Original Article

Clonal dissemination of KPC-2-producing *Klebsiella pneumoniae* ST11 and ST48 clone among multiple departments in a tertiary teaching hospital in Jiangsu Province, China

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Background: The world-wide prevalence of carbapenem-resistant *Klebsiella pneumoniae* (CRKP) poses a threat to the public health. The objective of this study was to determine the epidemiological and molecular patterns of KPC-producing *Klebsiella pneumoniae (K. pneumoniae)* clinical isolates.

Methods: In this study, a total of 82 non-duplicated CRKP isolates were analyzed for the prevalence of resistant determinants including carbapenemase, extended spectrum β-lactamase (ESBLs), and AmpC as well as integrons and cassette regions by polymerase chain reaction (PCR) and DNA sequencing. The genetic relatedness was investigated by pulsed field gel electrophoresis (PFGE) and multi-locus sequencing typing (MLST).

Results: Overall, $bla_{\text{KPC-2}}$ (n=75) was the predominant carbapenemase gene, followed by high prevalence of bla_{SHV} (92.7%) and $bla_{\text{CTX-M}}$ (90.2%). PFGE and MLST analysis revealed that 65 out of 68 KPC-2-producing CRKP belonged to the ST11 clone and were distributed mainly in the department of neurology ICU. Moreover, first report on clonal dissemination of KPC-2-producing CRKP ST48 clone and NDM-5-producing CRKP ST337 clone was also identified. Class I integron were detected in 17 (20.7%) of 82 isolates with *aadA2* being the most common cassette. And a novel cassette array of integron, *aac(6')-II*-$bla_{\text{CARB/PSE-1}}$ was identified.

Conclusions: All in all, KPC-2-producing CRKP ST11 and ST48 clone were widely disseminated in multiple departments of our hospital, which triggers the need for active surveillance and implementation of infection control measures.

Keywords: Carbapenemases; *Klebsiella pneumoniae (K. pneumoniae)*; KPC-2; NDM-5; clonal dissemination

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Introduction

Klebsiella pneumoniae (*K. pneumoniae*) is one of the most opportunistic pathogen responsible for numerous infections, including respiratory tract infection, urinary tract infection, bacteremia, skin and soft tissue infections. Carbapenem has long been regarded as the last resort against infections caused by *K. pneumoniae* resistant to the 3rd or 4th cephalosporin. However, in recent years, the rapid emergence of CRKP isolates poses a great challenge to public health because of the limited therapeutic regimen, high mortality and healthcare costs (1). Up to date, clonal dissemination and outbreak caused by CRKP isolates have occurred worldwide due to the endemic clone spread and rapid transmission of carbapenemase encoding gene mediated by plasmids, integrons and transposons (2,3). It has been reported that the production of carbapenemase is the predominant mechanism leading to carbapemen resistance (4,5), in addition to the presence of extended-spectrum β-lactamases (ESBLs)/AmpC β-lactamases with porin loss combination and the overexpression of efflux pumps (6,7). The high-risk clone, ST11, has been reported to be the dominant sequence type (ST) in CRKP isolates which frequently caused clonal dissemination and outbreaks in healthcare settings in China (8-10). Recently, due to the rapid evolution of such strain under selective pressure, hypervirulent ST11 and ST11 co-producing NDM and KPC have also been consistently reported (11). Moreover, the emergence of new sequence non-ST11 isolates also poses a great challenge to clinicians and microbiologists.

Therefore, more information on the molecular characterization of such strains is needed to effectively control the transmission of CRKP and prevent the outbreaks. In this study, CRKP isolates responsible for clonal dissemination were analyzed for resistant determinants and genetic relatedness as well as integrons to provide data on CRKP isolates.

Methods

Bacterial isolates

In total, eighty-two non-duplicated CRKP isolates were collected from the Department of Laboratory Medicine of the Affiliated Hospital of Xuzhou Medical University from June, 2015 to August, 2016. The strains were obtained from sputum (n=61), blood (n=9), urine (n=6), pus (n=3), secretion (n=1), cerebrospinal fluid (n=1), and catheter (n=1). Species confirmation was performed by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS, Bruker Daltonics, Bremen, Germany) and Vitek 2 Compact system (bioMérieux, France). Antimicrobial susceptibility testing towards tigecycline, meropenem, imipenem, cefazolin, cefoxitin, cefepime, ceftriaxone, aztreonam, amikacin, gentamicin, ciprofloxacin, levofoxacin, and piperacillin/tazobactam were also performed by the Vitek 2 Compact system according to the manufacturer's instructions. *Escherichia coli* ATCC25922 was used as the quality control. The interpretation of results was based on the Clinical and Laboratory Standards Institute 2016 (12). The breakpoint of Food and Drug Administration (FDA) was used for tigecycline. Clinical characteristics on clinical features and laboratory tests were retrieved from the electronic record. The informed consent was granted by all patients and this study protocols were approved by the Ethics Committee of Affiliated Hospital of Xuzhou Medical University (XYFY2019-KL112-03).

The identification of infection and colonization bacteria were based on the clinical symptoms and signs in individual patients, imaging findings. Moreover, patients must have had fever >38 ℃ without other recognized cause, or abnormal white blood cell count [leukopenia (<4,000 WBC/mm^3) or leukocytosis (≥12,000 WBC/mm^3)], and at least two of the following: new onset of purulent sputum or change in the sputum characteristics, increased respiratory secretions or increased suctioning requirements, new onset or worsening of a cough or dyspnea or tachypnea, rales or bronchial breath sounds, or worsening gas exchange.

