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Risk factors, and antibiotic resistance pattern of Escherichia coli with extended-spectrum β-lactamase enzyme in west of Iran

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Abstract:

Background: Extended-spectrum β-lactamases (ESBL) is present in drug-resistant enterobacteriaceae that is causing many epidemics in hospitals of developing countries; it remarkably limits therapeutic options and increases the rate of motility and morbidity. In order to choose the most appropriate treatment for urinary tract infections (UTI), it is necessary to know its risk factors. The aim of the present study is assessment of risk factors, and antibiotic resistance pattern of Escherichia coli (E. Coli) with ESBL (ESBL-EC) in the patients with UTI in west of Iran.

Methods: This case-control study was conducted on patients with UTI referred to Sanandaj Tohid Hospital in Iran during March 2015 to March 2017, who had positive E. coli culture.they were divided into two groups of case (posetive ESBL-EC) and control (negative ESBL-EC) . Demographic data and risk factors findings were compared in the two groups. Using disk diffusion method. Antibiogram test was performed. SPSS software version 21 was used to analyze the data and $p < 0.05$ was considered as the significant level.

Result: 98 patients with UTI and positive E. coli cultures (49 case and 49 control) were evaluated . Risk of UTI induced ESBL-EC was increased 3.33 times in patients with urinary catheterization in the last 12 months (OR = 3.33; 95% CI: 1.33 – 8.35; p = 0.010).

Conclusion: Based on the findings of this study, urinary catheterization in the last 12 months increased the risk of UTI induced ESBL-EC and cotrimoxazole and ciprofloxacin should not be administered for treating E. coli-induced UTI in Sanandaj.

Keyword: Escherichia Coli; Risk Factors; Antibiotic Resistance; β-lactamases Enzyme

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1. Introduction

Escherichia coli (E. coli) is the main cause of urinary tract infection (UTI) (1, 2). The inappropriate use of antibiotics leads to drug resistance. 38 years ago, an enzyme called extended-spectrum β-lactamases (ESBL) was discovered and today this resistance pattern has become a major health problem. This enzyme is present in E. Coli, Enterobacteriaceae, and Klebsiella and is the main cause of antibiotic resistance in these bacteria (3, 4). Therapeutic options are very limited for patients with ESBL-producing bacteria (ESBL-EC) and in most cases, they are admitted to the intensive care unit (4), thus the epidemics caused by these organisms increase the length of hospitalization, mortality, morbidity, and costs (5). Prevalence of ESBL-EC was reported in Iran (Kurdistan) 19.02% (6) and in Korea, Norway, India, Hong Kong, Singapore, and Egypt 1.5%, 13%, 60%, 48%, 33%, and

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60.9%, respectively (7-9). The risk factors for antibiotic resistance of ESBL-EC infection are age, sex (1, 6), food chain and use of antibiotics in animals, use of extended-spectrum antibiotics, especially cephalosporins and fluoroquinolones, underlying diseases (such as diabetes, heart failure, kidney failure, chronic kidney disease), cancers, burns, immunodeficiency, pregnancy, prolonged hospitalization, urinary catheter insertion, invasive procedures on urinary tract (8, 10, 11). Given the high cost of treating this type of infections and antibiotic resistance of ESBL-EC, identifying risk factors of ESBL-EC is important for choosing the best treatment.

Therefore, the aim of this study is assessment of risk factors, and antibiotic resistance pattern of ESBL-EC in west of Iran (Sanandaj) from March 2015 to March 2017.

2. Method

2.1. Study design and setting

This case-control study was done in Tohid Hospital, Sanandaj, Iran from March 2015 to March 2017. The protocol of the study was approved by the ethics committee of Kurdistan University of Medical Sciences (IR.MUK.REC.1394/252). All participants filled written informed consent. The researchers adhered to the principles of Helsinki Declaration.

