Phenotypic and molecular characterization of extended-spectrum β-lactamases and AmpC β-lactamases in *Klebsiella pneumoniae*

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Abstract: The incidence of resistance to extended-spectrum cephalosporins (ESC) among Egyptian isolates of *Klebsiella pneumoniae* has already been increasing and previously reported. This work devotes to investigate the genetic basis of resistance to ESC in *K. pneumoniae* isolates. Disc diffusion test, minimum inhibitory concentrations (MIC) determination and phenotypic screening for extended spectrum β-lactamases (ESBLs) and plasmid-mediated AmpC β-lactamases (PABLs) were carried out for 21 *K. pneumoniae* isolates, collected during 2011 at Sayed Galal Hospital, Cairo. Genes for ESBLs, PABLs and class 1 integrase were sought by PCR and DNA sequencing. Matting out assay was performed to determine the mobility of *bla* genes. Six (28.57%) of 21 clinical isolates *K. pneumoniae* were non-sensitive to ESC. ESBL and PABL phenotypes were identified in 5 and one *K. pneumoniae* isolates, respectively. PABL-producing isolate was found to carry bla_{CMY-2} and bla_{SHV-1}. All five ESBL-producing isolates carried $bla_{CTX-M-15}$. CTX-M-15 was associated with SHV-1 and SHV-12 in three isolates and two isolates respectively.TEM-1 was associated with CTX-M-15 and SHV in two isolates. Both CTX-M-15 and CMY-2 genes were located on conjugative plasmids and associated with class 1 integrase. Resistance to ESC was due to CTX-M-15, SHV-12 and CMY-2 in *K. pneumoniae*. This study represents the first report of CMY-2 and SHV-12 β-lactamase-producing *K. pneumoniae* isolates in Egypt.

Keywords: *Klebsiella pneumoniae*, ESBL, AmpC-plasmid mediated, Antimicrobial resistances, Egypt.

INTRODUCTION

β-Lactamase production is the predominant mechanism for resistance to β-lactams in Enterobacteriaceae. *Klebsiella pneumoniae* isolates are the major hosts for extended-spectrum β-lactamases (ESBLs). In particular, *K. pneumoniae* have also acquired plasmid-mediated AmpC β-lactamases (PABLs) (Tofteland et al., 2012). ESBLs and PABLs inactivate third generation cephalosporins, but PABLs are able to actively inactivate cefoxitin and cefotetan and also resistant to inhibition by β-lactamase inhibitors such as clavulanic acid, tazobactam and sulbactam (Livermore and Woodford. 2006). Prevalence of PABLs is less common than ESBL in most parts of the world (Jacoby, 2009). ESBLs have been reported global, most often in *Escherichia coli* and *K. pneumoniae* (Dallenne *et al*., 2010). ESBL-producing *K. pneumoniae* have spread quickly and pose a serious risk in healthcare-associated infections (Kiratisin *et al*., 2008). ESBLs have spread threateningly worldwide and now contain over three hundred variants (http://www.lahey.org/studies). Although the PABLs are less common than ESBLs, but they have been found in several areas of the world. Among them, CMY-2 has the broadest geographical spread. *E. coli* and *K. pneumoniae* producing ESBL have been well recognized in Egypt, but the appearance of bla_{CTX-M} determinants is a recent finding (Al-Agamy *et al*., 2006; Khalaf *et al*., 2009; AbdelGhani *et al*., 2009: Fam *et al*., 2011). PABLs are not being detected in Egypt except CMY-2 was recently detected in *Salmonella enterica* serovar Typhimurium (Ahmed and Shimamoto, 2011). The goals of the present study were to determine the prevalence rate of these resistance mechanisms in isolates of *K. pneumoniae* from Cairo, Egypt. The first identification of the CMY-2 PABLs in *K. pneumoniae* in Egypt is also described.

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MATERIALS AND METHODS

Bacterial strains

Between January and March 2011, 21 non-duplicate non-consecutive *K. pneumoniae* clinical isolates were collected from Sayed Galal Hospital, Cairo, Egypt. *E. coli* ATCC 25922 strain has been used as standard control in MIC. The isolates were preserved at −20 °C in trypticase soy broth with 20% glycerol.

