Evaluation of ESBL (Extended Spectrum Beta-Lactamase) Producing Escherichia Coli Isolated from Patients with Urinary Tract Infection by Phenotypic and Genotypic Methods

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Research article

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Abstract

Background: Urinary Tract Infection (UTI) is the most common bacterial infection in the world. E. coli is the predominant Pathogen. This study evaluates the prevalence of ESBL in E. colis isolated from patients with urinary tract infections with phenotypic and genotypic methods.

Methods: This descriptive-analytical study was done on 155 isolates of E. coli isolated from patients with urinary tract infection who had received the study consent. After accurate identification of E. coli strains. ESBL production for Escherichia coli isolates which are resistant to ceftriaxone or ceftazidime was evaluated by CDT method. TEM, SHV and CTX-M genes were identified by PCR.

Results: The results showed that 30 strains from 155 strains of E. coli had ESBL. Strains of ESBL producer were more in males was lower in educated persons. 38.9% of ESBL producer had antibiotic use, 29.9% -producing Escherichia hospitalization and 31.6% uti history. The highest level of drug allergy in the ESBL was related to nitrofurantoin, and the highest resistance was related to cefazolin, co-trimoxazole. The CTX-M and the CTX-M15 gene were found in 92.7% and 57.1% of cases, respectively; also the SHV and TEM genes were not found in any of ESBL-producing Escherichia coli strains. Most therapeutic response in patients was related to cefexime, ciprofloxacin and nitrofurantoin 27.4%, 26% 21.9%, respectively.

Conclusion: This study showed that the history of antibiotic use, hospitalization, uti related to increase of ESBL-producing in E. coli isolates., the CTMX-M gene is the most common gene in ESBL-producing E. coli strains.

1. Background

Urinary Tract Infection (UTI) is the most common bacterial infection in around the world with a high incidence and high annual treatment costs. UTI is a major cause of disease in older men and all age groups of women. The manifestations of UTI may be different from asymptomatic cystitis to pyelonephritis and septicemia[1]. More than 60% of women have experienced UTI, at least once in their lifetime. About 25–46% of them experience the second episode, 6 months after the primary UTI. A high percentage of recurrent UTIs are caused by that bacterium which causes the primary infection[2]. E. coli is a gram-negative bacillus and the dominant microorganism in UTI.

Since, 90% of UTI is because of E. coli, so experimental antibiotic treatment is mainly towards this pathogen. Experimental treatment begins before the result of culture is obtained, because the results of culture and antibiogram will be prepared 4 days after the test[3].

Choosing an appropriate antibiotic for the management of UTI depends on gender, clinical manifestations, prevalence of resistance in the society, and the presence of anatomical or functional disorders. However, overuse of antibiotics has been led to an increase in the production of ESBL (extended Spectrum beta-lactamase) which has been produced by the bacterium.Various methods have been used by bacterium to protect against the effects of antibiotics. Production of Extended-spectrum beta-lactamases (ESBL) enzymes is one of the most important mechanisms which are used in gram-negative bacteria against β-

lactamases antibiotics[4]. Mutation in TEM, HSV and CTX-M gene cause this resistance. These enzymes are capable of hydrolyzing all cephalosporins and azotronam[5]. Nowadays, there is a high prevalence of urinary tract infection caused by ESBL-producing pathogens around the world, and the antibiotic resistance of it has been converted to a serious challenge of public health which increases mortality and morbidity[6]. Therefore the present study was done to determine the prevalence of ESBL in E. colis which are agent of urinary tract infection and to evaluate its genotypic in outpatients who were referred to Imam Reza Hospital in order to use the results for reducing their medical costs, in addition to assisting physicians in choosing the correct treatment and avoiding unnecessary treatments.

2. Methods

This cross-sectional study from November, 2017 up to April, 2019 was done on 155 urinary isolates of E. coli isolated from patients with urinary tract infection who were referred to Imam Reza Hospital as the outpatient. A questionnaire was obtained including demographic information of patient such as age, gender, job, clinical symptoms, hospitalization history and UTI and history of antibiotic use. The bacterium specimen isolated from the patients was transferred to the Research Laboratory of the University of Medical Sciences, where the bacteria were identified by biochemical methods.

