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Characterize carbapenem-resistant *Klebsiella pneumoniae* isolates for nosocomial pneumonia and their Gram-negative bacteria neighbors in the respiratory tract

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Abstract

This study was conducted to perform an epidemiological survey of carbapenem-resistant *Klebsiella pneumoniae* (CRKP) isolates for nosocomial pneumonia (NP) and visit their Gram-negative bacteria neighbors (GNNs) from the respiratory tracts. Pulsed-field gel electrophoresis and multi locus sequence typing were performed to evaluate the clonal relationships of these isolates. Statistical methods were then used to understand their possible relationship between CRKP and their GNNs. Among the 63 CRKP isolates, 84.1% produced a KPC-2 carbapenemase gene, followed by *bla*NDM-1 and *bla*IMP-38 *like*. 13 different STs and 29 clones were identified. Less heterogeneous clone backgrounds were observed in 53 KPC-2 *K. pneumoniae* isolates, and 6 STs have been found to contain KPC-2, of which the predominant ST11 harbored 47 KPC-2-producing *K. pneumoniae* isolates. Particularly, the two infrequent isolates co-possessing *bla*KPC-2 and *bla*IMP-38 *like* shared a different clonal relationship. 87 GNNs isolates of CRKP were established, the analysis present that different strains showed significant or no difference with CRKP upon antibiotics susceptibility. Conclusion, ST11 harboring KPC-2-producing *K. pneumoniae* isolates were dominant for NP, and both clonal spread and horizontal transfer contributed to the dissemination of CRKP. However, additional studies are needed to explore the biological relationship with their GNNs.

Keywords Carbapenem-resistant *Klebsiella pneumoniae* · Nosocomial pneumonia · Gram-negative bacteria · Biological relationship

Introduction

Nosocomial pneumonia (NP) is one of the most common hospital-acquired infections and is associated with considerable morbidity and mortality [1]. Gram-negative pathogens (including enterobacteriaceae and nonfermenters) which are responsible for the respiratory infections in hospital

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patients that are difficult to treat with antimicrobial therapy, particularly carbapenem-resistant Gram-negative bacteria (CR-GNB) [2]. Of greater concerns are the populations at risk of acquiring respiratory infections for carbapenem-resistant *K. pneumoniae* (CRKP) which is regarded as the key pathogen in NP [3, 4]. A 7 years retrospective study on carbapenem-resistant enterobacteriaceae infections in three tertiary care hospitals of China showed that the patients of 68.8% diagnosed with hospital-acquired bacterial pneumonia were identified for CRKP infection, of which 25% died within 28 days [5]. Qureshi et al. [6] described that 10 of 41 patients with pneumonia from hospital or health care caused by CRKP for death. Currently, the prevalence and the high mortality associated with CRKP in NP also have been confirmed in other literature [7, 8]. In addition, *K. pneumoniae* is also considered as one of the most challenging multiple drug resistance organisms, especially when they are isolated with other co-pathogens from polymicrobial respiratory [9]. Acquired carbapenemases in intra-species and inter-species facilitate their spread among bacterial pathogens. Encoding

carbapenemase genes including serine-type enzymes (KPC, OXA-48) and metallo β-lactamases (NDM, IMP, VIM) in *K. pneumoniae* have been typically reported [10].

The epidemiology of CRKP strains were described worldwidely. The major clone of ST258 *K. pneumoniae* undeniably was reported in worldwide, while the high-risk clone of *K. pneumoniae* ST11 is frequently reported as a nosocomial pathogen in numerous geographic regions, including China [11]. Nevertheless, data on the molecular characterization of these CRKP isolates collected from NP is still limited, and the inter-reaction between the CRKP and the other Gram-negative pathogens, regarding as the neighbors of CRKP isolates from the same specimen category, scarcely been reported.

Gram-negative bacteria (GNB) consist of multiple species in commensal or symbiotic coexistence, and several studies had been recorded to monitor the effects of commensal species on the growth or survival of the target species [12, 13]. With antimicrobial resistance becoming a common development of microbial, GNB are often researched as multi-drug resistance (MDR), which has been recorded for several articles [14, 15], resulting in increased mortality for the limitation of treatment. Whether the species of microbial communities are co-infection or colonization, the sources of specimens are more focused on the gastrointestinal flora [16–18], few took insight into the respiratory tract.

