Phenotypic and Genotypic Characterization of IRS-Producing *Escherichia coli* Isolated from Patients with UTI in Iraq

Aliaa Z. AL-Tememy Alaa H. Al-Charrakh
College of Medicine, Babylon University, Iraq

Received 19 October 2014 **Accepted 26 October 2014**

Abstract

The aim of this work was to analyze the current of IRT β-lactamases in uropathogenic *Escherichia coli*. Isolates were prospectively collected in our hospital (2013 and 2014) from urine of hospitalized patients (100%). From a total of 60 *E. coli* isolates recovered during the study period, 22 showed reduced susceptibility to Beta lactam Beta lactamase inhibitor (BLBLI) combination antibiotics, with 17 of them producing IRT enzymes. These were mostly recovered from urine (100%). A high degree of IRT was detected (TEM-2, CTX-M, bla-SHV, SHV-2, TEM-1,OXA-1, and AMPC). The results of this study are recorded for the first time in Iraq.

الخلاصة

يهدف هذا البحث الى الكشف عن تواجد الانزيمات المقاومة لمثبطات البيتالاكتاميز في بكتريا اشريشيا القولون البولية. تم عزل السلالات البكتيرية من عينات الادار من المرضى الراقدين في ثلاثة مستشفيات رئيسة في مدينة الحلة، العراق، خلال الفترة من تشرين الثاني ٢٠٠٣ الى كانون الاول ٢٠٠٤. تم تشخيص العزلات بالاعتماد على الشكل المظهري والفحوصات الكيموحياتية، وتم تأكيد التشخيص باستخدام نظام الفايتيك-٢ ومورثة الحامض النووي الرايبوزي الرسول 16S . أظهرت النتائج الحصول على ٢٢ عزلة (من مجموع ٦٠ عزلة اشريشيا القولون البولية) حساسة لمزيج البيتالاكتاميز والمثبط، أظهرت ٢٢ عزلة منها القدرة على انتاج الانزيمات المقاومة لمثبطات البيتالاكتاميز. وجد ان نسبة عالية من هذه الانزيمات قد تم الكشف عنها وبانواعها المختلفة (TEM-2, CTX-M, bla-SHV, SHV-2, TEM-1, OXA-1, and AMPC) وان النوع TEM من الانزيمات المقاومة لمثبطات البيتالاكتاميز كان هو السائد في هذه الدراسة. أن نتائج هذه الدراسة تسجل لأول مرة في العراق.

Introduction

IRT enzymes represent an adaptive resistance mechanism specifically developed by bacteria to overcome the activity of β-lactamase inhibitors [1]. Resistance to β-lactam–β-lactamase inhibitor combinations in *Escherichia coli* may be due to different mechanisms, including TEM-1 penicillinase hyperproduction, constitutive AmpC over-production or plasmid AmpC production, OXA-type β-lactamase production, permeability deficiencies involving OmpF and/or OmpC porins, inhibitor-resistant TEM (IRT)- and complex mutant TEM (CMT)-like ß-lactamase production, and more recently, carbapenemase production [2]. IRT enzymes comprise a group of plasmid-encoding variants of TEM-1 and TEM-2 with decreased affinities for amino-, carboxy-, and ureidopenicillins and altered interaction with class A ß-lactamase inhibitors [3]. IRT-producing isolates remain susceptible to cephalosporins, cephamycins, carbapenems, and in most cases, piperacillin-tazobactam. They are usually resistant to ampicillin-sulbactam and intermediate or resistant to amoxicillin-clavulanate combinations. IRT enzymes have previously been reported in different organisms, such as *E. coli, Klebsiella spp., Enterobacter cloacae, Proteus mirabilis, Citrobacter freundii,* and *Shigella sonnei* [2]. But there are only a few recent epidemiological studies concerning these enzymes. Moreover, the population structure of IRT-producing *E. coli* isolates has not been addressed using a multilocus sequence typing (MLST) technique. They were originally named TRC (TEM enzymes resistant to clavulanic acid) [4], and later TRI (TEM resistant to β-lactamase inhibitors) [5], and were finally named IRT [6].

Materials and Methods

Study design:

At the beginning of this study, 100 urine samples were collected from patients suffering from significant bacteriurea during the period of November 2013 to the end of January 2014 from three main Hospitals in Hilla city/Iraq (Hilla Teaching hospital, Childhood and gynecology hospital, and general hospital of AL-Hashimiyeh, in addition to some private clinics. Each sample was immediately inoculated on MacConkey agar plates and EMB agar. The swab has been inoculated on culture media and incubated aerobically for 24 hours at 37∘C. Information about age, residence, antibiotic usage, and hospitalization of patients was taken into consideration.

Bacterial Isolates

Uropathogenic *E. coli* isolates were recovered and identified based on their morphology, Gram-staining, Indole test, MR-VP test, and motility test [7]. Identification was confirmed using specific 16S rRNA gene by PCR assay.

Antimicrobial Susceptibility Testing:

The antimicrobial susceptibility patterns of isolates to different antibiotics were determined using disk diffusion test and interpreted according to CLSI guidelines [8]. The following antibiotics were obtained (from Oxoid, UK, and Bioanalyse, Turkey) as standard reference disks as known potency for laboratory use: Ampicillin, AM (10 µg) Amoxillin, AX (25 µg), Amoxillin/ Clavulanic acid, AMC (30 µg), Ceftazidime/ Clavulanic acid, CAC (30/10 µg), Ceftriaxone-Tazobactam, CIT (30/10 µg) Cephalothin, KF (30 µg), Cephalxin, CL (30 µg), Cefoxitin, FOX (30 µg), Ceftizoxime, CZX (30 µg), Ceftazidime CAZ, (30 µg), Cefotaxime, CTX(30 µg), Ceftriaxone CRO (30 µg), Cefepime FEP (30 µg), Imipenem IMP (10 µg), Meropenem MEM (10 µg), Aztreonam ATM (30 µg), Gentamicin CN (10 µg), Nalidixic acid NA(30 µg), Ciprofloxacin CIP (30 µg), Tetracycline TE (30 µg), Trimethoprim/ Sulphamethoxazole SXT (25 µg) (1.25/23.75), Nitrofurantoin F (300µg).

Screening Test for β-Lactam Resistance

Ampicillin and amoxicillin were added, separately, from the stock solution to the cooled Muller-Hinton agar at final concentrations of 100 and 50 µg/ml, respectively. The medium was poured into sterilized Petri dishes, then stored at 4˚C. Preliminary screening of *E. coli* isolates resistant to both antibiotics was carried out using pick and patch method on the above plates [9]. Results were compared with *E. coli* ATCC 25922 as a negative control and *E. coli* ATCC 35218 as a positive control.

Detection of *β*-Lactamase Production:

Nitrocefin diagnostic disk (Fluka, Switzerland) was used to detect the ability of 15 isolates to produce *β*-lactamase. A number of required nitrocefin disks were placed into sterile empty Petri dish and moistened with one drop of sterile D.W.; then the disk was holed by sterile forceps and wiped across a young colony on agar plate. The development of a red color in the area of the disk where the culture was applied indicated a positive result.

Determination of MICs of *E. coli* isolates:

The two-fold agar dilution susceptibility method was used for determination of MICs of β-lactam antibiotics according to CLSI [8].

Initial screening AmpC β-Lactamase

All β-lactam resistant isolates were tested for cefoxitin susceptibility by using standard disk diffusion method [8]. The resistant isolates (≤18mm inhibition zone diameter) were consider as initially AmpC β-lactamase producers.

Initial screening for ESBL production:

All bacterial isolates that were β-lactamase producing were tested for ESBL production by initial screen test. The isolate would be considered potential ESBL producer, if the inhibition zone of ceftazidime (30 µg) disks was ≤ 22 mm [8].

Confirmatory test for ESBL production

All the β-lactamase producing isolates were tested also for confirmatory ESBL production by disk combination test (Recommended by CLSI, 2014) [8] as follows: Cefotaxime alone and in combination with clavulanic acid were tested. Inhibition zone of ≥ 5 mm increase in diameter for antibiotic tested in combination with clavulanic acid versus its zone when tested alone confirms an ESBL producing isolate.

