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First description of NDM-1-, KPC-2-, VIM-2- and IMP-4-producing *Klebsiella pneumoniae* strains in a single Chinese teaching hospital

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SUMMARY

A total of 180 non-duplicate carbapenem-resistant *Klebsiella pneumoniae* isolates were recovered from patients hospitalized between December 2010 and January 2012 at a Chinese hospital. Eight KPC-2, four NDM-1, one VIM-2, and five KPC-2 plus IMP-4 producers were identified and all were multidrug resistant due to the presence of other resistance determinants, including extended-spectrum β-lactamases (CTX-M-15, SHV-12), 16S rRNA methylases (*armA, rmtB*) and plasmid-mediated quinolone-resistance determinants (*qnr*A, B, S, *aac(6′)-Ib-cr*). Nine *K. pneumoniae* clones (Kpn-A1/ST395, Kpn-A3/ST11, Kpn-A2/ST134, Kpn-B/ST263, Kpn-C/ST37, Kpn-D/ST39, Kpn-E/ST1151, Kpn-F/ST890, Kpn-G/ST1153) were identified. $bla_{\text{KPC-2}}$ was located on transferable ~65 kb IncL/M (ST395, ST11, ST134, ST39) and ~100 kb IncA/C (ST37, ST1153, ST890) plasmids, respectively. On the other hand, $bla_{\text{NDM-1}}$ was associated with a ~70 kb IncA/C plasmid (ST263). However, non-typable plasmids of ~40 kb containing $bla_{\text{VIM-2}}$ were detected in the ST1151 clone. This work reports the first co-occurrence of four diverse types of carbapenemase of *K. pneumoniae* clones from a single hospital in China. IncA/C, IncL/M, and other successful plasmids may be important for the dissemination of carbapenemases, producing a complex epidemiological picture.

Key words: Carbapenemase, *Klebsiella*, metallo-β-lactamases, multilocus sequence typing, plasmid.

INTRODUCTION

Carbapenemase-producing *Klebsiella pneumoniae* (CPKP) is an ever-increasing clinical problem in hospitals which significantly limits treatment options for infections with these organisms. The most clinically significant carabapenemases are the KPC-type (Ambler class A), IMP-, VIM- and NDM-types (class B) and OXA-48 (class D), which have mostly been identified in *K. pneumoniae* isolates as sources of nosocomial outbreaks [1]. In Chinese hospitals there is an ongoing epidemic of *K. pneumoniae* clonal strains, predominantly sequence type (ST)11, harbouring class A (KPC) and/or class B (MBLs) carbapenemases [2]. The carbapenemases are often encoded by genes located on large plasmids which also carry genes for resistance to other antimicrobial agents such as aminoglycosides and fluoroquinolones [3]. The carbapenemase genes are associated with a variety of plasmid types, although commonly with broad range IncA/C elements [4]. As plasmids are

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the principal vehicles for the dissemination of a great variety of resistance genes, their study and understanding is critical for reversing the increasing trend in antibiotic resistance rates worldwide [5].

Until now, there have been no attempts to analyse potential connections between STs and plasmid replicon types of *K. pneumoniae* isolates producing carbapenemases. Moreover, there is little information regarding the association of carbapenemase genes with specific plasmid families. Such linkages are potentially important owing to the spread of the KPC-producing lineage of *K. pneumoniae* ST11 in China [6]. The aim of this study was therefore to determine the plasmid families and ST diversity of a collection of *K. pneumoniae* isolates producing carbapenemases in a large teaching hospital in China.

MATERIALS AND METHODS

Isolation and identification of bacterial strains

This study was conducted at the First Affiliated Hospital of Nanchang University, a 2900-bed teaching hospital with 400 adult intensive care unit beds and 3650000 annual inpatient discharges. Over a 13-month study period (December 2010 to January 2012), initiated with the identification of the first isolate under investigation, a total of 180 carbapenem-resistant *K. pneumoniae* isolates (imipenem, meropenem, or ertapenem resistant) were recovered from clinical specimens. Of these isolates, 18 were shown to be carbapenemase producers which were randomly selected for a battery of phenotypic tests and the molecular study. Identification of isolates was performed using an automated microbiology analyser (bioMérieux, France) according to the manufacturer's instructions. Semi-quantitative counts of isolates of $>10^7$ c.f.u./ml were considered indicative of pulmonary infection.

