

# Outbreak Of *Klebsiella pneumoniae* Carbapenemase-Producing *Klebsiella aerogenes* Strains In A Tertiary Hospital In China

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**Purpose:** This study aimed to evaluate the molecular characteristics and prevalence of clinical carbapenem-resistant *Klebsiella aerogenes* (CRKA), collected during an outbreak in a Chinese tertiary hospital.

**Methods:** Antimicrobial susceptibility test, using 17 antibiotics, was performed on 14 CRKA isolates. The strains were examined for the presence of  $\beta$ -lactamase genes by PCR, and efflux pump phenotype was determined by efflux pump inhibition test. Presence of outer-membrane porins was examined. Clonal relatedness among the isolates was investigated by pulsed-field gel electrophoresis (PFGE). S1 nuclease-PFGE and plasmid incompatibility group analysis were performed to determine plasmids, and the genetic environment of *bla*<sub>KPC-2</sub> was analyzed. Epidemiological data were collected via chart review.

**Results:** The 14 CRKA isolates were all resistant to carbapenems; five distinct groups (PFGE types A–E) were observed. All 14 isolates carried the *bla*<sub>KPC-2</sub> gene. S1 nuclease-PFGE indicated the size of *bla*<sub>KPC-2</sub>-carrying plasmids to range from 20 kb to 200 kb, and the 14 plasmids belonged to various incompatibility groups. The most frequent genetic environment of *bla*<sub>KPC-2</sub> was Tn1721-*bla*<sub>KPC-2</sub>- $\Delta$ Tn3-IS26. PFGE type A group, including 11 KPC-2-producing clinical isolates, was primarily responsible for dissemination.

**Conclusion:** Our findings suggest both transposons and vertical transmission to contribute to the transformation of *bla*<sub>KPC-2</sub>. The results strongly suggest strict implementation of infection control of CRKA, in healthcare facilities.

**Keywords:** carbapenem resistance, *Klebsiella aerogenes*, *Klebsiella pneumoniae* carbapenemase, plasmid, genetic environment, outbreak

## Introduction

Carbapenem is a broad-spectrum  $\beta$ -lactam antibiotic with high stability to  $\beta$ -lactamases mediated by plasmids and chromosomes. However, carbapenem-resistant *Enterobacteriaceae* (CRE) strains have recently been reported frequently worldwide. CRE isolates show multiple-drug resistance, and even pan-drug resistance, to existing antimicrobial agents, resulting in a serious clinical challenge, treatment failure, and high mortality rates.<sup>1–3</sup> *Klebsiella aerogenes*, formerly known as *Enterobacter aerogenes*, is one of the major bacterial species responsible for nosocomial infections. The prevalence of carbapenem-resistant *K. aerogenes* (CRKA) has reached 21% in a Chinese hospital in Shanghai<sup>4</sup> and 12.5% in the USA,<sup>5</sup> thus undoubtedly becoming a major public health concern.<sup>6,7</sup> Reported mechanisms of resistance to carbapenems<sup>8</sup> include production of carbapenemases,

overproduction of  $\beta$ -lactamases, efflux pumps, porin deficiency, and a change in penicillin-binding proteins. The most common carbapenemases that are overproduced by these strains include *Klebsiella pneumoniae* carbapenemase (KPC) and metalloenzymes.<sup>9–11</sup> KPC-type carbapenemases remain the major determinant of carbapenem resistance in *K. pneumoniae*, and in many other *Enterobacteriaceae*.<sup>12</sup> Plasmids harboring the *bla*<sub>KPC</sub> gene, in association with the mobile element Tn1721, are largely responsible for the effective spread of these genes among *K. pneumoniae* in People's Republic of China, especially among the ST11 isolates, which is in contrast to their spread via Tn4401 transposons reported in other countries. However, little is known about the genetic environment of *Enterobacteriaceae*, besides *K. pneumoniae*, in People's Republic of China.

In the present study, we evaluated the molecular characteristics and prevalence of clinical CRKA isolates in a tertiary teaching hospital in Shanghai, People's Republic of China. These data should provide valuable insight into the development of strategies for treatment and control of the spread of CRKA isolates.

## Materials And Methods

### Clinical Isolates

All the 61 clinical *K. aerogenes* strains, which were isolated from January 2013 to December 2013 at Huashan Hospital (Fudan University, Shanghai, People's Republic of China), were screened to identify carbapenem-resistant isolates based on disc diffusion method results (imipenem or meropenem inhibition zone diameters  $\leq 19$  mm, or ertapenem inhibition zone diameters  $\leq 18$  mm). All the isolates in this manuscript were part of the routine hospital laboratory procedure. They are obtained from our hospital bacteria bank. They were not specifically isolated for this research. Based on this screen, a total of 14 non-duplicate carbapenem-resistant isolates were detected, and species confirmation was performed by 16S RNA amplification.<sup>13</sup>