Table (1): Primers of monoplex PCR

Target	Primer name	Oligo Sequence (5′→3′)	Product Size (Pb)	Ref.
AmpC	*AmpC-F*	ATC AAA ACT GGC AGC CG	550	Al-Sehlawi [23]
	AmpC-R	GAG CCC GTT TTA TGC ACC CA	550	
TEM	*TEMU1*	ATG AGT ATT CAA CAT TTC CG	867	Reguera *et al* [40]
	TEML1	CTG ACA GTT ACC AAT GCT TA	867	
TEM	*TEMU2*	ACT GCG GCC AAC TTA CTT CTG	374	Kaye *et al.* [15]
	TEML2	CGG GAG GGC TTA CCA TCT G	374	
SHV	*SHV-F*	GGT TAT GCG TTA TAT TCG CC	867	Ferreira *et al.* [41]
	SHV-R	TTA GCG TTG CCA GTG CTC	867	
SHV	*SHVU2*	CCG CAG CCG CTT GAG CAA A	477	Kaye *et al.* [15]
	SHVL2	GCT GGC CGG GGT AGT GGT GTC	477	
OXA	*OXA-1F*	ACA CAA TAC ATA TCA ACT TCG C	813	Steward *et al.* [*34*]
	OXA-1R	AGT GTG TTT AGA ATG GTG ATC	813	
OXA	*OXA-10F*	CGT GCT TTG TAA AAG TAG CAG	651	Steward *et al* [34]
	OXA-10R	CAT GAT TTT GGT GGG AAT GG	651	
OXA	*OXA-2F*	TTC AAG CCA AAG GCA CGA TAG	702	Steward *et al.*, [34]
	OXA-2R	TCC GAG TTG ACT GCC GGG TTG	702	
universal *CTX-M*	*CTX-M-F*	CGC TTT GCG ATG TGC AG	550	Messai *et al.* [42]
	CTX-M-R	ACC GCG ATA TCG TTG GT	550	

Plasmid DNA extraction and purification:

A single colony of cultivated bacteria, which had been incubated overnight, transfer to 2 ml of sterile nutrient broth and incubate at 37 ◦C for 18-20 hours .The DNA extracted and purified using Quick Guide plasmid Mini Prep Kit DNA extraction (SolGent, Korea) according to manufacture instructions (SolGent, Korea). Plasmid DNA was used to detect *TEM-1, TEM-2, SHV, SHV-2, OXA-1, OXA-2, OXA-10, AMPC*, and CTX-M.

Preparing the primers suspension:

The DNA primers (Table-1) were resuspended by dissolving the lyophilized product after spinning down briefly with TE buffer molecular grad depending on manufacturer instruction as stock suspension. Working primer tube was prepared by diluted with TE buffer molecular grad. The final picomoles depended on the procedure of each primer.

Monoplex PCR thermocycling conditions:

The PCR tubes were placed on the PCR machine and the right PCR cycling program parameters conditions were installed as in Table-2.

Agarose gel electrophoresis:

The amplified PCR products were detected by agarose gel electrophoresis was visualized by staining with ethidium bromide. The electrophoresis result was detected by using Biometra gel documentation system.

Table (2): Programs of Monoplex PCR Thermocycling Conditions

Monoplex gene	Temperature (°C) /Time					Cycle No.
	Initial denaturation	Cycling condition			Final extension	
		Denaturation	Annealing	Extension		
bla-AmpC	94/3 min	94/45 sec	60/45sec	72/1 min	72/5 min	35
bla-SHV	96/5 min	96/1 min	60/1 min	72/1 min	72/10 min	35
bla-CTX-M	94/4.5 min	94/50 sec	58/50 sec	72/50 sec	72/7 min	35
TEM-1	95°C/ 5 min	94°C/ 1min	58°C/ 1min	72°C/1min	72°C/ 10min	35
TEM-2	95°C/5 min	94°C /30s	62°C/30 s	72°C/30 s	72°C/10min	30
SHV-2	95°C/5 min	94°C /30s	62°C/30 s	72°C/30 s	72°C/10min	30
OXA-1	96°C/ 5 min	96°C/1 min	61°C / 1 min	72°C/ 2 min	72°C/10min	35
OXA-2	96°C/5 min	96°C /1 min	65°C /1 min	72°C / 2 min	72°C/10min	35
OXA-10	96°C/5 min	96°C/1 min	61°C / 1 min	72°C / 2 min	72°C/10min	35

Results and discussion:

Isolation and Identification of isolates:

Out of 100 urine specimens, 90 (90%) showed culture growth positive and yielded 90 bacterial isolates. 60 (60%) were found to be uropathogenic *E. coli*

Frequency of β-lactam resistant uoro-pathogenic *E. coli*:

The frequency of β-lactam resistance was evaluated when the isolates primarily screened for resistance using ampicillin and amoxicillin [10, 11]. The results obtained in this study revealed that 33 (55%) *E. coli* isolates were resistant to both ampicillin and amoxicillin.

Production of β-Lactamase by nitrocefin disk method:

The results revealed that among the 33 isolates tested, 30 (90.9%) produced β-lactamase. This result revealed that β-lactamase producing *E. coli* isolates by nitrocefin method was significant. This may refer to the fact that nitrocefin is more sensitive to be hydrolyzed with all known β-lactamases produced by Gram-negative bacteria [12]. In addition, this method is useful for the detection of β-lactamase patterns from bacterial cell extracts and susceptible for detecting low level of β-lactamases produced constitutively or by induction in enteric bacteria.

Tuwaij [13], found that 21 (84%) and 24 (96%) *Serratia spp.* isolates were identified as β-lactamase producers with rapid iodometric and nitrocefin methods, respectively.

Susceptibility to β-lactam-β-lactamase Inhibitor (BLBLI) combinations

The results obtained in this study revealed that only 22 (66.6%) from 33 β-lactam resistant were still resistant or intermediate to one or more of BLBLI combinations antibiotic. In this study 21 (95.4%) of 33 *E. coli* isolates were resistant to ampicillin-sulbactam, 13 (59%) resistant to amoxicillin-clavulanic acid, and 11 (50%) resistant to pipracillin-tazobactam.

Miro *et al.,* [14] found that 7% of 7,252 non duplicated clinical *E. coli* strains from a Spanish hospital were showed reduced susceptibility to amoxicillin-clavulanate.

Kaye *et al*., [15] found that out of 283 isolates that tested resistant to ampicillin-sulbactam, 69 unique patient isolates were also resistant to amoxicillin-clavulanate by disk diffusion testing (zone diameter < 13 mm). Among the isolates, 12 were nosocomial (rate of amoxicillin-clavulanate resistance = 4.7%) and 57 were community acquired (rate of amoxicillin clavulanate resistance =2.8%). No predominant strain was identified.

Leflon-Guibout *et al* [16] found that Amoxicillin-clavulanate resistance (MIC >16 mg/ml) and the corresponding molecular mechanisms were prospectively studied in *E. coli* over a 3-year period (1996 to 1998) in 14 French hospitals. The overall frequency of resistant *E. coli* isolates remained stable at about 5% over this period. The highest frequency of resistant isolates (10 to 15%) was observed, independently of the year, among *E. coli* isolated from lower respiratory tract samples, and the isolation rate of resistant strains was significantly higher in surgical wards than in medical wards in 1998 (7.8 versus 2.8%).

Production of ESBL

According to CLSI [8] the isolate is considered to be a potential ESBL producers, if the inhibition zone of ceftazidime disks (30 µg) was ≤ 22 mm. The study found that 17 (77.2%) of the 22 *E. coli* isolates were ESBL positive during the initial screening using ceftazidime disk, which considered as suspected of ESBL-producing *E. coli.*

Out of the 22 *E. coli* isolates β-lactamase producers, 18 (81.8%) exhibited zones enhancement with clavulanic acid, confirming their ESBL production.