Antimicrobial susceptibilities

Minimum inhibitory concentration (MICs) for carbapenems (imipenem and meropenem) and other antimicrobial agents (amoxicillin/clavulanic acid, cefotaxime, ceftazidime, cefepime, aztreonam, cefoxitin, amikacin, gentamicin, tobramycin, ciprofloxacin, trimethoprim-sulfamethoxazole) were determined by a broth microdilution method (bioMérieux). Additional susceptibilities for ertapenem were performed using E-tests according to the manufacturer's instructions (AB BioDisk, Sweden). The Clinical and Laboratory Standards Institute (CLSI) M100-S22 interpretive breakpoints were used to interpret the MIC results for all antimicrobial agents studied [7]. Carbapenemase production was detected using the modified Hodge test (MHT) and a combined disc test using meropenem plus boronic acid or EDTA [8].

Detection of antimicrobial resistance determinants

KPC, OXA-48-like, VIM, IMP, and NDM carbapenemases were identified using polymerase chain reaction (PCR) amplification and sequencing as described previously and a microarray assay (Check-MDR CT101; Check-Points, The Netherlands) was used to detect additional β-lactamases. The amplification of the *qnr*A, *qnr*S, and *qnr*B genes was undertaken in all isolates with multiplex PCR [9]. Genes *aac(6')-Ib* and *qepA* were amplified in separate PCRs using published primers and conditions [10]. The variant *aac(6')-Ib-cr* was further identified by digestion with *Bst*F5I and sequencing [11] (New England Biolabs, USA). The amplification of genes encoding 16S rRNA methylases was determined using a multiplex PCR. Detection of *intI1, IS*CR1 and *complex class 1 integron* was accomplished using PCR and nucleotide sequencing with previously published primers [12, 13].

Transferability of carbapenemase genes and plasmid characterization

Plasmids were isolated using the QIAprep spin miniprep kit (Qiagen GmbH, Germany) and the incompatibility group was determined according to the PCR-based replicon typing scheme [14]. The size of the carbapenemase plasmids and association with replicon type were confirmed by hybridization of S1 nuclease-digested genomic DNA from *Escherichia coli* transconjugants (or wild-type strains in the absence of transfer) with appropriate probes. The potential for conjugational transfer of carbapenem resistance was examined using representative isolates and *E. coli* J53Az^R as the recipient strain. Transconjugants were selected on Luria–Bertani (LB) agar containing sodium azide (50 mg/l) supplemented with ceftazidime (50 mg/l) or imipenem (1 mg/l). In isolates unable to transfer carbapenemase by conjugation the gene was transferred by transformation using plasmid extracts purified using a Gene Pulser Xcell (Bio-Rad, USA) with *E. coli* DH5α as a recipient. Transformants were selected on LB agar plates with 100 mg/l ampicillin. Similarity between the carbapenemase plasmids

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was assessed by comparing *Eco*RI-, *Pst*I- and *Hpa*I-digested plasmid DNA profiles in all transconjugants.

Pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST)

PFGE of *Xba*I-digested genomic DNA of the isolates under investigation was performed with a CHEF-DR-III system (Bio-Rad, UK), with a running time of 23 h and pulse times ranging from 3 s to 20 s. MLST was performed using seven conserved housekeeping genes (*gapA, infB, mdh, pgi, phoE, rpoB, tonB*) (http://www.pasteur.fr/recherche/genopole/PF8/mlst/K.pneumoniae.html).

RESULTS

Patients' characteristics

Eighteen patients had proven or suspected acquisition of CPKP, including eight with invasive infection. The patients were mostly elderly (median age 64 years, range 26–85 years) with multiple underlying illnesses (Table 1). Infection was documented in all cases, the most prevalent being respiratory tract infection. All patients had a prolonged hospital stay (median 41 days, range 9–263 days) and the median number of days between admission and the first positive culture with CPKP was 21 days (range 8–86). All patients had a history of recent hospitalization in our institution and two patients had travelled abroad or been transferred from another hospital.

