Original Article

Clinical and resistance characterization of carbapenem-resistant *Klebsiella pneumoniae* isolated from intensive care units in China

Te Xu[1], Wei Feng[2], Fengjun Sun[2], Yan Qian[1]

[1]Department of Pharmacy, The Second Affiliated Hospital of Chongqing Medical University, Chongqing, China; [2]Department of Pharmacy, Southwest Hospital, Third Military Medical University (Army Medical University), Chongqing, China

Contributions: (I) Conception and design: F Sun; (II) Administrative support: F Sun, Y Qian; (III) Provision of study materials or patients: F Sun; (IV) Collection and assembly of data: T Xu; (V) Data analysis and interpretation: T Xu, W Feng; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Fengjun Sun. Department of Pharmacy, Southwest Hospital, Third Military Medical University (Army Medical University), Chongqing 400038, China. Email: fengj_sun@163.com; Yan Qian. Department of Pharmacy, The Second Affiliated Hospital of Chongqing Medical University, Chongqing 400010, China. Email: cqqianyan@hospital.cqmu.edu.cn.

Background: Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) is a serious threat to health, and the detection rate in intensive care units (ICUs) is relatively high. We compared regional differences in the clinical and molecular characteristics of CRKP from three ICUs in different hospitals, to make a reference and contribution for infection control and clinical medication.

Methods: A total of 150 CRKP strains from Chongqing, Beijing, and Nantong, as well as the clinical data of the infected patients, were collected between 2019 and 2021. The carbapenemase phenotype was determined by CarbaNP test, and the outer membrane porin (OMP) genes (*ompK35/ompK36*), multi-locus sequence typing (MLST) and resistance genes were identified by polymerase chain reaction (PCR) amplification and sequencing.

Results: Patients infected with CRKP were mainly elderly, with comorbidity, and had undergone invasive operation and multiple antibiotic therapy. All strains exhibited high-level resistance to most antibiotics except for polymyxin B and tigecycline. Among the CRKP strains, 100 had the $bla_{\text{KPC-2}}$ gene and 8 had $bla_{\text{NDM-1}}$ gene, which were distributed in all of the hospitals. Nearly all the strains harbored extended-spectrum beta-lactamase (ESBL) genes (bla_{SHV}, $bla_{\text{CTX-M}}$, and bla_{TEM}). Class C carbapenemase genes (bla_{CIT}, bla_{DHA}), and deletion and mutation of *ompK35/ompK36* existed in some strains. ST11 was the main MLST type, followed by ST15.

Conclusions: There were a few significant differences in the molecular epidemiology and clinical characteristics, but generally the features of CRKP from the three ICUs aligned fairly well, which might have resulted from dissemination through frequent personnel exchanges between regions.

Keywords: Carbapenem-resistant *Klebsiella pneumoniae* (CRKP); clinical characteristics; regional differences; resistance mechanism

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Introduction

Klebsiella pneumoniae (*K. pneumoniae*), one of the most common gram-negative bacteria of nosocomial infections, has shown generally upward trend in resistance rate to imipenem and meropenem over the past decade, according to data from the China Antimicrobial Surveillance Network (CHINET) (https://www.chinets.com/Data/GermYear). The prevalence of carbapenem-resistant *K. pneumoniae* (CRKP) isolates increased rapidly, with the national average up to 8.7%, and CRKP represents the growing

problem of antibiotic resistance (1). The nasopharyngeal and gastrointestinal colonization rates of *K. pneumoniae* could be around 19% and 20%, respectively (2). As one of the most common opportunistic pathogens, *K. pneumoniae* colonization is considered as a starting point for further infection, and for critically ill and immunocompromised patients, especially patients from intensive care unit (ICU), such risk increases. Alarmingly, the detection rate for CRKP patients in ICUs of tertiary hospitals surged from 0% in 2013 to 75% in 2016, which was far above that for non-ICU patients (3). CRKP from the ICU can cause serious respiratory and circulatory infections with high morbidity and mortality, and consequent poorer prognosis (4). Many studies have indicated that the mortality rate of patients with CRKP infection could increase to about 40–50%, and some risk factors, including ICU stays, higher Acute Physiology and Chronic Health Evaluation (APACHE) II scores, or comorbidity with septic shock and congestive heart failure have been identified to be associated with higher mortality of CRKP infection (5,6). Current research on the mechanism of carbapenem resistance has focused on carbapenemase, extended-spectrum beta-lactamases (ESBLs) or Class C carbapenemase (also known as AmpC) genes combined with abnormal expression of outer membrane porin (OMP), efflux pump and biofilm formation (7,8). Understanding the drug resistance mechanism is urgently required for the prevention and treatment of CRKP infection.

