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Distribution of CTX-M gene among *Escherichia coli* strains isolated from different clinical samples in Erbil City

Fattma A. Ali

College of Health Sciences-Hawler Medical University- Erbil -Iraq.

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Abstract: *Escherichia coli frequently causing urinary tract, wound and blood infection resulting in significant morbidity and mortality due to had plasmid encoded ESBLs lead to failure treatment* .Tow hundred samples were collected from (urine, wound, sputum), *Escherichia coli*. isolated and identified by using microscopical, morphological, biochemical tests and Vitek 2 compact system. Antibiotic susceptibility testing was screening according to the CLSI guideline and Vitek 2 compact system. Phenotypic screening of ESBLs was undertaken using (Double disk diffusion and Standard disk diffusion) Methods, also PCR technique was used for genotypic detection of ESBL genes (blaCTX-M) according to the standard protocol. We obtained in this study 60 (29.33%) total positive results of *Escherichia coli*.40 (20%) isolates for *Escherichia coli* isolated from urine, 15 (7.5%) wound,5 (2.5%) sputum,from patient attending e Rizgary hospital fromSemptemper 2015 to March 2016.Susceptibility profile has been done for all *Escherichia coli* isolated by using 13 antimicrobial agent,Our multifinding pointed out that highest resistance be Ceftriaxone 45(75%) Ceftazidime 44(73.3) Tobromycin 39(65%) Piperacillin 39(65%),most of *Escherichia coli* isolates were resistance to more than three antibiotics belonging to different classes used and these were considered to be multidrug resistant (MDR) isolates. The incidence rate of ESBL-producing *Escherichia coli* was 48 (80%) by Standard disk diffusion Method,46 (76.7%) by Double disk diffusion and 38(66.7%) of ESBL producer in urine samples. Remarkably, dissemination of blaCTX-M 44 (73.3%) genes among ESBLs-positive isolates and the length of amplified genes (550) bp for blaCTX-M genes. It can be said that the incidence rate of *Escherichia coli* carrying genes encoding for ESBL enzyme representing their commonness in our institute and multi resistance to many classes of antibiotic, resulting in limited treatment options.

Keywords: Escherichia coli, extend spectrum β-lactamase, bla CTX-M gene, PCR.

Corresponding author: should be addressed (Email: fattmaabeer@yahoo.com).

Introduction:

E*scherichia coli* is one of the most important pathogenic bacteria that share the events of microbial contamination and cause about 90% of the urinary tract infection (UTI) and recurrent UTI, particularly in women. However, the importance of this pathogen comes from its ability to elaborate a wide spectrum of virulence factors. *Escherichia coli* comprises a wide population of phenotypically and genetically highly variable organisms (1). The emergence of ESBL, as an important cause of transferable multidrug resistance in gram-negative bacteria, particularly in *E. coli* is a global health problem since 1995. ESBLs are a heterogeneous group of enzymes that confer resistance to 3 and 4 generation of cephalosporins and monobactams,ESBLs are grouped into four classes A, B, C and D on the basis of their amino acid sequences5. In comparison of other classes, classes A and C are the most common classes. Class A ESBLs hydrolyzes oxyimino-cephalosporins and aztreonam and generally susceptible to clavulanate,

sulbactam, tazobactam as beta lactamase inhibitors (2).

The major types of ESBLs are TEM, SHV and CTX-M and most of these enzymes evolved by point mutations around the active site of native β-lactamases, particularly TEM-1, TEM-2, and SHV-1 (3), In fact, the majority of the enterobacteria infections caused by ESBL producing organisms are *E. coli* and *K. pneumoniae* strains carrying the CTX-M gene responsible for community infections, especially the urinary tract infections, CTX-M-type ESBLs have recently become predominant among ESBL subtypes (4). Since the first report of CTX-M ESBL from a strain of *E. coli* in 1989, the CTX-M family of ESBLs has spread in many countries, and the global spread of CTX-M - producing *Enterobacteriaceae* is a major concern(5). Among the different CTX-M subtypes, those of the CTX-M-1 and CTX-M-9 groups were the most common in Asia as well as worldwide(6), the nature of ESBL dissemination has changed: *E. coli* is now the most frequently isolated ESBL-carrying bacterium,and CTX-Ms have become the most frequently isolated ESBLs. Moreover, in the last few years CTX-M–type ESBLs have emerged within the community, particularly among *E. coli* isolated from UTIs(7).

