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ORIGINAL ARTICLE

Detection of β-lactamase genes (blaTEM, bla CTX-M and bla SHV) in uropathogens isolated from patients with UTI

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Key words:
Beta-lactamase genes{bla(TEM), bla(CTX-M), and bla(SHV)} , uropathogens, and UTI

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ABSTRACT

Background: *The problem of antibiotic resistance has become worldwide health issue among hospitalized patients in developing countries, where the inappropriate usage of antibiotics is common. The widespread occurrence of multidrug resistant strains (MDRS) of Escherichia coli (E. coli) and klebsiella pneumoniae (K. pneumoniae) is currently considered avital medical challenge. The identification of ESβL genes and their MDRS in widely isolated uropathogens may provide a helpful tool for choosing effective antibiotic therapy and clinical improvement.* ***Objective:*** *The aim of this study is to determine the prevalence of (bla.TEM, bla.CTX-M, and bla.SHV) β-lactamase DNAs in E. coli and K. pneumoniae, and MDRS of uropathogens E. coli and k. pneumoniae isolated from patients urine UTI diagnosed who were admitted to the Urology & Nephrology Center.* ***Methodology:*** *Clinical E. coli and K. pneumoniae isolates were isolated from urine specimens of UTI diagnosed patients. Microbiological techniques were utilised to identify the isolates. Antibiotics susceptibility analysis was done utilising the Vitek2 system (bioMérieux, Marcy l'Etoile, France). The double disc diffusion technique (DDDT) was used for detecting phenotypic ESBL in isolates. Using the traditional PCR technique, the ESBL genes were identified.* ***Results:*** *Antibiotic resistance of E. coli's isolates to commonly used antibiotics was high (88.2% resistant to Amoxicillin/clavulanate, 83.5 % were resistant to Trimethoprim-sulfamethoxazole) while minimum resistance appeared against Amikacin (AK) 10.5% and Imipenem (IP) 14%. For isolates of K. pneumoniae, the resistance was higher for nitrofurantoin, ceftazidime, cefotaxime, ciprofloxacin 96.6%, 83.3 %,83.3%, 80% respectively .In our study, out of 115 isolates, E. coli and K. pneumoniae isolates, 81 (70.43%) were phenotypically positive for ESβL by Combination Disc Test (CDT). The most commonly molecularly detected ESBL genes were TEM 80% of K. pneumoniae isolates and 77.6% of E. coli isolates .followed by SHV 70% of K. pneumoniae isolates and 34.1% of E. coli isolates and finally, CTXM 67% of isolates of E. coli and 63.3% of isolates of K. pneumoniae.* ***Conclusions:*** *The current study concluded that a high rate of resistance has been developed in uropathogens with empiric antibiotic treatment. It emphasized the alarming role of β-lactamases, especially ESßLs in antibiotic*

INTRODUCTION

Urinary tract infections (UTIs) are considered to be one of the most important and frequent infectious diseases, especially among adults [1]. In both healthcare-associated and community-acquired UTIs, *E. coli* is the most prevalent bacterial pathogens accounts for *70–80* % of entirely uropathogens [1-3].

While most cases of UTIs are effectively treated as β-lactams, β-lactam/β-lactamase inhibitors, flouroquinolones, and carbapenems using antimicrobial therapy, many of extracted urological pathogens have become progressively resistant to majority of antibiotics[4-5].

ß-lactamases are enzymes that are a major reason of bacteriological resistance to antibiotics such as penicillins, cephalosporins, cephamicins and carbapenems in beta-lactam family. This enzymes catalyse hydrolysis of amide bond (-CO-NH-) of a four-membered beta-lactam ring, making antibiotics ineffective against the cell wall transpeptidase, which was the antibiotic's original target [6]. Beta-lactamases are categorized into four groups based on their primary structure: A, B, C, and D. Class (A), (C), and (D) enzymes are active-site serine enzymes, while class (B) enzymes are zinc-metalloenzymes [6-7]. By hydrolyzing antibiotics, beta-lactamase confers resistance to bacteria against penicillins, first, second and third generation cephalosporins, and aztreonam, and is blocked by β-lactamase inhibitors [8].