Detection of antimicrobial resistance determinants

DNA templates were extracted by absorption column method (Tiangen, China). All isolates that exhibited resistance to carbapenem (imipenem or meropenem) were screened for presence of the resistance genes including carbapenemase gene (*bla*$_{KPC}$, *bla*$_{SME}$, *bla*$_{GES}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{IMP}$, *bla*$_{OXA-48-like}$), extended spectrumβ-lactamase gene (*bla*$_{SHV}$, *bla*$_{TEM}$, *bla*$_{CTX-M1\ group}$, *bla*$_{CTX-M2\ group}$, *bla*$_{CTX-M8\ group}$, *bla*$_{CTX-M9\ group}$) and AmpC β-lactamase genes (*bla*$_{ACC}$, *bla*$_{FOX}$, *bla*$_{MOX}$, *bla*$_{DHA}$, *bla*$_{CIT/SPM}$, *and bla*$_{EBC}$) by polymerase chain reaction (PCR) as previously described (10). All purified positive amplicons were sequenced by GENEWIZ Company (Suzhou, China) and subtypes of β-lactamase genes were aligned on blast database.

PCR detection of integrons and RPLP analysis of cassette regions

PCR was performed to screen the presence of integrons

and integron cassette regions among the isolates using degenerate primers as described previously (13). Integrase products were digested with *Hinf*I to identify classes of integrons. The amplicons of cassettes with the same *Hinf*I pattern were considered to contain the same variable region. The representative amplicons were selected for DNA sequence by GENEWIZ Company (Suzhou, China). The results of sequencing were aligned in BLAST (http://blast.ncbi.nlm.nih.gov).

Pulsed-field gel electrophoresis

Pulsed-field gel electrophoresis (PFGE) was used for molecular typing and analysis of clone relatedness. Plugs containing genomic DNA were prepared according to previous protocol by Pereira *et al.* (14). The DNA fragments digested with restriction endonuclease *XbaI* (TaKaRa Biotechnology, Dalian, China) were separated by PFGE on 1% SeaKem Gold agarose (Lonza, Rockland, ME, United States) in 0.5× TBE (Vicmad, China) buffer using the CHEF Mapper XA PFGE system (Bio-Rad, United States) and the electrophoresis conditions were as follows: running time for 18 h at 6V/cm, temperature at 14 ℃, and electrophoretic switch times from 6 to 36 s. The similarity of PFGE patterns was calculated by Dice coefficients, and cluster analysis was performed by unweighted pair group method with arithmetic averages (UPGMA) by the BioNumerics software version 5.10. Isolates were considered to be of the same PFGE cluster when their dice similarity index was ≥80%.

Multi-locus sequence typing

MLST was performed according to the protocol shown on the *Klebsiella pneumoniae* MLST website (http://www.pasteur.fr/mlst/Kpneumoniae.html). Seven housekeeping genes (*gapA*, *infB*, *mdh*, *pgi*, *phoE*, *rpoB and tonB*) were amplified and sequenced for multilocus sequence typing of carbapenem-resistant *Klebsiella pneumoniae* (CRKP). Alleles and STs were identified using the MLST database.

Results

Clinical characteristics and antimicrobial susceptibility of CRKP isolates

The CRKP isolates were obtained from patients admitted to 15 wards in our hospital with majority being from neurology ICU (30.5%, 25/82), followed by emergency ICU (23.2%, 19/82), neurosurgery (11.0%, 9/82), critical medicine ICU (8.5%, 7/82), neurology (7.3%, 6/82), respiratory department (3.7%, 3/82), urology (3.7%, 3/82), and neonatal ICU (2.4%, 2/82). The other wards were department of bone marrow transplantation center (n=1), gastroenterology (n=1), orthopaedics (n=1), otorhinolaryngology (n=1), oncology (n=1), geriatric (n=1), and cardiology (n=1).

And all cases were identified as infected with CRKP strains.

All CRKP isolates exhibited resistance to penicillins, cefazolin, cefoxitin, cefepime, ceftriaxone, piperacillin/tazobactam, impenem and meropenem, whereas non-susceptible rates towards levofloxacin, ciprofloxacin, amikacin, and gentamicin were 96.6%, 97.7%, 74.7%, and 92.0% respectively. Furthermore, susceptibility of 100% to tigecycline was observed.

Prevalence of antibiotic resistance determinants

Carbapenemase encoding genes were identified in 80 out of 82 CRKP isolates. According to DNA alignment results, $bla_{\text{KPC-2}}$ (n=75) was predominant followed by $bla_{\text{NDM-5}}$ (n=4), and $bla_{\text{NDM-1}}$ (n=1). 2 of 4 NDM-5-producing isolates were obtained from neonatal ICU while 2 were from department of respiratory and emergency ICU respectively. 1 isolate with $bla_{\text{NDM-1}}$ gene was obtained from bone marrow transplantation center.