In this report, we sought to investigate an molecular epidemiology of CRKP isolates, targeting from NP, received at a China academic tertiary hospital from the year of 2015 to 2016. Besides, we payed a visit to the GNNs of CRKP isolates from the respiratory tract, to understand their distribution, antimicrobial resistance and the possibility of interspecies relevance with CRKP upon the antimicrobial susceptibility.

Materials and methods

Study settings and ethical statement

The study was designed as a retrospective analysis that included CRKP isolates acquired from NP in a tertiary hospital with 2800 beds. Antibiotic resistance data for relevant respiratory isolates in NP, during January 2015 to December 2016, was retrieved from electronic medical records. And the present study was approved by the Ethics Committee of the first affiliated hospital of Anhui Medical University.

Bacterial isolates in NP

Eligible isolates were included that not only showed resistance to carbapenems but also acquired from NP participants, including VAP (ventilator-associated pneumonia).

Hospital-acquired pneumonia was defined as occurring at least 48 h after admission to hospital, which based on clinical manifestation to diagnose, including a new or progressive and persistent infiltrate on chest radiographs, also at least one systemic sign and two respiratory symptoms [1, 19]. For this study not only respiratory samples such as sputum, tracheobronchial secretions and bronchoalveolar lavage were eligible for inclusion. In addition, blood samples were as well selected upon bacteremia, when the clinical manifestations of NP were also produced, but no respiratory specimens were detected.

Antibiotic susceptibility testing was performed using a Vitek 2 automated system. Resistance to imipenem and meropenem was evaluated using agar dilution or disc diffusion method and interpreted according to CLSI guidlines [20]. All CRKP isolates in NP were screened for the presence of carbapenemase genes (*bla*KPC, *bla*IMP, *bla*VIM, *bla*NDM and *bla*OXA-48) by polymerase chain reaction (PCR).

To evaluate and characterize the clonal relationships of the *K. pneumoniae* isolates, pulsed-field gel electrophoresis (PFGE) of the XbaI-digested genomic DNA was conducted using a CHEF-DRIII system (Bio-Rad, United Kingdom) and a unique PFGE profile was created for each clonal strain. Concomitantly, multi locus sequence typing (MLST) was performed for CRKP isolates according to the Institute Pasteur MLST website. And sequence types (STs) were further defined by eBURST.

Concurrent interspecies of CRKP neighbours

In this study, CRKP isolates were labeled as the host GNB in NP, furthermore, we payed a visit to their neighbours that defined as concurrent interspecies which were isolated simultaneously or successively from the same specimen source from the single patient during hospitalization and also screened in GNB including enterobacteriaceae and nonfermenters.

Sampling collection of bacterial species, specimen source, MDR, carbapenem-resistant MDR (CR-MDR) and drug susceptibility to 12 selected antibiotics were included in our study. By the way, the definition for MDR made reference to Magiorakos et al. [21]. Strains with MDR showing resistance to imipenem or/and meropenem, namely CR-MDR GNB, become a global healthcare concern. Among MDR isolates, natural resistant strains were only chose several antibiotics for drug-sensitive tests. The above materials were retrieved from electronic medical records.

Statistical analysis

Descriptive statistical data were presented in frequency and percentage. In addition, a comparison of drug susceptibility

adopted by CRKP isolates and their GNNs was conducted through independent-samples *t* test. All statistical analyses were performed using SPSS 21 software (IBM Corp., Armonk, NY).

Results

Of all the diagnosed cases of NP, a total of 63 CRKP isolates (only one strain was moderately resistance to imipenem) were isolated from 63 patients. The distribution of samples in NP was as follows: sputum samples ($n = 44$, 69.8%), blood samples ($n = 10$, 15.9%), tracheobronchial secretion samples ($n = 3$, 4.8%), bronchoalveolar lavage samples ($n = 3$, 4.8%) and the samples from VAP ($n = 3$, 4.8%). Among the 63 CRKP isolates diagnosed with NP, 25 samples had been isolated from ICU (25/63, 37.9%), 26 samples from medical wards including respiratory ward ($n = 11$, 17.5%), neurology ward ($n = 6$, 9.5%), haematology ($n = 4$, 6.3%), gastroenterology ($n = 3$, 4.8%), cardiology and oncology ($n = 1$, 1.6%, respectively). The others contained burn unit, general surgery and plastic surgery of surgical wards ($n = 3$, 4.8%) and emergency wards ($n = 9$, 14.3%).

