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ORIGINAL ARTICLE

Prevalence of Carbapenem-Resistance among Extended Spectrum Beta Lactamase-Producing *E. coli* at Menoufia University Hospitals: Comparison of Phenotypic and Molecular Characterization Methods

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Key words:

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ABSTRACT

Background: *Emergence and global spread of ESBLs and carbapenemase-producing E.coli isolates are of great concern especially in healthcare settings with resultant increases in the rates of morbidity and mortality. Carbapenems are the antimicrobials of last resort to treat serious infections especially in high risk patients. Unfortunately, the high incidence of carbapenemase production seriously threatens this class of life-saving drugs.* ***Objectives:*** *This study was carried out during the period from January to December 2016 involving a total of 172 E. coli isolates collected from the different departments of Menoufia University Hospitals (MUHs).* ***Methodology:*** *Antibiogram was done by the modified Kirby Bauer disk diffusion method. Phenotypic identification of ESBLs production was confirmed by the combined disk synergy test (CD-T) using ceftazidime/ceftazidime-clavulanic acid. PCR-confirmed ESBLs-producing isolates were subjected to amino-phenylboronic acid combined disk (APB-CD) and Ertapenem/EDTA combined disk tests for detection of class A and class B carbapenemases respectively. Conventional multiplex PCR was conducted for detection of ESBLs and carbapenem resistance genes (blaSHV, blaTEM, blaKPC and blaVIM). In vitro susceptibility of E.coli isolates to tigecycline was performed by disk diffusion test.* ***Results:*** *Out of 172 E.coli isolates, 106 (61.6%) were ESBLs-producers of which 29 (27.3%) were carbapenemase co-producers. The blaTEM gene was the most prevalent (77.4%), followed by blaSHV (22.6%).None of the isolates harbored blaTEM and blaSHV together. Co-existence of ESBLs and carbapenemase genes was detected in 27.3% of the ESBL E.coli isolates including blaTEM+blaVIM in 75.8%, blaTEM+blaKPC in 20.7% and blaSHV+blaKPC in 3.5% of E.coli isolates. Aztreonam identified most of ESBL producers, while ertapenem alone identified most of the carbapenemase producers. In relation to PCR results, the sensitivity, specificity and accuracy of the CD-T were 96%, 67% and 85% respectively. While for APB-CD and Ertapenem/EDTA combined disk test, the sensitivity, specificity and accuracy were 86%,98%, 97%,91%,94% and 93% respectively.100% and 93% of ESBLs-and combined ESBLs/carbapenemase-producing E.coli isolates were susceptible to tigecycline.* ***Conclusion:*** *The blaTEM-type ESBLs-producing E. coli are highly prevalent in our locality. Co-existence of blaTEM and blaVIM were also found. Adequate prevention and control of these strains is imperative. Tigecycline can be used for treatment of infection by these strains.*

INTRODUCTION

Nosocomial infections are health problems that reduce the quality of life in patients. They also increase the length of hospitalization, costs, and mortality and morbidity rates [1]. Hospitalized patients have been a tendency to colonization and infections with resistant microorganisms due to presence of chronic diseases; and the heavy use of broad-spectrum antibiotics and the more frequent exposure to invasive procedures such as mechanical ventilators, central venous catheters, nasogastric tubes and urinary catheters [2].

Beta-lactam antibiotics were the most successful treatment options for human bacterial infections. *Escherichia coli* strains are important causative agents in nosocomial infections [3,4]. Unfortunately, *E. coli* produces beta-lactamase enzymes that degrades and inactivates this life saving antibiotics [5]. The most clinically significant enzymes are the extended spectrum beta-lactamases (ESBLs), AmpC beta-lactamases, *Klebsiella pneumoniae* carbapenemases (KPCs), and the metallo beta-lactamases (MBLs). [6]

ESBLs are plasmid-mediated enzymes that hydrolyze and inactivate beta-lactam drugs, including third-generation cephalosporins, penicillins and

aztreonam, however, clavulanic acid and other beta-lactamase inhibitors can block their activities [4]. They can be classified into three main types, designated as TEM, SHV, and CTX-M. Several ESBLs of clinical relevance belong to the TEM and SHV families [7].

