Incidence of extended-spectrum beta-lactamases in uropathogenic *Escherichia coli*

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Abstract: In the present study, a total of 259*E.coli* isolates were recovered from patients suffering from urinary tract infections. Resistance to ceftazidime were screened by using agar dilution technique (breakpoint method) which revealed that 132 of the isolates were ceftazidime resistant. Susceptibility testing of these *E.coli* isolates to other 16 beta-lactams by disc diffusion technique reveled that 100% of isolates were resistant to pipracillin (PRL), penicillin G (P). and cefazolin (CZ). In contrast, only 3(2.27%) isolates were resistant to imipenem. On the other hand, the resistance to other drugs was ranged from 12.12% to 84.09%. MICs for 3rd and 4th generation cephalosporins (Ceftazidime, cefotaxime, ceftriaxone and cefipime) were determined by agar dilution technique, it was found that MICs of ceftazidime ranged from 16to 1024(µg/ml), cefotaxime ranged from 1 to 1024 (µg/ml), ceftriaxone ranged from 1 to 1024 (µg/ml) and cefipime ranged from 2 to 128 (µg/ml). A total of 106(80.30%), 72(54.54%), 82(62.12%) and 48(36.36%) of tested isolates had high level of resistance (≥64 µg/ml), (≥128 µg/ml), (≥128 µg/ml) and (≥64 µg/ml) to ceftazidime of, cefotaxime, ceftriaxone and cefepime respectively. β-lactamase (s) production was investigated by iodometric overlay test, it was found that 110 (83.3%) of tested isolates were β-lactamase (s) producers. This indicated that other mechanisms were involved in the resistance to the tested β-lactams among the remaining 22 (16.6%) isolates. ESBLs activitywas observed phenotypically in 79 out of 110 (71.81%) by using double disc synergy test and combined disc method. The data obtained from multiplex PCR showed that only 22 out of phenotypically ESBL positive isolates harbored one or two of typical ESBLs genes, which21.51% were *bla* TEM, 2.53% *were bla* SHV and 3.79% were *bla* CTX-M. The variation in results which in tested isolates were positive for ESBL in the phenotypic test but were negative (57 isolates) in the PCR can be explained firstly according to the types of primers used, secondly by the probable presence of another variants of ESBLs from the TEM such as TEM-1, SHV such as SHV-1 and CTX-M or other ESBL families (cephalosporinases1 and/or carpabenemases) were involved which are not regarded as extended spectrum β-lactamases, presence of these enzymes lead to reduced susceptibility to third-generation cephalosporins, that phenotypically ESBL production.
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Keywords: UTI; ESBL; *Escherichia coli*; CTX-M; TEM; SHV genes.

1. Introduction:

Urinary tract infections (UTI) are the most prevalent infections worldwide, mostly caused by *Escherichia coli***.** Accounting for more than 70% of uncomplicated cases both in outpatients and inpatients **(Gupta *et al*., 2001).** Clinically, UTIs are categorized as uncomplicated, complicated and recurrent infection. Uncomplicated UTIs typically affect individuals who are otherwise healthy and have no structural or neurological urinary tract abnormalities. These infections are differentiated into lower UTIs (cystitis) and upper UTIs (pyelonephritis) **(Hannan, 2012 & Hooton, 2012).** Complicated UTIs are defined as UTIs associated with factors that compromise the urinary tract or host defence, including urinary obstruction, urinary retention caused by neurologicaldisease, immunosuppression, renal failure, renal transplantation, pregnancy and the presence of foreign bodies such as calculi, indwelling catheters or other drainage devices **(Lichtenberger *et al*., 2008 & Levison *et al.*, 2013).** UTI is defined as 2 uncomplicated UTIs in 6 months or, more traditionally, as ≥ 3 positive cultures within the preceding 12 months **(Annette *et al*., 2015).** Several risk factors are associated with cystitis, including female gender, aprior UTI, sexual activity, vaginal infection, diabetes, obesity and genetic susceptibility **(Hannan, 2012 & Foxman, 2014).** UTIs are usually treated with broad-spectrum cephalosporins flouroquinolones and aminoglycosides. The rapid spread of resistance to broad-spectrum beta-lactams in pathogenic strains of bacteria has recently become a major health problem in the world. It causes antibiotics ineffectiveness, increased severity of illness and cost of treatment **(Yazdi *et al*., 2012 & Harada *et al*., 2013).** These bacteria produce enzymes called extended spectrum β-lactamases (ESBL) which render penicillin's and cephalosporins inactive**.** Rapid

detection of ESBL is important, not only for treatment guidelines but also to facilitate improved prevention of nosocomial infections **(Shah *et al*., 2009 & Nwakaeze *et al*., 2013).** ESBL can be detected using a standard screening test showing reduced susceptibility to five antibiotics, such as ceftazidime, ceftriaxzone, cefotaxime, aztreonam and cefpodoxime, as detected by standard disk diffusion and minimal inhibition concentration (MIC**) (NCCLS.2001).** Various phenotypic methods are recommended in the routine practice to detect the ESBL production in gram negative bacilli. Among them, the Double Disc Synergy Test (DDST) which uses the third generation cephalosporins (3GC), is a simple and a reliable method. But the coexistence of Amp C may give false negative results. In such cases, the ESBL detection can be improved by using cefepime along with the third generation cephalosporins in DDST **(Jaspal *et al*., 2013)** The ESBL genes are mostly plasmid encoded and most ESBLs can be divided into 3 genotypes: TEM, SHV, and CTX-M **(Paterson *et al*.,2005).** *E. coli* has now become an important ESBL carrier in Western countries. In addition, a genotype CTX-M has become more prevalent worldwide compared to the TEM and SHV genotypes **(Levison *et al*., 2013 & Harada *et al*.,2013)**

2. Material and Methods:

Between May 2014 and August 2016, one thousand fresh mid-stream urine samples from urinary tract infected patients were collected. The samples were as following 407 urine sample were collected from male patient and 593 urine sample were collected from female patient. Urine samples were collected aseptically in a sterile clean catch container.

