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RESEARCH ARTICLE

Genetic Study of Extended Spectrum Beta-Lactamase and Carbapenemase Producing *Escherichia Coli* Causing Sepsis among Egyptian Children

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Abstract:

Background:

Treatment failure of sepsis caused by *Escherichia coli* (*E. Coli)* is a leading cause of death of infants and children in intensive care units.

Objective:

To detect the prevalence of Extended-Spectrum Beta-Lactamase (ESBL) and carbapenemase-genes between *E. coli* isolates from infants and children with septicemia and to identify their antibiotic sensitivity pattern.

Methods:

This is a cross-sectional study performed on 88 patients with sepsis. The isolated *E. coli* were identified by Gram stain and biochemically by the Microscan automated system. ESBL and carbapenemase producing *E. coli* were isolated on double disk diffusion and EDTA double disk, respectively. Polymerase chain reaction for ESBL and carbapenemase producing *E. coli* genes were performed. Bacterial susceptibility to antibiotics was tested. The initial results were measured through the 30-days of hospital admission. IRB approved the study.

Results:

Of 88 patients with sepsis, 49 and 30 strains were ESBL producing and carbapenemase producing *E. coli*; respectively. Neither risk factors for infection nor clinical picture can differentiate between ESBL and carbapenemase producing *E. coli*. The most frequently detected gene of ESBL producing *E. coli* was *SHV*, it was more sensitive to Piperacillin/Tazobactam (90%) and cefepime (86.7%) while for carbapenemase-producing *E. coli*; *IMP* was the most frequent, its sensitivity was high to Piperacillin/Tazobactam and Ciprofloxacin (52.6% each).

Conclusion:

The commonest gene of ESBL producing *E. coli* is *SHV* whereas for carbapenemase-producing *E. coli* is *IMP*. Piperacillin/Tazobactam is the candidate drug to start in children with septicemia and suspected ESBL or carbapenemase-producing *E. coli* infection.

Keywords: Beta-lactamase, Carbapenemase, *E. coli*, Children, Sepsis, Egypt.

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1. INTRODUCTION

Development of antibiotics resistance is a major health problem all over the world. Among the common resistance pattern is the resistance of *Escherichia coli* (*E. coli*) to Extended-Spectrum Beta-Lactamase (ESBL). Recently, resistance to carbapenem has emerged both in community-acquired and in hospital-acquired infections. The resistance of *E. coli* to carbapenem confers resistance to most β-lactams, including carbapenems, and often carries additional antimicrobial resistance genes to other non-β-lactam antibiotics, making them resistant to most antibiotics [1 - 3].

The mechanisms of resistance to carbapenem are mediated by two pathways; the first pathway is associated with the reduced outer membrane permeability to the antibiotics mediated

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by porin loss. The second pathway is associated with the production of EBSL or of Amp C-type beta-lactamase; beta-lactamase production is capable of hydrolyzing carbapenems (carbapenemases) [1 - 3]. Carbapenem resistance pathways are linked to the presence of transferable genes carried on transferable elements such as plasmids and transposons that can be spread between the different species of Enterobacteriaceae [4].

Carbapenem resistance genes are classified to class A (*KPC*), B Metallo-beta-lactamases (*IMP, VIM, NDM*) and class D (*OXA*-48) serine carbapenemases. The resistance pattern of carbapenemase-producing *E. coli* (CPE) depends mainly on the type of gene responsible for carbapenemase enzyme production. Basically, carbapenem-resistant Enterobacteriaceae hydrolyze imipenem, ertapenem, meropenem, and doripenem [5]. Amber class A carbapenemase is inhibited by boronic acid, clavulanic acid and Tazobactam. The class B β-lactamases has a broad spectrum of hydrolytic activity, including all penicillins, cephalosporins, and carbapenems with sensitivity only to aztreonam [5]. The activity of this class of enzyme is not inhibited by clavulanic acid, Tazobactam, or sulbactam with inhibition of the enzyme by a chelator for zinc ions, a cofactor of its activity, such as Ethylenediaminetetraacetic acid (EDTA) [5, 6]. The amber class D has a peculiar pattern of resistance to ceftazidime and resistant to inhibition by clavulanic acid and Tazobactam [1].

The resistance pattern of CPE and the distribution of carbapenem resistance genes varies according to geographical regions [1 - 4]. *KPC* carbapenem resistance gene was found among USA, Greece, and Israel isolates [7], *VIM* in Greece [1, 6], *OXA*-48 in North Africa and Turkey [5], and *NDM* in the Indian subcontinent and the Balkans [7].

The determinations of CPE by phenotypic methods are difficult and time consuming. Moreover, clinical isolates with carbapenemase activity rarely have an isolated pattern of resistance as it is usually associated with an Extended-Spectrum β-Lactamases (ESBLs), leading to a broader composite resistance profile [5].

The development of molecular methods for genetic determination of CPE has evolved to be an accurate, sensitive and rapid method for detection of such strains. The used techniques either single Polymerase Chain Reaction (PCR), multiplex PCR and sequencing provide precise identification of carbapene-mase resistance [8].

There are data concerning the association of *E. coli* with carbapenemase resistance among serious types of infections in adults [9]. However, there is insufficient data about the association of CPE with sepsis in children. The aim of this study is to detect the prevalence of ESBL- and carbapenemase-genes among *E. coli* isolated from children with suspected septicemia.

2. MATERIALS AND METHODS

2.1. Design, Setting and Population Studied

This cross-sectional study was carried out on children with suspected or diagnosed sepsis after two calendar days of admission to the pediatric intensive care unit in a tertiary-care university hospital, Egypt from January 2016 until January 2018. The study was approved by Mansoura Ethical Committee (code number: R/ 18.08.250). Informed consent was taken from patients' parents who accepted to participate in the study. Confidentiality of the data was considered. Patients, aged from 1 day to 16 years old, diagnosed to have sepsis according to the International Pediatric Sepsis Consensus Conference 2005 [10] were recruited in the study.

2.2. Data Collection

The clinical data of each child was obtained from the medical records consisting of demographic data, comorbid conditions, duration of hospital stay and the presence of devices such as mechanical ventilator, Central Venous Catheter (CVC) or urinary catheter at the time of bacterial blood culture collection. All patients started empirical broad-spectrum antibiotics until the results of blood cultures. Patients' survival was registered through 30-days of hospital admission.

Blood samples for blood glucose, C-reactive protein and arterial blood gases were withdrawn to all patients and in addition, three ml venous blood was withdrawn for blood culture. Identification of *E. coli* was done by gram stain and biochemical identification using Microscan automated system. Antibiotics susceptibility test was performed by the disk diffusion method. The following disks were used: ceftazidime (30 μg), cefotaxime (30 μg), cefepime (30 μg), imipenem (10 μg), meropenem (10 μg), gentamicin (10 μg), amikacin (30 μg), ciprofloxacin (5 μg), levofloxacin (5 μg), sulfamethoxazole/trimethoprim (1.25/23.75 μg), and piperacillin/tazobactam (100 μg/10 μg) (Oxoid). The interpretations of the results were done according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [11].

