Review Article

Indian J Med Res 144, July 2016, pp 21-31
DOI:10.4103/0971-5916.193279

A structural, epidemiological & genetic overview of *Klebsiella pneumoniae* carbapenemases (KPCs)

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Received June 27, 2014

Klebsiella pneumoniae **carbapenemases (KPCs) are plasmid encoded carbapenem hydrolyzing enzymes which have the potential to spread widely through gene transfer. The instability of upstream region of** $\textit{\textbf{bla}}_{\textbf{KPC}}$ **accelerates emergence of different isoforms. Routine antibiotic susceptibility testing failed to detect KPC producers and some commercial kits have been launched for early identification of KPC producers. Notable among the drugs under development against KPC are mostly derivatives of polymixin; β-lactamase inhibitor NXL104 with combination of oxyimino cephalosporin as well as with ceftazidime; a novel tricyclic carbapenem, LK-157, potentially useful against class A and class C enzymes; BLI-489-a bicyclic penem derivative; PTK-0796, a tetracycline derivative and ACHN-490. Combination therapy might be preferable to control KPC infections in immediate future. Clinicians are likely to opt for unconventional combinations of antibiotics to treat KPC infections because of unavailability of alternative agents. The KPCs have become endemic in many countries but there is no optimal treatment recommendation available for bacteria expressing KPCs. Reports of outbreaks involving KPCs have focused mainly on laboratory identification, empirical treatment outcomes and molecular epidemiology. This review includes information on the emergence of KPC variants, limitations of phenotyping methods, available molecular methods for identification of the KPC variants and treatment options highlighting the drugs under development.**

Key words $\textit{bla}_{\text{KPC}}$ - carbapenamase - carbapenem resistance - KPC

Introduction

Emergence of multi-drug resistance (MDR) among *Enterobacteriaceae* is a threat observed in the last two decades. The most important and common therapeutic challenge posed by *Enterobacteriaceae* is resistance to carbapenems. Carbapenems are broad spectrum antibiotics structurally very similar to penicillin but contain a sulphur group at C1 position. Several Gram-negative pathogens have become resistant to these by acquiring any one or more of the following mechanisms: structural alterations in drug targets like penicillin binding proteins (PBPs), porin loss, upregulation of efflux pumps and expression of carbapenemases[1]. One of the most commonly observed mechanism among Gram-negative pathogens is production of carbapenem inactivating enzymes, called carbapenemases[2]. All carbapenemases are β-lactamases but not all

β-lactamases are carbapenemases. These periplasmic enzymes hydrolyze beta lactam antibiotics either by alteration in the target site of the antibiotic that reduces its binding capacity or modification of the antibiotic so that it is no longer recognized by the target. Carbapenemases are broadly divided into two major types based on the amino acid sequences: metallo β-lactamases (Class B) containing zinc at the active site whereas serine β-lactamases (Classes A, C and D) containing serine at the active site. The *Klebsiella pneumonia* carbapenemases (KPC) along with other members like SME, IMI, NMC, GES constitute the class A[1,3]. Among these, the carbapenemase is more important than others due to its prevalence and transmissibility through plasmids[4]. Class C enzymes are chromosomally encoded, which were mostly found in several Gram-negative pathogens such as *Acinetobacter* spp., *Aeromonas* spp. and *Enterobacter* spp. The combination of class A and class C β-lactamases is highly potent as these confer resistance to most cephamycins, penicillins, cephalosporins and are not inhibited by clinically used inhibitors such as clavulanic acid[5]. Most common type of carbapenemases seen in *Acinetobacter baumannii* is oxacillanases. The class B enzymes include IMP, VIM, SPM, GIM, NDM and SIM families detected primarily in *Pseudomonas aeruginosa*[1]. This review provides information on one of the class A carbapenemase called KPC named after the organism in which it was first identified[6,7]. Later, it was detected in various other Gram-negative bacteria like *Enterobacteriaceae* (*Escherichia coli, K. oxytoca*, *Enterobacter* spp., *Serratia* spp., *Salmonella* spp., and *Citrobacter freundii*) and *Pseudomonadaceae (Pseudomonas aeurignosa)*[2,8-12]. Class A carbapenemase production has become a progressively common mechanism of resistance across the world[3,13-19]. The KPCs are encoded on a conjugative plasmid, carried in Tn*3* (Tn*4401*) based transposon[9,19,20]. Though class A enzymes and Bush's 2f type of hydrolases[21] are comparable in many characteristics, the KPC enzymes have two unique features: *(i)* these are borne on transferable conjugative plasmids, and *(ii)* capable of hydrolyzing advanced derivatives of cephalosporins like cefotaxime[1,22]. Tazobactam and clavulanic acid are partial inhibitors of class A enzymes whereas boronic acid acts as complete inhibitor[1,9,23]. Thirteen variants of KPC (*bla*$_{\text{KPC 1-13}}$) have been identified till date, which differ from each other by non-synonymous mutations[12,24]. These variants also show differences in their substrate (carbapenem) profile and affinity. So, there is a need for specific identification of these KPC variants in such Gram-negative bacteria (GNB) that would allow clinicians make early treatment decisions for nosocomial infections[7].

Epidemiology

Initially KPC producing organisms were restricted to sporadic outbreaks, but the situation became difficult because of global explosion and endemicity in both developed and developing countries[8,18,25,26]. From 2001 till date, 13 KPC subtypes have been found among Gram-negative organisms in different geographical regions[3,4,10,11,16,19,26-31]. Dissemination of variants occurs through acquisition of stable genetic elements by gene transfer mechanisms. Genotyping and sequence characterization of KPC by pulsed field gel electrophoresis (PFGE) and multilocus sequence typing (MLST), conducted in different countries including India, have reported that all KPC expressing strains harbour a single complex clone C11 which further indicates the origin of KPC from the same[32-34]. The major clone belongs to a sequence type called ST258 which is possibly responsible for all endemic outbreaks of KPC and also of international dissemination[3]. Other sequence types of KPC were also identified, for example, ST14 in Midwest United States[6]. Interestingly, ST11 is the predominant type of *K. pneumonia* associated with KPC production in China, which is closely related to ST258 and the latter is found more commonly elsewhere in the world[25,35]. The overall mortality rate associated with KPC producing organisms is 50 per cent[36]. The awareness of the incidence of resistance to carbapenems from India is increasing[37-41]. Gupta *et al*[42] reported 17-22 per cent of hospital acquired infections among Gram-negative pathogens, while Wattal *et al*[43] reported 13-51 per cent prevalence of carbapenem resistant *Enterobacteriaceae* (CRE).