Although CRKP has emerged globally, there are regional differences in strains. An observational study in the USA reported geographic variation in the CRKP occurrence rate, which reflected the overall emergence and dissemination (9). In China, the prevalence of CRKP ranges from 0.9% to 23.6% in different provinces (10), and thus drug resistance status could vary endemically (11). Additionally, discrepancies among regions exist in molecular epidemiology. ST258 is the major sequencing type in Europe and the USA, whereas in East Asia, ST11 is dominant. Moreover, the rate of *K. pneumoniae* carbapenemase (KPC) in carbapenemase-producing strains is lower in Spain than in Greece, and the prevalence of New Delhi metallo-β-lactamase (NDM) in India is particularly high (12). Determining the current resistance situation is essential for guiding rational use of antibiotics, and it is significant to clarify drug resistance mechanism and epidemic trend for nosocomial prevention and control.

In this study, CRKP strains isolated from ICUs in Beijing, Chongqing and Nantong were compared in terms of carbapenem resistance mechanism, sequence types (STs) and genetic relationships. The clinical features of patients infected with CRKP in these three regions were also analyzed. We present the following article in accordance with the MDAR reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-4323/rc).

Methods

CRKP strain selection

Non-repetitive CRKP strains (at least resistant to one of imipenem, meropenem and ertapenem) from the first tested sample of each ICU patient from January 2019 to December 2021 were collected in three tertiary hospitals in Chongqing, Beijing, and Nantong in the southwest, north, and southeast of China, respectively. All strains were identified by the VITEK2 system; the antimicrobial susceptibility test was conducted by a VITEK2 fully automatic bacteria identification instrument and matching drug sensitivity card. Susceptibility was defined according to the Clinical and Laboratory Standards Institute (CLSI) breakpoints, and *Escherichia coli* American Type Culture Collection (ATCC) 25922 and *Pseudomonas aeruginosa* ATCC27853 were used as quality control strains.

Collection of clinical information

The clinical data of patients infected with CRKP were collected by searching online records, including demographic profile (age, sex, length of stay), comorbid conditions [hypertension, diabetes, chronic obstructive pulmonary disease (COPD), malignant tumor, etc.], invasive procedures (tracheotomy, venous catheter, mechanical ventilation, etc.), medications and other relevant information. This clinical research was a retrospective study, and the Ethics Committee of the Second Affiliated Hospital of Chongqing Medical University waived the requirement of ethical approval and informed consent, and patients' information was processed anonymously and confidentially. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Detection of carbapenemase

Class A, B and D types of carbapenemase in the CRKP strains were detected by a modified CarbaNP method (13). Carbapenemases were extracted from bacteria by ultrasonic

disruption and high-speed centrifugation. Substrates I–IV all contained red phenol plus $ZnSO_4$, and different type of carbapenemases caused color change in the different substrates [substrate I: no imipenem; substrate II: imipenem; substrate III: imipenem + tazobactam; substrate VI: imipenem + ethylene diamine tetraacetic acid (EDTA); substrate V: imipenem + tazobactam + EDTA]. *K. pneumoniae* ATCC BAA-1706, ATCC BAA-1705 and ATCC BAA-2146 were the quality control strains.

