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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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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*$_{CTX-M}$ and *bla*$_{TEM}$ 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*$_{CTX-M}$ and *bla*$_{TEM}$.

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*$_{CTX-M}$ was 25 (50%) while that of *bla*$_{TEM}$ 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*$_{CTX-M}$ and *bla*$_{TEM}$ 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

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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, pathogenic[2] and cause extra-intestinal and intestinal infections including septicemia, peritonitis, meningitis, UTI, and gastroenteritis.[3,4] 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 hospitals[6,7] 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.[11] ESBLs are often encoded on large plasmids, which can be exchanged between the bacterial species and the strains.[12] ESBL-producing strains mainly include *Klebsiella pneumonia* and *E. coli*.[13] 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.[14] 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*.[15,16]

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 bla_{TEM} and bla_{CTX-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.[1] All the fifty *E. coli* isolates of the urine cultures of the male and female patients procured were confirmed using standard biochemistry tests.[17,18]

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.[1] 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.[19]

Detection of bla_{TEM} and bla_{CTX-M} Genes

A modified boiling process[20] 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 15 000 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.[21,22] 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 bla_{CTX-M} amplification was performed for 40 seconds at 50°C and that of bla_{TEM} for 50 seconds at 55°C. The known clinical isolates of bla_{CTX-M}- and bla_{TEM}-producing *E. coli* were used as the quality control, and double-distilled water was used as the negative control instead of DNA in PCR.[23] The electrophoresis of the PCR

Table 1. Oligonucleotides Used for Detecting bla_{CTX-M} and bla_{TEM} Genes in the *E. coli* Isolates

Target Gene	Sequences (5'-3')	PCR Product Size (bp)	Reference
bla_{TEM}	ATGAGTATTCAACATTTCCG CCAATGCTTAATCAGTGAGG	858	21
bla_{CTX-M}	ATGTGCAGYACCAGTAARGT TGGGTRAARTARGTSACCAGA	593	22

Table 2. PCR Process for the Amplification of bla_{CTX-M} and bla_{TEM} Genes

Step	Temperature bla_{CTX-M} (bla_{TEM})	Duration bla_{CTX-M} (bla_{TEM})	Number of Cycles
Initial denaturation	94 (94) ºC	7 (5) minutes	1
Denaturation	94 (94) ºC	50 (55) seconds	35
Annealing	50 (55) ºC	40 (50) seconds	35
Extension	72 (72) ºC	1 (1) minutes	35
Final extension	72 (72) ºC	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 bla_{TEM} in the *E. coli* isolates was 16 (32%) and that of bla_{CTX-M} was 25 (50%). The frequency of both genes in the *E. coli* isolates were compared and observed that the prevalence of bla_{CTX-M} was significantly higher than that of bla_{TEM} ($P<0.001$).

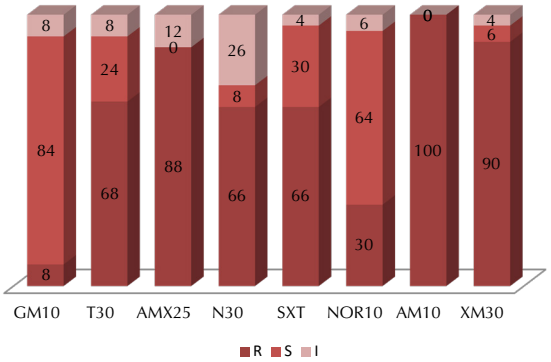

Figure 1. Frequency of Antimicrobial Resistance of *E. coli* Isolated From Patients With UTI
S: susceptible, I: intermediate, R: resistant, GM_{10}: gentamicin, T_{30}: tetracycline, AMX_{25}: amoxicillin, N_{30}: neomycin, SXT: co-trimoxazole, NOR_{10}: norfloxacin, AM_{10}: ampicillin, XM_{30}: cefuroxime.

