International Journal of Molecular and Clinical Microbiology 10(1) (2020) 1241-1247

International Journal of
Molecular and Clinical Microbiology

Antibiotic resistance pattern and frequency of beta lactamase and tetracycline resistance genes in *Escherichia coli* isolated from urinary tract infections in Tabriz

Yeganeh Poursaeed[1], Saman Mahdavi[2]*

1. Department of Microbiology, Sarab Branch, Islamic Azad University, Sarab, Iran
2. Department of Microbiology, Maragheh Branch, Islamic Azad University, Maragheh, Iran

ARTICLE INFO

Article history:
Received 3 April 2020
Accepted 27 May 2020
Available online 1 June 2020

Keywords:
Escherichia coli,
Urinary tract infection,
Antibiotic resistance,
PCR

ABSTRACT

Urinary tract infection (UTI) is one of the most prevalent diseases in human. Unfortunately, the indiscriminate use of antibiotics leads to resistance in bacteria gradually. The aim of this study was to determine the antibiotic resistance pattern and frequency of beta lactamase and tetracycline resistance genes in *Escherichia coli* isolated from urinary tract infections in women in Tabriz. In this cross-sectional descriptive study, 40 *E.coli* samples isolated from women with UTI were tested for determining the antibiotic resistance pattern as recommended by the Clinical Laboratory Standards Institute (CLSI) using disk diffusion method. Then, PCR assay was performed for identification of *tetA*, *tetB*, *tetC*, *TEM* and *SHV* genes. The most antibiotic resistance was related to ampicillin and cephalexin (100%) and the least antibiotic resistance was related to tetracycline (25%). The frequency of *tetA*, *tetB*, *tetC*, *TEM* and *SHV* genes was 5%, 0%, 0%, 30% and 0%, respectively. One *E.coli* isolate (2.5%) harbored *TEM* and *tetA* genes simultaneously. Antibiotic resistant *Escherichia coli* in UTI cases can indicate excessive use of antibiotics, the spread of antibiotic resistance gene cassettes and genetic transmission among the population. For definitive treatment and no resistance in pathogen strains, it is necessary to determine the resistance pattern to follow the resistance process.

1. Introduction

Urinary tract infection (UTI) is due to the presence of pathogenic bacteria in the urinary tract, which is one of the most common infections in outpatients (Coşkun et al., 2011). The frequency of urinary tract infection in women is more common than in men, so that nearly half of women experience at least once urinary tract infection during their lifetime (Dielubanza and Schaeffer, 2011). Previous studies conducted in various communities indicate that Gram-negative bacilli especially uropathogenic *Escherichia coli* are as the most common causes of urinary tract infection that is responsible for more than 80% of UTI (Dielubanza and Schaeffer, 2011). Different strategies are used by bacteria to protect the harmful effects of antibiotics. The production of beta-lactamase enzymes is one of the most important antibiotic resistance mechanisms in gram-negative bacteria against beta-lactam antibiotics (Li et al., 2002). The emergence of new antibiotics, such as broad-spectrum cephalosporins and aztreonam, and excessive use of them in the treatment of bacterial

*Corresponding author: Dr. Saman Mahdavi
Tel: +98-9144150454
E-mail address: S.mahdavi@iau-maragheh.ac.ir

infectious diseases, has led to production of a new class of antibiotic resistance enzymes by bacteria called broad-spectrum beta-lactamase (Tenover et al., 2003). In fact, occurrence of point mutations in amino acid sequences of the early TEM-1 and TEM-2 beta-lactamases has led to formation of these enzymes (Koneman et al., 2005). The beta-lactamases are classified in A, B, C and D groups, which broad-spectrum enzymes are located in A group and include derivatives of TEM and SHV mutated enzymes (Bush et al., 1995). The tetracycline resistant strains are highly prevalent among antibiotic resistant *E. coli* (Koo and Woo., 2011). Because of low cost and low side effects, this antibiotic is widely used in the treatment of livestock and human infections. Tetracycline resistant strains are related to the *tet* genes, which the *tetA*, *tetB*, and *tetC* genes are very important in antibiotic resistance (Tuckman et al., 2007; Ogeppaard et al., 2001). The aim of present study was to determine the antibiotic resistance pattern and frequency of beta lactamase (*SHV* and *TEM*) and tetracycline (*tetA*, *tetB* and *tetC*) resistance genes in *E. coli* isolated from urinary tract infection in women in Tabriz.