1- Isolation and Identification of Pathogens:

The pathogens were isolated by following standard protocol using sterile bacteriological media. Each sample were inoculated on MacConkey's agar using calibrated loop delivering 0.01ml of the sample. Then, plates were incubated overnight at 37^{o}C for 24 hrs. Identification of the organisms were done on the basis of Gram stain and routine biochemical tests including, reaction on triple sugar iron (TSI) producing acids, citrate utilization test, methyl red test, Voges Proskauer test and indole test. Microbact 12A^{TM} (API) was used as a confirmatory identification. Bacterial growth, only for strains of *E.coli* with clinically growth (>10^{5} CFU/m) were included in this study.

2- Ceftazidime resistant isolates among the tested *E.coli* isolates was screened by using breakpoint method:

Culture on Muller Hinton agar supplemented with 2 mg/liter Ceftazidime. The plates were incubated overnight at 35ºC in ambient air and then examined for any growth **(Khater *et al*., 2014).**

3- Antimicrobial susceptibility testing by disc diffusion method for Uropathogenic *E.coli* isolates for antimicrobials:

Routine disc diffusion susceptibility testing was performed by modified Kirby Bauer's disc diffusion method **(Yazdi *et al*., 2012).** Susceptibility of the tested isolates to16 different β-lactam antibiotics; (AX) Amoxicillin, (PRL) Pipracillin, (P) Penicillin G, (CFR) Cefadroxil, (AMC) Amoxicillin/Clavulinicacid, (CZ) Cefazolin, (CEC) Cefaclor,

(MA) Cefamandolin, (FOX) Cefoxetin, (CTX) Cefotaxime, (CAZ) Ceftazidime, (CRO) Ceftriaxone, (CFM) Cefixime, (FEP) Cefepime, (IPM) Imipenem, (MEM) Meropenem, (ATM) Azetreonam. The results were interpreted according the clinical and laboratory standards institute **(CLSI 2014).**

4- Determination of minimum inhibitory concentrations (MICs) of 3rd & 4th generation cephalosporins among *E.coli* isolates by using agar dilution method:

The minimum inhibition concentrations (MICs) of different antibiotics (Ceftazidime, Cefotaxime, Ceftriaxone and Cefepime) were determined by the agar diffusion method according to CLSI 2014 on Muller-Hinton agar (MHA) to determine the degree of resistance. Inoculation *E.coli* isolates which applied to the surface of dry MHA plates containing graded concentration (from 0.5-1024 µ g/ml) of the respective antibiotics by using multi-inoculators of 25 teeth (carries 25 isolates in one time) **(CLSI, 2014).**

5- Phenotypic detection of β-lactamase (s) production

5.1- Iodometric test for identification of b-lactamases producers:

For detection of β -lactamase producing isolates of *E. coli*, were tooth picked or applied with a multi-inoculators onto the surface of nutrient agar plates. After overnight incubation at 37°C, the plates were over laid with 1% molten agarose containing 0.2% soluble starch, 1% penicillin G and 0.2%toluene. The plates were incubated for 15 min at room temperature and iodine solution was poured onto the agar plates to cover the overlay uniform1y, After 10 sec, the residual iodine solution was damped out and the plates were incubated at room temperature until discoloration zones appeared around β-lactamase producing colonies **(Abo-Kamar *et al*., 1998).**

5.2Doubledisc synergy test for detection of ESBLs (DDST):

Disc of third generation cephalosporin Ceftazidime (30 mcg) and Cefotaxime (10 mcg) were placed 15mm apart from an Amoxicillin (20 mcg) & Clavulanic acid (10 mcg) combined disc, center to center. Incubated at 37°C for 18-24 hours. If inhibition

around for either third generation cephalosporins showed a clear extension towards augmentin disc, was said to be ESBL producer **(Mangaiyarkarasi *et al.*, 2013).**

5.3Combined disc method for detection of ESBLs:

ESBL testing requires use of both cefotaxime and ceftazidime, alone and in combination with clavulinic acid, ≥ 5-mm increase in a zone diameter for either antibiotic (ceftazidime or cefotaxime) tested in combination with clavulinic acid versus its zone when tested alone was determined to be ESBL producer **(Mangaiyarkarasi *et al.*, 2013).**

6- Determination of genes mediated ESBLs production in tested *E.coli* isolates by multiplex polymerase chain reaction (Multiplex PCR):

All phenotypically confirmed ESBL producing strains were investigated to determine the probable type of β-lactamase enzyme which was responsible for resistance. Extraction of DNA was performed using DNA extraction kit (Jena Bioscience, Germany). PCR amplification of *bla* genes, including *bla*TEM, *bla*SHV and *bla*CTX-M was performed with Taq master mix DNA polymerase (Jena Bioscience, Germany) using primers listed in table (1), under the following conditions: A- Initial denaturation step: 94°C for 5 min. B- 30 cycles consisting of: Denaturation: 94°C for 1 min, Annealing: 61°C for 1 min, DNA extension: 72°C for 1 min. C- Final extension: 72°C for 5 min **(Stürenburg *et al.*, 2004 and Kim *et al.*, 2009).**

Table (1): Multiplex PCR primers for detection of *bla*TEM, *bla*SHV and *bla*CTX-M.

