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Emergence of New Delhi Metallo Beta Lactamase *bla*$_{NDM-1}$ and Oxacillinases *bla*$_{OXA-48}$ Producing *Klebsiella pneumoniae* in an Egyptian Hospital

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THE RAPID dissemination of carbapenem resistant *Enterobacteriaceae* (CRE) all over the world represents a matter of concern. This study aims to investigate the prevalence of CRE in clinical isolates recovered from Al Kasr Al-Ainy hospital in the period between August 2015 and February 2017. The isolates were identified by conventional methods and Maldi-TOF spectroscopy. Phenotypic identification of CRE was carried out by Modified Hodge test and genotypic characterization for Extended-Spectrum Beta-Lactamase (ESBL) and CRE genes was also performed. Carbapenemase activity of CRE isolates was confirmed in 46% of the isolates of which, *bla*$_{NDM-1}$ and *bla*$_{OXA-48}$ were detected in 75%, 59% of the isolates respectively, while *bla*$_{VIM}$ was detected in 2.3% only. However, *bla*$_{IMP}$ and *bla*$_{KPC}$ were not detected in any isolate. All CRE isolates carried at least one ESBL gene, 95.4% of CRE isolates had *bla*$_{CTX-M-15}$, 88.6% *bla*$_{TEM-1}$ and 68.2% *bla*$_{SHV}$. The *bla*$_{SHV}$ gene showed different alleles in the CRE isolates. All the isolates were sensitive to polymyxin B and colistin while only 36.4% were sensitive to tigecycline. Consequently, microbiologists and clinicians should implement the necessary control measures to prevent the spreading of these resistant bacteria.

Keywords: *Klebsiella pneumoniae*, Carbapenem resistance, *bla*$_{NDM-1}$, *bla*$_{OXA-48}$, ESBLs, MHT.

Introduction

Post antibiotics era and super bugs are of great significant to the world. The global emergence of multidrug-resistant Gram-negative superbugs is a critical problem to public health. In 2016, Center for Disease Control and Prevention (CDC) reported Carbapenem resistant *Enterobacteriaceae* as an urgent threat that requires quick solutions to prevent emergence of antibiotic resistance and hospital acquired infection (CDC, 2016).

Enterobacteriaceae are rod-shaped, Gram negative bacteria that includes: *Klebsiella* spp., *E. coli* and *Enterobacter* spp. *Enterobacteriaceae* cause infection in both community and hospitals. Now some *Enterobacteriaceae* acquired resistance to all antibiotics even to the last resort carbapenems. Infections caused by carbapenem resistant *Enterobacteriaceae* (CRE) are increasing worldwide, and are associated with high rates of mortality nearly 50% of patients (van Duin & Paterson, 2016). Carbapenem resistant *Enterobacteriaceae* are usually spread person to person through contact with infected or colonized people, particularly contact with wounds or stool. They can also enter the body through medical devices like ventilators, intravenous catheters, urinary catheters, or surgical wounds. Patients with long term hospitalization or have compromised immune systems or have invasive devices through their bodies are more likely affected by CRE (CDC, 2015).

Carbapenem resistant *Enterobacteriaceae* (CRE) is defined as those *Enterobacteriaceae*

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which were non-susceptible to at least one antibiotic in carbapenems class and were resistant to third generation of cephalosporins (CDC, 2015). They are able to produce carbapenemase enzymes that hydrolyse β lactams. Carbapenemase enzymes are classified into three classes: Ambler class A (bla_{KPC}, and bla_{IMI}), Metallo β lactamase class B (bla_{VIM} and bla_{NDM}) and Oxacillinase class D (bla_{OXA-48}). The genes encoding β-lactamases (*bla)* are either on the chromosome such as bla_{IMI} and some of bla_{OXA-48} or on mobile genetic elements such as bla_{KPC}, bla_{VIM} and bla_{NDM} (Queenan & Bush, 2007 and Nordmann et al., 2011).

Until now, there is no standard treatment against CRE infections. Colistin sulfate, polymyxin B and tigecycline may have *in-vitro* antimicrobial activity against CRE but they have insufficient clinical efficacy and higher level of toxicity (Vidal et al., 2007; Paul et al., 2010; Yahav et al., 2011 and Kontopidou et al., 2014). In addition, overusing of colistin and tigecycline should be taken in concern as they are the last line of defense, leads to occurrence of resistant isolates (Kumar, 2016). This study aimed to investigate the distribution of CRE between inpatients in an Egyptian hospital and to evaluate the coexistence of CRE and ESBL genes.

Materials and Methods

Clinical specimens

A total of 112 clinical samples were collected from Al Kasr AL-Ainy hospital, Egypt in the period from August 2015 till February 2017. Clinical samples were collected from different sites: Wound, blood, urine, sputum, drain and cerebrospinal fluid, pus and central venous line.