Genetics of the KPC variants

Genes encoding versatile carbapenemases not only reside on chromosomes and but are also found on extrachromosomal DNA and transposons. Chromosomally encoded carbapenem inactivating enzymes evolved as defense mechanism in these bacteria also have a role in regulation of cell wall synthesis[1,22,44]. The expression of these plasmid encoded enzymes gets upregulated by various interacting bacterial factors like *(i)* intrinsic hydrolytic activities shown by KPC variants, *(ii)* the genetic relatedness and control of carbapenemase production, and *(iii)*

the presence or absence of other broad-spectrum β-lactamases and porin mutations[1,45]. It is necessary to establish how the bacterium is able to show resistance to all classes of antibiotics and the mutations that are responsible for resistance. The cause of rapid disseminatin of bla_{KPC} is the presence of transposable element Tn*4401* transposon which can integrate into various plasmids of non-clonally related organisms. Tn*4401* is found at various loci on plasmids, which differ in size and incompatibility group. Tn*4401* is approximately 10 kb in size characterized by the terminal two 39 bp imperfect inverted repeat sequences, harbours insertion sequences ISK*pn6* and ISK*pn7* and a set of site specific transposase and resolvase genes for insertion and excision of transposon[9,46]. It can integrate into different plasmids and evolve as isoforms. Three isoforms, a, b, and c of Tn*4401*, have been described by Naas *et al*[9], differing by a 99-215 bp sequence upstream of bla_{KPC}, while isoforms with 68 bp and 255 bp (Tn*4401* e) deletions have also been reported[46]. A 5.3 kb region consisting of insertion sequence ISK*pn7* and partial *tnpA* gene and more than half of the bla_{KPC} gene has been deleted from the transposon Tn*4401*. This novel truncated transposon was named as Tn*4401*d by Chen *et al*[46]. Tn*4401* can transpose with 5 bp target site duplication without any target site specificity (Fig.1). Mobility and plasticity of Tn*4401* lead to genetic diversity of plasmids and to the spread of KPC. Several studies have concluded[8,19,46] that bla_{KPC} gene has variable upstream region due to heterogeneous genetic environment and conserved downstream region. Genetic environment of each variant shows that instability in the upstream region of bla_{KPC} may be involved in variations of KPC. Several studies were conducted to elucidate the changes in non-conserved variable regions which were involved in the evolution of variants and the consequent changes in the spectrum of enzyme activity[9,46]. The enzyme activity decreases if the change is due to insertion sequences and increases if there are deletions[19]. Inconsistencies in detection of KPC variants may be due to acquisition of genes from other sources or due to indiscriminate mutational events. KPC genes are endowed with all molecular features such as transposon, self-transferable plasmids, competent Sequence Types (STs) which are responsible for their propagation between members of *Enterobacteriaceae* and other Gram-negative members. Phylogenetic tree for KPC subtypes was constructed (Fig. 2) which showed that most of the members were scattered throughout the phylogenetic tree and they were not grouped under single genetic lineage. This indicates that the distribution of KPC is through horizontal gene transfer (HGT) and not by clonal spread[44,47].

Characteristics of KPC variants

Life threatening nosocomial infections are associated with these KPC producing Gram-negative pathogens. KPC-2 is considered as the ancestral variant and it is observed mostly among the clinical isolates[11]. All variants arose from KPC2, which differ from one another by one or two point mutations[45]. Mostly these changes have been noticed frequently at four conserved amino acid positions, namely nucleotides

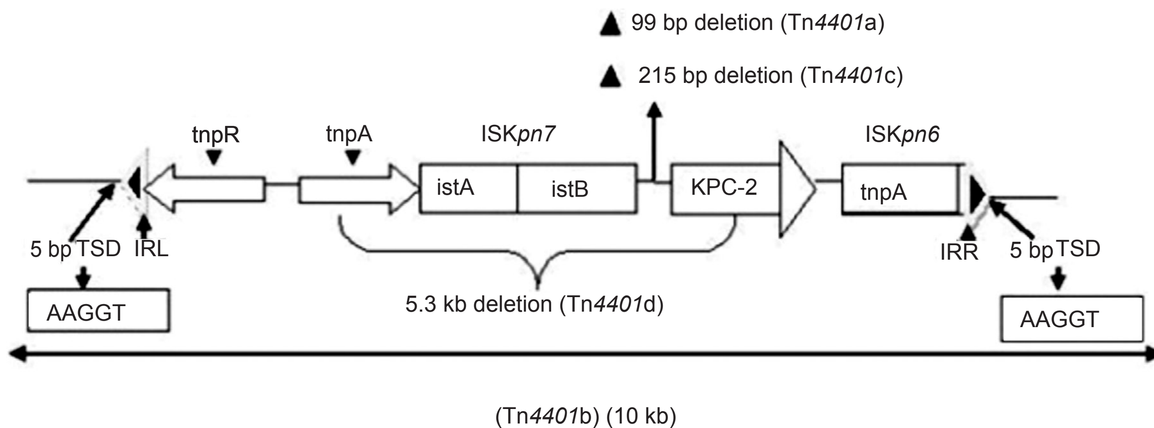

Fig. 1. Schematic diagram of Tn*4401b* and other isoforms on plasmids of *Klebsiella pneumoniae* isolates. IRR and IRL are inverted repeats (black triangles), flanked by 5 bp target site duplications (TSD). Insertion sequences: *ISKpn6*, *ISKpn7*; Transposase: tnpA, resolvase (tnpR). Dark triangles: deletions in isoforms a, c; Tn*4401d* truncated isoform with 5.3 kb deletion (flower bracket); Tn*4401e*: 255 bp deletion (entire putative promoter and transcription start site in upstream region[9,19,46].