Carbapenemases represent the most versatile family of β-lactamases capable of efficiently hydrolyzing penicillins, cephalosporins, monobactams, and even carbapenems. They are mostly found within the molecular class A (e.g. KPC, NMC, and SME), class B (e.g., IMP, VIM and NDM) and class D (e.g., OXA-23 to -27) [8]. Ambler's molecular class A plasmid- mediated serine β-lactamases (*bla*KPC-1) was first described in North Carolina in *Klebsiella pneumoniae* isolates, then it was reported in *E.coli* spp. Existence of other KPC genes (*bla*KPC-2 to *bla*KPC-11) do not only hydrolyze all β-lactams, but also carbapenems like imipenem, meropenem and ertapenem [9]. The rapid spread of KPC may be due to the presence of *bla*KPC gene on conjugative plasmids which is associated with resistance determinants for many antibiotics or carried on a Tn3-based transposon, Tn4401.Therefore, the potential for dissemination is significant [10].

Ambler's class B metallo-β-lactamases (MβLs) genes are usually carried on mobile genetic elements with a high capacity for dissemination especially from *P. aeruginosa* to *Enterobactericeae* members including *E.coli* spp. Most MβLs require zinc for their catalytic activity to hydrolyze all β-lactam antibiotics with exception of aztreonam [11]. The co-existence of different ESBLs and carbapenemase resistance genes on transposable genetic elements like plasmids in the same bacterial isolate are of major concern because it leads to widespread transfer of these elements between the same, as well as different bacterial species, and will result in failure of antimicrobial therapy [12].

With the spread of ESBLs and carbapenemase-producing bacteria, tigecycline became a lifeline for treatment of these infections [13]. Tigecycline is the novel glycylcycline that is often used in treatment of infections caused by carbapenemase-producing organisms and other multidrug-resistant Gram-negative bacteria. Using the Federal Drug Administration (FDA) susceptibility breakpoints, tigecycline has excellent *in vitro* activity against KPC-producing bacteria. The results of tigecycline use for this indication have ranged from failure to favorable when used as a monotherapy or in combination with colistin [14].

The aim of this study was to determine the prevalence and the potential co-association of ESBL and carbapenem resistance among *E.coli* strains isolated from hospitalized patients at Menoufia University Hospitals (MUHs), to compare the different phenotypic and molecular methods, and to determine genes responsible for ESBL and carbapenemase production and to identify the co-existence between these two genes. Detection of *in vitro* susceptibility of *E. coli* isolates to tigecycline was also tested as a therapeutic option.

METHODOLOGY

Collection of samples and identification of *E. coli* isolates:

The study was conducted at the Microbiology and Immunology Department, Faculty of Medicine, Menoufia University in collaboration with Shebin El-kom Teaching Hospital during the period from January to December, 2016 after obtaining written consents from all participants. Totally, 350 clinical samples (153 mid-stream urine, 60 discharge from surgical wounds, 47 sputum, 13 cervical discharge and 77 blood for culture) were received from Intensive Care Units (ICUs) and out-patient clinics (Chest, Urology, Surgery, Neonatology and Gynecology Departments). The patients already on antibiotics were excluded. All specimens were cultured on blood agar, Cysteine Lactose Electrolyte Deficient (CLED) and MacConkey's agar plates and incubated at 37°C for 24 hours at Microbiology laboratory. The grown *E. coli* isolates were identified by colonial morphology, Gram staining, standard biochemical tests and API-20E System (Oxoid, England). Confirmed *E.coli* isolates were suspended in nutrient broth supplemented with 16% glycerol and stored frozen at -80°C [15].