The bla (CTX-M) enzymes are a group of molecular class "A" extended spectrum β-lactamases that display a

global preference for ceftriaxone and cefotaxime, than towards ceftazidime, as well bla(CTX-M) has a higher susceptibility to tazobactam than to clavulanate[8] and are categorized by an active-site-serine, a molecular weight of around 29.0 KDa, and preferential penicillins hydrolysis [9].

Most β-lactamase are derivatives of TEM or SHV enzymes [10-11]. There are now adays more than 90 TEM-types of β-lactamases and >25SHV-type enzymes. β-lactamase of types TEM- and SHV- are mostly produced by *E. coli* and *K. pneumoniae*; however, they are present in *Proteus*, *Providence*, and other strains of *Enterobacteriaceae* species.

In Gram-negative bacteria, TEM-1 is the most encountered β-lactamase, since production of TEM-1 is accountable for approximately 90% of ampicillin resistance in species of *E. coli*[12]. *K. pneumoniae* is main species that producing SHV-1 β-lactamase, which is responsible for about 20% of plasmid‑mediated‑ampicillin resistance in *K. pneumoniae* species [13]. UPEC is also keeping an eye on the rise in resistance and appearance of MDR strains [14]. Therefore, there is requirement for periodic screening of common bacterial pathogens such as UPEC to control their antibiotic resistance profiles in different health care settings [15-16]. In addition, it is essential to monitor the distribution of genes accompanying the antibiotic resistance. This knowledge can allow us to prevent the spreading of strains with a high risk of MDR expression. In reference to this, current study targeted to investigate predominance of [bla(TEM), bla(CTX-M) and bla(SHV)] β-lactamase genes and evaluation of resistance pattern of urological pathogens *E.coli* and *K. pneumoniae*, which extracted from patients who diagnosed UTI hospitalized in Urology and Nephrology Center, at Mansoura.

METHODOLOGY

Bacterial strains

A total of 115 bacterial isolate 85 *E. coli* and 30 *K. pneumoniae* were isolated from urine samples of inpatients with UTI admitted during the period from August 2019 to August 2020. Identification of bacteriological isolates was performed utilizing conventional biochemical methods [17-18] and evaluating antimicrobial resistance pattern. Detecting β-lactamase genes (bla.CTX-M, bla.TEM, bla.SHV) of uropathogenics *E. coli* and *K. pneumoniae* isolated from urine specimens of inpatients using PCR method [17-18]. Also *E. coli* [ATCC 25922]and *K. pneumoniae* [ATCC BAA-1705] were used as controls.

Antimicrobial susceptibility and MIC investigation

All bacterial isolates were analysed for minimum inhibitory concentration (MIC) by Vitek2 system (bioMérieux, Marcy l'Etoile, France) utilizing card for Gram-negative strains (GN cards) and AST. The antimicrobial agents examined in the current study is listed in table 1. The cards were inoculated and incubated as stated by manufacturer's instructions. The interpretative criteria were applied according to CLSI recommendations [19]. *E. coli* [ATCC 25922] and *K. pneumoniae* [ATCC BAA-1705] were utilised as control strains (Table 1).

Table 1: Antibiotics used in this study

Antibiotic Types	MIC			Conc.range µg/ml
	S	I	R	
Amoxicillin/clavulanate (XL)	≤8	16	≥32	4 - 32
Cefotaxime (CT)	≤8	16	≥64	8 - 64
Ceftazidime (CAZ)	≤8	16	≥32	8 - 32
Imipenem (IP)	≤4	8	≥16	1 - 16
Amikacin (AK)	≤16	32	≥64	2 - 64
Ciprofloxacin (CIP)	≤1	-	≥4	0.25 - 4
Nitrofurantoin (NI)	≤32	64	≥128	16 - 128
Trimethoprim sulfamethoxazole (SXT)	≤2	-	≥4	2 - 4
Piperacillin/tazobactom (TZP)	≤16	32	≥128	8 - 128

S. Susceptible or [sensitive]; I, Intermediate; R, Resistant.

Susceptibility was performed as designated by CLSI that were applied to categorize strains as susceptible, intermediate, or resistant.

