Prevalence Rate Of ESBL Among *Enterobacteriaceae* Isolated From UTI Patients In Sulaimani Providence

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ABSTRACT

Background: ESBLs are enzymes that provide resistance agenst third-and fourth generation Cephalosporins and Monobactams and they are distributed among the *Enterobacteriaceae* family which are the main cause of urinary tract infections due to high rate of antibiotic therapy failure. In this study we describe the prevalence of ESBL among UTI cases in Sulaimani providence aiming to provide a clear data on ESBL distribution and antibiotic abuse in this area.

Methods: One hundred bacterial isolates of *Enterobacteriaceae* were collected from UTI patients admitted to Smart hospital inpatients and outpatients with signs and symptoms of UTI, and urine samples were inoculated onto different culture media. Colony morphology, gram staining, and BD Phoenix™ system were used for bacterial identification, antibiotic profile and ESBL were observed phenotypically by antibiotic profile results and double disk synergy test and confirmed by combined disk test methods and BD Phoenix™ system.

Results: Out of one hundred isolates of *Enterobacteriaceae E. coli* was the commonest isolate (89) followed by *Klebsiella pneumoniae* (10), and one isolates of *Proteus mirabilis*. According to the antibiotic profile, the most sensitive antibiotic among all three isolates were Imipenem, and nitrofurantoin, while most resistance antibiotic were nalidixic acid and third generation cephalosporin. The prevalence rate of ESBL-producing *Enterobacteriaceae* was 69% by the screening tests and 48% by the confirmatory tests.

Conclusion: In this study, ESBL prevalence was shown to be at an alarming rate that must be taken into consideration. The high priority of public health justifies further investigation to properly establish annual surveillance systems that can aid in the selection of an appropriate antibiotic upon ESBL detection.

***Key words**: ESBL, Enterobacteriaceae, UTI*

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INTRODUCTION

Urinary tract infection (UTI) is a term that refers to any infection affecting urinary system, including the kidneys, ureters, bladder, and urethra. UTI is classified into: upper (kidneys and ureters) and lower (bladder, urethra) infection or as uncomplicated or complicated UTI [(1)]. The possibility that asymptomatic colonization disappears spontaneously or escalates to symptomatic infection is influenced by both host and bacterial factors. host factors such as anatomical or functional defects, genetic factors and activities that promote uropathogen exposure or transfer bacteria into the bladder(sexual activities) while, bacterial factors include a number of virulence factors that allow the pathogen to invade and colonize the bladder such as adherence factors, siderophores, bacteriocins, toxins and biofilm [(2)].

In both the community and the health-care setting, *Enterobacteriaceae* species constitute the most prevalent cause of UTI. This family include *Escherichia, Klebsiella, Enterobacter, Citrobacter, Salmonella, Shigella, Proteus, Serratia,* etc [(3)]. Most members of this family are normal inhabitants of the gastrointestinal tract of the infected patient that carries different virulent factors aiding in there attachment to the uroepithelial cells [(4)].

Antibiotic resistance is the ability of an organism to withstand the effects of a certain antibiotic, which leads to treatment failure and other complications [(5)]. One of the well-defined enzymes of resistance is Extended-Spectrum β-Lactamase (ESBL). ESBLs are enzymes that hydrolyze third-and fourth generation Cephalosporins and Monobactams, but not Cephamycins or Carbapenems. ESBL are serine β-lactamases, belonging to Ambler molecular and structural classification as class A. They are inhibited by Clavulanic acid and There genetic requirements are mostly found on plasmids and transposons or insertion sequences which has enabled their spread [(6)]. It can spread globally since their identification in the early 1980s and are currently prevalent in *Enterobacteriaceae* isolated from both hospital-associated and community-acquired infections. It became a worldwide public health issue [(7)]. The first ESBL enzymes were TEM and SHV variants with amino acid substitutions that resulted in a shift in substrate profile to include expanded-spectrum cephalosporins [(8)].

METHODOLOGY

Type of study and study population; This cross-sectional study includes all patients who were admitted to Smart hospital as outpatient and inpatients presenting with the sign and symptoms of UTI according to what was established previously [9]. in a period of September 2021 to May 2022,
Exclusion criteria: pregnant women and pediatric age group (< 10 years old).

Ethical consideration; The study was approved by the ethical committee of the college of Medicine University of Sulaimany- Iraq, and the directorate health of Sulaimani and the ethics consideration was taken from each patient before sample collection.