2.3. Determination of ESBL Production by Double-Disc Synergy Test (DDST)

Escherichia coli strains resistant to ceftazidime (30 μg), cefotaxime (30 μg) were subjected to determination of ESBLs production by DDST. Briefly, overnight culture of *E. coli* adjusted to 0.5 McFarland standard was subculture on Muller-Hinton agar plate with application of disks containing ceftazidime, ceftazidime (30 μg) + clavulanic acid (10 μg), cefotaxime, and cefotaxime (30 μg + clavulanic (10 μg) pairs were placed with 20 mm space between them. Plates were incubated overnight at 37ºC. The increase of ≥5 mm inhibition zone of growth in ceftazidime/clavulanic acid and cefotaxime/-clavulanic compared to ceftazidime and cefotaxime was regarded as an ESBLs producing isolate [12]. The following strains were used as positive control strains for ESBL, *E. coli* ATCC 25922 for *blaCTX-M-* and *E. coli* AF427133.1 for TEM and *SHV* beta lactamase (Grenole Cedex2, France). *E. coli* ATCC 25922 was used as a negative control strain.

2.4. Determination of Carbapenemases Production by Combined Disk Test (CDT) and Boronic Acid Discs

Resistant strains of *E. coli* strains to imipenem (10 μg) and/or to meropenem (10 μg) were subjected to further determination of carbapenemase by CDT and boronic acid discs. The CDT with positive results determines the production of Metallo-β-Lactamase (MBL) while the positive boronic acid disc detects presence of class A carbapenemase as previously

described [13].

CDT was performed by the use of two plates of Muller –Hinton agar plated with standard suspension of *E. coli* as previously described in DDST, then four antibiotics discs imipenem (10 µg), meropenem (10 µg) were added to each plate. Then over two antibiotics discs on one plate, 10µL of EDTA was added and on the other plate 10 µL of aminophenyl boronic acid (APBA) was added. The plates were incubated overnight at 37ºC for 24 hours. The increase of ≥5 mm in zone diameter around discs with the β-lactamase inhibitor (APBA or EDTA), as compared with the carbapenem discs alone, was considered to be a positive result. The followings strains were used as positive control strains; *E. cloacae* JMI10526 for *bla*IMP, *Acinetobacter baumannii* AB5 for *bla*VIM, *K. pneumoniae* ATCC strain BAA-1705 for *bla* KPC, *K. pneumoniae* ATCC strain BAA-2146 for *bla*NDM-1, and *E. coli* ATCC BAA-2523 for *bla*OXA-48), *E. coli* ATCC 25922 for *blaCTX-M-* and *E. coli* AF427133.1 for TEM and SHV beta lactamase (Grenole Cedex2, France).

2.5. Polymerase Chain Reaction (PCR) for ESBLs and Carbapenemase Genes

DNA extraction of *E. coli* was performed by boiling method in sterile distilled water. Briefly, colonies were used from nutrient agar, washed with 1 ml of 1X Tris-EDTA buffer (10 mM Tris-HCl, 1 mM EDTA [pH 8]) and centrifuged. The deposit was suspended in 0.5 ml sterile distilled water then was boiled in a water bath at 100ºC for 15 minutes. After, another 10 minutes of incubation at room temperature, centrifugation was performed at 11,500 X g for 5 minutes. The supernatant was decanted and the pellet was stored at -20ºC until use for further DNA analysis. The used primers for determination of ESBLs and carbapenemase genes are listed in Table **1** [8, 14 - 16]. Positive control strains for each genotype were used by previous strains described in the phenotypic methods.

Ready to use kit for amplification was supplied from Qiagen. The total reaction volume was 50 µL with 0.2 µM concentration of each specific primer and 2 µL extracted DNA for each target gene, PCR amplification is carried out in a 50 µL reaction volume. Amplification was performed by PCR (Biosystem). Initial denaturation step was performed at 95°C for 10 minutes followed by, 35 cycles composed of 45 seconds of denaturation at 94°C, 45 seconds of primer annealing at the specific temperature for each primer pair, then 50 seconds for extension at 72°C and final extension was performed at 72°C for 7 minutes. The amplification products were held at 4°C. Electrophoresis was performed for 30 minutes by the use of 1.5% agarose gel stained with ethidium bromide. The products were visualized by ultraviolet light and compared to ladder marker a 100 bp DNA size marker (Invitrogen, UK).

2.6. Data Analysis

Statistical analysis was done using SPSS software version 21 (IBM SPSS Statistics for Windows, IBM Corp., Armonk, NY, USA). Qualitative variables were presented as number and percent and chi-square was used for the comparison between different groups. Continuous variables were presented as mean ± SD (standard Deviation) for parametric data and Student t-test was used to compare two means. P value < 0.05 was considered statistically significant.

Table 1. Primers for determination of ESBLs and carbapenemase genes.

Primer Name	Primer Sequence 5'-3'	Product Size, bp	References
SHV-UP SHV-LO	CGCCGGGTTATTCTTATTTGTCGC TCTTTCCGATGCCGCCGCCAGTCA	1,016	Mulvey *et al*., 2011 [14]
TEM-G TEM-H	TTGCTCACCCAGAAACGCTGGTG TACGATACGGGAGGGCTTACC	708	Mulvey *et al*., 2011 [14]
CTXMGp1	TTAGGAARTGTGCCGCTGYA CGATATCGTTGGTGGTRCCAT	688	Dallenne *et al*., 2010 [16]
KPC1 KPC2	ATGTCACTGTATCGCCGTC AATCCCTCGAGCGCGAGT	863	Mulvey *et al*., 2011 [14]
VIM1 VIM2	GTTTGGTCGCATATCGCAAC AATGCGCAGCACCAGGATAGAA	382	Mulvey *et al*., 2011 [14]
IMP1 IMP2	CCWGATTTAAAAATYGARAAGCTTG TGGCCAHGCTTCWAHATTTGCRTC	522	Mulvey *et al*., 2011 [14]
NDM-F NDM-R	GGTGCATGCCCGGTGAAATC ATGCTGGCCTTGGGGAACG	660	Mulvey *et al*., 2011 [14]
*bla*OXA-48-like	AACGGGCGAACCAAGCATTTT TGAGCACTTCTTTTGTGATGGCT	585	Mlynarcik *et al*., 2016 [15]
*bla*SME	TATGGAACGATTTCTTGGCG CTCCCAGTTTTGTCACCTAC	300	Mlynarcik *et al*., 2016 [15]
blaGIM GIM-F GIM-R	TCGACACACCTTGGTCTGAA AACTTCCAACTTTGCCATGC	477	Poirel *et al*., 2011 [8]
blaSIM SIM-F SIM-R	AAAATCTGGGTACGCAAACG ACATTATCCGCTGGAACAGG	271	Poirel *et al*., 2011 [8]