Of these 63 CRKP strains, they showed resistant rates of 100% to ampicillin/sulbactam, ceftriaxone and meropenem, 98.4% to ceftazidime and imipenem, more than 92% to ceftazidime, aztreonam, ciprofloxacin and piperacillin/tazobactam. Amikacin, gentamicin and levofloxacin showed activities against CRKP with susceptibility rates of 76.2%, 79.4% and 88.9%, respectively. And all isolates were susceptible to Tigecycline (Table 1). Among the 63 studied isolates, 53 strains (53/63, 84.1%) produced a KPC-2 carbapenemase. The other carbapenemases genes were of *bla*NDM-1 ($n = 5$) and *bla*IMP-38 *like* ($n = 2$). Of those, one isolate co-harbored *bla*KPC-2 and *bla*NDM-1, while two isolates co-possessed *bla*KPC-2 and *bla*IMP-38 *like*. Results of screening for VIM and OXA-48 carbapenemases genes were negative. And 6 isolates were not found correlating to the aforementioned genes.

The distribution of the STs among the CRKP isolates for NP showed a similarity and diversity. 13 different STs were identified including the dominating type ST11 ($n = 48$, 76.2%), ST23 ($n = 3$, 4.8%), ST15 ($n = 2$, 3.2%), and ST17, ST35, ST36, ST307, ST340, ST414, ST528, ST661, ST1334 and ST1688 obtaining one strain respectively. Surprisingly, less heterogeneous clone backgrounds were observed in 53 KPC-2 *K. pneumoniae* isolates in this study, and 6 STs have been found to contain KPC-2, of which the predominant ST11 harbored 47 KPC-2-producing *K. pneumoniae* isolates (47/48, 97.9%), only one strain identified ST11 was excluded for negative carbapenemases gene. The distribution of STs in the isolated place was as follow: 7 different STs were identified in ICU (18 ST11,

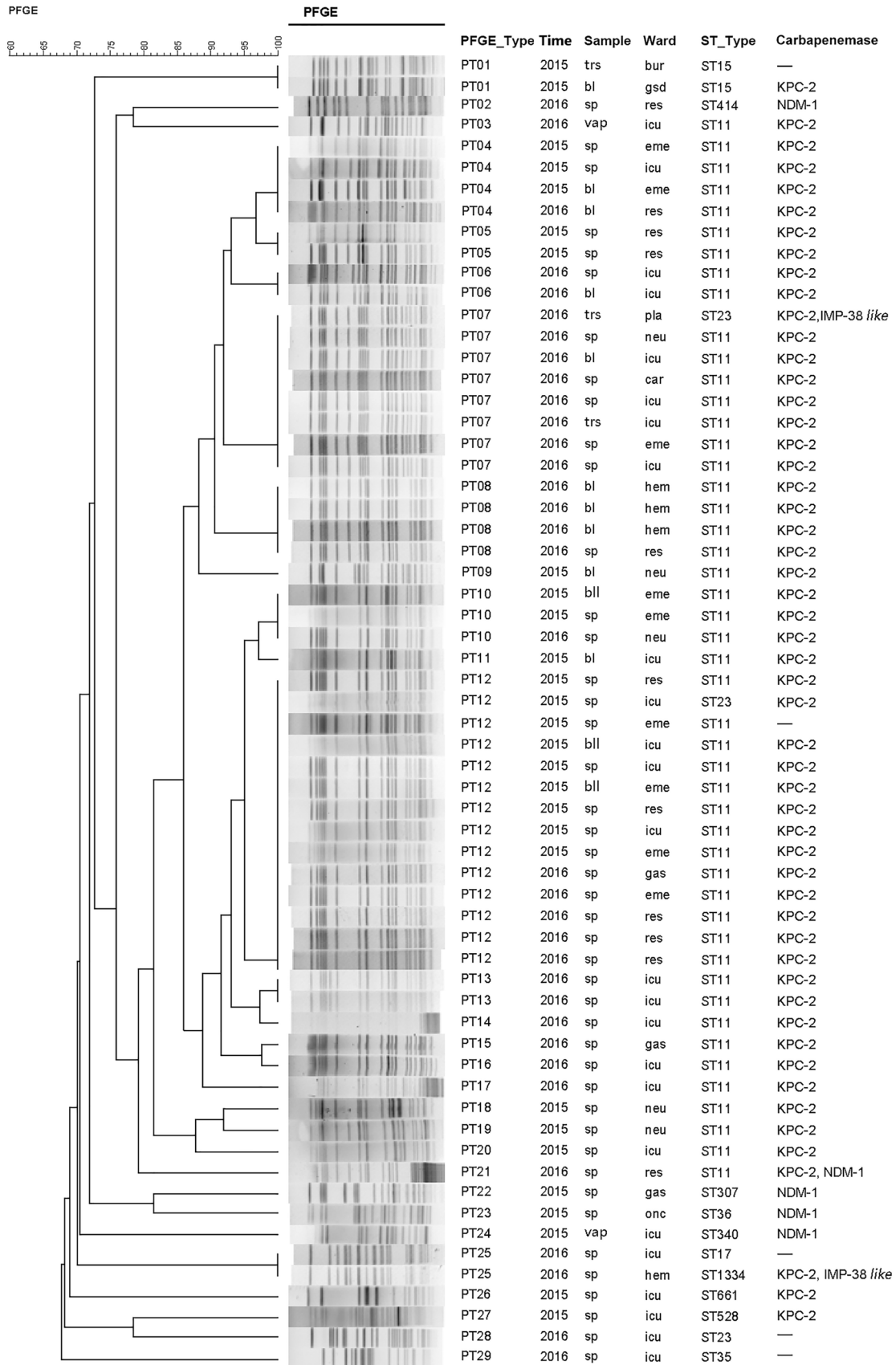**Table 1** Antibiotic susceptibilities of carbapenem-resistant *Klebsiella pneumoniae*