Phenotypic analysis of extended‑spectrum betalactamases (ESBLs)

Screening test:

According to CLSI,[20] criteria, microbial resistance to ceftriaxone, cefotaxime (CT), ceftazidime (CAZ), and cefepime, is defined by MICs ≥ 16 ug/ml.

Phenotypic confirmatory examinations for production of ESßL:

According to CLSI [20] ESßL confirmatory analysis was based upon phenotype necessitates using of both ceftazidime (CAZ) and cefotaxime (CT) alone and with clavulanate (XT). ESBL detection by double-disk diffusion test (DDDT) using Ceftazidime, 30µg; Ceftazidime-clavulanic acid, 30/10 µg; Cefotaxime, 30µg; Cefotaxime-clavulanic acid. 30/10µg was

performed. Briefly, after inoculating a Mueller-Hinton Agar plate as for routine DDDT, 30 mg disks of CT and CAZ were placed 30 mm (center to center) from a Ceftazidime 30µg discs+Clavulanic acid 10 µg and Cefotaxime (CT)30µg discs+ Clavulanic acid10 µg. After12-18 hrs at 37 ºC. Enhancement of inhibition zone of at least one of antibiotics towards the area containing clavulanate was taken to indicate the ESßL production.

Molecular detection of β –Lactamase Genes:

DNA was prepared using High Pure PCR Template Purification Kit, (Germany). Genes of TEM and SHV were examined using PCR technique of the related determinants, as mentioned previously [21-23]. The oligonucleotide primers and the base-pairs (bp) specific to SHV and TEM genes for amplified fragment size are described in (table 2).

Table 2: The Oligo-nucleotide primers and amplified fragment size base-pairs (bp) specific for SHV, TEM and CTX-M genes.

primer	Sequence (5′-3′)	Tm	Amplicon size (bp)
E. coli TEM-F TEM-R	**5`ATGAGTATTCAACATTTCCG 3`** **5`CTGACAGTTACCAATGCTCTC3`**	43°C	867bp
E. coli **SHV-F** **SHV-R**	**5`GGTTATGCGTTATATTCGCC 3`** **5`TTAGCGTTGCCAGTGCTC 3`**	46°C	867bp
Kl.pn. **TEM-F** **TEM-R**	**5`ATGAGTATTCAACATTTCCG 3`** **5`CCAATGCTTAATCAGTGAGC 3`**	43°C	717bp
Kl.pn. **SHV-F** **SHV-R**	**5`CTTTACTCGCTTTATCG 3`** **5`TCCCGCAGATAAATCACCA 3`**	42°C	867bp
Universal ***CTX- MA*** ***CTX-MB***	**5`CGCTTTGCGATGTGCAG 3`** **5`ACCGCGATATCGTTGGT 3`**	45°C	550 bp

PCR amplifications were performed in a DNA Thermal-Cycler-9700 instrument (Perkin-Elmer, Cetus, Norwalk, Conn.) with Gene Amp DNA amplification kit. The composition of the reaction mixture was as follows: 10 mMTris-HCl (pH 8.3), 50 mMKCl, 0.1% Triton X-100, 1.5 mM $MgCl_2$, each of 4 deoxynucleoside triphosphates at 0.20 mM a concentration , and 1.2 Unit of AmpliTaq in 50 µL as a total volume. PCR-cycling consisted of step of initial denaturation at 96°C for 5 mins, followed by 40 cycles of DNA-denaturation at 96 °C for 15 s, primer-annealing (Tm) depends on primer in use, and extension-cycle at 72 °C for 2 mins. After last cycle, the products were kept at +4°C. The amplicons were electrophoresing in 1% Agarose gel and visualized after staining with ethidium bromide (EtBr).

Statistical analysis of data

Statistical analyses were achieved employing Statistical Package for Social Sciences (SPSS) for Windows16.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

In our study, the majority of *E. coli* isolates displayed resistance against Amoxicillin/clavulanate (XL) (88.2%),Trimethoprim-sulfamethoxazole (SXT) (83.5%), Ciprofloxacin (78.8%), while minimum resistance was for Amikacin (AK) (10.5%) and imipenem (IP) (14%). For *K. pneumoniae*, most of extracted isolates showed resistance to Nitrofurantoin (NI) 96.6%, Ceftazidime (CAZ), Cefotaxime (CT) (83.3%) whereas minimum resistance was for Amikacin (AK) (33.3%) and imipenem (IP) (46.7%) Table 3.

