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Occurrence, Risk Factors and Antimicrobial Susceptibility Pattern of Extended-spectrum β-lactamases (ESBLs) Producing *Escherichia coli* and *Klebsiella pneumoniae* Isolated from Community-acquired Urinary Tract Infections

Aza Bahadeen Taha*
Medical Research Center, Hawler Medical University, Erbil, Iraq

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Abstract

The emergence of antimicrobial-resistant ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* among community-acquired uropathogens represents an important therapeutic challenge. Henceforth, the purpose of the study was to investigate the antibiotic susceptibility patterns of ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* among community-acquired urinary tract infections and then identify the risk factors. *Escherichia coli and Klebsiella pneumoniae* strains were isolated from patients with symptomatic community-acquired urinary tract infections. The ESBL-producing and antimicrobial susceptibility tests were then performed using the broth microdilution technique. Among 826 *Escherichia coli* and *Klebsiella pneumoniae* uropathogens, 17.43% were found to be ESBL producers. According to logistic regression analysis, the risk factors for ESBL-producing community-acquired urinary tract infections are urinary tract infections in the preceding 6 months and recurrent urinary tract infections. All ESBL producers were sensitive to meropenem, followed by imipenem (98.61%), amikacin sulfate (90.28%), and nitrofurantoin (80.56%). Significant ESBL-positive *Escherichia coli* and *Klebsiella pneumoniae* were identified which were found to be associated with recurrent UTIs. Meropenem was effective against both ESBL-positive and -negative bacteria.

Keywords: Antimicrobial drug resistance; ESBL production; Community-acquired infections; Recurrent urinary tract infections; Risk factors.

ظهور،عوامل الخطرونمط الحساسية لمضادات المايكروبات لبكتريا الاشريكية القولونية والكلبسيلا الرئوية المنتجة لانزيمات البيتا لاكتاميز الممتدة الطيف والمعزولة من التهابات المسالك البولية المكتسبة من المجتمع

آزا بهاءالدين طه *
مركز البحوث الطبية ، جامعة هولير الطبية ، أربيل ، العراق

الخلاصة

يمثل ظهور *Escherichia coli* و*Klebsiella pneumoniae* المنتجة للبيتا لالكتام واسعة الطيف من بين مسببات الأمراض البولية المكتسبة من المجتمع تحديًا علاجيًا مهمًا. الغرض من الدراسة هو

* Email: aza.taha@hmu.edu.krd

استقصاء عن أنماط الحساسية للمضادات الحيوية في *Escherichia coli* و*Klebsiella pneumoniae* المنتجة لـ ESBL في التهابات المسالك البولية المكتسبة من المجتمع ومن ثم تحديد عوامل الخطرة للعدوى. تم عزل سلالات *Escherichia coli* و *Klebsiella pneumoniae* من المرضى الذين يعانون من أعراض المسالك البولية المكتسبة من المجتمع. ثم تم اختبار انتاجها لـ ESBL و حساسيتها للمضادات الحيوية باستخدام تقنية المرق المجهري. من بين 826 *Escherichia coli* و *Klebsiella pneumoniae* مسببه الأمراض البولية كان 17.43٪ منتجين لـ ESBL. وفقًا لتحليل الانحدار اللوجستي ، فإن عوامل الخطر للسلالات المنتجة لـ ESBL لعدوى المسالك البولية المكتسبة من المجتمع هي التهابات المسالك البولية في الأشهر الستة السابقة والتهابات المسالك البولية المتكررة. كانت جميع السلالات المنتجة لـ ESBL حساسة لـ meropenem ، يليهم imipenem (98.61٪)،amikacin sulfate (90.28٪)، nitrofurantoin (80.56٪). في البحث تم تحديد أهمية *Escherichia coli* و *Klebsiella pneumoniae* الموجبة لـ ESBL والتي ترتبط مع عدوى المسالك البولية المتكررة و كان meropenem فعالاً ضد البكتيريا الموجبة والسالبة لـ ESBL.