2.2. Participants

The participants over 15 years old with posetive E. coli urine culture were included in the present study. If questionnaire could not be completed any causes, participant was excluded from the study. Finally, 98 patients were selected and entered into the study.

Posetive ESBL-EC patiants were selected as case group and negative ESBL-EC were considered as control group. The sampling method of present study were consecutive.

2.3. Measurements

A questionnaire was used to collection of data include age, sex, job status, underlying diseases (such as pulmonary diseases, kidney diseases and cardiovascular diseases, type 2 diabetes), malignancy, neurological disorder, rheumatologic or gastrointestinal diseases, urinary catheter insertion, antibiotic or immune suppressant drugs usage, hospitalization, dialysis, and any type of urinary intervention.

According to the The Clinical & Laboratory Standards Institute guidelines, antibiotic susceptibility test was performed by antibiogram kit (Accelerate PhenoTest™ BC kit, Germany). Brifly, petri dishes contain of cefotaxime and ceftazidime alone and combined with clavulanic acid (cefotaxime-clavulanate and ceftazidime-clavulanate petri dishes) were used to detect posetive ESBL-EC. The difference in the growth of halo was measured in alone and combined antibiotic petri dishes. This type of resistance was proved whenever the difference in halo diameter was larger than or equal to 5mm.

2.4. Sample size

The sample size was calculated based on the mean difference of outcomes from the study of Elgaml et al., (12) with effect size of 0.68 (difference of mean outcomes between the two study groups), power of 80%, and significance level of 5%. Sample size of 49 patients in each group was regarded to detect a clinically important difference between the outcome measures of the study groups. It was assumed that the outcome measures are normally distributed.

2.5. Statistical analysis

Statistical analyses were performed using SPSS 21.0 statistical software. Chi-square or Fisher exact test was used to assess the relationship between the variables and ESBL-EC. Then, in order to determine the independent factors affecting ESBL-EC, the variables with a $p < 0.1$ in the

Table 1: Baseline characteristics of studied patients

Variable	Negative ESBL-EC (n=49)	Posetive ESBL-EC (n=49)	Odds ratio	p
Age (year)				
18-40	12 (24.5)	7 (14.3)	*Ref.*	0.34
41-65	19 (38.8)	25 (51)	0.44 (0.15 1.34)	
> 66	18 (36.7)	17 (34.7)	0.69 (0.20-1.94)	
Sex (%)				
Female	26 (53.1)	34 (69.4)	*Ref.*	
Male	23 (46.9)	15 (30.6)	2.01 (0.88-4.58)	0.10
Marital status (%)				
Married	45 (91.8)	46 (93.9)	*Ref.*	
Unmarried	4 (8.2)	3 (6.1)	0.73 (0.15-3.46)	0.69
Occupation (%)				
Housewife	25 (51)	32 (65.3)	*Ref.*	0.04
Farmer and rancher	3 (6.1)	7 (14.3)	0.37 (0.15-0.93)	
Other occupations	21 (42.9)	10 (20.4)	0.20 (0.04-0.96)	

ESBL-EC: Escherichia coli with extended-spectrum β-lactamases; Ref.: Reference category

univariate analysis were entered into a multivariate logistic regression model and the data were reported as odds ratio (OR) with 95% confidence interval (95% CI). P-value less than 0.05 (statistically significant indicator) were used in this study.

3. Result

3.1. Demographic characterization

49 patients were posetive ESBL-EC (case group) and 49 patients were negative ESBL-EC (control group). Mean age of the patients was 57.81 ± 18.86. 61.2% were female and 92.9% were married. Demographic and baseline characteristics were presented in table 1.