bp: Base Pair

3. RESULTS

Data from 112 patients were collected; 88 patients were eligible to our study and 24 were excluded due to different causes as incomplete clinical data, improperly preserved samples or samples improperly taken. Samples were collected from admitted patients to neonatal ICU (39 patients with mean age is 12 days), PICU (21 patients with mean age 24 months) and surgical ICU (28 patients with mean age 3 months). Forty-six males (52.3%) and 42 females (47.7%) were included in the study. *Escherichia coli* strains resistant to ceftazidime (30 µg), cefotaxime (30 µg) were subjected to determination of ESBLs production *E. coli* and strains that were resistant to imipenem (10 µg) and/or to meropenem (10 µg), were subjected to determination of carbapenemase production and there was some cross-production as some of the isolates came from the same patient (Table **2**). Phenotypic testing shows that out of the 88 *E. coli* isolates, 49 (55.68%) were positive for ESBL production and 30 (34.09%) were positive for carbapenemase production and 20 isolates were positive for both ESBL and carbapenemas.

Clinical characteristics associated with infection with ESBL and carbapenemase, are summarized in Table **3**. There was significantly lower survival among patients infected with ESBL producing *E. coli* than those with ESBL non-producing *E. coli* and lower survival among patients infected with carbapenemase-producing *E. coli* than those with carbapenemase non-producing *E. coli*. Neither type of bloodstream infection, usage of CVC, urinary catheter nor endotracheal tube significantly differed between ESBL producing and non-producing *E. coli* infected groups, also they didn't significantly differ between carbapenemase producing and non-producing *E. coli* infected groups.

The clinical symptoms of sepsis are shown in Table **4**. There were non-significant differences between ESBL producing and non-producing *E. coli* and between carbapenemase producing and non-producing *E. coli* regarding clinical and laboratory signs of infection. Table **5** shows statistically non-significant correlations between infection with ESBL producing *E. coli* and infection with carbapenemase producing *E. coli* and the clinical data in the studied patients;

Table 2. ESBL producing *E. coli* and Carbapenemase producing *E. coli* isolates in the studied patients.

	ESBL Producing *E. coli*			**Total**	***P* value**
Carbapenemase producing *E. coli*	**Positive isolates**		**Negative isolates**		
	Positive Isolates	20	10	30	0.136
	Negative Isolates	29	29	58	
Total		49	39	88	

ESBL: Expanded Spectrum B-Lactamase Chi Square Test

Table 3. Clinical characteristics of the studied groups.

	Number (%)	**ESBL Producing *E. coli* Infected Group (n=49)**	**ESBL Non-Producing *E. coli* Infected Group (n=39)**	**P*- Value**	**Carbapenemase Producing *E. coli* Infected Group (n=30)**	**Carbapenemase Non-Producing *E. coli* Infected Group (n=58)**	***P**- Value**
Suspected places of infection **NICU** **PICU** **Surgical ICU**	39 (44.3) 21 (23.9) 28 (31.8)	18 13 18	21 8 10	0.272	14 6 10	25 15 18	0.829
CVC inserted	46 (52.3)	28	18	0.305	30	16	0.886
Urinary catheter inserted	28 (31.8)	15	13	0.785	22	6	0.087
ETT inserted	53 (60.2)	31	22	0.514	36	17	0.624
BSI type **Primary** **Secondary**	76 (86.4) 12 (13.6)	41 8	35 4	0.41	25 5	51 7	0.551
Duration of ICU admission (days)		18.94±4.56	11.67±4.34	0.001**	19.43±4.64	13.79±5.33	0.001**
Patient Survival **Survived** **Died**	57 31	27 22	30 9	0.033	24 6	33 25	0.032

NICU: Neonatal Intensive Care Unit, PICU: Pediatric Intensive Care Unit, ICU: Intensive Care Unit, CVC: Central Venous catheter, ESBL: Extended Spectrum B-Lactamase, ETT: Endo-Tracheal Tube, ICU; Intensive Care Unit.
Chi-Square test, ** Independent sample t-test

Table **6** demonstrates the frequency of detected genes of ESBLs producing *E. coli* isolates (49 patients). *SHV* was the most frequently detected gene. It was detected in 30 (61.22%) *E. coli* isolates; 20 (40.81%) had *SHV* only and 10 (20.41%) in combination with *CTX-M*. Meanwhile, among 30 patients with carbapenemase-producing *E. coli* isolates, *IMP* was the most frequent gene; it was detected in 19 isolates (63.33%); 8 (26.67%) with *IMP* gene only and 11 (36.67%) in combination with other carbapenemase-producing *E. coli* genes.

Table 4. Clinical signs and laboratory tests of sepsis in the studied groups.

–	Number (%)	ESBL producing *E. coli* Infected Group (n=49)	ESBL Non-Producing *E. coli* Infected Group (n=39)	*P*- Value	Carbapenemase Producing *E. coli* Infected Group (n=30)	Carbapenemase Non-Producing *E. coli* Infected Group (n=58)	*P**- Value
Fever	33(37.5)	21	12	0.204	12	21	0.616
Hypothermia	35(39.8)	19	16	0.810	10	25	0.314
Bradycardia	13(14.8)	8	5	0.840	3	10	0.314
Hypotension	34(38.6)	20	14	0.650	12	22	0.949
Oliguria	6(6.8)	3	3	0.833	1	5	0.132
Apnea	10(11.4)	4	6	0.590	4	6	0.763
Prolonged CRT	9(10.2)	2	7	0.083	2	7	0.317
Hyperglycemia	4(4.5)	3	1	0.238	2	2	0.540
Metabolic acidosis	15(17)	8	7	0.535	4	11	0.319
CRP	27(30.7)	12	15	0.563	9	18	0.548

CRT: Capillary Refill Time, ESBL: Extended Spectrum B-Lactamase, CRP: C-Reactive Protein
*Chi-Square test

Table 5. Correlations between infection with ESBL producing *E. coli* and infection with Carbapenemase producing *E. coli* and the clinical data in the studied patients.

