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Dissemination of *Klebsiella pneumoniae* ST11 isolates with carbapenem resistance in integrated and emergency intensive care units in a Chinese tertiary hospital

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Abstract

Purpose. The aim of the present study was to investigate the dissemination of carbapenem-resistant *Klebsiella pneumoniae* (CRKP) isolates in integrated intensive care units (IICUs) and emergency ICUs (EICUs) for controlling the spread of CRKP in different ICUs of the hospital.

Methodology. From January 2016 to April 2017, a total of 46 non-duplicate CRKP isolates were consecutively isolated from a tertiary hospital. The production of carbapenemases was determined by the modified carbapenem inactivation method (mCIM) test. The resistance and virulence-associated genes were detected by PCR and DNA sequencing. A hypermucoviscosity phenotype was identified by the string test. Bacterial clonal relatedness of the CRKP isolates tested was determined by multi-locus sequence typing (MLST) and PFGE.

Results. All CRKP isolates showed multiple drug resistance. All CRKP isolates harboured bla_{KPC-2}-encoding carbapenemase and at least one of the other β-lactamase genes tested, with positive rates of 89.1 % (41/46) for $bla_{CTX-M-65}$. *qnrS* was found among 76.1 % (35/46) of the CRKP isolates. A hypermucoviscosity phenotype was found in only two (4.3 %, 2/46) CRKP isolates. The virulence-associated genes with positive rates of more than 90 % among the 46 isolates tested included *wabG* (100 %, 46/46), *ycf* (100 %, 46/46), *ureA* (95.6 %, 44/46) and *fim H* (95.6 %, 44/46). MLST results showed that 46 CRKP isolates belonged to ST11 (95.6 %, 44/46) and ST86 (4.4 %, 2/46). PFGE patterns showed four clusters.

Conclusion. The CRKP ST11 clone with co-production of CTX-M-65 and KPC-2 disseminated in ICUs of this tertiary teaching hospital in central China. The emergence of CRKP with a hypermucoviscosity phenotype in ICUs should be of particular concern.

INTRODUCTION

Klebsiella pneumoniae can cause numerous infections in hospitals, long-term care facilities and communities worldwide [1]. Carbapenems including imipenem, meropenem, ertapenem and doripenem are effective agents for the treatment of severe infections caused by *K. pneumoniae*, especially for extended-spectrum β-lactamase (ESBL)-producing strains. However, carbapenem-resistant *K. pneumoniae* (CRKP) strains have rapidly increased in the last decade [2]. The emergence of CRKP has become a threat to public health. The mechanisms of carbapenem resistance typically include β-lactamase activity combined with structural mutations and production of carbapenemases that hydrolyse carbapenem antibiotics [3, 4]. Carbapenemases can be divided into metallo-carbapenemases (class B) and non-metallo-carbapenemases (classes A and D), according to their dependency on divalent cations for enzyme activation [5]. *K. pneumoniae* carbapenemases (KPCs) are the most common transmissible

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Keywords: *Klebsiella pneumoniae*; carbapenem resistance; ST11; KPC-2; Dissemination.

Abbreviations: CRKP, carbapenem resistance *Klebsiella pneumoniae*; ESBLs, extended-spectrum beta-lactamases; KPC-2, *K.pneumoniae* carbapenemase; mCIM, modified carbapenem inactivation method; MIC, minimal inhibitory concentration; MLST, multilocus sequence typing; NDM-1, New Delhi metallo-β-lactamase-1 enzymes; PFGE, pulsed-feld gel electrophoresis; ST, sequencing type.

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class A enzymes that circulate in CRKP isolates worldwide [1]. KPC-producing strains have a low-to-high level of carbapenem resistance, with MICs for carbapenems ranging from susceptible to >16 μg ml^{-1} [6].

