The Frequency and Antimicrobial Resistance of $bla_{TEM}$ and $bla_{CTX-M}$ Genes in *Escherichia coli* Isolated From Patients With a Urinary Tract Infection

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Published online June 30, 2020  
Accepted February 23, 2019  
Received December 3, 2018

**Abstract**  
**Introduction:** The production of β-lactamase in bacteria, especially in *Escherichia coli* as a prevalent opportunistic bacterium, has caused many problems in patient treatment. β-lactamases are encoded by extended-spectrum β-lactamase (ESBL) genes such as $bla_{TEM}$ and $bla_{CTX-M}$. We aimed to assess the prevalence and antibiotic sensitivity of β-lactamases encoded by $bla_{TEM}$ and $bla_{CTX-M}$ in *E. coli* isolated from patients suffering from urinary tract infections (UTIs).

**Methods:** *Escherichia coli* strains were isolated from the patients’ urine culture presented to medical diagnostic laboratories in Zabol, Iran. The agar disc-diffusion test was performed on Müller-Hinton agar to investigate the antibiotic resistance of these isolates using eight antimicrobial paper discs including gentamicin, tetracycline, co-trimoxazole, norfloxacin, cefuroxime, ampicillin, neomycin, and amoxicillin. A conventional polymerase chain reaction (PCR) was used to detect $bla_{TEM}$ and $bla_{CTX-M}$.

**Results:** The frequencies of resistance to cefuroxime, norfloxacin, co-trimoxazole, neomycin, amoxicillin, tetracycline, gentamicin, and ampicillin were found to be 45 (90%), 15 (30%), 33 (66%), 33 (66%), 44 (88%), 34 (68%), 4 (8%), and 50 (100%), respectively. Moreover, the prevalence of $bla_{TEM}$ was 25 (50%) while that of $bla_{CTX-M}$ was 16 (32%).

**Conclusion:** Based on the results, gentamicin and norfloxacin can be recommended as effective antibacterials for treating UTI caused by *E. coli* in the study population. Moreover, the frequency of resistant genes including $bla_{TEM}$ and $bla_{CTX-M}$ was high in the isolated *E. coli*. Effective control systems including appropriate treatments for ESBL-producing strains are therefore required for humans and food animals.

**Keywords:** ESBL, *E. coli*, Prevalence, Antimicrobial resistance

**Introduction**  
Urinary tract infection (UTI) caused by *Escherichia coli* is the second most common infection in humans.¹ *Escherichia coli* is a member of the normal intestinal microflora in many birds, animals, and humans. Some strains of *E. coli* can be, however, pathogenic² and cause extra-intestinal and intestinal infections including septicemia, peritonitis, meningitis, UTI, and gastroenteritis.³⁴ Despite having a long history of application as the first-line therapy for treating *E. coli* infections, antibiotics are gradually losing their effectiveness given the increasing resistance of bacteria to the treatments.⁵ Resistant bacteria cause major health problems, which can be exacerbated through the excessive usage of antibiotics in hospitals⁶,⁷ or in animal feed.⁸ These bacteria threaten both the public health and animal health, and can cause huge economic damage to animal husbandry.⁹ *Escherichia coli* can spread among different ecosystems through water and food chains.¹⁰ The transmission of *E. coli*,
including drug-resistant extended-spectrum β-lactamase (ESBL)-producing strains, from food animals to humans through food chains is a well-known phenomenon. ESBLs are often encoded on large plasmids, which can be exchanged between the bacterial species and the strains. ESBL-producing strains mainly include Klebsiella pneumonia and E. coli. According to Brook, β-lactamase-producing bacteria both survive penicillin treatments and protect other bacteria that are vulnerable to penicillin by releasing free enzymes into the environment. Clinical cases of resistance to β-lactams have been observed across the world owing to the excessive administration of β-lactam antibiotics for treating infections associated with Enterobacteriaceae.

Factors such as the amount and type of the antibiotics used appear significantly effective in the prevalence of ESBL in a geographical region. In our region, the data available is limited to the expression of antibiotic resistance genes in E. coli isolated from patients with UTI. The present research was therefore conducted to investigate the frequency and antimicrobial resistance of the β-lactamase-encoding genes, namely blaTEM and blaCTX-M in the E. coli isolated from patients with UTI in Zabol in the southeast of Iran.