In this investigation the Vitek2 compact system was also used for detection of ESBLs-production in 22 *E. coli* isolates. All these isolates were resistant to one or more BLBLI combination antibiotics. 18 (81.8%) of them were found to be ESBL producer.

In this study the Vitek2 compact system detected one isolate (No. 0023) that had positive result for ESBL with susceptible results to new cephalosporins and aztronam (Table 3).

Table (3) Antibiotic susceptibility of ESBL-producing uropathogenic *E. coli* isolates detected by Vitek 2 system

Isolate no.	β-lactamase	CTX	CZ	CAZ	CRO	ATM	IMP	AMC	SAM	TZP
003	+	R	R	R	R	R	S	R	R	I
004	+	R	R	R	R	R	S	I	R	I
005	+	R	R	R	R	R	S	R	R	R
007	+	R	R	R	R	S	S	I	R	S
0011	+	R	R	R	R	R	S	S	R	S
009	+	R	R	R	R	R	S	R	R	S
0010	+	R	R	R	R	R	S	I	R	R
0013	+	R	R	R	R	R	S	R	I	I
0014	+	R	R	R	R	R	S	S	I	S
0015	+	R	R	R	R	R	S	R	R	I
0016	+	R	R	R	R	R	S	S	R	S
0023	+	S	S	S	S	S	S	I	R	S

0024	+	R	R	R	R	R	S	S	R	S
0025	+	R	R	R	R	R	S	I	R	I
0027	+	R	R	R	R	R	S	R	R	R
0028	+	R	R	R	R	R	S	S	I	S
0030	+	R	R	R	R	R	S	S	R	S
0032	+	R	R	R	R	R	S	R	R	R

ESBL extend spectrum Beta- lactams, ATM aztronam, CAZ ceftazidume, CTX cefotaxime, CRO ceftraxone, CZ cefazolin, IMP impenem, AMC amoxillin-clavulanic acid, SAM ampicillin-sulbactam, TZP pepracillin-tazobactam.

Although strains that produce ESBL are characteristically resistant to new cephalosporins and/or aztronam, many strain producing these enzymes susceptible or intermediate to some or all of these agents in vitro, while expressing clinically significant resistance in infected patients [17]. Such strains are often not recognized as ESBLs producer, placing infected patients at risk of receiving an inappropriate therapy, and also making it difficult to implement effective infection control measures. For these reasons a rapid and accurate detection of ESBL-producing isolates has to become an important issue in clinical laboratories. The detection of organisms producing these enzymes can be difficult [18], because the presence of ESBLs in the bacterial cell does not always produces a resistance phenotype [19]. The majority of ESBL are derived through single amino acid substitutions in three non-ESBL parental β-lactamases enzymes, TEM-1, TEM-2 and SHV-1. Since TEM- and SHV-ESBLs had been uniformly susceptible to β-lactamase inhibitors (e.g. clavulante, sulbactam, and tazobactam), inhibitor/ β-lactam combination were advocated as potential therapeutic alternative [20].

Production of Ampc β-Lactamase

Vetik2 compact was applied to detect the production of AmpC β-lactamases in β-lactam resistant *E. coli* isolates. 5 from all 22 isolates showed susceptibility to 3rd generation cephalosporins (ceftazidime, ceftriaxone, cefazolin) and aztronam and 4 of these were recorded as negative for ESBL and one as positive.

This study showed that all 22 uropathogenic *E. coli* isolates were sensitive to cefoxitin and the inhibitor zone was more than 18 µg/ml according to CLSI [8].

Although, some of AmpC types producing Gram-negative bacteria are susceptible to cefoxitin, In general, cefoxitin readily detects hyper-production of AmpC in some *Enterobacteriaceae*. A low level of production yields negative results or marginally positive results. In a previous study, in India, Manchanda and Singh [21], mentioned that 61% of AmpC producers were found to be resistant to cefoxitin and 39% of them were susceptible to cefoxitin antibiotics disk.

The results of Tuwaij [13] revealed that 18 (72%) isolates were cefoxitin resistant while, 9 (28%) and 18 (72%) isolates were confirmed as AmpC producers by rapid iodometric method and nitrocefin disk, respectively.

AmpC β-lactamases are one of the most important β-lactamases in Gram-negative bacteria. Nevertheless, the knowledge about the AmpC β- lactamases is still limited at present. The capability to detect AmpC is important in all hospitals, to improve the clinical management of infections and provide sound epidemiological data. Reduced susceptibility to cefoxitin in the Entero-bacteriaceae may be an indicator of AmpC activity, but it should be confirmed by other tests. The detection of AmpC β-lactamase is a challenge for clinical laboratories, and there is no Clinical Laboratories Standards Institute (CLSI) guideline for its detection [22].

.

AmpC-β-lactamase producing bacterial pathogens may cause a major therapeutic failure if not detected and reported in time. AmpC β-lactamases have been associated with false *in vitro* susceptibility to cephalosporins. Thus, the type of β-lactamase produced by the organism should be detected along with the antibiogram before administering the β-lactam drug to the patient. The potential benefits would include better patient outcomes in terms of avoiding inappropriate therapy. Also failure to identify AmpC β-lactamase producers may lead to inappropriate antimicrobial treatment and may result in increased mortality. This is alarming and requires urgent action from both a therapeutic and infection control perspective [23].

Antibiotics susceptibility of *E. coli*:

Arrange of antibiotics have been used for the treatment of UTI caused by *E. coli* in Iraq and other countries. However, the widely spread use of this approach has criticized on the ground of drug toxicity and the risk of an increase spread antibiotic resistance [24].

Antibiotic susceptibility of all 22 *E. coli* isolates against 20 antibiotics showed multidrug resistance. Bacterial resistance to antibiotic is now widespread and possessed serious clinical threats.

The frequency of antibiotic resistance of 22 *E. coli* isolates that resistant to one or more of BLBL were determined. All these isolates (100%) were found to be resistant to ampicillin and amoxicillin. The susceptibility of 22 *E. coli* isolates against 20 selected antibiotics was studied. The results in figure-1 represent the antibiogram profile of the isolates, and indicate that isolates varied in their susceptibility to the antibiotics. All isolates were highly resistant (100%) to ampicillin, and amoxicillin. Also the results in this figure showed that all 22 isolates were sensitive to cefoxitin (100%). It was found that 81.8% of the isolates were resistant to cephalexin, cephatholin, and aztreonam. The percentages of resistance to third generation cephalosporins were as follows: 77.2% cefazolin, ceftraxone, and ceftazidime. Additionally, 22.7% of the isolates exhibited resistance to the fourth generation cephalosporin, cefepime. The lowest resistance rate was found against carbapenems. Resistance to carbapeneme antibiotics (represented by imipenem, ertapenem, and meropenem) were 0 (0%), also the lowest rate showed in Nitrofurantoin antibiotic was 0 (0%). Low percentages of resistance to aminoglycoside, gentamicin was detected 45.4%. The most active quinolones against all tested *E. coli* was levofloxacin, for which isolates had a resistance rate of 27.2% followed by ciprofloxacin which had a resistant rate of 31.8% and nalidixic acid 63.6%. The resistance rate of isolates to the remaining antibiotics was as follows: tetracycline 68.1% trimethoprim-sulfamethoxazole 77.2%, and tobramycin22.7%.