### Antimicrobial Susceptibility

Antimicrobial susceptibility test was performed with all 14 CRKA isolates using disc diffusion and broth microdilution methods based on Clinical and Laboratory Standards Institute (CLSI), 2019 guidelines.<sup>14</sup> The minimum inhibitory concentrations (MICs) of imipenem, meropenem, ertapenem, piperacillin-tazobactam, cefepime, cefotaxime, ceftazidime, amikacin, ciprofloxacin, aztreonam,

cefuroxime, levofloxacin, gentamicin, and fosfomycin were determined according to CLSI standards, whereas those of tigecycline and colistin were determined according to European Committee on Antimicrobial Susceptibility Testing guidelines. In addition, the MICs of imipenem were determined in the presence of 25 g mL<sup>-1</sup> of the efflux pump inhibitor Phe-Arg  $\beta$ -naphthylamide dihydrochloride (PA $\beta$ N) to investigate the role of efflux pump in the carbapenem resistance mechanism.<sup>15</sup>

### Pulsed-Field Gel Electrophoresis (PFGE)

PFGE of XbaI (Takara, Dalian, People's Republic of China)-digested genomic DNA samples of *K. aerogenes* isolates was performed according to the manufacturer's instructions. The DNA fragments were separated with a CHEF-Mapper XA PFGE system (Bio-Rad, USA). Electrophoresis was performed for 20 hrs at 14°C with pulse times ranging from 3 to 40 s at 6 V cm<sup>-1</sup>. The DNA fingerprints generated were analyzed according to the criteria proposed by Tenover et al<sup>16</sup>.

### Detection Of $\beta$ -Lactamase Genes

All *K. aerogenes* isolates were screened for carbapenemase-encoding genes (*bla*<sub>SME</sub>, *bla*<sub>KPC</sub>, *bla*<sub>NDM</sub>, *bla*<sub>IMP</sub>, *bla*<sub>VIM</sub>, and *bla*<sub>OXA-48-like</sub>), plasmid-mediated AmpC  $\beta$ -lactamase-encoding genes (*bla*<sub>AAC</sub>, *bla*<sub>FOX</sub>, *bla*<sub>MOX</sub>, *bla*<sub>DHA</sub>, *bla*<sub>CIT</sub>, and *bla*<sub>EBC</sub>) and  $\beta$ -lactamase-encoding genes (*bla*<sub>TEM</sub>, *bla*<sub>SHV</sub>, *bla*<sub>CTX-M</sub>, and *bla*<sub>OXA-1</sub>) by PCR using previously reported primers.<sup>17</sup> To identify all genes detected in the PCR assays, PCR amplicons were sequenced, and the sequences were compared with those available in the National Center for Biotechnology Information GenBank database using BLAST searches (<http://www.ncbi.nlm.nih.gov/BLAST>).

### Analysis Of Outer-Membrane Proteins

Outer-membrane proteins were extracted and quantified as described previously.<sup>18</sup> After purification, most proteins, except for outer-membrane porins, were removed, and subsequently loaded onto a 12% sodium dodecyl sulfate-polyacrylamide gel. After 150-min electrophoresis at 80 V in a Mini-PROTEAN Tetra set-up (Bio-Rad, People's Republic of China), the gel was stained with 0.1% Coomassie brilliant blue (Sangon, Shanghai, People's Republic of China). Based on the molecular weight of Omp35 and Omp36, porins could be easily identified on the gel. The coding sequences of *omp35* and *omp36*, along with their respective promoter regions, were amplified as

previously described,<sup>19</sup> and aligned with the respective sequences of *E. aerogenes* strain NCTC 10336 (GenBank accession no. KT894105.1 and KT894106.1, respectively).

## Transformation And SI Nuclease-PFGE

The transfer experiment was carried out in mixed lysogeny broth cultures. The 14 CRKA strains producing *bla*<sub>KPC-2</sub> served as the donors while *Escherichia coli* DH5 $\alpha$  served as the recipient strain. After a short incubation in ice, a mixture of chemically competent bacteria and plasmids, extracted from donor, was placed at 42°C for 45 s (heat shock) and then placed back in ice. SOC medium was added and the transformed cells were incubated at 37°C for 30 mins with shaking. Transformed bacteria were then plated and incubated at 37°C for 20 hrs.<sup>20</sup> Transformants were selected on lysogeny broth agar plates containing 50 mg L<sup>-1</sup> ampicillin. SI nuclease-PFGE was then performed to determine the location of *bla*<sub>KPC-2</sub> gene, as described previously.<sup>21</sup>

## Incompatibility Group Analysis Of *bla*<sub>KPC-2</sub>-Carrying Plasmids

Next-generation sequencing, using the Illumina Miseq system, was performed to sequence the 14 *bla*<sub>KPC-2</sub>-carrying plasmids.<sup>22</sup> Sequencing reads were assembled de novo into contigs using SPAdes,<sup>23</sup> and manually inspected thereafter using Geneious 9. Gene predictions were

conducted using the most popular ab-initio methods, Glimmer (v3.02) and Prodigal (v2.6.2). The consensus genes sets (with the same start or stop positions in parent scaffolds), predicted by both tools, were chosen as candidate gene models. The potential plasmids were identified by PlasmidFinder, and plasmid typing was performed using pMSI software.<sup>24</sup>

## Genetic Environment Of The *bla*<sub>KPC-2</sub> Gene

A PCR mapping approach was adopted to compare the genetic context of *bla*<sub>KPC-2</sub> gene in the 14 CRKA isolates with that of carbapenem-resistant *K. pneumoniae* strains reported in our hospital previously, using the same primers.<sup>25</sup> The obtained amplification products were sequenced and compared with those available in the National Center for Biotechnology Information GenBank database using BLAST searches (<http://www.ncbi.nlm.nih.gov/BLAST>).