Antibiogram was done by disk diffusion method if the bacteria belonged to Escherichia coli. Antibiotic resistance test was determined by Kirby-bauer method. First, 3–4 colonies were removed from the all colonies and were transferred to a sterile tube containing physiological serum and the tubes were placed in incubator at 37 ° C for 1–2 hours to obtain 0.5 McFarland suspension. The sterile swab was then smeared with the suspension and was cultured on Mueller Hinton Agar medium in all directions. Then we placed the antibiotic discs on a plate surface with the help of a forceps and next to the flame, at a distance from the edge of the plate. The plates were then transferred to an incubator at 37 ° C and the results were evaluated after 18–24 min. Results were reported based on measurement of non-growth zone diameter by a ruler sensitive and according to standard criteria of (CLSI) as sensitive, semi-sensitive and resistant. Combined disk method was used to identify the ESBL-producing Escherichia coli strains phenotypically .For this purpose, ceftazidime disc, combined disc of ceftazidime/clavulanic acid and combined disc of ceftriaxone and ceftriaxone/clavulanate were placed on a molar Hinton agar medium in a distance of 20 mm from each other. Increasing the diameter of the non-growth zone by more than 5 mm in the combined disk compared to the non-growth zone around the ceftazidime disk indicates the presence of extended spectrum beta-lactamase (ESBL) enzymes.

ESBL-producing bacteria were used for evaluating the genotypic and PCR was used to identify TEM, SHV and CTX-M genes. Heat shock method was used to extract DNA. In this method, we centrifuged the cc1 from 24-hour bacterium culture at 14000 rpm for 5 minutes. We emptied the supernatant and washed the sediments twice with PBS buffer (Phosphate buffered saline) and PH: 7. The cells were suspended in 50 µl of sterile water. The suspension was first incubated at 80 ° C for 5 minutes and then at 95 ° C for 10 minutes. Then, we centrifuged at 12,000 rpm for 4 min at 4 ° C.The supernatant was used as template for PCR. The PCR reaction was done by 20 µL mixture which contains 10 µL of master mix, 1 µL of each primer

with a concentration of 20 picomol, 2 µL of genomic DNA and 6 µL of deionized water. Finally, the reaction products were electrophoresed in 1.2% agarose gel to evaluate the results of the PCR reaction.

Statistical analyses were performed by SPSS version 16 software (SPSS Inc., Chicago, IL, USA). Statistical significance was considered with P values < 0.05.

3. Results

In this study, 151 patients were evaluated, that majority of whom were female (88.1%), and most of them were in the age group of 20–40 years (46.7%) and also older than 40 years (31.3%). 55% of people had a bachelor's degree or higher. 26% of them were housewives, 18.5% were students and 86.8% lived in the city. Among patients, 83% had antibiotic use history, 45.3% had hospitalization history, and 26% had a urinary tract infection history. Dysuria and frequency with 46.6% and 26.5% were the most common clinical symptoms, respectively. Pyuria with 48% and positive nitrite with 42.6% were the most common laboratory findings. The highest level of sensitivity was related to ceftazidime (74.1%), gentamicin (72.6%) and nitrofurantoin (72.6%) in evaluating the antibiogram of antibiotics. Ceftriaxone and ciprofloxacin were next ranks. Ampicillin with 20.4% and doxycycline with 29.9% had the lowest sensitivity.

30 strains from 155 E. coli strains were productive of ESBL which increased by increasing age ($p < 0.01$). But there was no significant difference in terms of gender education (Table 1). 38.9% of people with ESBL positive strains, had history of antibiotic use, 29.9% had history of hospitalization and 31.6% had history of UTI, which has significant relation between increasing the prevalence of ESBL with above according to $p < 0.05$ (Table 1). The highest resistance is related to cefazolin, co-trimoxazole and ampicillin and the lowest resistance is related to nitrofurantoin, gentamicin and ciprofloxacin, respectively, in the ESBL-producing E. coli strains (Table 2). In the distribution of ESBL-producing genes, the CTX-M gene with 92.9% had the most prevalence in the ESBL-producing E. coli strains (Table 3).