The global spread of KPC-producing *K. pneumoniae* is primarily due to clonal expansion of clonal group CG 258 (CG258), more specifically to sequence type (ST) 258 [7–9]. However, ST11, which is closely related to ST258, is the dominant clone responsible for the spread of KPC-producing *K. pneumoniae* in China [10–12]. Our previous study reported that an outbreak was caused by ventilator-associated ST11 *K. pneumoniae* with co-production of CTX-M-24 and KPC-2 in a surgical intensive care unit (ICU) of a tertiary-care teaching hospital in Central China [11]. Hypermucoviscous *K. pneumoniae* (HMKP) infection is now becoming a global public health problem [13]. Recently, we also reported an outbreak by HMKP ST11 isolates with carbapenem resistance in China [13]. Infection with hypermucoviscous CRKP will present severe challenges in clinical treatment of infections. Therefore, outbreaks caused by CRKP are of concern. Herein, we investigated the dissemination of this important pathogen in the integrated ICU (IICU) and emergency ICU (EICU) of a Chinese tertiary hospital in Nanchang, Central China.

METHODS

Collection and identification of *K. pneumoniae* clinical isolates

From January 2016 to April 2017, a total of 46 non-duplicate CRKP isolates were consecutively isolated from various specimens of patients admitted to the IICU and EICU of the Second Affiliated Hospital of Nanchang University, Nanchang, Central China. The *K. pneumoniae* isolates were identified using a VITEK-2 compact automated microbiology analyser system (BioMérieux, Marcy l'Etoile, France). All relevant clinical information of patients was obtained from their medical records, and the study was approved by the ethics committee of the hospital.

Antimicrobial susceptibility testing

The VITEK-2 compact automated microbiology analyser platform was used to test the susceptibility of CRKP isolates to the following antimicrobial agents: aztreonam, cefazolin, cefoxitin, ceftriaxone, cefepime, amoxicillin/clavulanic acid, piperacillin/tazobactam, imipenem, ciprofloxacin, levofloxacin, tigecycline, gentamycin, tobramycin, amikacin, trimethoprim/sulfamethoxazole and nitrofurantoin, in accordance with the manufacturer's instructions. Then, multidrug resistance profiles were further evaluated by the disk diffusion test using commercial disks according to Clinical and Laboratory Standards Institute (CLSI) [14]. *Escherichia coli* ATCC25922 was used as the control strain for antimicrobial susceptibility testing.

Detection of carbapenemases and resistance genes

The production of carbapenemases was determined by the modified carbapenem inactivation method test (mCIM) recommended by the CLSI [14]. The genes encoding carbapenemases and ESBLs were detected as described previously [15–17].

Phenotypical identification of HMKP isolates

The CRKP isolates with the hypermucoviscosity phenotype were identified by the string test described previously [18]. The isolates positive for the string test were identified as HMKP.

Capsular serotyping and detection of virulence-associated genes

Capsular serotypes (K1, K2, K5, K20, K54 and K57) were detected by the methods described previously [19, 20]. Seventeen virulence-associated genes including *kfuBC*, *ybtS*, *ureA*, *uge*, *rmpA*, *wcaG*, *alls*, *wabG*, *ycf*, *entB*, *iutA*, *vatD*, *magA*, *aerobactin*, *iroN*, *fimH* and *mrkD* were detected by PCR with primers described previously among these carbapenem-resistant isolates [19, 21–23]. The *K. pneumoniae* isolates with tested virulence-associated genes determined by PCR and DNA sequencing previously in our laboratory were selected as positive control strains for the PCR assays.

Multilocus sequence typing (MLST)

The DNA sequences of seven housekeeping genes including *gapA*, *infB*, *mdh*, *pgi*, *phoE*, *rpoB* and *tonB* of *K. pneumoniae* isolates were amplified by PCR and sequenced by DNA sequencing for MLST [24]. STs were identified by the online database on the Pasteur Institute MLST website http://bigsdb.pasteur.fr/klebsiella/klebsiella.html.

