Egyptian Journal of Medical Microbiology Volume 26 / No.1 / January 2017 49-59

ORIGINAL ARTICLE

Prevalence and Molecular Characterization of Extended Spectrum Beta Lactamases Producing *Escherichia coli* Isolates Causing Hospital - Acquired and Community - Acquired Urinary Tract Infections in Sohag University Hospitals, Egypt

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Key words:

Urinary tract infection, Escherichia coli, Antibiotic Resistance, Extended-Spectrum Beta-Lactamase (ESBL)

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ABSTRACT

Background: *Escherichia coli (E. coli) are the most common etiological agents of hospital acquired and community acquired urinary tract infections (UTIs) and the incidence of antibiotic resistant strains has been steadily increasing over the past few years resulting in limitation of therapeutic options.* ***Our objectives*** *were to investigate the prevalence and molecular characterization of extended spectrum β- lactamases (ESBLs) producing E. coli isolated from out-patients and hospitalized patients with UTI attending Urology Department of Sohag University Hospitals during the period from May 2014 to May 2015.* ***Methodology:*** *Forty four E. coli isolates were collected during the study period. The isolates were tested for antibiotic sensitivity by the disc diffusion method. All isolates were screened phenotypically for ESBL production by the double disc diffusion (DDD) test. The presence of* ^{bla}*CTX-M-I,* ^{bla}*TEM and* ^{bla}*SHV genes in ESBL- producing E. coli was tested by polymerase chain reaction (PCR).* ***Results:*** *showed high prevalence of ESBL-producing E. coli; as 88.6% of the collected isolates were ESBL producers. Twenty- eight (71.8 %) isolates were multidrug resistant (MDR). CTX-M-I gene was the most prevalent ESBL type (95 %); followed by TEM (53.8%) and lastly SHV gene which was detected only in 10.3% of cases.* ***Conclusions:*** *E. coli isolates carrying multiple ESBL genes are widespread in our locality. CTX-M-I -producing E. coli are currently a problem which may be related to the misuse of third generation cephalosporins, especially cefotaxime. Continuous monitoring, effective infection control measures and judicious antibiotic usage are required.*

INTRODUCTION

Urinary tract infection (UTI) is tied with pneumonia as the second most common type of healthcare-associated infection, second only to SSIs [1]. It is estimated that annually global economy spends more than six billion dollars on UTI [2]. Studies show that about 50% of women and 12% of men get UTI during their life. Also 20% to 30% of women experience recurrent infections within 6-12 months [3].

Therefore, it is a major public health problem. The commonest bacterial agent involved in UTIs is *Escherichia coli*, being the main pathogen both in the community as well as in the hospitals [4]. Antibiotic resistance of *E. coli* strains causing UTI is a major concern for treating UTIs and is increasing day by day and the evolution of multidrug-resistant (MDR) strains is a public threat of increasing magnitude. In the early 1980s, third-generation cephalosporins were introduced to the clinical practice as β-lactam antibiotics able to overcome resistance caused by the common β-lactamases produced by *E. coli*. However, within few years; extended spectrum β-lactamases (ESBLs) producing *E. coli* which make them resistant to expanded-spectrum cephalosporins, and monobactams had emerged [5].

ESBLs do not hydrolyze cephamycins (e.g., cefoxitin or cefotetan) or carbapenems (e.g., imipenem and meropenem), and they are inhibited by β-lactamase inhibitors such as clavulanic acid [6].

ESBL arise mainly due to mutation in β- lactamases encoded by the ^{bla}SHV, ^{bla}TEM, and ^{bla} CTX-M genes. More than 400 ESBLs have been described so far, that typically, are derived by point mutation from the TEM, SHV and CTX-M groups, with 183, 134 and 103 variants, respectively [7].

ESBLs are mostly encoded by large plasmids (up to 100 kb and even more) that are transferable from strain to strain and between bacterial species. Genes encoding ESBLs are frequently found on the same plasmid as genes encoding resistance for other classes of antibiotics such as Fluoroquinolones [8].

Plasmid mediated quinolone resistance (PMQR) was first described in 1998 [9]. Since then, five major groups of *qnr* determinants *(qnrA, qnrS, qnrB, qnrC* and *qnrD)* have been identified [10]. These molecular factors

have wide distribution and were mostly reported in ESBL producing Enterobacteriaceae [11]. A recently emerged, disseminated lineage of virulent *E. coli*, designated sequence type ST131 according to multilocus sequence typing is associated with CTXM-15 and is usually fluoroquinolone resistant [12]. Finally; the strains becoming more resistant to antibiotics as the plasmids enable the transfer of these resistance genes [8] and infections are gradually becoming more difficult to treat and may lead to therapeutic dead ends. So; understanding the spectrum and resistance patterns may help to guide effective empirical antibiotic therapies, decrease treatment failure and costs. It was on this background the present study was conducted to detect prevalence, molecular characterization and antimicrobial resistance pattern of ESBL producing ***E. coli*** isolates causing community –acquired and hospital-acquired UTI in Sohag University Hospitals.