Gene	Sequence of primers	Size bp	References
SHV-F SHV-R	5'GATGAACGCTTTCCCATGATG3' 5'CGCTGTTATCGCTCATGGTAA3'	214	**Kim *et al.*, 2009**
TEM-F TEM-R	5'AGTGCTGCCATAACCATGAGTG3' 5'CTGACTCCCCGTCGTGTAGATG3'	550	**Kim *et al.*, 2009**
CTX –F CTX –R	5'TCTTCCAGAATAAGGAATCCC3' 5'CCGTTTCCGCTATTACAAAC3'	909	**Stürenburg *et al.*, 2004**

F= Forward primer, R= Reverse primer

3. Results:

Isolation and Identification of Pathogens:

Urine samples were cultured on MacConkey's agar. Out of the developed colonies, the lactose fermenter; flat, dry, pink colonies were selected for further identifications using gram staining, traditional biochemical identification tests and Microbact ™ 12A Biochemical Identification Kit. It revealed that 132 uropathogenic *E.coli* isolates.

Ceftazidime resistant isolates among the tested *E.coli* isolates was screened by using breakpoint method

Antimicrobial susceptibility testing by disc diffusion method for uropathogenic *E.coli* isolates for 16 antimicrobials:

Resistance to tested antibiotics were distributed among recovered isolates as shown in Figure (1).

Determination of minimum inhibitory concentrations (MICs) of 3rd & 4th generation cephalosporins among *E.coli* isolates by using agar dilution method:

Most of isolates showed MIC values of 512 µg/ml in case of Ceftazidime (30 isolates), 128 µg/ml in case of Cefotaxime (28 isolates), 512 µg/ml in case of Ceftriaxone (40 isolates) and 32 µg/ml in case of Cefepime (46 isolates) as presented in table (2).

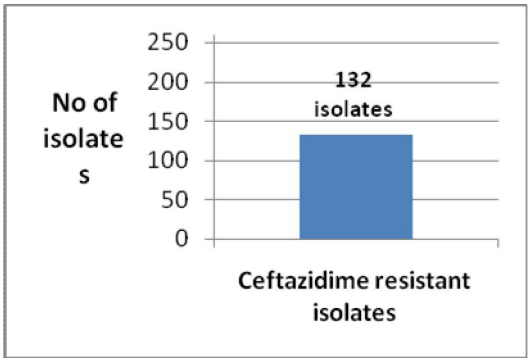

Figure (1): Incidence of Ceftazidime resistance among the detected *E.coli* isolates

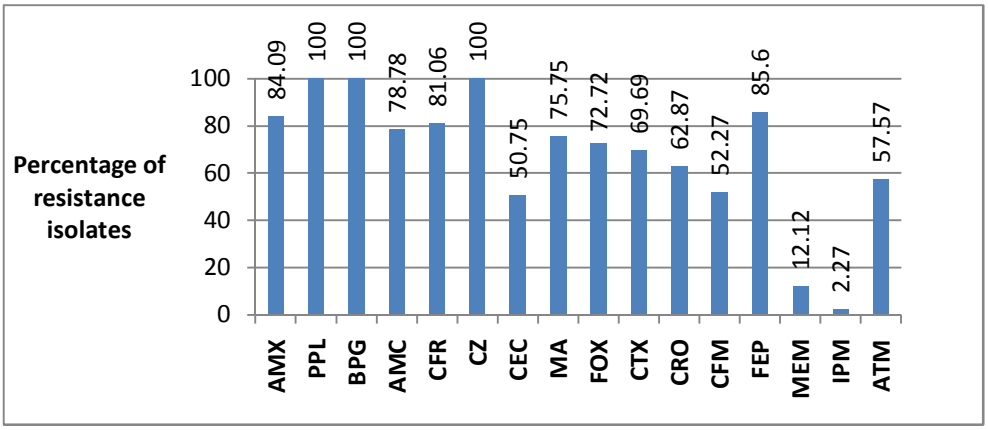

Figure (1): Histogram showing resistance of Ceftazidime resistant *E.coli* isolates to different β-Lactam antibiotics.

Table (2): Minimum inhibitory concentration (µg/ml) of ceftazidime resistant *E.coli* isolates (n=132) to 3rd and 4th generation cephalosporins

MICs (µg/ml)	Ceftazidime*			Cefotaxime*			Ceftriaxone*			Cefepime*		
	No of *E.coli* isolates	Susceptibility Group	No of isolates (%)**	No of *E.coli* isolates	Susceptibility group	No of isolates (%)**	No of *E.coli* isolates	Susceptibility Group	No of isolates (%)**	No of *E.coli* isolates	Susceptibility group	No Of isolates (%)**
1	0	Sensitive	0(0%)	13	Sensitive	13 (9.84%)	8	Sensitive	8 (6.06%)	0	Sensitive	8 (6.06%)
2	0			4	Intermediate	4 (3.03%)	7	Intermediate	7 (5.30%)	8		
4	0			5	Resistant	115 (87.12%)	6	Resistant	117 (88.63%)	7	Intermediate	13 (9.84%)
8	0	Intermediate	0(0%)	10			8			6		
16	11	Resistant	132 (100%)	20			4			17	Resistant	111 (84.09%)
32	15			7			6			46		
64	17			0			11			34		
128	25			27			16			14		
256	17			13			13			0		
512	30			26			40			0		
1024	17			6			13			0		

* Ceftazidime breakpoint ≥16, Cefotaxime breakpoint ≥4, Ceftriaxone breakpoint ≥4 and Cefepime breakpoint ≥16 according to CLSI 2014.
** Percentage was calculated relative to total no. of ceftazidime resistant *E.coli* isolates.

