



Determination of Antimicrobial Resistance Pattern and Detection of *bla*TEM Gene among Clinical Isolates of *Escherichia coli*

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ABSTRACT

Background: Unfortunately, antibiotic resistance has become an increasingly critical problem in many countries like Iran. Since there are very few published data on antibiotics resistance in Alborz province, the aim of this study was to survey the pattern of antimicrobial resistance and prevalence detection of TEM-type beta-lactamases among clinical isolates of *Escherichia coli* using universal primers.

Methods: The study was performed on 83 clinical *Escherichia coli* strains collected from hospitals and clinical laboratories. Antimicrobial susceptibility was performed using Kirby-Bauer disk diffusion method against common antibiotics. Isolates were also screened for the production of extended spectrum beta-lactamases (ESBLs) by double disk synergy test (DDST). Positive isolates were evaluated by PCR analysis for the TEM family of ESBLs genes.

Results: Isolates showed the highest resistance to amoxicillin (83%), whereas nitrofurantoin was the most effective drug, with only 8.4% resistance. The frequency of multi drug resistance (MDR) to more than 5 antibiotics was 79.5% (66 strains). ESBL screening of *E. coli* strains by DDST showed that out of 83 strains, 33 isolates were ESBL positive. Based on the PCR results 61 percent of phenotypic ESBL positive *E. coli* isolates possessed a single gene encoding a TEM type ESBL.

Conclusion: As the results of this study indicate, multidrug resistance is an increasing therapeutic concern and treatment requires further attention to the results of susceptibility tests. As antibiotic options in the treatment of ESBL-producing organisms are extremely limited, molecular screening by laboratories is suggested to reduce the risk of therapeutic defeat.

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Introduction

Escherichia coli is the most common cause of the urinary tract infections. In recent years, the threat of acquisition of antibiotic resistance is growing because of excessive and unregulated use of antibiotics. Antibiotic resistance and, in particular, multidrug resistance (MDR) are major problems for the empirical treatment of patients (1). Development of third generation cephalosporins in early 1980s was a considerable progress in the battle against beta-lactamase mediated bacterial resistance. However, a new plasmid encoded beta-lactamase capable of hydrolyzing the extended spectrum cephalosporins was reported after a short time (2-4). Extended spectrum beta lactamases (ESBLs) are a class of group A beta-lactamases which result in hydrolysis of first, second, and third generation cephalosporines but are inhibited by beta-lactamase inhibitors like acid clavulanic (5). The Enterobacteriaceae producers of ESBLs have become a serious problem of public health worldwide since 1995(6). These extended spectrum beta-lactamases are mutants derived from older, broad-spectrum beta-lactamases (e.g., TEM-1, TEM-2, SHV-1), and have an extended substrate profile which allows hydrolysis of all cephalosporins, penicillins, and aztreonam (7). Most of the ESBLs encoding genes are plasmid-borne and often located in the transposons and integrons (8). Thus they may be easily transferred between bacteria. TEM-types are the most prevalent beta-lactamases in enterobacteria. Up to 90% of ampicillin resistance in *E. coli* is due to the production of TEM-1. It was first reported in 1965 from an *E. coli* isolate from a patient in Athens, Greece, named Temoneira (hence the designation TEM) (9). Currently, more than 160 TEM-type ESBLs have been described (10). Emerging antimicrobial resistance rates and extended-spectrum beta-lactamase producing *E. coli* recovered from urinary tract infections (UTIs) is a growing problem in many regions, limiting therapeutic options (11). Antibiotics resistance is a frequently and vastly reported phenomenon in

different provinces of Iran; probably, the most important reason is that antibiotics are sold over the counter without prescription. Additionally, misuse and overuse of antibiotics have played a role in the development of multi drug-resistant bacteria and the spread of resistance between bacterial species. Since there are very few published data on antibiotics resistance in Alborz province, the aim of this study was to survey the pattern of antimicrobial resistance and prevalence detection of TEM-type beta-lactamases among clinical Isolates of *E. coli* using universal primers. This knowledge may help physicians to prescribe more effective antibiotics and prevent the spread of antibiotics resistance among clinically important strains within the province.

Materials and Methods

Bacterial strains

Between July and September 2010, 83 non-duplicate *E. coli* isolates were collected from four hospitals and two private clinical laboratories of Alborz province Karaj city. Isolates were identified as *E. coli* based on standard biochemical tests (12).

Antimicrobial susceptibility assay

The susceptibilities of all isolates to different antibiotics were determined by Kirby- Bauer disk diffusion method, as suggested by the CLSI (formerly the National Committee for Clinical Laboratory Standards) (13). The zone of inhibition of each isolate was tested on Muller-Hinton agar medium with commercial antimicrobial disks (Padtan Teb Co., Tehran, Iran). The antibiotic disks used in this study were gentamicin (10 µg), amikacin (30 µg), amoxicillin (10 µg), ceftazidime (30 µg), cephalothin (30 µg), imipenem (10 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), norfloxacin (10 µg), co-trimoxazole (25 µg), tetracycline (30 µg), chloramphenicol (30 µg) and nitrofurantoin (300 µg). *E. coli* (ATCC 25922) was

used as the reference strain for antibiotic susceptibility tests.