## Outbreak Characteristics

Outbreak data were collected via chart review for each patient from the hospital's uniform electronic database. The following parameters were assessed: 1) general demographics, such as age, sex, and other background information; 2) the ward to which the patient was assigned after

**Table 1** In-Vitro Activities Of Antimicrobial Agents Against CRKA Isolates

	Range	MIC50	MIC90	R%	S%	I%
Imipenem	0.125–16	16	>16	100	0	0
Ertapenem	0.125–32	16	32	100	0	0
Meropenem	0.125–16	16	>16	100	0	0
Cefepime	0.25–32	>32	>32	100	0	0
Cefotaxime	0.5–32	>32	>32	100	0	0
Ceftazidime	1–32	>32	>32	92.9	0	7.1
Piperacillin/tazobactam	8/4–256/4	>256	>256	100	0	0
Cefuroxime	4–64	>64	>64	100	0	0
Cefoxitin	4–128	>128	>128	100	0	0
Amikacin	8–128	>128	>128	78.6	21.4	0
Aztreonam	2–64	>64	>64	100	0	0
Ciprofloxacin	0.25–8	2	>8	85.7	0	14.3
Levofloxacin	0.5–16	2	>16	78.6	21.4	0
Gentamicin	2–32	>32	>32	85.8	14.2	0
Fosfomycin	8–512	>512	>512	100	0	0
Colistin	1–16	≤1	2	7.1	92.9	/
Tigecycline	0.25–8	≤0.25	0.5	0	100	/

**Note:** MIC presented as  $\mu\text{g/mL}$ .

**Abbreviations:** CRKA, carbapenem-resistant *Klebsiella aerogenes*; R, resistance; S, susceptibility; I, Intermediate; MIC, minimum inhibitory concentration.

admission; and 3) previous use of antibiotics, particularly carbapenems.

# Results

## Prevalence, Antibiotic Susceptibility, And Resistance Mechanism Of CRKA Isolates

A total of 14 isolates were screened out as CRKA based on the disc diffusion results and confirmed by broth micro-dilution method. This accounts for a rate of 23.0% of the 61 non-duplicated clinical *K. aerogenes* strains isolated during 2013.

MICs of the 14 isolates to the 17 antimicrobial agents tested are listed in Table 1. All the isolates were non-susceptible to ertapenem, imipenem, meropenem, cefotaxime, ceftazidime, cefoxitin, cefepime, piperacillin/tazobactam, cefuroxime, cefoxitin, aztreonam, and fosfomycin. Treatment with the efflux pump inhibitor PA $\beta$ N did not decrease the MICs of carbapenems in any of the CRKA isolates tested (Table 2).

Moreover, SDS-PAGE analysis revealed the loss of Omp36 porin from isolate A52, whereas no such loss was found in the other 13 CRKA isolates (Figure 1); *omp36* in isolate A52 failed to be amplified after several trials with different primers. No mutation in *omp35* or *omp36* was found in the other isolates.

## $\beta$ -Lactamase Characterization And Genetic Diversity

The PFGE results indicated that the 14 CRKA isolates belonged to five distinct PFGE groups (types A–E), with type A being the most dominant, including 71.4% (10/14) of the *K. aerogenes* isolates (Figure 2).

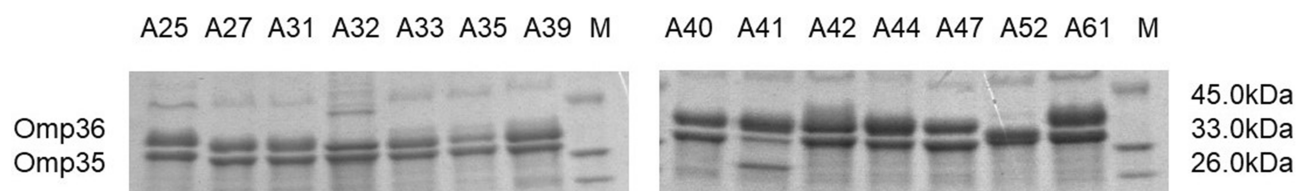
All 14 isolates carried the *bla*<sub>KPC-2</sub> gene. Six isolates (42.9%) carried the *bla*<sub>CTX-M-15</sub> gene, two isolates (14.3%) carried the *bla*<sub>CTX-M-14</sub> gene, and three isolates (21.4%) carried the *bla*<sub>SHV-1</sub> gene. No PCR product was obtained for any of the other genes investigated (Table 2). The 14 *bla*<sub>KPC-2</sub>-carrying plasmids, obtained in the present study, belonged to various incompatibility groups, with IncFII and IncN being the most dominant.