Iodometric test for identification of b-lactamases producers:

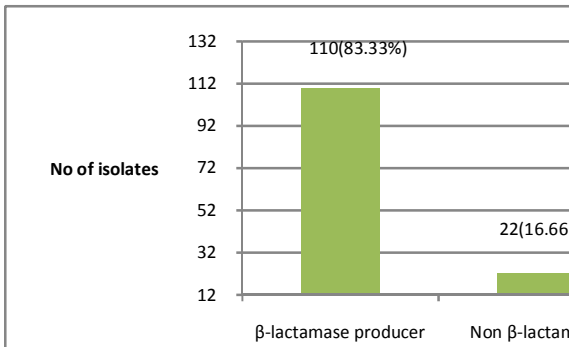

Figure (2): Out of 132 Ceftazidime resistant *E.coli* isolates, 110 (83.33%) were Iodometric over lay positive test.

Out of 132 Ceftazidime resistant *E.coli* isolates, 110 (83.33%) showed positive test indicating β-lactamase production as shown in figure (2).

Double disc synergy test for detection of ESBLs (DDST):

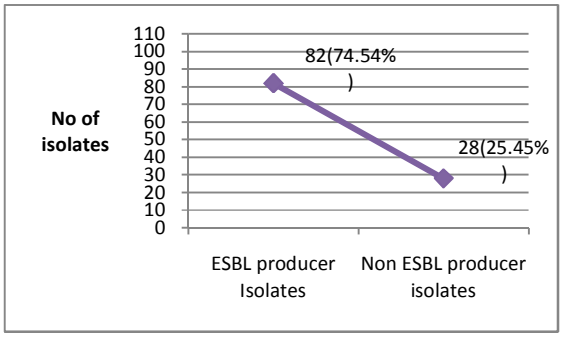

Figure (3): Double-disk synergy test

Using double disc synergy test, it was found that out of 110 potentially ESBL producer *E.coli* isolates, 82 (74.54%) were confirmed to be ESBL producer as indicated by enhancement of the inhibition zones of CTX and CAZ toward the AMC disk as shown in figure (3).

Combined disc method for detection of ESBLs:

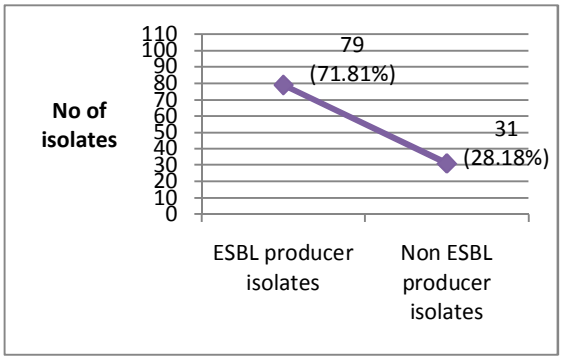

Figure (4): combined disc method.

Using combined disc method, it was found that out of 110 potentially ESBL producer *E.coli* isolates, 79 (71.81%) were confirmed to be ESBL producer indicated by anincrease of ≥5 mm in inhibition zone of the combination disk when compared to the corresponding cephalosporin disk alone as shown in figure (4).

Only 3 isolates showed positive test with double disc synergy test and negative with combined disc method. Therefore these isolates were excluded and a total of 79 isolates confirmed by both tests to be ESBL producer were selected for further studies.

Determination of genes mediated ESBLs production in tested *E.coli* isolates by multiplex polymerase chain reaction (Multiplex PCR):

ESBL encoding *bla* TEM, *bla* SHV and *bla* CTX-M genes exhibited molecular size of 550 bp, 214 bp and 909 bp respectively. As shown in Figure (5), a total of 22 (27.84%) isolates out of 79showed at least one of the ESBL mediated genes.

Incidence of each of the detected ESBLs mediated genes using79 positive ESBL producer *E.coli* isolates is shown in Table (3).

Table (3): Prevalence and pattern of ESBL-encoding genes among ESBL – producing *E.coli*.