PFGE

All CRKP isolates were typed by PFGE (Bio-Rad CHEF III system) with switch times of 6 and 36 s at 6 V cm^{-1}, using *XbaI* digestion for 4 h at 37 °C and electrophoresis for 20 h at 14 h, at a 120° angle. *Salmonella* serotype *Braenderup* strain H9812 was used as the molecular marker, and DNA bands were stained with ethidium bromide (0.5 μg ml^{-1}) prior to their identification through photography under ultraviolet (UV) light. Analysis of DNA band patterns was performed with Bionumerics software (Applied Maths, Sint-Martens-Latem, Belgium) using the Dice Similarity coefficient, with more than 80 % similarity defined as the same PFGE cluster.

RESULTS AND DISCUSSION

Records of the patients infected by CRKP

A total of 738 cases of *K. pneumoniae* were isolated from various specimens of patients admitted to the Second Affiliated Hospital of Nanchang University from January 2016 to April 2017, with CRKP prevalence of 27 %. Among CRKP isolates, 25 and 21 were isolated from the patients in the IICU and EICU. The records of the patients infected by CRKP from two ICUs were listed in Table 1. Forty-six CRKP isolates from two ICUs were obtained from specimens including sputum (16), blood (13), urine (7), drainage fluid (2), ascites (2),

Table 1. Clinical characteristics of 46 patients with CRKP infections

Bacterial strain	Gender/age (years)	Underlying conditions	Invasive procedures	Antimicrobial Treatment	Outcome
11	M/76	COPD	Yes	MEM, BIA, TZP	Discontinued treatment
12	M/53	ARDS	Yes	IMP, TZP	Discontinued treatment
22	F/85	Shock	Yes	IMP, TZP, MXF	Death
24	M/43	Spinal cord injury	Yes	LEV, TZP, SXT	Death
27	F/42	Acute left heart failure	Yes	IMP, BIA, AMK	Discontinued treatment
30	M/59	Diabetes; hypertension; auricular fibrillation	Yes	IMP, LEV, CSL, AMK	Death
39	F/48	Ovarian cancer coma	Yes	IMP, TZP	Death
45	M/45	Diabetes; acute pancreatitis; choledocholithiasis	Yes	BIA, IMP	Death
53	M/82	Severe pneumonia	Yes	MEM, LEV, AMK, TGC	Discontinued treatment
68	M/40	Severe pneumonia	Yes	IMP, BIA	Death
74	F/67	Poisoning with hypnotic	Yes	MXF, CTF	Discontinued treatment
77	F/87	Cerebral infarction; hypertension	Yes	TZP, CSL	Discontinued treatment
82	M/59	Hematencephalon; hypertension	Yes	MEM, TGC, CZO	Survived
84	M/73	Hypophysoma; coma	Yes	TZP, AZM	Survived
97	M/53	Head injury	Yes	BIA	Death
110	M/68	Auricular fibrillation	Yes	MEM, LEV, TGC	Death
112	M/18	Asystole	Yes	MEM	Survived
120	F/48	Diabetes; hypertension; cerebral infarction	Yes	MEM, TGC	Discontinued treatment
137	M/51	Head injury	Yes	MEM, CRO	discontinued treatment
145	M/84	Diabetes; hypertension; pulmonary infection	Yes	IMP, MXF, TGC	Death
156	M/89	Hypertension; urinemia	Yes	BIA, CSL, TGC	Death
157	M/73	Hypertension; cerebral contusion	Yes	MEM, TZP, CSL, AMK	Discontinued treatment
164	F/19	Encephaledema	Yes	MEM, IMP, SXT, TZP	Death
172	F/71	Diabetes; hypertension; head injury	Yes	MXF	Discontinued treatment
198	M/53	Severe acute pancreatitis	Yes	TZP, TGC, AMK, LEV	Survived
224	F/74	Intracranial infection	Yes	IMP, CRO, FOX, CSL	Survived
235	M/39	Multisite trauma	Yes	IMP, MXF, TZP	Discontinued treatment
237	M/39	Trauma	Yes	MXF, CIP, CSL	Discontinued treatment
279	M/75	Hypertension; COPD	Yes	IMP, MXF, TZP	Survived
283	M/94	Respiratory failure	Yes	BIA, IMP, TGC, CSL	Survived

