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Detection of Class I Integrons with Phenotypic ESBL Detection among *Enterobacteriaceae* Isolated from Patients with Urinary Tract Infection in Sulaimani Provenance/Iraq

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Abstract

Objectives: This study aims to evaluate class I integrons and ESBL detection in members of *Enterobacteriaceae* obtained from urinary tract infection samples.

Methods: Four hundred mid-stream urine sample were collected from patients admitting to Shar teaching hospital, Sulaimani teaching hospital, Anwar Shexa medical city hospital with signs and symptoms of UTI, and inoculated on different culture media. Colony morphology, gram staining, and VITEK 2 compact were used for bacterial identification, antibiotic profile and ESBL screened phenotypically by antibiotic profile results and double disk synergy test and confirmed by combined disk test methods. Conventional PCR was used to detect class I Integron by using a specific primer and the result was analyzed, sequenced and uploaded into NCBI.

Results: Among four hundred samples the prevalence rate of *Enterobacteriaceae* was 67.03 which includes 86 *E. coli,* 32 *Klebsiella pneumoniae,* and two isolates of *Proteus mirabilis,* according to the antibiotic profile, the most sensitive antibiotic among all three isolates were Imipenem, meropenem, and nitrofurantoin, while most resistance antibiotic were nalidixic acid and third generation cephalosporin. The prevalence rate of ESBL-producing *Enterobacteriaceae* was 56.6% by the confirmatory test which was a combined disk test, and the prevalence rate of class I integron was 54.2%.

Conclusion: A significant majority of the isolates under study had integrons gene and were ESBL positive. As a result, proactive antibiotic surveillance systems are required in both clinical and community settings in order to stop the occurrence and spread of antibiotic resistance genes among diverse bacterial species.

Keywords: Class I integron, ESBL, *Enterobacteriaceae*, UTI

Introduction

Urinary tract infection (UTI) is a collective term that defines any infection that involves different parts of the urinary system, such as the kidneys, ureters, bladder, and urethra,[1] and it is the second most prevalent clinical reason for empirical antibiotic therapy in primary and secondary health care.[2]

The role of specific uropathogenin in the identification of women with recurrent UTI is critical for targeted antibiotic therapy and adherence to the principles of antibiotic stewardship because women with a confirmed clinical history of recurrent UTI are frequently exposed to antibiotic treatment.[3]

The gold standard technique for diagnosing symptomatic UTIs is urine culture, which identifies the bacteria that caused the infection as well as the pathogen load.[4] *Enterobacteriaceae* is among the family of bacteria that causes UTI, with *E. coli* as the most abundant one. Depending on certain underlying circumstances; non-fermenters like *Acinetobacter*, *P. aeruginosa*, and gram-positive cocci like *S. aureus*, *S. saprophyticus*, and *E. faecalis* also are common isolates in causing urinary tract infection.[5]

Antibiotic resistance is more common among *Enterobacteriaceae* and it is mostly linked to the presence of ESBL enzyme, although additional resistance mechanisms such as multidrug resistance (MDR) are emerging also[6] that is confirmed by different phenotypic methods approved by Clinical and Laboratory Standards Institute (CLSI).[7]

One of the systems that aid in transmitting resistant genes is the integrons system, with a natural capture and assembly platform that allows bacteria to absorb gene cassettes and convert them to functional proteins through proper expression. As a result of the naturally large pool of gene cassettes, integrons may have an almost infinite ability to exchange and store functional gene cassettes, allowing for fast adaptation to selection pressure and perhaps increasing the host's fitness and advantage.[8] Antibiotic resistance has been linked to three different forms of integrons (IntI1) class 1 is one of them with its own set of integrons genes Integrons have been detected in around 9% of sequenced bacterial genomes, with the class 1 integrons platform being the most frequent and widely reported in clinical bacteria.[9]

Methods

This cross-sectional study includes all patients who were admitted to Shar teaching hospital, Sulaimani teaching hospital, Anwar Shexa medical city hospital, and an outpatient clinic in Shar teaching hospital who present with the sign and symptoms of UTI according to what was established previously from September 2021 to January 2022.

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.