The aim of this study was to identify *Escherichia coli* isolates harboring gene encoding for ESBL enzyme and multiresistance antibiotics such as *bla CTX-M.*

Materials and Methods:

This study was conducted in the Rizgary hospital in Erbil province at a period from (September 2015 – March 2016).

A total of 200 sample were collected from different clinical specimens (sputum, urine, wound) from patient attending public hospitals with age group up to70 years.

Isolation of microorganism:

The specimen was inoculated on Blood culture and MacConkey agar plates and were incubated aerobically at 37°C for (24-48) hours, were identified using Vitek 2 system (8)**.**

Antimicrobial susceptibility test by Vitek 2 system:

With its ability to provide accurate "fingerprint" recognition of bacterial resistance mechanisms and phenotypes, the AES is a critical component of Vitek 2 technology.The Vitek 2 card contains 64 microwells. Each well contains identification substrates or antimicrobial. Vitek 2 offers a comprehensive menu for the identification and antibiotic susceptibility testing of organisms (9). The Vitek 2 test card is sealed, which minimizes aerosols, spills, and personal contamination. Disposable waste is reduced by more than 80% over microtiter methods.

Phenotypic detection of ESBL enzyme:

All bacterial species were screened for ESBL enzyme production by the following methods:

Screening test for ESBL (Standard disk diffusion method):

ESBL detection was carried out by standard disk diffusion methods for all Gram negative isolates according to the Standard Institute of Antimicrobial Susceptibility Testing recommendation

(10) by using various antimicrobials. ESBL positive meant the organism shows comparatively high level co-resistance to third generation cephalosporin such as; Ceftazidime zone ≤ 22 mm, Aztreonam zone ≤ 27 mm, Cefotaxime zone ≤ 27 mm or Ceftriaxone zone ≤ 25 mm. A laboratory strain of *Escherichia coli* ATCC (13883) was used as a control.

Confirmatory test for detection of ESBL by double disc diffusion test:

A double disc diffusion test was performed with amoxicillinclavulanic acid surrounded by aztreonam and third generation cephalosporin discs cefotaxime and ceftazidime, the standard inhibition zone of amoxicillin-clavulanic acid, ceftazidime, cefotaxime and aztreonam as in table (1) (11).

Table (1): The standard inhibition zone of amoxicillin- clavulanic acid, ceftazidime, cefotaxime and aztreonam

Antibiotic discs	Concentration µg/ml	Resistance mm	Intermediate mm	Susceptibility mm
Amoxicillin-clavulanic acid (AMC)	20-10 (30)	≤ 13	14-17	≥ 18
Ceftazidime (CAZ)	30	≤ 14	15-17	≥ 18
Cefotaxime (CTX)	30	≤ 14	15-22	≥ 23
Aztreonam (ATZ)	30	≤ 15	16-21	≥ 22

Isolation of plasmid from bacterial cell:

The method that used for isolation of plasmid from *E.coli* was performed by using Prime Prep™ plasmid DNA isolation kit.

All bacterial plasmids were run on 1% agaros gel for detection of their pattern. Fifteen µl of extracted plasmid was mixed with 3 µl of loading dye (6 ×) and the mixture was loaded in to prepared agaros gel and the gel ran for 3 min at a voltage of 1 to 5 volts/cm^2 of the gel size. The results were read using ultra violet light in Gel Documentation System.

Polymerase Chain Reaction (PCR):

PCR reaction mixtures were prepared in duplicates; negative controls were included in each run to validated the reaction. Each reaction mixture was prepared to a volume of 50 µl in a sterile PCR tube.

lyophilized primers provided by Cinnage® were processed in order to product a stock concentration of 100 µM by mixing the concentrated Lyophilized primer with specific volumes (458.01, 528.40, 502.93, 568.43) of nuclease free de-ionized water according to the primer manufactures and concentration table (1). From this stock concentration a working primer concentration of 10 µM was prepared.

All isolated *E.coli* were selected for detecting multi resistant gene namely (*bla* CTX-M).