Clinical Data	ESBL Producing *E. coli*		Carbapenemase Producing *E. coli*	
	r*	*P*	r*	*P*
Fever	0.150	0.210	0.059	0.622
Hypothermia	-0.029	0.813	-0.120	0.321
Bradycardia	-0.035	0.846	-0.175	0.399
Hypotension	0.049	0.654	0.007	0.949
Oliguria	-0.055	0.847	-0.389	0.152
Apnea	-0.090	0.603	0.050	0.771
Prolonged CRT	-0.289	0.087	-0.167	0.330
Hyperglycemia	0.197	0.250	0.102	0.553
Metabolic acidosis	0.103	0.548	-0.166	0.333
CRP	-0.096	0.576	-0.100	0.561

CRT: Capillary Refill Time, ESBL: Extended Spectrum B-Lactamase, CRP: C-Reactive Protein
*Correlation coefficient

Table 6. Frequency of detected genes of ESBL and Carbapenemase producing *E. coli* among isolates positive by double discs diffusion and EDTA double discs.

Genes	Number (%)
ESBL producing *E. coli* genes (n=49)	
SHV	20 (40.81%)
TEM	19 (38.78%)
CTX-M	0
***SHV* + CTX-M**	10 (20.41%)
Carbapenemase producing *E. coli* genes (n=30)	
SME	1 (3.33%)
VIM	2 (6.67%)
IMP	8 (26.67%)

(Table 6) contd.....

Genes	Number (%)
OXA-48	3 (10%)
SIM	2 (6.67%)
GIM	1 (3.33%)
NDM	1 (3.33%)
KPC	0
IMP + NDM + KPC + VIM	1 (3.33%)
IMP + SIM + GIM	2 (6.67%)
IMP + OXA-48 + KPC + SME	1 (3.33%)
IMP + GIM	2 (6.67%)
IMP + NDM + SME	1 (3.33%)
IMP + VIM	2 (6.67%)
IMP + OXA-48	2 (6.67%)
VIM + SIM	1 (3.33%)

ESBL: Extended Spectrum B-Lactamase

Table 7. Bacterial sensitivity to antibiotics in different types of ESBL producing *E. coli* genes and different types of carbapenemase producing *E. coli* genes.

	Percentage of Bacterial Sensitivity to Antibiotics										
	AK	**FEP**	**CTX**	**FOX**	**CAZ**	**CIP**	**GEN**	**IPM**	**MEM**	**TZP**	**SXT**
Total *E. coli*	77.3	84.1	54.5	54.5	0	75	63.6	43.2	38.6	76.1	78.4
Types of ESBL producing *E. coli* genes											
Total ESBL	67.3	73.5	20.4	18.4	0	67.3	59.2	18.4	16.3	71.4	67.3
SHV	80	86.7	3.3	0	0	83.3	73.3	0	0	90	76.7
TEM	47.4	52.6	47.4	47.4	0	42.1	36.8	47.4	42.1	42.1	52.6
CTX-M	90	80	10	0	0	90	47.4	0	0	47.4	42.1
Types of Carbapenemase producing *E. coli* genes											
Total Carbapenemase	83.3	90	36.7	33.3	0	86.7	70	0	0	90	83.3
SME	100	100	33.3	33.3	0	100	100	0	0	100	66.7
VIM	83.3	83.3	33.3	33.3	0	83.3	83.3	50	33.3	50	66.7
IMP	47.4	42.1	5.2	0	0	52.6	47.4	47.4	42.1	52.6	42.1
OXA-48	83.3	83.3	33.3	33.3	0	83.3	66.7	50	50	66.7	50
SIM	60	60	0	0	0	60	60	40	20	40	20
GIM	20	20	20	20	0	20	20	80	60	0	20
NDM	100	100	0	0	0	100	66.7	0	0	100	100
KPC	100	100	0	0	0	100	100	0	0	100	50

ESBL: Extended Spectrum β-Lactamase; AK: Amikacin; FEP: Cefepime; CTX: Cefotaxime; FOX: Cefoxitin; CAZ: Ceftazidime; CIP: Ciprofloxacin; GEN: Gentamicin; IMP: Imipenem; MEM: Meropenem; TZP: Piperacillin/Tazobactam; SXT: Sulfamethoxazole/trimethoprim.

Antibiotic sensitivity pattern was shown in Table **7**. All ESBL producing *E. coli* were resistant to ceftazidime. High resistance was detected to cefotaxime, cefoxitin, imipenem and meropenem. Sensitivity to amikacin, ciprofloxacin, gentamicin, Piperacillin/Tazobactam and Sulfamethoxazole/trimethoprim was 67.3%, 67.3%, 59.2%, 71.4% and 67.3%; respectively. Meanwhile, all carbapenemase producing *E. coli* were resistant to imipenem, meropenem and ceftazidime in addition, high resistance to cefotaxime, cefoxitin was observed. Sensitivity to amikacin, ciprofloxacin, gentamicin, Piperacillin/Tazobactam and Sulfamethoxazole/trimethoprim was 83.3%, 86.7%, 70%, 90% and 83.3% respectively. Antibiotic sensitivity pattern among individualized ESBL producing *E. coli* and CPE genes revealed that isolates with *SHV* gene were more sensitive to Piperacillin/Tazobactam and Cefepime and isolates with *IMP* gene was more sensitive to Piperacillin/Tazobactam and Ciprofloxacin.

A logistic regression analysis was employed to create a model that can predict 30-days mortality for patients with ESBL producing *E. coli* and CPE infections. We assessed the presence of risk factors for infection as insertion of central venous catheter, urinary catheter or endotracheal tube, patient age, primary blood stream infection, positive ESBL producing *E. coli* colonization and positive CPE colonization. The model can correctly predict the fatal outcome for 61.8% of the patients and recovery for 88.9% with a total success rate of 78.4%. Table **8** shows the logistic regression coefficient, Wald chi-square test which tests the unique contribution of each variable, the odds ratio and 95% confidence interval for odds ratio for each of the risk factors. Patient age, insertion of CVC,

positive colonization with ESBL producing *E. coli* and CPE had statistically significant partial effects. Positive colonization with ESBL producing *E. coli* was found to be the most statistically significant risk factor for fatal outcome in the studied group.

4. DISCUSSION

ESBL producing *E. coli* and CPE strains are worldwide leading pathogens in community and hospital acquired infections [17, 18], that need continuous surveillance of antimicrobial resistance and sensitivity patterns. β-lactam antibiotics are the most common class of antibacterial agents used to treat bacterial infections due to their broad antibacterial spectrum and excellent safety profile [17]. Carbapenems are the first-choice treatment for ESBL producing *E. coli* especially with the increase of ESBL clinical isolates reports expressing multidrug resistance [19]. The development of CPE, is causing a threat to health, leaving few treatment options.

Table 8. Logistic regression analysis for associated factors of 30-days mortality in the studied group.