Identification of resistance genes and OMP genes

DNA templates were extracted by the boiling method. Polymerase chain reaction (PCR) amplification was performed to identify the most common ESBL genes (bla_{SHV}, bla_{CTX-M}, bla_{TEM}), AmpC genes (bla_{CIT}, bla_{DHA}, bla_{EBC}, bla_{MOX}, bla_{FOX}, bla_{ACC}), and carbapenemase genes positive for carbapenemase screening, of which the corresponding genes were Class A (bla_{GES}, bla_{KPC}, bla_{SME}, bla_{IMI}, bla_{BIC}), Class B (bla_{IMP}, bla_{VIM}, bla_{NDM}, bla_{SPM}, bla_{DIM}, bla_{GIM}, bla_{SIM}, bla_{AIM}, bla_{SMB}) and Class D (bla_{OXA-48}), using previously reported primers and conditions (14-16). PCR-positive products of resistance genes were sequenced for comparative analysis by NCBI BLAST. OMP genes *ompK35* and *ompK36* were detected, and the sequence of positive porin amplification products was compared with the wild type CI507 (GenBank: No. FJ577672) and VM522 (GenBank: No. FJ577673) (17).

MLST

The allelic profiles and STs were determined after PCR amplification of the internal fragments of seven *K. pneumoniae* housekeeping genes (*gapA*, *infB*, *mdh*, *pgi*, *phoE*, *rpoB*, *tonB*) and the allele number combination for gene mapping according to BIGSdb-Pasteur database (https://bigsdb.pasteur.fr/). Primer design and the PCR process were referred to the MLST website (https://bigsdb.pasteur.fr/klebsiella/primers_used.html), and genetic relationships were analyzed by PHYLOViZ software.

Statistical analysis

The regional comparison was performed by IBM SPSS26 (USA). Chi-square test were used to determine the differences in drug resistance rate, resistance genes and major STs, and P values were calculated by Pearson chi-square test, continuity correction or Fisher's exact test. Clinical profiles included in this study were analyzed retrospectively. All variables were described as qualitative data. Two-sided P values <0.05 were considered statistically significant, and the Bonferroni-corrected P value for pairwise comparisons was 0.05/3=0.0167.

Results

Comparison of clinical characteristics

The demographic and clinical profiles of patients infected with CRKP are shown in *Table 1*. Of these 135 patients (complete data of 15 patients was unavailable), 71.85% were ≥60 years, and the number of male patients was almost 3-fold more than that of female patients. Nearly half of the patients had been in the ICU for ≥7 days, and had been hospitalized within 2 months before ICU admission. The highest incidence of underlying disease was hypertension and chronic cardiac insufficiency in Chongqing and Beijing, respectively, and hypertension and diabetes in Nantong. Most of the patients had received ventilatory assistance, central venous catheterization, sputum suction and other operations. Carbapenems were the most frequently used antibiotics in all three hospitals, and more than 50% of the patients were co-infected with other bacteria, at higher rates in Chongqing and Beijing.

Antimicrobial susceptibility testing of CRKP strains

Totals of 52 strains from Chongqing, 51 strains from Nantong and 47 strains from Beijing were collected, and clinical samples were sputum (n=106), urine (n=22), ascites (n=10), pus (n=4) and other (n=8). In addition to 100% resistance to imipenem, CRKP strains were extensively resistant to other antibiotics (*Figure 1*). The resistance rates of strains to penicillins (ampicillin, ampicillin sulbactam, piperacillin tazobactam), cephalosporins (cefazolin, cefotetan, ceftazidime, cefepime), aztreonam and levofloxacin were extremely high (≥90%) in all ICUs. Different to Chongqing, the resistance rate of aminoglycosides (gentamicin, tobramycin, amikacin) was much lower in Nantong and Beijing. Moreover, tigecycline-resistant strains were only detected in Beijing and all strains were susceptible to polymyxin B.