Isolation and identification of bacterial isolates

The clinical isolates were identified by conventional microbiological methods. All isolates in this study were cultured on MacConkey and Eosin methylene blue media and preserved in 20% glycerol at -80°C. Positive CRE isolates were selected for further identification using MALDI/ TOF-TOF (Ultraflextreme), Bruker daltonics, Germany by Extended direct transfer method. A fresh pure single colony was smeared as a thin film onto a spot on MALDI plate. One µl of 70% formic acid was overlaid followed by 1µl of 70% α-Cyano-4-hydroxycinnamic acid. The plate was left to dry at room temperature then introduced for mass spectrometry measurement. The mass spectra were detected and compared with Maldi database (Zimmermann, 2015). The identification score values were interpreted according to Bruker Daltonics guidelines.

Antimicrobial susceptibility testing

Disk diffusion test were performed against imipenem 10µg discs and third generation of cephalosporins (Ceftazidime 30µg and cefotaxime 30µg) to distinguish between CRE isolates and non CRE isolates. The antibiotic resistant profile against various antibiotic classes were determined according to Kirby Bauer disk diffusion method (Bauer et al., 1966). Minimum inhibitory concentrations (MICs) of the resistant clinical isolates were determined using microdilution plates method (EUCAST, 2003)**.** A 10µl of 2, 3, 5 triphenyltetrazolium chloride (TTC 20mg/ ml) were added to each 100µl of broth culture for 30min after incubation, red color developed indicated the presence of viable cells (Kim et al., 2010). Interpretative breakpoint criteria were reviewed according to CLSI guidelines (CLSI, 2014).

Modified Hodge test (MHT)

A 0.5 McFarland of *E. coli* ATCC 25922 were streaked on Muller Hinton agar plates. Meropenem disk (10µg) was put on the center of a plate, a swab of the tested isolate was streaked from the edge of the disk to the edge of the plate. The plates were incubated for 16-24hr at 37°C, the appearance of clover leaf indentation inhibition zone is considered positive (CLSI, 2017).

Screening of carbapenemase genes

Colony PCR was performed as described by Ishikawa et al. (2000). The primers used for detection of carbapenemase genes bla_{KPC} (Tenover et al., 2006), bla_{VIM}, bla_{NDM}, bla_{IMP} and bla_{Oxa-48} (Poirel et al., 2001) and ESBLs encoding genes bla_{CTX-M} (Poirel et al., 2001), bla_{SHV} (Schlesinger et al., 2005), and bla_{TEM} (Schmiedel et al., 2014) are listed in Table 1. PCR was performed by thermal cycler (Applied Biosystem 337). The amplification reaction was as follow: 95°C initial denaturation for 5min., 30 cycles of (denaturation 95°C for 40sec, annealing for 40sec as recommended in Table 1, extension at 72°C for 40sec.) and a final extension step at 72°C for 7min. PCR products were loaded on 1% agarose gel stained with 0.5% ethidium bromide for electrophoresis.

TABLE 1. Primers used for detection of carbapenemase and ESBL genes.

Primers	Sequence (5'-3')	Target gene	Ta	Product size (bp)	References
KPC-F	CTTGCTGCCGCTGTGCTG	*bla*$_{KPC}$	57ºC	489	Tenover et al. (2006)
KPC-R	GCAGGTTCCGGTTTTGTCTC				
NDM-F	GGTTTGGCGATCTGGTTTTC	*bla*$_{NDM}$	57ºC	621	Poirel et al. (2011)
NDM-R	CGGAATGGCTCATCACGATC				
VIM-F	GATGGTGTTTGGTCGCATA	*bla*$_{VIM}$	53ºC	390	Poirel et al. (2011)
VIM-R	CGAATGCGCAGCACCAG				
IMP-F	GGAATAGAGTGGCTTAAYTC	*bla*$_{IMP}$	51ºC	232	Poirel et al. (2011)
IMP-R	TCGGTTTAAYAAAACAACCACC				
OXA-48 F	GCGTGGTTAAGGATGAACAC	*bla*$_{OXA-48}$	53ºC	438	Poirel et al. (2011)
OXA-48 R	CATCAAGTTCAACCCAACCG				
CTX-M-F	CGCTTTGCGATGTGCAG	*bla*$_{CTX-M}$	51ºC	550	Poirel et al. (2001)
CTX-M-R	ACCGCGATATCGTTGGT				
SHV-F	ATGCGTTATATTCGCCTGTG	*bla*$_{SHV}$	53ºC	747	Schlesinger et al. (2005)
SHV-R	TGCTTTGTTATTCGGGCCAA				
TEM-F	ATGAGTATTCAACATTTCCG	*bla*$_{TEM}$	51ºC	851	Schmiedel et al. (2014)
TEM-R	TTAATCAGTGAGGCACCTAT				

Ta: annealing temperature.

Selected positive PCR products were sequenced with an ABI3730 sequencer (Applied Biosystems) and the sequences were compared with the reported sequences from Gene Bank by Blast (www.ncbi.nlm.nih.gov/blast/) and submitted on Gene Bank.

In vitro susceptibility of CRE isolates against the antibiotics in therapeutic regimen

The bacterial isolates were screened for their susceptibility against colistin sulfate, polymyxin B and tigecycline using microdilution plate method. The antibiotics concentration ranged between 0.5-128µg/ml. The interpretative break point criteria were reviewed according to EUCAST recommendations (EUCAST, 2018).

