INTRODUCTION

Carbapenem-resistant *Enterobacteriaceae* (CRE) have disseminated widely since being first reported in 2001 and are usually resistant to all β-lactam agents as well as most other classes of anti-microbial agents. Carbapenem-resistant *Enterobacteriaceae* (CRE) often carry genes that confer high levels of resistance to many other antimicrobials. The treatment options for patients infected with CRE are very limited. Patients colonized with CRE are thought to be a source of transmission in the healthcare setting. Carbapenem-resistant *Enterobacteriaceae* (CRE) have been associated with high mortality rates (up to 40 to 50% in some studies) ***(Calfee and Jenkins, 2008)***.

Carbapenem resistance among *Enterobacteriaceae*, in particular among *Klebsiella pneumonia* and *Escherichia coli*, is an emerging problem world wide. Several resistance mechanisms have been reported to circumvent the efficacy of carbapenems, and carbapenemases are the most prominent enzymes that neutralize carbapenems. *Klebsiella pneumoniae* carbapenemase (KPCs) are the most prevalent of this group of enzymes ***(Hindiyeh et al., 2008).***

The increased worldwide spread of carbapenem resistant *Enterobacteriaceae* (CRE) emphasizes the need for a sensitive screening procedure to identify these micro-organisms. Gastrointestinal carriers may serve as the reservoir for cross-

transmission in the health care setting, and thus active surveillance is a key part in preventing the spread of such strains. Many methods used for identification of these strains, as agar-based methods which detect CRE directly from rectal swabs: CHROMagar KPC; MacConkey agar with imipenem; and MacConkey plates with imipenem, meropenem, and ertapenem disks ***(Adler et al., 2011).***

While molecular techniques remain the gold standard for the precise identification of carbapenemase gene, most of these techniques are based on PCR. A PCR technique can give results within 4-6h (or less when using real-time PCR technology) with excellent sensitivity and specificity. The main disadvantages of the molecular based technologies are their cost, the requirement for trained microbiologists and inability to detect novel unidentified genes ***(Nordmann and Poirel, 2013).***

AIM OF THE WORK

The aim of this work is to assess CHROMagar KPC for detection of KPC producing carbapenem resistant *Enterobacteriaceae (CRE)* from rectal swabs of ICU patients.

CARBAPENEMASE

Carbapenemases represent the most versatile family of β-lactamases. Earlier carbapenemases were mostly species specific and chromosomally encoded enzymes ***(Livermore and Woodford, 2006).*** This was true until approximately 2000, when the rapid dissemination of multidrug-resistant (MDR) *Klebsiella pneumoniae* strains producing carbapenemases encoded by transmissible plasmids occured. Later, other clinically important *Enterobacterial* species, including *Escherichia coli*, acquired carbapenemase genes. Thus, it appears probable that as in the ESBL "era," *K. pneumoniae* again functions as a pool of potent β-lactamases. The clinically most important carbapenemases in *Enterobacteriaceae* are the class A enzymes of the KPC type and the zinc-dependent class B metallo β-lactamases (MβLs), represented mainly by the VIM, IMP, and NDM types. The plasmid-expressed class D carbapenemases of the OXA-48 type complete the picture ***(Patel and Bonomo, 2011).***

Resistance to carbapenem among Gram negative bacteria may occure due to other mechanisms as:

1. Active transport of carbapenem drugs out of the cell (efflux pump)

2. One mechanism of resistance is mutation in or loss of outer membrane porins, preventing antibiotics from entering the cells ***(Meletis et al., 2012).***

Combinations of these mechanisms can cause high levels of resistance to carbapenems in bacteria such *as K. pneumoniae, P. aeruginosa,* and *A. baumanii*. Carbapenem resistance in Gram-positive cocci is typically due to the result of substitutions in amino acid sequences of PBPs or acquisition production of a new carbapenem resistant PBP ***(Meletis et al., 2012).***

Classification:

Carbapenemases can be classified on the basis of function or structure. The most commonly used classification is Ambler which based on molecular structure. In this classification, carbapenemases belong to classes A, B, or D. Class A and D are serine carbapenemases, meaning that they have a serine at their active sites, like ESBLs. In contrast, the class B enzymes are known as metallo β lactamases, because they require zinc as a cofactor. Ambler classified beta-lactamases according to the amino acid sequences. While according to Bush-Jacoby functional classification carbapenemases fall under groups 2f, 2df and 3, this classification depend on biochemical analysis of enzyme, determination of isoelectric point, determination of substrate hydrolysis, enzyme kinetics and inhibition profiles (table 1) ***(Sridhar, 2012).***

Table (1): Classification schemes for β-lactamases

Molecular class (subclasses	Bush–Jacoby group (2009)	Distinctive substrate(s)	Defining characteristic(s)	Representative enzyme(s)
A	2a	Penicillins	Greater hydrolysis of benzyl penicillin than cephalosporins	PC1
A	2b	Penicillins,early cephalosporins	Similar hydrolysis of benzyl penicillin and cephalosporins	TEM-1, TEM-2, SHV-1
A	2be	Extended-spectrum cephalosporins, monobactams	Increased hydrolysis of oxyimino-β-lactams (cefotaxime,ceftazidime,ceftriaxone,cefepime and aztreonam)	TEM-3, SHV-2, CTX-M-15, PER-1, VEB-1
A	2br	Penicillins	Resistance to clavulanic acid and sulbactam but not to tazobactam.	TEM-30, SHV-10
A	2ber	Extended-spectrum cephalosporins,monobactams	Increased hydrolysis of oxyimino-β-lactams combined with resistance to clavulanic acid,sulbactam and tazobactam	TEM-50
A	2c	Carbenicillin	Increased hydrolysis of carbenicillin	PSE-1, CARB-3
A	2ce	Carbenicillin, cefepime	Increased hydrolysis of carbenicillin,cefepime and cefpirome	RTG-4
A	2e	Extended-spectrum cephalosporins	Hydrolyzes cephalosporins Inhibited by clavulanic acid but not aztreonam	CepA
A	2f	Carbapenems	Increased hydrolysis of carbapenems,oxyimino-β-lactams and cephamycins	KPC-2, IMI-1, SME-1
B (B1)	3a	Carbapenems	Broad-spectrum hydrolysis including carbapenems but not monobactams, inhibited by EDTA and chelating agent of divalent cations	IMP-1, VIM-1, CcrA, IND-1
B (B2)	3b	Carbapenems	Preferential hydrolysis of carbapenems, inhibited by EDTA and chelating agent of divalent cations	CphA, Sfh-1
B (B3)				L1, CAU-1, GOB-1, FEZ-1
C	1	Cephalosporins	Greater hydrolysis of cephalosporins than benzylpenicillin; hydrolyzes cephamycins	*Escherichia coli* AmpC, P99, ACT-1, CMY-2, FOX-1, MIR-1
C	1e	Cephalosporinsfm c1	Increased hydrolysis of ceftazidime and often other oxyimino-β-lactams	GC1, CMY-37
D	2d	Cloxacillin	Increased hydrolysis of cloxacillin or oxacillin, poorly inhibited by clavulanic but strongly inhibited by Nacl	OXA-1, OXA-10
D	2de	Extended-spectrum cephalosporins	Hydrolyzes cloxacillin or oxacillin and oxyimino-β-lactams. Strongly inhibited by Nacl	OXA-11, OXA-15
D	2d	Carbapenems	Hydrolyzes cloxacillin or oxacillin and carbapenems. Inhibited by tazobactam and poorly inhibited by clavulanic	OXA-23, OXA-48

(Modified from Bassetti et al., 2011)

Molecular classification of carbapenemases:

A. Molecular Class A enzymes:

Characteristic properties of these enzymes include presence of an active site serine at position 70 and presence of a disulfide bond between Cys69 and Cys238 (changes the overall shape of the active site) ***(Sridhar, 2012).***