ESBL genes	bla TEM only	bla TEM + bla SHV	bla TEM + bla CTX-M	No genes	Total No of isolates
n (%)	17(21.51%)	2(2.53%)	3(3.79%)	57(72.15%)	79(100%)

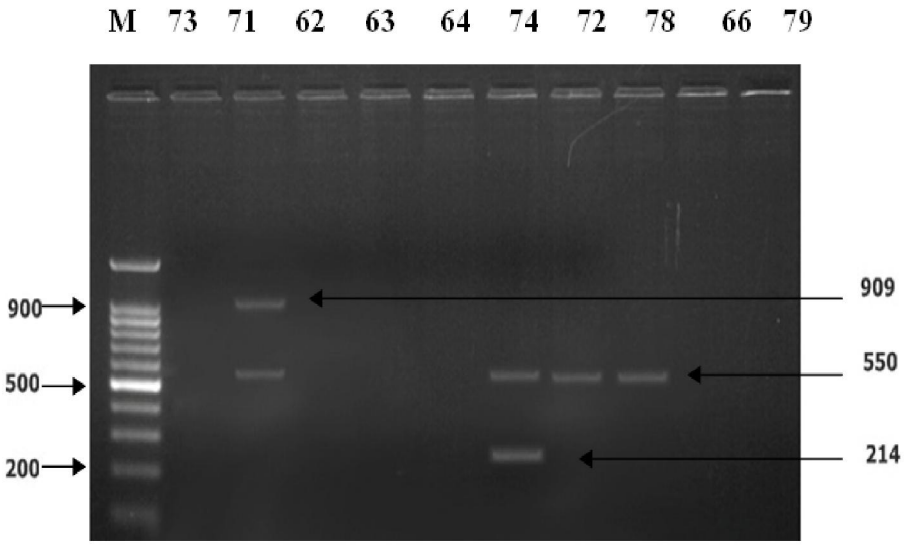

Figure (5): Polymerase chain reaction Shows:

M: the 100 bp DNA size marker.
Isolates 71, 74, 72 and 78 shows 550 pb fragment of bla TEM gene.
Isolate 71 shows 909 pb fragment of bla CTX-M gene.
Isolate 74 shows 214pb fragment of bla SHVgene.
Isolates 73, 62, 63, 64, 66 and 79 show no genes.

In present study, MICs of ceftazidime among positive ESBLs mediated genes among *E.coli* isolates ranged from 32 µg/ml to 512 µg/ml and MAR index values of these isolates ranged from 0.416 to 0.833 as shown in Table (4).

Table (4): Characteristics of positive ESBL encoding genes among *E.coli.*

ESBL producer *E.coli* isolates code	Gene detected	MICs of CAZ	Resistance Pattern
E17b	*bla*TEM	256	AMX-PRL-P-AMC-CFR-CZ-CEC-MA-FOX-CTX-CAZ-CRO-CFM-FEP-ATM-CIP-OFX-TE-C-SXT
E226	*bla*TEM	32	AMX-PRL-PG-AMC-CFR-CZ-CEC-MA-FOX- CAZ-CRO-CFM-FEP-ATM-TE-CM-SXT
E278	*bla*TEM	64	AMX-PRL-PG-AMC-CZ-FOX- CTX-CAZ-CRO-FEP-ATM-CIP-TE-CM
E291	*bla*TEM	32	AMX-PRL-PG-AMC-CFR-CZ-CEC-MA-FOX-CTX-CAZ-CRO-CFM-FEP-ATM-CIP-OFX-TE-CM
E294	*bla*TEM + *bla*CTX-M	32	AMX-PRL-P-AMC- CZ- MA-FOX-CTX-CAZ-CRO
E301	*bla*TEM	32	AMX-PRL-P-AMC-CZ-MA-FOX-CAZ-CRO-CFM-FEP
E324	*bla*TEM	256	AMX-PRL-P-AMC-CFR-CZ-CEC-MA-FOX-CTX-CAZ-CRO-CFM-FEP-ATM-CIP-OFX-TE-C-SXT
E533	*bla*TEM	32	AMX-PRL-PG-AMC-CFR-CZ-CEC-MA-FOX- CAZ-CRO-CFM-FEP-ATM-TE-CM-SXT
E585	*bla*TEM	64	AMX-PRL-PG-AMC-CZ-FOX-CTX-CAZ-CRO-FEP-ATM-CIP-TE-CM
E598	*bla*TEM	32	AMX-PRL-PG-AMC-CFR-CZ-CEC-MA-FOX-CTX-CAZ-CRO-CFM-FEP-ATM-CIP-OFX-TE-CM
E601	*bla*TEM + *bla*CTX-M	32	AMX-PRL-P-AMC- CZ- MA-FOX-CTX-CAZ-CRO
E608	*bla*TEM	32	AMX-PRL-P-AMC-CZ-MA-FOX- CAZ-CRO-CFM-FEP
E631	*bla*TEM	256	AMX-PRL-P-AMC-CFR-CZ-CEC-MA-FOX-CTX-CAZ-CRO-CFM-FEP-ATM-CIP-OFX-TE-C-SXT
E840	*bla*TEM	32	AMX-PRL-PG-AMC-CFR-CZ-CEC-MA-FOX- CAZ-CRO-CFM-FEP-ATM-TE-CM-SXT
E892	*bla*TEM	64	AMX-PRL-PG-AMC-CZ-FOX- CTX-CAZ-CRO-FEP-ATM-CIP-TE-CM
E905	*bla*TEM	32	AMX-PRL-PG-AMC-CFR-CZ-CEC-MA-FOX-CTX-CAZ-CRO-CFM-FEP-ATM-CIP-OFX-TE-CM
E908	*bla*TEM + *bla*CTX-M	32	AMX-PRL-P-AMC- CZ- MA-FOX-CTX-CAZ-CRO
E915	*bla*TEM	32	AMX-PRL-P-AMC-CZ-MA-FOX- CAZ-CRO-CFM-FEP
E933	*BlaTEM + blaSHV*	64	AMX-PRL-PG-AMC-CFR-CZ-CEC-MA-FOX-CTX-CAZ-CRO-CFM-FEP-ATM-CIP-OFX-TE-AK-CN-CM-SXT
E950	*blaTEM*	512	AMX-PRL-PG-AMC-CFR-CZ-CEC-MA-FOX-CTX-CAZ-CRO-CFM-FEP-MEM-ATM-CIP-TE-CN-SXT
E956	*BlaTEM + blaSHV*	32	AMX-PRL-PG-AMC-CFR-CZ-CEC-MA-FOX- CAZ-CRO-CFM-FEP-ATM-TE-CM-SXT
E987	*blaTEM*	32	AMX-PRL-PG-AMC-CFR-CZ-CEC-MA-FOX-CTX-CAZ-CRO-CFM-FEP-ATM-CIP-OFX-TE-SXT