In standard PCR, the reaction was prepared for two sets of primer separately worked cause of difference in annealing temperature was prepared by mixing the reverse with the forward of primer and addition to other component of the PCR reaction. PCR amplification conditions was modified according to the annealing temperature of the primer; while for denaturation and extension steps table (1).

Table (2): primers used in the study

Primer Name	primer Sequence	Ann. Temp.	Target Gene	Amplicon in
CTX-M F	GGAAGTGTGCCGCTGTATGCGC	55°C	*CTX-M*	550-bp
CTX-M R	CCGCCGACGCTAATACATCGCG			

The PCR protocol was ad-opted as follows: one single cycle of denaturation step for 10 minutes at 98°C followed by 32 repeating cycles, each consisting of a denaturation (30 seconds at 94°C), annealing (30 seconds at 54°C) and extension (1 min at 72°C), followed by a final extension at 72°C for 10 minutes. PCR ampilicons were visualized on 2% agarose gel using 1X TBE buffer which contains 0.5 µg/mL of ethedium promide and finally the PCR products were photographed under UV lamp (SYNGENE, UK) and bands sizes were compared to a 1000 bp DNA ladder. *E. coli* ATCC 25922 and were used as positive control (ESBL producers) in this project.

Agaros gel preparation:

The agaros gel was prepared according to what was performed by Ozer *et al.* (12).

- To prepare 1% agarose gel, 1 g of agarose powder was mixed with 1× TBE (Tris base-boric acid – EDTA) buffer to reach a final volume of 100 ml, the mixture was thoroughly mixed by swirling the flask.
- The mixture was melted in a microwave oven for about 2 minute until the mixture become clear; and after the mixture had been cooled to a temperature of appox 55°C. 3 µl of 10 mg/ml of the intercalating agent Ethidium Bromide was added and the suspension mixed thoroughly by gentle swirling.
- The agaros mixture was poured into the assembled gel support (10 × 20 cm); a comb was inserted and gel was allowed to set completely and solidify at the room temperature.
- The gel cast was placed on its support into the electrophoresis running apparatus and the tank was filled sufficient electrophoresis running buffer (1× TBE) to cover the gel completely, then the comb was removed.
- The DNA (PCR product) was mixed with 3 µl of loading dye and the samples were loaded in separate wells including a DNA size marker in one of the lane.
- The lid of the tank was closed, electrophoresis separation was started by running the electric current at a voltage of 1 to 5 volts/cm^2; bubbles arising from the anode pole and dye migration indicate the gel is in separation.
- After complete migration of markers to the other end, the gel was removed and visualized under UV light trans illuminator, the gel was photographed and documented.

Ethics approval:

The study was approved by the local ethics committee of Hawler Medical University.

Analysis:

Data were analyzed using the SPSS statistical software package, Version 16. Chi-square test was used to recognize differences within the data. All *p*-values < 0.05 were considered as statistically significant.

Results:

In present study a total of 200 samples collected from Rizgary hospital in Erbil. Only 60 (30%) obtained and isolated were identified as *Escherichia coli*. The results of this study indicate that the high percentage of *Escherichia coli* distribution in urine 40 (20) followed by 15 (7.5%) wound,5 (2.5%) sputum as in table (3).

Table (3): Distribution of *Escherichia coli* in different clinical specimens

Isolated Bacteria	*Clinical samples*			Total
	Urine No (%)	Sputum No (%)	Wound No (%)	
Escherichia coli	40 (20%)	5 (2.5%)	15 (7.5%)	60 (30%)
Total	120 (60%)	20 (10%)	60 (30%)	200 (100%)

Antimicrobial susceptibility testing for *Escherichia coli* :

In present study as shown in table (4) for *Escherichia coli* the most sensitive antibiotics were Imipenem 50(83.33%) followed by Meropenem 49(81.66),Etrapenem 48(80%) Amikacin 48 (80%)and Ciprofloxacin 48 (80%) while highest level of resistance in the current study was observed to be Ceftriaxone 45(75%) Ceftazidime 44(73.3%) Tobromycin 39(65%) Piperacillin 39(65%).