Identification of bacterial isolates

The bacterial isolates were identified using a commercial identification system of MERLIN Diagnostika, micronaut-IDS (Merlin, Bornheim, Germany). This system is used in the identification of *Enterobacteriaceae*, nonfermentative Gram-negative rods, staphylococci, enterococci, and streptococci. The system characterizes the bacterial isolates via 23 different biochemical reactions. The well-isolated colonies of the test organism were taken from 18 h culture. The colonies were suspended in 5 ml sterile 0.9 % (w/v) sodium chloride pH7 and mixed thoroughly until the turbidity was

equivalent to McFarland 2. Micronaut-IDS test plate was taken of its package just prior to use. The bacterial suspension of each was poured in one sector of a four-sector reservoir. Each 2 rows of the plate were inoculated (100 µl/well) with the bacterial suspension. The wells which contain urease, amino acids**,** decarboxylase and amino acid control tests were covered with 2 drops of sterile paraffin oil. The inoculated plate was incubated at 37 ^{0}C for 5-6 h. At the end of incubation period, 2 drops of indole reagent were added to the well of indole test and 2 drops of peptidase reagent were added to the wells which contain pyrase, hydroxyprolinamidase, and tripeptidase tests. The results were measured automatically using Micronaut multiscan software.

Antibiotic susceptibility testing

Susceptibility testing was performed according to CLSI recommendations (CLSI, 2006) by a micro-dilution procedure in Mueller-Hinton Broth (Becton Dickinson, USA). Microtiter plates containing panel of dehydrated antimicrobial agents in doubling dilutions (Merlin-Diagnostika, Germany) were inoculated with 100µl volume of an appropriate bacterial suspension ($5x10^5$ CFU/ml). MIC values were determined with a photometer for microtitre plates (Labystems Multiscan Multisoft, Helsinki, Finland) and evaluated with EXCEL (Microsoft).

Phenotypic characterization of β-lactamases

Phenotypic detection of ESBL and PABLs was done according to the methods described previously (Sabia *et al*., 2010). Mueller–Hinton agar plate was inoculated with 0.5 McFarland bacterial suspension in 0.9% sodium chloride. Three sets of antibiotic discs were placed onto the medium. The first set included cefotaxime (CTX) (30 µg) and ceftazidime (CAZ) (30 µg) discs; the second set contained CTX+clavulanate (CTX+CLA) and CAZ+CLA discs; the final set included CTX +boronic acid (CTX+BA) and CAZ+BA discs. After incubation period **of** 16–20 h at 35 °C the results were recorded. ESBL was detected if ≥5 mm increases of inhibition zone diameter around CTX and/or CAZ disks when combined with CLA and PABL was detected if ≥5 mm increases of inhibition zone diameter around CTX and/or CAZ disks when combined with BA.

Determination of molecular weight(s) of β-lactamases

The molecular weight**(s)** of β-lactamases was estimated by Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) according to method described by Sambrook *et al.* (1989). The acrylamide solution (13%) was poured into the gap between the glass plates. The gel was covered with water and kept in vertical position at room temperature for 30 minutes at room temperature. The β-lactamase samples were prepared by mixing the specimens with 1x SDS gel-loading buffer in a ratio of 1:2 v/v. The gel was mounted in the electrophoresis apparatus. Tris-glycine buffer, pH 8.3 was added to the top and the bottom reservoirs. Amounts of 15 µL of each of the sample as well as protein marker were loaded into the wells. The electrophoresis apparatus was connected to an electric power supply at 150 V for 2 h. The glass plates were removed from the electrophoresis apparatus. The gel was stained with nitrocefin solution and scanned. The protein marker, containing 175, 83, 62, 47.5, 32.5, 16.5 and 6.5 KD MW proteins was routinely used with the electrophoresis run.

Preparation of DNA template

Total bacterial DNA was extracted by the whole cell boiling procedure. Bacterial cells were pelleted by centrifugation, resuspended in 100 µL of DNA-free sterile water and then boiled for 10 min at 100^{o}C using heat block to get lysate. The lysate was collected by centrifugation and then the supernatant was transferred to a fresh tube. The cell lysate was used directly for PCR as DNA template.