Analysis of ESBL genes revealed that $bla_{\text{CTX-M}}$, bla_{SHV}, and bla_{TEM} were identified in 74, 76, and 65 isolates respectively. Of 74 $bla_{\text{CTX-M}}$ positive isolates, 55 isolates carried $bla_{\text{CTX-M-65}}$, 16 $bla_{\text{CTX-M-15}}$, 3 $bla_{\text{CTX-M-14}}$, 2 $bla_{\text{CTX-M-27}}$, and 1 $bla_{\text{CTX-M-9}}$. Among 76 bla_{SHV} isolates, the most prevalent ESBLs bla_{SHV} gene was $bla_{\text{SHV-12}}$ (n=47) followed by $bla_{\text{SHV-2a}}$ (n=8). The other non-ESBLs bla_{SHV} genes including $bla_{\text{SHV-11}}$ (n=18), $bla_{\text{SHV-1}}$ (n=2) and $bla_{\text{SHV-28}}$ (n=1) were also identified, all of which co-existed with ESBLs. Moreover, $bla_{\text{TEM-1}}$ subtype was identified in all bla_{TEM} isolates (n=65). Screening for AmpC β-lactamase genes revealed that $bla_{\text{DHA-1}}$ (n=46) were predominant followed by $bla_{\text{CMY-2}}$ (n=1) and $bla_{\text{CMY-42}}$ (n=1). The bla_{SME}, bla_{GES}, bla_{VIM}, bla_{IMP}, $bla_{\text{OXA-48-like}}$, $bla_{\text{CTX-M2 group}}$, $bla_{\text{CTX-M8 group}}$, bla_{ACC}, bla_{FOX}, bla_{MOX}, *and* bla_{EBC} were not detected.

Seventy-seven isolates co-carried 2 or more resistant determinants with the combination of $bla_{\text{KPC-2}}$, $bla_{\text{SHV-12}}$, $bla_{\text{TEM-1}}$, $bla_{\text{CTX-M-65}}$ and/or $bla_{\text{DHA-1}}$ being the most common type, accounting for 29.8%. Specifically, isolates co-

Table 1 The distribution of genotypes among 82 CRKP isolates

ST	No.	Genotypes [No.]				Integron	Ward
		Carbapenemase	ESBLs	Non-ESBLs	AmpC		
ST 11	68	KPC-2 [65], NDM-5 [2], NDM-1 [1]	SHV-12 [51], SHV-2a [9], CTX-M-15 [5], CTX-M-65 [60], CTX-M-14 [3], CTX-M-9 [1], CTX-M-27 [1]	SHV-11 [10], TEM-1 [70]	DHA-1 [50], CMY-42 [1]	aadA2 [8], dfrA16-aadA2 [2], dfrA17-aadA5 [1], dfrA12-orfF-aadA2 [1], aac [6']-II-bla$_{CRAB-1}$ [1], 5'CS-3'CS [1]	Neurology ICU [22], neurology [6], neurosurgery [8]; emergency ICU [15]; critical care medicine ICU [5]; Respiratory [3]; urinary surgery [3]; others[a] [6]
ST 48	7	KPC-2 [7]	CTX-M-15 [7]	SHV-11 [6], TEM-1 [6]	CMY-2 [1]	0	Neurology ICU [2]; critical care medicine ICU [2]; emergency ICU [2]; cardiology [1]
ST 337	2	NDM-5 [2]	CTX-M-15 [2]	SHV-11 [2], TEM-1 [2]	0	0	Neonatal ICU [2]
ST 1	1	0	CTX-M-15 [1]	TEM-1 [1]	0	0	Neurology [1]
ST 15	1	KPC-2 [1]	SHV-12 [1], CTX-M-15 [1]	TEM-1 [1]	0	dfrA12-orfF-aadA2 [1]	Geriatrics [1]
ST 37	1	0	SHV-2a [1], CTX-M-27 [1]	0	DHA-1 [1]	dfrA12-orfF-aadA2 [1], 5'CS-3'CS [1]	Emergency ICU [1]
ST 700	1	KPC-2 [1]	CTX-M-65 [1]	SHV-1 [1], TEM-1 [1]	0	0	Neurosurgery [1]

[a], geriatrics (n=2), gastroenterology (n=1), otolaryngology (n=1), oncology (n=1), and bone marrow transplantation center (n=1) were included. CRKP, carbapenem-resistant *Klebsiella pneumoniae*; ESBLs, extended spectrum β-lactamase.

production of *bla*$_{KPC-2}$, *bla*$_{SHV-12}$, *bla*$_{TEM-1}$, *bla*$_{CTX-M-65}$ were identified in 13 (56.5%) strains in neurology ICU, 5 (29.4%) in emergency ICU, 3 (37.5%) in neurosurgery.