Carbapenem resistance was successfully transferred from the 14 CRKA strains to *E. coli* DH5 $\alpha$  by transformation. S1 nuclease-PFGE indicated that the size of the *bla*<sub>KPC-2</sub>-carrying plasmid ranged from approximately 20 kb to 200 kb (Figure S1). Notably, the 14 transformants simultaneously harbored the *bla*<sub>KPC-2</sub> and *bla*<sub>CTX-M</sub> genes while *bla*<sub>SHV</sub> was not detected in any of the transformants (Table 2).

Table 2 Carbapenem MICs,  $\beta$ -Lactamase Detection, And *bla*<sub>KPC-2</sub>-Carrying Plasmid Analysis In The Isolates Studied

Isolates	ETP	MEM	IPM	IPM+PA $\beta$ N	CAZ	LVX	AMK	CT	TGC	$\beta$ -lactamases In Clinical Isolates	$\beta$ -lactamases In Transformants	PFGE Type	RFLP Type	Plasmid Size (bp)
A25	>32	>16	>16	>16	32	>16	>128	$\leq 1$	$\leq 0.25$	KPC-2, SHV-1, CTX-M-14	KPC-2, CTX-M-14	D	III	$\approx 150$
A27	32	>16	16	8	>32	8	>128	$\leq 1$	$\leq 0.25$	KPC-2, CTX-M-15	KPC-2, CTX-M-15	A	I	$\approx 40$
A31	32	>16	16	16	>32	2	>128	$\leq 1$	$\leq 0.25$	KPC-2, SHV-1, CTX-M-15	KPC-2, CTX-M-15	A	I	$\approx 40$
A32	32	>16	8	8	16	4	$\leq 8$	$\leq 1$	–	KPC-2	KPC-2	C	IV	$\approx 85$
A33	16	16	8	16	32	2	>128	$\leq 1$	$\leq 0.25$	KPC-2, CTX-M-15	KPC-2, CTX-M-15	A	I	$\approx 40$
A35	16	16	8	4	32	2	>128	$\leq 1$	$\leq 0.25$	KPC-2, CTX-M-15	KPC-2, CTX-M-15	A	I	$\approx 40$
A39	8	8	4	2	16	$\leq 0.5$	$\leq 8$	$\leq 1$	$\leq 0.25$	KPC-2	KPC-2	A	II	$\approx 95$
A40	8	8	16	16	>32	4	>128	$\leq 1$	$\leq 0.25$	KPC-2	KPC-2	A	II	$\approx 95$
A41	16	16	8	16	>32	2	>128	$\leq 1$	$\leq 0.25$	KPC-2	KPC-2	A	II	$\approx 95$
A42	8	8	8	8	8	$\leq 0.5$	$\leq 8$	8	$\leq 0.25$	KPC-2, CTX-M-14	KPC-2, CTX-M-14	A	V	$\approx 75$
A44	16	>16	8	8	>32	2	>128	$\leq 1$	$\leq 0.25$	KPC-2, CTX-M-15	KPC-2, CTX-M-15	A	I	$\approx 40$
A47	32	>16	>16	>16	>32	4	>128	$\leq 1$	$\leq 0.25$	KPC-2, CTX-M-15	KPC-2, CTX-M-15	A	I	$\approx 40$
A52	32	16	8	8	>32	$\leq 0.5$	>128	$\leq 1$	$\leq 0.25$	KPC-2, SHV-1	KPC-2	B	VI	$\approx 50$
A61	8	>16	8	4	>32	>16	>128	2	0.5	KPC-2	KPC-2	E	VII	$\approx 105$

Abbreviations: ETP, ertapenem; MEM, meropenem; IPM, imipenem; CAZ, ceftazidime; LVX, levofloxacin; AMK, amikacin; CT, colistin; TGC, tigecycline.



**Figure 1** Outer-membrane protein profiles of 14 clinical *K. aerogenes* isolates on a 12% sodium dodecyl sulfate-polyacrylamide gel.

**Abbreviations:** *K. aerogenes*, *Klebsiella aerogenes*; M, marker.

## Genetic Environment Of The *bla*<sub>KPC-2</sub> Gene

The *bla*<sub>KPC-2</sub>-bearing genetic structure of the 12 KPC-2-producing CRKA isolates was determined to be Tn1721-*bla*<sub>KPC-2</sub>-ΔTn3-IS26 with (6 isolates) or without (6 isolates) a truncated IS26 element fused at the left-most end of Tn1721. The other two strains only showed a partial version of this structure, which indicated variation in the Tn1721 region (Figure 3).