Urine culture and bacterial identification; Urine samples were inoculated to different culture media (sheep blood agar, MacConky agar, nutrient agar, and EMB) by striking methods [10].
For bacterial identification on different culture media; colonial morphology, characteristics growth, gram stain and BD Phoenix™ system were used [11].

Antibiotic susceptibility testing and ESBL detection; BD Phoenix™ Automated Microbiology System (BD \ US) was used for antibiotic susceptibility testing and for ESBL confirmation of all isolated *Enterobacteriaceae* according to the manufacturer recommendation.

The antimicrobial susceptibility pattern of the isolated organisms was confirmed by the Kirby-Bauer disc diffusion method using commercially available antibiotic discs (Liofilchem \ Italy) according to what was fixed by the Clinical and Laboratory Standards Institute [11]. The organisms were tested against different antibiotics and commonly used discs were Amoxiclav AMC (20 µg Amoxycillin/10 µg clavulanic acid), Ceftazidime CAZ (30 µg), Ceftriaxone CRO (30 µg), Cefotaxime CTX (30 µg), Cefepime CPM (30 µg), Ciprofloxacin CIP (5 µg), Trimethoprim-sulfamethoxazole SXT (5 µg), Nalidixic acid NA (10 µg), Nitrofurodantine NIT (10 µg), Gentamicin CN (10 µg), Imipenem IPM (10 µg) and Meropenem MEM (10 µg). Zone of inhibition was recorded as “Sensitive” “Resistant” or intermediate [11]. *K. pneumonia* ATCC 700603 was used as a positive control for ESBL and *E. coli* 25922 were used as negative quality control for antibiotic and ESBL detection [12].

Standard disk diffusion method and double-disk synergy tests were used for screening of ESBL. For both tests Amoxicillin/clavulanic acid (20/10µg), Ceftazidime CAZ (30 µg), Cefotaxime CTX (30 µg), Ceftriaxone CTR (30 µg) and Cefepime CPM (30 µg) discs were used. In the standard disk diffusion method positive ESBL meant the comparatively high-level co-resistance shown by *Enterobacteriaceae* to the third

generation cephalosporin (ceftazidime zone diameter ≤ 22 mm, cefotaxime zone diameter ≤ 27 mm, ceftriaxone zone diameter ≤25 mm, Cefepime zone diameter ≤25 mm) [13]. In the double-disk synergy tests any extension zone or keyhole phenomenon towards the disc of amoxicillin/clavulanic acid was considered to be a positive result for ESBL enzyme production [14].

Combined disk synergy test was used for ESBL confirmation. clavulanic acid and ceftazidime (CZC combined disk) was used with ceftazidime disk alone for this test. A combined inhibitor disks were placed at a distance of 20 mm apart from the ceftazidime disk on a lawn culture of the isolate on the Muller Hinton agar plate. The tested organism was considered to be positive for ESBL if the zone size around the CZC combined disk is >5 mm than the ceftazidime disk alone [15].

Statistical Analysis; Statistical analyses were performed by SPSS software version 26 (SPSS Inc., Chicago, IL, USA). The chi-square test was used to calculate the association between ESBL prevalence rate (screening & confirmation) and *Enterobacteriaceae* isolates. The significance level was defined as $P < 0.05$.

RESULTS

In this cross-sectional study, a total of 100 culture positive samples were collected from September 2021 to May 2022 form Smart hospital in Sulaimani providence.

The majority of the cases were females that account for 75(75%) of the cases which makes ¾ of the studied population and males account for 25(25%) of the studied population (Table-1).

Age of the patients were between 11-80 year and they were divided to 5 age groups in which they were labeled as the following; A:10-20, B:21-30, C:31-40, D:41-50 and E:51-older. The majority of the cases were from the age group E which account for 29(29%) of the studied population followed by group A which accounts for 23(23%). While, group D account for the minority in which they uptake 10(10%) of the population.

Another factor that was assessed among the studied population was repeated UTI which showed 20(20%) of the participants.

Catheterization was another factor that was assessed in the current study and data showed that 14(14%) were using it.

Comorbidities were also recorded and it was shown that 18% of the studied population had chronic illnesses, in which heart disease was shown to affect 9(9%) of the population followed by diabetes mellitus 5(5%), chronic kidney disease and only 1(1%) reported for hepatitis B virus. The demographic data are illustrated in Table-1.