Sample Collection

The early morning midstream urine specimens were requested from non-catheterized patients and collected in a sterile screw cup container and the samples were processed within 30 minutes. Separating 2–3 ml of urine into another disposable tube for dipstick urine analysis. In catheterized patients, urine samples were collected from the port of catheters by using a sterilized syringe when the catheter was cleaned with an alcohol pad and clamped for approximately 10–30 minutes and then transferred immediately to a sterile screw cup container.[10]

Urine samples were inoculated to different culture media (sheep blood agar, MacConkey agar, nutrient agar, and EMB) by using the standard bacteriological technique (striking methods) and the culture plates were incubated for 24–48 hours at 37°C under aerobic conditions.[11]

Observing colonial morphology on blood, MacConkey, EMB were analyzed and used to differentiate lactose fermenter from non-lactose fermenter isolates, the fast drop in pH of the EMB agar is a key element in the production of the green metallic sheen seen in *E. coli*, as well as rapid lactose fermentation and the generation of strong acids.[12]

VITEK 2 compact Biomeirux machine was used for the identification of all isolated *Enterobacteriaceae* according to the manufacturer's recommendation.

Antibiotic Susceptibility Testing

The antimicrobial susceptibility pattern of the isolated organisms was determined by the Kirby-Bauer disc diffusion method using commercially available antibiotic discs according to what was fixed by the Clinical and Laboratory Standards Institute CLSI.[7] 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), Cefixime CFM (5 µg), Cefepime CPM (30 µg), Ciprofloxacin CIP (5 µg), Trimethoprim-sulfamethoxazole SXT (5 µg), Nalidixic acid NA (10 µg), Nitrofurantoin 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 *Klebsiella pneumonia* ATCC 700603 was used as a positive control for ESBL and *E. coli* 25922 was used as negative quality control for antibiotic profiles.

ESBL Screening by Standard Disk Diffusion Method

ESBL detection was carried out by standard disk diffusion methods for all *Enterobacteriaceae*. Positive screen 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, Aztreonam zone diameter ≤16 mm).[13]

Double Disk Synergy Test

A double-disk synergy test had been used for screening of ESBL by preparing a suspension of the bacterial isolate with McFarland standard (0.5%) and streaking on Mueller Hinton agar. Amoxicillin/clavulanic acid (20/10 µg) disc was placed in the center of the plate and Ceftazidime CAZ (30 µg), Cefotaxime CTX (30 µg), Cefixime CFM (5 µg), Ceftriaxone CTR (30 µg), Aztreonam ATM (µg) and Cefepime CPM (30 µg), discs were placed 15 mm apart center to center to amoxicillin/clavulanic acid and incubated aerobically for 18–24 hours at 37°C. 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

A disc of clavulanic acid plus cefotaxime (CEC) and cefotaxime disk alone were used together. The disks were placed at a distance of 20 mm apart from the inhibitor disk on a lawn culture of the resistant isolate on the Muller Hinton agar plate. The tested organism was considered to be positive for ESBL if the zone size around the cephalosporin (third) plus clavulanic acid is >5 mm than the cefotaxime disk alone.[15]

DNA Extraction

Genomic DNA was extracted by boiling method (colony PCR) as done previously.[16]

PCR Reaction

The presence of class 1 integrons was tested among all *Enterobacteriaceae* by conventional PCR using primers specific for integrase genes of the integron, (intl1) as shown in Table 1. By using PCR condition shown in Table 2.

The total reaction volume was 20 µl. The amplicons were analyzed by electrophoresis on 1.0% agarose gel in TBE buffer. The PCR product was carried out in 1.0% agarose gel for 60 minutes and a voltage of 90. Then, the results were evaluated under UV light on the UV Trans illuminator.[17]

Sequencing of PCR Products

The purified PCR product of one positive sample (intlI) were sequenced by Macrogen Genome center 1001, 254, Beotekkot-ro, Geumcheon-gu, Seoul 08511, Republic of Korea. Using 10 pmol of specific primers and 5 µl of PCR products. The sequences were analyzed by Chromas Technelysium as well as. They had provided Genbank accession numbers for our nucleotide sequence ON745429 and approved to be class I integrons.