All the enzymes have the ability to hydrolyze penicillins, and extend-spectrum cephalosporins, aztreonam as wells as carbapenems. These enzymes are inhibited by clavulanic acid and tazobactam but not by EDTA. These enzymes are placed under functional 2f subgroup. Early classA carbapenemases were mostly chromosomal (SME, IMI, NMC) but plasmid mediated carbapenemases (KPC, GES) are on the rise ***(Queenan and Bush, 2007).***

1-Chromosomally encoded class A enzymes:

a) **SME (Serratia marcescens):** SME-1 was first discovered in *S. marcescens* isolate from England in 1982. There were only 3 SME types (SME-1, SME-2 and SME-3).These enzymes have been sporadically observed in *S. marcescens* isolates throughout the United States. No clonal spread among these isolates was observed ***(Nordmann et al., 2012a)***.

b) IMI (Imipenem hydrolyzing β-lactamase): IMI-1 enzyme was first observed in an *Enterobacter cloacae* isolate in United States during 1984. Since then these have been rarely observed in clinical isolates of *E.cloacae* in United States, France and Argentina. Subsequently IMI-2 was reported from China, which was plasmid encoded. These chromosomal β-lactamases can be induced in response to imipenem and cefoxitin ***(*Yu *et al., 2006)***.

c) NMC (Not metalloenzyme carbapenemase): NCM-A enzyme was isolated from *E. cloacae* isolate in France during 1990 and subsequently reported from Argentina and United States.

NMC-A and IMI have 97% amino acid homology and are related to SME-1 with 70% amino acid homology. Even though these enzymes revealed a broad hydrolysis spectrum that includes the penicillins, early cephalosporins, aztreonam, and carbapenems, Cefoxitin and extended-spectrum cephalosporins were inefficiently hydrolyzed ***(Radice et al., 2004).***

2- Plasmid encoded class A carbapenemases:

a) KPC (*Klebsiella pneumoniae* carbapenemase): This enzyme was first observed in *a K. pneumoniae* isolate from US in 1996. The resistance gene was associated

with a large plasmid. Although predominantly seen in *K. pneumonia* isolates, they have been observed in *Salmonella enterica, K. oxytoca, E. cloacae, E. coli, and P. aeruginosa.* These enzymes confer resistance to all penicillins, cephalosporins, aztreonam and carbapenem but remain susceptible to inhibition by clavulanic acid ***(Yigit et al., 2001).***

The hydrolysis rates for imipenem, meropenem, cefotaxime and aztreonam are ten fold lesser than those for penicillins and early cephalosporins. Even though these enzymes can hydrolyze carbapenems, the resistance is not apparent and in many cases the MIC values are less than the MIC breakpoints.This has resulted in under detection of several KPC producers ***(Queenan and Bush, 2007).***

There are two characteristics separate the KPC carbapenemases from the other functional group 2f enzymes. First, the KPC enzymes are found on transferable plasmids; second, their substrate hydrolysis spectrum includes the amino-thiazoleoxime cephalosporins, such as cefotaxime ***(Bratu et al., 2005)***.

Sixteen different variants (KPC-2 to KPC-17) in the KPC family have been reported, and most current studies are focusing on KPC-2 and KPC-3. The KPC-16 variant, which isolated from *Klebsiella pneumoniae* in a Chinese hospital, was a recently discovered KPC enzyme. To compare the characteris- tics of KPC-15 and KPC-2, the variants were

determined by susceptibility testing, PCR amplification and sequencing, and study of kinetic parameters (table 2) (***Wang et al., 2014).***

Table (2): KPC-variant carbapenemases.