Results

A total of 112 clinical specimens were collected from patients from Al Kasr AL-Ainy hospital. The specimens were collected from different sources (Fig. 1). About 27.6% (n= 31) of clinical isolates obtained from hospitalized patients in ICU. Most of the patients suffered from urinary tract infections 26.8% (n= 30) followed by respiratory tract infections 18.8% (n= 21), post-surgery infections 8% (n= 9), wound infections 7.1% (n= 8), blood stream infections 3.6% (n= 4) and other types of infection 8% (n= 9). Bacterial isolates were identified by conventional methods as 75% *Klebsiella* spp. (n= 84), 22% *E. coli* (n= 25) and 3% *Enterobacter* spp. (n= 3). All isolates were screened for their carbapenemase activity, about 46% (n= 52) of isolates were resistant to imipenem, 7% (n= 8) intermediate and 46% (n= 52) were sensitive.

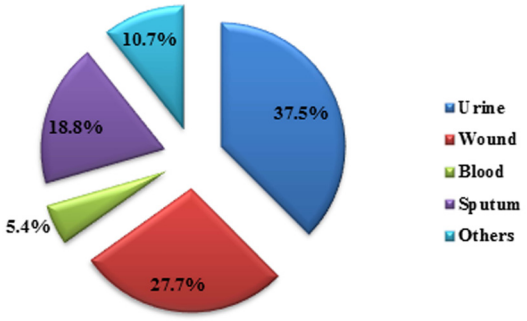

Fig. 1. Pie chart showing the percentages of collected specimens.

Carbapenem resistant isolates were selected for identification by Maldi-TOF. About 84.6% (n= 44) were identified as *Klebsiella pneumoniae*, 11.5% (n= 6) *E. coli,* 1.9% (n= 1) *Enterobacter aerogenes* and 1.9% (n= 1) *Enterobacter cloacae* with score value ranges between 2 to 2.6. In addition, CRE isolates showed

high resistance to different antibiotics classes, piperacillin (96%), cephalosporins (cefadroxil 96%, cefazolin 100%, cefuroxime 100%, cefoxitin 100%, ceftazidime 100%, cefepime 96%), amikacin (98%), nitrofurantoin (81%), levofloxacin (77%), tetracycline (65.3%) and ESBL inhibitor combinations which include amoxycillin/ clavulanic acid (100%) and trimethoprim/ sulfamethoxazole (94.2%). MICs values confirmed that the isolates were highly resistant to piperacillin and cephalosporins (Table 2). On the other hand, MIC values ranged between moderate resistant to carbapenems (MIC; ≥4 to 16µg/ml) to highly resistant isolates (MIC; ≥256µg/ml). Moreover, 77.3% of *K. pneumoniae* had MIC value≥ 128µg/ ml against at least one antibiotic of carbapenem class as shown in Table 2.

Modified Hodge test (MHT) revealed that 65.9% of the isolates were positive while 15.9 % were negative (Fig. 2 and Table 2). On the other hand, 18.2% of the isolates could not be detected using MHT

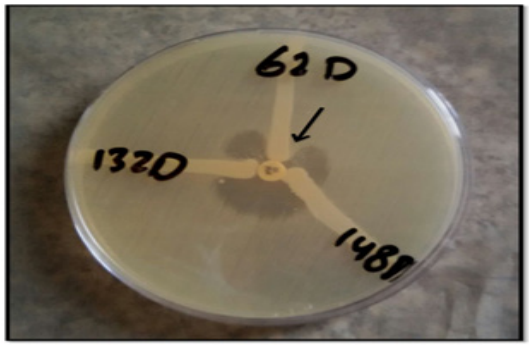

Fig. 2. Modified Hodge test. A lawn of *E. coli* ATCC 25922 (0.5 McFarland) streaked on Muller Hinton agar plates, meropenem disk (10µg) placed on the center of the plate. Three isolates were streaked, streaks 62D and 132D (represent isolates K-93 and K-94) showing positive MHT with the appearance of clover leaf indentation in inhibition zone. Streak148 represent isolate (K-84) showing negative unchanged inhibition zone.

PCR screening of *bla* genes revealed that the most prevalent carbapenemase encoding genes were bla_{NDM} (75%) followed by bla_{OXA-48} (59%). Moreover, 45.5% of *K. pneumoniae* harbored both bla_{NDM} and $bla_{OXA-48,}$ representative PCR amplicons for bla_{NDM} and bla_{OXA-48} are shown in Fig. 3. In this study, bla_{VIM} was recorded in only one isolate in addition; all the isolates were bla_{KPC} and bla_{IMP} genes negative. It was observed that all CRE isolates harbored at least one ESBL encoding gene (Fig. 4), 95.4% of the CRE isolates carried bla_{CTX-M}, 88.6% carried bla_{TEM} , and 68.2% carried bla_{SHV} (Table 2).

DNA sequencing for representative carbapenemase and *ESBL*s encoding genes ($bla_{NDM,}$ $bla_{VIM,}$ $bla_{CTX-M,}$ bla_{TEM} and bla_{SHV}) were submitted to GenBank with accession numbers: (MH673489 - MH673497) for bla_{NDM}, (MH660740-MH660749) for bla_{CTX-M}, and (MH673479-MH673488) for bla_{SHV} encoding genes of *K. pneumoniae,* respectively.