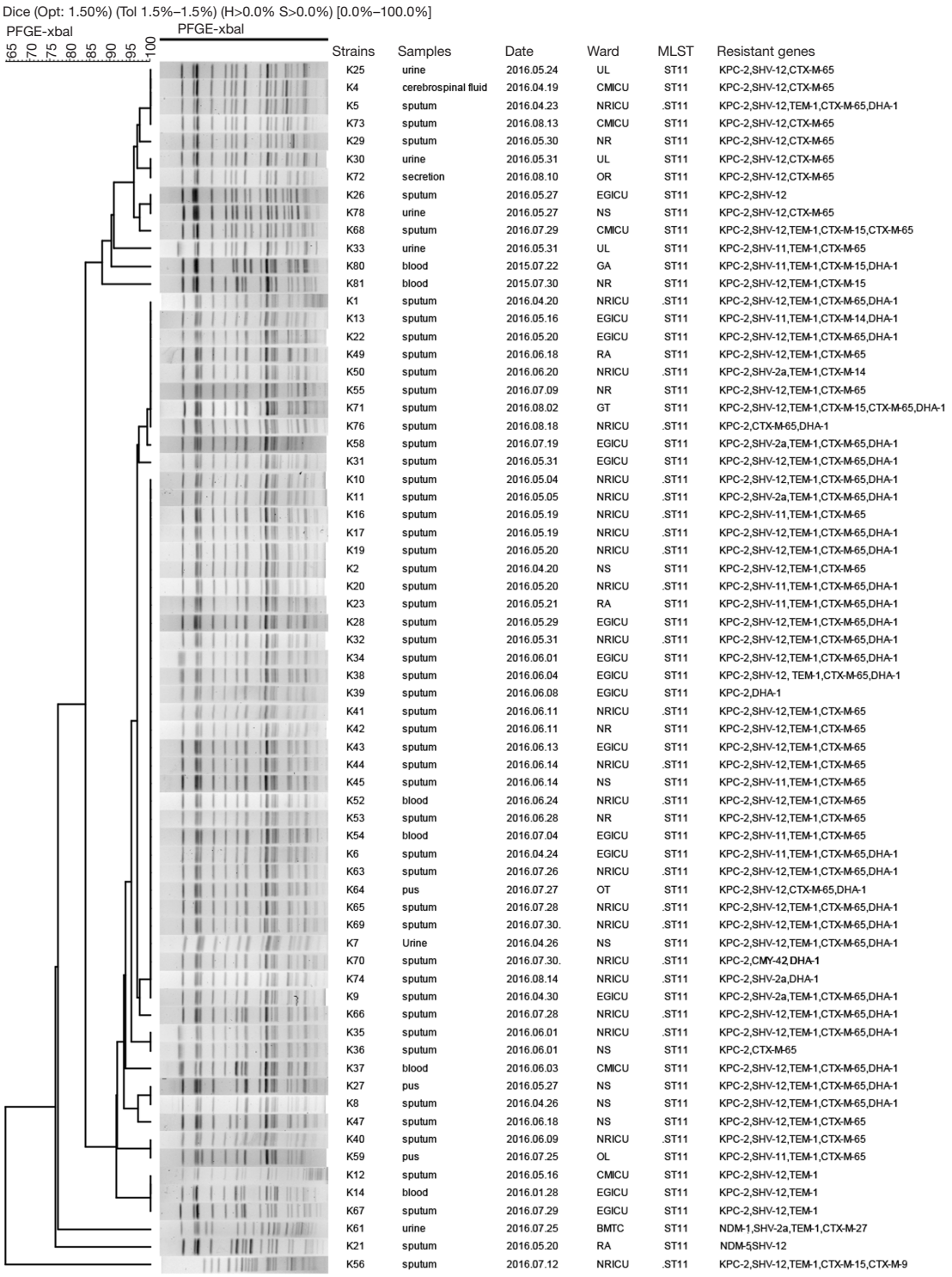
Integron identification

No class II and class III were detected. Class I of integrons were detected in 17 isolates: 14 ST11, 2 ST37, and 1 ST15 isolate (*Table 1*). The length of amplicons of 17 fragments varied from 0.15 kb to 1.9 kb. The DNA sequence analysis of gene cassette arrays revealed 6 distinct profiles with the *aadA2* (n=8) being the most prevalent array, and *dfrA12-orfF-aadA2* (n=3), *dfrA16-aadA2* (n=2), and *dfrA17-aadA5* (n=1) were also identified. Of note, we found a novel cassette arrays of integron, *aac(6')-II-bla*CARB/PSE-1. Additionally, we found integron with the amplicon size at 0.15kb in which not any gene cassettes were present but 5' and 3' conserved segments of class I integron.

Molecular typing of CRKP isolates

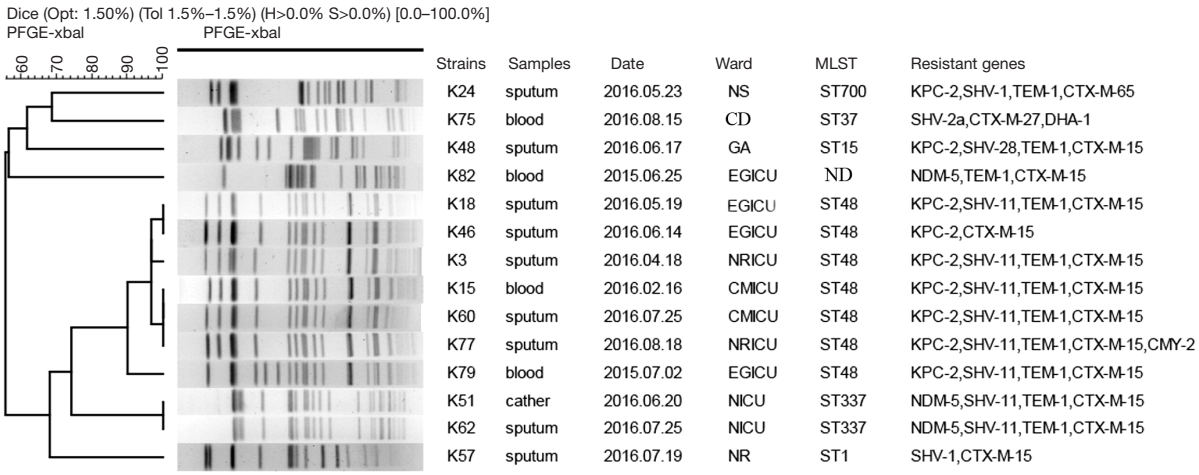
Seven STs were identified among 82 clinical isolates, and ST11 was the most prevalent sequence type accounting for 82.9%, followed by ST48 (n=7) (*Table 1*). The remaining isolates were identified as ST337 (n=2), ST15 (n=1), ST700 (n=1), ST1 (n=1), ST37 (n=1), and 1 isolate could not be typed.