*(AMX)Amoxicillin, (PRL) Pipracillin, (PG) PenicillinG, (AMC) Amoxicillin/ Clavulinicacid, (CFR) Cefadroxil, (CZ) Cefazolin, (CEC) Cefaclor, (MA) Cefamandolin, (FOX) Cefoxetin, (CTX) Cefotaxime, (CAZ) Ceftazidime, (CRO) Ciprofloxacin, (CFM) Cefixime, (FEP) Cefepime, (MEM) Meropenem, (IPM) Imipenem, (ATM) Azetreonam, (OFx) Ofloxacin, (TE) Tetracyclin, (AK) Amikacin, (CN)Gentamycin, (CM) Chloramphenicol, (CIP) Ciprofloxacin (SXT) Trimethoprim/Sulphamethaxole.

In present study out of 22 positive ESBLs mediated genes among *E.coli* isolates, 14 were exhibiting 32 µg/ml of MIC of ceftazidime, 4 isolates were 64 µg/ml, 3 isolates were 256 µg/ml and 1 isolate was 512 µg/ml as shown in Figure (6).

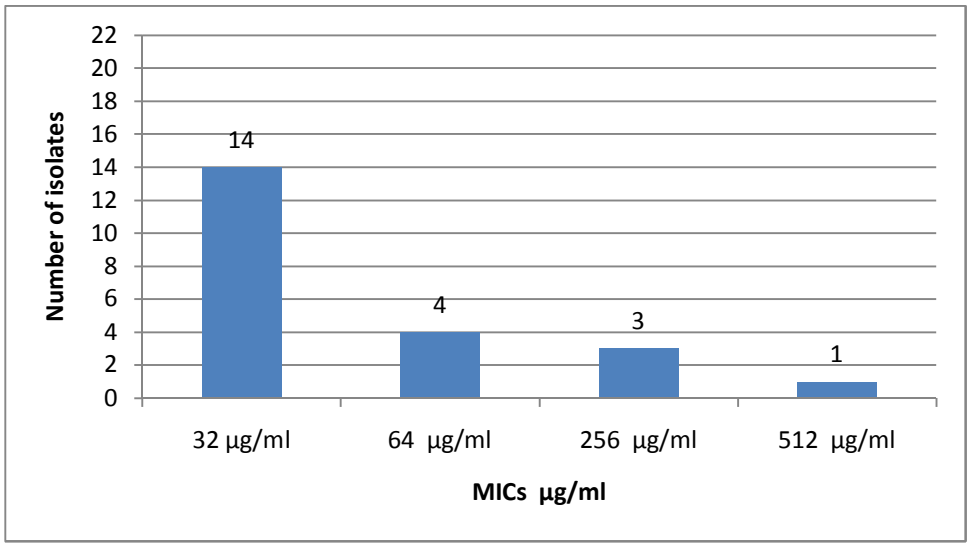

Figure (6): Relationship between MICs µg/ml and its exhibiting number of positive ESBLs mediated genes among *E.coli* isolates.

4. Discussion:

In present study, a total of 1000 urine samples collected from patients suffering from UTIs attending from Shebien El Kom Teaching Hospitals, and Monofeya University Hospitals from May 2014 till August 2016. Out of 1000 urine sample, 593 (59. 3%) from female patients and 407 (40.7%) from male patients. A total of 536 clinical isolates were recovered from 1000 urine samples by culturing on MacConkey's agar. A total of 259 isolates were

determined as *E.coli* by gram staining and conventional biochemical identification tests and confirmed by using Microbact 12A™ biochemical identification kits. This kits offers manual identification of microorganisms for: Infectious disease diagnosis and identification of important industrial microorganisms and strips give accurate identifications based on extensive databases and are standardized, easy-to-use test systems.

In present study, majority of isolates from females (59.23%) than males (40.77%). This finding agreed with other studies by **(Deshmukh *et al.,* 2014)** in Indiawhich reported the majority of isolates from females (68.57%) than males (31.42%), by **(Ullah *et al*., 2009)** in Pakistan which reported that more isolates were recovered from females (75%) than males (25%), by **(Manikandan *et al.,* 2014)** in India which reported that the prevalence of UTI in females (69.8%) is higher than males (30.2%) and by **(Hussein *et al*., 2012)** in Egypt which observed that the number of patients with urinary tracts infection was higher in females (70.9%) compared to males (29.1%). It has been reported that women have a higher prevalence of UTIs than men, principally due to anatomical factors **(Biswas *et al.,* 2014).**