In the current study, all bla_{NDM} sequences were $bla_{NDM-1,}$ bla_{CTX-M} were $bla_{CTX-M-15}$ type while different types of bla_{SHV} encoding genes (bla_{SHV-1}, $bla_{SHV-5,}$ bla_{SHV-11}, bla_{SHV-12} and $bla_{SHV-204}$) were recorded. Furthermore, coexistence of both carbapenemase and ESBLs encoding genes in the same isolate revealed the highest coexistence of bla_{NDM-1} and $bla_{CTX-M-15}$ in 77% of the isolates (Fig. 5).

By using microdilution plate method, both colistin sulfate and polymyxin B showed high potency on CRE isolates by 100%. On the other hand, CRE isolates were less sensitive against tigecycline only 36.4% of the CRE isolates were sensitive. Results showed that 29.5% of *K. pneumoniae* (n= 13/44) had intermediate resistance (MIC, 2µg/ml) and 34.1% (n= 15/44) were highly resistant isolates towards tigecycline (MIC, 4-128µg/ml).

Discussion

Detection of carbapenem resistant *Enterobacteriaceae* has become a necessary objective especially in developing countries where there is uncontrolled use of antibiotics. In the Middle East and Mediterranean region, there were limited reports about the prevalence of CRE; Most of the reports are from Turkey, Greece and Israel (Walsh, 2010; Schwaber et al., 2011 and Djahmi et al., 2014).

A total of 112 clinical isolates that include *K. pneumonia*, *E. coli*, *Enterobacter cloacae* and *Enterobacter aerogenes* were collected and investigated for their carbapenemase activity. The most common infection was urinary tract infection; it was predominant in males 27.9% (of total males) compared to 25% of females. In most reports, urinary tract infections (UTI) are the most commonly observed infection associated with CRE (Van Duin et al., 2014 and Guh et al., 2015).

TABLE 2. Minimal inhibitory concentration of *K. pneumoniae* against different classes of antibiotics, phenotypic (MHT) and genotypic characterizations of CRE isolates.

Isolate code	Source/ gender	MIC (µg/ ml)							MHT	PCR for *ESBL*s and carbapenemase genes							
		IPM	MEM	PRL	CFR	CXM	CTX	FEP		*bla*$_{NDM}$	*bla*$_{OXA-48}$	*bla*$_{VIM}$	*bla*$_{KPC}$	*bla*$_{IMP}$	*bla*$_{CTX-M}$	*bla*$_{TEM}$	*bla*$_{SHV}$
K- 2	Urine/M	64	128	>256	>512	>512	>128	256	+	+	+	-	-	-	+	+	+
K- 3	Wound/ F	128	128	>256	>512	>512	>128	64	+	+	-	-	-	-	+	+	+
K- 5	Urine/M	128	128	>256	>512	>512	>128	128	-	+	-	-	-	-	+	+	+
K- 7	Urine/F	256	64	>256	256	>512	>128	>128	+	-	+	-	-	-	+	+	+
K- 8	Sputum/M	256	16	>256	>128	>512	>128	>128	+	-	+	-	-	-	+	+	+
K-10	Urine/M	256	256	128	>128	>512	>128	>128	+	+	+	-	-	-	+	+	+
K-11	Blood/M	256	128	128	>128	>512	>128	>128	+	+	-	-	-	-	+	+	+
K-13	Urine/M	128	8	128	>128	>512	>128	>128	+	+	-	-	-	-	+	+	-
K-14	Sputum/M	16	≥4	>256	>128	>512	128	64	-	+	+	-	-	-	+	+	-
K-17	Sputum/M	16	≥4	>256	>128	>512	128	64	-	+	+	-	-	-	+	+	-
K-21	Blood/M	256	128	128	>128	>512	>128	>128	+	+	-	-	-	-	+	+	+
K-22	Wound/ F	128	128	128	>128	>512	>128	>128	-	-	-	+	-	-	-	+	+
K-23	Sputum/M	≥128	16	128	>128	>512	>128	>128	+	+	+	-	-	-	+	+	+
K-24	CVL/ M	≥128	256	128	>128	>512	>128	>128	+	+	-	-	-	-	+	+	+
K-51	Sputum/F	128	64	>256	>512	>512	>128	32	+	-	-	-	-	-	+	-	+
K-52	Wound/M	128	128	>256	>128	>512	>128	>128	+	+	-	-	-	-	+	+	-
K-53	Wound/M	64	64	>256	>128	>512	>128	>128	N	+	-	-	-	-	+	-	-
K-55	Sputum/F	≥128	64	>256	>128	>512	>128	>128	+	+	+	-	-	-	+	+	+
K-56	Wound/ F	32	64	>256	>128	>512	>128	>128	+	+	+	-	-	-	+	+	+
K-71	Sputum/M	≥128	128	>256	>128	>512	>128	>128	+	+	-	-	-	-	+	+	+
K-72	Wound/M	64	64	>256	>128	>512	>128	>128	N	+	-	-	-	-	+	-	-

TABLE 2. Cont.