METHODOLOGY

Study design and patients

This study was carried out in the Departments of Medical Microbiology & Immunology and Urology, Faculty of Medicine, Sohag University during the period from May 2014 to May 2015. Urine samples were collected from patients with suspected UTI whether admitted in the Department of Urology or out patients attending the Urology Outpatient Clinic of Sohag University Hospitals. Populations included in the study were inpatients had hospital acquired UTI developed 48 to 72 hours after patient's admission and outpatients had community acquired UTI only with positive urine cultures for *E. coli* regardless of the type of UTI. All individuals were subjected to complete history taking and clinical examination. Oral informed consents were obtained from the patients. The study was approved by the ethical committee of Sohag Faculty of Medicine.

Microbiological analysis:

- Midstream urine samples were obtained from the studied patients in sterile wide mouthed containers with cap tops after instructing them to clean the genital area with soap and water. Morning urine samples were obtained whenever possible. In case of catheterized patients; the urine sample was collected through a sterile syringe after 10 min of clamping the catheter. The needle inserted proximal to the site of clamping under aseptic precautions. Transferring it to a sterile cup and affixing the lid. The specimens were immediately transferred to the laboratory for microbiological examination. Urine samples were examined either immediately (within 2 hours) or, if not possible, refrigerated at 4°C to be examined within 24 h [13].
- Macroscopic examination of urine for its color, turbidity, or frank hematuria was done.
- Microscopic examination of wet mount of one loopful of uncentrifuged urine was done directly using ×10, ×40 lens for the presence of pus cells.
- Urine samples were inoculated on MacConkey agar *(Oxoid Ltd., Basingstoke UK)*, Cystine-Lactose-Electrolyte-Deficient (CLED) agar with bromo-thymol blue/Andrade's indicator *(Mast Group Ltd., UK)* and Eosin Methylene Blue agar (EMB agar) *(Oxoid Ltd., Basingstoke UK)* for selection and identification of *E. coli* using sterile disposable calibrated loops (1 µl &10 µl) to perform viable count. All inoculated plates were incubated aerobically at 37□c for 24-48 hours. After incubation, the colonies on plates were counted. Counts ≥ 10^5 CFU/ ml of uncentrifuged urine were considered UTI. Bacterial counts less than this were considered insignificant. (Figure 1)
- The isolated bacteria were then further identified as *E. coli* by colony morphology, Gram staining and their biochemical characteristics were detected using RapID ONE system kits *(Oxoid Ltd., Basingstoke UK)*. (Figure 2)

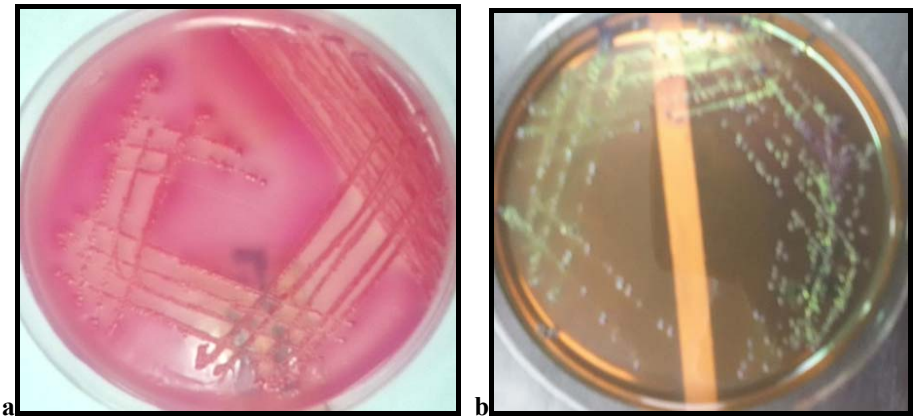

Fig. 1: a) Bright pink semi-translucent lactose fermenting *E. coli* colonies on CLED agar with Andrade's indicator medium. b) Green metallic sheen of *E. coli* colonies on EMB agar medium.

Fig. 2: RapID ONE test strip with a panel that was identified as *E. coli.*

Antibiotic Susceptibility Testing:

Antibiotic susceptibility of ***E. coli*** isolates was determined by modified Kirby-Bauer disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines *(CLSI,2014)*[14] using commercially available discs *(Oxoid Ltd., Basingstoke UK*): Penicillins {Ampicillin(10µg)}, β-Lactam/β-Lactamase inhibitor combinations {Amoxicillin- clavulanate (20/10µg), Ampicillin-sulbactam (10/10µg)}, Cephems {Cefazolin (30µg), Cefepime (30µg), Cefotaxime (30µg), Ceftriaxone (30µg), Cefuroxime sodium (30µg), Ceftazidime (30µg)}, Monobactams {Aztreonam (30µg)}, Carbapenems {Ertapenem (10µg), Imipenem (10µg), Meropenem (10µg)}, Aminoglycosides {Gentamycin (10µg),Amikacin (30µg)}, Fluoroquinolones {Ciprofloxacin (5 µg), Norfloxacin (10 µg)},Quinolones {Nalidixic acid (30µg)}, Folate pathway inhibitors {Trimethoprim-sulfamethoxazole (25µg)}, Fosfomycins {Sulbactam-sefoperazone (105µg)}, Nitrofurans {Nitrofurantoin (300 µg)}. After incubation at 37˚C for 24 h; inhibition zone diameters were read and the examined isolates were reported as susceptible, intermediate, or resistant to the antibiotics (Table 1). Multi Resistant strains were divided into MDR (Multidrug-Resistant), XDR (Extensively Drug-Resistant) and PDR (Pandrug-resistant). MDR bacteria are defined as resistant to at least three different classes of antibiotics. XDR bacteria are characterized by their sensitivity to only one class of antibiotics and the PDR bacteria are resistant to all classes of antibiotics recommended for treatment [15].