Table 3: Antibiotic resistance arrays

Organism	XL	CT	CAZ	IP	AK	CIP	NI	SXT	TZP
***E. coli* (85)**	(75/85) 88.2 %	(64/85) 75.3 %	(64/85) 75.3 %	(12/85) 14 %	(9/85) 10.5 %	(67/85) 78.8 %	(39/85) 46 %	(71/85) 83.5 %	(25/85) 29.5 %
***K. pneumoniae* (30)**	(22/30) 73.3 %	(25/30) 83.3 %	(25/30) 83.3 %	(14/30) 46.7 %	(10/30) 33.3 %	(24/30) 80 %	(29/30) 96.6 %	(23/30) 76.7 %	(16/30) 53.3 %

Analysis of 115 isolates of *E. coli* and *K. pneumoniae*, 89 (77.39%) displayed low sensitivity to any antibiotic of third generation cephalosporines (cefotaxime, ceftazidime). A phenotypic confirmatory test was used to examine production of ESßL. For *E. coli*, 64 strains were positive for production of ESßL by MIC screening test, while 58 strains gave positive results for Combination Disc Test (CDT) (Table 4)

Table 4: ESβL producers identified by screening and confirmatory tests

Bacterial isolates	ESBL positive by MIC screening test	ESßL positive combined disk method	*Percentage*	*P*a
***E. coli* (n=85)**	64	58	68.2%	<0.05
***K. pneumoniae*(n=30)**	25	23	76.6%	<0.05
Total isolates (n=115)	89	81	70.4%	

As shown in table (5), out of the 3 beta lactamase genes studied, bla TEM was detected among 24 (80%) of *K. pneumoniae* and 66 (77.6%)of *E. coli* isolates , SHV was detected in 21 (70%) of *K. pneumoniae* and 29(34.1%) of *E. coli* isolates ,while CTXM gene was detected in 57(67%) of *E. coli* and 19 (63.3%) of *K. pneumoniae.*

Table 5: Molecular genetics resistance results of studied isolates

ESβLS Genes	*E. coli* (85)	*K. pneumoniae* (30)	*E coli + K. pneumoniae (115)*
TEM (Temoniera)	(66) 77.6%	(24) 80%	(90) 78.2%
SHV(Sulphydrylvariable)	(29) 34.1%	(21)70%	(50)43.4%
CTXM (Cefotaximase)	(57) 67%	(19)63.3%	(76) 66%

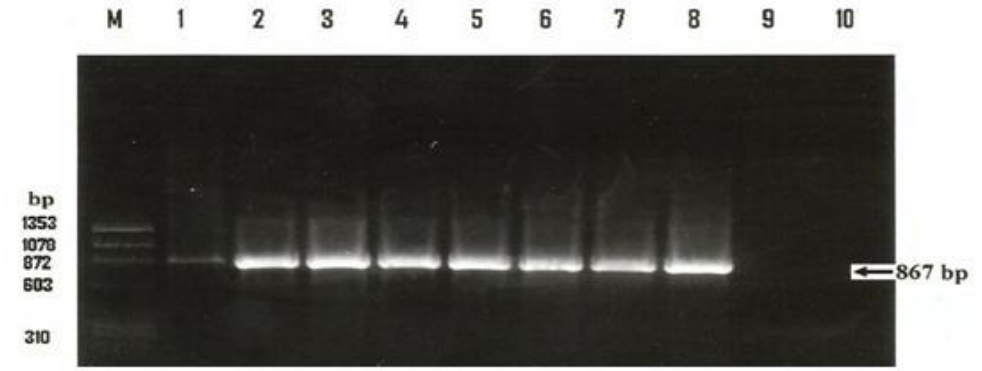

Fig. 1: PCR-Amplified DNA of a selection of TEM gene isolates of *E. coli*. Lane M: DNA molecular weight marker of *Hae.III* digested phage øx174.