Isolate code	Source/ gender	MIC (µg/ ml)							MHT	PCR for *ESBL*s and carbapenemase genes							
		IPM	MEM	PRL	CFR	CXM	CTX	FEP		bla_{NDM}	bla_{OXA-48}	bla_{VIM}	bla_{KPC}	bla_{IMP}	bla_{CTX-M}	bla_{TEM}	bla_{SHV}
K-74	Wound/M	128	128	>256	>128	>512	>128	>128	+	+	-	-	-	-	+	+	-
K-75	Wound/M	64	64	>256	>128	>512	>128	>128	N	+	-	-	-	-	+	-	-
K-76	Sputum/M	≥128	32	>256	>128	>512	>128	>128	+	-	+	-	-	-	+	+	+
K-79	ETT/ M	32	16	>256	>512	>512	>128	512	N	+	+	-	-	-	+	+	-
K-80	Wound/M	128	64	>256	>512	>512	>128	>512	+	+	+	-	-	-	+	+	+
K-81	Wound/M	128	32	>256	>512	>512	>128	64	+	+	+	-	-	-	+	+	+
K-82	Wound/M	128	128	>256	>128	>512	>128	>128	+	+	-	-	-	-	+	+	-
K-83	Sputum/M	≥128	32	>256	>128	>512	>128	>128	+	-	+	-	-	-	+	+	+
K-84	ETT/ M	32	16	>256	>512	>512	>128	512	N	+	+	-	-	-	+	+	-
K-87	Urine/M	64	16	>256	>512	>512	>128	>512	N	+	+	-	-	-	+	+	-
K-88	Blood/F	≥128	256	>256	128	>512	>128	>128	+	-	+	-	-	-	+	+	+
K-90	Wound/ F	128	128	128	>128	>512	>128	>128	-	-	-	+	-	-	-	+	+
K-91	Sputum/M	≥128	32	>256	>128	>512	>128	>128	+	-	+	-	-	-	+	+	+
K-93	Wound/M	128	64	>256	>512	>512	>128	>512	+	+	+	-	-	-	+	+	+
K-94	Sputum/ F	≥128	128	>256	>128	>512	>128	>128	+	+	+	-	-	-	+	+	+
K-95	Sputum/M	≥128	32	>256	>128	>512	>128	>128	+	-	+	-	-	-	+	+	+
K-96	Urine/M	128	128	>256	>512	>512	>128	128	-	+	-	-	-	-	+	+	+
K-99	ETT/ M	32	16	>256	>512	>512	>128	512	N	+	+	-	-	-	+	+	-
K-100	Wound/M	128	64	>256	>512	>512	>128	>512	+	+	+	-	-	-	+	+	+
K-101	Wound/M	128	64	>256	>512	>512	>128	>512	+	+	+	-	-	-	+	+	+
K-102	Urine/M	128	128	128	128	128	128	128	N	+	+	-	-	-	+	-	+
K-110	Wound/M	128	64	>256	>512	>512	>128	>512	+	+	+	-	-	-	+	+	+
K-111	Blood/M	≥256	≥256	≥256	≥256	≥256	≥256	≥256	+	+	+	-	-	-	+	+	+

M: Male, F: Female, CVL: Central venous line, ETT: Endotracheal tube, MIC: Minimal inhibitory concentration, IPM: Imipenem , MEM: Meropenem, PRL: Piperacillin, CFR: Cefadroxil, CXM: Cefuroxime, CTX: Cefotaxime, FEP: Cefepime, MHT: Modified Hodge test, (+): Positive,(-): Negative, N: Not confirmed, NDM: New Delhi metallo β lactamase , OXA-48: Oxacillinase, KPC: *Klebsiella pneumoniae carbapenemase,* IMP*:* Imipenemase metallo β lactamase, VIM: Verona metallo β lactamase, ESBLs: Extended spectrum β lactamase. Interpretive criteria according to CLSI (2014, 2017) IPM, MEM, CTX: ≤1 S, 2 I and ≥4 R; PRL: ≤16 S, 32–64 I and ≥128 R; CFR, CXM: ≤8 S, 16 I, ≥32R.