	B	Wald χ^2	P	OR	95% C.I. for OR
Age (month)	0.021	5.883	0.015	1.021	(1.004-1.038)
Gender	0.354	0.403	0.525	1.424	(0.478-4.242)
CVC insertion	-1.235	4.493	0.034	0.291	(0.093-0.911)
Urinary catheter insertion	0.668	1.107	0.293	1.950	(0.562-6.763)
ETT	0.041	0.005	0.945	1.042	(0.320-3.392)
Positive ESBL producing *E. coli* colonization	1.832	8.798	0.003	6.248	(1.862-20.969)
Positive Carbapenemase producing *E. coli* colonization	-1.443	4.745	0.029	0.236	(0.065-0.865)

BSI: Blood Stream Infection, CVC: Central Venous Catheter, ESBL: Expanded Spectrum B-Lactamase, ETT: Endo-tracheal Tube. B= logistic regression coefficient, P= *P* value calculated using Wald chi-Square test (Wald χ^2), OR= Odds Ratio, 95% CI = 95% Confidence Interval for odds ratio.

This study was conducted to detect the production of ESBL and carbapenemase among *E. coli* isolated from children with hospital acquired bloodstream infection, admitted to pediatric intensive care unit from January 2016 until January 2018.

Phenotypic testing of ESBL and carbapenemase production by the isolated *E. coli* showed that out of the 88 isolates; 49 patients (55.68%) were ESBL producers and 30 (34.09%) were carbapenemase producers. This finding is consistent with the high proportion of ESBL production reported in a study conducted in Egypt also, showing more than half (54.5%) of isolated *E. coli* positive for ESBL [20] and in New Zealand as about half of *E. coli* isolates produced ESBL [21]. Similar results in the USA [22] and India [23] have been reported as prevalence rates of ESBL were from 35.0 to 42% for *E. coli*. Higher rates were even reported in other studies from India (>80%), China (>60%) [24]. While in other researches, all the tested *E. coli* were ESBL-positive; in Saudi Arabia [25], Ethiopia [26] and Senegal [27]. In this study, carbapenemase production among isolated *E. coli* was higher than those previously studied from clinical isolates in New Zealand (15%) [21]. Meanwhile, in another study, carbapenemase production among *E. coli* was higher (45.2%) [27]. These high rates of ESBL producing *E. coli* and CPE in Egypt are probably related to the overuse of antibiotics, improper administration of antimicrobial agents in trial therapies for the management of any febrile illness or due to lack of proper infection control practices (that can lead to an emergence of resistant organisms). Moreover, the trained attitudes to use third-generation cephalosporins have been described as the most important precipitating factor in the emergence of ESBL and carbapenemase producing *E. coli* [28].

In the presenting study, it was found that risk factors for sepsis as CVC, urinary catheter or endotracheal tube insertion were not significantly associated with ESBL or carbapenemase-producing *E. coli* indicating that usage of these invasive devices is not a reliable significant risk factor for infection with ESBL producing *E. coli* or CPE. It may be due to hospital rules in practicing strict infection control measures or due to the small number of patients included in this study. Although some authors reported that, bladder catheterization or other invasive medical devices are important risk factors for infection with ESBL-producing *E. coli* [29 - 31], others reported urinary catheter insertion is not a risk factor for infection with ESBL producing *E. coli* [32]. Furthermore, endotracheal tube insertion and mechanical ventilation are independent risk factors for carbapenemase-producing *E. coli* [33].

In the existing study, none of the clinical manifestations of sepsis statistically differed between ESBL producing and non-producing *E. coli* infected groups and also didn't differ between carbapenemase producing and non-producing *E. coli* groups. Therefore, the physician, depending on the clinical examination alone, can't predict whether the patient is infected with ESBL or carbapenemase producing or non-producing *E. coli*. This is concordant with another study that revealed non-significant differences in patients' clinical characteristics [34]. However, in another study, there was a great difference concerning to disease severity and clinical signs between the ESBL-positive and ESBL-negative groups [35].

It was observed that a significant increase in mortality among ESBL producing *E. coli* infected patients versus non-infected. This is similar to other authors who explored a significantly high mortality rate in patients infected with ESBL producing *E. coli* [36, 37]. Also, in a survey that included 400 bloodstream isolated pathogens, more than 60% of neonates infected with ESBL producing *E. coli* died opposed to 35.7% who were infected with other isolates [38].

Widespread use of carbapenems has led to the appearance

of CPE isolates [39]. Gram-negative bacteria including *E. coli* develop carbapenems resistance by increasing the production of Carbapenemases and/or decrease in permeability of the outer membrane. *KPC* is Class A carbapenemase, while Metallo-beta-lactamases (MBL) include *VIM*, *IMP* and *NDM*. Class D Carbapenemases are *OXA* like enzymes [40, 41].

In this study, among the ESBL *E. coli* producers, the most frequently detected gene was *SHV*; it was in 30 isolates (61.22%); it was detected alone in 20 isolates (40.81%) and in combination with *CTX-M* in 10 isolates (20.41%). The next most frequent detected gene was *TEM* (38.78%) and then *CTX-M* comes (20.41%). In another study by Abdallah *et al*, *TEM* and *SHV* presented in about half, one fifth of isolates; respectively. In the same study, *CTX-M* type was the most common β-lactamase-encoding gene represent about 90% of the ESBL-producing *E. coli* [21] and this was higher than that in our study (20.41%). In contrast to this study, In a study conducted by Abdallah *et al*, the genetic analysis showed that *CTX-M* was present in 96% of ESBL *E. coli* in adult infections, also in a recent study the most common ESBLs genes detected were again *CTX-M* in multi-drug resistant gram negative bacilli in infected febrile neutropenic cancer patients [42]. These discrepancies can be attributed to the differences in age categories in the first study and the different patients' clinical criteria in the second one. It was observed that *TEM* and *SHV* are important factors in decreasing the susceptibility of ESBL *E. coli* producers to third-generation cephalosporin [43].

Among 30 carbapemase producing *E. coli*, *IMP* was the most frequent gene; it was detected in 19 isolates (63.33%); 8 isolates (26.67%) had *IMP* only and 11 (36.67%) in combination with other CPE genes. *OXA-48* and *NDM* were detected in 6 (20%) and 3 (10%) isolates; respectively. This was similar to a study by Kamel *et al*. [42], as one *E. coli* isolate harbored more than one type of metallo beta lactamase (*VIM, NDM*). The high prevalence of *IMP* gene in this study is probably due to excessive use of Imipenem in clinical therapy. A study conducted in Saudi Arabia, a near regional area to Egypt, showed different results as neither *IMP*, *VIM* or *KPC* was present in the carbapenemase-positive isolates. Meanwhile, the prevalence rates of *OXA*-48-like is 58.1% and of *NDM*-type is 41.9% and this was higher than in the present study [44].