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 integron classes and

Table 1. **Primer sequence for class 1 integrons (9)**

Gene name	Primer name sequence		Size bp
Intl1	Intl1-F	TCTCGGGTAACATCAAGG	296 bp
	Intl-R	AGGAGATCCGAAGACCTC	

Table 2. **PCR condition for integron detection (9)**

PCR condition	Number of cycles	
	Intl1	
Initial denaturation	95°C/5 min	1 cycle
Denaturation	95°C/30 sec	32 cycle
Annealing	58°C/30 sec	32 cycle
Extension	72°C/30 sec	32 cycle
Final extension	72°C/5 min	1 cycle

ESBL-positive *Enterobacteriaceae*. The significance level was defined as $P < 0.05$.

Results

A total of 400 urine samples were collected during the period of study from September 2021 to January 2022 from Shar teaching hospital, Sulaimani teaching hospital, and Anwar Shexa medical city hospital.

The age of patients were between 7–84 years old and were divided into 5 age groups as it is illustrated in Table 3. The greater age groups that attend hospitals complain of signs and symptoms of UTI were patients with >60 years old (36.75%), followed by 45–60 years (25.0%), while the age group of 31–45 recorded (22.5%), and (10.0)% recorded among 15–30 years old. The last age group which had been recorded in this study is the group of <15 years old (5.75%).

According to gender; females were more affected by UTIs than males in a ratio of 2/3 as 276 females (69%) were recorded to have symptoms in comparison to 124 males (31%).

Other factors were assessed among all participants such as frequency of UTI attacks and repeated UTI was recorded among 122 patients (30.5%).

Different comorbidities were recorded and it was found that most patients with diabetic militants were attending hospitals complaining of symptoms of UTI than other conditions as obvious in Table 3 diabetic patients were among the commonest group (35.0%) followed by hypertension (17.5%), while chronic kidney disease accounts for 5.5%.

According to the type of UTIs; cystitis was more common and recorded in 225 (56.3%) patients while symptoms of pyelonephritis were less and identified in only 125 (31.3%) patients in spite of that prostatitis was also recorded in 7 males (Table 3).

Among four hundred patients that were participated in the study; 229 (57.25%) samples were negative culture after 48 hr. of incubation in spite of having UTI symptoms, while 171 (42.75%) samples were positive for urine culture. Out of the total positive urine culture; 120 samples were *Enterobacteriaceae* and followed by 16 gram-positive bacteria, 24 non-fermenter gram-negative bacteria with 11 fungi were also isolated (Figure 1).

Antibiotic Susceptibility Testing

All *Enterobacteriaceae* samples were tested for antimicrobial susceptibility testing, by using Kirby-Bauer disc diffusion method, and the results were analyzed according to the Clinical and Laboratory Standard Institute (CLSI, 2022). The susceptibility result against 12 antimicrobial agents are shown in Table 4 as a percentage of resistance, sensitive, and intermediate among all confirmed *Enterobacteriaceae*. The most resistant antibiotics for *E. coli* were nalidixic acid (77.9%), ceftriaxone (75.6%), ceftazidime (74.7%), cefotaxime (72.1%), trimethoprim (72.1%), ciprofloxacin (59.3%), cefepime (50.0%), gentamycin (45.3%), amoxiclav (44.2%), nitrofurantoin (34.9%), meropenem (29.1%), and imipenem (16.3%). Antibiotic profile for *Klebsiella pneumoniae* was the same as *E. coli* in order of resistance to nalidixic acid (78.1%), ceftriaxone (71.9%), ceftazidime (71.9%), cefotaxime

Table 3. **Demographic characteristics of the patients with according to positive and negative urine culture**