In this study only culture isolates of *Enterobacteriaceae* family were included and the identity of all isolates were confirmed by BD Phoenix™ Automated Microbiology System. Three members of the *Enterobacteriaceae* family were isolated from the samples that includes; *E. coli* (89%), *K. pneumonia* (10%) and *P. mirabilis* (1%). Figure 1 illustrates the isolated organism percentage.

Antibiotic susceptibility testing results were generated by merging data from both BD Phoenix™ and Kirby-Bauer disc diffusion method. Results showed that imipenem was the most effective antibiotic in which only 12(12%) of isolates were resistant to it followed by nitrofurantoin 18(18%). On the other hand, 78% of the isolates were resistant to nalidixic acid.

The most resistant antibiotics for *E. coli* were nalidixic acid (77.5%), ceftazidime (70.8%), ceftriaxone (69.7%), cefotaxime (68.5%), ciprofloxacin (65.2%), trimethoprim (60.7%), cefepime (49.4%), amoxicillin/clavulanic acid (41.6%), gentamycin (39.3%), meropenem (28.1%), nitrofurantoin (12.3%), and imipenem (11.2%) as illustrated in Table-2.

Antibiotic profile for *K. pneumoniae* was as following nalidixic acid (80%), ceftriaxone (80%), ceftazidime (80%), cefotaxime (70%), trimethoprim (70%), cefepime (70%), nitrofurantoin (60%), amoxicillin/clavulanic acid (40%), ciprofloxacin (40%), meropenem (40%), gentamycin (30%), and imipenem (20%).

Only one isolate of *P. mirabilis* was isolated in urine culture as it was resistant to all antibiotics that had been used excluding cefepime and imipenem which were sensitive for the samples. These results are illustrated in Table-2.

Isolated bacteria were different in there ESBL enzyme range, the results of ESBL screen tests differs according to the type of test used. According to the antibiotics profile, 69(61 E. coli, 7 *K. pneumoniae* and 1 *P. mirabilis*) of the isolates were resistant to the third-generation cephalosporins**.** Out of these 69 isolates; 49 samples were positive by double-disk synergy test that were divided to 43 (48.3%) *E. coli* and 5 (50%) for *K. pneumoniae* and the only sample of *P. mirabilis* (100%) which were considered positive for ESBL screening (Table-3). There was no significant difference among all isolated *Enterobacteriaceae* in relation to the ESBL screening tests (P-value > 0.05).

According to the confirmation tests ESBL was produced by 48(48%) of the isolates in which 42(47.2%) was positive for *E. coli*, and 5(50%) for *Klebsiella* and 1(100%) for *P. mirabilis.* There was no significant difference among all isolated *Enterobacteriaceae* in relation to the ESBL confirmation tests (P-value > 0.05).

DISCUSSION

UTI is one of the most common bacterial infections that affects both genders at any given age which is caused by bacterial colonization of the sections of urinary tract [16].

In the current study, the data showed that the rate of UTI in females is higher than males which is mostly due to the fact that male possess longer urethra and poses prostatic fluid which have antibacterial activity that protect them from infection [4,17].

In this study age 51–older was the most infected age groups which agree with previous studies [18–20] results when they found a high prevalence of UTI in elder patients (50 to 75 years old). This is mostly caused by the decline in their immune system's ability to remove causes of infection.

E. coli is the most common cause of UTI globally which is due to its presence in normaly in the gastrointestinal tract and because it possess required adhesins, toxins, flagella, surface polysaccharides, iron-acquisition systems and factors that increase their ability to colonies urinary tract [21]. In the current study, *E. coli* was the most common isolate(89%) which was in agreement with studies done before [22,23] which were conducted in Zakho/Iraq and Wasit/Iraq while *K. pneumonia* was the second most common specie that was isolated which which was in agreement with studies done in Iraq [22,23]. This is due to that *K. pneumonia* is mostly the causes of complicated UTI and UTI in catheterized patients [24] but most of this studies population were uncomplicated and uncatheterized.

Only one isolate of *P. mirabilis* was obtained which disagreed with results of studies done previously [22,23] which were conducted in Zakho/Iraq and Wasit/Iraq. The differences are probably related to the difference in sample size and the studed population because *P. mirabilis* is most common in pediatric age group [25].

Resistance to antibiotics therapy is a global threat in medical field because it affects several essential variables in treatment including spreading of disease, hospitalization duration and increasing of treatment cost [26].

The current study shows that isolates were highly resistant to Nalidixic acid and third generation Cephalosporin which aligns with the results of previous studies [27–29] that were conducted in near regions including: Erbil/Iraq Thi-Qar/Iraq and Baghdad/Iraq. The high rate of resistance shown in this region is mostly due to frequency uses of these antibiotics as empirical treatment in this community and low restrictions on obtaining these antibiotics [30].

