

PREVALENCE RATE OF ESBL AMONG ENTEROBACTERIACEAE ISOLATED FROM UTI PATIENTS IN SULAIMANI PROVINCE

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Submitted: 28/8/2022; Accepted: 19/2/2023; Published: 21/6/2023

ABSTRACT

Background

Extended-Spectrum β -Lactamase are enzymes that provide resistance against third-and fourth generation Cephalosporins and Monobactams, and they are distributed among the Enterobacteriaceae family.

Objectives

To describe the prevalence of Extended-Spectrum β -Lactamase among Enterobacteriaceae causing urinary tract infections in Sulaimani province.

Patients and Methods

One hundred bacterial isolates of Enterobacteriaceae from patients with urinary tract infections attending Smart Hospital (inpatients and outpatients). Urine samples were inoculated onto different culture media. Colony morphology, gram staining, and BD Phoenix™ system were used for bacterial identification. Antibiotic profile and Extended-Spectrum β -Lactamase were observed phenotypically by antibiotic profile results, double disk synergy test, and confirmed by combined disk test methods and BD Phoenix™ system.

Results

Out of one hundred isolates of *Enterobacteriaceae*, *Escherichia coli* was the commonest isolate (89), followed by *Klebsiella pneumoniae* (10) and one isolate of *Proteus mirabilis*. According to the antibiotic profile, the most effective antibiotic among all three isolates was Imipenem and Nitrofurantoin, while the most resistant antibiotic was Nalidixic acid and third generation Cephalosporin. The prevalence rate of Extended-Spectrum β -Lactamase -producing *Enterobacteriaceae* was 69% by the screening tests and 48% by the confirmatory tests

Conclusion

In this study, Extended-Spectrum β -Lactamase prevalence was shown to be at an alarming rate that must be considered. The high priority of public health justifies further investigation to properly establish annual surveillance systems that can aid in selecting an appropriate antibiotic upon ESBL detection.

Keywords: *Cephalosporins, Extended-Spectrum β -Lactamase, ESBL, Enterobacteriaceae, UTI.*

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INTRODUCTION

Urinary Tract Infection (UTI) is a term that refers to any infection affecting the 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⁾. Both host and bacterial factors influence the possibility that asymptomatic colonization disappears spontaneously or escalates to symptomatic infection. The host factors such as anatomical or functional defects, genetic factors and activities that promote uropathogenic exposure or transfer bacteria into the bladder, while bacterial factors include the 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 healthcare setting, Enterobacteriaceae species constitute the most prevalent cause of UTI. This family includes Escherichia, Klebsiella, Enterobacter, Citrobacter, Salmonella, Shigella, Proteus and Serratia ⁽³⁾. Most of this family are normal inhabitants of the gastrointestinal tract of humans and animals. These bacteria carry different virulent factors aiding their attachment to the uroepithelial cells ⁽⁴⁾.

Antibiotic resistance is the ability of an organism to withstand the effects of a particular 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 Cephamecins or Carbapenems. ESBL are serine β -lactamases belonging to Ambler molecular and structural classification as class A. They are inhibited by Clavulanic acid, and Their genetic requirements are mostly found on plasmids and transposons or insertion sequences, which has enabled their spread ⁽⁶⁾. Extended-Spectrum β -Lactamase can spread globally since its identification in the early 1980s and are currently prevalent in Enterobacteriaceae isolated from hospital-associated and community-acquired infections. Extended-Spectrum β -Lactamase became a worldwide public health issue ⁽⁷⁾.

The first ESBL enzymes were TEM and SHV variants with amino acid substitutions, resulting in a substrate profile shift to include expanded-spectrum cephalosporins ⁽⁸⁾.

MATERIALS AND METHODS

Type of study and study population

This is a cross-sectional study on bacteria isolated from patients' urine attending Smart Hospital as outpatients and inpatients presenting with the sign and symptoms of UTI according to what was established previously ⁽⁹⁾ from September 2021 to May 2022, the 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 at the University of Sulaimani-Iraq and the Directorate Health of Sulaimani-Iraq and Sulaimani Directorate of Health. Participation permission was taken from each patient before sample collection.

Urine culture and bacterial identification

Urine samples were inoculated to different culture media (sheep blood agar, MacConkey agar, nutrient agar, and EMB) by striking methods ⁽¹⁰⁾. Clinical presentation of UTI includes dysuria, frequency, urgency, suprapubic pain, hematuria, fever, chills, flank pain and nausea⁽⁹⁾. A urine sample from catheterized patients was obtained through the sampling port of the catheter. Voided urine was collected from non-catheterized patients.