Categories		Patient with positive culture *N* (%)	Patient with negative culture *N* (%)	Total *N* (%)	*P*-value
Age	<15	9 (39.1)	14 (60.9)	23 (5.75)	> 0.05
	15–30	17 (42.5)	23 (57.5)	40 (10.0)	
	31–45	38 (42.2)	52 (57.8)	90 (22.5)	
	46–60	43 (43.0)	57 (57.0)	100 (25.0)	
	>60	64 (43.5)	83 (56.5)	147 (36.75)	
Gender	Male	45 (42.7)	79 (57.3)	124 (31.0)	> 0.05
	Female	126 (42.8)	150 (57.2)	276 (69.0)	
Catheterization	Catheterized	60 (88.2)	8 (11.8)	68 (17.0)	< 0.05
	Non-catheterized	111 (33.4)	221 (66.6)	332 (83.0)	
Hospitalization	Inpatients	90 (81.8)	20 (18.2)	110 (27.5)	< 0.05
	Outpatients	81 (27.9)	209 (72.1)	290 (72.5)	
Recurrence	First time UTI	76 (27.3)	202 (72.7)	278 (69.5)	< 0.05
	Repeated UTI	95 (77.9)	27 (22.1)	122 (30.5)	
Medical history	Diabetic	44 (31.4)	96 (68.6)	140 (35.0)	< 0.05
	Hypertension	42 (60.0)	28 (40.0)	70 (17.5)	
	Renal failure	21 (95.5)	1 (4.5)	22 (5.5)	
	None	64 (38.1)	104 (61.9)	168 (42.0)	
Type of UTI	Cystitis	78 (34.7)	147 (65.3)	225 (56.3)	< 0.05
	Pyelonephritis	76 (60.8)	49 (39.2)	125 (31.3)	
	Urethritis	11 (25.6)	32 (74.4)	43 (10.7)	
	Prostatitis	6 (85.7)	1 (14.3)	7 (1.7)	
Total		171 (42.7)	229 (57.3)	400	

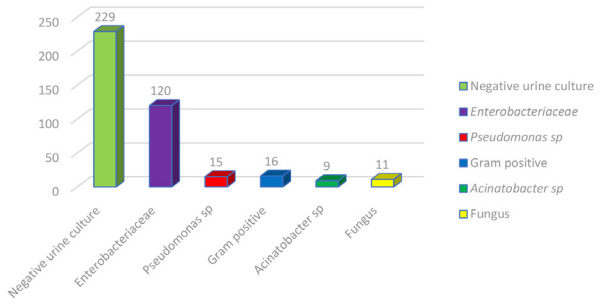

Fig. 1 **Percentage of positive and negative urine culture with isolated bacteria.**

(65.6%), trimethoprim (62.5%), amoxiclav (46.9%), ciprofloxacin (43.8%), cefepime (40.6), gentamycin (37.5%), nitrofurantoin (31.3%), meropenem (18.8%), and imipenem (6.3%). Only 2 *Proteus* species were isolated in urine culture as they were resistant to all antibiotic discs that had been used excluding cefepime and imipenem which were sensitive for both two samples. The most sensitive antibiotic for all three isolates were imipenem for *E. coli* (82.6%), *Klebsiella pneumoniae* (93.8%), and *Proteus mirabilis* (100%), and the seconed most sensitive one was meropenem and nitrofurantoin (65.1%) for *E. coli* and (78.1%), (65.6%) for *Klebsiella pneumoniae* and *Proteus mirabilis*.

Extended-Spectrum Beta-Lactamase Enzyme among Isolated *Enterobacteriaceae*

All isolated *Enterobacteriaceae* were screened for ESBL by screen test (antibiotics profile result and double-disk synergy test) and confirmed by combined disk test and VITEK system at the same time and the results were shown in Table 5.

ESBL enzyme had a different range for each isolated bacteria, the percentage of ESBL by screen test differs according to the type of test used. According to the antibiotics profile, 89 out of 120 gram-negative bacteria (74.2%) were resistant to the third-generation cephalosporins. Out of these 89 isolates; 66 samples were positive by double-disk synergy test and 51 (59.3%) were recorded for *E. coli* and 13 (40.6%) for *Klebsiella pneumoniae* and both samples of *Proteus mirabilis* (100%) were positive for ESBL and there was no significant difference among species *E. coli* and *Klebsiella pneumoniae* according to ESBL formation (*P*-value > 0.05).

According to the confirmation tests the prevalence rate of ESBL was 56.6% in which 61.6% was positive for *E. coli*, and 40.62% for *Klebsiella*. Statistically, there was no significant difference among all isolated *Enterobacteriaceae* in relation to the combined disk test (*P*-value > 0.05).