Antimicrobial susceptibility testing:

It was done for *E. coli* isolates by the Kirby-Bauer disk diffusion method against different antimicrobial agents (Oxoid) as recommended by CLSI, 2015; piperacillin (100µg), amoxicillin/clavulanic acid (20/10µg), piperacillin/tazobactam (100/10µg), cefazoline (30µg), cefoxitin (30µg), cefotaxime (30µg), ceftriaxone (30µg), cefepime (30µg), aztreonam (30µg), amikacin (30µg), gentamicin (10µg), cefoperazone (75µg), ceftazidime (30µg), levofloxacin (5µg), colistin (10µg), norfloxacin (10µg), trimethoprim/sulfamethoxazole (1.25/23.75µg), chloramphenicol (30µg) and tigecycline (30µg) [16].

Screening and phenotypic confirmation of ESBLs *E. coli* producers:

Suspected ESBLs-producing *E.coli* isolates (ceftazidime zone diameter ≤ 22 mm, cefotaxime zone diameter ≤ 27 mm, ceftriaxone zone diameter ≤ 25 mm and aztreonam zone diameter ≤ 27 mm) were subjected to combined-disk synergy test as a phenotypic confirmatory test for ESBLs detection. The test was performed using ceftazidime (30µg)/ ceftazidime-clavulanic acid (30µg/10µg). An increase in zone diameter by ≥ 5mm around the disk with ceftazidime and clavulanic acid versus the zone around disks with ceftazidime alone was interpreted as positive (Fig.1) as per CLSI guidelines 2015 [16].

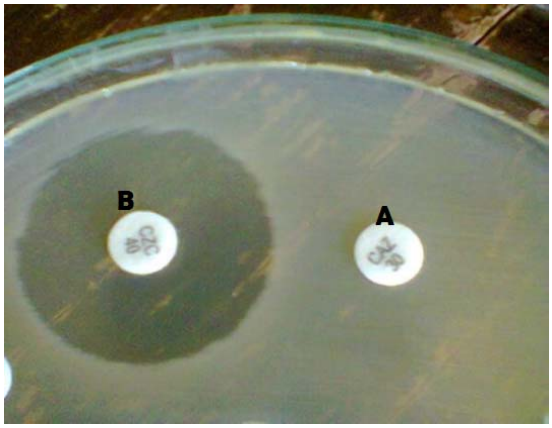

Fig.1 Detection of ESBL- producing *E.coli* using ceftazidime/ceftazidime-clavulanic acid combined disk test. Letter A represents ceftazidime disk alone, letter B represents ceftazidime disk combined with clavulanic acid. There was an expansion of the bacterial growth inhibition zone around the combined disk by ≥ 5mm in diameter.

Screening and phenotypic confirmation of class A and class B carbapenemases *E. coli* producers:

ESBL-producing *E. coli* isolates were tested against imipenem (IPM), meropenem (MEM), and ertapenem (ETP) (10µg for each disk) (Oxoid) by disk diffusion method. The average diameters of zones of inhibition were measured and interpreted according to CLSI guidelines (2015), (S≥23, R≤19 for imipenem and meropenem; and S≥22, R≤18 for ertapenem).3-amino-phenylboronic acid combined disk (APB-CD) and Ertapenem/EDTA combined disk (ETP/EDTA-CD) tests (inhibitor based methods) were evaluated as confirmatory tests for detection of class A (KPCs) and class B (MβLs) carbapenemases respectively. Phenylboronic was dissolved in dimethylsulfoxide to get a concentration of 30mg/ml. EDTA solution was prepared by dissolving 18.61gm of disodium EDTA 2H2O in 100 ml of distilled water, and the pH was adjusted to 8.0 by sodium hydroxide and autoclaved to prepare a sterile 0.5M EDTA solution. Sets of three ertapenem disks (10µg) were placed 25mm apart onto an Muller-Hinton agar plate inoculated with the tested *E. coli* strain, which was adjusted to a 0.5 McFarland turbidity standard, then10µL of a 30mg/ml APB (300µg of APB per disk) and 5µL of EDTA solutions were added to the first and third disks, respectively (Fig. 2). A difference of ≥5 mm in zone diameter (around the disks) between the disks containing the APB and EDTA solutions and that containing ertapenem alone was considered positive for class A and class B carbapenemases respectively, whereas an increase of <5 mm was considered negative [17,18].