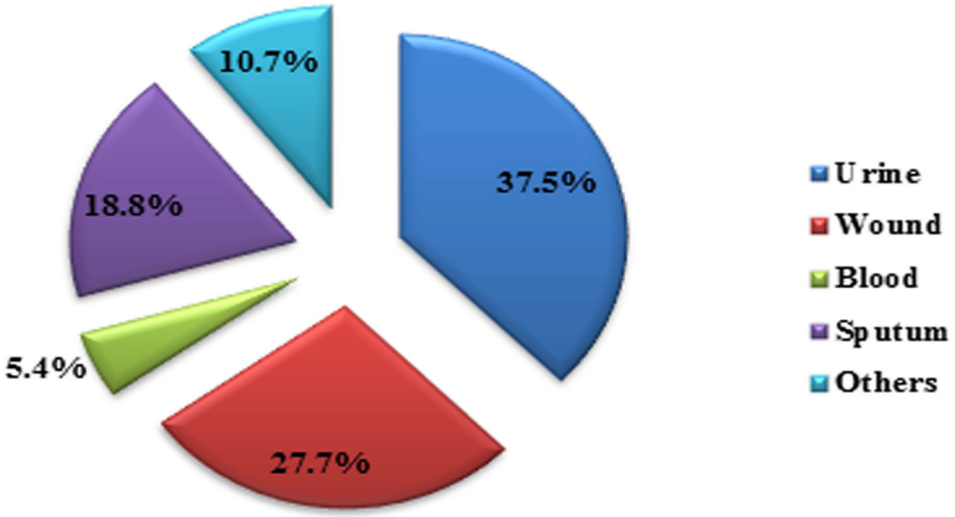

Fig. 3. Agarose gel electrophoresis of PCR amplicons of carbapenem resistance genes of the clinical isolates. A) PCR amplification of blaNDM gene, showing single band at 621bp, B) PCR amplification of blaOXA-48 gene, showing single band at 438bp [M is 1kb DNA marker].

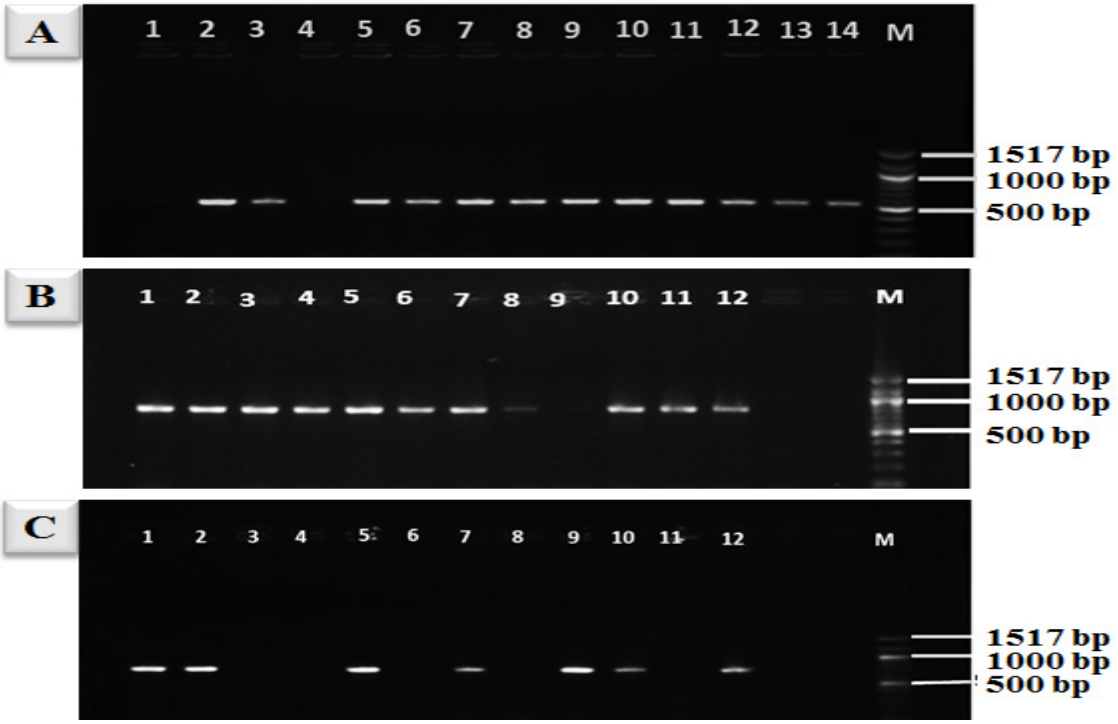

Fig. 4. Agarose gel electrophoresis of PCR amplicons of ESBL genes of the clinical isolates. A) PCR amplification of *bla*$_{CTX-M}$ gene, showing single band at 550bp, B) PCR amplification of *bla*$_{TEM}$ gene, showing single band at 851bp, C) PCR amplification of *bla*$_{SHV}$ gene, showing single band at 747bp [M is 1kb DNA marker].