Continued

Table 1. Continued

Bacterial strain	Gender/age (years)	Underlying conditions	Invasive procedures	Antimicrobial Treatment	Outcome
286	M/18	Lienal rupture	Yes	IMP, MXF, TZP	Death
313	M/40	Severe acute pancreatitis	Yes	IMP, MXF, TZP	Discontinued treatment
315	F/68	Hydronephrosis	Yes	IMP, TZP	Survived
346	M/54	Diabetes; hypertension; respiratory failure	Yes	MEM	Discontinued treatment
394	F/63	Encephalorrhagia	Yes	MEM, LEV, FOX	Death
395	M/75	Pulmonary infection	Yes	IMP, TGC, AMK	Discontinued treatment
399	M/28	Metabolic encephalopathy	Yes	BIA, CSL	Discontinued treatment
400	F/56	upper gastrointestinal hemorrhage	Yes	BIA, IMP	Survived
414	M/92	Choledocholithiasis	Yes	BIA, TZP, CSL	Survived
420	M/40	Encephalorrhagia	Yes	CRO, SXT	Death
437	M/27	Severe acute pancreatitis	Yes	IMP, CSL, TZP	discontinued treatment
444	F/54	Cardiac insufficiency	Yes	FOX	Survived
446	M/70	Ruptured abdominal aortic aneurysm	Yes	IMP, FOX, TZP, CSL	Discontinued treatment
460	M/62	Pancreatic fistula	Yes	TZP	Discontinued treatment
464	M/80	Hypertension; cancer of biliary duct	Yes	IMP, TZP, CTF	Death
465	F/67	Respiratory failure	Yes	MXF, AMK, CSL	Survived

AMK, amikacin; ARDS, acute respiratory distress syndrome; AZM, azithromycin; BIA, biapenem; CIP, ciprofloxacin; COPD, chronic obstructive pulmonary disease; CRO, ceftriaxone; CSL, cefoperazone sulbactam; CTF, cefotiam; CZO, cefazolin; FOX, cefoxitin; IMP, imipenem; LEV, levofloxacin; MEM, meropenem; MXF, moxifloxacin; SXT, sulfamethoxazole; TGC, tigecycline; TZP, piperacillin-tazobactam.

central venous catheter (2), wound secretion (2) and throat swab (2). The first isolate from every patient was selected for further investigation. The patients were aged from 18 to 94 years (median age of 53.7 years) and underwent multiple invasive procedures such as mechanical ventilation (46), central venous catheterization (44) and drainage (46). Moreover, all patients received treatment with multiple antimicrobial agents during the course of hospitalization. Among the 46 patients with CRKP infections, 15 died, 19 declined further treatment and 12 survived.

Antimicrobial susceptibility

The isolates with resistance to at least three types of antibacterial drugs were defined as multidrug-resistant isolates, while the isolates with resistance to many commonly used antibacterial drugs except only one or two kinds of antibacterial drugs were defined as pandrug-resistant isolates [14]. All CRKP isolates showed multiple drug resistance (100.0 %, 46/46) and even extensive drug resistance (39.1 %, 18/46) to the antimicrobials tested. More than 95 % of the CRKPs isolated were resistant to 11 tested antimicrobials including aztreonam, cefazolin, cefoxitin, ceftriaxone, cefepime, amoxicillin/clavulanic acid, piperacillin/tazobactam, imipenem, ciprofloxacin, levofloxacin and nitrofurantoin, with 100 % resistance to aztreonam, cefazolin, cefoxitin, imipenem, amoxicillin/clavulanic acid, piperacillin/tazobactam and nitrofurantoin (Table 2). The resistance pattern between the CRKP isolates from the EICU and IICU were also similar.