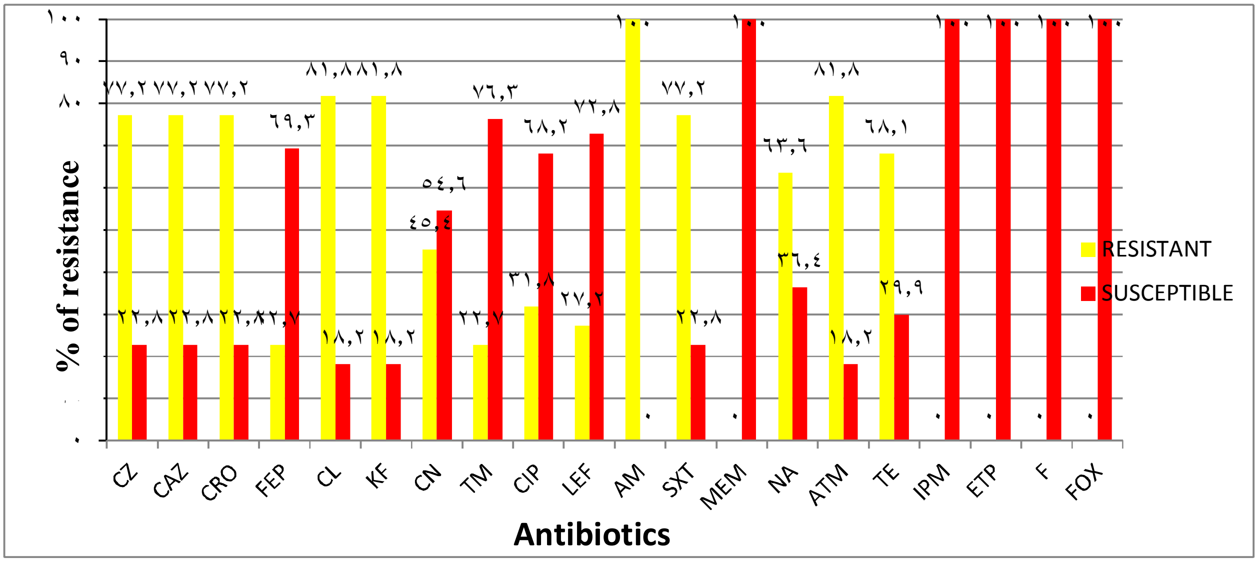

Figure (1): Antibiotics susceptibility profile of β-lactam resistant uropathogenic *E. coli* isolates by disk diffusion method.
CZ cefazolin, CAX ceftazidime, CRO ceftriaxone, FEP cefepeme, CL cephlexin, KF cephatholin, CN gentamicin, TM tobramicin, CIPciprofloxacin, LEF levofloxacin, AM ampicillin, SXT tremethoprin-sulfamethoxazole, MEM meropenem, NA naldix acid, AT Maztronam, TE tetracycline, IPM impenem, ETP ertapenem, F nitrofurantoin.

Suman *et al* [25] have reported that 54% of the isolates were sensitive to gentamicin followed by tobramycin (50%), co-trimoxazole (44%) and ciprofloxacin (44%), whereas in the present study, the uropathogenic *E. coli* isolates were less susceptible to the tested antibiotics.

Supriya *et al* [26] have reported that 82 and 79.6% of *E. coli* were resistant to co-trimoxazole, and ampicillin. Similar results were observed in the present study indicating maximum resistance to these drugs. *E. coli* with integrons are significantly more likely to exhibit MDR to gentamicin, ampicillin, tetracycline and nalidixic acid [27].

In this work, it was found that some of *E. coli* isolates were resistant to more than six antibiotics, which mean that an alternative choice of antibiotic is needed to eradicate *E. coli* associated with urinary tract infection.

E. coli as the commonest cause of UTI exhibiting high antibiotic resistance among the strains, so this sure that the need for judicious use of antibiotics. In chronic UTI, a slow growing *E. coli* with atypical colony morphology and MDR strain was reported by [28].

Penicillins, such as ampicillin and amoxicillin, were used previously as front-line therapies for UTIs. Resistance to these agents is mediated by β-lactamases which degrade them, and these enzymes play an important role in antibiotic-refractory UTIs [29].

The results in the present study were also similar to the results of Aiyegor *et al.* [24] who found that *E. coli* was the principal pathogen isolated from patients with significant bacteriuria, showing high resistance to amoxicillin and ampecillin. Other studies from other countries have reported an increasing resistance in *E. coli* strains to ampecillin [30].

Determination of MIC of IRs-producing isolates:

Results of determination of MIC of IRs-producing *E. coli* isolates revealed that all 22 isolates were highly resistant to ampicillin with concentrations beyond the breakpoint values. The MIC value of ampicillin was 32ug/ml that representing (100%).

The results presented in table 12 evaluate that the MIC of ceftazidime range from 1 to 64 µg/ml; 17% of isolates resistance to ceftazidime, only 5 of isolates had a minimum MIC values 1 µg/ml; 4 of these with negative ESBL and 1 with positive ESBL; The results presented in table -4 evaluate that the MIC of ceftriaxone range from 1 to 64 µg/ml, 77% of isolates resistance to ceftriaxone also had only 5 isolates with a minimum MIC values 1 µg/ml; also evaluate the MIC of cefazolin range from1 to 64 µg/ml which had only 3 isolates with a minimum MIC values 4, 86% of isolates resistance to cefazolin; on other hand the isolates had MIC of ampicillin-sulbactam range from 8 to 32 µg/ml with only one minimum MIC values 8 µg/ml, 86% of isolates resistance to ampicillin-sulbactam; also had MIC of pipracillin-tazobactam range from 4 to 128 with only 4 (18%) maximum MIC values 128 µg/ml; and finally the isolates had MIC for amoxcillin-clavulanis acid range from 4 to 32 only one had minimum values 4 µg/ml, 32% of isolates resistance to amoxcillin-clavulanis.

The results of this study indicated that only 5 isolates in table-4 were resistant or intermediate to one or more of BLBLI combinations and showed susceptible to cephalosporins so these isolates may had one or more of IRs enzyme such as (TEM, SHV, OXA, CTX-M, and AMPC); On other hand the other 17 isolates in this table had positive results for ESBL may had one or more of IRs that mutated to express ESBL such as (TEM-1, TEM-2, SHV-1, and CTX-M).

Table (4) MICs of Inhibitor Resistances of uropathogenic *E. coli* isolates

Isolate no.	AM* (>32ug/ml)	SAM*(>32/16ug/ml)	AMC*(>32/16ug/ml)	TZP*(>128/4 ug/ml)	CAZ* (>64ug/ml)	CRO* (>64ug/ml)	CZ* (>64ug/ml)
002	(-ve) ESBL 32(R)	32(R)	8(S)	4(S)	1(S)	1(S)	4(S)
003	32(R)	32(R)	32(R)	64(I)	16(R)	64(R)	64(R)
004	32(R)	32(R)	16(I)	64(I)	16(R)	64(R)	64(R)
005	32(R)	32(R)	32(R)	128(R)	16(R)	16(R)	64(R)
006	(-ve) ESBL 32(R)	32(R)	8(S)	4(S)	1(S)	1(S)	4(S)
007	3(R)	32(R)	16(I)	4(S)	2(R)	16(R)	64(R)
009	32(R)	32(R)	32(R)	8(S)	16(R)	64(R)	64(R)
0010	32(R)	32(R)	16(I)	128(R)	16(R)	64(R)	64(R)
0011	32(R)	32(R)	4(S)	4(S)	64(R)	64(R)	64(R)
0013	32(R)	32(R)	32(R)	64(I)	16(R)	64(R)	64(R)
0014	32(R)	16(I)	8(S)	4(S)	16(R)	64(R)	64(R)
0015	32(R)	32(R)	32(R)	64(I)	64(R)	64(R)	64(R)
0016	32(R)	32(R)	8(S)	64(I)	16(R)	64(R)	64(R)
0017	(-ve) ESBL 32(R)	32(R)	16(I)	64(I)	1(S)	1(S)	64(R)
0023	(+ve) ESBL 32(R)	32(R)	16(I)	4(S)	1(S)	1(S)	16(R)
0024	32(R)	32(R)	8(S)	4(S)	16(R)	64(R)	64(R)
0025	32(R)	32(R)	16(I)	64(I)	16(R)	64(R)	64(R)
0027	32(R)	32(R)	32(R)	128(R)	16(R)	64(R)	64(R)
0028	32(R)	16(I)	8(S)	4(S)	8(R)	64(R)	64(R)
0029	(-ve) ESBL 32(R)	16(I)	8(S)	4(S)	1(S)	1(S)	4(S)
0030	32(R)	32(R)	8(S)	4(S)	4(R)	64(R)	64(R)
0032	32(R)	32(R)	32(R)	128(R)	4(R)	64(R)	64(R)

AM ampicillin, SAM ampicillin-sulbactam, AMC amoxillin-clavulanic acid, TZP pepracillin-tazobactam, CAZ ceftazidime, CRO ceftriaxone, CZ cefazolin**.**

*Numbers between brackets refer to break points recommended by CLSIs [8]. R resistant, I intermediate, and S susceptible.