The prevalence rate of class I Integrons among all isolated Enterobacteriaceae

Class I integrons were tested among isolated *Enterobacteriaceae* the prevalence rate was 54.2%, and different prevalent rates were recorded according to isolated bacteria (Table 6) and it is obvious that 58.1% of all isolated *E. coli* harbor class I integrons (intl 1) gene, and 40.6% for *Klebsiella pneumoniae* harbor class I while both species of *Proteus mirabilis* were positive for class I integrons. Statistically this distribution is not significant (*P* value > 0.05). Figure 2 illustrate the accepted band of class 1 integron at 294 bp.

Table 4. **Antibiotic profile among all *Enterobacteriaceae***

Antibiotic		Bacterial name		
Name of antibiotics	**Susceptibility**	***E. coli* N (%)**	***Klebsiella pneumoniae* N (%)**	***Proteus mirabilis* N (%)**
Amoxiclav AMC	R (%)	38 (44.2)	15 (46.9)	1 (50.0)
	I	5 (5.80)	2 (6.3)	0.0
	S	43 (50.0)	15 (46.9)	1 (50.0)
Ceftazidime CAZ	R (%)	64 (74.4)	23 (71.9)	2 (100)
	I	2 (2.3)	2 (6.3)	0 (00.0)
	S	20 (23.3)	7 (21.9)	0 (00.0)
Cefotaxime CTX	R (%)	62 (72.1)	21 (65.6)	2 (100)
	I	3 (3.5)	0 (00.0)	0 (00.0)
	S	21 (24.4)	11 (34.4)	0 (00.0)
Ceftriaxone CRO	R (%)	65 (75.6)	23 (71.9)	2 (100)
	I	2 (2.3)	2 (6.3)	0 (00.0)
	S	19 (22.1)	7 (21.9)	0 (00.0)
Cefepime CPM	R (%)	43 (50.0)	13 (40.6)	0 (00.0)
	I	1 (1.2)	0 (00.0)	0 (00.0)
	S	42 (48.8)	19 (59.4)	2 (100)
Imipenem IPM	R (%)	14 (16.3)	2 (6.3)	0 (00.0)
	I	1 (1.2)	0 (00.0)	0 (00.0)
	S	71 (82.6)	30 (93.8)	2 (100)
Meropenem MEM	R (%)	25 (29.1)	6 (18.8)	2 (100)
	I	5 (5.8)	1 (3.1)	0 (00.0)
	S	56 (65.1)	25 (78.1)	0 (00.0)
Nitrofurantoin NIT	R (%)	30 (34.9)	10 (31.3)	2 (100)
	I	0 (00.0)	1 (3.1)	0 (00.0)
	S	56 (65.1)	21 (65.6)	0 (00.0)
Nalidixic acid NA	R (%)	67 (77.9)	25 (78.1)	2 (100)
	I	4 (4.7)	0 (00.0)	0 (00.0)
	S	15 (17.4)	7 (21.9)	0 (00.0)
Gentamicin GN	R (%)	39 (45.3)	12 (37.5)	2 (100)
	I	5 (5.8)	4 (12.5)	0 (00.0)
	S	42 (48.8)	16 (50.0)	0 (00.0)
Ciprofloxacin CIP	R (%)	51 (59.3)	14 (43.8)	2 (100)
	I	6 (7.0)	3 (9.4)	0 (00.0)
	S	29 (33.7)	15 (46.9)	0 (00.0)
Trimethoprim-sulfamethoxazole SXT	R (%)	62 (72.1)	20 (62.5)	2 (100)
	I	8 (9.3)	1 (3.1)	0 (00.0)
	S	16 (18.6)	11 (34.4)	0 (00.0)

S, susceptibility; R, resistance; I, intermediate.