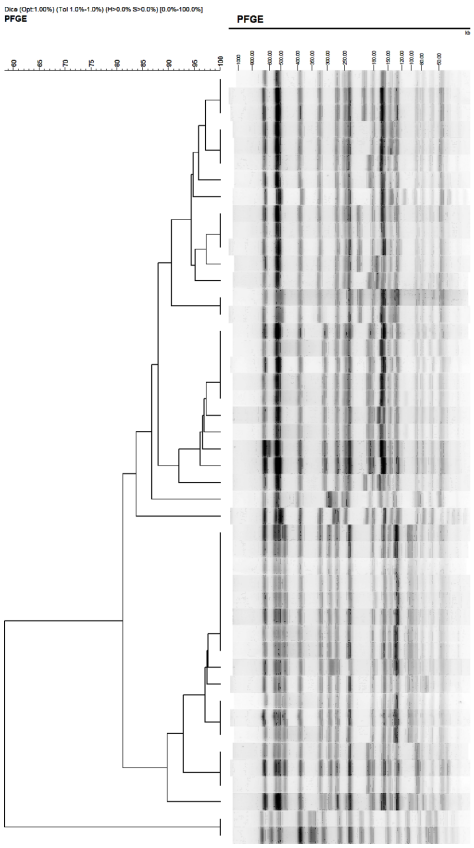
Detection of β-lactamases and resistance genes

All CRKP isolates produced carbapenemases. $bla_{\text{KPC-2}}$ gene encoding carbapenemase was identified among all carbapenemase-producing isolates (Fig. 1). Although the predominant genotype of carbapenemase genes among *K. pneumoniae* varies geographically, $bla_{\text{KPC-2}}$ was the predominant carbapenemase gene among CRKP isolates in China [25, 26]. The results of the present study corroborated this phenomenon. Our previous investigation found that 54.9 % (28/51) of carbapenem-resistant *Enterobacteriaceae* isolates from five teaching hospitals in Central China harboured

Table 2. Antimicrobial resistance rates of 46 CRKP isolates

Antimicrobials	No.	%
Aztreonam	46	100
Cefazolin	46	100
Cefoxitin	46	100
Ceftriaxone	46	100
Cefepime	46	100
Amoxicillin/clavulanic acid	46	100
Piperacillin/tazobactam	46	100
Imipenem	46	100
Ciprofloxacin	44	95.7
Levofloxacin	44	95.7
Macrodantin	44	95.7
Gentamycin	35	76.1
Tobramycin	35	76.1
Amikacin	35	76.1
Sulfamethoxazole	41	89.3
Tigecycline	20	43.5

bla_{KPC-2} [12]. The spread of KPC-2-producing *K. pneumoniae* varies geographically [1]. Other than in China, the endemic spread of KPC-2-producing *K. pneumoniae* has been reported in many countries including the USA, Greece, Israel, Italy, Poland, Brazil, Argentina and Colombia [1]. The coexistence of KPC-2 and other carbapenemases in *K. pneumoniae* was frequently identified worldwide, which poses a challenge for infection control [1, 27–31]. In the present study, all isolates were negative for other carbapenemase genes other than bla_{KPC-2}, such as bla_{NDM}, bla_{VIM}, bla_{IMP} and bla_{GES}. Coexistence of carbapenemases with other β-lactamases leads to resistance to nearly all clinically available β-lactams. In the present study, all CRKP isolates carrying bla_{KPC-2} also harboured at least one of the other β-lactamase genes tested, with positive rates of 89.1 % (41/46) for $bla_{CTX-M-65}$, 4.3 % (2/46) for $bla_{CTX-M-3}$, 2.2 % (1/46) for $bla_{CTX-M-90}$, 47.8 % (22/46) for bla_{SHV-11}, 36.9 % (17/46) for bla_{SHV-12}, 4.3 % (2/46) for bla_{SHV-28} and 41.3 % (19/46) for bla_{TEM-1} (Fig. 1). Overall, 95.6 % (44/46) of the tested isolates were found to harbour both bla_{CTX-M} and bla_{KPC-2}, with 84.8 % (39/46) having coexistence of bla_{CTX-M}, bla_{SHV} and bla_{KPC-2} (Fig. 1). Other than β-lactamase genes, *qnrS* was found among 76.1 % (35/46) of the CRKP isolates. In particular, ten isolates with bla_{KPC-2} were positive for three β-lactamase genes including bla_{CTX-M}, bla_{SHV}, bla_{TEM} and one quinolone resistance gene, *qnrS* (Fig. 1). The positive rates