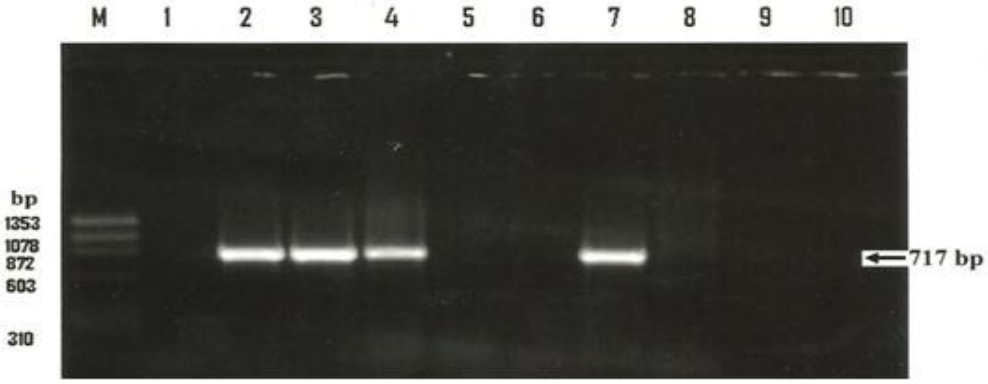

Fig. 2: PCR-Amplified DNA of a selection of TEM gene isolates of *K. pneumoniae*. Lane M: DNA molecular weight marker of *Hae.III* digested phage øx174.

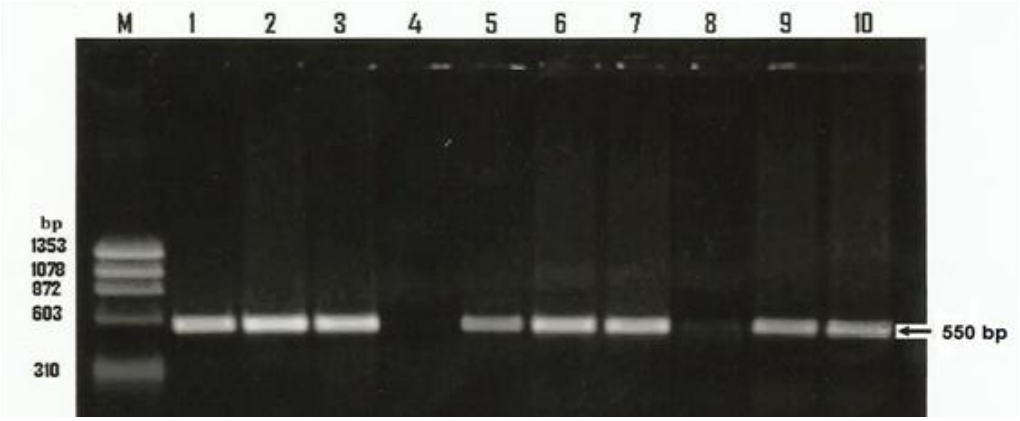

Fig. 3: PCR-Amplified DNA of a selection CTX-M gene isolates of *E. coli* and *K. pneumoniae*. Lane M: DNA molecular weight marker of *Hae.III* digested phage øx174.

DISSCUSSION

The problem of antibiotic resistance in uropathogens is increasing worldwide with limitation of antibiotic choices for treatment of UTI [24]. The rate of ESBLs production in bacterial species differs greatly all over the world, and rapidly changing from time to time. The ESßL's prevalence was established to be over 10% in east Europe, 3.5%in Canada, 7.5%-41% in Persian Gulf region and 20-48.8% in Asian countries [25-26].

In current study, rate of ESßL production among the studied uropathogens was (70.4%), which was higher than a previous research by Odwan et al. (32.7%)[27]. The rate of ESßL production was greater in *K. pneumoniae* (76.6 percent) than in *E. coli* (68.2%) among the ESßL-producing uropathogens isolated in our sample. These findings are consistent with other studies that found a higher rate of ESβL production in *K. pneumoniae* (54.9%) than in *E. coli* (42.4%) [28].