Molecular detection of β-lactamases genes and class 1 integrase

The primers (Operon Biotechnologies GmbH, Cologne, Germany) used in the current study to amplify and sequence of β-lactamase genes in *K. pneumoniae* are listed in Table 1. PCR methods were used to detect TEM, SHV and CTX-M genes according to the previously described methods (Al-agamy *et al*., 2006, Nüesch-Inderbinen *et al*., 1997, Woodford *et al*., 2006). Multiplex PCR was used to detect PABL genes. Multiplex PCR was performed according to the previously described method (Pérez-Pérez and Hanson, 2002). Amplification of class 1 integrase was performed by using the forward int1L primer (5'-ACATGTGATGGCGACGCACGA-3') and backward int1R primer (5'-ATTTCTGTCCTGGCTG GCGA-3'). PCR protocol of class 1 integrase was performed as mentioned: initial denaturation at 94°C for 10 min followed by 35 cycles of 94°C for 1 min, 60°C for 1 min, 72°C for 1 min and then final elongation at 72°C for 10 min (Pan *et al*., 2006). All PCR assays were run in Techne Flexigene Thermocycler (Techne, Duxford, Cambridge, UK).

Gel electrophoresis

PCR amplicons were separated electrophoretically in a 1-2% agarose gel using 1**x** TBE, visualized by staining with 0.5 µg/ml ethidium bromide, and examined in UV light and photographed using video documentation system.

DNA sequencing

To identify the β-lactamase genes detected in the PCR assays, DNA sequence analyses of the amplicons were performed. Amplified PCR products were purified using the Qiagen purification kit (Qiagen, Hilden, Germany) and bidirectional sequencing was performed using

Table 1: Primers used to amplify and sequencing of β-lactamase genes in this study

Target	Primer	Sequence (5'-→3')	bp	reference
TEM	T1	ATT CTT GAA GAC GAA AGG GCC TC	1073	Al-Agamy *et al.*, 2006
	T3	CGC AAC GTT GTT GCC ATT GCT G		
SHV	NI1	GCC CGG GTT ATT CTT ATT TGT CGC	1016	
	NI2	TCT TTC CGA TGC CGC CGC CAG TCA		
CTX-M	Group-1F	AAA AAT CAC TGC GCC AGT TC	415	Woodford *et al.*, 2005
	Group-1R	AGC TTA TTC ATC GCC ACG TT		
	Group-2F	CGA CGC TAC CCC TGC TAT T	552	
	Group-2R	CCA GCG TCA GAT TTT TCA GG		
	Group-9F	CAA AGA GAG TGC AAC GGA TG	205	
	Group-9R	ATT GGA AAG CGT TCA TCA CC		
	Group-8F	TCG CGT TAA GCG GAT GAT GC	666	
	Group-25F	GCA CGA TGA CAT TCG GG	327	
	Group-8/25R	AAC CCA CGA TGT GGG TAG C		
MOX-1, MOX-2, CMY-1, CMY-8 to CMY-11	MOXMF	GCT GCT CAA GGA GCA CAG GAT	520	Pérez-Pérez and Hanson 2002
	MOXMR	CAC ATT GAC ATA GGT GTG GTG C		
LAT-1 to LAT-4, CMY-2 to CMY-7, BIL-1	CITMF	TGG CCA GAA CTG ACA GGC AAA	462	
	CITMR	TTT CTC CTG AAC GTG GCT GGC		
DHA-1, DHA-2	DHAMF	AAC TTT CAC AGG TGT GCT GGG T	405	
	DHAMR	CCG TAC GCA TAC TGG CTT TGC		
ACC	ACCMF	AAC AGC CTC AGC AGC CGG TTA	346	
	ACCMR	TTC GCC GCA ATC ATC CCT AGC		
MIR-1T ACT-1	EBCMF	TCG GTA AAG CCG ATG TTG CGG	302	
	EBCMR	CTT CCA CTG CGG CTG CCA GTT		
FOX-1 to FOX-5b	FOXMF	AAC ATG GGG TAT CAG GGA GAT G	190	
	FOXMR	CAA AGC GCG TAA CCG GAT TGG		

sequencing kit on a 3130x1 DNA Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Nucleotide- and deduced protein sequences were analyzed with public access software (www.ncbi.nlm.nih.gov).

Matting out assay

Matting out assay was performed with *K. pneumoniae* isolates as donors and sodium azide resistant *E. coli* JM109 as the recipient. The broth mating technique was carried out as previously described (Sambrook *et al*., 1989). Transconjugants were selected on MacConkey agar plates containing sodium azide 200 mg/L and CTX 2 mg/L. PCR assays were done for the obtained transconjugants as described above.