Ampicillin AM, ampicillin-sulbactam SAM, amoxicillin-clavulanic acid AMC, pipracillin-tazobactam TZP, ceftazidime CAZ, ceftriaxone CRO, and cefazolin CZ MICs of the 22 E. coli isolates were established according to clinical and laboratory standards institute criteria [8] by a standard agar dilution method on Muller-Hinton medium containing antibiotics and by Vitek2 compact system.

Kaye [15] showed by agar dilution testing, 67 of *E. coli* isolates were non susceptible (39 resistant and 28 intermediate) to amoxicillin-clavulanate and 37 were piperacillin-tazobactam resistant but only 8 were ceftazidime resistant (ceftazidime MIC > 32 _g/ml). Two isolates were susceptible to amoxicillin-clavulanic acid by agar dilution, although they were resistant by disk diffusion testing.

Molecular screening for IR enzymes

PCR technique has been used to screen and detect IR genes carrying plasmid primer. The results are illustrated as follows:

Molecular characterization of TEM-1, TEM-2:

This molecular method was used to detect the most common kinds of IRs; *TEM-1*, and TEM-2. Distribution of IRs genes among uropathogenic *E. coli* isolates is shown in fgure-2and-3. One to two genes for IRs were present in some isolates. In this study 3 (13.6%) isolates had TEM-1 (Figure 2) and 7 (31.8%) isolates had TEM-2 (Figure 3).

In this study, results revealed that high percentage of inhibitor-resistant TEM (IRT) isolates were detected. This result is a first record in Iraq.

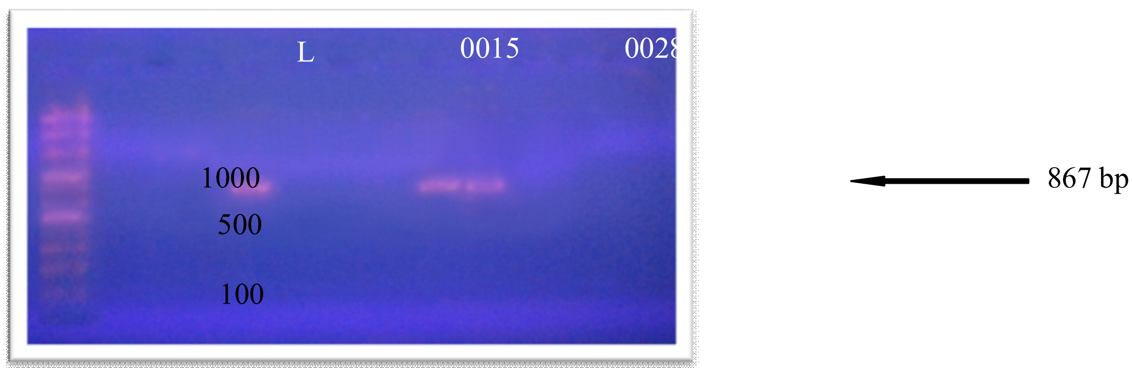

Figure (2) Ethidium bromide-stained agarose (2.1%) gel of PCR amplified products from extracted plasmid DNA of *E. coli* isolates and amplified with primer TEML-1 forward and *TEMU-1* reverse. The electrophoresis was performed at 70 volt for 1.5-2 hr. lane (L), DNA molecular size marker (100bp ladder). Lanes (0015, 0028, and 0029) show positive results with TEM-1 gene (867 bp).

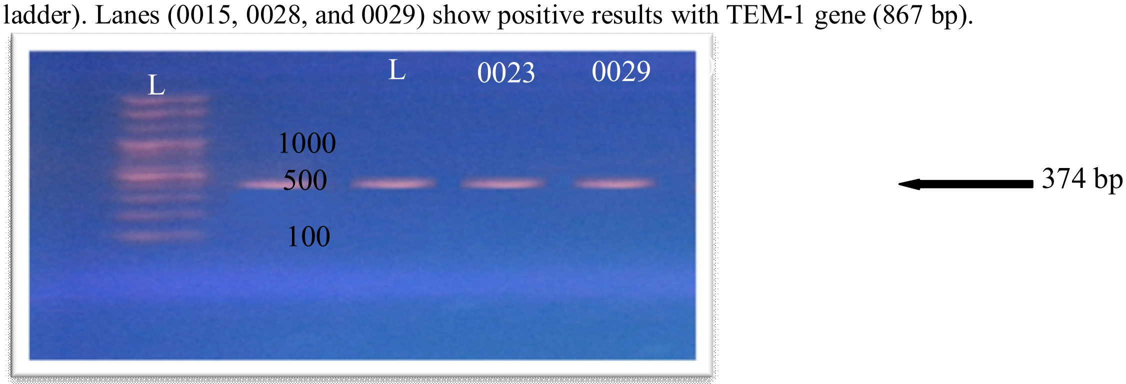

Figure (3) Ethidium bromide-stained agarose (2.1%) gel of PCR amplified products from extracted plasmid DNA of *E. coli* isolates and amplified with primer *TEM*L-2 forward and *TEMU-2* reverse .The electrophoresis was performed at 70 volt for 1.5-2 hr. lane (L),DNA molecular size marker (100bp ladder). Lanes (0023, 0029, 0030, and 0032) show positive results with *TEM*-2 gene (374 bp) .

Kaye *et al.* [15] analysed *E. coli* isolates in the microbiology laboratory of a US tertiary care hospital, From October1998 to December 1999, and revealed that the TEM type alone was found in 52 isolates; the TEM type with CMY-2 were found in 2 isolates. Also, there was one isolate had the TEM type and the SHV type. On other hand found one isolate had two enzyme, the first was the TEM type and the second was unidentified.

Miro *et al.* [14] revealed that out of 7,252 nonduplicated clinical *Escherichia coli* strains from a Spanish hospital showed reduced susceptibility to amoxicillin-clavulanate, 0.8% were probable TEM-1 hyperproducers.

Martín *et al.* [31] found that from a total of 3,556 *E. coli* isolates recovered during the study period, 18 of them producing IRT enzymes (0.5%). These were mostly recovered from urine (77.8%). A high degree of IRT diversity was detected (TEM-30, -32, -33, -34, -36, -37, -40, and -54).

The PCR results show that 74 isolates of *E. coli* (57.8%) had the TEM gene. This study showed that the majority of the ESBL positive clinical isolates of *E. coli* carried the TEM gene.

Fèria *et al* [32] showed that the resistance of uropathogenic *E. coli* isolates from animals to β-lactamase inhibitors was showed in TEM-1 alone (6/ 26) or together with AmpC (4 /26).

Molecular characterization of *bla*-SHV

The study showed that 6 (27.2%) isolates had ***bla*-$_{\text{SHV}}$** (Figure 4) and 3 (13.6%) isolates had SHV-2 (Figure 5).