In the present study, all ESBL and carbapenemase-producing *E. coli* were resistant to ceftazidime and all carbapenemase producers were resistant to Imipenem and Meropenem. High resistance was detected to cefotaxime, cefoxitin also (79.6% and 81.6% among ESBL producers respectively and 36.7% and 33.3% among carbapenemase producers; respectively). A study in Senegal showed that all strains were resistant to ceftriaxone or cefotaxime (99.9%) [27]. In the present study low rates of resistance to amikacin was detected, which is consistent with findings from Korea [45], Taiwan [31], Japan [46] and Senegal [27].

In the present study, high resistance rates were observed among ESBL producers towards Imipenem and Meropenem; 81.6% and 83.7% respectively. This was comparable to resistance rates observed for Imipenem (60%), and Meropenem (30%) in Saudi Arabia [45]. Meanwhile, all Carbapenemase producers in our study were resistant to Imipenem and Meropenem. Whereas, relatively high sensitivity of ESBL *E. coli* producers to sulfamethoxazole/trimethoprim complex (78.4%), and ciprofloxacin (75%) were observed in contrast to other authors that showed high resistance rates with sulfamethoxazole/trimethoprim complex (85.7%) and ciprofloxacin (72%) [27]. Colistin, amikacin, fosfomycin, and temocillin are the only few remaining antibiotics used to treat infections caused by carbapenemase-producing Gram-negative bacilli. Proper combination therapy with two or more drugs is better than using monotherapy associated with a better survival rate [47, 48].

In the current study, a logistic regression analysis was employed to create a model that can predict 30-days mortality for patients with ESBL producing *E. coli* and CPE infections. Risk factors for infection were assessed as the presence of invasive devices as CVC, urinary catheter or endotracheal tube, patient age, primary blood stream infection, positive ESBL producing *E. coli* colonization and positive CPE colonization. The model can correctly predict the fatal outcome for 61.8% of the patients and recovery for 88.9% with a total success rate of 78.4%. Positive colonization with ESBL producing *E. coli* was 6 times risk of mortality when compared to carbapenemase producing so should be cautiously and vigorously managed. According to our knowledge, this is the first study to do this analysis. A recent study by Komatsu and coauthors performed only univariate analysis, revealing multiple significant risk factors of death but they couldn't complete the multivariate analysis because there were only ten deaths [49]. In agreement to these results, other studies observed that patients with infection due to ESBL producing *E. coli* tended to have poorer outcomes [49, 50].

CONCLUSION

Neither risk factors for infection nor clinical manifestations can differentiate between ESBL and carbapenemase producing *E. coli*. *SHV* is the most frequently detected gene of ESBL producing *E. coli* and *IMP* the most frequently detected for carbapenemase production.Piperacillin/Tazobactam is the candidate drug to start within children with sepsis and suspected ESBL or carbapenemase-producing *E. coli* infection. Infection with ESBL *producing E. coli* was 6 times associated with mortality when compared to carbapenemase producing *E. coli.* Future studies on multicenter hospitals on a larger number of patients are recommended to validate the results.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The protocol and methods employed were reviewed and approved by the Institutional Review Board, Faculty of medicine, Mansoura University, Mansoura, Egypt (code number: R/ 18.08.250).

HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Informed consent was taken from patients' parents who accepted to participate in the study.

AVAILABILITY OF DATA AND MATERIALS

The authors confirm that the data supporting the findings of this research are available within the article.

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None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

[1] Nordmann P, Dortet L, Poirel L. Carbapenem resistance in Enterobacteriaceae: Here is the storm! Trends Mol Med 2012; 18(5): 263-72. [http://dx.doi.org/10.1016/j.molmed.2012.03.003] [PMID: 22480775]

[2] Cantón R, Akóva M, Carmeli Y, *et al.* Rapid evolution and spread of carbapenemases among Enterobacteriaceae in Europe. Clin Microbiol Infect 2012; 18(5): 413-31. [http://dx.doi.org/10.1111/j.1469-0691.2012.03821.x] [PMID: 22507109]

[3] Temkin E, Adler A, Lerner A, Carmeli Y. Carbapenem-resistant Enterobacteriaceae: biology, epidemiology, and management. Ann N Y Acad Sci 2014; 1323: 22-42. [http://dx.doi.org/10.1111/nyas.12537] [PMID: 25195939]

[4] Mathers AJ, Peirano G, Pitout JD. The role of epidemic resistance plasmids and international high-risk clones in the spread of multidrug-resistant Enterobacteriaceae. Clin Microbiol Rev 2015; 28(3): 565-91. [http://dx.doi.org/10.1128/CMR.00116-14] [PMID: 25926236]

[5] Nordmann P, Naas T, Poirel L. Global spread of Carbapenemase-producing Enterobacteriaceae. Emerg Infect Dis 2011; 17(10): 1791-8. [http://dx.doi.org/10.3201/eid1710.110655] [PMID: 22000347]

[6] Miriagou V, Papagiannitsis CC, Tzelepi E, Casals JB, Legakis NJ, Tzouvelekis LS. Detecting VIM-1 production in Proteus mirabilis by an imipenem-dipicolinic acid double disk synergy test. J Clin Microbiol 2010; 48(2): 667-8. [http://dx.doi.org/10.1128/JCM.01872-09] [PMID: 20007383]

[7] Nordmann P, Cuzon G, Naas T. The real threat of *Klebsiella pneumoniae* carbapenemase-producing bacteria. Lancet Infect Dis 2009; 9(4): 228-36. [http://dx.doi.org/10.1016/S1473-3099(09)70054-4] [PMID: 19324295]

[8] Poirel L, Walsh TR, Cuvillier V, Nordmann P. Multiplex PCR for detection of acquired carbapenemase genes. Diagn Microbiol Infect Dis 2011; 70(1): 119-23. [http://dx.doi.org/10.1016/j.diagmicrobio.2010.12.002] [PMID: 21398074]

[9] Martin A, Fahrbach K, Zhao Q, Lodise T. Association between carbapenem resistance and mortality among adult, hospitalized patients with serious infections due to Enterobacteriaceae: Results of a systematic literature review and meta-analysis. Open Forum Infect Dis 2018; 5(7)ofy150 [http://dx.doi.org/10.1093/ofid/ofy150] [PMID: 30046639]

[10] Calandra T, Cohen J. The international sepsis forum consensus conference on definitions of infection in the intensive care unit. Crit Care Med 2005; 33(7): 1538-48. [http://dx.doi.org/10.1097/01.CCM.0000168253.91200.83] [PMID: 16003060]

[11] Performance standards for antimicrobial susceptibility testing. USA: Wayne: M100-S23 2019.