3.2. Risk factors

The analysis showed that there was significant relationship between posetive ESBL-EC and hospitalization in last 12 months (p= 0.002), UTI in the last 12 months (p= 0.044), history of kidney disease (p= 0.010), and urinary

Table 2. Comparison of characteristics in patients with posetive or negative ESBL-EC

Variables	Negative ESBL-EC N (%)	Posetive ESBL-EC N (%)	p
Hospitalization in the past year			
No	34 (69.4)	19 (38.8)	0.002
Yes	15 (30.6)	30 (61.2)	
Comorbidities[1]			
No	10 (20.4)	4 (8.2)	0.074
Yes	39 (79.6)	45 (91.8)	
Cardiovascular disease[2]			
No	30 (61.2)	36 (73.5)	0.196
Yes	19 (38.8)	13 (26.5)	
Type 2 diabetes			
No	35 (71.4)	32 (65.3)	0.515
Yes	14 (28.6)	17 (34.7)	
Kidney disease[3]			
No	37 (75.5)	25 (51)	0.010
Yes	12 (24.5)	24 (49)	
Cerebrovascular accidents			
No	40 (81.6)	41 (83.7)	0.790
Yes	9 (18.4)	8 (16.3)	
Pulmonary disease[4]			
No	45 (91.8)	42 (85.7)	0.337
Yes	4 (8.2)	7 (14.3)	
Gastrointestinal disease			
No	47 (95.9)	45 (91.8)	0.399
Yes	2 (4.1)	4 (8.2)	
Infectious disease			
No	49 (100.0)	46 (93.9)	0.121
Yes	0 (0)	3 (6.1)	
UTI in the past year			
No	42 (85.7)	34 (69.4)	0.044
Yes	7 (14.3)	15 (30.6)	
Urinary catheter insertion			
No	40 (81.6)	28 (57.1)	0.008
Yes	9 (18.4)	21 (42.9)	
Use of immunosuppressive drugs			
No	48 (98.0)	48 (98.0)	>0.99
Yes	1 (2.0)	1 (2.0)	
Dialysis			
No	48 (98.0)	47 (95.9)	0.558
Yes	1 (2.0)	2 (4.1)	
Antibiotic use in the past 3 months			
No	41 (83.7)	36 (73.5)	0.222
Yes	8 (16.3)	13 (26.5)	

1, Comorbidities: Cardiovascular diseases, type 2 diabetes, neurological disease, pulmonary diseases, gastrointestinal diseases, infectious diseases, inactivity, and other comorbidities; 2, Cardiovascular disease: Hypertension and heart failure; 3, Kidney disease: Chronic kidney disease, renal anatomical disorder and nephrolithiasis; 4, Pulmonary disease: Chronic respiratory failure system and chronic obstructive pulmonary disease; ESBL-EC: Escherichia coli with extended-spectrum β-lactamases; OR: Odds Ratio; UTI: Urinary tract infection

Table 3: Independent risk factors of posetive ESBL-EC

Variables	OR	95% CI	p
Urinary catheterization	3.33	1.33 – 8.35	0.010

OR: Odds Ratio

catheterization in the last 12 months (p= 0.008) (Table 2). The results of multivariate regression analysis showed that the urinary catheterization in last 12 months increased the risk of posetive ESBL-EC about four times. (OR = 3.33; 95% CI: 1.33 – 8.35; p = 0.010) (Table 3).

3.3. Antibiotic resistance and susceptibility

In 98 studied cases with E. coli (negative and posetive ESBL-EC), the lowest antibiotic resistance to nitrofurantoin and amikacin and the highest antibiotic resistance to ciprofloxacin, imipenem, and cotrimoxazole was recorded. Also, lowest susceptibility in nitrofurantoin and amikacin and highest susceptibility in ciprofloxacin and imipenem was observed.

In the case group (posetive ESBL-EC), the lowest antibiotic resistance to nitrofurantoin, amikacin, and penicillin G and the highest antibiotic resistance to imipenem and ceftizoxime was recorded. Also, lowest susceptibility in nitrofurantoin and amikacin and highest susceptibility in ceftizoxime and imipenem was observed.