Table 1: Performance standards for antimicrobial susceptibility testing according to CLSI guidelines *(CLSI, 2014)*

Antimicrobial agent	Conc.	Zone diameter interpretive criteria		
		Sensitive	Intermediate	Resistant
Ampicillin	10 µg	≥ 17	4-16	≤ 13
Amoxicillin/clavulanate	20/10 µg	≥ 18	14-17	≤ 13
Ampicillin/ sulbactam	10/10 µg	≥ 15	12-14	≤ 11
Cefazolin	30 µg	≥23	20-22	≤ 19
Cefepime	30 µg	≥25	19-24	≤ 18
Cefotaxime or ceftriaxone	30 µg	≥26	23-25	≤ 22
Cefuroxime sodium	30 µg	≥18	15-17	≤ 14
Ceftazidime	30 µg	≥21	18-20	≤ 17
Aztreonam	30 µg	≥21	18-20	≤ 17
Ertapenem	10 µg	≥22	19-21	≤ 18
Imipenem	10 µg	≥23	20-22	≤ 19
Meropenem	10 µg	≥23	20-22	≤ 19
Gentamycin	10 µg	≥15	13-14	≤ 12
Amikacin	30 µg	≥17	15-16	≤ 14
Ciprofloxacin	5 µg	≥31	21-30	≤ 20
Norfloxacin	10 µg	≥17	13-16	≤12
Nalidixic acid	30 µg	≥19	14-18	≤13
Trimethoprim/ sulfamethoxazole	25 µg	≥16	11-15	≤ 10
Sulbactam/sefoperazone	105 µg	≥16	13-15	≤12
Nitrofurantoin	300 µg	≥17	15-16	≤14

Phenotypic Detection of extended – spectrum β - Lactamase (ESBL) producing isolates using double disc diffusion test (DDDT):

All tested ***E. coli*** isolates were screened for ESBL production using the double disc diffusion test (DDDT). ESBL Kits were obtained from *(Oxoid Ltd., Basingstoke UK*) and they included: cefotaxime (30 µg), cefotaxime/clavulanic acid (30/10 µg), ceftazidime (30 µg), ceftazidime/clavulanic acid (30/10 µg), cefpodoxime (30ug) and cefpodoxime/ clavulanic acid (30/10 µg). The organism was interpreted as ESBL producer if there was an increase of ≥ 5 mm in the inhibition zone diameter of the combined disc when compared to the corresponding cephalosporin disc alone. The performance and interpretation were based on the recommendations of CLSI guidelines *(CLSI, 2014)* [14]. (Figure 3)

Fig. 3: DDDT with increase of inhibitory zone diameter around ceftazidime/clavulanic acid disk ≥5 mm as compared to the ceftazidime disk alone indicating ESBL producing *E. coli* isolate.

Genotypic detection of ESBL genes (CTX-M-I, SHV and TEM genes) by PCR:

Sample treatment & DNA extraction were done according to the manufacturer's instructions by the use of QIAamp DNA Kits *(QIAGEN GmbH, Germany)*. The primers sequences *(synthesized by Metabion International AG, Germany)*, sizes of the expected amplification products and thermal cycling conditions used for PCR amplification for the detection of *bla* $_{\text{CTX-M-I}}$, *bla* $_{\text{SHV}}$ and *bla* $_{\text{TEM}}$ genes in this study were listed in Table 2.

Table 2: Primers sequences and thermal cycling conditions used for PCR amplification

Target genes	Primers used (sequence 5´-3´)	Thermal cycling condition	PCR product size
CTX-M-I Forward Reverse	GACGATGTCACTGGCTGAGC AGCCGCCGACGCTAATACA [16]	1 min of denaturation at 95°C (1 cycle), followed by 30 cycles of amplification; each of heat denaturation at 95 °C for 45 s, primer annealing at 56.9 °C for 45 s, and DNA extension at 72 °C for 1 min then one cycle for final extension at 72°C for 10 minutes.	499 bp
SHV Forward Reverse	TCAGCGAAAAACACCTTG CCCGCAGATAAATCACCA [17]	1 min of denaturation at 95°C (1 cycle), followed by 30 cycles of amplification; each of heat denaturation at 95 °C for 45 s, primer annealing at 56.9 °C for 45 s, and DNA extension at 72 °C for 1 min then one cycle for final extension at 72°C for 10 minutes.	471 bp
TEM Forward Reverse	GAGTATTCAACATTTCCGTGTC TAATCAGTGAGGCACCTATCTC [17]	1 min of denaturation at 95°C (1 cycle), followed by 30 cycles of amplification; each of heat denaturation at 95 °C for 45 s, primer annealing at 57.4 °C for 45 s, and DNA extension at 72 °C for 1 min then one cycle for final extension at 72°C for 10 minutes.	861 bp