Resistance mechanism of carbapenems

In this study, 100 strains producing Class A carbapenemases carried bla_{KPC-2} and 8 strains producing Class B

Table 1 Comparison of clinical characteristics

Characteristics	Total (n=135)	Chongqing (n=52)	Nantong (n=36)	Beijing (n=47)	P value[a]	P value[b]	P value[c]	P value
Sex (male)	100 (74.07)	38 (73.08)	28 (77.78)	34 (72.34)	0.617	0.935	0.572	0.836
Age (≥60 years)	97 (71.85)	31 (59.62)	27 (75.00)	39 (82.98)	0.134	0.011*	0.372	0.032*
Hospital stay (in 2 months)	78 (57.78)	26 (50.00)	21 (58.33)	31 (65.96)	0.441	0.109	0.477	0.275
Length of ICU stay before CRKP isolation (≥7 days)	72 (53.33)	26 (50.00)	18 (50.00)	28 (59.57)	1.000	0.339	0.384	0.569
Comorbidities								
Hypertension	47 (34.81)	19 (36.54)	16 (44.44)	12 (25.53)	0.456	0.238	0.071	0.19
Diabetes mellitus	44 (32.59)	8 (15.38)	16 (44.44)	20 (42.55)	0.003*	0.003*	0.863	0.003*
Chronic heart failure	62 (45.93)	19 (36.54)	7 (19.44)	36 (76.60)	0.084	<0.001*	<0.001*	<0.001*
COPD	14 (10.37)	4 (7.69)	3 (8.33)	7 (14.89)	1.000	0.255	0.569	0.459
Chronic renal disease	26 (19.26)	12 (23.08)	5 (13.89)	9 (19.15)	0.283	0.633	0.526	0.561
Chronic hepatopathy	29 (21.48)	18 (34.62)	3 (8.33)	8 (17.02)	0.004*	0.047	0.406	0.008*
Malignant tumor	22 (16.30)	5 (9.62)	4 (11.11)	13 (27.66)	1.000	0.02	0.064	0.043*
Impaired immune system	12 (8.89)	6 (11.54)	1 (2.78)	5 (10.64)	0.233	0.887	0.227	0.349
Neutropenia	10 (7.41)	5 (9.62)	1 (2.78)	4 (8.51)	0.394	1.000	0.382	0.502
Invasive operations								
Tracheotomy	46 (34.07)	12 (23.08)	24 (66.67)	10 (21.28)	<0.001*	0.83	<0.001*	<0.001*
Mechanical ventilation	102 (75.56)	42 (80.77)	24 (66.67)	36 (76.60)	0.133	0.612	0.317	0.311
Central venous catheter	117 (86.67)	46 (88.46)	29 (80.56)	42 (89.36)	0.304	0.887	0.258	0.448
Urinary catheter	122 (90.37)	46 (88.46)	33 (91.67)	43 (91.49)	0.896	0.869	1.000	0.873
Sputum suction	102 (75.56)	38 (73.08)	24 (66.67)	40 (85.11)	0.517	0.144	0.048	0.133
Enteral nutrition	97 (71.85)	34 (65.38)	30 (83.33)	33 (70.21)	0.063	0.608	0.166	0.841
Parenteral nutrition	–	10 (19.23)	4 (11.11)	–	0.306	–	–	–
Immunosuppressant	–	2 (3.85)	0 (0.00)	–	0.511	–	–	–
Hormonotherapy	–	34 (65.38)	–	–	–	–	–	–
Antibiotics								
Cephalosporins	45 (33.33)	20 (38.46)	11 (30.56)	14 (29.79)	0.445	0.364	0.94	0.292
Carbapenems	69 (51.11)	34 (65.38)	20 (55.56)	15 (31.91)	0.352	0.001*	0.031	0.003*
Aminoglycosides	7 (5.19)	3 (5.77)	1 (2.78)	3 (6.38)	0.642	1.000	0.629	0.793
Quinolones	29 (21.48)	13 (25.00)	3 (8.33)	13 (27.66)	0.046	0.764	0.027	0.077
Penicillins	48 (35.56)	20 (38.46)	17 (47.22)	11 (23.40)	0.413	0.107	0.023	0.064
Glycopeptides	31 (22.96)	17 (32.69)	3 (8.33)	11 (23.40)	0.007*	0.306	0.069	0.028*
Linezolid	32 (23.70)	19 (36.54)	0 (0.00)	13 (27.66)	<0.001*	0.346	0.001*	<0.001*
Tigecycline	28 (20.74)	11 (21.15)	7 (19.44)	10 (21.28)	0.845	0.988	0.838	0.975
Antifungal agents	44 (32.59)	29 (55.77)	9 (25.00)	6 (12.77)	0.004*	<0.001*	0.151	<0.001*
Infection								
Fungal infection	21 (15.56)	12 (23.08)	6 (16.67)	3 (6.38)	0.464	0.021	0.255	0.056
Other bacterial infections	108 (80.00)	46 (88.46)	20 (55.56)	42 (89.36)	<0.001*	0.887	<0.001*	<0.001*