Bacterial identification on different culture media, colonial morphology, characteristics growth, gram stain and BD PhoenixTM system was used ⁽¹¹⁾.

Antibiotic susceptibility testing and ESBL detection

The antimicrobial susceptibility pattern of the isolated organisms was made 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 μ g Amoxicillin/10 μ g clavulanic acid), Ceftazidime CAZ (30 μ g), Ceftriaxone CRO (30 μ g), Cefotaxime CTX (30 μ g), Cefepime CPM (30 μ g), Ciprofloxacin CIP (5 μ g), Trimethoprim-sulfamethoxazole SXT (5 μ g), Nalidixic acid NA (10 μ g), Nitrofurantoin NIT (10 μ g), Gentamicin CN (10 μ g), Imipenem IPM (10 μ g) and Meropenem MEM (10 μ g). The zone of inhibition was recorded as "Sensitive", "Resistant", or intermediate ⁽¹¹⁾. *K. pneumoniae* ATCC 700603 was used as a

positive control for ESBL, and *E. coli* 25922 was used as negative quality control for antibiotic and ESBL detection⁽¹²⁾.

Standard disk diffusion method and double-disk synergy tests were used to screen 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 a positive result for ESBL enzyme production⁽¹⁴⁾. (Figure 1. a)

BD Phoenix™ Automated Microbiology System (BD \ US) was used for ESBL confirmation of all isolated Enterobacteriaceae according to the manufacturer's recommendation.

A combined disk synergy test was used for ESBL confirmation. Clavulanic acid and Ceftazidime (CZC combined disk) were used with the Ceftazidime disk alone for this test. Combined inhibitor disks were placed 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 positive for ESBL if the zone size around the CZC combined disk was >5 mm higher than the Ceftazidime disk alone⁽¹⁵⁾. (Figure 1. b)

Statistical Analysis

Statistical analyses were performed by SPSS software version 26 (SPSS et al., USA). The chi-square test calculated 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 Enterobacteriaceae isolates were investigated from a period of September 2021 to May 2022 from Smart Hospital in Sulaimani Providence.

The majority of the cases were females, that account for 75(75%) of the cases, which makes $\frac{3}{4}$ of the studied population, and males account for 25(25%) of the

studied population (Table 1).

The age of the patients was between 11-80 years, and they were divided into five age groups in which they were labelled as follows; A:10-20, B:21-30, C:31-40, D:41-50 and E:51-older. Most of the isolates were from the age group E which accounted for 29 (29%) of the studied population, followed by group A which accounted for 23 (23%). While group D account for the minority in which they uptake 10 (10%) of patients.

Another factor that was assessed among the studied population was repeated UTI, which showed 20 (20%) of the participants. Fourteen (14%) bacterial isolates were from catheterised patients. Among the studied bacterial isolates, 78 (78%) were from outpatients, and 22 (22%) were from inpatients. The demographic data are illustrated in Table-1.

In this study, only culture isolates of the Enterobacteriaceae family were included, and BD Phoenix™ Automated Microbiology System confirmed the identity of all isolates. Three members of the Enterobacteriaceae family were isolated from the samples that include; *Escherichia. coli* (89%), *Klebsiella pneumoniae* (10%) and *Proteus mirabilis* (1%).

Antibiotic susceptibility testing showed that imipenem was the most effective antibiotic in which only 12(12%) of isolates were resistant, 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%), Table 2.

Antibiotic profile for *K. pneumoniae* was as follows 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, excluding Cefepime and Imipenem, which were sensitive to the

samples, Table 2.

Isolated bacteria were different in their ESBL enzyme range; the results of ESBL screen tests differ 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 was divided into 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 concerning 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%) were positive for *E. coli*, 5(50%) for *K. pneumoniae* and 1(100%) for *P. mirabilis*. There was no significant difference among all isolated Enterobacteriaceae concerning the ESBL confirmation tests (P-value > 0.05).

Table 1. Demographic characteristics.