RESULTS

K. pneumoniae clinical isolates (n= 21) were analyzed to characterize phenotypic and genotypic resistance profile. Resistance rates to β-lactams among the 21 *K. pneumoniae* isolates, by MIC, were: piperacillin 80.95%, piperacillin/tazobactam 28.75%, CAZ 28.75%, CAZ/CLA 4.76%, CTX 28.75%, CTX/CLA 4.76%, cefepime 23.8%, and aztreonam 28.75%; while those for non-β-lactam antibiotics were gentamicin 23.8%, amikacin 14.28%, ciprofloxacin 23.8%, trimethoprim 76.19%, sulphamethoxazole/ trimethoprim 61.9%, and chloramphenicol 85.71%. All isolates were resistant to amoxicillin 100%, ampicillin 100%, ticaricillin 100%, and sulphamethoxazole 100%. No resistance was detected for imipenem and tigecycline.

Six (28.57%) out of 21 isolates were resistant to CTX. These isolates were undertaken for phenotypic and genotypic investigation of the β-lactamases.
MICs of CTX-resistant isolates are shown in table 2.

The results of SDS-PAGE (fig. 1) for six CTX-resistant isolates revealed that all isolates had β-lactamase band at 28.5 KD; however only one isolate had extra β-lactamase band at 39 KD.

The prevalence of ESBL and PABLs were 23.81% (5/21) and 4.76% (1/21) respectively. Isolates found to be ESBL and PABL positive were subjected to PCR.

The results of PCR (table 2, figs. 2 and 3) showed *bla* genes in six CTX-resistant isolates were: bla_{TEM} 2/6 (33.33%), bla_{SHV} 6/6 (100%), bla_{CTX-M} 5/6 (83.33%) and bla_{CMY} 1/6 (16.66%). Among five ESBL positive isolates, 2 of them produced TEM gene; however all the five isolates produced SHV and CTX-M genes. DNA sequencing of TEM, SHV, and CTX-M genes revealed that *bla* genes belonging to TEM-1, SHV-1, SHV-12 and CTX-M-15. The identified genes in the isolates are: KP16 isolate harbors TEM-1+SHV-12+CTX-M-15, KP18 isolate harbors TEM-1+SHV-1+ CTX-M-15, KP20 isolate harbors SHV-12+ CTX-M-15 and KP7 and KP9 isolates harbor SHV-1+CTX-M-15. On the other hand KP1 isolate which produced PABL, contained CMY-2 +SHV-1.

The results of the matting out assay showed that all CTX resistance determinants were encoded on conjugative plasmid. PCR experiments showed that $bla_{CTX\text{-}M\text{-}15}$ and $bla_{CMY\text{-}2}$ were transferable however $bla_{SHV\text{-}12}$ was not transferable and encoded on non-conjugative plasmid.

Table 2: MIC and phenotypic and genotypic characterization of ceftazidime-resistant *Klebsiella pneumoniae* isolates