Data are present as n (%). P value[a]: Chongqing *vs.* Nantong; P value[b]: Chongqing *vs.* Beijing; P value[c]: Nantong *vs.* Beijing; P value: Chongqing *vs.* Nantong *vs.* Beijing; –: not available. *P<0.05. ICU, intensive care unit; CRKP, carbapenem-resistant *Klebsiella pneumoniae*; COPD, chronic obstructive pulmonary disease.

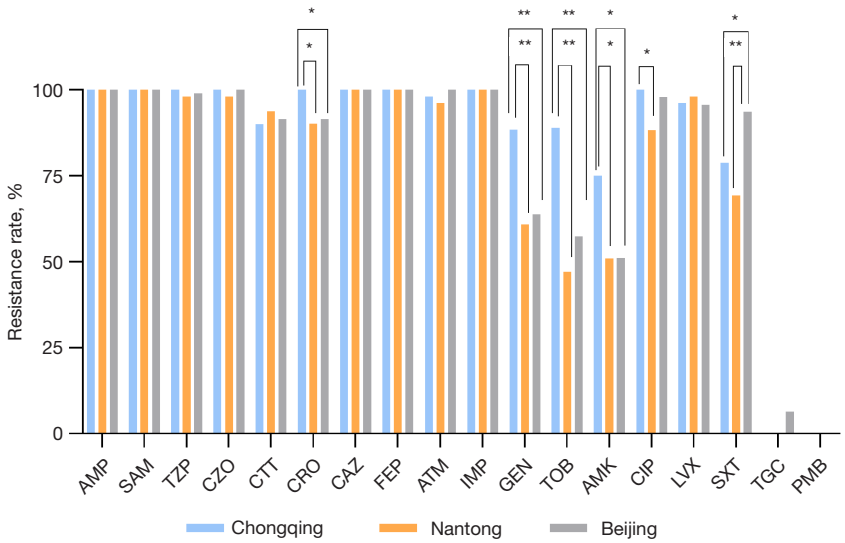

Figure 1 Susceptibility of CRKP to common antibiotics: AMP, SAM, TZP, CZO, CTT, CRO, CAZ, FEP, ATM, IMP, GEN, TOB, AMK, CIP, LVX, SXT, TGC, PMB. *P<0.05; **P<0.01. AMP, ampicillin; SAM, ampicillin sulbactam; TZP, piperacillin tazobactam; CZO, cefazolin; CTT, cefotetan; CRO, ceftriaxone; CAZ, ceftazidime; FEP, cefepime; ATM, aztreonam; IMP, imipenem; GEN, gentamicin; TOB, tobramycin; AMK, amikacin; CIP, ciprofloxacin; LVX, levofloxacin; SXT, cotrimoxazole; TGC, tigecycline; PMB, polymyxin B; CRKP, carbapenem-resistant *Klebsiella pneumoniae*.