In the control group (negative ESBL-EC), the lowest antibiotic resistance to ceftriaxone and ceftizoxime and the highest antibiotic resistance to ciprofloxacin and cotrimoxazole was observed. Also, lowest susceptibility in ceftriaxone and ceftizoxime and highest susceptibility in ciprofloxacin and cotrimoxazole was observed (Figure 1 and 2).

4. Discussion

The result of present study showed that, the urinary catheterization in the past year increased the risk of UTI induced ESBL-EC. Also, antibiogram test showed that cotrimoxazole and ciprofloxacin should not be administered for treating UTI induced E. coli in Sanandaj.

In our study, there was no significant relationship between age and ESBL-EC infection. Many study showed that, age is not a significant risk factor (3, 12-14). So, although the prevalence of UTI is higher at older ages, the prevalence of ESBL resistance is not higher. In our study, there was no statistically significant relationship between sex and posetive ESBL-EC. In many studies, sex had no significant relationship with this type of resistance. In our study, urinary catheterization in the last 12 months was associated with increasement of ESBL-EC infection. However, in several studies, it was not identified as a risk factor (11-14). Perhaps, our finding might be due to lack of precautions regarding safe contact during the procedure in the studied hospital. Antibiotic use is the only risk factor that can be controlled and prevented through interventions. In our study in both groups, resistance to nitrofurantoin was 4% or less and sensitivity to this antibiotic was 69%. The results of our study are consistent with the results of EI-Kersh et al., and Moyo et al.,(15, 16). Due to the high sensitivity of nitrofurantoin and high resistance of E. coli, it is recommended that, nitrofurantoin to be used instead of other antibiotics as the first line of treatment (17). In our study, E. coli resistance to cotrimoxazole was 58.8%. The high resistance to this antibiotic might be attributed to the excessive administration of it due to the high prevalence of brucella in the studied province. In conditions that E. coli resistance to cotrimoxazole in a community is above 10-20%, it is not recommended to use this as an empiric treatment for community acquired UTI (18). In our study, amikacin resistance was lower than gentamicin (4% and 20.4%, respectively) and its susceptibility was higher than gentamicin (56% and 20%, respectively). In case and control group resistance to amikacin was approximately the same, but case group resistance to gentamicin was higher (36.7%) than control group (4%). Therefore, gentamicin is less favorable than amikacin for UTI. The differences in the sensitivity and resistance to various types of aminoglycosides can be attributed to the higher rate of gentamicin consumption in the current studied area. Perhaps the differences might be also attributed to amikacin resistance to the aminoglycoside N-acetyltransferases, which is the main mechanism of resistance to aminoglycosides (19). In our study, in the control group resistance to imipenem was 34.6% and in case group it was 77.5%. The higher resistance in the case group was not expected. In Mohsenpour et al.,'s study in Sanandaj, the emergence of infection resistant to carbapenem (includ imipenem) was reported as a major concern; as they reported one of the major risk factors was the high and irrational consumption of extended-spectrum antibiotics in this region (20). Based on the results of antibiogram test in our study, all the tested E. coli samples had resistance to more than two types of antibiotics that, based on the existing definitions, this condition is recognized as multiple drug resistance (21).

This is the first study to investigate the risk factors for

ESBL-EC in Kurdistan. It was better that mutations leading to this type of resistance was investigated by standard method such as polymerase chain reaction.

5. Conclusion

Based on the findings of this study, the urinary catheterization in the last 12 months increased the risk of UTI induced ESBL-EC and cotrimoxazole and ciprofloxacin should not be administered for treating E. coli-induced UTI in Sanandaj.