Variables No. (%)	Age groups No. (%)					Total No. (%)	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
Catheterisation		2 (14.2)	4 (28.5)	3 (21.4)	2 (14.2)	3 (21.4)	14 (14)	
Hospitalisation	Inpatients	3 (13.6)	6 (27.2)	4 (18.1)	4 (18.1)	5 (22.7)	22 (22)	> 0.05
	Outpatients	20 (25.6)	11 (14.1)	17 (21.7)	6 (7.6)	24 (30.7)	78 (78)	
Total No. (%)		23 (23)	17 (17)	21 (21)	10 (10)	29(29)	> 0.05	

Table 2. Antibiotic susceptibility results for isolated Enterobacteriaceae.

Antibiotic discs	Susceptibility pattern	Bacterial isolates			Total No. (%)
		<i>E. coli</i> No. (%)	<i>K.pneumoniae</i> No. (%)	<i>P.mirabilis</i> No. (%)	
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)
Nitrofurantoin	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)

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Table 2 continued ..

	R	58 (65.2)	4 (40)	1(100)	63 (63)
Ciprofloxacin	I	1(1.1)	1(10)	0	2 (2)
	S	30 (33.7)	5 (50)	0	35(35)
	R	54 (60.7)	7 (70)	1 (100)	62 (62)
Trimethoprim-sulfamethoxazole	I	1 (1.1)	0	0	1 (1)
	S	34 (38.2)	3 (30)	0	37 (37)
	R	37 (41.6)	4 (40)	1 (100)	42 (42)
Amoxicillin/clavulanic acid	I	8 (8.9)	1 (10)	0	9 (9)
	S	44 (49.4)	5 (50)	0	49 (49)
	R	63 (70.8)	8 (80)	1 (100)	72 (72)
Ceftazidime	I	0	1 (10)	0	1 (1)
	S	26 (29.2)	1 (10)	0	27 (27)
	R	61 (68.5)	7 (70)	1(100)	69 (69)
Cefotaxime	I	2 (2.2)	0	0	2 (2)
	S	26 (29.2)	3 (30)	0	29 (29)
	R	62 (69.7)	8 (80)	1(100)	71(71)
Ceftriaxone	I	1 (1.1)	1 (10)	0	2(2)
	S	26 (29.2)	1 (10)	0	27(27)
	R	44 (49.4)	7 (70)	0	51(51)
Cefepime	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 by different screen and confirmatory tests.

Isolates	ESBL screening No. (%)		ESBL confirmation No. (%)	
	Standard disk diffusion (Screening 1)	Double-disk synergy (Screening 2)	BD Phoenix™	Combined disk synergy
<i>E. coli</i>	61 (68.5)	43 (48.3)	42 (47.2)	42 (47.2)
<i>K. pneumoniae</i>	7 (70)	5(50)	5(50)	5 (50)
<i>P. mirabilis</i>	1 (100)	1(100)	1(100)	1(100)
P value	> 0.05	> 0.05	> 0.05	> 0.05



Figure 1. a. Double-disk synergy tests; extension zone from Cephalosporine discs towards Amoxicillin/Clavulanic acid disc was considered to be a positive result for this test.

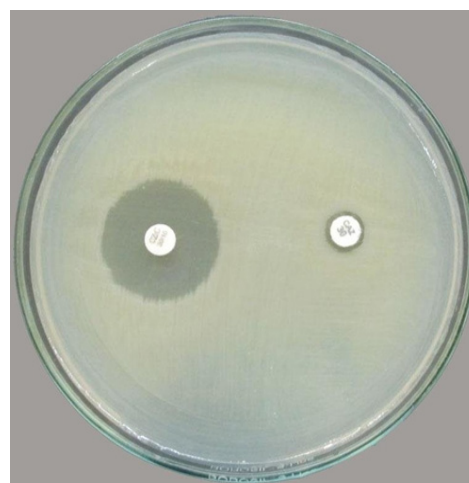


Figure 1. b. Combined disk synergy test; the inhibition zone size around the CZC combined disk must be at least larger by 5 mm than the CAZ disk alone to consider this test positive.

DISCUSSION

Urinary Tract Infection UTI is one of the most common bacterial infections that affect both genders at any given age, which is caused by bacterial colonisation of the sections of the urinary tract ⁽¹⁶⁾.

In the current study, the data showed that the rate of UTI in females is higher than in males, which is mostly because males possess longer urethra and possess prostatic fluid, which has antibacterial activity that protects them from infection ^(4,17).