carbapenemases carried $bla_{\text{NDM-1}}$ (*Figure 2*). There were no strains producing both A and B, or D carbapenemases. For CRKP from Chongqing, 35 strains carried $bla_{\text{KPC-2}}$ and 4 strains carried $bla_{\text{NDM-1}}$; for strains from Nantong, 28 carried $bla_{\text{KPC-2}}$ and 1 carried $bla_{\text{NDM-1}}$; and for strains from Beijing, 28 carried $bla_{\text{KPC-2}}$ and *1* carried $bla_{\text{NDM-1}}$. Other related carbapenemase genes were not found in our study. Both AmpC and ESBL genes were identified, of which the prevalence rates were higher in Nantong. A total of 3 strains with *ompK35* deletion were found from Nantong, and *ompK36* deletion was detected, comprising 2 from Chongqing and 2 from Nantong. Notably, 1 strain simultaneously producing KPC carbapenemase, AmpCs and ESBLs did not express the *ompK36* gene, and another strain producing NDM carbapenemase and ESBLs was missing OmpK35. Both these strains were highly resistant to imipenem. Resistance mechanisms showed few differences except for expression of AmpCs and ESBLs genes (*Table 2*).

Molecular epidemiology

In this study, there were altogether 28 STs among the 150 CRKP strains from the three regions, mainly ST11 (76/150, 50.67%), ST15 (33/150, 22%), ST13 (6/150, 4%), ST1 (3/150, 2%), ST48 (3/150, 2%), and several other types (*Figure 3*). Among 52 CRKP strains from Chongqing, ST11 was major (31/52, 59.6%), followed by ST15 (9/52, 17.3%). For the 51 strains from Nantong, ST11 (21/51, 41.8%) and ST15 (21/51, 41.8%) were the most common. And of the 47 strains from Beijing, ST11 was dominant (24/47, 51.06%). There was no difference among the three regions in the composition ratio of ST11, but the difference of ST15 was statistically significant between Nantong and both Chongqing and Beijing (P=0.008 & P<0.001). BURST analysis showed that ST11, ST340, ST859, ST1326, ST1953, ST690 and ST1883 belonged to clonal complex 11, whereas ST37 and ST2020, ST1561 and ST147, ST48 and ST2361 belonged to three different sequence groups respectively, and the other types were singletons (*Figure 4*).

Discussion

Admission to an ICU is one of the risk factors for CRKP infection (18), and the morbidity of CRKP infection in ICU patients is higher than for other patients (19). A 4-year report revealed that, without paying attention to hand hygiene and environmental cleaning and disinfection, it was ineffective to isolate patients infected with CRKP. The hand hygiene and environmental cleaning were however, important, because CRKP could be cross-

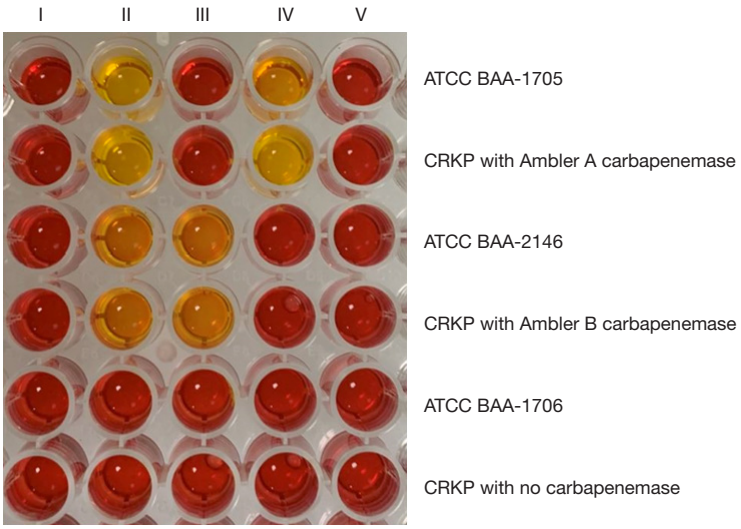

Figure 2 Detection of carbapenemase ATCC BAA-1706 produced no carbapenemases but was resistant to carbapenems. ATCC BAA-1705 and ATCC BAA-2146 had Class A and B carbapenemase activity respectively. ATCC, American Type Culture Collection; CRKP, carbapenem-resistant *Klebsiella pneumoniae*.