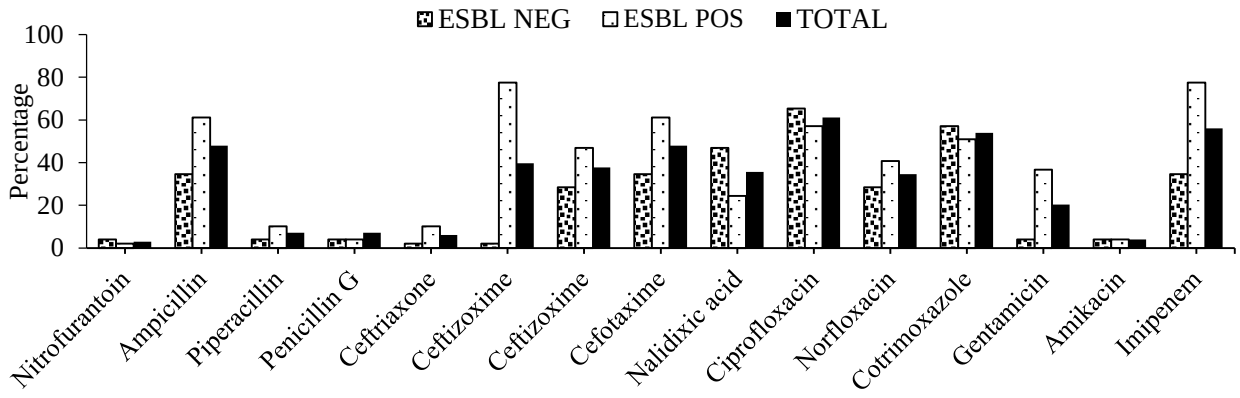

Figure 1: Antibiotic resistance in patients with E. coli-induced urinary tract infection in the two groups with posetive extended-spectrum β-lactamases (ESBL POS) and negative ESBL (ESBL NEG).

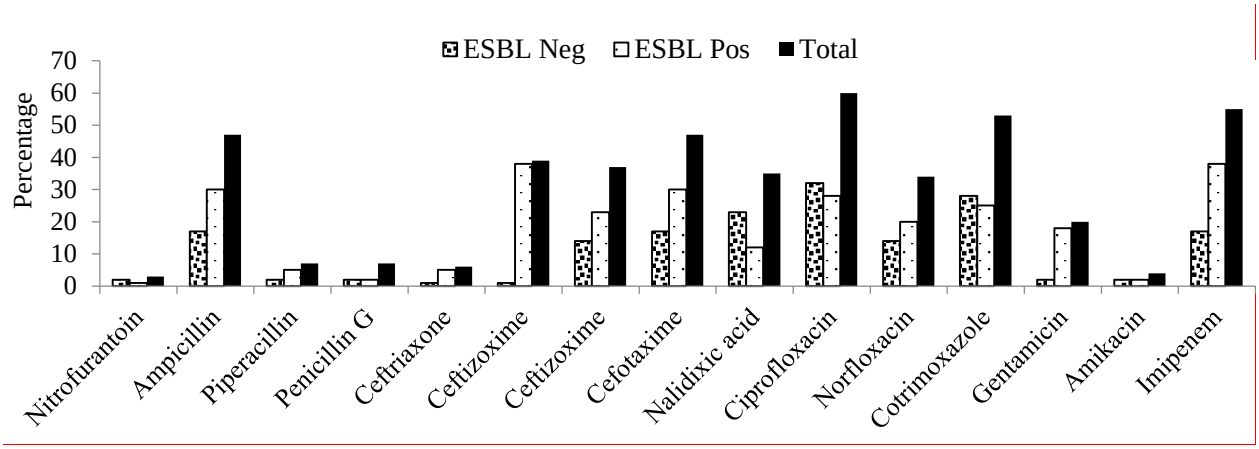

Figure 2: Antibiotic susceptibility in patients with E. coli-induced urinary tract infection in the two groups with posetive extended-spectrum β-lactamases (ESBL Pos) and negative ESBL (ESBL Neg).

6. Acknowledgment

We thank the patients who participated in the study.

7. Conflict of interest

No conflict of interest was declared.

8. Funding source

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9. Author contribution

B.M and, A.A contributed to study design. B.M, and S.A contributed to the selection of patients and data gathering. K.H performed data analysis. M.B write the manuscript. D.R and F.G edit the manuscript.

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