E. coli is the most common cause of UTI globally due to its presence normally in the gastrointestinal tract and because it possesses required adhesins, toxins, flagella, surface polysaccharides, iron-acquisition systems and factors that increase its ability to colonise the urinary tract ⁽¹⁸⁾. In the current study, *E. coli* was the most common isolate (89%), which was in agreement with studies done before ^(19,20) conducted in Zakho/Iraq and Wasit/Iraq. At the same time, *K. pneumoniae* was the second most common species isolated, which was in agreement with studies done in Iraq ^(19,20); this is because *K. pneumoniae* is mostly the cause of complicated UTI in catheterised patients ⁽²¹⁾, and most of the data of this study were uncomplicated UTI.

Only one isolate of *P. mirabilis* was obtained, which disagreed with the results of studies done previously ^(19,20), which were conducted in Zakho/Iraq and Wasit/Iraq. The differences are probably related to the sample size and the studied population because *P. mirabilis* is

most common in the pediatric age group ⁽²²⁾.

Resistance to antibiotics therapy is a global threat in the medical field because it affects several essential variables in treatment, including spreading disease, hospitalisation duration and increasing treatment cost ⁽²³⁾.

The current study shows that isolates were highly resistant to Nalidixic acid and third-generation Cephalosporin, which aligns with the results of previous studies ^(24–26) that were conducted in nearby regions, including Erbil/Iraq Thi-Qar/Iraq and Baghdad/Iraq. This region's high resistance rate is mostly due to the frequent use of these antibiotics as empirical treatment in this community and low restrictions on obtaining these antibiotics ⁽²⁷⁾.

On the other hand, 87% of isolates were sensitive to imipenem; this result is in agreement with the study done in India ⁽²⁸⁾, which showed that Imipenem susceptibility was (97.85%), also in Iran ⁽²⁹⁾ proved that Imipenem susceptibility was (96.8%), this is mostly imipenem is less used as an empirical treatment for UTI cases. After all, imipenem is expensive and requires intravenous administration.

Secondly, 76% of isolates were susceptible to Nitrofurantoin, which agreed with a previous study ⁽³⁰⁾ conducted in Duhok/Iraq; this is mainly because most urologist does not use Nitrofurantoin as a treatment for UTI.

The spread of ESBL-producing organisms threatens public health due to the limited therapeutic options for specific organisms. Dissemination of ESBL-producing bacteria could be connected to the presence of multiple risk factors such as inappropriate use of broad-spectrum antibiotics, inappropriate prescription, long duration of hospitalisation and transfer of ESBL genes by transposable elements such as plasmid and integron in health care settings⁽⁸⁾.

ESBL production was confirmed in 48% of isolates, which aligns with a previous study⁽³¹⁾ 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, another study⁽³²⁾ that was conducted in Wasit/Iraq showed that 37.8% of the isolates were positive for ESBL production. Studies conducted in Iran, a neighbouring country, show a similar prevalence of ESBL-positive isolates shown in studies conducted in Shahrud/Iran⁽³³⁾ that found 50% of isolated *K. pneumoniae* were positive for ESBL. Also, Tehran/Iran⁽³⁴⁾ showed that 55% of isolated *E.coli* were positive for ESBL. These results align with the current studies.

In contrast, a higher prevalence (61%) of ESBL was detected in a study conducted in Turkey⁽³⁵⁾ by collecting data from 29 provinces. Similar prevalence were found in Spain⁽³⁶⁾ and China⁽³⁷⁾. The current study used an antibiotics profile and double-disk synergy test for ESBL screening and BD Phoenix™ and combined disc synergy for ESBL confirmation. In contrast, in the other studies, only an antibiotics profile was used for screening and a double-disk synergy test was used as a confirmation test. Numerous screening and confirmation tests provide higher sensitivity and specificity in the overall data and immunity against false results⁽³⁸⁾. However, the current study results differ slightly from other studies' results. The minor differences in ESBL detection can be explained by the differences in sample size and the fact that two screening and two confirmation methods were used to evaluate the ESBL ratio in the current research. In Iraq, lack of control over antibiotic use, especially β -lactams, can explain these high rates of ESBL production by clinical isolates from the Iraqi population.

In 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 selecting an

appropriate antibiotic upon ESBL detection.

Acknowledgment

This study was supported financially by Razga Company for Trading, General Contracting and Quality Control. We are grateful for their support. Also, we would like to thank the head and staff of the Department of Microbiology, College of Medicine of the University of Sulaimani, for their cooperation in completing this research. Special thanks to the Laboratory Department of Smart Hospital of Sulaimani / Iraq staff.

Conflict of Interest

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

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