1. Introduction

Urinary tract infections are still the most prevalent bacterial infections in the communities [1]. The incidence of community-acquired (CA) urinary tract infections (UTIs) caused by ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* (ESBL-EK) has spread rapidly worldwide [2, 3] and has emerged as one of the most serious problems not only in hospital settings but also in the communities [4, 5]. The spread of ESBL-EK outside of the hospital setting may jeopardize the future availability of effective broad-spectrum β-lactam antimicrobial agents for UTI treatment [6].

The ESBLs are rapidly expanding group of β-lactamases that can inactivate third-generation cephalosporins and aztreonam [7] which are inhibited by β-lactamase inhibitors such as clavulanic acid [8]. Furthermore, ESBL-positive (ESBL-P) bacteria are frequently resistant to the non-β-lactam antimicrobial drugs such as aminoglycosides, trimethoprim-sulfamethoxazole, and quinolones [9], resulting in multidrug resistance and clinical treatment failure, making the UTIs difficult to treat [10-12]. In addition, ESBL is difficult to diagnose using routine antimicrobial susceptibility methods [13]. The therapeutic options for ESBL-P uropathogens are limited and have become an important challenge for clinicians [14].

There is limited information on the impact of treatment outcomes on ESBL-causing CA-UTIs, particularly in the Middle Eastern countries [3]. This study therefore documented the occurrence of ESBL-EK causing CA-UTIs and found a risk factor associated with ESBL-producing strains to explore the influential role of antimicrobial agents as treatments for CA-UTIs. In this undertaken study, the antimicrobial susceptibility profiles of ESBL-EK were determined and were then compared these with the susceptibilities of the ESBL-producing strain.

2. Material and Methods

A prospective cross-sectional study was performed from November 2016 to January 2021. The urine specimens were collected from patients with UTI symptoms attending the outpatient department at Rizgary and Hawler teaching hospitals in Erbil, Kurdistan Region, Iraq. The infection was diagnosed based on signs and symptoms of an UTI and a microbiologically significant count in the urine [15]. Patients with at least two episodes of UTIs during the last six months are considered to have recurrent UTIs [16]. Data collected from the patients included the following: gender, age, use of an antimicrobial in the previous two months, UTIs in the preceding six months and recurrent UTIs. The ethical committee approved this study and the participants gave verbal informed consent for this research.

2.1 Inclusion and Exclusion Criteria

The study included non-hospitalized patients aged between 18 to 80 years with symptomatic CA-UTIs caused by *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae* (*K. pneumoniae*). Only urine specimens with significant bacterial growth ($\geq 10^5$ colony forming units /mL) were included in this study. Exclusion criteria included the following: patients who had been hospitalized within the past month, complicated UTIs, diabetes, cancer, pregnant or lactating women, children, current use of non-steroidal anti-inflammatory or immunosuppressive drugs, previous urinary surgery, and patients whose urine samples showed no significant bacterial growth. During the COVID-19 pandemic, patients with COVID-19 infection were also excluded from the research.

2.2 Bacterial Isolation and Identification

All urine samples were collected by midstream clean-catch, and were then cultured on nutrient, blood and MacConkey agars (Lab M Ltd., UK) plates with a standard loop and incubated at 37°C for 24 h. The diagnosis of UTIs was based on a positive urine culture of $\geq 10^5$ colony-forming units/mL of the same bacterial species [17, 18]. Identification of the two isolates was performed by standard bacteriological techniques and API 20E strips (bioMérieux).

2.3 The Antimicrobial Susceptibility Method

The antimicrobial susceptibility tests for both isolates were performed by the broth microdilution method according to Clinical Laboratory Standard Institute (CLSI) document M07-A10 [19]. The MICs were then analyzed and interpreted using CLSI susceptibility breakpoints [20]. The following antimicrobial agents were used: amikacin sulfate, amoxicillin-clavulanate potassium, cefepime, cefotaxime sodium, ceftazidime, ceftriaxone, ciprofloxacin hydrochloride, gentamicin sulfate, imipenem, levofloxacin, meropenem, nitrofurantoin, piperacillin sodium-tazobactam sodium, tobramycin sulfate and trimethoprim sulfate-sulfamethoxazole (Sigma-Aldrich).