Antimicrobial susceptibility

Based on the results of initial antibiotic susceptibility tests, all 18 CPKP strains were highly resistant to gentamicin, tobramycin, ciprofloxacin, cefepime, cefotaxime and carbapenems. The majority (83·3%) proved resistant to aztreonam, 72·2% to amoxicillin/clavulanic acid, 66·7% to trimethoprim-sulfamethoxazole, 61·1% to levofloxacin, 61·1% to amikacin, and 55·6% to ceftazidime. The MHT and the carbapenem/boronic acid combination disc test were positive for all strains.

β-Lactamase identification

The carbapenemase genes $bla_{\text{KPC-2}}$, $bla_{\text{IMP-4}}$, $bla_{\text{VIM-2}}$, and $bla_{\text{NDM-1}}$ were identified by PCR and subsequent sequencing and all of the 18 patients' representative carbapenemase-producing strains tested positive for at least one of the *β*-lactamase-encoding genes by PCR with $bla_{\text{KPC-2}}$ being the most frequent (Table 2). However, extended-spectrum *β*-lactamase (ESBL) genes could be sequenced in only 13/18 strains and the remainder contained only $bla_{\text{TEM-1}}$ ($n=4$) or $bla_{\text{SHV-11}}$ ($n=1$), which are not ESBL enzymes (Table 2). The dominant ESBL types detected were $bla_{\text{SHV-12}}$ ($n=6$), $bla_{\text{CTX–M-14}}$ ($n=4$) and $bla_{\text{CTX-M-15}}$ ($n=4$).

Association of other resistance genes with carbapenemase production

All 18 CPKP strains harboured at least one of the five plasmid-mediated quinolone resistance (PMQR) genes tested; *qnr*A, *qnr*B, *qnr*S and *aac(6′)-Ib-cr* genes were detected in eight, nine, 11 and 10 of the strains, respectively. The *qnr* genes included eight *qnr*A1, eight *qnr*B4, one *qnr*B6 and 11 *qnr*S1 genes; all were negative for *qepA*. Three KPC-producing strains were positive for *qnr*S1, three for *acc(6′)-Ib-cr* with *qnr*A1 and *qnr*S1, one each for *qnr*A1 and *acc(6′)-Ib-cr* with *qnr*B4. Four NDM producers were positive for *qnr*B4 with *qnr*S1. Two each of strains that co-produced KPC and IMP were positive for *acc(6′)-Ib-cr* with *qnrB*4 and *acc(6′)-Ib-cr* with *qnr*A1 and *qnr*S1, one was positive for *acc(6′)-Ib-cr* with *qnr*A1 and *qnr*B4. Only one VIM producer was positive for *acc(6′)-Ib-cr* with *qnr*A1 and *qnr*B6 (Table 2).

All 18 strains had at least one of the three aminoglycoside resistance determinant (ARD) genes tested. The *aac(6′)-Ib, armA* and *rmtB* genes were detected in eight, three, and 13 of the strains, respectively. Six KPC strains were positive for *rmtB*, three were positive for *armA*. Two each of NDM producers were positive for *acc(6′)-Ib* and *rmtB* and five co-producers of KPC and IMP were positive for *rmtB* with *acc(6′)-Ib*. Only one VIM producer was positive for *armA* with *acc(6′)-Ib*. Mobile elements *IS*CR1, class 1 integron and the complex class 1 integron were detected in 12, three, and four strains, respectively (Table 2); four KPC producers harboured the complex class 1 integron; two with *IS*CR1 and two with class 1 integron. All NDM producers and co-producers of KPC and IMP were positive for *IS*CR1. Only one VIM producer was positive for class 1 integron.

Genetic context of carbapenemase and plasmid analysis

The carbapenemase genes were successfully transferred from all strains either by conjugation or

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Table 1. *Clinical features of the carbapenemase-producing* Klebsiella pneumoniae *strains and patients' characteristics*