Table 1

Frequency of ESBL-producing E. coli strains based on patient characteristics and epidemiological variables

Variables		Esbl				P
		Positive		Negative		
		Frequency	Percentage	Frequency	Percentage	
Gender	Male	4	22/2	14	77/8	1
	Female	26	19/5	107	80/5	
Educational Level	High School Or Less	14	28	36	72	0/216
	High School Diploma	6	19/4	25	80/6	
	Bachelor's Degree	9	17/3	43	82/7	
	Ma And/Or Higher	1	5/6	17	97/4	
Age	Under 20	7	10	63	90	0/01
	20–40	8	24/2	25	75/8	
	Above 40	15	31/9	32	68/1	
Occupation	Employee	3	11/5	23	88/5	0/4
	Manual Worker	0	0	7	100	
	Housewife	9	22/5	31	77/5	
	Student	5	17/9	23	82/1	
	Self Employed	3	16/7	15	83/3	
	Other	10	31/3	22	68/8	
Place Of Residence	Urban	25	19/1	106	80/9	-
	Rural	5	25	15	75	
Antibiotic Consumption History	Positive	7	38/9	11	61/1	0/043
	Negative	20	16/7	100	83/3	
Hospitalization History	Positive	20	29/9	47	70/1	0/007
	Negative	10	12/3	118	79/7	
Urinary Infection History	Positive	12	31/6	26	68/4	0/045
	Negative	18	16/7	90	83/3	

Table 2
Antibiotic resistance pattern in ESBL-producing E. coli strains

A	Sensetive (Percentage) Frequency	Resistant (Percentage) Frequency	Unknown (Percentage) Frequency
Cefazolin	2 (7/6%)	25 (3/83%)	3 (10%)
Cotrimoxazole	4 (3/13%)	24 (80%)	2 (7/6%)
Ampicillin	5 (7/16%)	25 (3/83%)	5 (7/16%)
Ceftriaxone	5 (7/16%)	24 (80%)	1 (3/3%)
Ceftazidime	7 (3/23%)	22 (3/73%)	1 (3/3%)
Cefixime	3 (10%)	20 (7/66%)	7 (3/23%)
Doxycycline	10 (3/33%)	18 (60%)	2 (7/6%)
Nalidixic Acid	8 (7/26%)	16 (3/53%)	6 (20%)
Cefotaxime	2 (7/6%)	13 (3/43%)	15 (50%)
Ciprofoxacin	9 (30%)	8 (7/26%)	12 (40%)
Gentamicin	19(3/63%)	3 (10%)	8 (7/26%)
Nitrofurantoin	27 (90%)	2 (7/6%)	1(3/3%)

Table 3
Frequency distribution table of ESBL
Producing Genes

Gene	Frequency	Percentage (%)
CTX – M	28	92/9
CTXM – 15	16	57/1
SHV	0	0
TEM	0	0

The results of univariate regression model showed that age, antibiotic consumption history, hospitalization history use predicted ESBL. Those with a hospitalization history have a higher odds of being positive ESBL by 3.02 units.

The results of multiple regression model also showed that variables hospitalization history and antibiotic consumption history have significant effect on ESBL. Those with a hospitalization history and antibiotic consumption history have a higher odds of being positive ESBL by 4.08 and 8.41 units (Table 4).

Table 4
logistic regression model for ESBL-producing E. coli strains based on patient characteristics and epidemiological variables

Variable		Univariate			Multiple		
		B(SE)	OR(95%CI)	p-value	B(SE)	OR(95%CI)	p-value
Gender	Male	Reference					
	Female	0.16(0.61)	1.18(0.36-3.86)	0.79	----	------	------
Education level	Ma And/or Higher	Reference					
	High School Or Less	1.88(1.07)	6.61(0.80-54.48)	0.08	----	------	------
	High School Diploma	1.41(1.12)	4.08(0.45-37.00)	0.21	----	------	------
	Bachelor's Degree	1.27(1.09)	3.56(0.42-30.27)	0.24	----	------	------
Age	Above 40	Reference					
	Under 20	-0.38(0.51)	0.68(0.25-1.86)	0.45	0.68(0.73)	1.98(0.47-8.33)	0.35
	40-20	-1.44(0.51)	0.24(0.08-0.64)	0.004	-1.05(0.56)	0.35(0.12-1.06)	0.06
Occupation	Other	Reference					
	Employee	-1.25(0.72)	0.29(0.07-1.18)	0.08	----	------	------
	Housewife	-0.45(0.54)	0.64(0.22-1.83)	0.40	----	------	------
	Student	-0.74(0.62)	0.48(0.14-1.62)	0.24	----	------	------
	Self Employed	-0.82(0.74)	0.44(0.10-1.87)	0.27	----	------	------
Place Of Residence	Rural	Reference					
	Urban	-0.35(0.56)	0.71(0.23-2.13)	0.54	----	------	------
Antibiotic Consumption History	Negative	Reference					
	Positive	2.13(0.75)	8.44(1.92-	0.005	2.13(0.78)	8.41(1.82-	0.006