2.4 Detection of ESBLs

ESBL-EK detection was performed by broth microdilution method using ceftazidime (0.25–128 μg/mL) and ceftazidime combined with clavulanic acid (0.25/4–128/4 μg/mL) [21]. The ESBL production was indicated by at least a three-fold concentration decrease in the MIC for ceftazidime in the presence of clavulanic acid compared to its MIC when tested alone, according to CLSI guidelines [20].

2.5 Analysis and Reporting of Results

Categorical variables were summarized as percentages and compared using the Chi-square test. The risk factors for ESBLs were performed and reported by univariate analysis by Chi-square, and multivariate logistic regression calculated the adjusted odds ratio (OR) with a 95% confidence interval (CI). The SPSS software, version 25 (SPSS, Chicago, IL), was used to analyze the data and p-value < 0.05 was considered significant.

2. Results

2. 1 ESBLs-producing Escherichia coli and Klebsiella pneumoniae

Among the 993 uropathogen bacteria isolated from CA-UTIs, 749 (75.43%) were *E. coli*, 77 (7.75%) were *K. pneumoniae*, and 167 (16.82%) were other uropathogenic bacteria (Figure 1). The production of ESBL in *E. coli* was found to be 123/749 (16.42%) and 21/77 (27.27%) in *K. pneumoniae*, with an average prevalence of 17.43%. The difference between *E. coli* and *K. pneumoniae* in ESBL production was significant (p= 0.017) (Table 1).

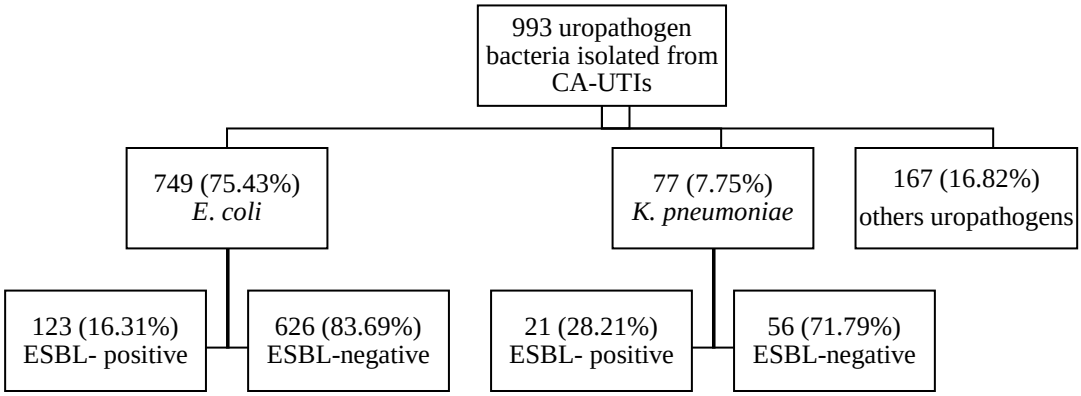

Figure 1: Distribution of uropathogenic bacteria among community-acquired urinary tract infections.

Table 1: ESBL production among uropathogenic *E. coli* and *K. pneumoniae*

ESBL Production	*E. coli*		*K. pneumoniae*		Total	
	n	**%**	***n***	**%**	***n***	**%**
ESBL- Positive	123	16.42	21	27.27	144	17.43
ESBL- Negative	626	83.58	56	72.73	682	82.57
Total	749	100	77	100	826	100

No significant difference was detected between *E. coli* and *K. pneumoniae* in ESBL production (*p*-value = 0.062, Pearson Chi-Square).

2. 2 The Risk Factors

The data from 144 patients with ESBL-P uropathogens and 682 patients with ESBL-negative (ESBL-N) uropathogens are listed in Table 2 which shows that the univariate analysis indicated that the ESBL-EK was more likely to be associated with the following: age ≥60 years (p= 0.013); an antimicrobial used in the past 2 months (p= 0.001); UTI in the preceding 6 months (p< 0.001); and recurrent UTI (p< 0.001). The logistic regression model indicated that UTIs in the preceding 6 months and recurrent UTIs were significant risk factors in determining UTIs due to ESBL-EK, of which the ESBL-producing strains were approximately 3.5 times more likely to cause UTIs than ESBL-N strains in recurrent UTIs (odds ratio 3.439% confidence interval 2.472-4.783, p< 0.001) (Table 2).