Patient no.	Age (gender)	Hospital unit	Underlying disease	Travel	LOS (days)	LOS until first positive culture (date of isolation)	Site of isolation	Treatment	Outcome
1	68 (F)	RU	Nosocomial pneumonia	No	65	23 (16 March 2011)	Respiratory	IPM+FEP	Survived
2	72 (F)	ICU	Breast carcinoma	No	108	42 (22 December 2010)	Respiratory +blood	SCF	Died
3	75 (M)	RU	DM, hypertension	Yes	41	12 (31 March 2011)	Respiratory	MEM	Died
4	65 (F)	BU	DM, UTI	No	35	20 (6 May 2011)	Urine	FEP	Survived
5	72 (M)	ICU	Acute pancreatitis	No	67	50 (29 June 2011)	Blood	SCF	Died
6	45 (F)	BU	Hypertension	No	13	8 (05 August 2011)	Urine	AMK+MEM	Survived
7	68 (M)	RU	Bronchiectasis	Yes	78	35 (07 July 2011)	Respiratory +blood	IPM+TGC +AMK	Died
8	44 (M)	BU	UTI	No	263	86 (11 August 2011)	Urine	TGC+SXT +AMK	Survived
9	28 (M)	BU	COPD	No	23	15 (22 January 2011)	Urine	MEM+TZP	Survived
10	64 (F)	CCU	Colon adenocarcinoma	No	115	45 (13 December 2010)	Abscess	MEM+SCF	Died
11	36 (M)	TCU	Liver transplant	Yes	56	8 (5 March 2011)	Peritoneal fluid	SCF	Died
12	43 (M)	BU	Ulcerative colitis	No	9	8 (25 August 2011)	Wound	None	Died
13	35 (F)	BU	ARDS	No	53	25 (07 June 2011)	Respiratory	AMK+MEM	Died
14	65 (F)	ICU	DM, sepsis	No	38	32 (21 January 2012)	Blood	TGC	Survived
15	32 (M)	BU	VAP	No	41	25 (9 December 2011)	Respiratory	None	Died
16	85 (F)	OD	DM	No	22	21 (12 February 2011)	Urine	TGC	Survived
17	26 (F)	BU	Septic shock	No	26	21 (23 September 2011)	Blood	AMK+MEM	Died
18	73 (F)	ED	Perianal abscess	No	28	12 (12 November 2010)	Abscess	TGC+AMK	Survived

LOS, Length of stay; M, male; F, female; RU, respiratory unit; ICU, intensive care unit; BU, burns unit; CCU, coronary care unit; TCU, transplantation care unit; OD, orthopaedics department; ED, emergency department; DM, diabetes mellitus; UTI, urinary tract infection; COPD, chronic obstructive pulmonary disease; ARDS, acute respiratory distress syndrome; VAP, ventilator-associated pneumonia; IPM, imipenem; FEP, cefepime; SCF, cefoperazone/sulbactam; MEM, meropenem; AMK, amikacin; TGC, tigecycline; SXT, trimethoprim-sulfamethoxazole; TZP, piperacillin/tazobactam.

Table 2. *Characteristics of the carbapenemase-producing* Klebsiella pneumoniae *clones*

CPKP isolates	PFGE type/ST	Case no.	Carbapenemasegene	Plasmids*		Associated resistance determinants†	Associated mobile elements	Carbapenem MICs (μg/ml)		
				Size (kb)	Inc group			MEM	IPM	ERT
KPC-2 producer ($n=8$)	Kpn-A1/395	Kp6	bla_{KPC-2}	~65	L/M	*qnr*S1, *acc(6′)-Ib-cr*, *rmtB*, $bla_{CTX-M-15}$	*IS*CR1	8	16	32
	Kpn-A2/134	Kp9, Kp15	bla_{KPC-2}	~65	L/M	bla_{SHV-12}, *rmtB*	Complex class1 integron	16	16	>32
	Kpn-A3/11	Kp4	bla_{KPC-2}	~65	L/M	*qnrS1*, *rmtB*, bla_{TEM-1}	*IS*CR1	4	8	16
	Kpn-C/37	Kp7	bla_{KPC-2}	~100	A/C	*acc(6′)-Ib-cr*, *armA*	Class 1 integron	16	32	>32
	Kpn-D/39	Kp1, Kp18	bla_{KPC-2}	~65	L/M	*acc(6′)-Ib-cr*, *qnr*S1, *rmtB*, $bla_{CTX-M-15}$, bla_{TEM-1}	Complex class1 integron	8	32	32
	Kpn-G/1153	Kp10	bla_{KPC-2}	~100	A/C	*qnr*S1, *armA*	Class 1 integron	32	16	>32
IPM-4 and KPC-2 co-producer ($n=5$)	Kpn-A1/395	Kp8, Kp12, Kp13, Kp17	bla_{KPC-2}	~65	L/M	*acc(6′)-Ib-cr*, bla_{TEM-1}	*IS*CR1	>32	>32	>32
	Kpn-F/890	Kp16	bla_{KPC-2}	~100	A/C	*qnr*S1, *rmtB*, $bla_{CTX-M-15}$	*IS*CR1	32	>32	>32
NDM-1 producer ($n=4$)	Kpn-B/263	Kp2, Kp3, Kp5, Kp14	bla_{NDM-1}	~70	A/C	*rmtB*, bla_{TEM-1}	*IS*CR1	4	16	>32
VIM-2 producer ($n=1$)	Kpn-E/1151	Kp11	bla_{VIM-2}	~20	n.t.	*acc(6′)-Ib-cr*, *armA*, bla_{TEM-1}	Class 1 integron	4	4	16