Table (4): Antimicrobial susceptibility tests for *Escherichia coli*

Antibiotics			*Escherichia coli*		
			R	I	S
Ciprofloxacin	CIP 10	No	10	2	48
		%	16.66	3.33	80
Amikacin	AK 10	No	11	1	48
		%	18.33	1.67	80
Levofloxacin	DO 10	No	43	10	8
		%	71.7	16	13.3
Ceftriaxone	CRO 10	No	45	1	14
		%	75	1.67	23.33
Meropenem	MPM 30	No	5	6	49
		%	8.3	10	81.66
Imipenem	IPM 10	No	3	7	50
		%	5	11.66	83.33
Ceftazidime	CAZ 10	No	44	5	11
		%	73.33	8.3	18.33
Gentamicin	CN 10	No	15	9	36
		%	25	15	60
Piperacillin	PRL 30	No	39	9	36
		%	65	15	60
Aztreonam	AX 25	No	15	10	35
		%	25	16.66	58.33
Etrapenem	EPM 10	No	1	10	48
		%	1.66	16.66	80
Tobromycin	TbM 30	No	39	0	21
		%	65	0	35
Ampicillin -Sulbacam	AMP+SbM10	No	10	9	41
		%	16.66	15	68.33

Table (5): Phenotypic detection of ESBL producing by *Klebsiella spp.* isolated from different clinical specimens

Isolated bacteria	ESBL production			
	Standard disk diffusion (Screen test)		Double disk diffusion (DDST)	
	Positive No.(%)	Negative No.(%)	Positive No.(%)	Negative No.(%)
Escherichia coli	48 (80%)	12(20%)	46 (76.7%)	14 (23.4%)

Phenotypic detection of ESBL producing by *Klebsiella spp.* isolated from different clinical specimens:

In current study a total of 60 isolates of *Escherichia coli* were screened for Extended Spectrum β-Lactamases (ESBL) enzyme production by using two method standard disk diffusion method as in figure (1) and double disk diffusion method as in figure (2) and the result showed there was difference in the detection of ESBL between screening test and confirmatory method in the aspect that lower prevalence rate of ESBLwas recorded by double disk synergy test46 (76.7%%) compared to standard disk diffusion test 48 (80%) , the results reflected significantly high rate of resistance among ESBL isolates compared with non-ESBL producers ($P < 0.05$) as shown in Table (5).

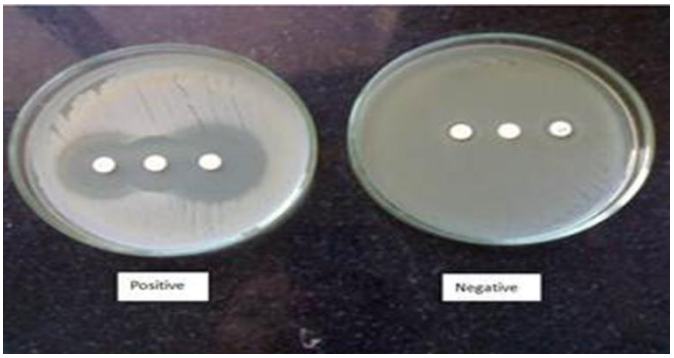
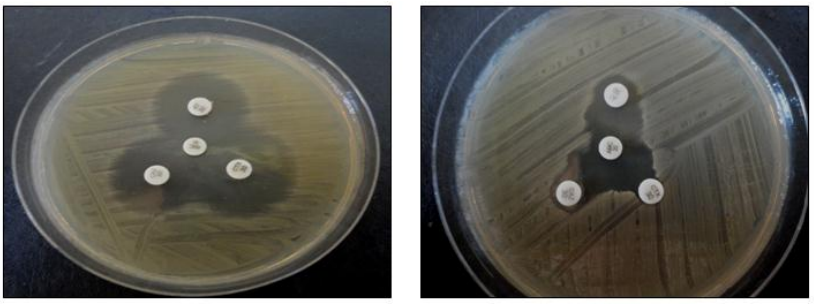


Figure (1): Stander disk diffusion method for ESBL production *by Escherichia coli.*



(A) **(B)**

Figure (2): Double- disk diffusion test used for the detection of ESBL production (A) ESBL negative (B) ESBL positive

Extended spectrum β-lact-amases producers (ESBLs) were more frequent in urine specimens (61.7%), followed by wound (25%), sputum (8.33%), as illustrated in table (6).