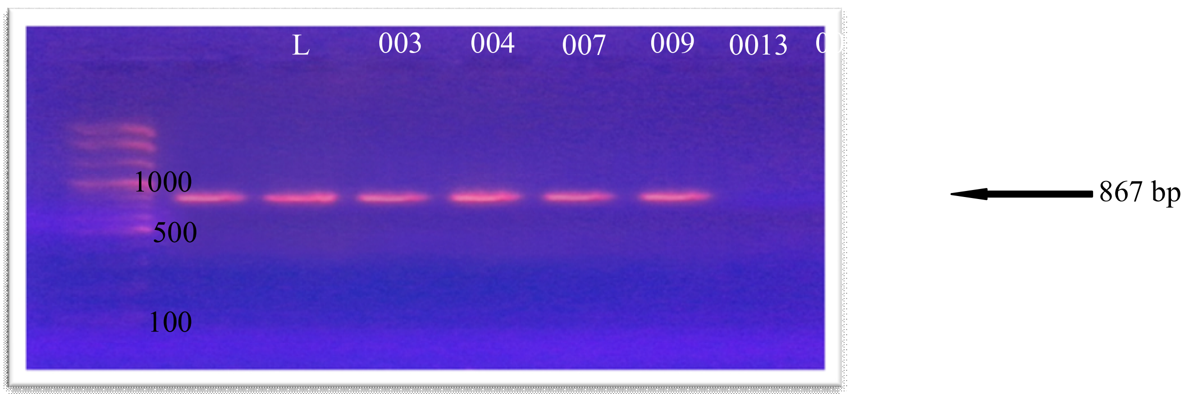

Figure (4) Ethidium bromide-stained agarose gel (0.7%) of PCR amplified products from extracted plasmid DNA of *Serratia* spp. isolates and amplified with primer *bla*-$_{\text{SHV}}$ forward and *bla*-$_{\text{SHV}}$ reverse .The electrophoresis was performed at 70 volt for 1.5-2 hr. lane (L), DNA molecular size marker (100bp ladder). Lanes (003, 004, 007, 009, 0013, 0027,) show positive results with *bla*-$_{\text{SHV}}$ gene (867 bp) .

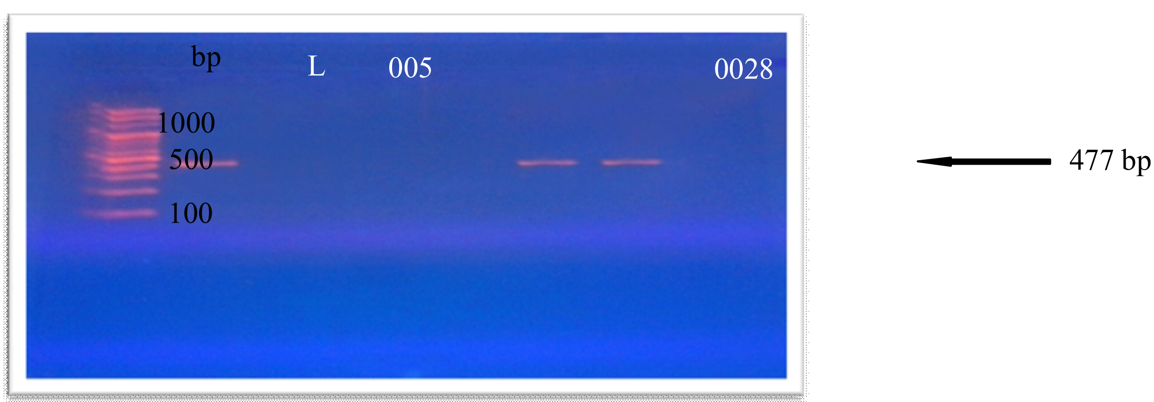

Figure (5) Ethidium bromide-stained agarose gel (2.1%) of PCR amplified products from extracted plasmid DNA of *E. coli* isolates and amplified with primer *SHV-2* forward and *SHV-2* reverse .The electrophoresis was performed at 70 volt for 0.5-1 hr. lane (L), DNA molecular size marker (100bp ladder). Lanes (005, 0028, and 0029) show positive results with *SHV-2* gene (477 bp).

In a study from Spain, Miro *et al* [14] revealed that out of 7,252 nonduplicated clinical *Escherichia coli* strains from a Spanish hospital showed reduced susceptibility to amoxicillin-clavulanate, 0.15% were over expressed SHV-1.

Soltan *et al* [33] showed that PCR was performed for all 128 resistant *E. coli* isolates, and only seven (5.5%) of the strains tested were shown to express bla-SHV.

Fèria *et al.* [32] showed that the resistance to β-lactamase inhibitors uropathogenic *E.coli* isolates from animals in portugal was found to expressed SHV (1/26).

Kaye *et al* [15] studied the molecular epidemiology of amoxicillin-clavulanate-resistant *E. coli* isolated of a US tertiary care hospital and showed that one isolate in the same time had SHV type and TEM type enzyme.

Molecular characterization of OXA-1, OXA-2, and OXA-10:

In this study OXA-1 was detected only in 2 (9%) of the isolates (Figure 6). On the other hand, no isolate (of all tested isolates) showed expression of OXA-2 and OXA-10 genes.

Figure (6) Ethidium bromide-stained agarose gel of PCR amplified products from extracted plasmid DNA of *E. coli* isolates and amplified with primer *OXA-1* forward and *OXA-1* reverse. The electrophoresis was performed at 70 volt for 0.5-1 hr. lane (L), DNA molecular size marker (100 bp laddar). Lanes (0011, and 0029) show positive results with *OXA-1* gene (813 bp).

Miro´ *et al*. [14] found that out of 7,252 non-duplicated clinical *E. coli* strains from a Spanish hospital showed reduced susceptibility to amoxicillin-clavulanate, 0.18% of isolates were produced OXA-30.

Fèria *et al* [32] revealed that the resistance to β-lactamase inhibitors of uropathogenic *E. coli* isolates was mediated by(OXA, TEM, SHV, and AmpC) and the OXA-1 enzymes was found to expressed (2/26).

The molecular epidemiology of amoxicillin-clavulanate-resistant *E. coli* isolated in the microbiology laboratory of a US tertiary care hospital was study by Kaye *et al* [15] and showed that the OXA enzyme type was found in 1 isolate.

Steward *et al.* [34], studied the presence of ESBLs in *K. pneumoniae, K. oxytoca*, and *E. coli* in Spain. using isoelectric focusing (IEF), and they showed that 7 of the 23 isolates contained a β-lactamase with a pI of >8.3 suggestive of an *AmpC*-type β-lactamase; 6 of the 7 isolates were shown by PCR to contain both *bla*-OXA and *ampC*-type genes.

3.10.4. Molecular Characterization of CTX-M enzymes:

In this study CTX-M enzymes were detected in 6 (27.2%) uropathogenic *E. coli* isolates (Figure 7) which had positive results for ESBL and resistance for cefotaxime and ciprofloxacin.

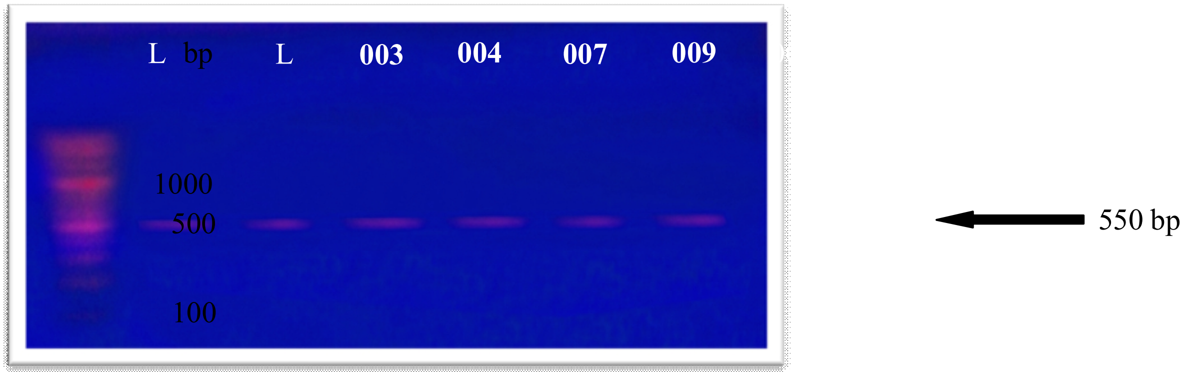

Figure (7) Ethidium bromide-stained agarose gel (0.7mg) of PCR amplified products from extracted plasmid DNA of *E.coli* isolates and amplified with primer *bla*-$_{CTX-M}$ forward and *bla*-$_{CTX-M}$ reverse. The electrophoresis was performed at 70 volt for 1.5-2 hr. lane (L), DNA molecular size marker (100 bp ladder). Lanes (003, 004, 007, 009, 0013, 0027) show positive results with *bla*-$_{CTX-M}$ gene (550 bp).