PFGE patterns of *XbaI*-digested genomic DNA of 82 CRKP isolates revealed 10 different clusters. A predominant cluster consisting of 65 KPC-2-producing CRKP ST11 clone isolates was identified. These isolates were mainly obtained from neurology ICU (n=22), emergency ICU (n=15), neurosurgery (n=8), critical care medicine ICU (n=5) and neurology (n=6) (*Figure 1*, *Table 1*). Furthermore, PFGE profiles of other 14 non-ST11 CRKP isolates displayed six different patterns (*Figure 2*). Among them, 7 KPC-2-producing CRKP ST48 clone displayed the same profiles. All ST48 CRKP isolates were found to harbor *bla*$_{KPC-2}$ and



Strains	Samples	Date	Ward	MLST	Resistant genes
K25	urine	2016.05.24	UL	ST11	KPC-2,SHV-12,CTX-M-65
K4	cerebrospinal fluid	2016.04.19	CMICU	ST11	KPC-2,SHV-12,CTX-M-65
K5	sputum	2016.04.23	NRICU	.ST11	KPC-2,SHV-12,TEM-1,CTX-M-65,DHA-1
K73	sputum	2016.08.13	CMICU	ST11	KPC-2,SHV-12,CTX-M-65
K29	sputum	2016.05.30	NR	ST11	KPC-2,SHV-12,CTX-M-65
K30	urine	2016.05.31	UL	ST11	KPC-2,SHV-12,CTX-M-65
K72	secretion	2016.08.10	OR	ST11	KPC-2,SHV-12,CTX-M-65
K26	sputum	2016.05.27	EGICU	ST11	KPC-2,SHV-12
K78	urine	2016.05.27	NS	ST11	KPC-2,SHV-12,CTX-M-65
K68	sputum	2016.07.29	CMICU	ST11	KPC-2,SHV-12,TEM-1,CTX-M-15,CTX-M-65
K33	urine	2016.05.31	UL	ST11	KPC-2,SHV-11,TEM-1,CTX-M-65
K80	blood	2015.07.22	GA	ST11	KPC-2,SHV-11,TEM-1,CTX-M-15,DHA-1
K81	blood	2015.07.30	NR	ST11	KPC-2,SHV-12,TEM-1,CTX-M-15
K1	sputum	2016.04.20	NRICU	.ST11	KPC-2,SHV-12,TEM-1,CTX-M-65,DHA-1
K13	sputum	2016.05.16	EGICU	ST11	KPC-2,SHV-11,TEM-1,CTX-M-14,DHA-1
K22	sputum	2016.05.20	EGICU	ST11	KPC-2,SHV-12,TEM-1,CTX-M-65,DHA-1
K49	sputum	2016.06.18	RA	ST11	KPC-2,SHV-12,TEM-1,CTX-M-65
K50	sputum	2016.06.20	NRICU	.ST11	KPC-2,SHV-2a,TEM-1,CTX-M-14
K55	sputum	2016.07.09	NR	ST11	KPC-2,SHV-12,TEM-1,CTX-M-65
K71	sputum	2016.08.02	GT	ST11	KPC-2,SHV-12,TEM-1,CTX-M-15,CTX-M-65,DHA-1
K76	sputum	2016.08.18	NRICU	.ST11	KPC-2,CTX-M-65,DHA-1
K58	sputum	2016.07.19	EGICU	ST11	KPC-2,SHV-2a,TEM-1,CTX-M-65,DHA-1
K31	sputum	2016.05.31	EGICU	ST11	KPC-2,SHV-12,TEM-1,CTX-M-65,DHA-1
K10	sputum	2016.05.04	NRICU	.ST11	KPC-2,SHV-12,TEM-1,CTX-M-65,DHA-1
K11	sputum	2016.05.05	NRICU	.ST11	KPC-2,SHV-2a,TEM-1,CTX-M-65,DHA-1
K16	sputum	2016.05.19	NRICU	.ST11	KPC-2,SHV-11,TEM-1,CTX-M-65
K17	sputum	2016.05.19	NRICU	.ST11	KPC-2,SHV-12,TEM-1,CTX-M-65,DHA-1
K19	sputum	2016.05.20	NRICU	.ST11	KPC-2,SHV-12,TEM-1,CTX-M-65,DHA-1