Table (6): Frequency of *ESBL producers* in *E.coli* isolated from different clinical specimens by using standard disk diffusion

Isolated Bacteria	ESBL producers			Total
	Urine No (%)	Sputum No (%)	Wound No (%)	No (%)
Escherichia coli	38 (66.7%)	2 (8.33%)	8 (25%)	48 (80%)
Total	40	5	15	60(100%)

Molecular Detection of ESBL enzyme in *E.coli* :

Detection of *blaCTX-M* :

PCR reaction were performed for all studies bacteria, PCR detection of ESBL genes yielded many positive results. Obtaining the expected size of amplicon was considered as in indicator for the gene presence. These amplicon sizes were consistent and the same results were obtained when PCR reported on the same sample. In our study 60 *Escherichia coli* plasmid were run on gel electrophoresis for detecting plasmid pattern PCR reaction for detecting *bla*CTX-M gene. Out of 60 *Escherichia coli, blaCTX-M genes was recorded in 44* (73.4%) samples and 16 (26.7%) samples were negative result as in table (7) with lengths of amplified gene was (550) bp as in figure (3).

Table (7): Frequency of *blaCTX-M gene* in *E.coli* isolated from different clinical specimens

Isolated Bacteria	PCR result			Total
	Urine No (%)	Sputum No (%)	Wound No (%)	No %
Escherichia coli	36 (60%)	2 (3.33%)	6 (10%)	44 (73.3%)
Total No %	40(66.7%)	5(8.3%)	15(25%)	60(100%)

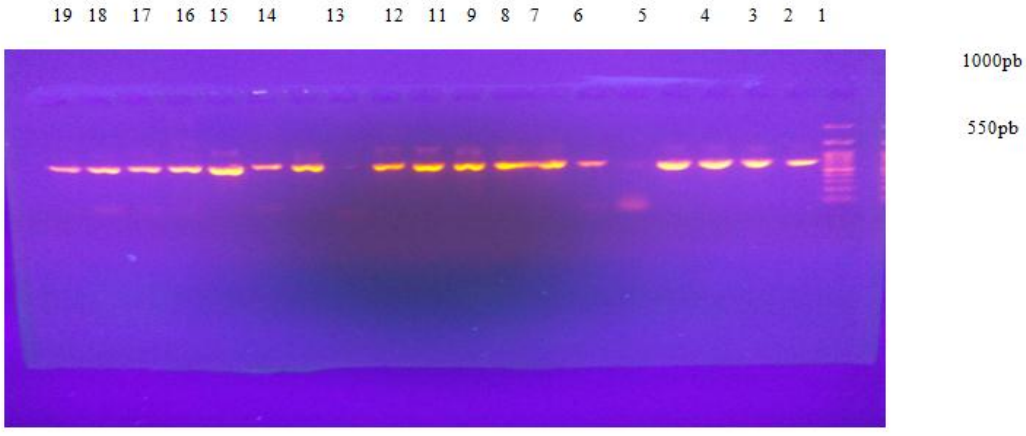


Figure (3): PCR product for *bla* CTX-M gene (550) bp resolved using 1% agarose gel electrophoresis.

L: Ladder (1500bp), +ve: positive control *E.coli* ATCC, Lane1,2,,4 , 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 17, 18, 19: amplified PCR product of *blaCTX-M* gene (550) bp for *E.coli* and Lane 5,Lane 13:*E.coli* negative for *blaCTX-M* gene

Discussion:

The result of this study indicate that the high percentage of *E. coli* was 60(30%%) distribution between40 (20%) urine, 16 (7.5%) wound,8 (2.5%) sputum. These results were in agreement with the results obtained from other studies conducted worldwide which approved that *E. coli* is the major pathogen that cause UTIs This result comparable to a study conducted by Polse *et al*. (13) from Zakho, Iraq they found that the higher percentage of *E. coli* isolates106 (51.7%) *E. coli* isolates among the 205 isolates, *E. coli* is a major normal flora in the gut and most of the times poor hygiene will lead to cross contamination and then urinary tract infections (14).