On the other hand 87% of isolates were sensitive to Imipenem, this result is in agreement with the study done in India [31], they showed that Imipenem susceptibility was (97.85%), also in Iran [32] they proved that Imipenem susceptibility was (96.8%). These is mostly due to the fact that Imipenem is less used as an empirical treatment for UTI cases because imipenem is expensive and requires intravenous administration.

Secondly, 76% of isolates were susceptible to Nitrofurantoin which was in agreement to a study done previously [33] which was conducted in Duhok/Iraq. This is mostly due to the fact that most of the urologist do not use Nitrofurantoin as a treatment for UTI.

ESBL production was confirmed in 48% of isolates which aligns with a previous study [34] that was performed in Erbil/Iraq, ESBL production was found among 54% of *K. pneumoniae* isolates from different clinical samples (urine, wound swab, sputum and blood). Also in another study [35] that was conducted in Wasit/Iraq showed 37.8% of the isolates were positive for ESBL production. similar prevalence were found in Spain [36] and China [37]. The minor differences in the ESBL detection can be explained by the differences in sample size and the fact that in the current research two screening and two confirmation methods were used to evaluate ESBL ratio. In Iraq, lack of control over antibiotic use and prescription and the extensive use of antibiotics in our community, especially β-lactams, explained these high rates of ESBL production by clinical isolates from Iraqi population.

CONCLUSION

In this study, ESBL prevalence was shown to be at an alarming rate that must be taken into consideration. Our study's findings led us to the conclusion that patients who initially report with UTI should be provided with modified initial empiric antibiotic treatment. The high priority of public health justifies further investigation to properly establish annual surveillance systems that can aid in the selection of an appropriate antibiotic upon ESBL detection.

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CONFLICT OF INTEREST

The authors declare no conflict of interest to this current study.

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Table-1 Demographic characteristics

Variables N(%)		**Age groups** N(%)					**Total** N(%)	**P value**
		A	**B**	**C**	**D**	**E**		
Gender	Male	7(28%)	3(12%)	5(20%)	5(20%)	5(20%)	25(25%)	> 0.05
	Female	16(21.3%)	14(18.6%)	16(21.3%)	5(6.6%)	24(32%)	75(75%)	
Recurrent UTI		7(35%)	1(5%)	5(25%)	2(10%)	5(25%)	20(20%)	> 0.05
Catheterization		2(14.2%)	4(28.5%)	3(21.4%)	2(14.2%)	3(21.4%)	14(14%)	> 0.05
Chronic diseases	Heart disease	0	2(22.2%)	1(11.1%)	2(22.2%)	4(44.4%)	9(9%)	> 0.05
	Diabetes	0	0	2(40%)	0%	3(60%)	5(5%)	> 0.05
	Hepatitis B virus	0	0	1(100%)	0%	0	1(1%)	> 0.05
	Chronic kidney disease	0	1(33.3%)	1(33.3%)	1(33.3%)	0	3(3%)	> 0.05
		23(23%)	17(17%)	21(21%)	10 (10%)	29(29%)	**Total** N(%)	

Table-2: Antibiotic susceptibility results for isolated *Enterobacteriaceae*

Antibiotics	**Susceptibility pattern**	**Bacterial isolates**			**Total** N(%)
		E. coli N(%)	*K. pneumonia* N(%)	*P. mirabilis* N(%)	
Imipenem	R	10(11.2%)	2(20%)	0	12(12%)
	I	1(1.1%)	0	0	1(1%)
	S	78(87.6%)	8(80%)	1(100%)	87(87%)
Meropenem	R	25(28.1%)	4(40%)	1(100%)	30(30%)
	I	2(2%)	1(10%)	0	3(3%)
	S	62(69.6%)	5(50%)	0	67(67%)
Nalidixic acid	R	69(77.5%)	8(80%)	1(100%)	78(78%)
	I	3(3.3%)	0	0	3(3%)
	S	17(19.1%)	2(20%)	0	19(19%)
Nitrofuranition	R	11(12.3%)	6(60%)	1(100%)	18(18%)
	I	4(4.5%)	2(20%)	0	6(6%)
	S	74(83.1%)	2(20%)	0	76(76%)
Gentamicin	R	35(39.3%)	3(30%)	1(100%)	39(39%)
	I	2(2.2%)	0	0	2(2%)
	S	52(58.4%)	7(70%)	0	59(59%)
Ciprofloxacin	R	58(65.2%)	4(40%)	1(100%)	63(63%)
	I	1(1.1%)	1(10%)	0	2(2%)
	S	30(33.7%)	5(50%)	0	35(35%)
Trimethoprim-sulfamethoxazole	R	54(60.7%)	7(70%)	1(100%)	62(62%)
	I	1(1.1%)	0	0	1(1%)
	S	34(38.2%)	3(30%)	0	37(37%)
Amoxicillin/clavulanic acid	R	37(41.6%)	4(40%)	1(100%)	42(42%)
	I	8(8.9%)	1(10%)	0	9(9%)
	S	44(49.4%)	5(50%)	0	49(49%)
Ceftazidime	R	63(70.8%)	8(80%)	1(100%)	72(72%)
	I	0	1(10%)	0	1(1%)
	S	26(29.2%)	1(10%)	0	27(27%)