Table 2: Univariate and multivariate logistic regression analysis of risk factors associated with *E. coli* and *K. pneumoniae.*

Variable		ESBL-P (*n* =144)		ESBL-N (*n*= 682)		Total (*n*= 826)		Chi-Square	Logistic Regression	
		n	**%**	***n***	**%**	***n***	**%**	***p*-value**	**OR (95% CI)**	***p*-value**
Gender	Male	65	45.14	254	37.24	319	38.62	0.077	1.235 (0.995-1.532)	0.055
	Female	79	54.86	428	62.76	507	61.38			
Age (Years)	≥60	35	24.31	107	15.69	142	17.19	0.013	1.075 (0.826-1.399)	0.590
	<60	109	75.69	575	84.31	684	82.81			
Use of antimicrobials in the past 2 months	Yes	48	33.33	141	20.67	189	22.88	0.001	0.986 (0.769-1.265)	0.911
	No	96	66.67	541	79.33	637	77.12			
UTI in the preceding 6 months	Yes	67	46.53	66	9.68	133	16.10	<0.001	1.6 (1.201-2.132)	0.001
	No	77	53.47	616	90.32	693	83.90			
Recurrent UTI	Yes	58	40.28	21	3.08	79	9.56	<0.001	3.439 (2.472-4.783)	<0.001
	No	86	59.72	661	96.92	747	90.44			

Abbreviations: CI: confidence interval; ESBL-N: ESBL-negative; ESBL-P: ESBL-positive; OR: Odds ratio.

2. 3 Antimicrobial Susceptibility Patterns

The comparative analysis of the antimicrobial sensitivity profiles is shown in Table 3. Statistically, all *E. coli* ESBL-P strains showed significantly lower sensitivity to fifteen antimicrobial agents than the ESBL-N. Fortunately, all isolates of ESBL-P and ESBL-N strains were sensitive to meropenem.

Table 3: Comparison of antimicrobial sensitivity rates (% S) between ESBL-P and ESBL-N (MIC data interpreted as sensitive according to CLSI breakpoints).

Antimicrobial	*E. coli*			*K. pneumoniae*			Total		
	S (%)		*p*-value	S (%)		*p*-value	S (%)		*p*-value
	ESBL-P (*n*=123)	ESBL-N (*n*=313)		ESBL-P (*n*=21)	ESBL-N (*n*= 28)		ESBL-P (*n*=144)	ESBL-N (*n*= 341)	
Amikacin sulfate	88.62	100	<0.001	100	100	ND	90.28	100	<0.001
Amoxicillin-clavulanate potassium	57.72	78.91	<0.001	23.81	67.86	0.002	52.78	78.01	<0.001
Cefepime	9.76	88.50	<0.001	9.52	78.57	<0.001	9.72	87.68	<0.001
Cefotaxime sodium	2.44	88.18	<0.001	0.00	78.57	<0.001	2.08	87.39	<0.001
Ceftazidime	34.15	89.14	<0.001	9.52	78.57	<0.001	30.56	88.27	<0.001
Ceftriaxone	4.07	84.98	<0.001	0.00	75.00	<0.001	3.47	84.16	<0.001
Ciprofloxacin hydrochloride	30.08	76.04	<0.001	28.57	78.57	<0.001	29.86	76.25	<0.001
Gentamicin sulfate	52.03	86.90	<0.001	42.86	92.86	<0.001	50.69	87.39	<0.001
Imipenem	98.37	100	0.024	100	100	ND	98.61	100	0.029
Levofloxacin	22.76	72.84	<0.001	28.57	75.00	0.001	23.61	73.02	<0.001
Meropenem	100	100	ND	100	100	ND	100	100	ND
Nitrofurantoin	87.80	94.89	<0.001	38.10	85.71	0.001	80.56	94.13	<0.001
Piperacillin sodium-tazobactam sodium	74.80	94.89	<0.001	61.90	85.71	0.055	72.92	94.13	<0.001
Tobramycin sulfate	60.16	89.14	<0.001	57.14	82.14	0.107	59.72	88.27	<0.001
Trimethoprim sulfate-sulfamethoxazole	55.28	85.94	<0.001	47.62	75.00	0.049	54.17	85.04	<0.001