ID	specimens	ward	ST	KPC	CTX-M	TEM	SHV	qnr
399	sputum	Emergency ICU	11	KPC-2	CTX-M-65	TEM-1	SHV-11	qnrS
420	sputum	Emergency ICU	11	KPC-2	CTX-M-65	TEM-1	SHV-11	qnrS
444	sputum	Emergency ICU	11	KPC-2	CTX-M-65	TEM-1	SHV-11	qnrS
235	sputum	Emergency ICU	11	KPC-2	CTX-M-65	TEM-1	SHV-11	qnrS
313	ascites	Integrated ICU	11	KPC-2	CTX-M-65	TEM-1	SHV-11	-
84	sputum	Integrated ICU	11	KPC-2	CTX-M-65	TEM-1	SHV-11	-
346	sputum	Emergency ICU	11	KPC-2	CTX-M-65	-	SHV-11	qnrS
465	sputum	Emergency ICU	11	KPC-2	CTX-M-65	TEM-1	SHV-11	-
237	blood	Emergency ICU	11	KPC-2	CTX-M-65	-	SHV-11	qnrS
279	throat swab	Emergency ICU	11	KPC-2	CTX-M-65	TEM-1	SHV-11	qnrS
414	drainage fluid	Integrated ICU	11	KPC-2	CTX-M-65	-	-	qnrS
315	blood	Integrated ICU	11	KPC-2	CTX-M-65	-	SHV-11	qnrS
157	CVP catheter	Emergency ICU	11	KPC-2	CTX-M-65	-	SHV-12	qnrS
11	blood	Integrated ICU	11	KPC-2	CTX-M-65	TEM-1	-	qnrS
437	drainage fluid	Integrated ICU	11	KPC-2	CTX-M-90	TEM-1	SHV-11	qnrS
12	blood	Integrated ICU	11	KPC-2	CTX-M-65	TEM-1	-	-
30	sputum	Integrated ICU	11	KPC-2	CTX-M-65	TEM-1	SHV-11	-
394	blood	Emergency ICU	11	KPC-2	CTX-M-65	TEM-1	SHV-11	qnrS
45	fester	Integrated ICU	11	KPC-2	-	TEM-1	SHV-11	-
74	CVP catheter	Emergency ICU	11	KPC-2	CTX-M-65	-	SHV-11	-
120	sputum	Emergency ICU	11	KPC-2	CTX-M-65	-	SHV-11	qnrS
53	sputum	Emergency ICU	11	KPC-2	CTX-M-65	-	SHV-11	-
77	blood	Emergency ICU	11	KPC-2	CTX-M-65	TEM-1	SHV-11	-
27	blood	Integrated ICU	11	KPC-2	CTX-M-65	TEM-1	-	-
283	urine	Integrated ICU	11	KPC-2	CTX-M-3	-	SHV-11	qnrS
22	urine	Integrated ICU	11	KPC-2	CTX-M-65	-	SHV-11	qnrS
460	blood	Integrated ICU	11	KPC-2	CTX-M-65	-	SHV-12	-
137	urine	Emergency ICU	11	KPC-2	CTX-M-65	-	SHV-12	qnrS
156	blood	Integrated ICU	11	KPC-2	CTX-M-65	-	SHV-12	qnrS
164	throat swab	Integrated ICU	11	KPC-2	CTX-M-65	-	SHV-12	qnrS
172	urine	Integrated ICU	11	KPC-2	CTX-M-65	-	SHV-12	qnrS
198	blood	Integrated ICU	11	KPC-2	CTX-M-65	-	SHV-12	qnrS
286	ascites	Emergency ICU	11	KPC-2	CTX-M-65	-	SHV-11	qnrS
68	sputum	Emergency ICU	11	KPC-2	CTX-M-65	-	SHV-12	qnrS
82	sputum	Emergency ICU	11	KPC-2	CTX-M-65	-	-	qnrS
224	sputum	Integrated ICU	11	KPC-2	CTX-M-65	-	SHV-12	qnrS
395	blood	Integrated ICU	11	KPC-2	-	-	SHV-12	qnrS
110	sputum	Emergency ICU	11	KPC-2	CTX-M-65	-	SHV-12	qnrS
400	blood	Emergency ICU	11	KPC-2	CTX-M-65	TEM-1	SHV-12	qnrS
97	fester	Integrated ICU	11	KPC-2	CTX-M-65	-	SHV-12	qnrS
145	urine	Integrated ICU	11	KPC-2	CTX-M-65	-	SHV-12	qnrS
446	blood	Integrated ICU	11	KPC-2	CTX-M-65	-	SHV-12	qnrS
464	urine	Integrated ICU	11	KPC-2	CTX-M-65	TEM-1	SHV-12	qnrS
112	sputum	Emergency ICU	11	KPC-2	CTX-M-65	TEM-1	SHV-12	qnrS
24	urine	Integrated ICU	86	KPC-2	CTX-M-3	-	SHV-28	qnrS
39	sputum	Integrated ICU	86	KPC-2	CTX-M-3	-	SHV-28	qnrS