2. Methods and Materials

2.1. Sample Collection

In current descriptive cross-sectional study, 40 *E. coli* isolates (from 100 patients with urinary tract infection signs) which had been collected from women with UTI (from April to September 2018 in Asadabadi hospital in Tabriz, located in northwest of Iran) were identified with microscopic, cultural and biochemical tests (Catalase, Oxidase, Indole, Citrate Utilization, Methyl-Red and Voges-Proskauer tests).

2.2. Antibiogram Test

The antimicrobial susceptibility testing of all identified isolates were done according to the criteria of the Clinical and Laboratory Standards Institute method (CLSI 2017) (Padtan teb, Iran) (Kirby-Bauer method). The antibiotic disks such as tetracycline, oxytracycline, ampicillin, cefalotin, cefalexin, ceftazidime, ceftriaxone and cefotaxime (Padtan Teb, Iran) were used to evaluation of antibiotic resistance in studied *E. coli* isolates. *E. coli* PTCC1163 was used as positive control.

2.3. Polymerase Chain Reaction (PCR)

The extraction of genomic DNA from cultured *E. coli* isolates in heart-brain broth (Merck, Germany) was performed using DNA extraction kit (Pak Gene Yakhteh, Iran), according to manufacturer's instructions. The polymerase chain reaction (PCR) method was used to confirm the presence of *tetA*, *tetB*, *tetC*, *TEM* and *SHV* genes in *E. coli* isolates. The sequences and characteristics of used primers to amplify *tetA*, *tetB*, *tetC*, *SHV* and *TEM* genes were showed in Table 1.

Table 1. The sequences and characteristics of used primers to amplify *tetA*, *tetB*, *tetC*, *SHV* and *TEM* genes

Gene	Sequences	Amplicon size (bp)	Reference
tetA	F: AAGCGAGCGGGTTGAGAG R: GCCTTTCCTTTGGGTTCTC	326	Sarshar et al., 2012
tetB	F: ACTTCGGTATCTGTATTATCACG R: TTATCTTTGCTCCTTGGCTTG	415	Sarshar et al., 2012
tetC	F: TTGTTTCGGCGTGGGTATG R: CTGACTGGGTTGAAGGCTCTC	188	Sarshar et al., 2012
SHV	F: GATGAACGTTTCCCATGATG R: CCCTGTTATCGCTCAGGTAA	214	Kim et al., 2009
TEM	F: ATGAGTATTCAACATTTCCG R: CTGACAGTTACCAATGCTTA	874	Kim et al., 2009

The polymerase chain reaction (PCR) method was done in 25 µl, including 11µl of Master mix PCR, 1 µl of each specific primers (25 nano moles), 1 µl (50 ng) of DNA template and 11 µl of double distilled water. Timetable and thermal schedule for each gene is presented in Table 2.

PCR products were electrophoresed on 1.5% agarose and photographed using a gel document instrument.

The results of this study were analyzed by using of SAS (Version 9.2, 2011) software and Chi-square test and 0.05 was used as the significance level.

Table 2. PCR test conditions for *E. coli* samples for replication of the tested genes

Stage	Number of cycles	Tested genes Time	Temperature (℃)
		tetA/tetB/tetc/SHV/TEM	
Primary denaturation	1	3′/4′/3′/4′/4′	94
Denaturation	36	54"	94
Annealing	36	56"/56"/56"/56"/55"	63/63/66/63/58
Extension	36	52"/55"/52"/55"/55"	72
Terminal extension	1	6′/6′/6′/6′/7′	72

3. Results

3.1. Antibiotic Resistance Pattern

The results showed that all studied *E. coli* isolates were resistant to ampicillin and cefalexin. Much of the sensitivity of *E. coli* strains was to tetracycline (75%) and oxytetracycline (70%), respectively. The antibiotic resistance pattern of studied *E. coli* isolates was showed in Table 3.