K2	sputum	2016.04.20	NS	ST11	KPC-2,SHV-12,TEM-1,CTX-M-65
K20	sputum	2016.05.20	NRICU	.ST11	KPC-2,SHV-11,TEM-1,CTX-M-65,DHA-1
K23	sputum	2016.05.21	RA	ST11	KPC-2,SHV-11,TEM-1,CTX-M-65,DHA-1
K28	sputum	2016.05.29	EGICU	ST11	KPC-2,SHV-12,TEM-1,CTX-M-65,DHA-1
K32	sputum	2016.05.31	NRICU	.ST11	KPC-2,SHV-12,TEM-1,CTX-M-65,DHA-1
K34	sputum	2016.06.01	EGICU	ST11	KPC-2,SHV-12,TEM-1,CTX-M-65,DHA-1
K38	sputum	2016.06.04	EGICU	ST11	KPC-2,SHV-12, TEM-1,CTX-M-65,DHA-1
K39	sputum	2016.06.08	EGICU	ST11	KPC-2,DHA-1
K41	sputum	2016.06.11	NRICU	.ST11	KPC-2,SHV-12,TEM-1,CTX-M-65
K42	sputum	2016.06.11	NR	ST11	KPC-2,SHV-12,TEM-1,CTX-M-65
K43	sputum	2016.06.13	EGICU	ST11	KPC-2,SHV-12,TEM-1,CTX-M-65
K44	sputum	2016.06.14	NRICU	.ST11	KPC-2,SHV-12,TEM-1,CTX-M-65
K45	sputum	2016.06.14	NS	ST11	KPC-2,SHV-11,TEM-1,CTX-M-65
K52	blood	2016.06.24	NRICU	.ST11	KPC-2,SHV-12,TEM-1,CTX-M-65
K53	sputum	2016.06.28	NR	ST11	KPC-2,SHV-12,TEM-1,CTX-M-65
K54	blood	2016.07.04	EGICU	ST11	KPC-2,SHV-11,TEM-1,CTX-M-65
K6	sputum	2016.04.24	EGICU	ST11	KPC-2,SHV-11,TEM-1,CTX-M-65,DHA-1
K63	sputum	2016.07.26	NRICU	.ST11	KPC-2,SHV-12,TEM-1,CTX-M-65,DHA-1
K64	pus	2016.07.27	OT	ST11	KPC-2,SHV-12,CTX-M-65,DHA-1
K65	sputum	2016.07.28	NRICU	.ST11	KPC-2,SHV-12,TEM-1,CTX-M-65,DHA-1
K69	sputum	2016.07.30.	NRICU	.ST11	KPC-2,SHV-12,TEM-1,CTX-M-65,DHA-1
K7	Urine	2016.04.26	NS	ST11	KPC-2,SHV-12,TEM-1,CTX-M-65,DHA-1
K70	sputum	2016.07.30.	NRICU	.ST11	KPC-2,CMY-42,DHA-1
K74	sputum	2016.08.14	NRICU	.ST11	KPC-2,SHV-2a,DHA-1
K9	sputum	2016.04.30	EGICU	ST11	KPC-2,SHV-2a,TEM-1,CTX-M-65,DHA-1
K66	sputum	2016.07.28	NRICU	.ST11	KPC-2,SHV-12,TEM-1,CTX-M-65,DHA-1
K35	sputum	2016.06.01	NRICU	.ST11	KPC-2,SHV-12,TEM-1,CTX-M-65,DHA-1
K36	sputum	2016.06.01	NS	ST11	KPC-2,CTX-M-65
K37	blood	2016.06.03	CMICU	ST11	KPC-2,SHV-12,TEM-1,CTX-M-65,DHA-1
K27	pus	2016.05.27	NS	ST11	KPC-2,SHV-12,TEM-1,CTX-M-65,DHA-1
K8	sputum	2016.04.26	NS	ST11	KPC-2,SHV-12,TEM-1,CTX-M-65,DHA-1
K47	sputum	2016.06.18	NS	ST11	KPC-2,SHV-12,TEM-1,CTX-M-65
K40	sputum	2016.06.09	NRICU	.ST11	KPC-2,SHV-12,TEM-1,CTX-M-65
K59	pus	2016.07.25	OL	ST11	KPC-2,SHV-11,TEM-1,CTX-M-65
K12	sputum	2016.05.16	CMICU	ST11	KPC-2,SHV-12,TEM-1
K14	blood	2016.01.28	EGICU	ST11	KPC-2,SHV-12,TEM-1
K67	sputum	2016.07.29	EGICU	ST11	KPC-2,SHV-12,TEM-1
K61	urine	2016.07.25	BMTC	ST11	NDM-1,SHV-2a,TEM-1,CTX-M-27
K21	sputum	2016.05.20	RA	ST11	NDM-5SHV-12
K56	sputum	2016.07.12	NRICU	.ST11	KPC-2,SHV-12,TEM-1,CTX-M-15,CTX-M-9