Materials and Methods
Design and Area of the Study
The present descriptive cross-sectional study was performed during 2015-2016 at Department of Pathobiology, Faculty of Veterinary Medicine, University of Zabol, Sistan and Baluchistan Province, Iran. All the urine samples were collected from patients with a suspected UTI presented to the medical diagnostic laboratories. UTI was confirmed if the CFU/mL of the urine cultures calculated was at least equal to $10^5$. All the fifty E. coli isolates of the urine cultures of the male and female patients procured were confirmed using standard biochemistry tests.

Susceptibility Testing
The present study tested 8 antimicrobial paper discs including neomycin (30 µg), ampicillin (10 µg), cefuroxime (30 µg), norfloxacin (10 µg), amoxicillin (25 µg), co-trimoxazole (1.25/23.75 µg), tetracycline (30 µg), and gentamicin (10 µg). All these discs were purchased from Padtan Teb Company, Tehran, Iran. All the isolates underwent antimicrobial susceptibility tests on Mueller Hinton agar using the disc-diffusion technique. The bacterial colony suspension was prepared in a glass tube, vortexed, and visually matched with the turbidity of a 0.5 McFarland standard. After applying the antimicrobial discs and performing incubation for 24 hours at 37°C, the susceptibility or resistance of the isolates was determined by comparing the diameters of the inhibition zones measured in mm with internationally-recognized values.

Detection of blaTEM and blaCTX-M Genes
A modified boiling process was adopted for DNA extraction. In summary, the bacterial isolates were inoculated in five mL Luria-Bertani broth and centrifuged for 5 minutes at 3600 g. The supernatant was decanted, and the pellet was then re-suspended in 0.2 mL double-distilled water, boiled in a thermomixer for 10 minutes at 95°C, and ultimately centrifuged for 10 minutes at 15000 g. The obtained supernatants were stored at -80°C in microtubes.

A 35-cycle amplification was performed in a thermos thermal cycler using a conventional polymerase chain reaction (PCR). Table 1 presents the oligonucleotides used for the reaction. The primers and the master mix solution were purchased from Pishgam Industrial Company, Tehran, Iran. The PCR volume was 25 µL and consisted of 1 µL of each of the reverse and forward primers, 7.5 µL of distilled water, 13.5 µL of the master mix solution and two µL of sample DNA. According to Table 2, the annealing step of blaCTX-M amplification was performed for 40 seconds at 50°C and that of blaTEM for 50 seconds at 55°C. The known clinical isolates of blaCTX-M and blaTEM-producing E. coli were used as the quality control, and double-distilled water was used as the negative control instead of DNA in PCR. The electrophoresis of the PCR

| Table 1. Oligonucleotides Used for Detecting blaTEM and blaCTX-M Genes in the E. coli Isolates |
|---------------------------------------------------------------|--------------------------|-----------------|
| Target Gene | Sequences (5’-3’) | PCR Product Size (bp) | Reference |
| blaTEM | ATGAGTATTCACATTCCCG CCAATGCAATAGGTAGG | 858 | 21 |
| blaCTX-M | ATGTCGAGCAGCAATGCT | 593 | 22 |

| Table 2. PCR Process for the Amplification of blaTEM and blaCTX-M Genes |
|------------------------|----------------|----------------|--------------|
| Step | Temperature (°C) | Duration (seconds) | Number of Cycles |
| Initial denaturation | 94 (94) | 7 (5) minutes | 1 |
| Denaturation | 94 (94) | 50 (55) seconds | 35 |
| Annealing | 50 (55) | 40 (50) seconds | 35 |
| Extension | 72 (72) | 1 (1) minutes | 35 |
| Final extension | 72 (72) | 5 (7) minutes | 1 |
products was conducted on 1.5% agarose gel.

**Statistical Analysis**

The data were expressed using descriptive statistics and analyzed in SPSS using the chi-square test and the Fisher exact test.