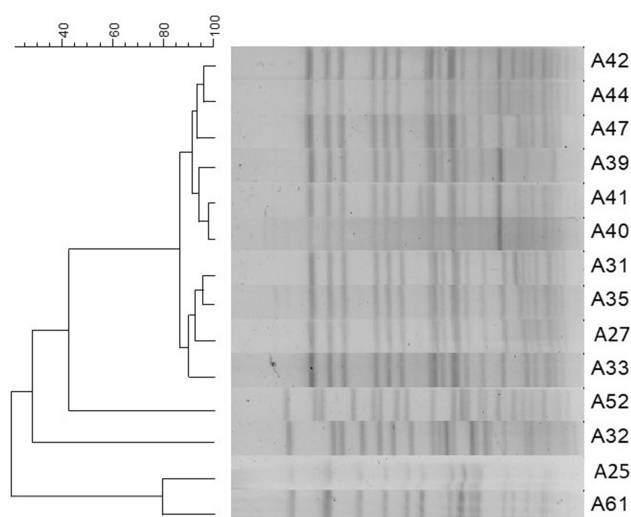
## Outbreak Characteristics

The epidemiological data of three patients (A25, A31, and A32) were not available since they had been hospitalized in a branch hospital. The remaining 11 patients (eight males and three females, aged 14–67 years) were all hospitalized in the neurosurgery ward of Huashan Hospital, except for one patient (A61) that was hospitalized in the intensive care unit. The patients' clinical characteristics are listed in Table 3. Patient 1, from whom the CRKA isolate A27 was identified (from a

sputum sample), had undergone surgery in February 2013 and developed post-operative pneumonia. Nearly 3 weeks after this, the first CRKA isolate was identified. Patient 2, who was in the same ward, was diagnosed with an invasive infection with CRKA (A33, PFGE type A). After 3 months, five additional patients successively tested positive for CRKA isolates within 12 weeks (PFGE type A). All these patients experienced actual pneumonia caused by CRKA. Overall, 63.6% of the patients (7/11) had undergone surgical procedures and 81.8% (9/11) had received mechanical ventilation.

## Discussion

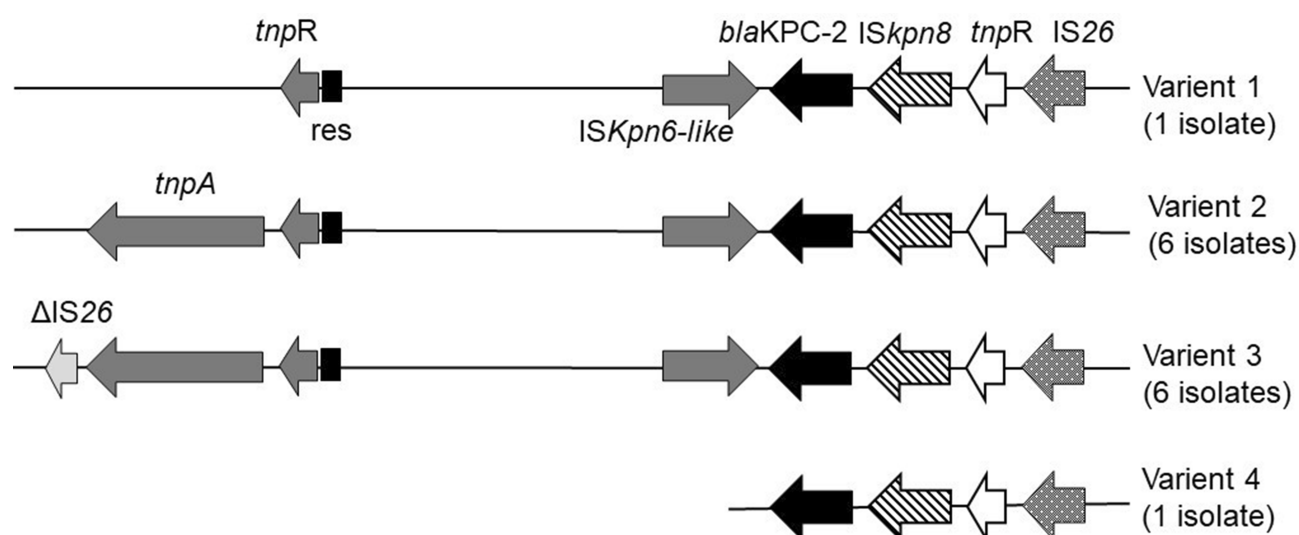
Although CRKA generally represents a smaller proportion of CRE, compared to carbapenem-resistant *K. pneumoniae* and *E. coli*, our findings demonstrated a quite relevant proportion of such strains, thus demanding more attention in this regard. Resistance rates against amikacin and gentamicin were higher than 75%. One isolate was found to be resistant to colistin; there were, indeed, some reports of colistin



**Figure 2** PFGE patterns for CRKA. Isolates with 85% similarity were considered the same type.

**Abbreviations:** PFGE, pulsed-field gel electrophoresis; CRKA, carbapenem-resistant *Klebsiella aerogenes*.





**Figure 3** Genetic environment of the *bla*<sub>KPC-2</sub> gene from clinical isolates. Genes are depicted as arrows according to the direction of transcription. Variant 1 included isolate A25. Variant 2 included isolates A27, A31, A33, A35, A44, and A47. Variant 3 included isolates A32, A39, A40, A41, A42, and A61. Variant 4 included isolate A52.

resistance in *K. aerogenes* during this time.<sup>26,27</sup> Since colistin is generally considered a “last-line” antibiotic, this emerging resistance is highly concerning and worth highlighting.