Antimicrobial agents	S (*n*, %)	I (*n*, %)	R (*n*, %)
Ampicillin/sulbactam (ABPC/SBT)	0	0	63 (100)
Ceftazidime (CAZ)	1 (1.6)	0	62 (98.4)
Ceftriaxone (CRO)	0	0	63 (100)
Ceftazidime (FEP)	1 (1.6)	4 (6.3)	58 (92.1)
Aztreonam (ATM)	2 (3.2)	0	61 (96.8)
Amikacin (AN)	15 (23.8)	0	48 (76.2)
Gentamicin (GEN)	13 (20.6)	0	50 (79.4)
Ciprofloxacin (CIP)	2 (3.2)	2 (3.2)	59 (93.7)
Levofloxacin (LVX)	3 (4.8)	4 (6.3)	56 (88.9)
Piperacillin/tazobactam (TZP)	1 (1.6)	1 (1.6)	61 (96.8)
Imipenem (IMP)	0	1 (1.6)	62 (98.4)
Meropenem (MEM)	0	0	54 (100)[a]
Tigecycline (TGC)	63 (100)	0	0

S susceptible, *I* intermediate, *R* resistant

[a]Only these isolates were tested for meropenem

72%; 2 ST23, 8%; 1 ST17/ST340/ST35/ST528/ST661, respectively, 4%), 6 STs in medical wards (21 ST11, 80.8%; 1 ST1334/ST1668/ST307/ST36/ST414, respectively, 3.8%), 2 STs in surgical wards (2 ST15, 66.7%; 1 ST23, 33.4%) and 1 ST in emergency wards (9 ST11, 100%) (Fig. 1).

Clonal relationship has been further analyzed using PFGE which showed a moderation upon the 2 year study. 29 clones (PFGE type, PT01–PT29) were identified out of the 62 strains, because one *K. pneumoniae* isolate showed nucleic acid degradation whose sample from VAP of neurology ward belonging to ST1688 with negative carbapenemases gene. 48 CRKP isolates from clones PT03 to PT21 corresponded to the same type of ST11, of those, the high percentage clones were named PT12 ($n = 13$), PT07 ($n = 7$), PT04 ($n = 4$), PT08 ($n = 4$) and PT10 ($n = 3$). The other 14 strains contained 10 types of PT. From the isolated 2 years, the same types of ST11 and ST23 as well as the same PT types such as PT04, PT10 and PT12 were discovered, of which the ST11 and the PT10/PT12 were all isolated from the departments the ICU, medical ward and emergency ward. Besides theses, PT07 corresponding to ST11 or ST23 only appeared in 2016, but existed in most departments of the hospital including the wards of ICU, medical, surgical and emergency. In addition, the three CRKP strains co-harbored two carbapenemase genes were all isolated in 2016. Of which, one isolate co-harboring *bla*KPC-2 and *bla*NDM-1 was determined for ST11 possessing clone PT21, and the two strains isolated co-possessing *bla*KPC-2 and *bla*IMP-38 *like* shared a different clonal relationship, one strain was associated to PT07 corresponding to ST11, while the other PT25 corresponding to ST1334 (Fig. 1).