PFGE, Pulsed-field gel electrophoresis; ST, sequence type; MIC, minimum inhibitory concentration; MEM, meropenem; IPM, imipenem; ERT, ertapenem; n.t., non-typable.
* Plasmids from Kpn-A1/395, Kpn-A2/134, Kpn-A3/11, and Kpn-D/39 clones were transferred by conjugation and showed a similar RFLP pattern.
† Resistance markers were co-harboured by the carbapenemase gene- (bla_{KPC-2}, bla_{VIM-2} or bla_{NDM-1}) carrying plasmids.

transformation into recipient *E. coli*, suggesting plasmid localization (Table 2). S1 nuclease PFGE and in-gel hybridization of all isolates (data not shown) showed that diverse carbapenemase genes were located on plasmids ranging from ~20 to ~100 kb. Co-transfer of several carbapenemase genes with other resistance determinants and mobile elements was observed. The co-transfer of bla_{KPC-2}, $bla_{CTX-M-15}$ and *rmtB* as observed in isolate Kpn-A/ST395, resulting in resistance to all β-lactams and aminoglycosides, is notable as dissemination of such multidrug resistance plasmids could have serious consequences for treatment options.

The bla_{KPC-2} gene was transferable for strains of six genotypes and this gene was located either on transferable ~65 kb IncL/M and ~100 kb IncA/C plasmids (Table 2). On the other hand, bla_{NDM-1} was located on a ~70 kb plasmid showing a highly similar restriction fragment length polymorphism (RFLP) pattern in all transconjugants. The nucleotide sequence of these replicons was 99% identical to those of the pMR0211 plasmid (GenBank accession nos. JF826284·1), which belongs to the A/C group. The bla_{VIM-2} gene was associated with a non-typable and non-transferable ~20 kb plasmid (Table 2).

Molecular epidemiology

Based on an 80% similarity as the cut-off to discriminate between DNA profile clusters, seven PFGE types were evident in the 18 strains; the most common type A was further discriminated into three subtypes. The linkage between PFGE type and MLST type is shown in Table 3. Three genotypes, Kpn-A1/ST395, Kpn-B/ST263 and Kpn-D/ST39 accounted for five, four and two strains respectively and the remaining strains were characterized by unique genotypes. With the exception of ST11, a single-locus variant of ST258, none of the strains belonged to the CG258 group.

Table 3 also shows that regarding carbapenemase types and resistance gene content, four NDM-1 producers fell in genotype Kpn-B/ST263, which is the first identification of this ST in China having been originally reported in Korea [15]. Thirteen strains in seven different STs (11, 37, 39, 134, 395, 890, 1153) harboured the bla_{KPC-2} gene. Interestingly, five KPC-2 strains were additionally positive for the metallo-β-lactamase bla_{IMP-4}, an association recently reported in China [16, 17]. MLST assigned the VIM-2-producing isolates to ST1151. Only four *K. pneumoniae* genotypes (Kpn-A1/ST395, Kpn-A2/ST134,

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Table 3. *Genotypic characteristics, PFGE patterns and their correspondence with MLST profiles of the 18 CPKP strains*

