%) and ceftazidime(57%) has been reported (24). Rajat Rakesh et al. (2012) have reported the resistance of it to the antibiotics of ciprofloxacin (49%), gentamicin (63%) and imipenem (14%)in their study. ChanderAnli has reported the resistance of it to the antibiotics of amikacin (25%), ciprofloxacin (75%) (25). Kianpour et al. (2010) have reported the resistance of it to the antibiotics of amikacin (58.14%), ciprofloxacin (42.58%) and imipenem (14.8%) (26). The production of ESBL in the isolates of P. aeruginosa is on the rise in the last few years. The rate of its increase was 20.6% in Thailand in 2003 (27). 25.4% in 2005 in Korea (28), 23.4% in 2006 in Bolivia and 45.3% in China in 2006 (29).
In the study by Mirsalehian et al. (2008), the production of ESBL in the clinical strains isolated in Tehran, has been reported 40% which is consistent with the results of present study (30). Shakibaie et al. (2008) (31) have been reported that 41 (34%) of 120 isolates of P. aeruginosa were ESBL-producing strains. Shahcheraghi et al. (2009) have been reported that 234 (39%) of 600 isolates of P. aeruginosa were Extended-Spectrum beta-lactamases producing strains (32). In the study by Shahcheraghi et al. (2010), the presence of the genes of VEB, OXA-10,CTX-M,PER-1,GES-1,OXA-1,OXA-4 in the P. aeruginosa strains isolated from the hospitals in Iran has been confirmed (33). performed in Shiraz, the frequencies of CTX-M1, CTX-M2 and CTX-M3 have been reported 49.9%, 135%, 23.1%, respectively (34). In the study of Mirzaee, which was performed on 160 strains of E. coli from different clinical specimens, 35.78% had the CTX-M-1 gene (35). 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 (36). Masjedian and colleagues examined 51% of the 148 E. coli strains reported producing ESBL (37). Mirsalehian and colleagues reported 59.3% of the samples as ESBL producers (38). In a study by Soltan Dallal et al., on 200 E. coli isolates, 64% of the isolates reported ESBL-producing (39-40). Amirmozafari and colleagues showed that 2018 out of 167 E. coli isolates, 38.9% were ESBL positive (41).

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.

References:


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