Fig. 1. PFGE results for 46 CRKP isolates

Table 3. Positive rates of virulence gene rates of 46 CRKP isolates

Genes	No.	%
wabG	46	100
ureA	46	100
entB	45	97.8
fimH	44	95.7
ybtA	44	95.7
ycf	43	93.5
uge	20	43.5
kfuB	14	30.4
rmpA	8	17.4
wcaG	0	0
mrkD	0	0
iroN	0	0
magA	0	0
alls	0	0
vatD	0	0
aerobactin	0	0

of resistance genes (bla_{KPC-2}, bla_{CTX-M}, bla_{SHV}, bla_{TEM} and *qnrS*) among CRKP isolates from the IICU and EICU were similar (P>0.05) (data not shown).

Prevalence and capsular serotyping of CRKP isolates

Among the 46 CRKP isolates, only two (4.3 %, 2/46) CRKP isolates from blood were positive for the string test and were identified as HMKP. Zhan *et al.* reported that 21 (15.0 %) of 140 CRKP isolates from Wenzhou, Eastern China, were determined as HMKP by the string test [13]. A previous report from China suggested that the prevalence of HMKP among CRKP isolates was 17.9 % (5/28) [32]. The prevalence of HMKP among CRKP isolates in the present study was lower than the reports mentioned above [13, 32]. However, the HMKP phenotype was not found among CRKP isolates in many previous reports [33–35]. All CRKP isolates were not typed successfully by capsular serotyping used in the present study and were defined as K-nontypable. Altogether, 61.9 % of HMKP with carbapenem resistance were not also successfully typed by capsular serotyping in the previous report [13].