Antimicrobial agents		MIC (mg/L)					
		KP1	KP7	KP9	KP16	KP18	KP20
Ampicillin		>2048	>2048	>2048	>2048	>2048	>2048
Ampicillin/sulbactam		>256	>256	>256	>256	>256	>256
Amoxicillin		>128	>128	>128	>128	>128	>128
Amoxicillin/Clavulante		>128	>128	>128	>128	>128	>128
Piperacillin		>256	>256	>256	>256	>256	>256
Piperacillin/Clavulanic acid		>128	64	128	32	64	32
Ticaricillin		>256	>256	>256	>256	>256	>256
Cefpodoxime		>64	>64	>64	>64	>64	>64
Cefpodoxime /Clavulanic acid		>16	2	0.5	<0,25	8	4
Ceftazidime		>32	>32	>32	>32	>32	>32
Ceftazidime/Clavulanic acid		>32	4	1	0.5	1	4
Cefotaxime		>32	2	1	2	0.5	4
Cefotaxime/Clavulanic acid		>32	8	2	<0,5	<0,5	2
Cefepime		>64	>64	>64	>64	>64	>64
Cefepime/Clavulanic acid		64	<0,5	<0,5	<0,5	1	2
Aztroenam		>32	16	>32	>32	>32	>32
Cefoxitin		>32	16	<4	<4	8	8
Imipenem		<0,25	1	<0,25	<0,25	<0,25	0.5
Meropenem		<0,0625	1	<0,0625	<0,0625	<0,0625	1
Amikacin		16	4	64	8	128	128
Gentamicin		64	0.25	32	128	64	32
Tobramycin		64	4	4	16	8	4
Netilimicin		128	8	32	32	4	2
Neomycin		32	8	8	32	64	32
Kanymycin		>256	>256	>256	>256	>256	>256
Streptomycin		>256	256	>256	>256	>256	256
Ciprofloxacin		>32	0.03125	32	>32	>32	32
Enoxacin		>32	0.125	>32	>32	>32	>32
Fleroxacin		>32	0.125	>32	>32	>32	>32
Norfloxacin		>64	0.125	>64	>64	>64	>64
Ofloxacin		>64	0.0625	64	32	>64	>64
Pefloxacin		>32	0.125	>32	>32	>32	>32
Sparfloxacin		32	0.03125	32	32	32	32
Pimpedic acid		>128	2	>128	>128	>128	>128
Tetracycline		>64	>64	>64	>64	>64	>64
Doxycycline		16	16	32	32	32	32
Minocycline		8	4	32	64	>64	>64
Tigecycline		≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25
Sulphamethoxazole		>512	>512	>512	>512	>512	>512
Trimethoprim		>64	>64	32	>64	>64	>64
Sulphamethoxazole/ Trimethoprim		>256	>256	8	>256	>256	>256
Nitrofurantoin		64	>256	>256	256	>256	>256
Chloramphenicol		>128	>128	>128	>128	>128	>128
Phenotypic characterization of β-lactamase		AmpC	ESBL	ESBL	ESBL	ESBL	ESBL
PCR	TEM	-	-	-	TEM-1	TEM-1	-
	SHV	SHV-1	SHV-1	SHV-1	SHV-12	SHV-1	SHV-12
	CTX-M	-	CTX-M-15	CTX-M-15	CTX-M-15	CTX-M-15	CTX-M-15
	AmpC	CMY-2	-	-	-	-	-
	Int1	+	+	+	+	+	+

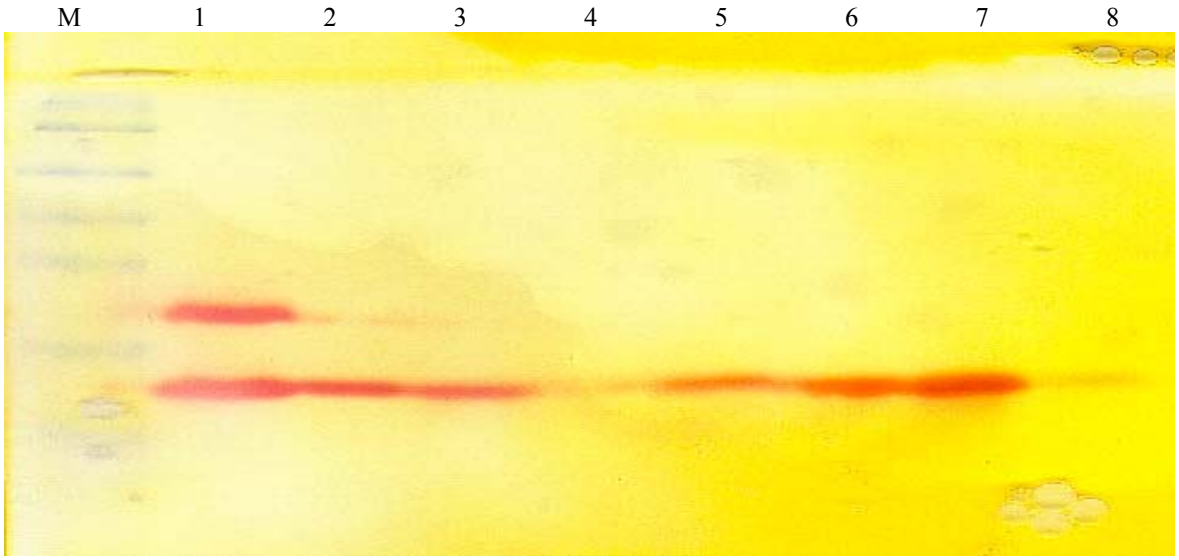

Fig. 1: Nitrocefin stained SDS-PAGE of ultrasonic free cellular extracts of ESBL-producing *K. pneumoniae* strains containing β-lactamase(s). *Lane M*: contained 15µl of prestained protein marker. *Other lanes*: contained 15µl/slot (each) of ultrasonic free cellular extracts of the tested ESBL-producing *K. pneumoniae* strains**.**