17 (42.5%) samples of *E. coli* isolates were resistant to all 8 antibiotics tested. The frequency and percentage distribution of resistant *E. coli* isolates has been shown in Table 4.

3.2. Antibiotic Resistance Genes Frequency

The results showed that 12 (30%) and 1 (2.5%) samples of *E. coli* isolates harbored *TEM* and *tetA* genes, respectively (Figures 1 and 2). *tetB*, *tetC* and *SHV* genes were not found in any of the *E. coli* isolates.

Also, the presence of *tetA* and *TEM* genes was observed only in one *E. coli* isolate, simultaneously. The frequency of *TEM* gene showed statistical significance in comparison with other genes studied ($P< 0.05$) (Table 5, 6).

Table 3. Antibiogram results obtained from *E. coli* samples isolated from women with UTI

Antibiotic	Abbreviation	Concentration (µg)	Susceptibility		
			S (%)	R (%)	I (%)
Tetracycline	TE	30	30 (75)	10 (25)	0 (0)
Oxytetracycline	OTC	30	28 (70)	12 (30)	0 (0)
Ampicillin	AM	10	0 (0)	40 (100)	0 (0)
Cefalotin	CF	30	6 (15)	22 (55)	12 (30)
Cephalexin	CN	30	0 (0)	40 (100)	0 (0)
Ceftazidime	CAZ	30	8 (20)	24 (60)	8 (20)
Ceftriaxone	CRO	30	0 (0)	26 (65)	14 (35)
Cefotaxime	CTX	30	2 (5)	34 (85)	4 (10)

R: Resistant, S: Sensitive, I: Intermediate

Table 4. Frequency and percentage distribution of resistant *E. coli* isolates

Resistant isolates (%)	1 fold	2 fold	3 fold	4 fold	5 fold	6 fold	7 fold	8 fold
	40 (100)	40 (100)	36 (90)	30 (75)	27 (67.5)	22 (55)	19 (47.5)	17 (42.5)

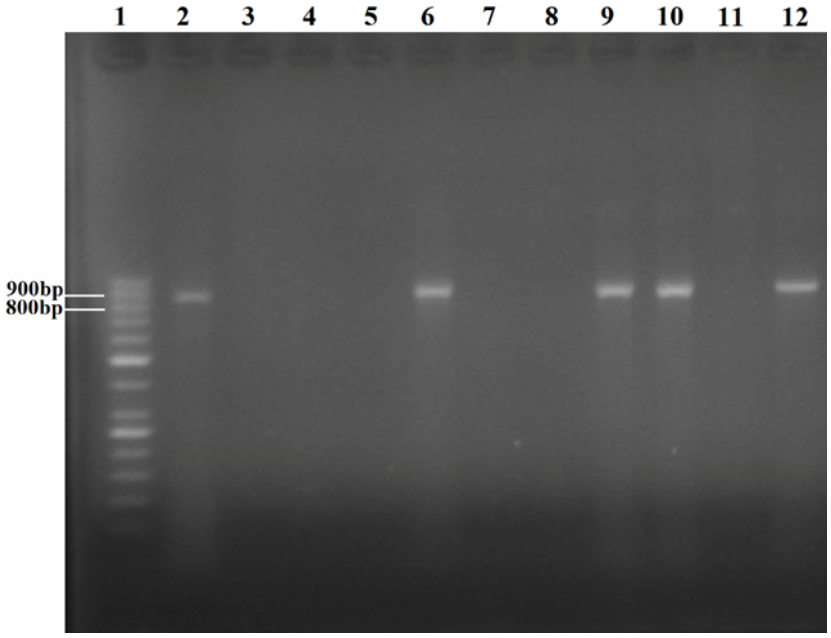

Figure 1. Electrophoresis of the amplified *TEM* gene (874bp) on 1.5% agarose gel. Lane 1: Ladder 50bp; Lane 2: positive control (*E. coli* ATCC 25922); Lane 3: negative control (double distilled water); Lanes 6, 9, 10, 12: PCR products of *TEM* gene; Lanes 4, 5, 7, 8, 11: negative samples of *TEM* gene.