PCR and DNA amplification:

In a sterile thermal cycler tube, 25µl PCR reaction mix containing 12.5µl PCR Master Mix *(Jena Bioscience GmbH, Lobstedter, Germany)*, 8 µl PCR grade water, 1.25 µl of each primer and 2 µl of the extracted DNA sample was added. In each set of experiments, a negative control was included. The negative control was prepared by replacing the DNA template with PCR grade water. Biometra thermal cycler *(T Gradient software PCR system version 4 - Biometra Whatman Company, Goettingen, Germany*) was used for amplification of DNA. Agarose gel electrophoresis was done as 10µl of each amplified DNA & 100bp ladder (molecular weight marker) *(GeneDireX)* were separated on 2% agarose gel stained with ethidium bromide using *Electrophoresis power supply (Biometra Whatman Company, Goettingen, Germany)*. The bands were visualized using UV transillumination and photographed using *InGenius3; gel documentation system (Syngene, Synoptics Ltd).*

Statistical Analysis

Data were analyzed using IBM-SPSS version 22 *(Chicago, USA, 2013)*. Qualitative data were expressed as number and percentage and quantitative data were expressed as mean ± standard deviation (SD). Chi square test was used to compare frequencies of qualitative data and Student's t test was used to compare means in quantitative data. *P* values < 0.05 were considered significant.

RESULTS

- A total of 200 urine samples were collected from; outpatients (72, 36%) and inpatients (128, 64%); 69 (34.5%) catheterized and 59 (29.5%) non catheterized had symptoms suggestive of UTI. The age of patients ranged from 2 - 80 years (mean 41 years). These patients were 130 (65%) males and 70 (35%) females.

- Of the total samples collected; 44 isolates (22%) were identified as *E. coli* by conventional and biochemical methods. *E. coli* was the causative agent of significant bacteriuria for 22.2% of outpatients and for 21.8% of inpatients (29% catheterized patients and 13.6% non catheterized patients). However, these differences were not statistically significant ($P > 0.05$). *E. coli* isolates were statistically significantly detected in female patients (22 cases (31.4%)) compared to male patients (22 cases (16.9%)) ($P < 0.05$). (Table 3)

Table 3: Characteristics of the studied patients

Variable		Total	*E. coli*		Chi square	*P* value
			Negative	Positive		
Sex	**Male**	130 (65%)	108(83.1%)	22(16.9%)	5.579	**0.018* (S)**
	Female	70(35%)	48(68.6%)	22(31.4%)		
Hospital admission	**Community acquired infection**	72(36%)	56(77.8%)	16(22.2%)	4.414	0.110 (NS)
	Hospital acquired infection – Catheterized patients	69(34.5%)	49(71%)	20(29%)		
	Hospital acquired infection - non catheterized patients	59(29.5%)	51(86.4%)	8(13.6%)		

* S: Significant, NS: Non Significant

Antimicrobial susceptibility testing of *E. coli* isolates:

Only nitrofurantoin had 100% efficacy against *E. coli* isolates. Imipenem, gentamycin, sulbactam-sefoperazone and amikacin showed over 90% efficacy while ertapenem and meropenem showed over 80% efficacy. On the other hand; *E. coli* isolates showed statistically significant higher drug resistance rates among inpatients (hospital acquired infections) than outpatients (community acquired infections) and among catheterized than non catheterized inpatients especially for ampicillin, amoxicillin-clavulanate and ceftazedime ($P < 0.05$). The highest resistance to antibiotics in patients with community-acquired infections was obtained for Cefazolin and Cefuroxime sodium (100%), followed by norfloxacin and nalidixic acid (87.5%), and ciprofloxacin (81.2%). The highest resistance to antibiotics in catheterized patients with nosocomial infections was obtained for ampicillin, cefuroxime sodium, ciprofloxacin and norfloxacin (100%), followed by Ceftazidime and nalidixic acid (95%), and Cefotaxime/Ceftriaxone (90%). The highest resistance to antibiotics in non catheterized patients with nosocomial infections was obtained for Cefuroxime sodium (100%), followed by ampicillin, cefazolin, cefepime ciprofloxacin, norfloxacin and nalidixic acid (75%), and cefotaxime/ceftriaxone, ceftazidime and aztreonam (50%). (Table 4)

Table 4: Antimicrobial resistance pattern of *E. coli* isolates among different patients groups