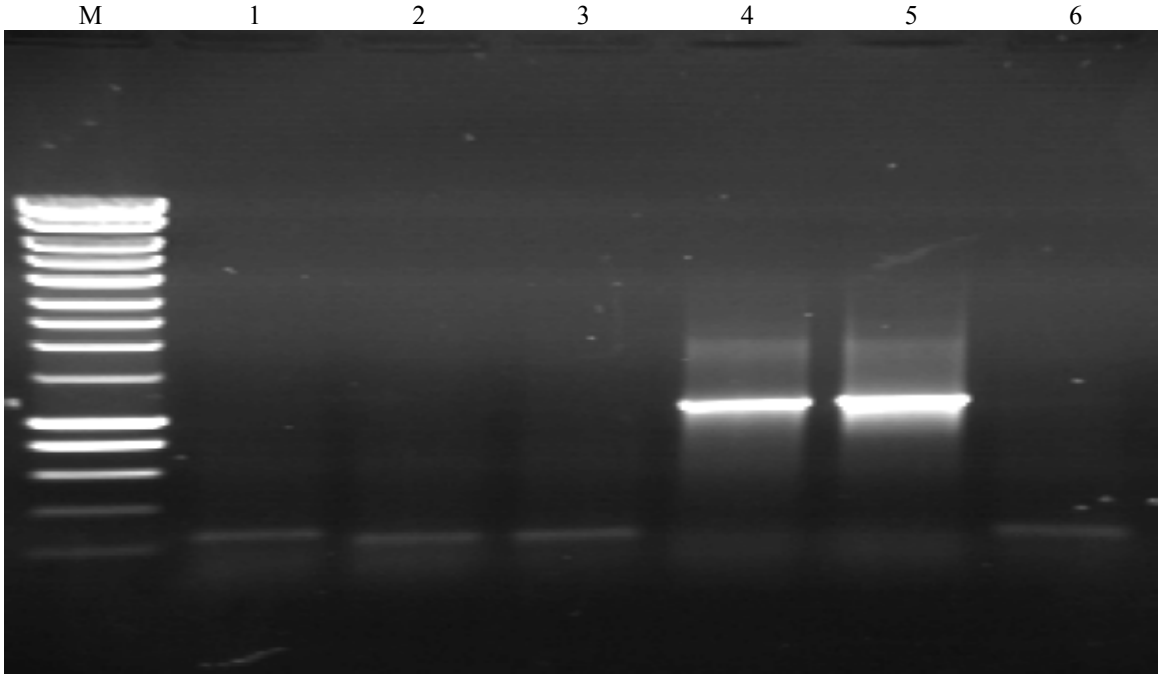

Fig. 2: TEM-PCR of ESBL-producing *K. pneumoniae* strains using T1 and T3 primer.

PCR studies for integrons (table 2) revealed the presence of class 1 integron in all six CTX resistant *K. pneumoniae* isolates and their transconjugants either ESBL- or PABL-producing isolates.

DISCUSSION

Resistance to ESC among members of the family Enterobacteriaceae occurs worldwide; however, little is known about ESC resistance in *K. pneumoniae* isolates from Egypt so this study was devoted to characterize molecular resistance mechanisms to CTX in *K. pneumonaie* isolates from Egypt.