In study from 28 Russian hospitals, Edelstein *et al.* [35] revealed a total of 904 consecutive nosocomial isolates of *E. coli* and *K. pneumoniae* were screened for production of ESBLs. The ESBL phenotype was detected in 78 (15.8%) *E. coli* and 248 (60.8%) *K. pneumoniae* isolates. 115 isolates carried the genes for CTX-M-type β-lactamases, which, as shown by PCR-RFLP analysis.

A previous study [36] reported higher (43/84, 51%) urinary *E. coli*- ESBL producers harboring both *bla*CTX-M, and *bla*TEM, and the *bla*-SHV gene detected in one of their isolates was not detected in our isolates.

Nimri and Azaizeh [37] out of the 165 isolates, 83 (50.3%) were ESBL-producing isolates, 67 (80.7%) of these had at least one ESBL gene (either *bla*-CTX-M or *bla*-TEM, or both), 16 (19.3%) isolates didn't have any of the three ESBL genes, and *bla*-SHV was not detected in any of the isolates. Out of the 67 isolates 47(70.1%) had either *bla*-CTX-M (28 isolate), or *bla*-TEM gene (19 isolates), while 20 (29.9%) isolates had both *bla*-CTX-M and *bla*-TEM genes.

Khosravi, *et al* [38] revealed that out of 500 tested *Entrobacteriaceae* isolates were identified as *K. pneumoniae* possessing 26 (47.27%) ESBL positives amongst them. Also found that the prevalence of SHV-1, TEM-1 and CTX-M-1 genes among ESBLs-positive isolates was 12 (46.15%), 9 (34.61%), and 7 (26.92%) respectively.

Molecular Characterization of *AmpC*

Only 1 (4.5%) of uropathogenic *E. coli* isolates had AmpC enzyme (Figure8). This isolate was designated as negative for ESBL expression in phenotypic assays (Index-1; 0023, Table 3-6) and found to express the TEM-2gene.

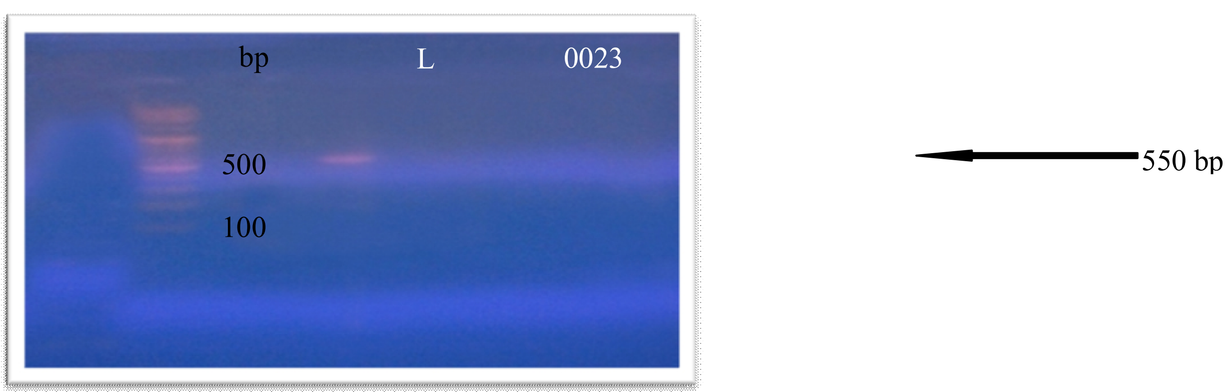

Figure (8): Ethidium bromide-stained agarose (0.7) gel of PCR amplified products from extracted total DNA of *E. coli* isolates and amplified with primer *bla*-$_{AmpC}$ forward and *bla*-$_{AmpC}$ reverse .The electrophoresis was performed at 70 volt for 1.5-2 hr. lane (L), DNA molecular size marker (100 bp laddar). Lane (0023) shows positive result with *bla*-$_{AmpC}$ gene (550 bp).

Coque *et al*. [39] revealed that two isolates of *E. coli* isolates were designated as negative for ESBL expression in phenotypic assays were found to express the TEM gene, possibly due to expression of novel β-lactamase enzymes, such as AmpC. Therefore, the use of molecular methods coupled with phenotypic tests is essential for the definitive identification of these types of β-lactamase enzymes.

Fèria *et al.* [32] showed that the resistant to β-lactamase inhibitors was mediated mainly by (AmpC, OXA, TEM, and SHV) and the AmpC type alone (1/26) or together with TEM-1(4 / 26).

Kaye *et al.* [15] showed that *E. coli* isolates resistant to amoxicillin-clavulanate in a tertiary care hospital was mediated by *(TEM, SHV, OXA,* and *AmpC*), *AmpC* enzyme was found in 4 isolates (2 identified as containing CMY-2).

References

1- Nicolas-Chanoine, M.H. (1997). Inhibitor-resistant β-lactamases. J. Antimicrob. Chemother., 40: 1–3.

2- Canto´n, R., Morosini, M. I., de la Maza, O. M., and de la Pedrosa, E. G. (2008). IRT and CMT β-lactamases and inhibitor resistance. Clin. Microbiol. Infect., 14 (Suppl. 1): 53–62.

3- Chaibi, E.B., Sirot, D., Paul, G., and Labia, R. (1999). Inhibitor-resistant TEM β-lactamases: phenotypic, genetic and biochemical characteristics. J Antimicrob Chemother., 43: 447–458.

4- Thompson C, Amyes S. (1992) TRC-1: emergence of a clavulanic acid-resistant TEM β-lactamase in a clinical strain. FEMS Microbiol Lett., 43: 113–117.

5- Vedel, G., Belaaouaj, A., Gilly, L., et al. (1992). Clinical isolates of Escherichia coli producing TRI β-lactamases: novel TEM enzymes conferring resistance to b-lactamase inhibitors. J Antimicrob Chemother., 30: 449–462.

6- Bla´zquez, J., Baquero, M.R., Canto´n, R., Alo´s, I., and Baquero, F. (1993). Characterization of a new TEM-type β-lactamase resistant to clavulanate, sulbactam, and tazobactam in a clinical isolate of Escherichia coli. Antimicrob Agents Chemother., 37: 2059–2063.

7- Forbes, B.A.; Daniel, F.S. and Alice, S.W. (2007). Baily and Scott's Diagnostic microbiology. 12th ed., Mosby Elsevier Company, USA.

8- Clinical and Laboratory Standards Institute (CLSI). (2014). Performance Standards for Antimicrobial Susceptibility Testing; 24ed. M100-S24. Informational Supplement. PA, USA.

9- National Committee for Clinical Laboratory Standards (2007). Performance standards for antimicrobial disc susceptibility testing. Disc diffusion. 12 ed. Informational supplement. M100-S17. NCCLS, Wayne, Pa.

10- Bush, K., Jacoby, G. A., and Medeiros, A. A. (1995). A functional classification scheme for β-lactamases and its correlation with molecular structure. Antimicrob. Agents Chemother., 39:1211–1233.

11- Al-Charrakh, A.H., Yousif, S.A. and Al-Janabi, H.S. (2011). Occurrence and detection of extended spectrum β-lactamase in *Klebsiella* isolates in Hilla, Iraq. Afr. J. Biotechnol., 10 (4):657-665.

12- Bebrone, C., Moali, C., Mahy, F., Rival, S., Docquier, J.D., Rossolini, G.M., Fastrez, J., Partt, R.F., Frere, J., and Galleni, M. (2001). CENTA as a chromogenic substrate for studying-lactamases. J. Antimicrob. Agents Chemother., 45:1868-1871.

13- Tuwaij, N. S. S. (2014). Phenotypic and Molecular Characterization of β-Lactamase Producing Serratia spp. Isolates in Najaf Hospitals. Ph.D. thesis. College of Science, Kufa University.

14- Miro, E., del Cuerpo, M., Navarro, F., Sabate, M., Mirelis, B., and Prats, G. (1998). Emergence of clinical *Escherichia coli* isolates with decreased susceptibility to ceftazidime and synergic effect with co-amoxiclav due to SHV-1 hyperproduction. J Antimicrob Chemother., 42: 535–538.