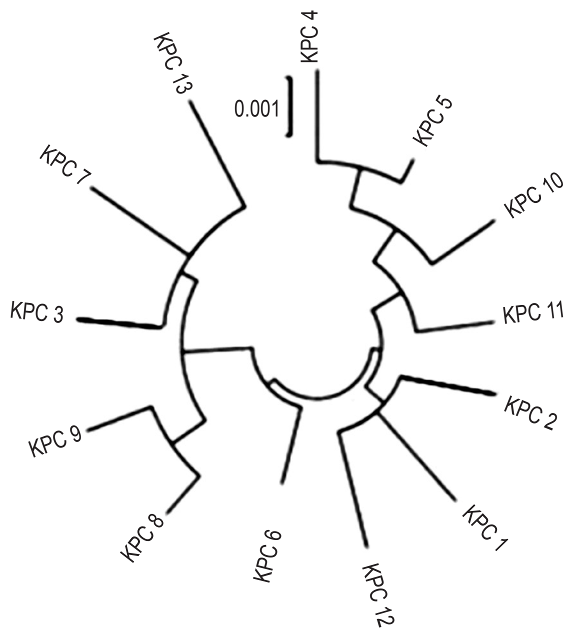

Fig. 2. Circular phylogenetic tree of currently known KPC enzymes, showing common ancestry (KPC-2) of all other variants. Amino acid sequences of KPC enzymes (GenBank- *http://www.ncbi.nlm.nih.gov*) aligned with Clustal W (*http://www.ebi.ac.uk/Tools/msa/clustalw2/*) using default parameters. The phylogenetic tree was constructed using MEGA 5.16.0 and the NJ (Neighbor Joining) method and the vertical bar in between KPC-13 - KPC-4 indicates measure for amino acid sequence diversity.

147, 308, 716, and 814 which encode for different amino acids[1,11,20,24]. Nucleotide changes in KPC-2 in comparison with other variants are compiled with the help of EMBOSS tools (*www.http://www.ebi.ac.uk/Tools/emboss/*) (Table I). Single amino acid substitutions allow lactamases to increase their substrate profile and gradually alter the ability of lactamases to hydrolyze lactams before they reach their target sites (Table II). Structural analysis has revealed that the characteristic feature of class A enzymes is that they possess S-X-X-K, S-D-N and K-T-G motifs at positions 70, 130 and 234, respectively in the protein which are responsible for efficient carbapenem hydrolytic activity compared to other classes[1]. The bicine buffer molecule of KPC-2 interacts through its carboxyl group to conserved active site residue of K234. Within the class A group, KPC enzymes evolved through various modalities including variation in primary sequence, selective pressure by β-lactam antibiotics, mutation rates, changes in three dimensional structure and protein stability[1,11,49]. Inhibitor profiling of KPC could help in accurate identification of the variants and hence an effective treatment strategy. Overall features of all KPC variants have been assembled and presented (Table III)[17,32].

Methods for KPC identification

The dissemination and emergence of KPC producers is a serious threat to public health because it drastically limits the treatment options[20,50]. It not only confers resistance to carbapenems, but also to almost all other β-lactams. Production of these enzymes could be confirmed by several phenotypic tests (*e.g.* modified Hodge tests) whereas genotypic methods such as multiplex PCR are being used to conform bla_{KPC} genes. Identifying KPCs is still a challenge for many clinical microbiology laboratories because bacteria expressing KPC do not always show high minimum inhibitory concentration (MICs), on the contrary, many isolates remain within susceptible or intermediate levels, frequently missed by technicians. Several methods have been developed so far for identification but each has its own limitations. A novel microtitre plate based chromogenic method called Carba NP has been developed and reported as one of the screening tests for carbapenemases[51,52]. Several phenotypic and molecular methods have been introduced for KPC identification but phenotypic methods are known to be time consuming. Though automation has reduced the time to report and enabled easy handling of multiple samples simultaneously, many such systems frequently yield false positive/false negative results due to errors in identification of carbapenamase production[53]. For phenotypic identification of KPC, diffusion of meropenem disk with aminophenylboronic acid, dipicolinic acid and cloxacillin was developed[54]. Broth microdilution method (BMD) is one of the accurate and reliable methods for the identification of KPC-mediated carbapenem resistance[51,55]. Among impienem, meropenem and ertapenem based automated systems like Microscan and Vitek-2, ertapenem based automated system proved to be more reliable and rapid for the detection of carbapenemases[51]. When compared with other phenotypic methods CHROMagar™ method showed 98 per cent efficiency in the detection of carbapenamases (KPC, VIM, NDM, *etc*.)[11]. In antimicrobial susceptibility tests higher MICs of the antibiotic used could be due to increased levels of expression of these enzymes and low penetration of drug into periplasmic space. In India, resistance to available carbapenems among *K. pneumonia* was reported as 43.6 per cent (meropenem), 32 per cent (imipenem), 20.3 per cent (ertapenem) and 60 per cent