**Results**

Figure 1 shows the distribution of the antimicrobial resistance of the *E. coli* isolates. The highest resistance of the isolates was against ampicillin [50 (100%)] and the lowest was against gentamicin [4 (8%)]. The frequency of resistance to other antibiotics were as follows: cefuroxime [45 (90%)], norfloxacin [15 (30%)], co-trimoxazole [33 (66%)], neomycin [33 (66%)], amoxicillin [44 (88%)], and tetracycline [34 (68%)]. The frequency of resistance patterns was statistically compared between all the antimicrobial agents in the *E. coli* isolates. There were significant differences in the prevalence of resistance between the tested antibiotics ($P < 0.001$).

According to Figures 2 and 3, the prevalence of $\text{bla}_{\text{TEM}}$ in the *E. coli* isolates was 16 (32%) and that of $\text{bla}_{\text{CTX-M}}$ was 25 (50%). The frequency of both genes in the *E. coli* isolates were compared and observed that the prevalence of $\text{bla}_{\text{CTX-M}}$ was significantly higher than that of $\text{bla}_{\text{TEM}}$ ($P < 0.001$).

**Discussion**

Antimicrobial resistance is considered a major public health issue, given the decrease in the effectiveness of antimicrobial treatments on infectious diseases.24 We examined the prevalence of antimicrobial resistance and identified certain ESBL genes, that are $\text{bla}_{\text{CTX-M}}$ and $\text{bla}_{\text{TEM}}$ in the *E. coli* isolated from the patients with UTIs. Production of ESBLs is a main route for bacterial resistance to β-lactam antibiotics.25,26 The present research was conducted to investigate the prevalence and antibiotic resistance of $\text{bla}_{\text{TEM}}$ and $\text{bla}_{\text{CTX-M}}$ genes in *E. coli* isolated from patients with UTIs given the lack of studies on this subject in the study area. The highest and the lowest resistance was noted against ampicillin (100%) and gentamicin (8%), respectively. Furthermore, *E. coli* resistance rates to tetracycline, amoxicillin, neomycin, co-trimoxazole, norfloxacin, and cefuroxime were 68%, 88%, 66%, 66%, 30%, and 90%, respectively. Out of fifty *E. coli* strains isolated by Yektadoust et al from patients with UTIs in Varamin, Iran, in 2017, 10% were found resistant to imipenem, 24% were resistant to ceftiraxone, 24% were resistant to ciprofloxacin, 64% were resistant to co-amoxiclav, and 62% were resistant to co-trimoxazole.27 Investigating 87 *E. coli* isolates of patients with UTIs in Zahedan, Iran, found the frequency of resistance to co-trimoxazole to be 66.6%, to nalidixic acid 63%, to ceftazidime 44.8%, to nitrofurantoin 26.1%, to amikacin 19.5%, to gentamicin 13.7%, and to imipenem 4.5%.1 The disparity between the present findings and the results of other studies can be ascribed to the diverging application of antibiotics for treating the patients in different areas of Iran.

The frequencies of $\text{bla}_{\text{TEM}}$ and $\text{bla}_{\text{CTX-M}}$ in the *E. coli* isolates were respectively 32% and 50% in the present study. Increases in the prevalence of ESBLs, especially $\text{bla}_{\text{CTX-M}}$, have been reported in recent years.25,28 The distribution of $\text{bla}_{\text{CTX-M}}$ in *E. coli* has also been reported as 77.34%,29 37.8%,30 and 84.1%30 in different areas of Iran. Doosti et al found that 58.06% of 31 ESBL-positive *E. coli*...
coli contain β-lactamase-encoding *bla*<sub>TEM</sub>. Furthermore, according to a recent molecular analysis, the frequency of ESBL-producing genes, including *bla*<sub>CTX-M</sub> and *bla*<sub>TEM</sub>, were 70.32% and 9.64%, respectively. Additionally, according to a study conducted by the authors of the present study, the frequency of *bla*<sub>CTX-M</sub> in the *E. coli* strains isolated from turkey was 23.3% and that of *bla*<sub>TEM</sub> was 16.6%. In the present study, the frequency of ESBL genes was lower compared to the results obtained from human isolates of *E. coli*. Given that the cephalosporins are rarely used to treat birds, the β-lactamase produced in bird isolates was expected to be lower compared to that in human isolates.

**Conclusion**

Based on the results of the present study, gentamicin and norfloxacin can be recommended as effective antibacterials for treating UTIs caused by *E. coli*. A high frequency was observed for ESBL-encoding genes such as *bla*<sub>TEM</sub> and *bla*<sub>CTX-M</sub>. Excessive administration of antibiotics to humans and food animals can develop resistance in bacterial strains. Effective control measures are, therefore, required to be taken into account including proper treatments for ESBL-producing strains.