Antibiotics	Total resistant isolates		Resistance among community acquired infection	Resistance among hospital acquired, catheterized patients	Resistance among hospital acquired non catheterized patients	*P* value
	No.	%				
Ampicillin	35	79.5%	9(56.2%)	20 (100%)	6(75%)	**0.005* (S)**
Amoxicillin-clavulanate	22	50.0%	4(25%)	16(80%)	2(25%)	**0.001* (S)**
Ampicillin-sulbactam	18	40.9%	4(25%)	16(80%)	2(25%)	0.063 (NS)
Cefazolin	39	88.6%	16(100%)	17(85%)	6(75%)	0.150 (NS)
Cefepime	33	75.0%	10(62.5%)	17(85%)	6(75%)	0.301 (NS)
Cefotaxime/Ceftriaxone	32	72.7%	10(62.5%)	18(90%)	4(50%)	0.051 (NS)
Cefuroxime sodium	44	100%	16(100%)	20(100%)	8(100%)	-
Ceftazidime	35	79.5%	12(75%)	19(95%)	4(50%)	**0.024* (S)**
Aztreonam	28	63.6%	10(62.5%)	14(70%)	4(50%)	0.606 (NS)
Ertapenem	8	19.2%	2(12.5%)	6(30%)	0	0.135 (NS)
Imipenem	4	9.1%	2(12.5%)	2(10%)	0	0.593 (NS)
Meropenem	6	13.6%	2(12.5%)	4(20%)	0	0.374 (NS)
Gentamycin	2	4.5%	0	1(5%)	1(12.5%)	0.379 (NS)
Amikacin	1	2.3%	0	1(5%)	0	0.541 (NS)
Ciprofloxacin	39	88.6%	13(81.2%)	20(100%)	6(75%)	0.086 (NS)
Norfloxacin	40	90.1%	14(87.5%)	20(100%)	6(75%)	0.097 (NS)
Nalidixic acid	39	88.6%	14(87.5%)	19(95%)	6(75%)	0.316 (NS)
Trimethoprim- sulfamethoxazole	21	47.7%	6(37.5%)	13(65%)	2(25%)	0.094 (NS)
Sulbactam-sefoperazone	2	4.5%	2(12.5%)	0	0	0.160 (NS)
Nitrofurantoin	0	0	0	0	0	-
Total number	44	100%	16	20	8	

**P* value was significant if less than 0.05

The prevalence of ESBL producing *E. coli* isolates among different patients groups:

High prevalence level of ESBL producing *E. coli* isolates was detected in the present study by DDDT. Of the collected 44 *E. coli* isolates; 39 (88.6%) were ESBL producers. The rate of ESBL producing *E. coli* was statistically significantly higher in hospitalized patients than out-patients and it was statistically significantly higher among catheterized hospitalized patients reaching up to 100% of cases; compared to only 81% and 75% among out-patients and non catheterized hospitalized patients; respectively ($P < 0.05$). (Table 5)

Table 5: The prevalence of ESBL producing *E. coli* isolates among different patients groups

Variable	ESBL		Total
	Positive	Negative	
Community acquired infection	13 (81%)	3 (19%)	16 (100%)
Hospital acquired infection, catheterized patients	20 (100%)	0 (0%)	20 (100%)
Hospital acquired infection, non catheterized patients	6 (75%)	2 (25%)	8 (100%)
Total	39 (88.6%)	5 (11.4%)	44 (100%)

Chi square = 4.908, *P* value = 0.032 (S)

Antimicrobial resistance pattern of ESBL-producing *E. coli* isolates:

The ESBL-producing *E. coli* isolates had significantly increased antibiotic resistance rate compared to non–ESBL producers to; amoxicillin-clavulanate, ampicillin-sulbactam, cefotaxime/ ceftriaxone, ceftazidime, aztreonam, ciprofloxacin, norfloxacin, nalidixic acid and trimethoprim-sulfamethoxazole ($P < 0.05$). However; there was no significant difference in the resistance level to gentamicin and amikacin between ESBL producing and non ESBL producing *E. coli* ($P > 0.05$). Nitrofurantoin followed by amikacin was the most effective drugs against ESBL producing isolates. In the present study, *E. coli* isolates expressed multi drug resistance (MDR) phenotype at high level, as twenty- eight (71.8 %) of the ESBL-producing *E. coli* isolates were found to be MDR; resistant to 3 or more antibiotic classes. (Table 6)

Table 6: The antimicrobial resistance percentages of ESBL-producing and non ESBL-producing *E. coli* isolates