Figure 1 Dendrogram of PFGE profiles of *XbaI*-digested DNA restriction fragments from 68 carbapenem-resistant *Klebsiella pneumoniae* ST11 isolates. The UPGMA algorithm was performed to conduct dendrogram based on the Dice similarity coefficient. Isolates were categorized to be of the same cluster when their dice similarity index was ≥80%. NRICU, neurology ICU; EGICU, emergency ICU; NS, neurosurgery; CMICU, critical care medicine ICU; NR, neurology; RA, respiratory; UL, urology; BMTC, bone marrow transplantation center; GT, gastroenterology; OT, otolaryngology; OL, oncology; OR, orthopaedics; GA, geriatrics.



Strains	Samples	Date	Ward	MLST	Resistant genes
K24	sputum	2016.05.23	NS	ST700	KPC-2,SHV-1,TEM-1,CTX-M-65
K75	blood	2016.08.15	CD	ST37	SHV-2a,CTX-M-27,DHA-1
K48	sputum	2016.06.17	GA	ST15	KPC-2,SHV-28,TEM-1,CTX-M-15
K82	blood	2015.06.25	EGICU	ND	NDM-5,TEM-1,CTX-M-15
K18	sputum	2016.05.19	EGICU	ST48	KPC-2,SHV-11,TEM-1,CTX-M-15
K46	sputum	2016.06.14	EGICU	ST48	KPC-2,CTX-M-15
K3	sputum	2016.04.18	NRICU	ST48	KPC-2,SHV-11,TEM-1,CTX-M-15
K15	blood	2016.02.16	CMICU	ST48	KPC-2,SHV-11,TEM-1,CTX-M-15
K60	sputum	2016.07.25	CMICU	ST48	KPC-2,SHV-11,TEM-1,CTX-M-15
K77	sputum	2016.08.18	NRICU	ST48	KPC-2,SHV-11,TEM-1,CTX-M-15,CMY-2
K79	blood	2015.07.02	EGICU	ST48	KPC-2,SHV-11,TEM-1,CTX-M-15
K51	cather	2016.06.20	NICU	ST337	NDM-5,SHV-11,TEM-1,CTX-M-15
K62	sputum	2016.07.25	NICU	ST337	NDM-5,SHV-11,TEM-1,CTX-M-15
K57	sputum	2016.07.19	NR	ST1	SHV-1,CTX-M-15

Figure 2 Dendrogram of PFGE profiles of *XbaI*-digested DNA restriction fragments from 14 carbapenem-resistant *Klebsiella pneumoniae* non-ST 11 isolates. The UPGMA algorithm was performed to conduct dendrogram based on the Dice similarity coefficient. Isolates were categorized to be of the same cluster when their dice similarity index was ≥80%. NRICU, neurology ICU; EGICU, emergency ICU; CMICU, critical care medicine ICU; NICU, neonatal ICU; NS, neurosurgery; GA, geriatrics, NR, neurology; CD, cardiology.

$bla_{\text{CTX-M-15}}$ genes indicating clonal dissemination of isolates from neurology ICU (n=2), critical care medicine ICU (n=2), emergency ICU (n=3). In addition, 2 NDM-5-producing CRKP ST337 clone also shared the same PFGE pattern, both of which were isolated from neonatal ICU.

Discussion

CRKP is of increasing clinical concern due to its high transmission capacity and pathogenicity (15). In this study, we described the clonal dissemination of KPC-2-producing CRKP ST11 and ST48 isolates in multiple departments and also provide the first report on clonal spread of NDM-5-producing CRKP ST337 clone.

All CRKP isolates exhibited resistance to all β-lactams and cephalosporins, in addition to high resistance rate to amikacin (>70%), which is consistent with a previous report in China (16). Moreover, amikacin has been reported to show a higher rate of microbiologic clearance than polymyxin B or tigecycline (17), which can still be considered for the infections caused by amikacin-susceptible CRKP isolates. A 100% of sensitivity toward tigecycline observed in our study is in accordance with previous study indicating that tigecycline may be considered as the basis of combination treatments for infections caused by such strains although tigecycline-resistant isolates have been reported (18). Tigecycline exhibited high susceptibility towards carbapenem-resistant *K. pneumoniae*, however, there is still some limitations of tigecycline therapy. Because of the wide distribution of tissues and low blood concentration, it is often used in combination with other antibiotics.

The high prevalence of $bla_{\text{KPC-2}}$ gene among our isolates is in line with previous reports, suggesting that $bla_{\text{KPC-2}}$ gene remains to be the most common enzyme among carbapenemase in *K. pneumoniae* isolates (15). The success of KPC is based on both gene and plasmid dissemination and on the clonal spread of K. pneumoniae ST258 and its variants (e.g., ST11). The dissemination of mobile elements may be attributed to high prevalence of $bla_{\text{KPC-2}}$ gene due to frequently reports presence of different sizes of plasmids harboring $bla_{\text{KPC-2}}$ gene (19). Moreover, the bla_{KPC} gene is located on a highly mobile Tn3-related transposon, Tn4401, that can be carried by different transferable plasmids of various incompatibility groups (20). Previous studies demonstrated that the emergence of $bla_{\text{KPC-2}}$ was characterized by two patterns of dispersion: the occurrence of *K. pneumoniae* harboring $bla_{\text{KPC-2}}$ in the IncL/M transferable plasmid, and the clonal spread of K. pneumoniae harboring $bla_{\text{KPC-2}}$ in Tn4401 different isoforms (21). In addition to KPC enzyme, New Delhi metallo-β-lactamase (NDM) was the only metallo-β-lactamase identified in our study, which included $bla_{\text{NDM-1}}$ and $bla_{\text{NDM-5}}$ subtypes. The NDM-1 enzyme, first identified in *K. pneumoniae* from Swedish patient with history of hospitalization in India, could hydrolyze all β-lactams besides monobactams (22). To our knowledge, $bla_{\text{NDM-1}}$

gene has been frequently detected in different species of *Enterobacteriaceae* from multiple countries such as Spain, Dutch, Algeria, and Korea (15). However, the emergence of NDM variants that exhibit high resistance poses a great challenge to treatment of isolates with $bla_{\text{NDM-1}}$ gene. For $bla_{\text{NDM-5}}$, it has been reported that substitutions at positions 88 and 154 on $bla_{\text{NDM-1}}$ resulted in increased resistance to carbapenems and broad-spectrum cephalosporins, moreover, it is also reported that $bla_{\text{NDM-5}}$ gene were carried by higher virulent strain (23). Noteworthy, $bla_{\text{NDM-5}}$ gene has been found to be co-carried with *mcr-1* in *Escherichia coli* from Spain (24) and *K. pneumoniae* from China (25). Moreover, isolates co-production of NDM-5 and OXA-181 enzyme have also been identified in *Escherichia coli* from Egypt (26) and *K. pneumoniae* from Singapore (27). Thus, the prevalence of $bla_{\text{NDM-5}}$ among *K. pneumoniae* in this study increases the awareness and urgency to implement surveillance on these strains to avoid the outbreaks, especially in the neonatal ICU.