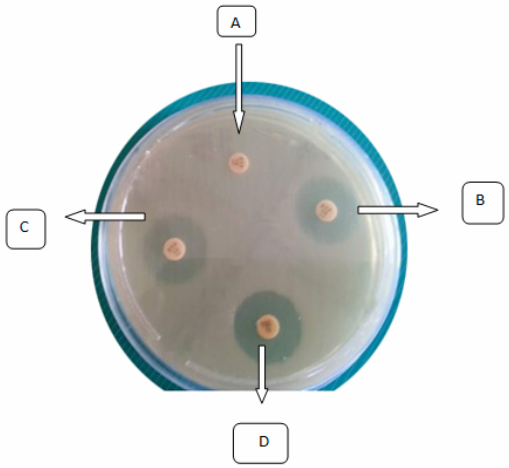

Fig.2 Detection of class A and class B carbapenemase-producing *E.coli* using APB-CD and Ertapenem/EDTA combined disk tests respectively. Letter A represents ertapenem disk alone, letter B represents ertapenem disk combined with amino-phenylboronic acid and letter C represents ertapenem disk combined with EDTA. There was an expansion of the bacterial growth inhibition zone around the combined disks by ≥ 5mm in diameter. Letter D represents tigecycline disk.

Genotype confirmation of ESBLs and carbapenemases:

- ***DNA extraction:*** purified colonies of ESBLs-producing *E. coli* isolates were inoculated into trypticase soy broth, incubated for 24h at 37°C, centrifuged and then the pellet was re-suspended in distilled water. The cells were lysed by heating (95°C for 10min) and cellular debris was removed by centrifugation. The supernatant was used as a source of template DNA for amplification [19].

- ***Conventional multiplex PCR reaction was performed to detect blaSHV and blaTEM genes.*** Specific primers for the genes were used for PCR amplification. The PCR program involved an initial denaturation at 95°C for 2 min and 35 cycles of 1 min at 95°C, 30 sec at 60°C (annealing), 1 min at 72°C (extension) and five min at 72°C as the final extension step. [20].

ESBLs-producing *E. coli* isolates were tested for the possibility of co-existence of carbapenemases genes. The PCR program involved an initial denaturation step at 94°C for 5min, followed by repeated 30 cycles of DNA denaturation at 94°C for 1 min, primer annealing for 1min, and primer extension at 72°C for 1.5min. The optimum annealing temperature was determined for each primer pair in the optimization step. Finally, an extended 72°C step for 10min was recorded to ensure that all of the products are full-length **(21).**For both multiplex PCR reactions, each reaction mixture (50 µl) consisted of 25µl Taq green PCR Master Mix, 1µl forward primer, 1µl reverse primer (Qiagen, Germany), 1µl template DNA in addition to 22µl nuclease-free distilled water. The PCR programs were performed in a thermal cycler (Biometra, Germany).The amplified

DNA products were detected on 1.5% agarose gels by ethidium bromide staining (Sigma, USA). A DNA ladder (100-1000bp) (Fermentas, Germany) was used to estimate allele sizes in base pairs (bp) for the gel (Fig. 3,4) [20, 21].

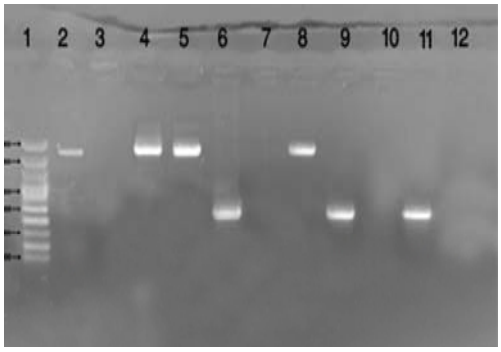

Fig.3 Agarose gel electrophoresis for the multiplex PCR amplified products of *E.coli bla*TEM and *bla*SHV genes. Lane 1: DNA molecular size marker (1000 bp). Lanes 2, 4, 5 and 8 represent positive *bla*TEM gene (861 bp). Lane 6, 9 and 11 represent positive *bla*SHV gene (498 bp). Lane 3,7,10 and 12 represent negative samples.