**Ethical Approval**

The Ethics Committee of University of Zabol reviewed and approved the protocol of the present research (IRUOZ.ECRA.2015.001).

**Conflict of Interest Disclosure**

The authors declare no competing interests.

**Acknowledgments**

The authors would like to express their gratitude to the staff of the Microbiology Laboratory of the Faculty of Veterinary Medicine, University of Zabol. (Grant No. UOZ-GR-9618-56).

**References**

1. Keikha M, Rava M. Trend of antibiotic resistance of *Escherichia coli* strains isolated from urinary tract infections in outpatient patients from Zahedan. Journal of Paramedical Sciences & Rehabilitation. 2017;6(4):73-78. doi:10.22038/jpsr.2017.21755.1556
2. Kaper JB, Nataro JP, Mobley HL. Pathogenic *Escherichia coli*. Nat Rev Microbiol. 2004;2(2):123-140. doi:10.1038/nrmicro818
3. Sodha SV, Lynch M, Wannemuehler K, et al. Multistate outbreak of *Escherichia coli* O157:H7 infections associated with a national fast-food chain, 2006: a study incorporating epidemiological and food source traceback results. Epidemiol Infect. 2011;139(2):309-316. doi:10.1017/s0950268810000920
4. von Baum H, Marre R. Antimicrobial resistance of *Escherichia coli* and therapeutic implications. Int J Med Microbiol. 2005;295(6-7):503-511. doi:10.1016/j.ijmm.2005.07.002
5. Singer RS, Hofacre CL. Potential impacts of antibiotic use in poultry production. Avian Dis. 2006;50(2):161-172. doi:10.1637/7569-033106r.1
6. van den Bogaard AE, Stobberingh EE. Antibiotic usage in animals: impact on bacterial resistance and public health. Drugs. 1999;58(4):589-607. doi:10.2165/00003495-199958040-00002
7. Witte W. Medical consequences of antibiotic use in agriculture. Science. 1998;279(5353):996-997. doi:10.1126/science.279.5353.996
8. Alexander TW, Yanke LJ, Topp E, et al. Effect of subtherapeutic administration of antibiotics on the prevalence of antibiotic-resistant *Escherichia coli* bacteria in feedlot cattle. Appl Environ Microbiol. 2008;74(14):4405-4416. doi:10.1128/aem.00489-08
9. Tadesse DA, Zhao S, Tong E, et al. Antimicrobial drug resistance in *Escherichia coli* from humans and food animals, United States, 1950-2002. Emerg Infect Dis. 2012;18(5):741-749. doi:10.3201/eid1805.111153
10. Skurnik D, Ruimy R, Andremont A, et al. Effect of human vicinity on antimicrobial resistance and integrons in animal faecal *Escherichia coli*. J Antimicrob Chemother. 2006;57(6):1215-1219. doi:10.1093/jac/dkl122
11. Hammerum AM, Heuer OE. Human health hazards from antimicrobial-resistant *Escherichia coli* of animal origin. Clin Infect Dis. 2009;48(7):916-921. doi:10.1086/597292
12. Jacoby GA, Medeiros AA. More extended-spectrum beta-lactamases. Antimicrob Agents Chemother. 1991;35(9):1697-1704. doi:10.1128/aaac.35.9.1697
13. Hashim RB, Husin S, Rahman MM. Detection of betalactamase producing bacterial genes and their clinical
features. Pak J Biol Sci. 2011;14(1):41-46. doi:10.3923/pjbs.2011.41.46
14. Brook I. The role of beta-lactamase-producing bacteria in mixed infections. BMC Infect Dis. 2009;9:202. doi:10.1186/1471-2334-9-202
15. Dierikx C, van Essen-Zandbergen A, Veldman K, Smith H, Mevius D. Increased detection of extended spectrum beta-lactamase producing Salmonella enterica and Escherichia coli isolates from poultry. Vet Microbiol. 2010;145(3-4):273-278. doi:10.1016/j.vetmic.2010.03.019
16. Gniadkowski M. Evolution and epidemiology of extended-spectrum beta-lactamases (ESBLs) and ESBL-producing microorganisms. Clin Microbiol Infect. 2001;7(11):597-608. doi:10.1046/j.1198-743x.2001.00330.x