Table I. Results of pair-wise alignment (Emboss) between KPC enzymes

Accession No.	Nucleotide changes among variants of bla_{KPC}	Variant	Reference
AF297554.1	**601**CACGTTCCGTCTGGACCGCTGGGAGCTGGAGCTGAACTCCGCCATCCCA**A650**	KPC-1	47
AF395881.1	**796**CCTAACAAGGATGACAAG**T**ACAGCGAGGCCGTCATCGCCGCTGCGGCTAG **845**	KPC-3	32
AY700571.1	**295** ATGCGCTGGTTC**G**GTGGTCACCCATCTCGGAAAAATATCTGACAACAGGC **344**	KPC-4	11
AY700571.1	**695** CAAAACCGGAACCTGCGGAG**G**GTATGGCACGGCAAATGACTATGCCGTCG **744**	KPC-4	11
EU400222.2	**2451**AAAAATGCGCTGGTTC**G**GTGGTCACCCATCTCGGAAAAATATCTGACAAC**2500**	KPC-5	11
EU555534.1	**696** CAAAACCGGAACCTGCGGAG**G**GTATGGCACGGCAAATGACTATGCCGTCG **745**	KPC-6	30
EU729727.1	**146** T**A**GATACCGGCTCAGGCGCAACTGTAAGTTACCGCGCTGAGGAGCGCTTC **195**	KPC-7	10
EU729727.1	**796** CCTAACAAGGATGACAAG**T**ACAGCGAGGCCGTCATCGCCGCTGCGGCTAG**845**	KPC-7	10
FJ234412.1	**696** CAAAACCGGAACCTGCGGAG**G**GTATGGCACGGCAAATGACTATGCCGTCG **745**	KPC-8	13
FJ234412.1	**796** CCTAACAAGGATGACAAG**T**ACAGCGAGGCCGTCATCGCCGCTGCGGCTAG **845**	KPC-8	13
FJ624872.1	**683** CAAAACCGGAACCTGCGGAG**C**GTATGGCACGGCAAATGACTATGCCGTCG **732**	KPC-9	28
FJ624872.1	**783** CCTAACAAGGATGACAAG**T**ACAGCGAGGCCGTCATCGCCGCTGCGGCTAG **832**	KPC-9	28
GQ140348.1	**296** ATGCGCTGGTTC**G**GTGGTCACCCATCTCGGAAAAATATCTGACAACAGGC **345**	KPC-10	45
GQ140348.1	**796** CCTAACAAGGATGACAAG**T**ACAGCGAGGCCGTCATCGCCGCTGCGGCTAG **845**	KPC-10	45
HM066995.1	**296** ATGCGCTGGTTC**T**GTGGTCACCCATCTCGGAAAAATATCTGACAACAGGC **345**	KPC-11	27
HQ641421.1	**496** CTGGAG**A**TGAACTCCGCCATCCCAGGCGATGCGCGCGATACCTCATCGCC **545**	KPC-12	29
AEA73284.1	**246** CAGCCAGCAGCAGGCCGGCTTGCTGG**G**CACACCCATCCGTTACGGCAAAA **295**	KPC-13	31
AEA73284.1	**296** A**C**GCGCTGGTTCCGTGGTCACCCATCTCGGAAAAATATCTGACAACAGGC **345**	KPC-13	31
AEA73284.1	**496** CTGGAGCTGAACTCCGC**T**ATCCCAGGCGATGCGCGCGATACCTCATCGCC **545**	KPC-13	31
AEA73284.1	**796** CCTAACAAGGATGACAAG**T**ACAGCGAGGCCGTCATCGCCGCTGCGGCTGG **845**	KPC-13	31

Nucleotide changes of *KPC* gene which resulted in various *KPC* variants have been highlighted
Source: EMBOSS (*www.http://www.ebi.ac.uk/Tools/emboss/*)

Table II. Substrate profile for variants of *bla*$_{KPC}$

Variant	KPC-1			KPC-2			KPC-3			KPC-6			KPC-9		
Parameter Antibiotic	K_{cat} (persec)	*Km/Ki* (µM)	K_{cat}/Km (per µM/ sec)	K_{cat} (per sec)	*Km/Ki* (µM)	K_{cat}/Km (per µM/ sec)	K_{cat} (per sec)	*Km/Ki* (µM)	K_{cat}/Km (per µM/ sec)	K_{cat} (per sec)	*Km/Ki* (µM)	K_{cat}/Km (per µM/ sec)	K_{cat} (persec)	*Km/Ki* (µM)	K_{cat}/Km (per µM/ sec)
Ampicillin	130	239	65±5	280 ±10	NA	110	210	77±4	130 ±10	NA	0.9	0.9	1.2	$(2.2\pm0.2)10^6$	NA
Cephalothin	53	82	44±3	>100	NA	75	69	153±5	>100	NA	1.4	0.8	3.5	$(2.4\pm0.1)10^6$	NA
Cefuroxime	160	220	95±8	>50	NA	14	22	52±4	>75	NA	0.1	0.1	0.5	$(6.9\pm0.1)10^5$	NA
Ceftazidime	94	NA	88±1	>0.6	32±6	0.1	0.1	3±0.06	>80	47.4 ±5.3	0.001	NA	0.03	$(9.1\pm0.1)10^3$	1.47 ±0.05
Cefotaxime	160	220	95±8	>39	NA	14	22	52±4	>150	NA	0.1	0.1	0.5	$(3.3\pm0.1)10^5$	NA
Cefoxitin	120	180	970±6	>0.2	NA	0.3	0.3	0.05	>100	NA	0.002	0.002	0.5	$(1.9\pm0.1)10^3$	NA
Imipenem	81	51	23±6	18± 1	34±9	12	15	45±0.04	42 ±4	101.5 ±11.9	0.2	0.3	1.9	$(4.4\pm0.4)10^5$	3.02 ±0.7
Meropenem	12	15	4±1	3.3 ±0.1	NA	3	4	6 ±0.09	7±1	NA	0.3	0.3	1.4	$(4.5\pm0.7)10^5$	NA
Ertapenem	NA	NA	47.5 ±10.9	2.8 ±0.1	52.8 ±5.4	NA	NA	177 ± 41	10±1	221 ±56	NA	NA	0.27± 0.11	$(2.9\pm0.3)10^5$	0.24 ±0.08
Nitrocefin	NA	NA	42± 5	210 ±10	NA	NA	NA	107±5	37±1	NA	NA	NA	2.6	$(5.6\pm0.2)10^6$	NA
Sulbactam	NA	NA	30±0.9	NA	NA	NA	NA	4±0.1	600±100	NA	NA	NA	0.1	NA	NA

Substrate kinetic values of various substrates as reported by several authors[28, 30, 48]. Though 12 variants have been reported till now, kinetic parameters are available only for five variants

NA, not available. Values are mean ± SD; K_i, inhibition constant; K_m, Michaelis constant; K_{cat}, turn over number