Antibiotic	Resistance among ESBL positive cases	Resistance among ESBL negative cases	*P* value
Ampicillin	32(82.1%)	3(40%)	0.250 (NS)
Amoxicillin-clavulanate	22(56.4%)	0	**0.018* (S)**
Ampicillin-sulbactam	18(46.2%)	0	**0.048* (S)**
Cefazolin	34(87.2%)	5(100%)	0.395 (NS)
Cefepime	31(79.5%)	2(40%)	0.055 (NS)
Cefotaxime/Ceftriaxone	32(82.1%)	0	**<0.001* (HS)**
Cefuroxime sodium	39(100%)	5(100%)	-
Ceftazidime	35(89.7%)	0	**<0.001* (HS)**
Aztreonam	28(71.8%)	0	**0.004* (S)**
Ertapenem	8(20.5%)	0	0.263 (NS)
Imipenem	4(10.3%)	0	0.453 (NS)
Meropenem	6(15.4%)	0	0.345 (NS)
Gentamycin	2(5.1%)	0	0.604 (NS)
Amikacin	1(2.6%)	0	0.717 (NS)
Ciprofloxacin	38(97.4%)	1(20%)	**<0.001* (HS)**
Norfloxacin	39(100%)	1(20%)	**<0.001* (HS)**
Nalidixic acid	38(97.4%)	1(20%)	**<0.001* (HS)**
Trimethoprim- sulfamethoxazole	21(53.8%)	0	**0.031* (S)**
Sulbactam-sefoperazone	2(5.1%)	0	0.604 (NS)
Nitrofurantoin	0	0	-
Total	39	5	-

*HS: Highly Significant ($P < 0.001$)

Prevalence and distribution of ESBL Genes in *E. coli* isolates:

PCR data of ESBL-producing *E. coli* isolates revealed that; CTX-M-I gene was the most frequent ESBL type detected as it was found in 37 (95%) out of the 39 (100%) ESBL positive cases with highly statistically significant *P* value ($P < 0.001$), followed by TEM gene which was positive in 21 cases (53.8%) with a statistically significant *P* value ($P < 0.05$) and lastly SHV gene which was detected in 4 cases (10.3%). CTX-M-I was detected in all (100%) of ESBL-producing *E. coli* isolated from outpatients and non-

catheterized hospitalized patients, while it was detected in 90% of those isolated from catheterized hospitalized patients. TEM gene was detected more in ESBL-producing *E. coli* isolated from catheterized hospitalized patients (65%) than non-catheterized hospitalized patients (50%) and outpatients (30.8%). SHV gene was only detected in ESBL-producing *E. coli* isolated from hospitalized catheterized patients (20%). (Table 7) (Figure 4)

Table 7: Prevalence and distribution of ESBL genes among different patients groups

ESBL positive *E. coli* isolates	Total	Prevalence of TEM gene	Prevalence of SHV gene	Prevalence of CTX-M-I gene
Community acquired infection	13(100%)	4 (30.8%)	0	13 (100%)
Hospital acquired infection, catheterized patients	20(100%)	13 (65%)	4 (20%)	18 (90%)
Hospital acquired infection, non catheterized patients	6(100%)	3 (50%)	0	6 (100%)
Total	39(100%)	21 (53.8%)	4 (10.3%)	37 (95%)
value		**0.023*(S)**	0.453	**<0.001*(HS)**

Twenty- one (53.8%) out of the 39 ESBL-producing ***E. coli*** isolates carried more than one type of ESBL genes. The majority of ESBL positive cases contained *bla* $_{\text{CTX-M-I}}$ either alone (16 cases, 41%), combined with *bla* $_{\text{TEM}}$ (17 cases, 43.6%), combined with *bla* $_{\text{SHV}}$ (2 cases, 5.1%) or combined with both (2 cases, 5.1%). *bla* $_{\text{TEM}}$ alone was found only in 2 cases (5.1%). *bla* $_{\text{SHV}}$ was not found alone or combined with *bla* $_{\text{TEM}}$ only. None of the ESBL positive cases by DDDT was negative for the three genes by PCR. Regarding ESBL negative cases by DDDT; only one case (20%) was positive for *bla* $_{\text{CTX-M-I}}$ gene by PCR. (Table 8)

Table 8: Distribution of *CTX-M-I, TEM*, and *SHV* genes in ESBL-producing *E. coli* isolates

ESBL genes detected by PCR	ESBL by DDDT	
	Positive	**Negative**
CTX-M-I alone	16(41%)	1(20%)
SHV alone	0	0
TEM alone	2(5.1%)	0
CTX-M-I + SHV	2(5.1%)	0
CTX-M-I + TEM	17(43.6%)	0
SHV + TEM	0	0
CTX-M-I + SHV + TEM	2(5.1%)	0
None	0	4(80%)
Total	39 (89%)	5 (11%)