Study of the resistance mechanism indicated that efflux pumps are not involved in the resistance, despite a previous

report of their important role in carbapenem resistance.<sup>28</sup> Moreover, porin dysfunction is probably not the main mechanism of resistance in these isolates.

A study conducted in Italy demonstrated *bla*<sub>KPC</sub> as the major mechanism (97%) of carbapenem resistance in *K.*

**Table 3** Clinical Characteristics Of Patients Infected With CRKA

Patient	Isolate No.	Sex (Age, Years)	Underlying Condition	Ward	Days Of Hospital Stay Before Infection/total Days	Specimen	Antimicrobial Therapy
1	A27	Female (64)	Craniocerebral trauma	Neurosurgery Department	13/19	Sputum	AMK, TZP, LVX
2	A33	Male (39)	Craniocerebral trauma	Neurosurgery Department	23/66	Sputum	TGC, MEM, FOS, CSL
3	A35	Male (67)	Craniocerebral trauma	Neurosurgery Department	12/50.5	Sputum	MEM, VAN, AMK, TGC
4	A39	Male (34)	Craniocerebral trauma	Neurosurgery Department	13/14.5	Sputum	MEM, VAN
5	A40	Male (14)	Cerebellar hemorrhage	Neurosurgery Department	18/33.5	Sputum	MEM, FOS, TGC
6	A41	Female (61)	Subarachnoid hemorrhage	Neurosurgery Department	23/42	Sputum	CFZ, MEM, VAN, SAM
7	A42	Male (50)	Craniocerebral trauma	Neurosurgery Department	5/18.5	Sputum	TZP
8	A44	Male (62)	Craniocerebral trauma	Neurosurgery Department	5/23.5	Sputum	CSL, VAN, AMK
9	A47	Male (62)	Craniocerebral trauma	Neurosurgery Department	4/24	Sputum	CSL, MEM, VAN
10	A52	Male (55)	Cervical spinal fractures	Intensive Care Unit	5/44	Sputum	CSL, AMK, VAN, IMP, TZP, FOS
11	A61	Female (44)	Craniocerebral trauma	Intensive Care Unit	7/31	Sputum	MEM, FOS, CSL

**Abbreviations:** AMK, amikacin; TZP, piperacillin/tazobactam; LVX, levofloxacin; TGC, tigecycline; MEM, meropenem; FOS, fosfomycin; CSL, cefoperazone/sulbactam; VAN, vancomycin; CFZ, cefazolin; SAM, ampicillin/sulbactam; IMP, imipenem.

*pneumoniae*.<sup>29</sup> In contrast, in Thailand and Hong Kong, the rate of KPC-producing isolates was reported to be very low among CRE isolates.<sup>30,31</sup> Therefore, although KPC-producing isolates have clearly spread worldwide, there are still regional differences in their prevalence. KPC was first discovered in the USA in 1996<sup>32</sup> and has spread worldwide since. Till date, KPC has been divided into 22 types, with KPC-2 dominating in People's Republic of China.<sup>33,34</sup>

Transposable element Tn1721 is a proven transposon that has often played an important role in the dissemination of *bla*<sub>KPC-2</sub> gene in People's Republic of China. On the contrary, Tn4401 is the dominant transposon in Western countries. Most KPC-2 carrying Tn1721 transposons were reported in ST11 *K. pneumoniae*. Since studies of Tn1721 transposon acting in other species are rare,<sup>35</sup> in this study, we investigated Tn1721-*bla*<sub>KPC-2</sub>- $\Delta$ Tn3-IS26 transposon in CRKA that shared the same genetic background with KPC-2-producing *K. pneumoniae* isolates, collected from the same hospital during the same period, as determined previously,<sup>25</sup> thus suggesting a common genetic structure for *Enterobacteriaceae*. Results indicated that Tn1721 transposons could not only transfer horizontally through *K. pneumoniae*, but could also transfer across different species, in this case through *K. pneumoniae* to *K. aerogenes*. Since IncFII and IncN are two dominant plasmid incompatibility groups detected in 14 isolates, the possibility of contribution of transposon as well as vertical transmission is stressed for the transformation of *bla*<sub>KPC-2</sub>.

The 11 CRKA strains in the dominant subgroup A that produced KPC-2 were likely to be primarily responsible for the first isolation and subsequent dissemination in the neurosurgery ward. The outbreak characteristic data showed mechanical ventilation, and previous use of broad-spectrum antibiotics could be potential risk factors for the acquisition of CRKA across the hospitalized patients.

Thus, the increased prevalence of carbapenem-resistant isolates in our hospital may have been caused by a failure to control the spread of these resistant strains, pointing at the importance of raising awareness of this situation in People's Republic of China, as well as worldwide.