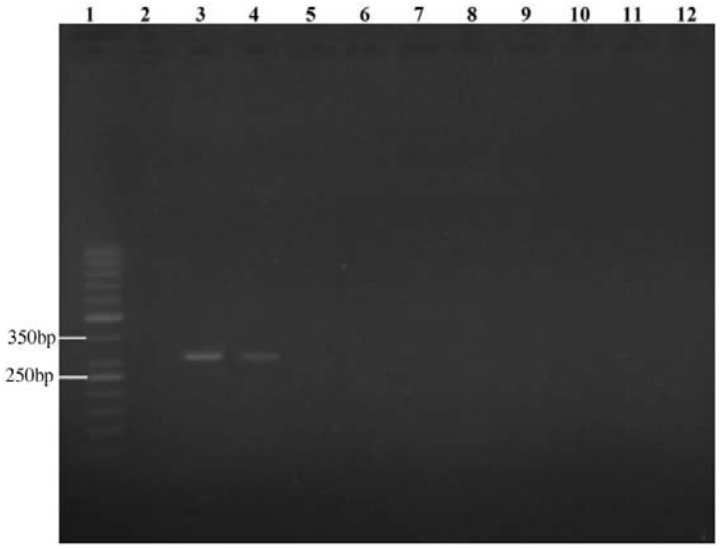

Figure 2. Electrophoresis of the amplified *tetA* gene (326bp) on 1.5% agarose gel. Lane 1: Ladder 50bp; Lane 2: negative control (double distilled water); Lane 3: positive control (*E. coli* PTCC 1163); Lane 4: PCR products of *tetA* gene; Lanes 5-12: negative samples of *tetA* gene.

Table 5. Frequency of intended genes in the studied bacterial samples

Gene	*TEM*	*tetA*	*tetB*	*tetC*	*SHV*
Yes	12	1	0	0	0
No	28	39	40	40	40
Total	40	40	40	40	40
P value	0.0119	0.9998	0.9999	0.9999	0.9999

Table 6. Comparison of the frequency of the studied genes in *E. coli* samples isolated from UTI

Gene	Prediction
TEM	0.3^{a}
tetA	0.025^{b}
tetB	0^{b}
tetC	0^{b}
SHV	0^{b}
Chi square	37.98
P Value	< 0.0001

a,b: Different letters in each row indicate a statistically significant difference($p< 0.05$).