Source: Refs 28, 30, 48

Table III. Biochemical characteristics of KPC variants identified till date

Variant	Year	Distribution	Organism	Accession number	PI values	Mol. weight (Daltons)	Nucleotide change	Amino acid change	Ref.
KPC-1	1996	USA	*Klebsiella pneumoniae*	AF297554	NR	31,115	CA**A**(523)	Gly_{174}→Ser	10
KPC-2	**1998 -1999**	**USA, Israel**	***K. pneumoniae***	**AY034847**	**6.7**	**31,115**	**-**	**Reference gene**	**17**
KPC-3	2000-2001	USA, Israel	*K. pneumoniae*	AF395881	6.7	31,141	**T**AC(814)	His_{272} →Tyr	32
KPC-4	2003	Puerto Rico, Scotland	*Enterobactercancerogenus*	AY700571	7.65	31,132	CG**C**(308) G**G**G(716)	Pro_{103}→Arg & Val_{239}→Gly	11
KPC-5	2006	Puerto Rico	*Pseudomonas aeruginosa*	EU400222	7.65	30,174	CG**C**(308)	Pro_{103}→Arg	11
KPC-6	2003	Puerto Rico	*K pneumoniae*	EU555534	6.7	31,073	G**G**G(716)	Val_{239}→Gly	30
KPC-7	2007-2008	USA	*K. pneumoniae*	EU729727	NR	31,123	**T**AC(814) AT**A** (149)	His_{272} →Tyr & Met $_{49}$→ Ile	10
KPC-8	2008	Puerto Rico	*K. pneumoniae*	FJ234412	NR	31,099	**T**AC(814) G**G**G(716)	His_{272} →Tyr & Val_{239}→Gly	13
KPC-9	2009	Israel	*Escherichia coli*	FJ624872	NR	30,064	G**G**G(716)	Val_{239}→Ala	28
KPC-10	2009	Puerto Rico	*Acinetobacter baumanii*	GQ140348	NR	31,200	CG**C**(308) **T**AC(814)	Pro_{103}→Arg His_{272} →Tyr	45
KPC-11	2010	Greece	*K. pneumoniae*	HM066995	NR	31,131	C**T**G(308)	Pro_{103}→Lys	27
KPC-12	2010	Thailand	*K. pneumoniae*	HQ342889	NR	31,133	G**A**T(507)	Lys_{167}→Met	31
KPC-13	2010	Thailand	*E. cloacae*	HQ342890	NR	31,083	**T**AC(814) C**T**A(518) G**G**C(227)	His_{272} →Tyr Gly_{91}→Asp Arg_{187} → Cys	31

NR, not reported

(colistin)[27,40]. Ertapenem resistance has been shown to be more accurate for detection of KPC producers. This heterogeneous expression of carbapenemases by microorganisms makes it difficult to give uniform values for phenotypic methods[53]. Modified Hodge test (MHT) one of the phenotypic methods to identify carbapenemase producers among *Enterobacteriaceae,* demonstrated high levels of sensitivity and specificity for detection of KPC activity[51]. However, MHT is prone to give false positive results particularly with strains producing CTX-M extended-spectrum β-lactamases (ESBLs) and AmpC β-lactamase[47,49,51,53]. Hence, in geographical areas where ESBL is frequently reported, alternative methods are warranted. Melt curve analysis in real time PCR gives accurate results but requires high expertise and is also expensive[24,56]. Though few commercial kits are available for the identification of KPC directly from the clinical samples, but discrepancies exist among these kits[57-60]. Real time PCR is being explored as a platform for rapid identification of bla_{KPC} variants[57- 60] .

Emerging treatment options and clinical outcomes

Nearly 89 per cent of nosocomial infections are caused mainly by *K. pneumonia* and its foremost site for infection is blood (52%), secondary respiratory related problems comprise 30 per cent, whereas urinary tract infections (UTI) contribute 10 per cent of infections in both acute care as well as tertiary care facilities[37]. Specific factors which play a role in pathogenesis have not yet been identified in KPC producing organisms. Major risk factors for infections are *(i)* long term hospital stay, *(ii)* mechanical ventilation, and *(iii)* previous antibiotic therapy and other factors[20]. Treatment failures occur due to intrinsic (genetic factors) and external factors interfering with proper identification of KPC gene. Resistance to other antibiotics includes fluoroquinolones, trimethoprim/ sulphamethoxazole and aminoglycosides. Current options for treatment of KPC include tigecycline and colistin. However, each of these drugs and polymixin B as monotherapy results in frequent failures in treatment compared to combination therapy. Therapeutic options under development against KPC are mostly derivatives of polymixin (*e.g*. NAB739, NAB740); others include β-lactamase inhibitor NXL104 with combination of oxyimino cephalosporin as well as with ceftazidime[4,61]. A novel tricyclic carbapenem LK-157 has emerged as a potential drug against class A and class C enzymes[62]. BLI-489 is a bicyclic penem derivative which has shown inhibitory effect against several enzymes though has not been extensively tested on KPC producing microbes[63]. Two other antibiotic derivatives are under development against KPCs, one is a tetracycline derivative called PTK-0796 and other is aminoglycoside derivative ACHN-490 called neoglycoside[4,61,64]. Clinicians are likely to opt for unconventional combinations of antibiotics to treat KPC infections because of unavailability of alternative agents. U.S. Food and Drug Administration (FDA) approved a promising agent Doribax (doripenem) which has broad spectrum activity[65]. In Greece, one cohort study reported that 88 per cent of the patients who underwent combination therapy, almost 22 per cent showed failures[6]. Improvements in combination therapy might be preferable for control of KPC infections for immediate future[66].

Conclusion

KPC producing *Enterobacteriaceae* members have become a major concern causing serious health problems worldwide. Accumulation and transfer of KPC resistance determinants rapidly through horizontal gene transfer leads to an increase in mortality and morbidity. Variations in resistance determinants add to the complication in identification of bla_{KPC} gene. The reduced susceptibility to carbapenems makes it necessary to add inhibitors to carbapenemases like clavulanic acid in the treatment regimen or include inhibitors like EDTA in culture characterization. Evaluation and characterization of Tn*4401*, plasmids and transposons may help in identification of natural reservoir for bla_{KPC} which might facilitate to control the dissemination of resistance. Further research is required to understand the genetic relatedness among variants. There is also a need to explore the heterogeneity in the genetic environment of bla_{KPC} and to implement accurate detection methods. It is essential that hospitals strictly administer screening and implement control measures for reducing emergence and spread of carbapenem resistant bacteria.