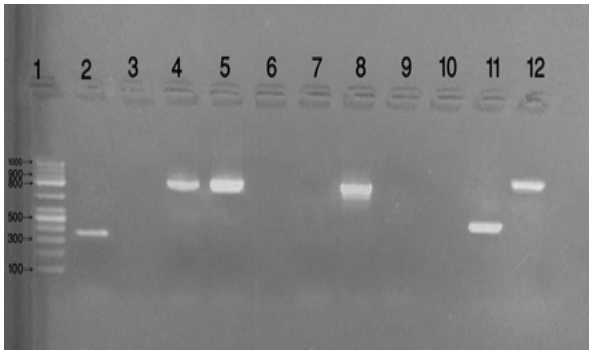

Fig.4 Agarose gel electrophoresis for the multiplex PCR amplified products of *E.coli bla*KPC and *bla*VIM genes. Lane 1: DNA molecular size marker (1000 bp). Lanes 4, 5, 8 and 12 represent positive *bla*KPC gene (785 bp). Lane 2 and 11 represent positive *bla*VIM gene (382 bp). Lane 3,6,7,9 and 10 represent negative samples

Primers used in this study

Name of the primer	Amplicon (bp)	Sequence 5′-3′
*bla*SHV	498	F5′-TCAGCGAAAAACACCTTG -3′ R5′-CCCGCAGATAAATCACCA -3′
*bla*TEM	861	F5′-GAGTATTCAACATTTCCGTGTC-3′ R5′-TAATCAGTGAGGCACCTATCTC -3′
Class A-*bla*KPC	785	F5′-TCGCTAAACTCGAACAGG-3′ R5′-TTACTGCCCGTTGACGCCCAATCC-3′
Class B-*bla*VIM	382	F5′-GTTTGGTCGCATATCGCAAC-3′ R5′-AATGCGCAGCACCAGGATAG-3′

Statistical analysis:

Computer SPSS program version 17 was used. The results were expressed as ranges and mean± SD. Chi-square test was done and p value <0.05 was considered as significant.

RESULTS

During the study, 172 *E.coli* isolates were collected from 350 different clinical specimens as described in table 1. *E.coli* was derived mostly from urine samples (21.5% from ICUs and 33.2% from the Urology Department). All isolates were resistant to piperacillin, cefazoline and cefoxitin by disk diffusion test. On the other hand, 98, 8% of *E. coli* isolates were sensitive to tigecycline. The resistance to aztreonam, cefotaxime, ceftriaxone and ceftazidime was 93%, 91.8%, 90.6% and 85.5% respectively. Moreover, the resistance pattern of *E. coli* isolates to imipenem, meropenem and ertapenem was 47.7%, 43% and 46.5% respectively as shown in table 2. Out of 172 *E.coli* isolates, 106 (61.6%) harbored ESBLs genes by multiplex PCR assay of which *bla*TEM was the most prevalent (77.4%; 82/106) followed by *bla*SHV (22.6%; 24/106). However, none of the isolates expressed both genes together. Out of 106 ESBLs-producing *E.coli* isolates, 27.3% (29/106) were also carbapenemase co-producers of which 75.8% (22/29) expressed both *bla*TEM and *bla*VIM; 20.7% (6/29) expressed *bla*TEM and *bla*KPC and only one isolate (3.5%) expressed *bla* SHV and *bla*KPC genes as shown in table 3. Considering PCR as the gold standard, the sensitivity, specificity and accuracy of the combined disk test using ceftazidime/ceftazidime-clavulanic acid for ESBLs detection were 96%, 67% and 85% respectively in relation to PCR results. For class A carbapenemase detection, the sensitivity, specificity and accuracy of the APB-CD test were 86%, 98% and 97% respectively.