4. Discussion

Urinary tract infection includes infections of the kidneys and bladder, and is the second most common bacterial infection after respiratory tract infections. *Escherichia coli* is the most common cause of urinary tract infections (Foxman, 2003). Today, one of the major health problems in the world is the increasing prevalence of antibiotic resistance of pathogens in various human and animal populations. The main cause of increased resistance of pathogenic bacteria is the overuse of antibiotics. This leads to the emergence and spread of resistant pathogens and resistance genes in them. In human and animal populations, antibiotics are used to treat and prevent infectious diseases. Due to the high use of antimicrobial drugs, the prevalence and spread of resistant bacterial clones and resistance genes in hospitals have increased (Van den Bogaard and Stobberingh., 2000). The results of current study revealed that the percentages of *TEM*, *tetA*, *tetB*, *tetc* and *SHV* genes in *Escherichia coli* strains isolated from urinary tract infections in women in Tabriz were 30%, 2.5%, 0%, 0% and 0%, respectively. In a study by Doosti et al. (2016) in Farsan, it was reported that the percentage of *TEM* and *SHV* genes in *E. coli* samples isolated from UTI in women and children was 62.1% and 8.42%, respectively (Doosti et al., 2016), which is much higher than our results in present study. In another study by Miraalami et al. (2015) in Tehran, it was reported that the percentage of *TEM* and *SHV* genes in *E. coli* samples isolated from UTI was 47.27% and 0.0%, respectively (Miraalami et al., 2015), which is consistent with the findings of our study. Sarshar et al. (2012), reported that the frequency of *tetA*, *tetB* and *tetC* genes in *E.coli* samples isolated from children with diarrhea in Shiraz was 76.7%, 62.7% and 13.9%, respectively (Sarshar et al., 2012). Nazemi et al. (2010), reported that the frequency of *TEM* and *SHV* genes in *E.coli* samples isolated from UTI in Tehran was 87.1% and 70.6%, respectively (Nazemi et al., 2010), which does not agree with the results of the recent study. Kamrani Hemat et al. (2017) reported that the percentage of *tetA* and *tetB* genes in *E. coli* samples isolated from UTI was 86.27% and 81.37%, respectively (Kamrani Hemat et al., 2017). The results of the current study indicate the difference in dispersion of *TEM*, *SHV*, *tetA*, *tetB* and *tetC* genes in *E. coli* isolates; this difference probably originates from geographical diversities and also differences in the ecological origin of the isolated strains (milk, human and different animals). In present study, the antibiogram results showed that all *E. coli* isolates (100%) were resistant to both ampicillin and cephalexin and 17 (42.5%) isolates were resistant to all of the tested antibiotics. The highest antibiotic susceptibility of *E. coli* isolates was observed against tetracycline (75%) and oxytetracycline (70%), respectively. In a study, Sarshar et al. (2012)

reported that the highest antibiotic resistance (55.8%) in *E.coli* samples isolated from children with diarrhea in Shiraz was related to tetracycline (Sarshar et al., 2012). In another study, Nazemi et al. (2010) reported that the resistance to ceftazidime and cefotaxime in *E. coli* samples isolated from UTI in Tehran was 47.1% and 39.2%, respectively (Nazemi et al., 2010). Miraalami et al. (2015) reported that the resistance to tetracycline, ampicillin and cefotaxime in *E. coli* samples isolated from UTI in Tehran was 69.1%, 87.2% and 76.3%, respectively (Miraalami et al., 2015). The excessive use of tetracycline creates tetracycline resistant strains, and ultimately transfer of tetracycline resistance genes through plasmids and transposons to other strains can be one of the most important causes of high prevalence of tetracycline resistance. The excessive use of this antibiotic in animals and transfer of resistance genes from animal strains to human strains are other reasons for increased resistance to tetracycline (Karami et al., 2006; Ogeppaard et al., 2001). Today, antibiotic resistance is a serious problem in treatment of infections caused by bacteria. One of the most important causes of infection is extended spectrum β-lactamase (ESBL) producing *E. coli* such that about 60% of the bacteremia caused by ESBL producing *E. coli* leads to death. It seems that one of the most important causes of increased antibiotic resistance in *E. coli* strains is excessive consumption of antibiotics, especially the beta-lactam group (Melzer and Petersen, 2007). The presence of *TEM* and *SHV* genes and alteration in membranes and impermeability to drugs may also lead to resistance to carbapenems (Livermore et al., 2003). The mismatch between the results of the antibiogram test in several studies is probably due to differences in geographical areas, type of treatment regimens and measure of antibiotic use in different regions.

Conclusion

The results of the current study showed high antibiotic resistance in *Escherichia coli* samples isolated from UTI in women in Tabriz. This may be due to the exchange of resistance genes within and across species and with commensal bacteria of the human and animals. Despite the high antibiotic resistance in *E. coli* samples, the frequency of the relevant genes was very low. Other genes may be involved in the resistance of *E. coli* samples to antibiotics.

Conflicts of interest

There are no conflicts of interest.

Funding

This work was financially supported by authors.

Refereces

Bush K., Jacoby GA., Medeiros AA. (1995) A functional classification scheme for beta-lactamases and its correlation with molecular structure. Antimicrob. Agents. Ch. 39(6): 1211-33.

Coşkun Ö., Erdem H., Avci A. (2011) Management of community-acquired acute bacterial cystitis in Turkey. Turk. J. Med. Sci. 41: 149-157.

