Research Article

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 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 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' GGTGGTATTGCCTTTCATCC 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

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

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 tis 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-I 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:

1. AL-Jasser A. Extended-spectrum beta-lactamases (ESBLs): A global problem, Jour. Kuwwait Medical. 2006;38(3):171-185.

2. Medeiros AA. Evolution and dissemination of β-lactamases accelerated by generations of β-lactam antibiotics. Clinical Infectious Diseases. 1997 Jan 1;24(Supplement_1):S19-45.

3. Ensor VM, Livermore DM, Hawkey PM. A novel reverse-line hybridization assay for identifying genotypes of CTX-M-type extended-spectrum β-lactamases. Journal of Antimicrobial Chemotherapy. 2007 Mar 1;59(3):387-95.

4. Dizaji AS, Fathi R, Sales AJ. Molecular study of extended-spectrum beta-lactamase (TEM-1) gene in Escherichia Coli isolates collected from Ostad Alinasab Hospital in Tabriz Iran. MMJ. 2016 Jan 1;29:35-40.

5. Jafari-Sales A, Shadi-Dizaji A. Molecular analysis of CTX-M genes among ESBL producing in Pseudomonas aeru-ginosa isolated from clinical samples by Multiplex-PCR. HOZAN J Environment Sci. 2018;2(5):17-29.

6. Sales A, Fathi R, Mobaiyen H. Molecular Study of the Prevalence of CTX-M1, CTX-M2, CTXM3 in Pseudomonas aeruginosa Isolated from Clinical Samples in Tabriz Town, Iran. Electronic J Biol. 2017;13(3):253-9.

7. Montso KP, Dlamini SB, Kumar A, Ateba CN. Antimicrobial Resistance Factors of Extended-Spectrum Beta-Lactamases Producing Escherichia coli and Klebsiella pneumoniae Isolated from Cattle Farms and Raw Beef in North-West Province, South Africa. BioMed Research International. 2019;2019.

8. Jafari Sales A, Mobaiyen H, Farshbafi Nezhad Zoghi J, Nezamdoost Shadbad N, Purabdollah Kaleybar V. Antimicrobial Resistance Pattern of Extended-Spectrum β-Lactamases (ESBLs) producing Escherichia coli Isolated from Clinical Samples in Tabriz city, Iran. Adv Environ Biol. 2014;8(16):179-82.

9. Jafari-Sales A, Bagherizadeh Y, Khalifehpour M, Abdoli-senejan M, Helali-Pargali R. Antibiotic resistance pattern and bla-TEM gene expression in Acinetobacter baumannii isolated from clinical specimens of Tabriz hospitals. Zanko Journal of Medical Sciences. 2019 Jul 10;20(65):20-9.

10. Jafari-Sales A. Study of Antibiotic Resistance and Prevalence of bla-TEM gene in Klebsiella pneumoniae Strains isolated from Children with UTI in Tabriz Hospitals. Focus On Medical Sciences Journal. 2018 Nov 12;4(1).

11. Wagenlehner FM, Naber KG, Weidner W. Rational antibiotic therapy of urinary tract infections. Medizinische Monatsschrift fur Pharmazeuten. 2008 Oct;31(10):385-90.

12. Jafari Sales A, Mobaiyen H. Frequency and resistance patterns in clinical isolates of Escherichia coli Extended Spectrum Beta Lactamase producing treatment Centers in Marand city, Iran. New Cellular and Molecular Biotechnology Journal. 2017 Apr 15;7(26):19-26.

13. De Francesco MA, Ravizzola G, Peroni L, Negrini R, Manca N. Urinary tract infections in Brescia, Italy: etiology of uropathogens and antimicrobial resistance of common uropathogens. Medical science monitor. 2007 May 31;13(6):BR136-44.

14. Al-Jasser AM. Extended-Spectrum Beta-Lactamases (ESBLs): A Global problem. Kuwait Med J. 2006;38(3):171-185.

15. Gold HS, Moelleing RC. Antimicrobial-drug resistance. N Engl J Med. 1996;335(19):1445-53.

16. Kollef MH, Fraser VJ. Antibiotic resistance in the intensive care unit. Ann Intern Med. 2001;134(4):298-314.

17. Wagenlehner FM, Naber KG, Weidner W. Rational antibiotic therapy of urinary tract infections. Med Monatsschr Pharm. 2008;31(10):385-390.

18. De Francesco MA, Giuseppe R, Laura P, Riccardo N, Nin M. Urinary tract infections in Brescia, Italy: Etiology of uropathogens and antimicrobial resistance of common Uropathogens. Med Sci Moni. 2007;13(6):136-144.

19. Sanchez U M, Bello T H, Dominguez Y M, Mella M S, Zemelman Z R, Gonzalez RG. Transference of extendedspectrum beta-lactamases from nosocomial strains of Klebsiella pneumoniae to other species of Enterobacteriaceae. Rev Med Chil. 2006;134(4):415-420.

20. Bell JM, Turnidge JD, Gales AC, Pfaller MA, Jones RN. Prevalence of extended spectrum β-lactamase (ESBL)-producing clinical isolates in the Asia-Pacific region and South Africa: regional results from SENTRY Antimicrobial Surveillance Program (1998–99). Diagn Microbiol Infect Dis. 2002;42(3):193-8

21. Acton A.Pseudomonas aeruginosa: new insights for the healthcare professional. ScholarlyBrief. Scholarly Editions. 2013; pp: 11-32.