The ERT/EDTA combined disk test showed sensitivity of 91%, specificity of 94% and accuracy of 93% for class B carbapenemase detection as described in table 4. The tested *E.coli* isolates showed excellent *in vitro* susceptibility to tigecycline. All ESBLs-producing and 93% of the combined ESBLs/carbapenemase-producing *E.coli* isolates were susceptible to tigecycline by disk diffusion test as shown in table5.

Table 1: Distribution of *E. coli* isolates (n=172) among the different studied specimens and hospital departments.

The studied Department	Specimens types (n=350)		*E. coli* isolates (n=172)	
	Type	Total	No.	%
ICUs	Urine	65	37	21.5
	Blood	37	18	10.5
	Sputum	15	5	2.9
Chest	Sputum	32	8	4.6
Urology	Urine	88	57	33.2
Surgery	Septic wound swab	60	21	12.2
Neonatology	Blood	40	20	11.7
Gynecology	Cervical swab	13	6	3.4

Table 2: Antimicrobial susceptibility patterns of *E.coli* isolates (n=172) by disk diffusion test.

Antimicrobial agents	Disk content (µg)	*E. coli* isolates (n=172)			
		S		R	
		No.	%	No.	%
Piperacillin (PRL)	100	0	0	172	100
Amoxicillin/clavulanic acid (AMC)	20/10	75	43.6	97	56.4
Cefazoline (CL)	30	0	0	172	100
Cefoxitin (FOX)	30	0	0	172	100
Cefotaxime (CTX)	30	14	8.2	158	91.8
Ceftriaxone(CRO)	30	16	9.4	156	90.6
Cefoperazone(CRP)	75	17	9.8	155	90.2
Ceftazidime(CAZ)	30	25	14.5	147	85.5
Cefepime (CPM)	30	40	23.3	132	76.7
Aztreonam (ATM)	30	12	7	160	93
imipenem (IPM)	10	90	52.3	82	47.7
Meropenem (MEM)	10	98	57	74	43
Ertapenem (ETP)	10	92	53.5	80	46.5
Amikacin (AK)	30	128	74.5	44	25.5
Gentamicin (GM)	10	116	67.5	56	32.5
Levofloxacin (LEV)	5	122	71	50	29
Norfloxacin (NOR)	10	132	76.8	40	23.2
Trimethoprim/sulfamethoxazole (SXT)	1.25/23.75	100	58.2	72	41.8
Chloramphenicol (C)	30	119	69.2	53	30.8
Tigecycline	30	170	98.8	2	1.2

Table 3: Prevalence of ESBLs (*bla*SHV and *bla*TEM) and carbapenemase (*bla*KPC and *bla*VIM) genes in *E. coli* isolates by multiplex PCR.

The studied genes	*E.coli* isolates (n=172)	
	No.	%
ESBLs genes		
***bla* TEM**	82	77.4
***bla* SHV**	24	22.6
***bla* TEM+ *bla* SHV**	0	0
Total +ve ESβL genes	106/172	61.6
Combined ESβL+ carbapenemase genes		
***bla*TEM+*bla*VIM**	22	75.8
***bla*TEM+*bla*KPC**	6	20.7
***bla* SHV+ *bla*KPC**	1	3.5
Total combined +ve ESBLs& carbapenemase genes	29/106	27.3

N.B Total class A carbapenemases = 7 Total class B carbapenemases = 22

Table 4: Sensitivity, specificity and accuracy of the phenotypic methods in relation to PCR as the gold standard for detection of ESBLs and carbapenemase genes among *E. coli* isolates.