Abbreviations: ESBL-N, ESBL-negative; ESBL-P, ESBL-positive; ND: Not done.

4. Discussion

ESBL-EK is the most worrisome bacterial strain that causes CA-UTIs [22, 23]. This study identified the prevalence of ESBL-EK in CA-UTIs and their susceptibility patterns to fifteen antibiotics. It also identified the risk factors associated with ESBL-EK in CA-UTIs. The CA-UTIs caused by ESBL-EK were also reported in this study, of which the prevalence was found to be lower than those found in Turkey [24] and the Western Province of Sri Lanka [25] but higher than those found in the Pays de la Loire and Bretagne in the west of France [26]. The disparties between these results were due to varying rates of ESBL-EK from one country to another and from region to region, and the fact that they rapidly change over time [27, 28].

The univariate analysis found that there was a risk factor association between CA-UTIs due to ESBL-EK and age over 65 years, use of antimicrobials in the past 2 months, UTI in the preceding 6 months, and recurrent UTI. The risk factors might need further investigation as the multivariate analysis proved the UTIs only in the preceding 6 months and the recurrent UTIs. The recurrent UTI was a crucial risk factor observed in the present study and has also been considered a risk factor for ESBL-EK in multiple studies [29-31]. The risk factors identified in this study will help guide physicians in the diagnosis of patients at high risk of UTI due to ESBL-EK.

Patients with recurrent ESBL infections are even more challenging to treat and are often prescribed long-term antimicrobial agents as in such cases the bacteria frequently develop resistance to major antibiotic classes. Therefore, increasing the resistance to routinely used antimicrobials in ESBL-EK infections constitute a problem for the treatment of UTIs and could result in increased costs due to the use of alternative antimicrobials. In this research, all isolated *E. coli* and *K. pneumoniae* remained sensitive to meropenem, a result that agrees with some other studies [32, 33]. These results could be due to the limited use of this antimicrobial in regionally. Resistance to meropenem resistance has been reported in Saudi Arabia [34], Pakistan [35] and the north of Iran [36]. As amikacin and imipenem were the most effective for these isolates, they have therefore become a choice in treating ESBL-EK strains. Fortunately, nitrofurantoin maintained its sensitivity and had been an effective option for treating CA-UTIs brought on by ESBL-EK. The antimicrobial-resistance rates of all ESBL-EK strains in the present study were higher than those of ESBL-N pathogens which provide important information for the choice of antimicrobial therapy in urologic practice. Furthermore, the bacteria that produce ESBLs continue to be the leading cause of failure in cephalosporin-based UTI treatment and have high rates of resistance to other antimicrobials. Therefore, successful detection of ESBL is required to treat UTIs. Further study is highly recommended to find the effects of antibiotic combinations on the resistant strains of ESBL-EK.

5. Conclusion

The current study showed that CA-UTI caused by ESBL-EK is a risk factor for recurrent UTI. The ESBL-P uropathogens showed a higher degree of resistance to all antimicrobial agents than the ESBL-N pathogens. Meropenem, imipenem, amikacin sulfate, nitrofurantoin and piperacillin sodium-tazobactam sodium showed significant activity against the ESBL-P strain. In this study, no uropathogenic bacteria were found to be resistant to meropenem making iy a strong antimicrobial option for treating recurrent UTIs caused by ESBL-EK.

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Ethical Approval: Ethics committee approval was obtained from Hawler Medical University, Erbil. The study was explained to the participants who then provided verbal informed consent for participation in the study. Their information would remain confidential and would be used exclusively for this study.

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