***Conflicts of Interest*:** None.

References

1. Queenan AM, Bush K. Carbapenemases: the versatile β-lactamases*. Clin Microbiol* 2007; *20* : 440-58.

2. Gupta V. An update on newer beta-lactamases. *Indian J Med Res* 2007; *126* : 417-27.

3. Cuzon G, Naas T, Truong H, Villegas MV, Wisell KT, Carmeli Y, *et al.* Worldwide diversity of *Klebsiella pneumoniae* that produces β-lactamase bla_{KPC-2}. *Emerg Infect Dis* 2010; *16* : 1349-56.

4. Sacha P, Ostas A, Jaworowska J, Wieczorek P, Ojdana D, Ratajczak J, *et al*. The KPC type -lactamases: new enzymes

that confer resistance to carbapenems in Gram-negative bacilli. *Folia Histochem Cytobiol* 2009; *47* : 537-43.

5. Jung HL, Sang HL. Carbapenem resistance in Gram negative pathogens: emerging non-metallo -carbapenemases. *Res J Microbiol* 2006; *1* : 1-22.

6. Arnold RS, Thom KA, Sharma S, Phillips M, Kristie Johnson J, Morgan DJ. Emergence of *Klebsiella pneumoniae* carbapenemase (KPC)-producing bacteria. *South Med J* 2011; *104* : 40-5.

7. Campos AC, Albiero J, Ecker AB, Kuroda CM, Meirelles LE, Polato A, *et al*. Outbreak of *Klebsiella pneumonia* carbapenemase-producing *K. pneumoniae*: A systematic review. *Am J Infect Control*. In press 2016.

8. Chen LF, Anderson DJ, Paterson DL. Overview of the epidemiology and the threat of *Klebsiella pneumonia* carbapenemases (KPC) resistance. *Infect Drug Resist* 2012; *5* : 133-41.

9. Naas T, Cuzon G, Truong HV, Nordmann P. Role of IS*Kpn7* and deletions in $bl_{a\mathrm{KPC}}$ gene expression. *J Antimicrob Chemother* 2012; *56* : 4753-9.

10. Perez F, Endimiani A, Ray AJ, Decker BK, Wallace CJ, Hujer KM, *et al*. Carbapenem-resistant *Acinetobacter baumannii* and *Klebsiella pneumoniae* across a hospital system: impact of post-acute care facilities on dissemination. *J Antimicrob Chemother* 2010; *65* : 1807-18.

11. Wolter DJ, Kurpiel PM, Woodford N, Palepou MF, Goering RV, Hanson ND. Phenotypic and enzymatic comparative analysis of the novel KPC variant KPC-5 and its evolutionary variants, KPC-2 and KPC-4. *Antimicrob Agents Chemother* 2009; *53* : 557-62.

12. Patel G, Bonomo RA. "Stormy waters ahead": global emergence of carbapenemases. *Front Microbiol* 2013; *4* : 48.

13. Gregory CJ, Liata E, Stine N, Gould C, Santiago LM, Vazquez GJ, *et al*. Outbreak of carbapenem-resistant *Klebsiella pneumoniae* in Puerto Rico associated with a novel carbapenemase variant. *Infect Control Hosp Epidemiol* 2010; *31* : 476-84.

14. Lascols C, Hackel M, Hujer AM, Marshall SH, Bouchillon SK, Hoban DJ, *et al*. Using nucleic acid microarrays to perform molecular epidemiology and detect novel β-lactamases: a Snapshot of extended-spectrum β-lactamases throughout the World *J Clin Microbiol* 2012; *50* : 1632-9.

15. Naas T, Cuzon G, Nordmann P. Genetic structures at the origin of acquisition of the β lactamase bla_{KPC} gene. *Antimicrob Agents Chemother* 2008; *52* : 1257-63.

16. Robledo IE, Aquino EE, Sante MI, Santana JL, Otero DM, Leon CF, *et al*. Detection of KPC in *Acinetobacter* spp in Puerto Rico. *Antimicrob Agents Chemother* 2010; *54* : 1354-7.

17. Moland ES, Hanson ND, Herrera VL, Black JA, Lockhart TJ, Goering RV, *et al*. Plasmid-mediated, carbapenem-hydrolysing β-lactamase, KPC-2, in *Klebsiella pneumoniae* isolates. *J Antimicrob Chemother* 2003; *51* : 711-4.

18. Virgincar N, Iyera S, Stacey A, Maharjan S, Pike R, Perry C, *et al*. *Klebsiella pneumoniae* producing KPC carbapenemase in a district general hospital in the UK. *J Hosp Infect* 2011; *78* : 293-6.

19. Cuzon G, Naas T, Nordmann P. Functional characterization of Tn*4401*, a Tn*3*-based transposon involved in bla_{KPC} gene mobilization. *J Antimicrob Chemother* 2011; *55* : 5370-3.

20. Ahmad N, Chong TM, Hashim R, Shukor S, Yin WF, Chan KG. Draft genome of multidrug-resistant *Klebsiella pneumoniae* 223/14 carrying KPC-6, isolated from a General Hospital in Malaysia. *J Genomics* 2015; *3* : 97-8.

21. Bush K, Jacoby GA, Medeiros AA. A functional classification scheme for β-lactamases and its correlation with molecular structure. *Antimicrob Agents Chemother* 1995; *39* : 1211-33.

22. Mandrawa CL, Cronin K, Buising KL, Poy Lorenzo YS, Waters MJ, Jeremiah CJ. Carbapenemase-producing *Klebsiella pneumoniae*: a major clinical challenge. *Med J Aust* 2016; *204* : 277-8.