[12] Thomson KS. Extended-spectrum-β-lactamase, AmpC, and Carbapenemase issues. J Clin Microbiol 2010; 48(4): 1019-25. [http://dx.doi.org/10.1128/JCM.00219-10] [PMID: 20181902]

[13] Maurer FP, Castelberg C, Quiblier C, Bloemberg GV, Hombach M. Evaluation of carbapenemase screening and confirmation tests with Enterobacteriaceae and development of a practical diagnostic algorithm. J Clin Microbiol 2015; 53(1): 95-104. [http://dx.doi.org/10.1128/JCM.01692-14] [PMID: 25355766]

[14] Mulvey MR, Grant JM, Plewes K, Roscoe D, Boyd DA. New Delhi metallo-β-lactamase in *Klebsiella pneumoniae* and *Escherichia coli*, Canada. Emerg Infect Dis 2011; 17(1): 103-6. [http://dx.doi.org/10.3201/eid1701.101358] [PMID: 21192866]

[15] Mlynarcik P, Roderova M, Kolar M. Primer evaluation for PCR and its application for detection of carbapenemases in entero-bacteriaceae. Jundishapur J Microbiol 2016; 9(1)e29314 [http://dx.doi.org/10.5812/jjm.29314] [PMID: 27099689]

[16] Dallenne C, Da Costa A, Decré D, Favier C, Arlet G. Development of a set of multiplex PCR assays for the detection of genes encoding important β-lactamases in Enterobacteriaceae. J Antimicrob Chemother 2010; 65(3): 490-5. [http://dx.doi.org/10.1093/jac/dkp498] [PMID: 20071363]

[17] Paterson DL, Bonomo RA. Extended-spectrum beta-lactamases: A clinical update. Clin Microbiol Rev 2005; 18(4): 657-86. [http://dx.doi.org/10.1128/CMR.18.4.657-686.2005] [PMID: 16223952]

[18] Tzouvelekis LS, Markogiannakis A, Psichogiou M, Tassios PT, Daikos GL. Carbapenemases in *Klebsiella pneumoniae* and other enterobacteriaceae: An evolving crisis of global dimensions. Clin Microbiol Rev 2012; 25(4): 682-707. [http://dx.doi.org/10.1128/CMR.05035-11] [PMID: 23034326]

[19] Morosini M-I, García-Castillo M, Coque TM, *et al.* Antibiotic coresistance in extended-spectrum-β-lactamase-producing Enterobacteriaceae and *in vitro* activity of tigecycline. Antimicrob Agents Chemother 2006; 50(8): 2695-9. [http://dx.doi.org/10.1128/AAC.00155-06] [PMID: 16870760]

[20] Abdallah HM, Wintermans BB, Reuland EA, *et al.* Extended-spectrum β-Lactamase- and carbapenemase-producing enterobacteriaceae isolated from egyptian patients with suspected blood stream infection. PLoS One 2015; 10(5)e0128120 [http://dx.doi.org/10.1371/journal.pone.0128120] [PMID: 26001049]

[21] Myat TO, Hannaway RF, Zin KN, *et al.* ESBL- and carbapenemase-producing enterobacteriaceae in patients with acteremia, Yangon, Myanmar, 2014. Emerg Infect Dis 2017; 23(5): 857-9. [http://dx.doi.org/10.3201/eid2305.161100] [PMID: 28418298]

[22] Ajao AO, Johnson JK, Harris AD, *et al.* Risk of acquiring extended-spectrum β-lactamase-producing Klebsiella species and *Escherichia coli* from prior room occupants in the intensive care unit. Infect Control Hosp Epidemiol 2013; 34(5): 453-8. [http://dx.doi.org/10.1086/670216] [PMID: 23571360]

[23] Taneja J, Mishra B, Thakur A, Dogra V, Loomba P. Nosocomial blood-stream infections from extended-spectrum-beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumonia* from GB Pant Hospital, New Delhi. J Infect Dev Ctries 2010; 4(8): 517-20. [http://dx.doi.org/10.3855/jidc.668] [PMID: 20818104]

[24] Molton JS, Tambyah PA, Ang BS, Ling ML, Fisher DA. The global spread of healthcare-associated multidrug-resistant bacteria: A perspective from Asia. Clin Infect Dis 2013; 56(9): 1310-8. [http://dx.doi.org/10.1093/cid/cit020] [PMID: 23334810]

[25] Al-Agamy MH, Shibl AM, Hafez MM, Al-Ahdal MN, Memish ZA, Khubnani H. Molecular characteristics of extended-spectrum β-lactamase-producing *Escherichia coli* in Riyadh: Emergence of CTX-M-15-producing E. coli ST131. Ann Clin Microbiol Antimicrob 2014; 13: 4. [http://dx.doi.org/10.1186/1476-0711-13-4] [PMID: 24397567]

[26] Legese MH, Weldearegay GM, Asrat D. Extended-spectrum beta-lactamase- and carbapenemase-producing *Enterobacteriaceae* among Ethiopian children. Infect Drug Resist 2017; 10: 27-34. [http://dx.doi.org/10.2147/IDR.S127177] [PMID: 28182124]

[27] Camara M, Mane MT, Ba-Diallo A, *et al.* Extended-spectrum beta-lactamase- and carbapenemase-producing Enterobacteriaceae clinical isolates in a Senegalese teaching hospital: A cross sectional study. Afr J Microbiol Res 2017; 11(44): 1600-5. [http://dx.doi.org/10.5897/AJMR2017.8716]

[28] Levy Hara G, Gould I, Endimiani A, *et al.* Detection, treatment, and prevention of carbapenemase-producing Enterobacteriaceae: Recommendations from an International Working Group. J Chemother 2013; 25(3): 129-40. [http://dx.doi.org/10.1179/1973947812Y.0000000062] [PMID: 23783137]

[29] Ben-Ami R, Rodríguez-Baño J, Arslan H, *et al.* A multinational survey of risk factors for infection with extended-spectrum beta-lactamase-producing enterobacteriaceae in nonhospitalized patients. Clin Infect Dis 2009; 49(5): 682-90.
[http://dx.doi.org/10.1086/604713] [PMID: 19622043]

[30] Lytsy B, Sandegren L, Tano E, Torell E, Andersson DI, Melhus A. The first major extended-spectrum beta-lactamase outbreak in Scandinavia was caused by clonal spread of a multiresistant *Klebsiella pneumoniae* producing CTX-M-15. APMIS 2008; 116(4): 302-8.
[http://dx.doi.org/10.1111/j.1600-0463.2008.00922.x] [PMID: 18397465]

[31] Liu HY, Lin HC, Lin YC, Yu SH, Wu WH, Lee YJ. Antimicrobial susceptibilities of urinary extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* to fosfomycin and nitrofurantoin in a teaching hospital in Taiwan. J Microbiol Immunol Infect 2011; 44(5): 364-8.
[http://dx.doi.org/10.1016/j.jmii.2010.08.012] [PMID: 21524974]