PFGE_Type	Time	Sample	Ward	ST_Type	Carbapenemase
PT01	2015	trs	bur	ST15	—
PT01	2015	bl	gsd	ST15	KPC-2
PT02	2016	sp	res	ST414	NDM-1
PT03	2016	vap	icu	ST11	KPC-2
PT04	2015	sp	eme	ST11	KPC-2
PT04	2015	sp	icu	ST11	KPC-2
PT04	2015	bl	eme	ST11	KPC-2
PT04	2016	bl	res	ST11	KPC-2
PT05	2015	sp	res	ST11	KPC-2
PT05	2015	sp	res	ST11	KPC-2
PT06	2016	sp	icu	ST11	KPC-2
PT06	2016	bl	icu	ST11	KPC-2
PT07	2016	trs	pla	ST23	KPC-2,IMP-38 *like*
PT07	2016	sp	neu	ST11	KPC-2
PT07	2016	bl	icu	ST11	KPC-2
PT07	2016	sp	car	ST11	KPC-2
PT07	2016	trs	icu	ST11	KPC-2
PT07	2016	sp	eme	ST11	KPC-2
PT07	2016	sp	icu	ST11	KPC-2
PT08	2016	bl	hem	ST11	KPC-2
PT08	2016	bl	hem	ST11	KPC-2
PT08	2016	bl	hem	ST11	KPC-2
PT08	2016	sp	res	ST11	KPC-2
PT09	2015	bl	neu	ST11	KPC-2
PT10	2015	bll	eme	ST11	KPC-2
PT10	2015	sp	eme	ST11	KPC-2
PT10	2016	sp	neu	ST11	KPC-2
PT11	2015	bl	icu	ST11	KPC-2
PT12	2015	sp	res	ST11	KPC-2
PT12	2015	sp	icu	ST23	KPC-2
PT12	2015	sp	eme	ST11	—
PT12	2015	bll	icu	ST11	KPC-2
PT12	2015	sp	icu	ST11	KPC-2
PT12	2015	bll	eme	ST11	KPC-2
PT12	2015	sp	res	ST11	KPC-2
PT12	2015	sp	icu	ST11	KPC-2
PT12	2015	sp	eme	ST11	KPC-2
PT12	2016	sp	gas	ST11	KPC-2
PT12	2016	sp	eme	ST11	KPC-2
PT12	2016	sp	res	ST11	KPC-2
PT12	2016	sp	res	ST11	KPC-2
PT12	2016	sp	res	ST11	KPC-2
PT13	2016	sp	icu	ST11	KPC-2
PT13	2016	sp	icu	ST11	KPC-2
PT14	2016	sp	icu	ST11	KPC-2
PT15	2016	sp	gas	ST11	KPC-2
PT16	2016	sp	icu	ST11	KPC-2
PT17	2016	sp	icu	ST11	KPC-2
PT18	2015	sp	neu	ST11	KPC-2
PT19	2015	sp	neu	ST11	KPC-2
PT20	2015	sp	icu	ST11	KPC-2
PT21	2016	sp	res	ST11	KPC-2, NDM-1
PT22	2015	sp	gas	ST307	NDM-1
PT23	2015	sp	onc	ST36	NDM-1
PT24	2015	vap	icu	ST340	NDM-1
PT25	2016	sp	icu	ST17	—
PT25	2016	sp	hem	ST1334	KPC-2, IMP-38 *like*
PT26	2015	sp	icu	ST661	KPC-2
PT27	2015	sp	icu	ST528	KPC-2
PT28	2016	sp	icu	ST23	—
PT29	2016	sp	icu	ST35	—

◄**Fig. 1** The genetic relatedness of carbapenem-resistant *K. pneumoniae* clones in nosocomial pneumonia during the years 2015 and 2016. Isolates were clustered into 29 different clusters (29 PT, PFGE types). And carbapenemase gene alleles, sequence types (STs), time, ward, sample and the date of isolation were presented on the right. (*sp* sputum, *bl* blood, *trs* tracheobronchial secretion, *bll* bronchoalveolar lavage, *vap* the samples from ventilator-associated pneumonia (VAP), *icu* intensive care unit, *res* respiratory, *neu* neurology, *hem* haematology, *car* cardiology, *onc* oncology, *gas* gastroenterology, *eme* emergency, *bur* burn unit, *gsd* general surgery department, *pla* plastic surgery)