Discussion

UTI is one of the most common infections that affect both men and women and it is developed due to the presence and duplication of bacteria in the different portions of the urinary system.[18]

Table 5. **ESBL detection among *Enterobacteriaceae* by different tests**

Bacterial isolates	Number of isolated *N* (%)	Screen 1 *N* (%)	Double disk test *N* (%)	Confirmation of ESBL	
				CD1 (%)	VITEK system (%)
E. coli	86 (71.7%)	64 (74.4)	51 (59.3%)	53 (61.6%)	50 (58.1%)
Klebsiella pneumoniae	32 (26.7%)	23 (71.9)	13 (40.6%)	13 (40.62%)	13 (40.62%)
Proteus mirabilis	2 (1.7%)	2 (100)	2 (100%)	2 (100%)	1 (50%)
Total	120 (100)	89 (74.2)	66 (55%)	68 (56.6%)	64 (53.3%)
P value	–	>0.05	>0.05	>0.05	>0.05

CD1, Combined Disk 1 (cefotaxime plus clavulanic); Screen 1, Resistance to third generation cephalosporin; DDT, double disk test.

Table 6. **Prevalence rate of class I integrons among three isolated *Enterobacteriaceae* species**

Isolated *Enterobacteriaceae*	Class I	
	Positive *N* (%)	Negative *N* (%)
E coli	50 (58.1)	36 (41.9)
Klebsiella pneumoniae	13 (40.6)	19 (59.4)
Proteus mirabilis	2 (100.0)	00 (00.0)
Total	65 (54.2)	55 (45.8)
P value	>0.05	

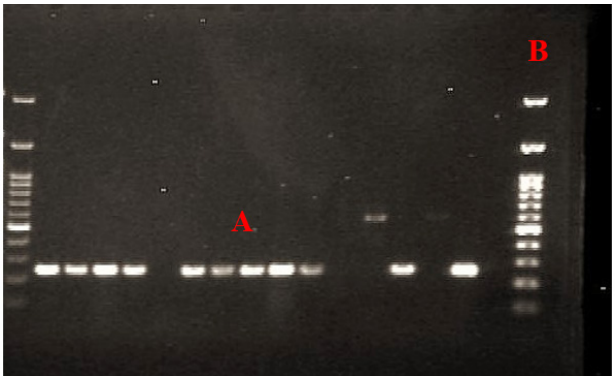

Fig. 2 **Gel electrophoresis for class 1 integron. A: Class 1 integron (294 bp) B: Ladder.**

The demographic data in this study concluded that female are more infected than male due to the fact that female possess short urethra and lack of prostatic fluid which have antibacterial activity that prone here to infection more,[19] and patients with age group > 60 years old were the most affected groups. These findings are consistent with studies done in Erbil, and Al Najaf/Iraq, that they found a significant frequency of UTI in elderly adults,[19,20] due to their propensity for frequent hospital stays which exposes them to nosocomial pathogens and frequent using antibiotics and urogenital catheters, a decline in adaptive and innate immunity, and a history of repeated urinary tract infections.[21]

In our study, 42.7% of participants have positive urine cultures and 57.3% had negative urine cultures despite having UTI symptoms, different percentages were recorded in different locations all over the world,[22–24] as each study record, a percentage with different sample size and source of infection as the positive urine culture is much more among hospitalized patients and among those with comorbidities, or failure to isolate anaerobic bacteria that require specific conditions to grow or prior antibiotic use before 24–48 hours of doing urine culture that decrease the number of bacteria in the urine.

One of the comorbidities in UTI is diabetic patients and the frequency of UTI, there are some finding that conduct UTI, particularly upper UTI is mainly associated with diabetic militants, this is because diabetes alters the typical host system, which might lead to the development of UTIs,[25] our result conduct that 140 patients with UTI had diabetic militants and among them, 49 patients had bacterial growth or positive for urine culture this finding is in agreement with studies done in Zakho/Iraq, and Pakistan.[22,26]

Using urinary catheter is regarded as the main risk factor for UTI especially among hospitalized patients,[27] in this study 68 patients were catheterized in which 88.2% of them had positive urine culture proving the fact that catheter is a risk factor that colonized by bacteria especially gram-negative bacteria.[28]

Another factor that aids in positive urine culture among UTI cases is a recurrent attack of cystitis that account for 77.9%, this result was in agreement with a study done in India,[29] the inadequate antibiotic treatment of previous UTI or failure of antibiotics to kill the causative bacteria either related to the wrong diagnosis or giving antibiotics without sensitivity results may be a risk factors.