In the present study, most of *E. coli* extracted isolates displayed resistance towards Amoxicillin/ clavulanate (88.2%), Trimethoprim-sulfamethoxazole (83.5%), Ciprofloxacin (78.8%), while minimum resistance was for Amikacin (10.5%) and imipenem (14%). For *K. pneumoniae*, most of the isolates were resistant to Nitrofurantoin 96.6%, Ceftazidime ,Cefotaxime (83.3%) while minimum resistance was for resistance was for Amikacin (33.3%) and imipenem (46.7%)these results showed higher resistance towards imipenem and amikacin in comparison to other related studies that showed imipenem resistance of 6% in *E. coli* and 15 % in *K. pneumoniae*, and amikacin resistance percentage of 4% and 10% for *E. coli* and *K. pneumoniae* respectively[29]. This may be clarified by improper antibiotic usage and prior exposure to several antibiotics in patients with urology problems, and we should keep imipenem as an alternative treatment for difficult cases and when other antibiotics fail to function.

Various studies have reported similar findings of elevating rate of resistance toward ceftriaxone, cefepime and ceftazidime among ESßL producers (93-100%) compared to non ESßL producer s (2.2-4.7%). Elevated resistances were also detected to ciprofloxacin 67.7% and cefotaxime 64.8% [30]. Although incidence of ESßLs-producing bacteria is a worldwide problem, identification of these isolates can vary by countries and institutions within a country [31].

In the present study we investigated 115 isolates of uropathogenic *E. coli* and *K. pneumoniae* isolated from hospitalized patients diagnosed as UTI , for presence of beta lactamase genes bla.TEM, bla.SHV, bla.CTX-M molecular genetic analysis revealed that the most predominant genes was TEM (78.2%) followed by bla.CTX-M ((66%) and bla.SHV 43.4%,

The predominant ESßL genes were bla.TEM in 80% *K. pneumoniae* and 77.6% of *E. coli* isolates. These results are in accordance with the results of a study by Yazdi et al., [32] (87.1% bla.TEM,) but disagreed with the results of studies by Eftekhar et al. [33], in which SHV (43.10%) exceeded bla.TEM (35.20 %). And the results of Ahmad et al 2013 who revealed bla.CTX-M 71.40 % in *E. coli* and 68.40 % in klebsiella exceeded bla.TEM 55.1% E coli and 58% klebsiella [34].

In the present study, bla.SHV detected in 70% of *K. pneumoniae* isolates and 34.10% of *E. coli* while bla.CTX-M was detected in 67% in *E. coli* and 63.3 % of *K. pneumoniae* isolates. When we correlate these results with that of related studies, we found that In Arab countries, the first description of bla.CTX-M was in Egypt and then in United Arab Emirates and Kuwait[35-36]. In these countries, the predominant ESßL was bla.CTX-M-15. After 2000, predominance of bla.CTX-M elevated steadily in Tunisian healthcare setting particularly CTX-M-15, as many studies considered CTX-M-15 most prevalent amongst ESßLs[37-38]. Mnif et al, [39] reported that majority (72.0%) of ESßL are positive in isolates of *E. coli*. High rates were demonstrated in Saudi Arabia; bla.CTX-M-type

ESßLs enzymes accounted for 100% of all ESßLs, and the predominant ESBL was bla.CTX-M-15 (92.1%). A similar trend (98%) was observed in Iran [40].

It is worth mentioning that it is important to determine localization of ESßLs producing strains and extended spectrum genes frequency for establishing local resistance pattern in order to begin with effective empiric antimicrobial treatment.

CONCLUSION

The current study concluded that a high rate of resistance has been developed in uropathogens with empiric antibiotic treatment, including carbapenems and aminogycosides, Therefore, the proper screening is needed for ESßLs detection in laboratories. ESßL-producing uropathogens are an emerging threat with few effective antibiotics. It emphasized the alarming role of β-lactamases, especially ESßLs in antibiotic resistance in uropathogenic *E. coli* and *K. pneumoniae* strains focusing the light into the current prevalence of genetic backgrounds of these strains.

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- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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