22. Quinn P, Markey B, Leonard F, Hartigan P, Fanning S, Fitzpatrick E. Veterinarymicrobiology and microbial disease. 2ndedition. John Wiley & Sons, 2011; pp: 225-243.

23. Vanhems P,lepape A,Savey A,JambouP,Fabry J. Nosocomial pulmonary infectionby antimicrobial resistant bacteria ofpatientshospitalized in intensive care unitsrisk factors and survival. J Hosp Infect2000; 45(2):98-106

24. Rajat Rakesh M, Ninama Govind L, Mistry Kalpesh, Parmar Rosy, Patel Kanu, Vegad MM. National Journal of Medical Research Natl J Med Res. 2012; 2(2):156-9.

25. Ryall B, Davies JC, Wilson R, Shoemark A, Williams HD. Pseudomonas aeruginosa, cyanide accumulation and lung function in cystic fibrosis and non-cystic fibrosis bron-chiectasis patients.Eur Respir J. 2008;32:740-7.

26. Hacker J, Carniel E. Ecological fitness, genomic islands and bacterial pathogen-icity–a Dar-winian view of the evolution of microbes. EMBO Rep. 2001;2:376-81.

27. Tramper-Stranders GA, van der Ent CK, Wolfs TF. Detection of Pseudomonas aeruginosa in patients with cystic fibrosis. J Cyst Fibros. 2005;54:37-43.

28. Falagas ME, Karageorgopoulos DE. Extended-spectrum β-lactamase-producing organisms. Journal of Hospital infection. 2009 Dec 1;73(4):345-54.

29. Wesam, A. and Hassanein. Molecular Identification of Resistant Pseudomonas Aeruginosa Wt. Aust. j. basic appl. sci. 2009;3, 2144-53.

30. Taghvaee R, Shojapour M, Sadeghi A, Pourbabaie A. The study of antibiotic resistance pattern and the frequency of Extended spectrum beta-lactamases (ESBL) in Pseudomonas aeruginosa strains isolated from medical centers in Arak city, Iran. Qom Univ Med Sci J. 2013;7(4):36-41 Ahadi A, Sharif Zadeh A, Golshani Z. Identification of antibiotic resistance patterns of Pseudomonas aeruginosa isolated from patients admitted with multiple resistances . Journal of Veterinary Laboratory Research. 2012;4(1):119-22.

31. Ahadi A, Sharif Zadeh A, Golshani Z. Identification of antibiotic resistance patterns of Pseudomonas aeruginosa isolated from patients admitted with multiple resistances . Journal of Veterinary Laboratory Research. 2012;4(1):119-22.

32. Anil C, Shahid RM. Antimicrobial Suseptibility Patterns of pseudomonas aeroginosa clinical isolates at tertiary care hospital in kathmando, nepal. Asian J Pharm Clin Res. 2013;6(3): 235-8.

33. Kianpour F, Havaei SA, Hosseini MM. Evaluation of Pseudomonas aeroginosa isolated from cutaneous infections and determination of drug resistance pattern in patients of Alzahra hospital in Esfahan. J Isfahan Med Sch. 2010; 28(110):503-9

34. Lee S, Park YJ, kim M, Lee HK, Han K. Prevalence of Ambler class A and D beta - lactamases among clinical isolates of Pseudomonas aerugionsa in Korea. J Antimicrob Chemother. 2005;56: 122-7.

35. Mirzaee M, Pourmand MR, Chitsaz M, Mansouri S. Antibiotic resistance to third generation cephalosporins dueto CTX-M-Type extended-spectrum β-lactamases in clinical isolates of Escherichia coli.Iranian J Publ Health 2009;38(1):10-7.

36. Sarshar MH, Akya A. The frequency of extended spectrum β-lactamase genes of SHV-2a, SHV-5 and SHV-12 in clinical isolates of klebsiella pneumoniae isolated from Kermanshah medical centers in 2014. Majallah-i dānishgāh-ǐ ulūm-i pizishkī-i Arāk. 2016;19(2):59-67.

37. Masjedian GF, Valehi F, Talebi RL, Rastegar L. Moulecular evaluation of resistance to espanded antibiotics in Escherichia coli and Klebsiella pneumoniae. Iran J Med Microbiol. 2007;1(2):27-34.

38. Mirsalehian A, Akbari-Nakhjavani F, Peymani A, Kazemi B, Jabal Ameli F, Mirafshar SM. Prevalence of Extended Spectrum β - Lactamase-Producing Enterobacteriaceae by phenotypic and genotypic methods in intensive care units in Tehran. Daru 2008;16(3):169-173.

39. Dallal MM, Sabbaghi AY, Fallah JA, Aghamirzaei HM, Lari AR, Eshraghian MR, Sanei AF. Evaluation of presence of the bla-SHV and bla-AmpC (CITM, FOX) β-lactamase genes in clinical isolates of Escherichia coli. Journal of Medical Council of Islamic Republic of Iran. 2010;28(3).

40. Soltan Dallal MM, Aghamirzaei HM, Mehrabadi JF, Lari AR, Sabbaghi A, Eshraghian MR, Sanei AF, Bakhtiari R, Abdar MH. Molecular detection of TEM and AmpC (Dha, mox) broad spectrum β-lactamase in clinical isolates of Escherichia coli. Tehran University Medical Journal. 2010 Sep 1;68(6).

41. Amirmozafari N, BabaieKasmaie Z, Mohsenpour M. The Frequency of Beta Lactamase genes in Escherichia coli isolates from outpatient suffering from urinary tract infections in Guilan province. Yafteh. 2018 Feb 10;19(5):43-52.