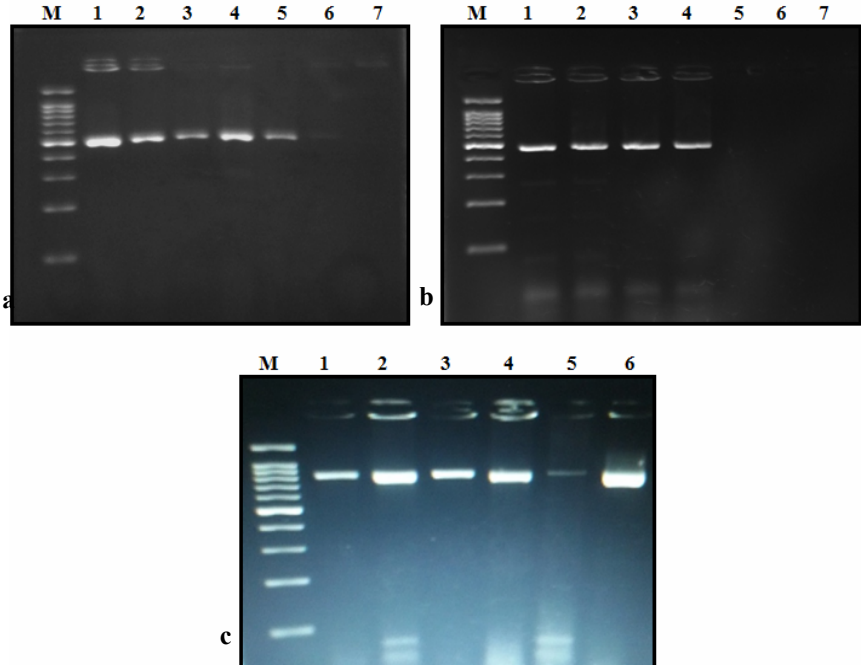

Fig. 4: Gel electrophoresis of PCR results of ESBL genes; M: M.W. marker 100 bp a) CTX-M-I-β-lactamase gene: 499 bp (lanes 1- 5). b) SHV-β-lactamase gene: 471 bp (lanes 1- 4). C) TEM-β-lactamase gene: 861 bp (lanes 1- 6).

DISCUSSION

Urinary tract infections (UTIs) are among the most common infectious diseases encountered in clinical practice all over the world with a high rate of morbidity and economic burden to health care systems [18]. In the present study of the total 200 urine samples collected from patients had symptoms suggestive of UTI; 44 isolates (22%) were identified as *E. coli. E .coli* was reported as the commonest isolated uropathogen either in community or hospital acquired UTI by many studies as that reported by Al Sweih et al.[19] in kwauit who found *E .coli* in 47% of cases, by Abujnah et al. [20] in lypia (12%) and by Daoud et al. [21] in Lebanon (60.5%). In our study *E. coli* was statistically significant isolated from female patients (31.4%) compared to male patients (16.9%) *(P* < 0.05) as expected; because of the well-known anatomical and physiological risk factors predispose females to UTI like shorter urethra, close proximity of the urethra to the perianal region and sexual activity. This was in agreement with results which were reported by previous studies like Linhares et al. [22]. Farajnia et al. [23] added that; males are less prone to UTI, not only because of their longer urethra but also because of the presence of antimicrobial substances in the prostatic fluid. Of much concern in our study was the finding of high antimicrobial resistance levels of the *E. coli* isolates and the majority of these isolates (28, 63.6%) were multi-drug resistant. There was a higher drug resistance rates among inpatients than outpatients and among catheterized than non catheterized inpatients especially for ampicillin, amoxicillin-clavulanate and ceftazedime (*P* < 0.05).

These high antibiotic resistance rates of the isolated *E. coli* from our clinical samples can be explained by the fact that; there was extensive use of broad spectrum antibiotics especially third generation cephalosporins in our hospitals and also in the community, which is usually empirical and without documented proof of infection besides the lack of strict infection control practices which predisposed to persistence of the resistant strains in our health care facilities. So, as bacteria have developed different strategies to counter the effects of antibiotics, the identification of the resistance mechanism may help in the control of such resistant pathogens. One of the most important antibiotic resistance mechanisms is the emergence of ESBL producing organisms. In the present study; high prevalence level of ESBL producing *E. coli* was recorded by DDDT, as of the collected 44 *E. coli* isolates; 39 (88.6%) were ESBL producers. The rate of ESBL producing *E. coli* was statistically significantly higher in hospitalized patients than out-patients and it was statistically significantly higher among catheterized inpatients reaching up to 100% of cases; compared to only 81% and 75% among non hospitalized and non catheterized inpatients; respectively (*P*<0.05). The overall data on ESBL-producing *E.coli* in our country are extremely worrisome as similar high ESBL rates were reported in studies from Egypt; as in an Egyptian study targeting catheter associated urinary tract infection; ESBLs were detected in 78.6% (11/14) of isolated *E.coli* [24].

Prolonged use of the catheters without any preventing measures for catheter associated infections. The lack of antibiotic policy and overuse of extended-spectrum cephalosporins in hospitals and absence of strict hospital infection control policies explain this high rate. Another Egyptian study by Al-Agamy et al. [25] reported high ESBL rate, 60.9% in *E.coli* isolated from nosocomial UTI. In our study; the rate of ESBL in the isolated *E.coli* from community acquired UTI was (81%) which was alarming to the spread of ESBL in the community. A lower rate were reported in Iran 22%[26], in Nebal (13.51%) [27] and in Denmark (1.5%) [28].