It has been demonstrated that the production of ESBLs/AmpC β-lactamases in combination with porin loss and overexpression of efflux pumps contribute to carbapenem resistance. This might have happened in 2 of our isolates which co-carried bla_{SHV} and bla_{CTX} genes, although no carbapenemase encoding genes were detected. Of note, the $bla_{\text{CTX-M-65}}$ gene was the dominant ESBLs gene in our study, which is inconsistent with other regions of China, where $bla_{\text{CTX-M-15}}$ or $bla_{\text{CTX-M-14}}$ genes were the predominant types of $bla_{\text{CTX-M}}$ variant (28,29). Although there is a low prevalence of $bla_{\text{CTX-M-65}}$ gene in China, outbreak of infection caused by CTX-M-65-producing strains has been reported (9). Moreover, the prevalence of $bla_{\text{CTX-M-15}}$ gene (18.2%) herein is higher than other regions of China (10,28), which is similar to those in Europe and America (30), indicating the rapid dissemination of $bla_{\text{CTX-M-15}}$. The $bla_{\text{CTX-M-15}}$ gene was frequently found to be associated with outbreaks caused by multidrug resistant *K. pneumonia* worldwide (31,32). Meanwhile, high prevalence of CRKP-ST11 isolates co-carrying $bla_{\text{KPC-2}}$, $bla_{\text{SHV-12}}$, $bla_{\text{TEM-1}}$, $bla_{\text{CTX-M-65}}$ and/or $bla_{\text{DHA-1}}$ in our hospital was in line with previous studies (33), which may be mainly attributed to spread of elements such as plasmids or clonal dissemination of such strains. Altogether, multiple resistance determinants among CRKP isolates suggested that rapid spread of mobile genetic elements such as plasmids or transposons may play a key role in the resistant determinants under the selective pressure produced by the widely used antimicrobial agents in clinical therapy.

Furthermore, class I integron is the most common and widespread between different genera (34). However, this study found a quite lower prevalence of Class I integron in CRKP than that found in ESBL-producing *Enterobacteriaceae* in China (35). Existence of atypical integrons and regional differences may explain the imbalance of this phenomenon. A novel cassette arrays of integron, *aac(6')-II*-$bla_{\text{CARB/PSE-1}}$, as far as we know, has not been identified.

Different from the high prevalence of ST258 in America, ST11 is the dominant sequence type in China which spreads rapidly around the healthcare settings (36). The ST11 clone is a single-locus variant (*tonB*) from ST258, which has been identified worldwide, especially in Asian regions such as Singapore, Korea, and Japan. Andrade *et al.* (11) reported that ST11 clone exhibited multidrug resistance phenotype with high prevalence of virulence factors favoring the colonization, biofilm formation, and defense against phagocytosis, which can explain the persistence and clonal spread successfully worldwide. Up to date, the outbreaks caused by ST11 have been reported in China and abroad, with cabapenemase coding gene being central to its rapid dissemination, especially $bla_{\text{KPC-2}}$ and bla_{NDM} (37). In our study, clonal dissemination of KPC-2-producing CRKP ST11 isolates were identified in multiple departments, mostly in the department of neurology and ICU, which is in line with previous studies (38). Evidence that neurology and ICU are the main departments where CRKP isolates spread rapidly, which may result from medical equipment used for invasive therapy, hence, high-risk wards such as neurology and ICU might be the focus of active surveillance.

Notably, this is the first report on clonal dissemination of KPC-2-producing CRKP ST48 clone. Albeit the CRKP ST48 clone has been identified in Korea and Thailand (39,40) and some of them exhibited tigecycline resistance (41), the clonal dissemination of ST48 CRKP isolates have never been reported. Furthermore, the clonal dissemination of such strains demonstrated that ST48 is a potential high-risk clone that needs close attention. Noteworthy, a minor clonal dissemination of NDM-5-producing ST337 clone isolates were also identified in neonatal ICU ward in our study, so far, this is the first report, indicating rapid evolution of bla_{NDM} gene and CRKP isolates.

Some limitations of this study exist. Firstly, active surveillance was not performed during the study period, otherwise the isolation rate of CRKP could be higher than we found and the sample size of study could be expanded to further realize the clinical characteristics. Moreover, fecal samples from healthy carrier were not included in our study,

which could provide an extensive description of clonal dissemination of such strains.

Conclusions

In summary, the clonal dissemination of KPC-2-producing CRKP ST11 clone was identified in multiple departments with neurology ICU being the most common, indicating extensive cross-transmission of CRKP isolates among high-risk departments in our hospital. This first report on the clonal dissemination of KPC-2 producing CRKP ST48 clone and a minor clonal dissemination of NDM-5-producing ST337 clone isolates hints at the potential occurrence of outbreak caused by such strains. Due to limited selective clinical treatment for infections caused by these strains in our hospital, active surveillance and implementation of infection control measures are therefore urgently needed.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The informed consent was granted by all patients and this study protocols were approved by the Ethics Committee of Affiliated Hospital of Xuzhou Medical University (XYFY2019-KL112-03).

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