ESBL detection

Using β -lactam and β -lactamase-inhibitor disks is a widely accepted method of detecting extended-spectrum β -lactamase-producing gram-negative bacilli. Isolates were screened for ESBL production using the double disc synergy test. The disks of extended-spectrum cephalosporins (cefotaxime, ceftazidime, ceftriaxone, and ceftizoxime) were placed around an amoxicillin (20 μ g)-clavulanate (10 μ g) disk at a distance of 25-30 mm center to center. Plates were incubated at 37 °C for 18 hrs. ESBL production was deduced when the zone of cephalosporins was expanded by clavulanate (14). *K. pneumoniae* ATCC 700603 and *E. coli* ATCC 35218 were used respectively as a positive ESBL control and a negative ESBL control.

Detection of blaTEM gene by PCR

Genomic DNA of isolates with an ESBL phenotype was extracted by boiling method. Briefly, 2-3 fresh colonies were suspended in 300 μ l of TE buffer and centrifuged at 15,000 g for 15 min. The supernatant was discarded and the pellet was resuspended in 50 μ l of deionized water, boiled at 100 °C in a water bath for 10 min, cooled on ice, and centrifuged at 15,000 g for 10 s before it was stored at -20 °C (15). 1 μ l of template DNA were added to 25 μ l of PCR mixture for amplification of blaTEM genes. As beta lactamase genes have several subfamilies, with following sequences universal primers were used to amplify all subfamilies: F: 5'-KACAATAACCCTGRTAA ATGC-3' and R: 5'AGTATATATGAGTAAACT TGG-3'. The length of the expected amplified fragment was about 936 bp. The PCR reaction was performed in a total volume of 25 μ l. Each reaction contained 2.5 μ l of 10X PCR buffer, 0.5 μ l of 10 mM dNTPs, 0.7 μ l of each primers (20 pM), 2 μ l of template DNA and 0.2 μ l of 5u/ μ l Taq polymerase. Amplification conditions were as

follows: early denaturation at 95 °C for 3 minutes followed by 35 cycles of 94 °C for 1 minute, 52 °C for 1 minute and 72 °C for 90 seconds, with a final elongation step of 10 minutes at 72 °C.

Results

This study was performed on 83 clinical *E. coli* strains that were collected from urine samples of hospitalized patients and two private clinical laboratories of Alborz province, Karaj city. Isolates were identified as *E. coli* based on standard biochemical tests. Total isolates were subjected to antibiotic susceptibility test by Kirby-Bauer disk diffusion method. Percentage of antimicrobial resistance of *E. coli* isolates has been shown in Table 1. Isolates showed the highest resistance to amoxicillin (83%) and high sensitivity to nitrofurantoin (8.4%). Among the isolates, 92.77% (77 strains) were resistant to more than two unrelated antibiotics and the frequency of MDR to more than 5 antibiotics was 79.5% (66 strains). Table 2 show the most commonly identified combinations of antimicrobial agents in multidrug-resistant isolates of *E. coli*. Moreover, 39.7% (33 strains) of the isolates were ESBL positive. Out of 83 isolates, 33 *E. coli* strains were found to be ESBL positive with DDST, indicates the total prevalence of 39.7%. The antibiotic susceptibility results showed that all ESBL producing isolates were resistant to amoxicillin. Determination of ESBL type in ESBL-producing bacteria could provide useful epidemiological information. As beta-lactamase genes have several subfamilies a set of universal primer was used to amplify all genes within the TEM cluster.

Of the 33 ESBL positive *E. coli* isolates, 32 harbored TEM genes as detected by PCR (Figure 1). Based on the PCR results, 96 percent of phenotypic ESBL positive *E. coli* isolates possessed a single gene encoding a TEM-type ESBL.

Table 1. Percentage of antimicrobial resistance of *E. coli* isolates.

Antibiotic	Sensitive strains	Resistant strains	Resistance (%)	Antibiotic	Sensitive strains	Resistant strains	Resistance (%)
Nitrofurantoin	76	7	8.4	Tetracycline	30	53	63.8
Amoxicillin	14	69	83	Ofloxacin	58	25	30
Levofloxacin	58	25	30	Co-trimoxazole	34	49	59
Amikacin	68	15	18	Imipenem	62	21	25.3
Choloramphenicol	69	14	16.9	Cephalexin	32	51	61.4
Gentamycin	60	23	28.2	Norfloxacin	55	28	33.7
Nalidixic acid	36	47	56.6	Cephalothin	43	40	48.2
Ciprofloxacin	56	27	32.5	Ceftazidime	60	23	27.7
Ceftriaxone	52	31	37.3	Ceftizoxime	69	14	16.9
Cefotaxime	51	32	38.5	Amoxycillin/ Clavulanic acid	26	57	68.7

Table 2. Percentage of antimicrobial resistance of *E. coli* isolates.