Isolate	PFGE type	Carbapenemase	PMQR genes	ARD genes	β-lactamases	MLST
Kp1	D	KPC-2	*qnr*A1, *qnr*S1, *acc(6′)-Ib-cr*	*rmtB*	CTX-M-15, TEM-1	ST39
Kp2	B	NDM-1	*qnr*B4, *qnr*S1	*rmtB, acc(6′)-Ib*	TEM-1	ST263
Kp3	B	NDM-1	*qnr*B4, *qnr*S1	*rmtB, acc(6′)-Ib*	TEM-1	ST263
Kp4	A3	KPC-2	*qnr*A1, *qnr*S1, *acc(6′)-Ib-cr*	*rmtB*	SHV-12	ST11
Kp5	B	NDM-1	*qnr*B4, *qnr*S1	*rmtB*	CTX-M-14, TEM-1	ST263
Kp6	A1	KPC-2	*qnr*A1, *qnr*S1, *acc(6′)-Ib-cr*	*rmtB, acc(6′)-Ib*	CTX-M-15, -14	ST395
Kp7	C	KPC-2	*qnr*B4, *acc(6′)-Ib-cr*	*armA*	SHV-12, TEM-1	ST37
Kp8	A1	KPC-2, IPM-4	*qnr*A1, *qnr*S1, *acc(6′)-Ib-cr*	*rmtB, acc(6′)-Ib*	CTX-M-14, TEM-1	ST395
Kp9	A2	KPC-2	*qnr*A1	*rmtB*	SHV-12	ST134
Kp10	G	KPC-2	*qnr*S1	*armA*	SHV-11	ST1153
Kp11	E	VIM-2	*qnr*A1, *qnr*B6, *acc(6′)-Ib-cr*	*armA, acc(6′)-Ib*	TEM-1	ST1151
Kp12	A1	KPC-2, IPM-4	*qnr*B4, *acc(6′)-Ib-cr*	*rmtB, acc(6′)-Ib*	SHV-12, TEM-1	ST395
Kp13	A1	KPC-2, IPM-4	*qnr*A1, *qnr*B4, *acc(6′)-Ib-cr qnr*A1, *qnr*B4, *acc(6′)-Ib-cr*	*acc(6′)-Ib*	CTX-M-14, TEM-1	ST395
Kp14	B	NDM-1	*qnr*B4, *qnr*S1	*rmtB*	TEM-1	ST263
Kp15	A2	KPC-2	*qnr*S1	*rmtB*	SHV-12	ST134
Kp16	F	KPC-2, IPM-4	*qnr*S1	*rmtB*	CTX-M-15	ST890
Kp17	A1	KPC-2, IPM-4	*qnr*B4, *acc(6′)-Ib-cr*	*acc(6′)-Ib*	SHV-12, TEM-1	ST395
Kp18	D	KPC-2	*qnr*A1, *qnr*S1, *acc(6′)-Ib-cr*	*rmtB*	CTX-M-15, TEM-1	ST39

PFGE, Pulsed-field gel electrophoresis; MLST, multilocus sequence type; PMQR, plasmid-mediated quinolone resistance; ARD, aminoglycoside resistance determinant.

Kpn-B/ST263, Kpn-D/ST39) were isolated for more than a 1-month period in the hospital, while others were more sporadic in occurrence.

DISCUSSION

Of 180 *K. pneumoniae* isolates with decreased susceptibility to carbapenems, 18 were shown to be carbapenemase producers. For the other isolates, no carbapenemase activity or carbapenemase genes were identified, suggesting a non-carbapenemase-related resistance mechanism. Reduced susceptibility to carbapenems may have been due to combined mechanisms such as overproduction of the chromosomal cephalosporinase or ESBL associated with decreased permeability of the outer membrane. In this scenario, PCR and sequencing analysis of the 18 strains revealed four diverse carbapenemase genes ($bla_{\text{NDM-1}}$, $bla_{\text{KPC-2}}$, $bla_{\text{IMP-4}}$, $bla_{\text{VIM-2}}$), with the $bla_{\text{KPC-2}}$ genes being the most common. The strain co-producing IMP-4 and KPC-2 enzymes was fully resistant to all carbapenems, whereas all other KPC-2-producing strains exhibited variable susceptibility to carbapenems, with only a slight increase in carbapenem MICs (Table 2). NDM-1-producing and VIM-2-producing strains also exhibited variable susceptibility to carbapenems. As frequently observed for carbapenemase producers, all of the 18 strains studied exhibited a multidrug resistance phenotype. All types of carbapenemase were highly associated with other resistance genes (PMQR, ESBLs, quinolone resistance determinant genes) and various highly efficient mobile elements (*IS*CR1 and class 1 integron), which is in keeping with the finding of Huang *et al*. [18].