23. Pournaras S, Poulou A, Tsakris A. Inhibitor-based methods for the detection of KPC carbapenemase producing *Enterobacteriaceae* in clinical practice by using boronic acid compounds. *J Antimicrob Chemother* 2010; *65* : 1319-21.

24. Chen L, Mediavilla JR, Endimiani A, Rosenthal ME, Zhao Y, Bonomo RA, *et al*. Multiplex real-time PCR assay for detection and classification of *Klebsiella pneumonia* carbapenemase gene (bla_{KPC}) variants. *J Clin Microbiol* 2011; *49* : 579-85.

25. Endimiani A, Hujer AM, Perez F, Bethel CR, Hujer KM, Kroeger J, *et al*. Characterization of bla_{KPC}-containing *Klebsiella pneumoniae* isolates detected in different institutions in the Eastern USA. *J Antimicrob Chemother* 2009; *63* : 427-37.

26. Jacome JR, Alves LR, Cabral AB, Lopes AC, Maciel MA. First report of KPC-producing *Pseudomonas aeruginosa* in Brazil. *Antimicrob Agents Chemother* 2012; *56* : 4990.

27. Hawser SP, Bouchillon SK, Lascols C, Hackel M, Hoban DJ, Badal RE. Susceptibility of *Klebsiella pneumonia* isolates from intra-1 abdominal infections, and molecular characterization of ertapenem-resistant isolates. *Antimicrob Agents Chemother* 2011; *55* : 3917-21.

28. Hidalgo- Grass C, Warburg G, Temper V, Benenson S, Moses AE, Block C. KPC-9, a novel carbapenemase from clinical specimens in Israel. *Antimicrob Agents Chemother* 2012; *56* : 6057-9.

29. Walsh F. The multiple roles of antibiotics and antibiotic resistance in nature. *Front Microbiol* 2013; *4* : 255.

30. Lamoureaux TL, Frase H, Antunes NT, Vakulenko SB. Antibiotic resistance and substrate profiles of the class A carbapenemase KPC-6. *Antimicrob Agents Chemother* 2012; *56* : 6006-8.

31. Netikul T, Sidjabat H, Paterson D, Kiratisin P. Emergence of novel $bla_{\mathrm{KPC-13}}$ among carbapenem-resistant *Enterobacteriaceae* in Thailand. *Int J Antimicrob Agents* 2014; *44* : 568-9.

32. Woodford N, Tiemo PM Jr, Young K, Tysall L, Palepou MF, Ward E, *et al.* Outbreak of *Klebsiella pneumonia* producing a new carbapenem-hydrolyzing class A beta-lactamase, KPC-3, in a New York Medical Center. *Antimicrob Agents Chemother* 2004; *48* : 4793-9.

33. Kitchel B, Rasheed JK, Patel JB, Srinivasan A, Navon-Venezia S, Carmeli Y, *et al* Molecular epidemiology of KPC-producing *Klebsiella pneumonia* isolates in the United States: clonal expansion of multilocus sequence type 258. *J Antimicrob Chemother* 2009; *53* : 3365-70.

34. Balm MN, Ngan G, Jureen R, Lin RT, Teo J. Molecular characterization of newly emerged $bla_{\mathrm{KPC-2}}$-producing

Klebsiella pneumoniae in Singapore. *J Clin Microbiol* 2012; *50* : 475-6.