## Conclusion

In this study, we identified KPC-2 production as the major carbapenem-resistance mechanism of CRKA. Moreover, we confirmed that Tn1721 transposon could transfer through the same species or via different ones. KPC-producing isolates can readily cause clonal spread and transposon transformation/vertical transmission across different species in a hospital environment. However, the proportion

of transposon transformation and plasmid vertical transmission in KPC-2 dissemination would require further study. Our current study investigated KPC-2 dissemination in *K. aerogenes* and shed light on transposable element and plasmid transmission for antibiotic resistance.

## Author Contributions

All authors contributed to data analysis, drafting or revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

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## Disclosure

The authors report no conflicts of interest in this work.

## References

1. Munoz-Price LS, Poirel L, Bonomo RA, et al. Clinical epidemiology of the global expansion of *Klebsiella pneumoniae* carbapenemases. *Lancet Infect Dis*. 2013;13(9):785–796. doi:10.1016/S1473-3099(13)70190-7
2. Nordmann P, Naas T, Poirel L. Global spread of carbapenemase producing *Enterobacteriaceae*. *Emerg Infect Dis*. 2011;17(10):1791–1798. doi:10.3201/eid1710.110655
3. Bornet C, Davin-Regli A, Bosi C, Pages JM, Bollet C. Imipenem resistance of *Enterobacter aerogenes* mediated by outer membrane permeability. *J Clin Microbiol*. 2000;38(3):1048–1052.
4. Qin X, Yang Y, Hu F, Zhu D. Hospital clonal dissemination of *enterobacter aerogenes* producing carbapenemase KPC-2 in a Chinese teaching hospital. *J Med Microbiol*. 2014;63(2):222–228.
5. Guh AY, Bulens SN, Mu Y, et al. Epidemiology of carbapenem-resistant *enterobacteriaceae* in 7 US communities, 2012–2013. *J Am Med Assoc*. 2015;314(14):1479–1487.
6. Voulgari E, Poulou A, Koumaki V, Tsakris A. Carbapenemase-producing *Enterobacteriaceae*: now that the storm is finally here, how will timely detection help us fight back? *Future Microbiol*. 2013;8(1):27–39.
7. Lee CR, Lee JH, Park KS, Kim YB, Jeong BC, Lee SH. Global dissemination of carbapenemase-producing *Klebsiella pneumoniae*: epidemiology, genetic context, treatment options, and detection methods. *Front Microbiol*. 2016;13(7):895.
8. Papp-Wallace KM, Endimiani A, Taracila MA, Bonomo RA. Carbapenems: past, present, and future. *Antimicrob Agents Chemother*. 2011;55(11):4943–4960.
9. Ahn C, Syed A, Hu F, O'Hara JA, Rivera JI, Doi Y. Microbiological features of KPC-producing *Enterobacter* isolates identified in a U.S. hospital system. *Diagn Microbiol Infect Dis*. 2014;80(2):154–158.
10. Pollett S, Miller S, Hindler J, Usulan D, Carvalho M, Humphries RM. Phenotypic and molecular characteristics of carbapenem-resistant *Enterobacteriaceae* in a health care system in Los Angeles, California, from 2011 to 2013. *J Clin Microbiol*. 2014;52(11):4003–4009. doi:10.1128/JCM.01397-14
11. Wang X, Chen G, Wu X, et al. Increased prevalence of carbapenem resistant *Enterobacteriaceae* in hospital setting due to cross-species transmission of the bla<sub>NDM-1</sub> element and clonal spread of progenitor resistant strains. *Front Microbiol*. 2015;16(6):695.