These result were in agreement with finding of Al-Jebouri1 and Mdish (15) from Tikrit, Iraq who found *E. coli* was the most prevalent in urine samples(31%) but not concordance with a study by Banadkar *et al.*(16) from India reported prevalence rate of *E. coli* was 37.5% in post-operative wound infections.

The results in this study showed that lawest percentage of *E.coli in* sputum samples lower than results recorded by other researcher who founded Pneumonia in 16.2% (37/228) of *E. coli* (17) .The presence of this bacteria in large present in UTI might be attributed to the fact that these bacteria are often part of the resident flora and different virulence factors contributing to their pathogenicity and the difference in the result with others might be attributed to the number of taken sample size and the difference in the time of the study.

Antimicrobial resistance in *E. coli* is of major concern worldwide due to its increasing resistance to several commonly prescribed antibiotics. In our study, *E. coli* isolates were various in their susceptibility to different antibiotics belonging to different groups ,the most sensitive antibiotics were Imipenem followed by Meropenem, Etrapenem, Amikacin and Ciprofloxacin This results was comparable to different locally and worldwide studies and these result relatively similar with the founding Polse *et al*. (13).

From our country from Zakho, Iraq in which reported tha all isolates were 100% resistant to penicillin, ampicillin and aztreonam, whereas, all isolates were 100% susceptible to imipenem and meropenem (18). The carbapenems (imipenem and meropenem) are known to be stable against ESBL enzymes and effective in the treatment of infections caused by ESBL - producing bacteria(19).

The result showed in table (4) high rates of resistance in *E .coli* to antibiotics highest level of resistance in the current study was observed to Ceftriaxone 45(75%) Ceftazidime 44(73.3) Tobromycin 39(65%) Piperacillin 39(65%) while Al-Jebouri1and Mdish (15) from Tikrit, Iraq reported that overall percentages of resistance of all isolates to the antimicrobial agents were 90.8% to amoxycillin, 66.5% to ampicillin, , 46.5% to gentamicin, , 42.8% levofloxacin, 42.2% to cefoxitin, 45% to nitro-furantoin, 35% to clorompheni‌col and 4.3% to amikacin, 19.3% to ciprofloxacin, The present study showed a high elevation in antibiotic resistance of pathogens isolated from Iraqi patients compared to previous years. This might be due to misuse of antibiotics,usage of

antibiotics from unknown origin, from uncontrol source of production, utilizing of inactivated antimicrobials, selective pressure of antibiotics and lacking of quality control on some sources of antibiotics entering Iraq.

Somily *et al.* (20) reported similar susceptibility rates among *E. coli* and *K. pneumonia*e isolates from a tertiary care hospital at Riyadh, Kingdom of Saudi Arabia. However, susceptibility to piperacillin and ciprofloxacin were lower in our study compared with isolates from a hospital at Dammam, Kingdom of Saudi Arabia (21). Furthermore, in Sudan, *E. coli* were highly resistant to trimethoprim/ sulfamethoxazole and ciprofloxacin but less resistant to amoxicillin/clavulanic acid compared with the present study, but similar susceptibility rates were observed to amikacin and gentamicin (22). The resistance observed with etrapenem and imipenem compared with merepenem could possibly result from carbapenemase production and/or resistance owing to the loss of porins and/or hyper-production of AmpC.

The result showed that 48 (80%) ESBL producer, while 12 (205%) total non ESBL producer of *E. coli* by Standard disk diffusion while by Double disk diffusion method 46 (76.7%) ESBL producers, 14 (23%) non ESBL producer similar to that recorded in Wasit/ Iraq by Al-Mayahie(22) phenotypically, 80.2% and 64.8% of the isolates were ESBL producers by screen and confirmatory tests, respectively.

In the Middle East area, notable differences regarding ESBL-producing by *E. coli* prevalence were apparent. For example, in Saudi Arabia (24), 20.3% of ESBL-produciyng by *E. coli* isolates from hospitalized patients . Reports from Egypt, Iran, and Turkey revealed that the distribution ESBL-producing *E. coli* was 78.8% (25), 56% (26), and 36.7% (27), While, the prevalence rate of ESBL-producing *E. coli* was 65.5% from vaginal *E. coli* isolates form pregnant and non-pregnant women living in Al-Kut, Wasit province, Iraq (29). Such geographical differences in the rates of ESBL production from country to country and even within countries from hospital-to-hospital were reviewed These geographical variations may be affected by local practices of antibiotic use in humans and animal husbandry. Therefore, the prevalence of ESBLs differs among patient groups and clinical and geographic settings (28).