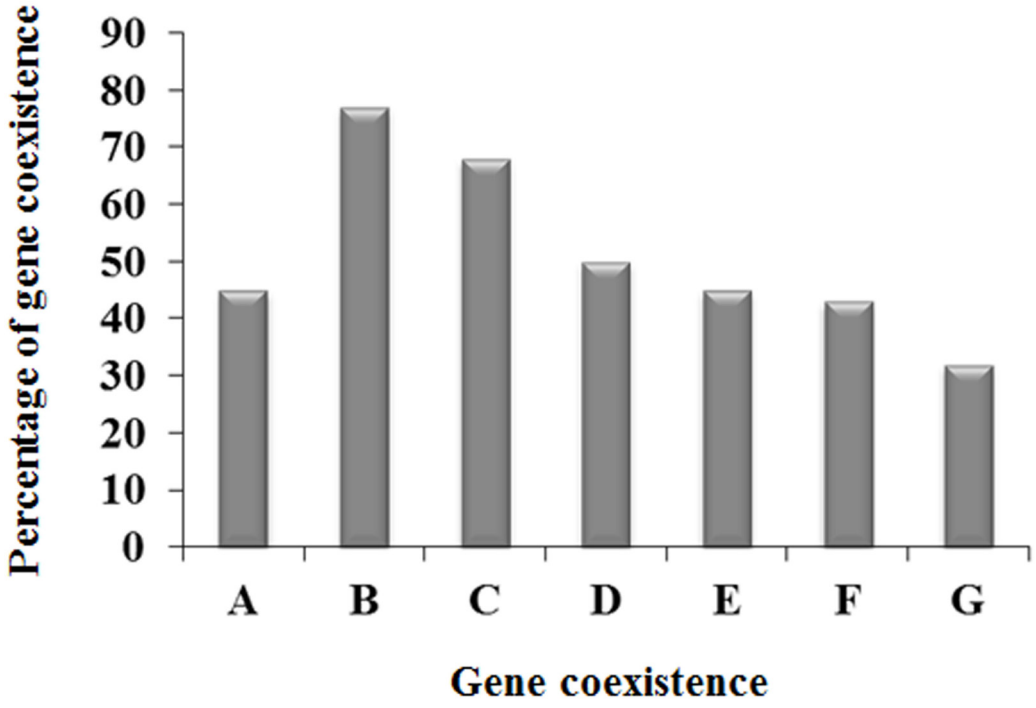

Fig. 5. Percentage of the genes coexistence in CRE clinical isolates. A) *bla*$_{\text{NDM-1 \& OXA-48}}$, B) *bla*$_{\text{NDM-1}}$ & *bla*$_{\text{CTX-M-15}}$, C) *bla*$_{\text{NDM-1}}$ & *bla*$_{\text{TEM-1}}$, D) *bla*$_{\text{NDM-1}}$& *bla*$_{\text{SHV}}$, E) *bla*$_{\text{NDM-1}}$ & *bla*$_{\text{OXA-48}}$ & *bla*$_{\text{CTX-M-15}}$, F) *bla*$_{\text{NDM-1}}$& *bla*$_{\text{OXA-48}}$ & *bla*$_{\text{TEM-1}}$, G) *bla*$_{\text{NDM-1}}$& *bla*$_{\text{OXA-48}}$ & *bla*$_{\text{SHV}}$

The percentage of CRE isolates in this study was 46%, antimicrobial susceptibility profile revealed that CRE isolates were associated with other types of resistance. All CRE isolates were multidrug resistant (MDR), 73% were extensively drug resistant (XDR), while there were no pan drug resistant (PDR) isolates recorded. Many MDR bacteria produce multiple β lactamase enzymes, including combinations of ESBLs and carbapenemases, as well as deficiency of porins expression (Marsik & Nambiar, 2011).

Clinical isolates were identified by Maldi-TOF with score value ranges between 2 to 2.6. According to manufacturer recommendations, the valid identifications score were ≥2.0. Scores below 1.69 were reported as non-reliable identification (ID), scores of 1.70–1.99 considered as probable genus ID, scores of 2.00–2.29 were secure genus, probable species ID and scores of 2.30–3.00 revealed highly probable species ID (Nagy et al., 2012). The Maldi- TOF is a new reliable technology that enhance the identification of bacterial isolates in more accurate, fast and inexpensive way than the conventional methods of bacterial identification and diagnosis (Singhal et al., 2015).

Modified Hodge Test is a simple reliable phenotypic test that used to detect CRE (CLSI, 2017). In our study, MHT could predict the carbapenemase activity for 81.8% while 18.2% remained undetected. Although MHT is a highly recommended test by CLSI for CRE detection, it might give false indications, in addition to the difficulty of the interpretation of the clover leaf for weak carbapenemase producers (Pasteran et al., 2009). Therefore, genetic screening was demonstrated to maximize the sensitivity and discriminate types of CRE encoding genes.

The current study reported that *bla*$_{\text{NDM}}$ and *bla*$_{\text{oxa-48}}$ were the most prevalent among carbapenemase encoding genes. In accordance to our study, Khalifa et al. (2017) and ElMahallawy et al. (2018) demonstrated that *bla*$_{\text{NDM}}$ and *bla*$_{\text{oxa-48}}$ were the most predominant carbapenemase encoding genes in Gram negative bacteria recovered from Egyptian hospitalized patients (Khalifa et al., 2017 and ElMahallawy et al., 2018).