The used method		Multiplex PCR for ESBLs genes in *E.coli* isolates (n=172)						
		+ve (n=106)		-ve (n=66)		Sensitivity %	Specificity %	Accuracy %
		No.	%	No.	%			
CD -test	+ve (n= 124)	102	96.2	22	33.3	96	67	85
	-ve (n= 48)	4	3.8	44	66.7			
The used method		**Multiplex PCR for class A carbapenemases in ESBLs-producing *E.coli* isolates (n=106)**						
		+ve (n=7)		-ve (n=99)		Sensitivity %	Specificity %	Accuracy %
		No.	%	No.	%			
APB-CD	+ve (n= 8)	6	85.7	2	2.1			
	-ve (n= 98)	1	14.3	97	97.9	86	98	97
The used method		**Multiplex PCR for class B carbapenemases in ESBLs-producing *E. coli* isolates (n=106)**						
		+ve (n=22)		-ve (n=84)		Sensitivity %	Specificity %	Accuracy %
		No.	%	No.	%			
ERT/EDTA	+ve (n= 25)	20	90.9	5	6.0			
	-ve (n= 81)	2	9.1	79	94.0	91	94	93

Table 5: Susceptibility patterns of ESBLs-producers and combined ESBLs/carbapenemase co-producers of *E. coli* isolates to tigecycline.

Susceptibility pattern	Multiplex PCR results							
	ESBLs-producing *E.coli* isolates (n=106)				Combined ESBLs and carbapenemase-producing-*E.coli* isolates (n=29)			
	S		R		S		R	
	No.	%	No.	%	No.	%	No.	%
Tigecycline (30µg)	106	100	0	0	27	93	2	7

DISCUSSION

The worldwide emergence of ESBLs- and carbapenemase-producing *E. coli* poses a great challenge for the clinical society. These organisms are usually multidrug-resistant with extremely limited therapeutic agents leading to increased morbidity and mortality [20]. Carbapenems represent the last resort for serious infections especially in high risk patients. Their clinical usage is under threat due to growing incidence of resistance due to production of carbapenemases [22].

In this study, a total of 172 *E. coli* strains were isolated from 350 different clinical specimens (49.1%) collected from patients admitted to different departments and ICUs at MUHs. Of these infections, 61.6% (106/172) were caused by ESBLs-producing *E.coli* of which 27.4% (29/106) were also carbapenemase-producers. Isolation of *E. coli* was common in both ICUs and Urology Department (34.9% and 33.2% respectively).This observation was in accordance with Sharma et al[23], who stated that, long hospital stay in ICU, the presence of catheterization and immuno-compromised status of most ICUs patients; provide optimum chance to catch infections.

In the current study, all *E. coli* isolates were resistant to piperacillin, cefazoline and cefoxitin by disk diffusion test. On the other hand, 99.8% of *E. coli* isolates were sensitive to tigecycline. The resistance to aztreonam, cefotaxime, ceftriaxone and ceftazidime was 93%, 91.8%, 90.6% and 85.5% respectively. Moreover,

the resistance pattern of *E. coli* isolates to imipenem, meropenem and ertapenem was 47.7%, 43% and 46.5% respectively. This observation is probably due to heavy pressure of antibiotic usage especially of β-lactam drugs. In a study done by Helal et al [24], it was found that history of β-lactam intake was observed in 73.3% and history of carbapenem antibiotic intake was found among 46.7% of cases infected with carbapenemase-producing-isolates. It was also reported that, the previous use of cephalosporins and carbapenems were identified as independent risk factors for acquisition of carbapenem-resistant isolates [25].

CLSI recommends using multiple agents, including aztreonam, ceftazidime, and cefotaxime for ESBLs screening, which is consistent with our findings [26]. This observation was in accordance with that of Muhammad and Swedan [20], about ESβL and carbapenem resistance among uropathogenic *E.coli*. It was recommended using multiple agents for ESBLs [20].

Phenotypic confirmation of ESBLs production was done in this study by the combined disk synergy test (CDST) using ceftazidime & ceftazidime/clavulanate. The ability of CDST to detect ESBLs-producing *E. coli* was very satisfactory; it was able to detect 102/106 (96.2%) of the PCR-confirmed ESBL-producers with a sensitivity, specificity and accuracy of 96%, 67% and 85% % respectively. These results were matched with that of Singh and Singh [27], who found that the sensitivity of ceftazidime & ceftazidime/clavulanate was 91.8%. They also demonstrated that the sensitivity of the test for ESBLs detection may reach 100% when testing both cefotaxime and cefepime [27].