The result of current study showed that highest percentage of ESBL producer in urine samples38(66.7%),these result were in agreement with finding of Abdul-Aziz shows that ESBL producing by *E. coli* uropathogen isolates was high 61.5 % (30).

Similarly, high percentages of ESBL-producing *E. coli* from outpatients with recurrent UTIs were reported from Spain (31).

However, the rate was within the range reported globall. The ESBL producing *E. coli* uropathogen frequency varied regionally. In USA, New York, Spandafino *et al* .(32) found ESBL producing *E. coli* rate of 12.5%.

In our country does not have any systematic program for studying the antibiotic resistance pattern. Proper use of antibiotics is ensured by formulating an antibiotic policy. ESBL screening as a routine test has not yet been practiced in Iraq. ESBL occurs at an alarming rate among Enterobactericeae isolates among the hospitalized patients which can result in an outbreak in the community that may be difficult to treat.

An interested finding in this part of the study there was an association of positive culture of *E.coli* with number of antibiotic resistant and with ESBL production. Therefor in current study found that most isolates multi resistance to more than 5 antibiotic (55.68%) and most of isolates ESBL producers. The majority of ESBL positive isolates showed high resistance to most of the tested antibiotics with highest rate of resistance to Amoxicillin and also Cefoxitin, Cefuroxime and Ceftazidime.

In the current study, our result revealed that the prevalence of ESBL genes *bla CTX-M gene* by using PCR was *44* (73.4%),all *E. coli* isolates were successfully amplified a single band of the CTX-M as the species specific locus in all strains with a molecular weight of about 550 bp On the other hands, In this content, it was clear that the gene are present especially in urine sample. . In similar study that done in Zakho Iraq the prevalence and molecular characterization of ESBLs genes; including *bla CTX-M i*t has been found that (159/169) isolates carried CTX-M type enzymes accounting (94.1%) (13).

Based on the PCR results, all *E. coli* isolates possessed one or more ESBL gene, CTX-M type ESBL was the most dominant ESBL (87.2%) among the isolates in Duhok, Iraq(33). This result indicates that this genes was present in Kurdistan Iraq and is regarded as a source of international disseminations and may be this MDR gene is transmitted to our locality from tourists of other neighboring countries such as Iran, Turkey and south and middle of Iraq and transmission mostly occurs by plasmid caring genes encoding for ESBL enzyme.

Indeed, CTX-M type represents the most rapidly enzymes spreading among *Enterobacteriaceae* worldwide and nowadays it is the most prevalent ESBLs in many parts of the world particularly increased in *E. coli* isolated from both community and nosocomial settings compared to TEM and SHV types (34). Two reports from Turkey have shown that the CTX-M enzyme is common among ESBL positive isolates accounting (76.5%) (35). Mahboobeh *et al.,* (36) in Iran found that CTXM type β-lactamases are widespread in the studied community (96.3%) .In Spain and some regions of Asia, CTX-M-9 is the predominant group (37). In China, the presence of CTX-M-Producing *E. coli,* mainly the group CTX-M-9, in urinary tract infections was documented (38).In South America, the group CTX-M has been found to reach endemic proportion (39). In Colombia, there are reports of CTX-M-1 (40) .In a local study of our countery done by Auda (41), the result revealed that the most common ESBL in *E. coli* were bla CTX-M .

The presence of ESBL genes *E.coli* indicates that there might be a hidden ESBL gene among isolated strains which cannot be diagnosed by classical antibiotic susceptibility tests leading to the dissemination of these genes in the hospital silently among patients even with in normal health workers who act as carriers for ESBL genes in future.

Regarding these isolates of *E.coli.* that give negative results by PCR reaction for ESBL genes, the causes might be due to the presence of other genes of ESBL that are responsible for ESBL producer such as *bla* (SHV**,** OXA**,** AmpC)

In conclusion, ESBL *E. coli* local isolates are found to exist relatively at a high level among clinical isolates derived from UTI patients.

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