In present study, the incidence of *E.coli* isolates that recovered and confirmed to be etiologic agent of urinary tract infection was 48.32%. This result was agreed with other study by **(Thabit *et al*., 2011)** in Assuit (54.28%). Similar values were obtained abroad, where *E.coli* isolates occurred with prevalence of (55.56%) in **(Kumar *et al.,* 2016)** and (36.89%) in India **(Singh *et al.,* 2014)**. In contrast, A lower incidence of *E.coli* (8.6%) was reported in Egypt by **(Ahmed *et al*., 2009)**, and (25%) and (20%) in Iran by **(Taher *et al*., 2009)** and **(Mirsalehian, *et al*., 2008)** respectively. Also, a higher incidence of *E.coli* (85.2%) was reported in Pakistan **(Ilyas *et al*.,2014),** (64.57%) and (64.28%) in India by (**Pratap *et al*., 2016)** and **(Chander, 2016**) respectively, (63.4%) by **(Kaur *et al.,* 2013)**, (60%) in Nigeria **(Akanbi *et al.,* 2013)** and (59.32%) by **(Pandey *et al*., 2016)**. This variation in results was due to number of samples used in each study, sex and age.

Screening of ceftazidime resistant isolates among the tested *E.coli* isolatesby using breakpoint method were used for detection of potentially ESBL producer isolates **(Khater *et al*., 2014).** In present study, out of 259 *E.coli* isolates, 132 isolates (50.96%) showed resistance to Ceftazidime which described as potential ESBL producer *E.coli* isolates. This result was agreed with the studies done by (**Khater *et al*., 2014**) in Benha reported that (65.4%) were ceftazidime resistant isolates and by **(Yazdi *et al*., 2012)** in Turkey reported that (47.1%) were ceftazidime resistant isolatesand (59%) ceftazidime resistant isolates in India **(Chauhan *et al*., 2014).** In contrast, a higher incidence of resistance of *E.coli* isolates to ceftazidime was (76.47%) reported in Assuit by **(Thabit *et al. (*2011).**

The present study focused on assessment of the efficacy of beta-lactam antibiotics by using disc diffusion method ((Amoxicillin, Penicillin G and Pipracillin), beta-lactam/ beta-lactam inhibitors (Amoxicillin/Clavulanic acid), 1st generation cephalosporin (Cefadroxil and Cehazolin), 2nd generation cephalosporin (Cefaclor, Cefamandolin and Cefoxitin), 3rd generation cephalosporin (Cefotaxime, Ceftriaxone and Cefixime), 4th generation cephalosporin (Cefepime), Carbapenem (Imipenem and Meropenem), Monobactam (Azetreonam).

In present study, recorded that the high rate of resistance of ceftazidime 100%, cefotaxime 82.12%, ceftriaxone 88.68% and cefepime 84.09% for Ceftazidime resistant *E.coli* isolates, and this findings were agreed with findings carried by **(Mangaiyarkarasi*et al*.,2013**, which record that Cefotaxime 87.7%, Ceftriaxone 87.7% and record low resistance rate of ceftazidime (73.2%). By **(Saeidi *et al., 2014*)**.

In present study, beta-lactamase (s) enzyme producers *E.coli* isolates were detected by using Iodometric overlay test **(Abo-Kamar *et al.,* 1998)**, the result recorded that 83.33% of ceftazidime resistant *E.coli* isolates were positive beta-lactamase (s) producer. This result was comparable to study done by **(Abo-Kamar *et al.,* 1998)** in Tanta (72.36%). Similar values were also obtained abroad, where beta-lactamase producer *E.coli* isolates occurred with prevalence of (90.47%) in India **(Gajul *et al.*,2012**) *and* 87.0% in Cotonou, Benin **(Anago *et al*., 2015)**. The non beta-lactamase (s) enzyme producers of ceftazidime resistant *E.coli* isolates could be due to others mechanisms which including outer membrane and efflux mechanisms.

In present study, two different techniques (double disc synergy test and combined disc method) were used for confirming ESBL production among beta-lactamases producer *E.coli* isolates **(CLSI 2014)** which observed thatthe incidence of ESBL production was 30.50% (79 isolates) which was comparable to the study done by **(Saied *et al*., 2011)** in Egypt (39%), by **(Thabit *et al.,* 2011)** in Assiut (39.47%) and by **(*Abdallah et al*., 2015)** in Zagazyg (31.91%). Similar values were also obtained abroad, where potential ESBL production among isolates of *E.coli* occurred with a rate of 30% in India **(Agrawal *et al*., 2008),** 31% in Nigeria **(Akanbi *et al.,* 2013),** 35.5% in Cotonou, Benin (*Anago et al., 2015*), 31.9% in Dhaka, Bangladesh **(*Islamet al*., 2015)**, 33.3% in Thailand **(Chayakulkeeree *et al*., 2005) and** 30.5% in Iran **(*Rezai et al.,2015*).**