***bla*$_{KPC}$ gene**	**KPC enzyme**	**Species**	**Yr isolated**	**Location**	**GenBank accession no.**
bla$_{KPC-1}$[b]	KPC-1[b]	*Klebsiella pneumonia*	1996	North Carolina	AF297554
bla$_{KPC-2}$	KPC-2	*K. pneumonia*	1998–1999	Maryland	AY034847
bla$_{KPC-3}$	KPC-3	*K. pneumonia*	2000–2001	New York	AF395881
bla$_{KPC-4}$	KPC-4	*Enterobacter cancerogenus*	2003	Scotland	AY700571
bla$_{KPC-5}$	KPC-5	*Pseudomonas aeruginosa*	2006	Puerto Rico	EU400222
bla$_{KPC-6}$	KPC-6	*K. pneumonia*	2003	Puerto Rico	EU555534
bla$_{KPC-7}$	KPC-7	*K. pneumoniae*	2007–2008	Ohio	EU729727
bla$_{KPC-8}$	KPC-8	*K. pneumoniae*	2008	Puerto Rico	FJ234412
bla$_{KPC-9}$	KPC-9	*Escherichia coli*	2009	Israel	FJ624872
bla$_{KPC-10}$	KPC-10	*Acinetobacter baumannii*	2009	Puerto Rico	GQ140348
bla$_{KPC-11}$	KPC-11	*K. pneumoniae*	2010	Greece	HM066995

(Wang et al., 2014)

b: *bla*$_{KPC-1}$ and KPC-1 are no longer considered valid designations, as their sequences are identical to those of *bla*$_{KPC-2}$ and KPC-2, respectively

b) GES (Guiana extended spectrum): This enzyme was first observed in *a K. pneumonia* isolate from

French Guiana in 2000. The enzymes of the GES family differ from each other by 1-4 amino acid substitutions. The genes encoding GES family of enzymes are located in integrons on plasmid ***(Poirel et al., 2007)***. There are 22 known GES types. These have been observed in *K. pneumoniae, E. coli* and *P. aeruginosa* isolates from several nations (Greece, France, Portugal, South Africa, French Guiana, Argentina, Japan and Korea) ***(Ciobotaro et al., 2011)***.

In one *E. coli* isolate, GES gene (bla_{GES-7}) is chromosomally located, and is the only bla_{GES} gene that has not been identified on an integron. Because of their ability to hydrolyze extended spectrum cephalosporins they were initially considered to be ESBLs ***(Nordmann et al., 2012a).***

B. Molecular Class B enzymes:

These are metallo-β-lactamases (MBLs), which are characterized by its resistance to all penicillins, cephalosporins, beta-lactamase inhibitors, and carbapenems but are susceptible to inhibition by aztreonam and metal ion chelators (EDTA***) (Frere et al., 2005)***.

Inhibition by EDTA can be reversed by adding Zn^{2+} ions. Most MBL genes (including *VIM, IMP*) are found as gene cassettes on class 1 integrons; few *IMP* genes are located on class 3 integrons. Genetic analysis of regions around the *SPM-1*

gene revealed that it was not part of an integron but instead was associated with common regions that contain a new type of transposable structure with potential recombinase and promoter. Mechanism of carbapenem hydrolysis is complex and varies from one MBL to another (table 3) ***(Walsh et al., 2005)***.

Table (3): Substrate and inhibition profiles of the carbapenemases.

Molecular Class	Functional Group	Enzyme	Hydrolysis profile					Inhibition profile	
			Penicillin	Early cephalos-porin	Extended spectrum cephalosp-orin	Aztreonam	Carbapenem	EDTA	Clavulanic acid
A	2f	NMC	+	+	+	+	+	-	+
		IMI	+	+	+	+	+	-	+
		SME	+	+	±	+	+	-	+
		KPC	+	+	+	+	+	-	+
		GES	+	+	+	-	±	-	+
B1	3a	IMP	+	+	+	-	+	+	-
		VIM	+	+	+	-	+	+	-
		GIM BCII	+	+	+	-	+	+	-
		SPM	+	+	+	-	+	+	-
B2	3b	CphA Sfh-1	– –	– –	– –	– –	+ +	+	-
B3	3a	L1 FEZ-1 Gob-1	– – –	– – –	– – –	– – –	+ + +	– + –	– –- –
D	2d	OXA	+	+	±	-	±	-	±

(Queenan and Bush, 2007)

1- Chromosome borne MBLs:

The first metallo-β-lactamases were detected in environmental and opportunistic pathogenic bacteria *(Bacillus cereus* (BCII), *Aeromonas spp* (CphA) and *Stenotrophomonas maltophilia* (L1) as chromosomally encoded enzymes. These bacteria also produced additional serine beta-lactamases and the both enzymes were inducible by exposure to β-lactams. Chromosomal MBL was also found in few strains of *Bacteroides fragilis* (CcrA). Fortunately, with the exception of *S. maltophilia*, these bacteria have not been frequently associated with serious nosocomial infections, as they are generally opportunistic pathogens, and the chromosomal metallo-β-lactamase genes are not easily transferred ***(Queenan and Bush, 2007).***

Phylogenetic analysis suggests the existence of three MβL lineages: B1, B2, and B3. These three subclasses are classified based on a combination of structural features, zinc affinities for the two binding sites, and hydrolysis characteristics. Subclasses B1 and B3, divided by amino acid homology, bind two zinc atoms for optimal hydrolysis, while enzymes in subclass B2 are inhibited when a second zinc is bound ***(Queenan and Bush, 2007)***.

Subgroup B1 includes the acquired enzymes of the VIM, IMP, GIM, SPM, SIM, AIM, DIM, and NDM types. Several

variants of the VIM, IMP, and NDM types have been encountered in *K. pneumoniae* and other *Enterobacteriaceae* including *E. coli, Enterobacter cloacae* (mainly VIM and IMP), *Serratia marcescens* (mainly IMP), *and Proteus mirabilis* (mainly VIM) ***(Tzouvelekis et al., 2012)***. Also, *Bacillus cereus (BcII)* are the well-characterized MBLs which was the first to be discovered, and *Bacteroides fragilis* (*CcrA*). Subclass B1 enzymes are encoded by mobile genetic elements, posing the greatest threat of all the MBLs. Subclasses B1 and B3 have broad spectrum substrate profile that include penicillins, cephalosporins and carbapenems ***(Horsfall et al., 2011)***.

Subclass B3 includes the tetrameric enzyme L1 from the opportunistic pathogen *Stenotrophpmonas maltophilia* (*S. maltophilia*), GOB-1 from *Chryseobacterium meningo-septicum*, THIN-B from *Janthinobacterium lividum*, FEZ-1 from *Fluoribacter gormanii* and Mbl1b from *Caulobacter crescentus* ***(Horsfall et al., 2011)***.

Subclass B2 has a narrow substrate spectrum limited to carbapenems. It contains the very similar *Aeromonas* chromosomal enzymes, CphA and ImiS ***(Horsfall et al., 2011)***.

2- Plasmid borne MBLs*:*

Plasmid mediated MBLs have now attained significance because of its global spread. The majority of the mobile MBL

genes are found as gene cassettes.These include bla_{IMP}, bla_{VIM}, bla_{GIM}, *and* bla_{SIM} ***(Carattoli et al., 2010)***.

a. **IMP (Active on imipenem):** Transferable carbapenem resistance was first detected in *a P.aeruginosa* isolate from Japan in 1990 (IMP-1). They were subsequently reported in four *S. marcescens* isolates in Japan. This was followed by another report describing such a resistance in an isolate of *B. fragilis*. IMP-2 was observed in *A. baumannii* in Italy ***(Daoud et al., 2008)***.

There are 48 known IMP types. While these are more commonly seen in *P. aeruginosa* and *A. baumannii* isolates, they have been reported from most *Enterobacteriaceae* members ***(Bedenic et al., 2014)***. This enzyme hydrolyzed imipenem, penicillins, and extended-spectrum cephalosporins but not aztreonam. The hydrolytic activity was inhibited by EDTA and restored by the addition of Zn^{2+} ***(Mathers et al., 2009).***

b. **VIM (Verona integron encoded metallo-β-lactamase):** This class 1 integron associated MBL was first observed in *a P. aeruginosa* isolate from Italy in 1997. It is most closely related in BCII with only 39% amino acid homology. VIM-2 was reported in a clinical isolate of *P. aeruginosa* from France in 1996 ***(Poirel et al., 2000).***