[32] Chung H-C, Lai C-H, Lin J-N, *et al.* Bacteremia caused by extended-spectrum-β-lactamase-producing *Escherichia coli* sequence type ST131 and non-ST131 clones: Comparison of demographic data, clinical features, and mortality. Antimicrob Agents Chemother 2012; 56(2): 618-22.
[http://dx.doi.org/10.1128/AAC.05753-11] [PMID: 22123694]

[33] Chitnis AS, Caruthers PS, Rao AK, *et al.* Outbreak of carbapenem-resistant enterobacteriaceae at a long-term acute care hospital: Sustained reductions in transmission through active surveillance and targeted interventions. Infect Control Hosp Epidemiol 2012; 33(10): 984-92.
[http://dx.doi.org/10.1086/667738] [PMID: 22961017]

[34] Ikeda Y, Mamiya T, Nishiyama H, Koseki T, Mouri A, Nabeshima T. Risk factors for extended-spectrum beta-lactamase-producing Escherichia coli infection in hospitalized patients. Nagoya J Med Sci 2012; 74(1-2): 105-14.
[PMID: 22515116]

[35] Chen CH, Huang CC. Risk factor analysis for extended-spectrum β-lactamase-producing *Enterobacter cloacae* bloodstream infections in central Taiwan. BMC Infect Dis 2013; 13: 417.
[http://dx.doi.org/10.1186/1471-2334-13-417] [PMID: 24010678]

[36] Kim R, Khachikian D, Reboli AC. A comparative evaluation of properties and clinical efficacy of the echinocandins. Expert Opin Pharmacother 2007; 8(10): 1479-92.
[http://dx.doi.org/10.1517/14656566.8.10.1479] [PMID: 17661730]

[37] Zaoutis TE, Goyal M, Chu JH, *et al.* Risk factors for and outcomes of bloodstream infection caused by extended-spectrum beta-lactamase-producing *Escherichia coli* and Klebsiella species in children. Pediatrics 2005; 115(4): 942-9.
[http://dx.doi.org/10.1542/peds.2004-1289] [PMID: 15805368]

[38] Jain A, Roy I, Gupta MK, Kumar M, Agarwal SK. Prevalence of extended-spectrum beta-lactamase-producing Gram-negative bacteria in septicaemic neonates in a tertiary care hospital. J Med Microbiol 2003; 52(Pt 5): 421-5.
[http://dx.doi.org/10.1099/jmm.0.04966-0] [PMID: 12721319]

[39] Wang X, Chen G, Wu X, *et al.* Increased prevalence of carbapenem resistant Enterobacteriaceae in hospital setting due to cross-species transmission of the bla NDM-1 element and clonal spread of progenitor resistant strains. Front Microbiol 2015; 6: 595.
[PMID: 26136735]

[40] Sonnevend Á, Ghazawi A, Alqahtani M, *et al.* Plasmid-mediated colistin resistance in *Escherichia coli* from the Arabian Peninsula. Int J Infect Dis 2016; 50: 85-90.
[http://dx.doi.org/10.1016/j.ijid.2016.07.007] [PMID: 27566913]

[41] Jeon JH, Lee JH, Lee JJ, *et al.* Structural basis for carbapenem-hydrolyzing mechanisms of carbapenemases conferring antibiotic resistance. Int J Mol Sci 2015; 16(5): 9654-92.
[http://dx.doi.org/10.3390/ijms16059654] [PMID: 25938965]

[42] Kamel NA, El-Tayeb WN, El-Ansary MR, Mansour MT, Aboshanab KM. Phenotypic screening and molecular characterization of carbapenemase-producing Gram-negative bacilli recovered from febrile neutropenic pediatric cancer patients in Egypt. PLoS One 2018; 13(8)e0202119
[http://dx.doi.org/10.1371/journal.pone.0202119] [PMID: 30157188]

[43] Wu T-L, Siu LK, Su L-H, *et al.* Outer membrane protein change combined with co-existing TEM-1 and SHV-1 β-lactamases lead to false identification of ESBL-producing *Klebsiella pneumoniae*. J Antimicrob Chemother 2001; 47(6): 755-61.
[http://dx.doi.org/10.1093/jac/47.6.755] [PMID: 11389107]

[44] Al-Agamy MH, Aljallal A, Radwan HH, Shibl AM. Characterization of carbapenemases, ESBLs, and plasmid-mediated quinolone determinants in carbapenem-insensitive *Escherichia coli* and *Klebsiella pneumoniae* in Riyadh hospitals. J Infect Public Health 2018; 11(1): 64-8.
[http://dx.doi.org/10.1016/j.jiph.2017.03.010] [PMID: 28462854]

[45] Lee SY, Park YJ, Yu JK, *et al.* Prevalence of acquired fosfomycin resistance among extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* clinical isolates in Korea and IS26-composite transposon surrounding fosA3. J Antimicrob Chemother 2012; 67(12): 2843-7.
[http://dx.doi.org/10.1093/jac/dks319] [PMID: 22893681]

[46] Wachino J, Yamane K, Suzuki S, Kimura K, Arakawa Y. Prevalence of fosfomycin resistance among CTX-M-producing *Escherichia coli* clinical isolates in Japan and identification of novel plasmid-mediated fosfomycin-modifying enzymes. Antimicrob Agents Chemother 2010; 54(7): 3061-4.
[http://dx.doi.org/10.1128/AAC.01834-09] [PMID: 20404116]

[47] Tumbarello M, Trecarichi EM, De Rosa FG, *et al.* ISGRI-SITA (Italian Study Group on Resistant Infections of the Società Italiana Terapia Antinfettiva). Infections caused by KPC-producing *Klebsiella pneumoniae*: Differences in therapy and mortality in a multicentre study. J Antimicrob Chemother 2015; 70(7): 2133-43.
[http://dx.doi.org/10.1093/jac/dkv086] [PMID: 25900159]

[48] Daikos GL, Tsaousi S, Tzouvelekis LS, *et al.* Carbapenemase-producing *Klebsiella pneumoniae* bloodstream infections: Lowering mortality by antibiotic combination schemes and the role of carbapenems. Antimicrob Agents Chemother 2014; 58(4): 2322-8.
[http://dx.doi.org/10.1128/AAC.02166-13] [PMID: 24514083]

[49] Komatsu Y, Kasahara K, Inoue T, *et al.* Molecular epidemiology and clinical features of extended-spectrum beta-lactamase- or carbapenemase-producing *Escherichia coli* bacteremia in Japan. PLoS One 2018; 13(8)e0202276
[http://dx.doi.org/10.1371/journal.pone.0202276] [PMID: 30157275]

[50] Schwaber MJ, Carmeli Y. Mortality and delay in effective therapy associated with extended-spectrum β-lactamase production in *Enterobacteriaceae bacteraemia*: A systematic review and meta-analysis. J Antimicrob Chemother 2007; 60(5): 913-20.
[http://dx.doi.org/10.1093/jac/dkm318] [PMID: 17848376]