The most common uropathogens that cause UTI is *E. coli* which accounts for approximately 75–90% of all UTIs all over the world.[30] In this study, *E. coli* is the main pathogen that was isolated, the same results were obtained in studies done in Zakho, Duhok, Erbil and Wasit Province/Iraq.[31-34] The abundance of *E. coli* among UTI refers to the presence of adhesins, toxins, flagella, surface polysaccharides, and iron-acquisition systems and factors which back to their capacity to occupy uroepithelial cells and form intracellular bacterial communities.[35]

The second most common *Enterobacteriaceae* which was isolated in this study is *Klebsiella pneumonia* (26.6%) which is in the agreement with many local studies done in Wasit Province and Zakho[26,32] in which they isolated *Klebsiella* as a second most common causative agent for UTI.

The third isolated species in this study was *Proteus mirabilis* with a frequency of 1.7%, that has lower than that detected in studies done in Wasit Province/Iraq and Zakho.[26,32] This difference might be related to the difference in the sample size and difference in the age group as the most common age in which *Proteus mirabilis* colonizes the urinary tract and cause infection is children below 10 years old.[36]

Several antibiotic discs were used to find the response of *Enterobacteriaceae* and different results were recorded. In our study the most sensitive antibiotic for all isolated *Enterobacteriaceae* was the carbapenem group which include

imipenem as a first choice and meropenem, this result was in agreement with the recent study done in Tikrit, Erbil, and Zakho when they record the high frequency of sensitivity to imipenem and meropenem.[17,26,37] This finding might be related to the fact that carbapenem groups are intravenous antibiotics and are more expensive in comparison to other oral antibiotics in this location, hence they are used less as empirical treatment.

While the most resistant antibiotics is nalidixic acid (77.9%), and third-generation of cephalosporins, these results were in agreement with studies done in Erbil, as the results of resistance to the third generation cephalosporin was approximately 76%.[38] The greater resistance rate to cephalosporins among isolated species might be related to the propagation of resistance genes specific for cefotaxime and ceftriaxone resistance among *Enterobacteriaceae*, resulting in the generation of more aggressive strains,[35] or higher frequency uses of these antibiotics as empirical treatment in this community and people easily get these antibiotics without doctor's prescription from health workers and pharmacy.

One of the mechanisms of resistance among *Enterobacteriaceae* is ESBLs which are β-lactamases enzymes that can hydrolyze β-lactam antibiotics such as second, third, and fourth generation Cephalosporins.[6]

ESBL enzymes were analyzed in this study by different phenotypic methods and different prevalence rates were recorded by each test; the prevalence rate was 56.6% by CD1 which was a confirmatory test approximately lower than a recent finding done in Erbil,[38] and in Egypt,[39] as they recorded higher percentage, this could be due to geographical distribution or difference in sample population that participated. Whereas slightly higher than the other recent study done in Northwest Iran.[40] The recording of higher rates of ESBL enzyme among isolated species in Iraqi persons were explained by a lack of control over antibiotic use and prescription, as well as the widespread use of antibiotics in our population, particularly β-lactams.

One of the mobile genetic elements in *Enterobacteriaceae* is the Integron system.[41] Recent research has revealed that integrons play a significant role in the acquisition of resistance genes in bacteria as they transfer a variety of resistance genes especially among gram-negative bacteria.[42] About 9% of the sequenced bacterial genomes contain integrons, and the class I integron platform is the most prevalent one and had received the most reports among clinical bacteria, and is still the subject of several investigations.[43] The prevalence rate of class I integron in this study was 54.2%, there are many studies all over the world that record different prevalence rates of integron classes specifically class I integron among *Enterobacteriaceae*[44-48] this variation might be due to difference in the sample population, geographical distribution, and groups which had been selected for taking urine samples.

The high prevalence rate of class I integrons may be due to integron's exceptional capacity to capture numerous drug resistance genes, and various gene cassette arrays encoding various resistance genes that may confer resistance against various drugs have emerged, As a result of the acquisition of one class of integrons which is class 1 by several mobile genetic elements, as well as the subsequent colonization of various bacterial species and a wide variety of animal hosts.[49]

Conclusion

This research suggests that a significant portion of the *Enterobacteriaceae* found were ESBL positive and have integrons specifically class I integron. As a result, proactive antibiotic surveillance systems are required in both clinical and community settings.

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Conflicts of Interest

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

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