			37.16)			38.98)	
Hospitalization History	Negative	Reference					
	Positive	1.11(0.43)	3.02(1.29-7.02)	0.01	1.41(0.60)	4.08(1.24-13.40)	0.02
Urinary Infection History	Negative	Reference					
	Positive	0.84(0.43)	2.31(0.98-5.40)	0.05	0.62(0.50)	1.86(0.69-4.97)	0.22

4. Discussion

In this study, 155 isolates of E. coli isolated from patients with urinary tract infection were evaluated. Of them (133 cases) were females and 10.5% (18 cases) were males. The level of UTI in the age group of less than 20 years, 20–40 years and over 40 years was obtained 22%, 46.7% and 31.3%, respectively .According to the results of this study, the highest level of antibiotic resistance in patients with urinary tract infection was related to ampicillin and doxycycline and the lowest level of resistance was related to gentamicin, cefotaxime and nitrofurantoin. In a study by Abdulaziz Qassim (2018), the highest level of antibiotic resistance was related to ampicillin, amoxicillin clavulanate and the lowest level of drug resistance was for imipenem, gentamicin, cefotaxime, and nitrofurantoin[7].

In this study, by evaluating the symptoms of urinary tract infection, it was determined that the prevalence of clinical symptoms showed the most prevalence in dysuria 46.4%, flank pain 27.2% and frequency 26.5% in the patients, respectively. Hematuria, diarrhea, vomiting, and fever were observed in less than 15% of cases. In the study of Mishra (2010), dysuria, frequency, and frequent urination (Orgency) were the most common symptoms, which are consistent with our study[8] .

Among the laboratory factors, nitrite in 41.7%, pyuria in 47% and hematuria in 15% of cases were positive. In the Schippmann's (2018) study, the prevalence of nitrite and pyuria was reported 41% and 38%, respectively[9]. Also in a study by Jennifer (2001) pyuria and nitrite was obtained 47.5% and 44%, respectively[10]. There is a little difference between these studies and the conducted study, according to comparing the results.

In this study, 30 E. coli isolates were productive of ESBL from 155 isolates. Among the strains with positive ESBL, 19.5% were related to females (26 cases of 133 female patients) and 22.5% were related to males (4 cases of 18 male patients) male. In the Shakia's study (2017), the prevalence of ESBL was 81.8% in females and 18.2% in males[11]. In the Sakina's study (2018), the prevalence of ESBL has been reported 40% in females and 30% in males[12]. Also in a study by Stefano (2014), the prevalence of ESBL in females was reported 15% higher than males[13]. One reason for the discrepancy between the results of our study and the conducted study was the overuse of antibiotics, which may result in a higher prevalence of ESBL.

The prevalence of ESBL in the age group of over 40 years, 20–40 years and less than 20 years was 31.9%, 24.2% and 10%, respectively. In the Sakina's study (2018), the level of ESBL in the age groups of 16–30 years, 31–45 years and 46–60 years were reported 34.6%, 37.5% and 30%, respectively[12]. In the Shakia's study (2017), the most prevalence of ESBL was reported 27.3% and 24.2%, respectively, in the two age

groups of 21–30 years and 31–40 years[11]. In this study, 11.5% of people with positive ESBL were employees, 0% workers, 22.5% housewives, 17.5% students, and 31.3% had other jobs. The results show that there is no relationship between job and prevalence of ESBL and all strata get the ESBL. In the statistical population under study, among those who had ESBL positive, 28% were under diploma (14 cases of 50 patients), 19.4% were diploma (6 cases of 31 patients) and 17.3% were bachelor (9 cases of 52 patients) and 5.6% had master's degree or higher (1 case of 18 patients).The results show that the level of ESBL in people with higher education is significantly lower than people with low education. The prevalence of ESBL in the urban population is 19.1% (25 cases of 131 patients) and in the rural population is 25% (5 cases of 20 patients), this issue indicated a higher ESBL prevalence in the rural population.

According to the results of this study, in ESBL-producing E. coli strains, 31.6% of patients reported a history of UTI (6 cases of 30 patients). History of hospitalization in patients with ESBL-producing E. coli strains has been reported 29.9% (20 out of 67 patients) and 12.3% of the patients (10 cases of 81 patients) did not report hospitalization history.

In this study, 38.9% (7 cases of 18 patients) reported a history of antibiotic use during one past month. In the study of Abyaneh (2018), the recurrent urinary tract infection and the use of more than two antibiotics per year have been proven as the factors risk of ESBL-producing[14]. In the koksal's study (2019), the prevalence of ESBL in patients with a history of hospitalization in the past 3 months was 27.3%, while it was 8% in other patients[15], which is consistent with the results of our study.