In the present study, 77.4% (82/106) of ESBLs-producing *E.coli* expressed *bla*TEM and 22.6% (24/106) of the isolates expressed *bla*SHV. However, none of the isolates co-expressed the two genes. In another study [28] it was found that 69%, 55% and 1% of the isolates expressed *bla*TEM, *bla*CTX and *bla*SHV respectively and that also none of the isolates harbored *bla*TEM and *bla*SHV together. The predominance of *bla*TEM gene in this study may be due to its presence on highly mobile genetic elements that facilitates its spread among different isolates and that it is one of the first genes that mediate resistance to ESBLs drugs [20].

The important finding in this study was the co-existence of some ESBLs and carbapenemase genes in the same *E. coli* isolate. Interestingly, 29/106 (27.3%) *E. coli* isolates co-expressed ESBLs and carbapenemases of which 75.8 % (22/29) had *bla*TEM and *bla*VIM genes, 20.7 % (6/29) were *bla*TEM and *bla*KPC and finally 3.5% (1/29) were *bla*SHV and *bla*KPC genes. The co-existence of different ESBLs and carbapenemase enzymes in the same *E. coli* isolates has similarly been reported in other studies in Taiwan and Nepal [28, 29]. Infections with such highly resistant organisms may result in repeated hospitalization or even death. Thus, it is imperative to apply better screening protocols to detect these isolates and to devise an empirical therapy policy for patients at high risk of infection by such isolates [20].

In the current study, phenotypic class A carbapenemase (*bla* KPC) detection, was performed by APB-CD test. The test was able to detect 6/7 (85.7%) of *bla*KPC-positive isolates. Its sensitivity, specificity and diagnostic accuracy was 86%, 98% and 97 %, respectively as compared to PCR as a golden standard method. Our findings are similar to that reported by Tsakris et al [30], who reported that APB was an effective inhibitor of KPC enzymes, and its use in CD tests with ertapenem or meropenem may give the most easily interpreted results. The false-positive results (positive by APB-CD test and negative by PCR) were explained by Pournaras et al [31], who stated that isolates that overproduced a plasmid-mediated AmpC also showed a weak synergy when synergy test using a carbapenem and phenylboronic acid was applied [31].

For class B carbapenemase detection, the sensitivity, specificity and accuracy of ETP/EDTA-CD test were 91%, 94% and 93% respectively. The test was able to detect 20/22 (90.9%) of *bla*VIM-positive isolates. The same observations were found by Pournaras et al [32], who investigated 189 clinical isolates of *Enterobacteriaceae* with reduced susceptibility to carbapenems for MβL production by using CDT with EDTA. They observed that, EDTA test was able to detect 94.8% of MβL-positive isolates by using a breakpoint of ≥ 5mm. They reported that, an initial screening by combined susceptibility testing of ertapenem followed by a confirmatory EDTA test represents a valid and less expensive alternative to the molecular investigation of MβL genes. This aspect is particularly important because it makes MβL detection can be possible not only in reference laboratories but also in routine diagnostic microbiology laboratories [32].

The current study evaluated *in vitro* susceptibility of tigecycline as a therapeutic option for both ESBLs and combined ESBLs/carbapenemase producing- *E.coli* isolates. Our results showed that 100% and 93% of the isolates were sensitive to tigecycline respectively by disk diffusion test. These results are similar to those obtained by Livermore et al [33] as they found that tigecycline was active against 100% of carbapenem-resistant *E.coli* isolates. A similar result was also reported by Nandi et al [34], who revealed that tigecycline had good activity and potency against *E.coli* isolates as 100% of their isolates were susceptible to tigecycline [34].

CONCLUSION AND RECOMMENDATION

High prevalence of *bla*TEM-type ESBLs and co-existence of *bla*TEM and *bla*VIM are found in our Hospital. Continuous monitoring of this ESBLs-producing *E.coli* will through light on its dissemination. It is important to find a strategy to improve prevention and control of spreading of these strains. Tigecycline

could be a valuable therapeutic option for the treatment of infections caused by *E. coli*.

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