Overuse of extended-spectrum cephalosporins as first choice treatment in community acquired UTI, adding to this improper use and incomplete course were the main reasons for such high resistance rate. In our study, ESBL-producing isolates had significantly (*P* < 0.05) increased resistance compared to non–ESBL producers to Amoxicillin-clavulanate, Ampicillin-sulbactam, Cefotaxime/Ceftriaxone, Ceftazidime, Aztreonam, Ciprofloxacin, Norfloxacin, Nalidixic acid, and Trimethoprim- sulfamethoxazole. The obtained results revealed co-resistance to non-β-lactam antibiotics among ESBL producers, especially for fluoroquinolones, co-trimoxazole and/or aminoglycosides. This could be explained by the fact that; the genes encoding ESBLs are usually located in transferable plasmids allowing them to spread between bacteria and many of these plasmids carry other resistance determinants, such as those for resistance to aminoglycosides, tetracyclines, chloramphenicol, Trimethoprim- sulfamethoxazole and quinolones so; acquisition of a single plasmid leads to multi-drug resistance [29].

One of the most prominent and concerning findings in our study was the high resistance rate to Fluoroquinolones which was the highest among hospitalized catheterized patients reaching up to 100% of cases, compared to only 87.5% and 75% among those had community acquired infections and hospitalized non catheterized patients respectively. ESBL isolates were markedly associated with Flouroquinolones resistance (100%) the link between the resistance of the two groups together was reported by many studies and was linked to plasmid transfer of quinolone resistance genes (PMQR) as most enzymes from ESBL-producing organisms can be horizontally transmitted by the same plasmids harboring qnr genes [30&31].

And because fluoroquinolones are increasingly relied on for empirical therapy of UTI [32]; fluoroquinolone resistance in *E. coli* poses a substantial threat among our patients, increasing the risk of treatment failure. In the present study, although, there were high resistance rates to different groups of antibiotics in the ESBL producing *E.coli* isolates; but still there was 100% sensitivity to nitrofurantoin and good sensitivity to aminoglycosides (95%) and carbapenems (85%) making them available options for the treatment of those patients. Of much concern was our finding that; ^{bla}CTX-M-I was the most prevalent β-lactamase-encoding gene in ESBL producing *E.coli* isolates. It was detected in almost 95% of isolates. However; ^{bla}TEM and ^{bla}SHV genes were detected in about 53.8% and 10.3% of isolates, respectively. Many studies also reported that ^{bla}CTX-M-I was the most prevalent type among ESBL producing *E. coli* in Egypt. Like the study done by Al-Agamy et al.[25] who reported that; CTX- M was detected in 100% of isolated ESBL producing *E.coli* [33].

To the best of our knowledge, this is the first report in our locality that has identified ^{bla} CTX-M ESBL type in our clinical samples. Worldwide; several studies reported that ^{bla}CTX-M was the most prevalent ESBL type among ESBL producing *E. coli* [34, 35 & 36] . In the present study; 21 (53.8%) out of 39 ESBL producing *E. coli* isolates carried more than one type of β-lactamase genes as, ^{bla}CTX-M was found combined with bla_{TEM} in 43.6% of cases, combined with bla_{SHV} in 5.1% of cases or combined with both in 5.1% of cases. So, we can deduce that; ^{bla}CTX-M-producing *E. coli* strains are currently a problem not only in our hospitals but also in the community which may be related to the misuse of third generation cephalosporins, especially cefotaxime. In the present study an important finding which should be discussed was the high resistance rate of ESBL producing *E. coli* isolates to β-Lactams/β-lactamase inhibitor combinations as amoxicillin-clavulanate (56.4%) and ampicillin-sulbactam (46.2%). This could be explained by the fact that; ß-lactam / ß-lactmase inhibitors are usually active against organisms producing a single ESBL type, however their effectiveness may be reduced in organisms producing multiple ESBLs [37] which was recorded in our study. Therefore, isolation and detection of ESBL producing strains are essential for the selection of the most effective antibiotic for treatment. We can say that our study was an alarming sign as ESBL producers are resistant to many classes of antibiotic resulting in limited treatment options. Overall, continuous monitoring and effective infection control measures, rational use of drugs and also the use of aminoglycosides and carbapenems instead of extended-spectrum cephalosporins and quinolones for treating infections in which ESBL-producing strains are likely to emerge could be effective.

CONCLUSION

Our data pointed out that the prevalence of ESBL-producing *E. coli* is high in our locality whether causing community or hospital- acquired UTI in Sohag University Hospitals which necessitate urgent restriction of the consumption of extended-spectrum cephalosporins. ^{bla}CTX-M-I group was the most prevalent ESBL type, especially in combination with ^{bla}TEM enzymes. Our data showed a significant trend toward flouroquinolone resistant *E. coli* emergence. This trend should be taken into consideration when treating UTI. Continued antibiotics resistance surveillance, determination of molecular characteristics of ESBL isolates, implementation of strict infection control measures especially for catheterized patients and the judicious use of antimicrobial drugs are recommended to decrease this threatening high antibiotic resistance rate.

Conflict of Interests

The authors stated that they have no conflict of interests.

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