Pattern of resistance	No. of resistant strains (%)
TE-AMX-SXT-AMC	18 (33.37)
FM-TE-AMX-SXT-SXT-IPM-CN	5 (6.40)
TE-AMX-SXTCN-CF-CRO	4 (5.10)
TE-AMX-OFX-LOM-IPM-CN-CF-NOR-CRO-CTX	6 (7.79)
TE-AMX-CN-NA-CF-CAZ-CRO-CTX-AMC	7 (9.09)
AMX-OFX-LOM-NOR-NA-CP	4 (5.19)
AMX-AN-CN-GM-AMC	5 (6.49)
TE-CN-GM-CF-CAZ-CRO-CT-CTX-AMC	4 (5.19)
AMX-AN-CTX-AMC	6 (7.79)
LOM-CN-NOR-CF-CP	7 (9.09)
CN-CF-AMC	3 (3.89)
Others	8 (10.38)

Discussion

Microbial infection of the urinary tract is one of the most common infectious diseases worldwide. Approximately 1 in 3 women will require antimicrobial treatment for a UTI before age 24, and 40% to 50% of women will suffer from UTI during their lifetime (16). With regards to change in drug resistance pattern among bacterial uropathogens, notably *E. coli*, yearly determination of this pattern in populations is recommended. As described, *E. coli* isolates showed the highest resistance to

amoxicillin followed by amoxycillin/clavulanic acid, tetracycline and cephalexin. Nitrofurantoin and ceftizoxime by highest susceptibility were the most effective drugs in vitro. These findings are comparable to those of Khoshbakht *et al.* (2013) that their study was conducted in Karaj city from November 2009 to August 2010 and have reported high resistance to gentamicin, ampicillin, trimethoprim/sulfamethoxazole, and cephalothin, and higher sensitivity to imipenem, ciprofloxacin, nitrofurantoin, and ceftizoxime (16). In 2010 another study was performed by Mansouri *et al.* on clinical isolates of *Enterobacteriaceae* from patients in three major

hospitals in southeast Iran. They showed that the most frequent resistance was to trimethoprim/sulfamethoxazole, amoxicillin, and tetracycline. Imipenem and ceftizoxime were the most active agents (17). Moreover, it seems that the antibiotics like ampicillin, tetracycline, trimethoprim/sulfamethoxazole and cotrimoxazole should be prescribed cautiously and other antibiotics such as ceftizoxime and nitrofurantoin should be considered in light of antibiogram.

As the results of this study indicate, multidrug resistance is a major therapeutic concern. Since, there is a high rate of co-resistance to more than two unrelated antibiotics (92.77%) and the frequency of MDR to more than 5 antibiotics is increasing (79.5%) treatment requires further attention to the results of susceptibility tests. Production of extended-spectrum β -lactamases (ESBLs) by Enterobacteriaceae, specifically *E. coli*, has caused a major concern in several countries (18). ESBLs producing organisms are clinically relevant and remain an important cause for failure of therapy with cephalosporins (19). Detection of ESBL producing isolates is a critical requirement for tracking of drug resistance in different geographic regions. Phenotypic tests for ESBL detection only confirm whether an ESBL is produced but cannot detect the ESBL subtype. Since the phenotypic method cannot efficiently differentiate ESBL type, molecular techniques, such as PCR, are necessary in detecting different ESBL genes. This is the first time, to our knowledge, that ESBL-producing *E. coli* isolates have been detected in the immigration friendly city of Karaj. In our study, among 83 screened *E. coli* isolates with DDST, 33 isolates were found to be ESBL positive.

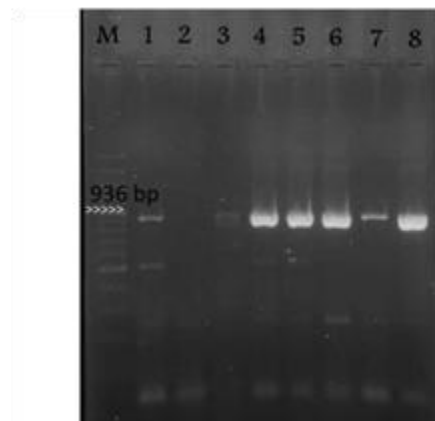


Figure 1. PCR product of TEM amplicon of *E. coli*, M: 1000 bp marker, Lane 1: positive control (*K. pneumoniae* ATCC 700603), Lane 2: Negative control (*E. coli* ATCC 35218), Lane 3 to 8: *E. coli* test isolates.

This rate (39.7%) was a little higher than that reported by Shayanfar *et al.* (28.6%- 2010) (20) and was lower than that reported by Mansouri *et al.* (63%- 2010) (17) and by shahcheraghi *et al.* (49%-2009) (21). The resulting prevalence rate of bla_{TEM} in this study (96%) was higher than that reported in different cities of Iran in the past decade.

Conclusion

Results show that as ESBL-producing organisms exhibit co-resistance to many other classes of antibiotics, especially the fluoroquinolones, then antibiotic options in the treatment of ESBL-producing organisms are extremely limited (22). Failure to treatment of ESBL producing organisms is largely due to the lack of clinical awareness; therefore molecular screening by laboratories is suggested to reduce the risk of therapeutic defeat.

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Conflict of interest

None declared conflicts of interest.

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