Among the 63 patients diagnosed with NP for CRKP infection, 32 patients had been screened out for concurrent interspecies which were all recovered from respiratory specimens during hospitalization. A total of 120 isolates were recovered, of which 33 were CRKP isolates as mentioned above (n = 32) for a patient owning two CRKP isolates (Table S1). And the 87 remaining isolates of GNB contained nonfermenters (n = 64) and enterobacteriaceae (n = 23). Of the 64 nonfermenters isolates, the predominant species was *Acinetobacter baumannii* (31/87, 35.6%), followed by *Pseudomonas aeruginosa* (17/87, 19.5%), *Acinetobacter* spp., (10/87, 11.5%), *Stenotrophomonas maltophilia* (3/87, 3.4%), and *Acinetobacter haemolyticus, Burkholderia cepacia* and *Flavobacterium indologenes* (1/87, 1.1%, respectively). Among enterobacteriaceae, isolated strains of *Klebsiella oxytoca* (7/87, 8%), *Escherichia coli* (5/87, 5.7%), *Enterobacter aerogenes* (3/87, 3.4%), *Enterobacter cloacae* (3/87, 3.4%), *Serratia marcescens* (3/87, 3.4%) and *Proteus mirabilis* (2/87, 2.3%) were collected from respiratory specimens in our study (Table 2).

Susceptibility testing was performed for 12 antibiotics (ceftazidime, ceftriaxone, cefepime, cefoperazone-sulbactam, aztreonam, amikacin, gentamicin, ciprofloxacin, levofloxacin, piperacillin-tazobactam, imipenem and meropenem) on these respiratory pathogens (Table S1). Excluded the 33 CRKP isolates and 5 strains of intrinsic resistance for nonfermenters, the remainder 82 strains, of which 64 isolates were considered as multidrug-resistant bacteria, species of *Acinetobacter* spp., *K. oxytoca, E. coli* and *P. mirabilis* all represented 100%, *A. baumannii* represented 96.8% (30/31), while *P. aeruginosa* showed 35.3% (6/17). Among these, 40 CR-MDR GNB isolates were recovered from the four dominant species including 2 nonfermenters isolates (*A. baumannii* and *P. aeruginosa*) and 2 enterobacteriaceae isolates (*K. oxytoca* and *E. coli*) and the predominant species was *A. baumannii* (n = 29, 29/30), followed by *K. oxytoca* (n = 6, 6/7), *P. aeruginosa* (n = 5, 5/6) and *E. coli* (n = 0, 0/5) (Table 1). Due to understand more about the relationship between concurrent interspecies, we used CRKP as a host isolate, which compared to the above four dominant species upon the results of 12 antibiotics susceptibilities. Univariate analysis showed that *P. aeruginosa* and *E. coli* were significant difference with CRKP for antibiotics susceptibility, while *A. baumannii* and *K. oxytoca* showed no difference with it (Table 3).

Discussion

NP is accepted as the most common of all healthcare-associated infections [22] and is associated with the particular morbidity and mortality. GNB are often known as the primary cause of NP, with the main pathogens isolated being *Klebsiella* spp., *P. aeruginosa, Enterobacter* spp., and *Acinetobacter* spp. [23, 24], which almost showed the commonly antimicrobial resistance. In this study, we described the molecular epidemiology of *K. pneumoniae* in NP that present the resistance to carbapenem. By the way, the distribution, MDR and the possible relationship of interspecies were carried out among GNB from the same respiratory specimen category.

The epidemiology survey on CRKP isolates associated with NP were not restricted to specific wards in this single-center study. Conversely, all patients with NP were included from ICU (37.9%), medical (17.5% respiratory ward, 9.5% neurology, 6.3% haematology, 4.8% gastroenterology, 1.6% oncology and cardiology, respectively), surgical (4.8%) and emergence department (14.3%) of this tertiary hospital, which allowed the surveillance of the data conclusion on all patients at risk of infection with NP. And obviously, ICU still play a major role in acquisition of carbapenem resistant isolates [25]. In our study, the top type of STs that accounted for CRKP in ICU was ST11 (72%), which also occupied the most important position in the wards of medical and emergency. The same type of ST11 was not only found in the different departments, but also appeared across the 2 years. Therefore, such clonal dissemination may be explained that nosocomial transmission was quite frequent in our hospital. Our medical workers should be aware of the issue and intervene the prevalence.