On the other hand, results showed low incidence of VIM (2.3%, n= 1/44) and there were no KPC and IMP detected. The lower detection rate of *bla*$_{VIM}$ gene compared with *bla*$_{OXA-48}$ and *bla*$_{NDM-1}$ may be attributed to the higher prevalence of this gene in Europe than Africa (Khalifa et al., 2017 and Sekyere et al., 2016). *Enterobacteriaceae* carried VIM type (1-4) recorded high incidence in two regions, the Mediterranean region particularly Greece and Turkey and the Indian subcontinent particularly Pakistan (Yildirim et al., 2007; Cagnacci et al., 2008 and Falcone et al., 2009). According to epidemiological and geographical studies, IMP were found mainly in Japan, Taiwan, and Eastern China, that may explain their potential absence in our study and reduced prevalence in other studies in the region (Nordmann & Poirel, 2014). In Africa, *K. pneumoniae* producing IMP-1 was detected only in Morocco and Tunisia (Chouchani et al., 2011 and Barguigua et al., 2012). Despite *bla*$_{KPC}$ wasn't detected in our study, other studies showed high prevalence of KPC in Egypt and suggested that it is underestimated problem (Helal et al., 2014 and Abdulalla et al., 2014). However, Djahmi et al. (2014) reported that sporadic outbreak or single cases of KPC producers *K. pneumoniae* in North African countries and the Middle East regions excluding Israel. KPC was first described in USA in the early 2000s, so far disseminated to other countries mainly Israel, Greece, China and South America (Tzouvelekis et al., 2012).

Our results showed that all carbapenemase producing isolates harbouring at least one *ESBL* encoding genes. The co-presence of CTX, TEM and SHV had been reported in 59% (n= 26/44) of *K. pneumoniae*. In addition, 29.5% of *K. pneumoniae* (n= 13/44) coproduce OXA-48, NDM-1, CTX-M-15, TEM and SHV which alarming a pandemic situation. we conducted a 2-year surveillance of CRE among 12,741 clinical isolates of Enterobacteriaceae at the largest university hospital in Thailand with molecular characterization of beta-lactamase (blaMost carbapenem resistant *K. pneumoniae* harbored multiple *bla* genes, especially in the TEM, SHV and CTX-M families (Netikul & Kiratisin, 2015). Similarly, Zhang et al. (2018) demonstrated the co-production of at least two *ESBL*s genes (SHV-1, SHV-11, CTX-M-14, CTX-M-15, TEM-1, and OXA-1) in a study included 41 carbapenem resistant *K. pneumoniae* isolates.

The most optimal treatment for cases suffering from CRE infections is still unknown. A mono-therapy of colistin sulfate, polymyxin B and tigecycline were demonstrated as a last defense strategy (Morrill et al., 2015 and Taneja & Kaur, 2016). In the present study, Colistin sulfate and polymyxin B showed high antimicrobial activity against almost all CRE isolates, while about 60% were resistant to tigecycline. Resistance of CRE against colistin, tigecycline, and aminoglycosides were reported (Ni et al., 2015). Moreover the percentage of mortality between CRE infected cases that treated by mono-therapy regimens exceeds 40% (Morrill et al., 2015). Combination therapy for CRE infections may decrease mortality compared with monotherapy. Many combinations that includes meropenems with colistin, avibactam and tigecyline are still under trials (Tzouvelekis et al., 2012 and Karaiskos & Giamarellou, 2014).

Conclusion

Carbapenem resistant *Enterobacteriaceae* is a serious issue that the entire world should act together to overcome. This study makes a warning alarm to the prevalence of CRE. The treatment and stopping emergence of CRE is a challenge that could be achieved by studying epidemiology, resistance mechanisms and a new methodology for fast clinical detection of them.

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ظهور الكلبسيلا الرئوية المنتجة لجين نيودلهي ميتالو بيتا لاكتاميز bla_{NDM-1} والاوكساسيلينيز bla_{OXA-48} في مستشفى مصرية

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إن الإنتشار السريع للبكتريا المعوية المضادة للكاربابينيم (CRE) في جميع أنحاء العالم يعد مصدراً للقلق. تهدف هذه الدراسة إلى البحث عن انتشارها في العزلات السريرية المعزولة من مستشفى القصر العيني في الفترة ما بين أغسطس 2015 وفبراير 2017.

تم تعريف العزلات بالطرق التقليدية والتحليل الطيفي Maldi-TOF. وقد تم التعريف للشكل الظاهرى للعزلات المعوية المضادة للكاربابينيم CRE بإستخدام اختبار هودج و التوصيف الوراثي لجينات البيتا لاكتاماز الممتدة الطيف (ESBL) وجينات المقاومة للكاربابينيم CRE.

تم التأكد من النشاط المضاد للكاربابينيم CRE في 46% من العزلات كما تم التأكد من وجود bla_{NDM-1} و bla_{OXA-48} في 75%، 59% من العزلات على التوالي. بينما تم اكتشاف bla_{VIM} في 2.3% فقط من العزلات. ومع ذلك، لم يتم اكتشاف bla_{IMP} و bla_{KPC} في أي من العزلات. وجد أن جميع العزلات المقاومة للكاربابينيم CRE تحمل جين ESBL واحد على الأقل، وأن 95.4% من العزلات لديها 88.6%، $bla_{CTX-M-15}$ تحمل جين bla_{TEM-1} و 68.2% تحمل جين bla_{SHV}. و أظهر جين bla_{SHV} أليلات مختلفة في العزلات المعوية المضادة للكاربابينيم.

كانت جميع العزلات حساسة للبوليميكسين B والكوليستين بينما كان 36.4% منها فقط حساسة للتيجيسيكلين. وتبعا لذلك، ينبغي أن علماء الأحياء الدقيقة والأطباء تنفيذ ما يلزم من تدابير الرقابة لمنع انتشار هذه البكتيريا المقاومة.