The most level of resistance in ESBL-producing E. coli strains was related to cefazolin, ampicillin, co-trimoxazole, ceftriaxone and ceftazidime with 83.3%, 83.3%, 80%, 80% and 73.3%, respectively, and the lowest level of resistance was related to nitrofurantoin and gentamicin with 6.7% and 10%, respectively. In the study of Abdulaziz Qassim (2018), the most level of resistance in ESBL-producing E. coli strains was related to ampicillin, amoxicillin clavulanate and ceftazidime with 100%, 88% and 85%, respectively, and the lowest level of resistance was for imipenem, nitrofurantoin and gentamicin with 0%, 21% % and 27%, respectively[7]. In the study of Dash (2018), ampicillin, cefuroxime and ceftriaxone showed the most level of resistance and meropenem, amikacin and nitrofurantoin showed the lowest level of resistance[16]. In the koksal's study (2019), the most level of resistance in ESBL-producing E. coli strains, was related to ceftriaxone, ceftazidime, and cefepime with 100% resistance and ampicillin with 95.5% resistance. The lowest level of resistance was reported for imipenem, meropenem and nitrofurantoin[15]. The results of conducted study are consistent with our study and indicate a high level of family of β-lactam antibiotics resistance in ESBL-producing E. coli strains.

Evaluating the genes generated in ESBL specimens using PCR in this study showed that CTX-M gene was found in 86.7% and CTX-M15 gene was found in 53.3% of the strains (16 from 28). The TEM gene and the SHV gene were not found in any of the strains. In the study of Haba Maged (2018), TEM, SHV and CTX-M genes were found in 42.8%, 36.7% and 38.6% of cases, respectively[17]. According to the study by Elizabeth Gaviral (2018), 92% of ESBL-containing strains had the CTX-M 15 gene and the SHV gene was found in only two isolates, which indicates a high degree of consistent with this study[18]. In the Abdulaziz Qassim (2018) study, 93.94% of the strains also contained the CTX-M gene, and TEM was found in 12.12% of the strains,

which is opposed with the results of this study regarding the TEM gene[7]. Al-Jami et al., identified the distribution of ESBL-producing genes on 121 isolates in 2019[19]. Among the 75 ESBL-producing strains, the SHV gene was in 30.7% and the TEM gene in 20.7% of cases, which is not consistent with our study.

In this study, the most response to treatment in antibiotics was reported for nitrofurantoin (100%), ciprofloxacin (94%), cefexime (80%), cotrimoxazole (80%) and doxycycline (64%), respectively. According to Ghazala's study (2019), nitrofurantoin was introduced as the first treatment with 100% response[20]. Thomas Hutton in 2012 also introduced nitrofurantoin, fosfomycin, and trimethoprim-sulfamethoxazole as the first treatment[21].

Overall, the conducted study shows that overuse of antibiotics and recurrent infections and timely and inadequate non-treatment can lead to ESBL-producing E. coli strains in urinary tract infections and the following drug resistance will have a serious challenge. Patients' awareness and education about the principles of hygiene, referring to a physician and not arbitrary use of antibiotics are effective in reducing multidrug resistance.

The antibiotic use, hospitalization, and UTIs history lead to increasing ESBL-producing E. coli strains in urinary tract infections.

5. Conclusion

In this study, ceftazidime, gentamicin, nitrofurantoin showed the highest sensitivity level, and ampicillin and doxycycline showed the highest resistance level, which can be a good guide for physicians in choosing a drug for the treatment of urinary tract infection.

Evaluation of the urine specimens of ESBL-producing genes show that the CTX-M gene is the most common and most important factor in causing drug resistance.

Nitrofurantoin as a cheap drug, with high sensitivity and low side effects, can be a suitable treatment for urinary tract infections.

Abbreviations

ESBL: extended spectrum beta-lactamase
UTI: Urinary Tract Infection

Declarations

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Contributions

MHN, HH and AE conceived and designed the study. HH, MM collected the data. MHN and AE analyzed the data. MHN, HH and AE wrote the manuscript. All authors read and approved the final version of the manuscript.

Ethics approval

This study was approved by the institutional review board and ethics committee of Birjand University of Medical Sciences, Birjand, Iran (ethical approval code, ir. bums. REC. 1397. 184). All participants completed the consent form.

Consent for publication

Not applicable

Competing interest

The authors declare that they have no competing of interest.

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