In the current study the resistance to CTX was moderately high (28.57%). Ambler class A and class C β-lactamases contributed to CTX resistance in the *K. pneumoniae* isolates. Phenotypic characterization of six isolates of

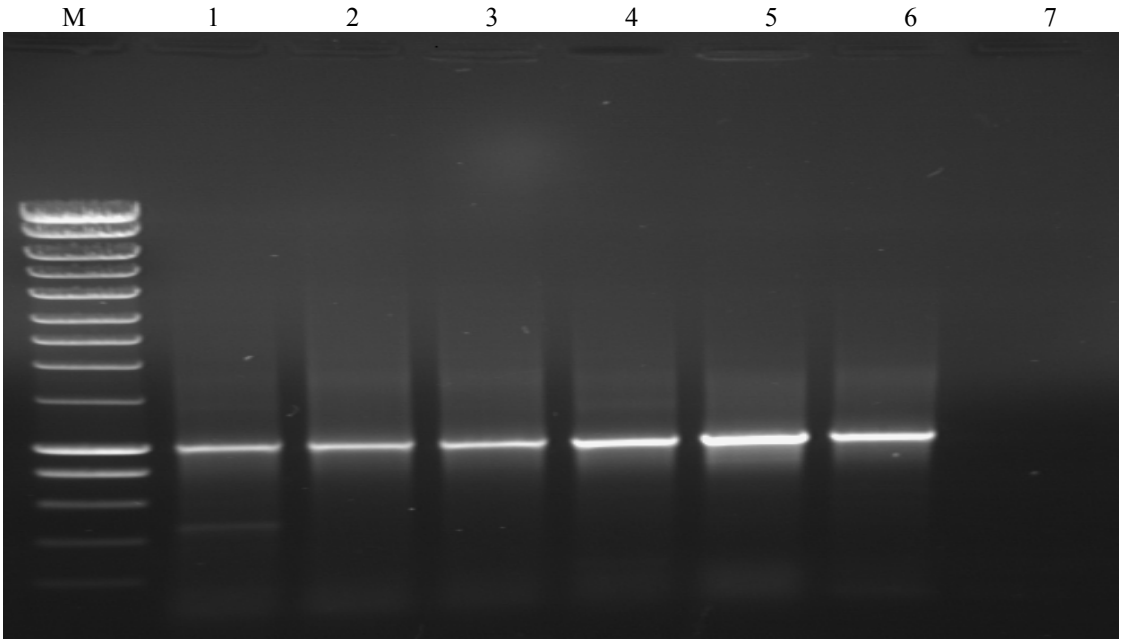

Fig. 3: SHV-PCR of ESBL-producing *K. pneumoniae* strains using NI1 and NI2 primer.

CTX-resistant *K. pneumonia*e was done by using SDS-PAGE to determine the molecular weights of β-lactamase and by combined disk test. SDS-PAGE revealed that CTX-resistant isolates had β-lactamase band at 28.5 KD; however only one isolate had extra β-lactamase band at 39 KD. These results indicated that the six isolates harbor β-lactamase band at 28.5 KD belong to ambler class A such as TEM, SHV and CTX-M while the isolates KP1 harbor an extra band at 39 KD which belong to PABL**.** In this study ESBLs and PABL production were 23.81% and 4.76% respectively. PABL is less common than ESBL production in most parts of the world (Jacoby, 2009) and this was in agreement with the present study. ESBLs production was 23.81% however PABL production was found to be 4.76% in this study. All ESBL- and PABL-positive isolates were resistant to CTX. On the other hand, ESBL positive isolates were sensitive to cefoxitin while PABL-positive isolate was resistant to it. The results of our study showed that imipenem and tigecycline are the most effective drugs against both the ESBL- and PABL- producing *K. pneumoniae*.

The current study showed significantly lower ESBLs production than other studies in Egypt (Ahmed *et al*., 2009, Abdel-Hady *et al*., 2008, Zaki, 2007, Moore *et al*., 2005). The frequency of CTX resistance in Egypt either due to ESBL- or PABL production was high (Ahmed *et al*., 2009, Abdel-Hady *et al*., 2008, Zaki, 2007, Moore *et al*., 2005). This may be explainable, in part, due to uncontrolled use of antibiotic in Egypt, since use of antibiotics and injectable formulations were high and also higher number of prescriptions for antibiotics. ESBL-and PABL-encoding genes are generally encoded on plasmids, which also carry resistance for other antibiotics such as aminoglycosides, fluoroquinolones, sulfonamides, tetracyclines, and chloramphenicol. The high resistance to gentamicin, ciprofloxacin, and sulfonamides in this study is not surprising because of its extensive use, particularly in UTIs. This phenomenon was also observed in other studies in Egypt (Al-Agamy *et al*., 2006), Saudi Arabia (Tawfik *et al*., 2011), Pakistan (Hussain *et al*., 2011), India (Shahid *et al*., 2008) and Brazil (Kiffer *et al*., 2006). The high rate of ESBLs and PABL among hospitalized patients is a global problem. It is generally thought that patients infected by ESBL- and/or PABL- producing organism are at an increased risk of treatment failure with ESC (Hussien *et al*., 2011).