17. Gillespie SH, Hawkey PM. Principles and Practice of Clinical Bacteriology. England: Wiley; 2002
18. Quinn PJ, Markey BK, Carter ME, Donnelly WJ, Leonard FC. Veterinary Microbiology and Microbial Disease. USA: Wiley-Blackwell; 2002
19. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing; Twenty-First Informational Supplement. CLSI document M100-S21. Wayne, PA: CLSI; 2011.
20. Sandalli C, Özgümüş OB, Sevim A. Characterization of tetracycline resistance genes in tetracycline-resistant Enterobacteriaceae obtained from a coliform collection. World J Microbiol Biotechnol. 2010;26(11):2099-2103. doi:10.1007/s11274-010-0381-z
21. Arlet G, Brami G, Décrè D, et al. Molecular characterisation by PCR-restriction fragment length polymorphism of TEM beta-lactamases. FEMS Microbiol Lett. 1995;134(2-3):203-208. doi:10.1111/j.1574-6968.1995.tb07938.x
22. Pagani L, Dell'Amico E, Migliavacca R, et al. Multiple CTX-M-type extended-spectrum beta-lactamases in nosocomial isolates of Enterobacteriaceae from a hospital in northern Italy. J Clin Microbiol. 2003;41(9):4264-4269. doi:10.1128/jcm.41.9.4264-4269.2003
23. Shahbazi P, Jahantigh M, Salari S. Antibiotical resistance pattern and prevalence of some extended-spectrum beta-lactamase genes in Escherichia coli isolated from Turkey. Veterinary Researches & Biological Products. 2018;31(4):2-8. [Persian].
24. Martinez JL, Baquero F. Interactions among strategies associated with bacterial infection: pathogenicity, epidemicity, and antibiotic resistance. Clin Microbiol Rev. 2002;15(4):647-679. doi:10.1128/cmrl.15.4.647-679.2002
25. Manouchehr M, Ahanjan M. Detection of CTX-beta-lactamase gene in Escherichia coli isolated from urinary tract infection using polymerase chain reaction. Journal of Mazandaran University of Medical Sciences. 2015;25(129):36-45. [Persian].
26. Tenover FC, Raney PM, Williams PP, et al. Evaluation of the NCCLS extended-spectrum beta-lactamase confirmation methods for Escherichia coli with isolates collected during Project ICARE. J Clin Microbiol. 2003;41(7):3142-3146. doi:10.1128/jcm.41.7.3142-3146.2003
27. Yektadoust F, Kazemi A, Yalfani R. Comparison of antibiotic susceptibility testing of Escherichia coli and Klebsiella pneumonia isolated from urinary tract infections against five antibiotics by disc diffusion and microdilution methods. Jundishapur Scientific Medical Journal. 2017;16(5):525-534. doi:10.22118/jsmj.2017.54016
28. Mirzae M, Pourmand MR, Chitsaz M, Mansouri S. Antibiotic resistance to third generation cephalosporins due to CTX-M-type extended-spectrum beta-lactamases in clinical isolates of Escherichia coli. Iran J Public Health. 1970;38(1):10-17.
29. Soltan Dallal MM, Mobasser G, Mehrabadi JF, et al. Detection of CTX-M-1 beta lactamase gene in Escherichia coli isolated from clinical samples by polymerase chain reaction (PCR). Tehran University Medical Journal. 2011;69(1):16-21. [Persian].
30. Soltan Dallal MM, Azarsa M, Shirazi MH, et al. The prevalence of extended-spectrum beta-lactamases and CTX-M-1 producing Escherichia coli in urine samples collected at Tabriz city hospitals. Tehran University Medical Journal. 2011;69(5):273-278. [Persian].
31. Doosti B, Rashidian E, Shams N. Phenotypic and molecular detection of ESBL-producing genes TEM-1 and SHV-1 in uropathogenic Escherichia coli isolates. Medical Journal of Tabriz University of Medical Sciences. 2017;39(1):44-49. [Persian].
32. Amirmozafari N, BabaeiKasmaie 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;19(5):43-52. [Persian].