In this study, the most frequent carbapemases were KPC-2, followed by NDM-1 and IMP-38 *like*. And no carbapenemase VIM and OXA-48 producing *K. pneumoniae* was detected. Carbapenem resistance of six isolates was not found the aforementioned carbapemases genes that may due to the combination of AmpC or ESBL with porin defects [10]. We found these carbapemases-producing *K. pneumoniae* isolates were highly resistant to most antibiotics examined, even if amikacin and gentamicin had some susceptibility to these isolates. However, Tigecycline is remain the useful alternative in patients with serious infections caused by CR-GNB [26].

K. pneumoniae carbapenemase (KPC)-producing *K. pneumoniae* strains of the hyperepidemic clonal complex 258 (CC258) are detected worldwide as hospital-acquired

Table 2 Species distribution of Gram-negative bacteria neighbors of CRKP from the respiratory tracts

Species	Strains tested N (%)	MDR N (%)	CR-MDR N (%)
Nonfermenters	64 (73.6)	46 (71.9)	44 (95.7)
A. baumannii	31 (35.6)	30 (96.8)	29 (96.7)
P. aeruginosa	17 (19.5)	6 (35.3)	5 (83.3)
Acinetobacter spp.	10 (11.5)	10 (100)	10 (100)
S. maltophilia	3 (3.4)	0 (0)	–
A. haemolyticus	1 (1.1)	0 (0)	–
B. cepacia	1 (1.1)	0 (0)	–
F. indologenes	1 (1.1)	0 (0)	–
Enterobacteriaceae	23 (26.4)	18 (78.3)	8 (44.4)
K. oxytoca	7 (8)	7 (100)	6 (85.7)
E. coli	5 (5.7)	5 (100)	0 (0)
E. aerogenes	3 (3.4)	2 (66.7)	1 (50)
E. cloacae	3 (3.4)	1 (33.3)	0 (0)
S. marcescens	3 (3.4)	1 (33.3)	1 (100)
P. mirabilis	2 (2.3)	2 (100)	0 (0)

CRKP carbapenem-resistant *Klebsiella pneumoniae, MDR* multidrug resistance, *CR-MDR* carbapenem-resistant multidrug resistance

Table 3 Comparition between CRKP and their topic Gram-negative bacteria neighbors upon antibiotics susceptibility in our study

Microorganism	T value	95% CI	P value
A. baumannii	1.26	− 0.06 to − 0.28	0.21
P. aeruginosa	6.39	0.68 to − 1.35	< 0.001
K. oxytoca	1.36	− 0.11 to − 0.58	0.18
E. coli	4.79	0.45 to − 1.10	< 0.001

CI confidence interval

P value < 0.05 was considered statistically significant

pathogens. In our study, the frequency of KPC-2 enzymes approximated to 80% among carbapenemase producers, which was higher than the previous report [27]. While ST258 is the predominant clone observed in European countries, ST11, belonging to CC258 (closely related to ST258), is the most frequently detected KPC-producing *K. pneumoniae* clone in China [28, 29]. Surprisingly, less heterogeneous were observed in 53 KPC-2 *K. pneumoniae* isolates in our study, and 6 STs have been found to contain KPC-2, of which the predominant ST11 harbored 47 KPC-2-producing *K. pneumoniae* isolates. High identity of the epidemic KPC-2 *K. pneumoniae* ST11 lineage in our study is mainly resulted from the clone spread, the others has been discovered in different *K. pneumoniae* strains, presumably due to the Tn4401-containing plasmid capable of mobilization through transposition [30].

The carbapenemases of NDM-1 and IMP-type belonging to metallo-β-lactamases (MBLs) were frequently identified in Enterobacteriaceae [31]. NDM-1-producing *K. pneumoniae* was first detected in a patient from India and then the sporadic spread has been reported in China [32, 33]. However, NDM-1 has been detected mostly in *Acinetobacter* spp., but the previous data [32] showed a proportional NDM-1-producing CRE isolates, including *K. pneumoniae*, suggesting the possible transmission of bla_{NDM-1}-containing sequences from *Acinetobacter* spp. to Enterobacteriaceae. IMP was first found in *S. marcescens* in 1991, following IMP-producing *K. pneumoniae* have spread in Europe and Asia, including China [33, 34]. IMP-38, maybe a point mutation different from the common IMP-4, was rarely reported [35]. In our present study, IMP-38-*like* was found in two *K. pneumoniae* isolates, as well co-harbored *bla*KPC-2, which was first reported as far as we all know. Contrary to all expectations we carried of the result, the two IMP-38-*like K. pneumoniae* isolates belonged to the different clone, of which associated to ST11 and ST1334, respectively, and further been documented by PFGE.