Considerable genetic diversity was evident in the characterized strains with seven clusters defined by PFGE and nine STs. Of the KPC-2-producing *K. pneumoniae*, five different STs were identified, underlining the genetic diversity of the KPC-2-positive backgrounds circulating in the same hospital (Table 3). Kpn-A/ST395 was the most common genotype with six representatives. Thus far, reports of STs of outbreak or nosocomial dissemination of NDM-1-producing *K. pneumoniae* around the world have involved ST231 and ST340 [19]. However, the ST of the NDM-1 clone described herein is ST263, which has not yet been reported to harbour NDM-1. This finding supports the conclusion that $bla_{\text{NDM-1}}$ occurs in *K. pneumoniae* belonging to diverse phylogenetic lineages and also emphasizes the need to study the plasmids carrying this gene in the species. Two novel sequence types (ST1151 and ST1153) were first described in our study and have been deposited in the *K. pneumoniae* MLST database.

In addition to the successful clones, previous studies have demonstrated that the spread of diverse carbapenemase genes is also linked to different types of transferable IncA/C, IncL/M and IncN plasmids [20]. The $bla_{\text{KPC-2}}$ gene was transferred from *K. pneumoniae* isolates of STs 11, 37, 39, 134, 395 and 1153, albeit at relatively low frequencies ($1{\cdot}8\times10^{-6}$ to $5{\cdot}2\times10^{-7}$ transconjugants per donor cell), and fell into incompatibility groups, IncL/M and IncA/C. Such $bla_{\text{KPC-2}}$-harbouring plasmids have been mainly described in *K. pneumoniae* ST11 in different countries including China, Greece and the USA [21–23]. Our work therefore underscores the fact that, intraclonal spread of $bla_{\text{KPC-2}}$-containing plasmids and STs occurs as previously reported by Huang *et al*. [18]. Notably, determination of the sequences flanking $bla_{\text{KPC-2}}$ revealed that the genetic environments of the gene for most strains were consistent with the genetic structure of $bla_{\text{KPC-2}}$ on the plasmid pKP048 [23]. In four CPKP strains, the $bla_{\text{NDM-1}}$ gene was carried on a 70 kb self-conjugative plasmid with an IncA/C-type backbone (Table 3). In addition, we identified the same plasmid with the $bla_{\text{NDM-1}}$ gene in both *K. pneumoniae* and *E. coli* isolates recovered sequentially from the same patient (data not shown), which is suggestive of *in vivo* plasmid transfer. Such transfer of a NDM-1-encoding IncA/C plasmid from *K. pneumoniae* ST263 to *E. coli*, which might act as an accidental recipient present in the patients' microbiota has previously been documented [24]. The $bla_{\text{VIM-2}}$ gene was carried on an untypable and non-conjugative plasmid. Most often, several resistance markers and mobile elements were co-harboured by the plasmids that carried the carbapenemase genes.

To our knowledge, this is the first systematic molecular survey reporting prevalence and characteristics of four diverse types of carbapenemase in a single Chinese hospital. Although the number of isolates studied was small the findings are disturbing as 18 distinct CPKP strains were recovered from different patients over 13 months. Such patterns imply wider persistence, although it is impossible to be certain of this without active surveillance. In addition, we have described the rapid penetration of four types of carbapenemase genes into different unrelated *K. pneumoniae* clones and highlighted the importance of horizontal gene transfer in the dissemination of these genes and the role of the local clonal pool as a

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potential substrate for their acquisition. Microbiologists and clinicians need to be made aware of this threat and implement the necessary control measures to prevent further spread in the wider population. Finally our study underlines the importance of surveillance programmes supported by powerful molecular epidemiological techniques to identify the transmission of STs and their respective plasmids.

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DECLARATION OF INTEREST

None.

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