The prevalence of ESBL producing isolates of *K. pneumoniae* varies in different countries (Shah *et al*., 2004). Frequency of ESBL-producing *K. pneumoniae* isolates reported in the TEST surveillance study (2004-2006) of different geographic areas showed that the most prevalence was in South America and the lowest prevalence was in North America and Europe (Valverde *et al*., 2008). The prevalence of *K. pneumoniae* isolates with ESBL phenotype has been reported from 0% in Iceland to 83.3% in Romania (Valverde *et al*., 2008).

Our results revealed high prevalence of bla_{SHV} (100%) and $bla_{CTX\text{-}M}$ (100%) and low frequency of bla_{TEM} (40%) in ESBL-producing *K. pneumoniae* isolates. However, $bla_{CMY,}$ the sole gene was detected among PABL genes tested in the KP1 isolate which phenotypically produce PABL. CTX-M β-lactamases play a significant role in resistance to ESC worldwide. The activity of these

enzymes against CTX is markedly higher than that against CAZ (Tzouvelekis *et al*., 2000). CTX-Ms have been reported in Africa, Asia, Europe and America, and the prevalence is increasing faster than TEM- and SHV-type ESBLs (Coque *et al*., 2008; Pitout and Laupland, 2008; Livermore *et al*., 2007; Bonnet 2004). PCR and DNA sequencing identified $bla_{\text{CTX-M-15}}$ in all ESBL positive isolates and $bla_{\text{SHV-12}}$ in two clinical isolate (KP16 and KP20). The most genes responsible for ESBLs production were found in $bla_{\text{CTX-M-15}}$ (100%) followed by $bla_{\text{SHV-12}}$ (40%) in the present study. CTX-M-15 is the predominant ESBL genes in Egypt (Fam *et al*., 2011; Ahmed *et al*., 2009; Khalaf *et al*., 2009; Al-Agamy *et al*., 2006).

Until now, 81 CMY alleles are currently known, 10 varieties of FOX; 9 varieties of ACT, 8 varieties of DHA,8 varieties of MOX, 5 varieties of MIR, 4 varieties of ACC and one variety of LAT (http://www.lahey.org/Studies/). The most frequent PABLs is CMY**,** and CMY-2 was the most frequent CMY plasmidic cephalosporinase in Enterobacteriaceae worldwide (Jacoby, 2009). In the present study $bla_{\text{CMY-2}}$ was detected in the isolate (KP1) produced PABL. Other PABL genes were not detected in the present study. Plasmids carrying genes for PABLs often carry multiple other resistances including genes for resistance to aminoglycosides, chloramphenicol, quinolones, sulfonamide, tetracycline, and trimethoprim as well as genes for other β-lactamases (Jacoby, 2009). In the present study CMY-2 producing isolate showed resistance to ciprofloxacin, gentamicin, sulphamethoxazole, trimethoprim, sulphamethoxazole/Trimethoprim, and chloramphenicol. Also, this isolate carry SHV-1 β-lactamase.

The presence of $bla_{\text{CTX-M-15}}$ and $bla_{\text{CMY-2}}$ on conjugative plasmids enhances the spread of these genes in Egyptian isolates however the presences of $bla_{\text{SHV-12}}$ on non-conjugative plasmids hinder the spread of this gene among Egyptian isolates.

In conclusion, both class A and class C β-lactamases contributed to cephalosporin resistance in the *K. pneumoniae* isolates, thereby limiting therapeutic options. The most reliable and effective antimicrobial treatment for infections caused by *K. pneumoniae* are imipenem and tigecycline. The $bla_{\text{CTX-M-15}}$ was found as a predominant gene followed by $bla_{\text{SHV-12}}$ which are responsible for ESBLs production and $bla_{\text{CMY-2}}$ was found as a sole gene responsible for PABL production. Future study needs to determine the prevalence and type of PABLs in *K. pneumoniae* and other genus of *Enterobacteriaca*e.

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