15- Kaye, K. S., Gold, H. S., Schwaber, M. J., Venkataraman, L., Qi, Y., De Girolami P., C., Samore, M. H., Anderson, G., Rasheed, J. K., and Tenover, F. C. (2004) Variety of β-Lactamases Produced by

Amoxicillin-Clavulanate-Resistant *Escherichia coli* Isolated in the Northeastern United States. Antimicrob. Agents Chemother., 48 (5): 1520–1525.

16- Leflon-Guibout, V., Speldooren,V., Heym, B., and Nicolas-Chanoine, M. H. (2000). Epidemiological survey of amoxicillin-clavulanate resistance and corresponding molecular mechanisms in Escherichia coli isolates in France: new genetic features of blaTEM genes. Antimicrob. Agents Chemother., 44 (10): 2709–2714.

17- Paterson, D. L., and Bonomo, R. A. (2005). Extented-spectrum beta-lactamases: Clinical Up tade. Clin. Microbial. Rev., 18 (4): 657-986.

18- Tenover, F.C. (1999). Detection and reporting of organisms produsing extended-spectrum beta-lactamases: survey of laboratories in Connecticut. J. Clin. Microbiol., 37: 4065-4070.

19- Meyer, K. S., Urban, C., Eagan, J. A., Berger, B.J. (1993). Nasocomial outbreak of Klebsiella infection resistant to late generation cephalosporins. Ann. Intern. Med., 19:353-358.

20- Jacoby, G. A., and Munoz-price, L. S. (2005). The new β-lactamases. N. Engl. J. Med., 352:380-391.

21- Manchanda V., and Singh N. P. (2003). Occurrence and detection of AmpC β-lactamases among Gram-negative clinical isolates using a modified three-dimentional test at Guru Tegh Bahadur Hospital, Delhi, India. J. Antimicrob. Chemother., 51: 415-418.

22- Nasim, K., Elsayed S., Pitout J. D., Conly, J., Church D.L. and Gregson D.B. (2004). New method for laboratory detection of AmpC-lactamases in *Escherichia coli* and *Klebsiella pneumoniae*. J. Clin. Microbiol., 42:4799-4802.

23- Al-Sehlawi, Z. S. R. (2012). Occurrence and characterization of AmpC β-lactamases in *Klebsiella pneumoniae* isolated from some medical centers in Najaf. Ph.D thesis, College of Science, University of Babylon. Iraq.

24- Aiyegoro, O. A., Igbinosa, O. O., Ogunmwonyi, I. N., Odjadjare, E. E., Igbinosa, O. E., and Okoh, A.I. (2007). Incidence of urinary tract infection among children and adolescents in lle-lfe, Nigeria. Afr. J. Microbiol. Res., pp., 013-019.

25- Suman, E., Jose, S., Varghese, Kotian, S. (2009). Study of biofilm production in *Escherichia coli* causing Urinary tract infection. Indian. J. Med. Microbiol., 25: 305-306.

26- Supriya, S., Tankhiwale., V., Suresh., V., Jalgaonkar., D., Sarfraz, A., Umesh, H. (2004). Evaluation of extended spectrum beta lactamase in urinary isolates. Indian J. Med. Res., 120: 553-556.

27- Elizabeth, M., Malin, G., and Kronvall, G. R. (2004). Integrons and multidrug resistance among Escherichia coli causing community-acquired urinary tract infection in southern India. APMIS., 112: 159–164.

28- Trilzsch, K., Hoffman, K., Christain., N., Schub, Luts, R. (2003). Highly resistant metabolically resistant dwarf mutant of Escherichia coli is the cause of chronic urinary tract infraction. J. Clin. Microbiol., 41: 5689-5694.

29- Schito, G.C., Naber, K.G., Botto, H., Palou, J., Mazzei, T., Gualco, L., and Marchese, A. (2009). The ARESC study: an international survey on the antimicrobial resistance of pathogens involved in uncomplicated urinary tract infections. Int. J. Antimicrob. Agents, 34(5):407-413.

30- Navaneeth, B. V., Belwadi, S., and Suganthi, N. (2002). Urinary tract pathogens resistance to common antibiotics: a retrospective analysis. Trop. Doct., 32: 20-22.

31- Martín, O., Valverde, A., Morosini, M. I., Rodríguez-Domínguez,M., Rodríguez-Ban˜os, M., Coque, T. M., Canto´n, R., and Campo, R. D. (2010). Population Analysis and Epidemiological Features of Inhibitor-Resistant-TEM- β-Lactamase-Producing *Escherichia coli* Isolates from both Community and Hospital Settings in Madrid, Spain. J. Clin. Microbiol., 48(7): 2368.

32- Feria, C., Ferreira, E., Correia, J. D., Goncalves,J., and Canica, M. (2002). Patteerns and mechanisms of resistance to β-lactams and β-lactamase inhibitors in uropathogenic *E. coli* isolated from dogs in Portugal., 49:77-85.

33- Soltan D., M. M., Molla Aghamirzaei, H., Fallah Mehrabadi, J., Rastegar Lari, A., Sabbaghi, A., Eshraghian, M. R., Sharifi Yazdi, M. K., Kalantar, E., and Avadisians, E. (2012). Prevalence of SHV β-lactamases in *Escherichia coli*. Tehran Univ. Med. J., 68 (6): 315-320.

34- Steward, C. D., Rasheed, J. K., Hubert, S. K., Biddle, J. W., Raney, P. M., Anderson, G. J., Williams, P. P., Brittain, K. L., Oliver, A., McGowan, J. E., and Tenover, F. C. (2001). Characterization of clinical isolates of *Klebsiella pneumoniae* from 19 laboratories using the National Committee for Clinical Laboratory Standards extended-spectrum-β-lactamase detection methods. J. Clin. Microbiol., 39:2864–2872.

35- Edelstein M., Pimkin M., Palagin I., Edelstein I., and Stratchounski L. (2003). Prevalence and Molecular Epidemiology of CTX-M Extended-Spectrumβ-Lactamase-Producing Escherichia coli and Klebsiella pneumonia in Russian Hospitals. Antimicrob. Agents Chemother., 47 (12): 3724–3732.

36- Bindayna, K., Khanfar, H. S., Senok, A. C., and Botta, G. A. (2010). Predominance of CTX-M genotype among extended spectrum beta-lactamase isolates in a tertiary hospital in Saudi Arabia. Saudi Med J., 30: 859-863.

37- Nimri, L. F., and Azaizeh, B. A. (2012). First Report of Multidrug-Resistant ESBL-Producing Urinary *Escherichia coli* in Jordan. Brit. Microb. Res. J., 2(2): 71-81.

38- Khosravi, A. D., Hoveizavi, H., and Mehdinejad, M. (2013). Prevalence of *Klebsiella pneumoniae* Encoding Genes for Ctx-M-1, Tem-1 and Shv-1 Extended-Spectrum Beta Lactamases (ESBL) Enzymes in Clinical Specimens. Jundishapur J. Microbiol., 6(10): e8256.

39- Coque, T. M., Baquero, F., and Canton, R. (2008). Increasing prevalence of ESBL-producing Enterobacteriaceae in Europe. Euro. Surveillance, 13 (47): 1-40.

40- Reguera, J. A., Baquero, F., Pe´rez-De´az, J. C., and Martınez, J. L. (1991). Factors determining resistance to β-lactam combined with β-lactamase inhibitors in *Escherichia coli*. J. Antimicrob. Chemother., 27: 569–575.

41- Ferreira, C. M., Ferreira, W. A., Cristina, N., Almeida, O., Naveca, F. G., das Graças, M., and Barbosa, V. (2011). Extended spectrum beta-lactamase-producing bacteria isolated from hematologic patients in Manaus, State of Amzonas, Brazil. Braz. J. Microbiol., 42: 1076-1084.

42- Messai, Y., Benhassine T., Naim M., Paul, G., and Bakour, R. (2006). Prevalence of β-lactams resistance among *Escherichia coli* clinical isolates from a hospital in Algiers. Rev. Esp. Quimioterap., 19 (2): 144-151.