Isolates from the different STs displayed a scientific moderation PFGE profiles, further demonstrating their clonal dissemination. However, it's not just a single inheritance, as well present mutation. 29 clones (PT01–PT29) were identified out of the 62 strains, among these CRKP clones, 48 from clones PT03 to PT21 corresponded to the same type of ST11. PT12 as the dominant PFGE type that appeared across the year of 2015 and 2016, while the other tufted bands (i.e., PT07) that only appeared in 2016 but generally existed in each department of the hospital. Our study indicated that which contributed to the dissemination of CRKP in the tertiary hospital, both clonal spread and horizontal transfer should be considered, as well that seem meet with the rule of natural development.

Not only insight into the knowledge of *K. pneumoniae*, there is a need to understand the relationship with co-pathogens or commensals, for the ongoing emergence of antimicrobial resistance. In this study, species of *K. pneumoniae* were targeted as the host strains isolating from the respiratory tract which coexisting with other gram-negative bacteria, of which *A. baumannii* was the most frequently isolated species, followed by *P. aeruginosa* in the nonfermenters isolates, and the highest in Enterobacteriaceae was *K. pneumoniae* and *E. coli*. This distribution is similar to the list of priority pathogens for NP in previous articles [23, 24]. Surprisingly, they all showed a shocking MDR, except *P. aeruginosa* showed 35.3%, the rest three species almost 100%. Additionally, MDR-*A. baumannii, -P. aeruginosa* and -*E. coli* almost all showed resistance to carbapenems, but none of the 5 MDR-*E. coli* isolates was CR-MDR strain. Except the species of *E. coli*, the pathogens of carbapenem-resistant *A. baumannii*,

carbapenem-resistant *P. aeruginosa*, carbapenem- and 3rd generation cephalosporin-resistant Enterobacteriaceae including *K. pneumoniae* were grouped into the critical priority tiers according to the release of World Health Organization in 2017 [36]. To further understand whether there is an association between the multidrug resistant strains and the host strain, we just could take a univariate analysis upon the results of 12 antibiotics susceptibilities. The results of which may show that *K. pneumoniae* was close to *A. baumannii* and *K. oxytoca*, and a significant difference from *P. aeruginosa* and *E. coli* under the antibiotic resistance. Possibly, in polymicrobial communities, the former shared the same metabolite to promote each other in the context of antibiotic resistance [37, 38], and thus commensal organisms in lung may carry the antibiotic resistance elements that could be transferred to opportunistic bacteria or respiratory pathogens [39]. Oppositely, the latter implied competition among bacterial species. Hafza et al. [40] suggested that competition between ESBL-production genes in *E. coli* and *K. pneumoniae* confer a fitness cost may provide us a new perspective. And also, the differences in the main type of carbapenemases production among resistant bacteria should be considered. The most prevalent carbapenemases production of *P. aeruginosa* was MBLs [41, 42], particularly IMP, which had significantly higher resistance to most antibiotics [41]. Due to evidence of cooperation or competition between bacteria was so few, our understanding about how relationships between different bacterial species occur still remains confusion. Therefore, plenty of studies are need to explore in the future research.

The key limitations of this study were as follow: It is a retrospective analysis that does not allow to draw profound conclusions on the relationships between CRKP isolates and their neighbours for unable to perform analyses of resistance mechanisms. In addition, we did not perform plasmid detection and conjugation analysis on CRKP isolates, particularly co-harbored *bla*KPC-2 and *bla*NDM-1 and *bla*KPC-2 and *bla*IMP-38 *like*, which would be explored in a subsequent study.

In conclusion, this is the first study on the molecular assessments of CRKP isolates with NP from the tertiary hospital of China. A significant finding from such study is that the different STs displayed a scientific moderation PFGE profiles and both clonal spread and horizontal transfer contributed to the dissemination of CRKP. ST11 harboring KPC-2-producing *K. pneumoniae* isolates were dominant for NP. Notably, as far as we all know, the two CRKP isolates co-harbored *bla*KPC-2 and *bla*IMP-38 *like* was first reported, and more unexpected both belonged to a different clone. In addition, our understanding about how relationships between the host CRKP isolates and their GNNs occur still remain obscure. Therefore, additional studies are needed to explore their biological relevance.

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