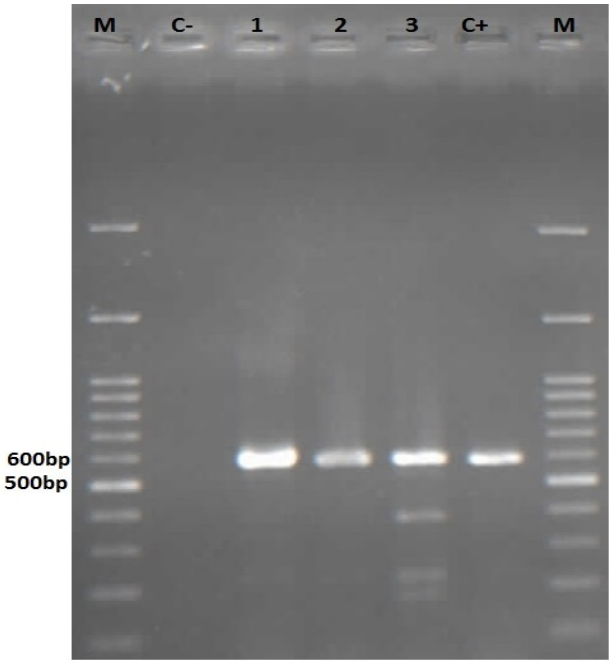

Figure 2. Electrophoresis Results for Detecting the bla_{CTX-M} Gene.
1, 2, 3: Positive samples; C^+: Positive control; C^-: Negative control; M: 100 bp-DNA ladder.

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 bla_{CTX-M} and bla_{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 bla_{TEM} and bla_{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 ceftriaxone, 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 bla_{TEM} and bla_{CTX-M} in the *E. coli* isolates were respectively 32% and 50% in the present study. Increases in the prevalence of ESBLs, especially bla_{CTX-M}, have been reported in recent years.[25,28] The distribution of bla_{CTX-M} in *E. coli* has also been reported as 77.34%,[29] 37.8%,[28] and 84.1%[30] in different areas of Iran. Doosti et al found that 58.06% of 31 ESBL-positive *E.*

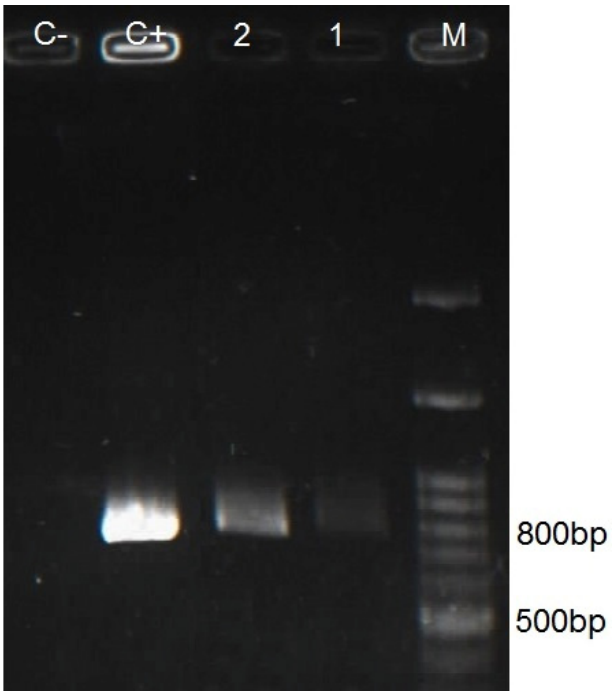

Figure 3. Electrophoresis Results for Detecting the bla_{TEM} Gene. 1, 2: Positive samples; C^+: Positive control; C^-: Negative control; M: 100 bp-DNA ladder.

coli contain β-lactamase-encoding bla_{TEM}.[31] Furthermore, according to a recent molecular analysis, the frequency of ESBL-producing genes, including bla_{CTX-M} and bla_{TEM}, were 70.32% and 9.64%, respectively.[32]

Additionally, according to a study conducted by the authors of the present study, the frequency of bla_{CTX-M} in the *E. coli* strains isolated from turkey was 23.3% and that of bla_{TEM} was 16.6%.[23] 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_{TEM} and bla_{CTX-M}. 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.

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