Cefotaxime	R	61(68.5%)	7(70%)	1(100%)	69(69%)
	I	2(2.2%)	0	0	2(2%)
	S	26(29.2%)	3(30%)	0	29(29%)
Ceftriaxone	R	62(69.7%)	8(80%)	1(100%)	71(71%)
	I	1(1.1%)	1(10%)	0	2(2%)
	S	26(29.2%)	1(10%)	0	27(27%)
Cefepime	R	44(49.4%)	7(70%)	0	51(51%)
	I	1(1.1%)	0	0	1(1%)
	S	44(49.4%)	3(30%)	1(100%)	48(48%)
		89(89%)	10(10%)	1(1%)	**Total**

S: susceptibility, R: resistance, I: intermediate

Table-3: ESBL detection

Isolates	**ESBL screening** N(%)		**ESBL confirmation** N(%)	
	Standard disk diffusion (screening 1)	Double-disk synergy (screening 2)	BD Phoenix™	Combined disk synergy
E. coli	61(68.5%)	43(48.3%)	42(47.2%)	42(47.2%)
K. pneumonia	7(70%)	5(50%)	5(50%)	5(50%)
P. mirabilis	1(100%)	1(100%)	1(100%)	1(100%)
P value	> 0.05	> 0.05	> 0.05	> 0.05

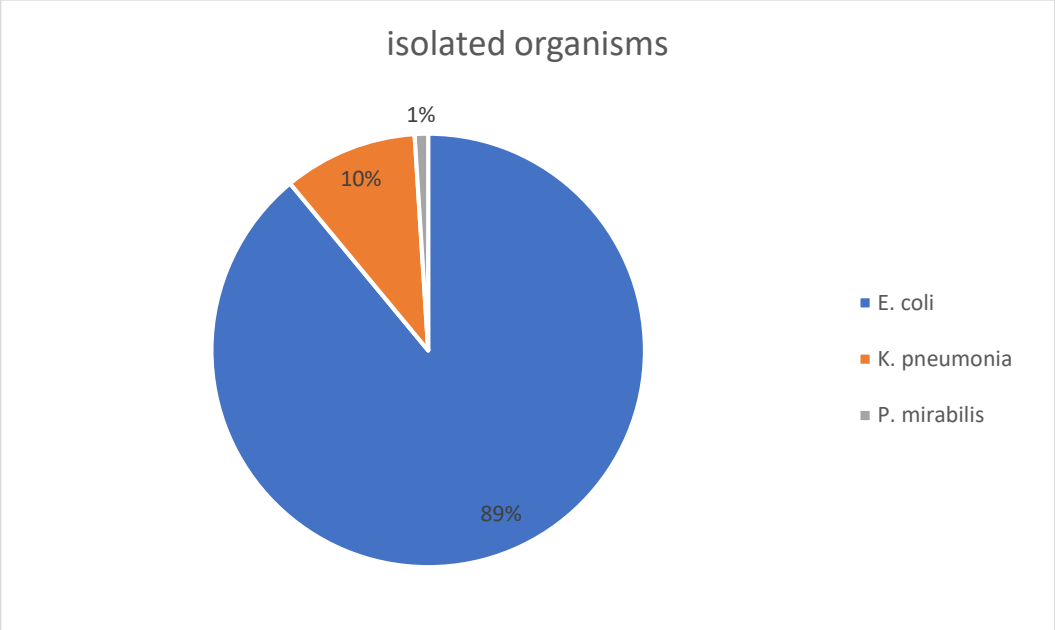

Figure 1: isolated organisms