Prevalence of virulence-associated genes among CRKP isolates

The positive rates of virulence-associated genes among CRKP isolates were shown in Table 3. The virulence-associated genes with more than 90 % of positive rates among 46 isolates tested included *wabG* (100 %, 46/46), *ycf* (100 %, 46/46), *ureA* (95.6 %, 44/46) and *fimH* (95.6 %, 44/46) (Table 3). These genes are closely related to the cell adhesion ability and iron uptake ability of *K. pneumoniae*. The positive rates of *uge, kfuBC*, *rmpA* and *entB* were 43.5 % (20/46), 30.4 % (14/46), 17.4 % (8/46) and 2.2 % (1/46), respectively (Table 3). *kfuBC* is also an important iron uptake system for *K. pneumoniae*, which is related to liver abscess and endophthalmitis caused by *K. pneumoniae*. The remaining virulence-associated genes tested including *magA*, *mrkD, iroN*, *alls*, *vatD* and *aerobactin* were not detected in any of the CRKP isolates. The *mrkD* gene is mainly expressed in type III pili, which is closely related to liver abscess and endophthalmitis. The absence of *mrkD* among these CRKP isolates in the present study may result from patients without the liver abscess and endophthalmitis. A previous report showed that *rmpA* was found among all *K. pneumoniae* isolates causing pyogenic liver abscesses (PLA) [36]. Interestingly, in the present study, 17.4 % (8/46) of the CRKP isolates not associated with PLA harboured *rmpA*. *magA* was associated with the hypermucoviscosity phenotype of *K. pneumoniae* in previous studies [37–39]. *aerobactin* was used as the marker for the identification of hvKP [40]. While two CRKP isolates with the hypermucoviscosity phenotype were negative for *rmpA* and *aerobactin* in our multiple investigation.

Bacterial clonal relatedness

MLST results showed that 46 CRKP isolates belonged to ST11 (44, 95.6 %) and ST86 (2, 4.4 %) (Fig. 1). ST11 was demonstrated to be the predominant ST among KPC-2-producing *K. pneumoniae* in China [41]. Other than China, ST11 was also found to be prevalent in Brazil [42]. The dissemination of KPC-2-producing *K. pneumoniae* in China is largely caused by the spread of the ST11 clone [41]. Our previous study found an outbreak caused by ventilator-associated KPC-2 *K. pneumoniae* ST11 isolates producing KPC-2 carbapenemase in a surgical ICU of a tertiary teaching hospital in Central China [11]. In the present study, we found that the ST11 clone with carbapenem resistance disseminated in the EICU and IICU. PFGE patterns showed four PFGE clusters (Fig. 1). PFGE results showed that 44 ST11 isolates belonged to different PFGE clusters, with clusters A, B, and C accounting for 15, 10 and 16 isolates. Cluster A, B and C isolates were found both in the IICU and EICU, indicating these clones disseminated in these two ICUs. Two CRKP isolates with hypermucoviscosity phenotype belonged to ST11. Zhan *et al.* reported a hospital outbreak and dissemination of ST11 HMKP with carbapenem resistance caused by KPC-2 [13]. Two ST86 isolates belonged to a specific PFGE cluster (D) different from the PFGE cluster representing the ST11 isolates, indicating that the ST86 isolates were not clonally related to the ST11 isolates.

Conclusion

Our data indicated that the dissemination of ST11 CRKP with co-production of CTX-M-65 and KPC-2 in the integrated and emergency ICUs of this tertiary teaching hospital in Central China. It is of concern that this important clone spreads in these ICUs. Particularly, emergence of CRKP with the hypermucoviscosity phenotype in ICUs should be of concern. To

control the spread of carbapenemase-producing *K. pneumoniae* in different ICUs of the hospital, strict infection control measures including contact precautions, reinforcement of hand hygiene, disinfection of contaminated environment around patients and disinfection of equipment should be implemented.

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Author contributions
F.Y., L.H.H., Q.S.Z., Y.P.H., Y.L.L., H.D., Y.H.C., X.Y.F. and X.H.X. collected bacteria and performed the experiments. F.Y.Y. made substantial contributions to conception and design. F.Y.Y. and L.H.H. revised the manuscript critically for important intellectual content. L.H.H. and X.Y.H. participated in experimental design and data analysis. F.Y.Y. drafted the manuscript. All authors read and approved the final manuscript.

Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
The study protocol was approved by the Second Affiliated Hospital of Nanchang University ethics committee for research in health. All patients signed informed consent at the time of admission.

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