12. Tzouveleakis LS, Markogiannakis A, Psychogiou M, Tassios PT, Daikos GL. Carbapenemases in Klebsiella pneumoniae and other Enterobacteriaceae: an evolving crisis of global dimensions. *Clin Microbiol Rev*. 2012;25(4):682–707. doi:10.1128/CMR.05035-11
13. Wilson KH, Blichington RB, Greene RC. Amplification of bacterial 16S ribosomal DNA with polymerase chain reaction. *J Clin Microbiol*. 1990;28(9):1942–1946.
14. CLSI. *M100 Performance Standards for Antimicrobial Susceptibility Testing*. Wayne, PA: Clinical and Laboratory Standards Institute. 2019.
15. Yang FC, Yan JJ, Hung KH, Wu JJ. Characterization of ertapenem-resistant Enterobacter cloacae in a Taiwanese University Hospital. *J Clin Microbiol*. 2012;50(2):223–226. doi:10.1128/JCM.01263-11
16. Tenover FC, Arbeit RD, Goering RV, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol*. 1995;33(9):2233–2239.
17. Dallenne C, da Costa A, Decré D, Favier C, Arlet G. Development of a set of multiplex PCR assays for the detection of genes encoding important  $\beta$ -lactamases in Enterobacteriaceae. *J Antimicrob Chemother*. 2010;65(3):490–495. doi:10.1093/jac/dkp498
18. Gayet S, Chollet R, Molle G, Pagès JM, Chevalier J. Modification of outer membrane protein profile and evidence suggesting an active drug pump in Enterobacter aerogenes clinical strains. *Antimicrob Agents Chemother*. 2003;47(5):1555–1559. doi:10.1128/aac.47.5.1555-1559.2003
19. Doumith M, Ellington MJ, Livermore DM, Woodford N. Molecular mechanisms disrupting porin expression in ertapenem-resistant Klebsiella and Enterobacter spp. clinical isolates from the UK. *J Antimicrob Chemother*. 2009;63(4):659–667. doi:10.1093/jac/dkp029
20. Bergmans HEN, Van Die IM, Hoekstra WPM. Transformation in Escherichia coli: stages in the process. *J Bacteriol*. 1981;146(2):564–570.
21. Barton BM, Harding GP, Zuccarelli AJ. A general method for detecting and sizing large plasmids. *Anal Biochem*. 1995;226(2):235–240. doi:10.1006/abio.1995.1220
22. Du H, Chen L, Chavda KD, et al. Genomic characterization of Enterobacter cloacae isolates from China that coproduce KPC-3 and NDM-1 carbapenemases. *Antimicrob Agents Chemother*. 2016;60(4):2519–2523. doi:10.1128/AAC.03053-15
23. Bankevich A, Nurk S, Antipov D, et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol*. 2012;19(5):455–477. doi:10.1089/cmb.2012.0021
24. Carattoli A, Zankari E, García-Fernández A, et al. In Silico detection and typing of plasmids using plasmidfinder and plasmid multilocus sequence typing. *Antimicrob Agents Chemother*. 2014;58(7):3895–3903. doi:10.1128/AAC.02412-14
25. Wang L-H, Wei -D-D, Wan L-G, Yu Y, Deng Q, Liu Y. Diversity of the genetic environment of the bla KPC-2 gene among Klebsiella pneumoniae clinical isolates in a Chinese Hospital. *Microb Drug Resist*. 2015;22(1):15–21. doi:10.1089/mdr.2014.0281
26. Diene SM, Merhej V, Henry M, et al. The rhizome of the multidrug-resistant enterobacter aerogenes genome reveals how new “Killer Bugs” are created because of a sympatric lifestyle. *Mol Biol Evol*. 2013;30(2):369–383. doi:10.1093/molbev/mss236
27. Zeng KJ, Doi Y, Patil S, Huang X, Tian GB. Emergence of the plasmid-mediated mcr-1 gene in colistin-resistant Enterobacter aerogenes and Enterobacter cloacae. *Antimicrob Agents Chemother*. 2016;60(6):3862–3863. doi:10.1128/AAC.00345-16
28. Szabó D, Silveira F, Hujer AM, et al. Outer membrane protein changes and efflux pump expression together may confer resistance to ertapenem in Enterobacter cloacae. *Antimicrob Agents Chemother*. 2006;50(8):2833–2835. doi:10.1128/AAC.01591-05
29. Conte V, Monaco M, Giani T, et al. Molecular epidemiology of KPC-producing Klebsiella pneumoniae from invasive infections in Italy: increasing diversity with predominance of the ST512 clade II sublineage. *J Antimicrob Chemother*. 2016;71(12):3386–3391. doi:10.1093/jac/dkw337
30. Netikul T, Kiratisin P, Nguyen MH. Genetic characterization of carbapenem-resistant enterobacteriaceae and the spread of carbapenem-resistant klebsiella pneumonia ST340 at a university hospital in Thailand. *PLoS One*. 2015;10(9):e0139118. doi:10.1371/journal.pone.0139116
31. Ho PL, Cheung YY, Wang Y, et al. Characterization of carbapenem-resistant Escherichia coli and Klebsiella pneumoniae from a health-care region in Hong Kong. *Eur J Clin Microbiol Infect Dis*. 2016;35(3):379–385. doi:10.1007/s10096-015-2550-3
32. Yigit H, Queenan AM, Anderson GJ, et al. Novel carbapenem-hydrolyzing  $\beta$ -lactamase, KPC-1, from a carbapenem-resistant strain of Klebsiella pneumoniae. *Antimicrob Agents Chemother*. 2001;45(4):1151–1161. doi:10.1128/AAC.45.4.1151-1161.2001
33. Dai W, Sun S, Yang P, Huang S, Zhang X, Zhang L. Characterization of carbapenemases, extended spectrum  $\beta$ -lactamases and molecular epidemiology of carbapenem-non-susceptible Enterobacter cloacae in a Chinese hospital in Chongqing. *Infect Genet Evol*. 2013;14:1–7. doi:10.1016/j.meegid.2012.10.010
34. Zhou T, Zhang X, Guo M, et al. Phenotypic and molecular characteristics of carbapenem-non-susceptible Enterobacteriaceae from a teaching hospital in Wenzhou, Southern China. *Jpn J Infect Dis*. 2013;66(2):96–102.
35. Tang Y, Li G, Liang W, Shen P, Zhang Y, Jiang X. Translocation of carbapenemase gene blaKPC-2 both internal and external to transposons occurs via novel structures of Tn1721 and exhibits distinct movement patterns. *Antimicrob Agents Chemother*. 2017;61(10):e01151–17.

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