Dielubanza EJ., Schaeffer AJ. (2011) Urinary tract infections in women. Med. Clin. N. Am. 95: 27-41.

Doosti A., Daruoshi M., Borza R., Pasand M. (2016) Antibiotic resistance and distribution of beta-lactamase resistance genes in Escherichia coli strains isolated from urinary tract infection in women and children in Farsan town. J. Shahrekord. Univ. Med. Sci. 17: 53-61.

Foxman B. (2003) Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. Am. J. Med.49: 53-70.

Kamrani Hemat N., Mirzaee M., Najar-peerayeh S. (2017) Prevalence of tetracycline resistance genes (tetA, tetB) and antibiotic resistance pattern in uropathogenic Escherichia coli isolates. N. C. M. B. J. 7(25): 9-18.

Karami N., Nowrouzian F., Adlerberth I., Wold AE. (2006) Tetracycline resistance in Escherichia coli and persistence in the infantile colonic microbiota. Antimicrob. Agents. Ch. 50: 156-61.

Kim J., Semi J., Rhie H., et al. (2009) Rapid detection of extended spectrum B-lactamases (ESBL) for entrobacteriaceae by use of a multiplex PCR-based method. Infect. Chemother. 41(3): 181-184.

Koneman EW., Allen SD., Dowell VR., et al. (2005) Color atlas and text book of diagnostic microbiology. 6th ed. Lippincott Williams & Wilkins.

Koo HJ., Woo GJ. (2011) Distribution and transferability of tetracycline resistance determinants in Escherichia coli isolated from meat and meat products. Int. J. Food. Microbiol. 145: 407-413.

Li Q., Lee JY., Castillo R., et al. (2002) A novel antibacterial agent with broad-spectrum activity and enhanced potency against beta-lactamase-producing strains. Antimicrob. Agents. Ch. 46(5): 1262-8.

Livermore DM., Sefton AM., Scott GM. (2003) Properties and potential of ertapenem. J. Antimicrob. Chemoth. 52: 331-44.

Melzer M., Petersen I. (2007) Mortality following bacteraemic infection caused by extended spectrum beta-lactamase (ESBL) producing E. coli compared to non-ESBL producing E. coli. J. Infect. 55: 254-9.

Miraalami GH., Parviz M., Khalajzadeh S. (2015) Evaluation of antibiotic resistance in extended-spectrum betalactamase (ESBL) genes in the E. coli isolates of urinary infections. J. Babol. Univ. Med. Sci. 17: 19-26.

Nazemi A., Yazdi M., Miri Nargasi M., et al. (2010) Prevalence of SHV/CTX-M/TEM (ESBL) Beta-lactamase resistance genes in Escherichia coli isolated from urinary tract infections in Tehran, Iran. Med. Lab. J. 4: 48-54.

Ogeppaard H., Steinum MT., Wasteson Y. (2001) Horizontal transfer of a multi-drug resistance plasmid between coliform bacteria of human and bovine origin in a farm environment. Appl. Environ. Microbiol. 67: 3732-34.

Sarshar M., Tavafi H., Ghorbani-Dalini S., et al. (2012) Tetracycline resistance genes of diarrhogenic E. coli in pediatric patients. Iranian. J. Infect. Dis. Trop. Med. 17: 13-18.

Tenover FC., Raney PM., Williams PP., et al. (2003) Evaluation of the NCCLS extended-spectrum beta-lactamase confirmation methods for Escherichia coli with isolates collected during Project ICARE. J. Clin. Microbiol. 41: 3142-6.

Tuckman M., Petersen PJ., Howe AY., et al. (2007) Occurrence of tetracycline resistance genes among Escherichia coli isolates from the phase 3 clinical trials for tigecycline. Antimicrob. Agents. Ch. 51(9): 3205-11.

Van den Bogaard AE., Stobberingh EE. (2000) Epidemiology of resistance to antibiotics links between animals and humans. Int. J. Antimicrob. Ag. 14: 327-35.