In contrast, comparing this incidence with that obtained in Egypt and other countries by other workers a controversary was obvious. A lower incidence of ESBL producer *E.coli* isolates was reported in Cairo (16%) by **(*Fam et al.,2011*)** and (16.4%) in Upper Egypt by **(*Saedii et al., 2017*)**, (19.02%) in Iran by **(Mobaleghi *et al.*,2012),** (6.7%) in Northwestern Libyaby **(*Abujnah* A *et al*., 2015),** (7.1%) in Nigeriaby **(Adeyankinnu *et al*., 2014),** (6.3%) in Saudi Arabiaby **(*Somily et al., 2014*),** (1.3%) in Morocco by **(Barguigua *et al.,* 2011)**, (12.8%) in Nigeria by **(Aibinu *et al*., 2012),** (9.2%) in USA by **(*Bhusal et al., 2011*)**, (11%) from 44 hospitals in different European countries (i.e. France, Germany, Greece, Romania, Spain, Turkey, Estonia, Italy, Latvia, Lithuania, Portugal, and UK) by **(Hawser *et al.,* 2011)** and (4.7%) in Taiwan **(Hsieh *et al.,* 2010).**

A higherincidence of ESBL producer *E.coli* isolates was reported in Egypt (71.25%) by **(Abdul Rahman *et al.,2011*),** (76.47%) in Assiut by **(*Thabit et al.,2011*),** (52%) in Egyptby **(Abdel-Moaty *et al*., 2016),** (64.7%) in Egypt by **(Ahmed *et al.*,2009)**, (60.9%) in Egypt by **(Al-Agamy *et al*.,2006)**, (61.7%) in Indiaby **(Mohanalakshmi *et al.,* 2014),** (54%) in Jordan by **(Muhammad *et al.*,2015),** (56.9%) in Pakistan by **(Ullah *et al.*,2009)**, and (63.4%) by **(Kaur *et al.,2013*).**

In present study, out of 79 isolates were ESBL producer phenotypically, 22 isolates (27.84%) were ESBL mediated genes and this result was in harmony with other studies, which found that, 50% (15/30) were positive ESBL mediated genes *(bla* TEM and *bla* SHV) by (**Abdallah *et al*.,2015)** in Egypt, (32.5%) by **(Saeidi *et al*., 2014)** in Iran, (71.15%) by **(Thabit *et al.*,2011**) in Assiut-Egypt, (93.3%) by (**Goudarzi *et al.*,2014)** in Iran and, (89.4%) by **(Harada *et al*., 2013)** in Japan**,** (45%) in India by **(Bajpai *et al.,2017*)** and (50%) by **(Pérez-Pérez *et al*.,2002)** and **(Hand*a et al*., 2013)**.

In present study, (72.15%) of phenotypically positive ESBL strains lacked TEM, SHV, and/or CTX□M genes and this finding in the line with other studies done by **(Shahid *et al*., 2011)**, **(Eftekhar *et al*., 2012)** and **(Ahmed *et al*., 2013)**. Also, the current result was agreed with other studies done by **(Abdallah *et al*.,2015)** which recorded (50%) of phenotypically positive ESBL strains lacked TEM, SHV, and/or CTX□M genes, (28.85%) in Assiut-Egyptby **(Thabit *et al.,* 2011),** (55%) in India by **(Bajpai *et al.*,2017)**, (6.7%) in Iran by (**Goudarzi *et al.*,2014)**, and (10.6%) in Japan by **(Harada *et al*., 2013)** and (50%) by **(Pérez-Pérez *et al*.,2002)** and **(Hand*a et al*., 2013).**

The variation in results between phenotypic and genotypic detection among the tested isolates explained as follow; first, the probable presence of another variants of ESBLs from the TEM, SHV, CTX-M or other ESBL families in these isolates and this is in line with other studies done by **(Oliveira *et al*., 2010), (Shahid *et al*., 2011)**, **(Eftekhar *et al*., 2012)**, **(Ahmed *et al*., 2013)** and **(Bajpai *et al.,* 2017),** because these extended spectrum beta-lactamases (ESBLs) are mutation within the structural gene (plasmid-mediated beta-lactamases derived from narrow-spectrum beta-lactamases e.g.; bla TEM-1, bla TEM-2, bla SHV-1) **(Bajpai *et al.*,2017)**; so, more than 350 different natural ESBL variants are known that have been classified into different distinct structural and evolutionary families based upon their amino acid sequence comparisons such as TEM, SHV, CTX□M **(Bajpai *et al.*,2017)**. Second, broad spectrum beta-lactamase (*bla* TEM-1 and *bla* SHV-1) are not regarded as extended spectrum β-lactamases and the presence of these enzymes, combined with changes in the outer membrane proteins lead to reduced susceptibility to third-generation cephalosporins, that phenotypically suggest ESBL production **(Wu. 2001).** Also, other cephalosporinases and /or carpabenemases were involved in development ceftazidime resistant isolates. finally, genotyping detection of ESBLs (*bla* TEM, *bla* SHV and *bla* CTX-M) is depending on the selected primers used in PCR amplification. Primers used are not universal so, not cover all variants of genes mediated ESBLs, and the different variant of genes mediated ESBLs present in isolates lead to different affinities to antimicrobial agents, so give higher or lower MICs values.

Conclusion:

Urinary tract infection is one of the important causes of morbidity and mortality, affecting all age groups across the life span. This study has demonstrated that emergence of ESBLs producer among *E.coli* has increased in Monofeya area. It is essential to report ESBL production along with routine susceptibility testing, which will help the clinicians in prescribing proper antibiotics. To optimize the use of empirical therapy, evidence-based guidelines for antibiotics use, guided by surveillance studies of target bacteria, need to be developed and implemented. Antimicrobial therapy should be narrowed as much as practicable effectively to treat only the causative organism. So should be activating antibiotic policy according to each hospital. The most efficient antibiotics were Imipenem followed by gentamicin for CAZ resistant *E.coli* isolates.

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