35. Cao X, Xu X, Zhang Z, Shen H, Chen J, Zhang K. Molecular characterization of clinical multidrug-resistant *Klebsiella pneumoniae* isolates. *Ann Clin Microbiol Antimicrob* 2014; *13*: 16.
36. Wei DD, Wan LG, Deng Q, Liu Y. Emergence of KPC-producing *Klebsiella pneumonia* hypervirulent clone of capsular serotype K1 that belongs to sequence type 11 in Mainland China. *Diagn Microbiol Infect Dis* 2016; *85* : 192-4.
37. Lee GC, Burgeess DS. Teatment of *Klebsiella pneumonia* carbapenemase (KPC) infections: a review of published case series and case reports. *Ann Clin Microbiol Antimicrob* 2012; *11* : 32.
38. Savita J, Rabindranath M, Nageshawari G, Mahadev U, Purbasha G, Kalpana, *et al.* Increasing incidence of multidrug resistance *Klebsiella pneumoniae* infections in hospital and community settings. *Int J Microbial Res* 2012; *4* : 253-7.
39. Sikarwar AS, Barta HV. Prevalence of antimicrobial drug resistance of *Klebsiella pneumoniae* in India. *Int J Biosci Biochem Bioinforma* 2011; *1* : 211-4.
40. Parveen M, Harish BN, Parija SC. Emerging carbapenem resistance among nosocomial isolates of *Klebsiella pneumonia* in south India. *Int J Pharm Biol Sci* 2010; *1* : 1-11.
41. Rakesh A, Kalpana S. Prevalence and antimicrobial susceptibility pattern of *Klebsiella pneumoniae* causing urinary tract infection and issues related to the rational selection of antimicrobials. *J App Med Sci* 2013; *1* : 395-9.
42. Gupta E, Mohanty S, Sood S, Dhawan B, Das BK, Kapil A. Emerging resistance to carbapenems in a tertiary care hospital in north India*. Indian J Med Res* 2006; *124* : 95-8.
43. Wattal C, Goel N, Oberoi JK, Raveendran R, Datta S, Prasad KJ. Surveillance of multidrug resistant organisms in a tertiary care hospital in Delhi, India. *J Assoc Physicians India* 2010; *58* : 32-6.
44. Del Franco M, Paone L, Novati R, Giacomazzi CG, Bagattini M, Galotto C, *et al*. Molecular epidemiology of carbapenem resistant *Enterobacteriaceae* in Valle d'Aosta region, Italy, shows the emergence of KPC-2 producing *Klebsiella pneumoniae* clonal complex 101 (ST101 and ST1789). *BMC Microbiol* 2015; *15* : 260.
45. Robledo IE, Vazquez GJ, Moland ES, Aquino EE, Goering RV, Thomson KS, *et al*. Dissemination and molecular epidemiology of KPC-producing *Klebsiella pneumoniae* collected in Puerto Rico Medical Center Hospitals during a 1-year period. *Epidemiol Res Int* 2011; *2011* : Article ID 698705, (8 pages).
46. Chen L, Chavda KD, Mediavilla JR, Jacobs MR, Levi MH, Bonomo RA, *et al*. Partial excision of bla_{KPC} from Tn*4401* in carbapenem-resistant *Klebsiella pneumonia*. *J Antimicrob Chemother* 2012; *56* : 1635-8.
47. Yigit H, Queenan AM, Anderson GJ, Domenech-Sanchez A, Biddle JW, Steward CD, *et al*. Novel carbapenem-hydrolyzing β-lactamase, KPC-1, from a carbapenem-resistant strain of *Klebsiella pneumoniae*. *J Antimicrob Chemother* 2001; *45* : 1151-61.
48. Alba J, Ishii Y, Thomson K, Moland ES, Yamaguchi K. Kinetics study of KPC-3, a plasmid-encoded class A carbapenem-hydrolyzing β-lactamase. *Antimicrob Agents Chemother* 2005; *49* : 4760-2.
49. Papp-Wallace KM, Taracila M, Hornick JM, Huijer KM, Distler AM, Endimiani A, *et al*. Subsrate selectivity and a novel role in inhibitor discrimination by residue 237 in the KPC-2 beta-lactamase. *Antimicrob Agents Chemother* 2010; *54* : 2866-77.
50. Kerbauy G, Vivan AC, Simões GC, Simionato AS, Pelisson M, Vespero EC, *et al*. Effect of a metallo antibiotic produced by *Pseudomonas aeruginosa* on *Klebsiella pneumoniae* carbapenemase (KPC)-producing *K. pneumoniae*. *Curr Pharm Biotechnol* 2016; *17* : 389-97.
51. Anderson KF, Lonsway DR, Rasheed JK, Biddle J, Jensen B, McDougal LK, *et al*. Evaluation of methods to identify the *Klebsiella pneumonia* carbapenemase in *Enterobacteriaceae*. *J ClinMicrobiol* 2007; *45* : 2723-5.
52. Bernabeu S, Poirel L, Nordmann P. Spectrophotometry based detection of carbapenamase producers among *Enterobacteriaceae*. *Diagn Microbiol Infect Dis* 2012; *44* : 88-90.
53. Bratu S, Mooty M, Nichani S, Landman D, Gullans C, Pettinato B, *et al*. Emergence of KPC-possessing *Klebsiella pneumonia* in Brooklyn, New York: epidemiology and recommendations for detection. *Antimicrob Agents Chemother* 2005; *49* : 3018-20.
54. Giske CG, Gezelius L, Samuelsen O, Warner M, Sundsfjord A, Woodford N. A sensitive and specific phenotypic assay for detection of metallo-blactamases and KPC in *Klebsiella pneumoniae* with the use of meropenem disks supplemented with aminophenylboronic acid, dipicolinic acid and cloxacillin. *Clin Microbiol Infect* 2011; *17* : 552-6.
55. da Silva KE, Maciel WG, Sacchi FP, Carvalhaes CG, Rodrigues-Costa F, da Silva AC, *et al*. Risk factors for KPC-producing *Klebsiella pneumoniae*: Watch out for surgery. *J Med Microbiol* 2016; *65* : 547-53.
56. Hindiyeh M, Smollen G, Grossman Z, Ram D, Davidson Y, Mileguir F, *et al*. Rapid detection of bl_{aKPC} carbapenemase genes by Real-time PCR. *J Clin Microbiol* 2008; *46* : 2879-83.
57. Spanu T, Fiori B, D'Inzeo T, Canu G, Campoli S, Giani T, *et al*. Evaluation of the New NucliSENS EasyQ KPC test for rapid detection of *Klebsiella pneumonia* carbapenemase genes (*bla*KPC). *J Clin Microbiol* 2012; *50* : 2783-5.
58. DiaPlexC™ CRE Detection Kit. Available from: *http:// www.solgent.com/english*, accessed on August 29, 2015.
59. Patel G, Huprikar S, Factor SH, Jenkins SG, Calfee DP. Outcomes of carbapenem-resistant *Klebsiella pneumoniae* infection and the impact of antimicrobial and adjunctive therapies. *Infect Control Hosp Epidemiol* 2008; *29* : 1099-106.
60. *Klebsiella pneumoniae*: KPC detection kit and genotyping, Hy-KPC PCR detection kit (KI 5040-I). Available from: *http:/www.hylabs.co.il/,* accessed on December 1, 2013.
61. Nordmann P, Cuzon G, Naas T. The real threat of *Klebsiella pneumonia* carbapenemase producing bacteria. *Lancet Infect Dis* 2009; *9* : 228-36.
62. Paukner S, Hesse L, Prezeli A, Solmaier T, Urleb U. *In vitro* activity of LK-157, a novel tricyclic carbapenem as broad spectrum {beta}- lactamase inhibitor. *Antimicrob Agents Chemother* 2009; *53* : 505-11.

63. Peterson PJ, Jones CH, Venkatesan AM, Mansour TS, Projan SJ, Bradford PA. Establishment of *in vitro* susceptibility testing methodologies and comparative activities of piperacillin in combination with the penem β-lactamase inhibitor BLI-489. *Antimicrob Agents Chemother* 2009; *53*: 370-84.

64. Endimiani A, Hujer KM, Hujer AM, Armstrong ES, Choudhary Y, Aggen JB, *et al*. ACHN-490, a neoglycoside with potent *in vitro* activity against multidrug-resistant *Klebsiella pneumoniae* isolates. *Antimicrob Agents Chemother* 2009; *53* : 4504-7.

65. Hilas O, Ezzo DC, Jodlowski TZ. Doripenem (Doribax), a new carbapenem antibacterial agent. *PT* 2008; *33* : 134-80.

66. Hirsch EB, Tam VH. Detection and Treatment options for *Klebsiella pneumoniae* carbapenemases (KPCs): an emerging cause of multi drug resistant infection. *J Antimicrob Chemother* 2010; *65* : 1119-25.

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