transmitted through the hands of medical staff and patients' surroundings (20). Moreover, for patients with coronavirus disease 2019 (COVID-19) patients, the prevalence of CRKP ranged from 0.35–53%, and because of mechanical ventilation and central catheters in ICUs, pulmonary and bloodstream infection were the main types (21). In this study, the characteristics of patients infected with CRKP were roughly similar to those in previous studies (22,23). There was no significant difference in most of the patients in the three study hospitals, apart from the underlying diseases and use of antibiotics, which were mainly due to the hospital admission and antibiotics available. Other than *K. pneumoniae*, the most common bacteria detected in this study were *P. aeruginosa* and *Acinetobacter baumannii*, and the isolation rates of the latter two species were higher in Chongqing and Beijing. Despite the advanced medical equipment and better infection control in tertiary hospitals, the patients admitted to the ICU of a tertiary hospitals generally suffer more serious disease or have been transferred from secondary hospitals, thus the risk of co-infection increased (24). In their analysis of the risk factors for colonization of ICU pathogens, a Greek study reported that KPC-producing *K. pneumoniae* and vancomycin-resistant *Enterococcus* were important risk factors for each other's colonization, which proved the risk of CRKP infection for the colonization of other multidrug-resistant pathogens in that institute (25). In general, patients from three ICUs were critically ill and treated with multiple antibiotics, increasing the risk of CRKP infection. At the same time, it also increased the risk of infection by other drug-resistant bacteria and fungal infection.

The CRKP strains in this study were highly resistant to most antibiotics, which may be related to the duration and various types of antibiotic exposure, and widespread of antibiotic-resistance genes (26). Moreover, resistance rates basically showed a slight increase from previous local reports (27,28). It is worth noting that the resistance rate of CRKP to aminoglycosides was comparatively lower, consistent with the current status of drug resistance (29). The low resistance rate of aminoglycosides may be due to their adverse reactions and thus lower use in clinical practice, suggesting that scheduled shifting or switching of empirical medication might alleviate antibiotic resistance (30). Furthermore, tigecycline and polymyxin B are still the most effective drugs against CRKP infection (31). A previous report showed that the resistance rates of *K. pneumoniae* in the Jiangzhe area were higher than in the southwest regions of China, which was contrary to this study's findings (32). As to the drug resistance in the three study hospitals, there were different antibiotics available and used, especially simultaneously, the locality of positioning of the hospitals was different, and the admitted patients were also different in their characteristics. Under these many different external conditions, there was no significant difference in the

Table 2 Resistance genes and mechanism of carbapenem resistance

Resistance mechanism	Chongqing (n=52)	Nantong (n=51)	Beijing (n=47)	P value[a]	P value[b]	P value[c]	P value
Resistance genes							
bla_{KPC-2}	35 (67.31)	28 (54.9)	37 (78.72)	0.196	0.203	0.013*	0.044*
bla_{NDM-1}	4 (7.69)	1 (1.96)	3 (6.38)	0.363	1.000	0.347	0.437
bla_{DHA}	2 (3.85)	16 (31.37)	8 (17.02)	<0.001*	0.066	0.099	0.001*
bla_{CIT}	1 (1.92)	0 (0.00)	0 (0.00)	1.000	1.000	–	1.000
bla_{SHV}	48 (92.31)	51 (100.00)	33 (70.21)	0.118	0.004*	<0.001*	<0.001*
bla_{TEM}	50 (96.15)	51 (100.00)	37 (78.72)	0.495	0.008*	0.001*	<0.001*
bla_{CTX-M}	38 (73.08)	50 (98.04)	31 (65.96)	<0.001*	0.441	<0.001*	<0.001*
OMP loss	2 (3.85)	5 (9.80)	0 (0.00)	0.269	0.496	0.057	0.06
Carbapenemases+							
Class A, AmpCs+, ESBLs+, OMP loss	1 (1.92)	0 (0.00)	0 (0.00)	1.000	1.000	–	1.000
Class B, ESBLs+, OMP loss	0 (0.00)	1 (1.96)	0 (0.00)	0.495	–	1.000	0.653
Class A, AmpCs+, ESBLs+	2 (3.85)	9 (17.65)	1 (2.13)	0.023	1.000	0.028	0.013*
Class A, AmpCs+, ESBLs–	0 (0.00)	0 (0.00)	1 (2.13)	–	0.475	0.48	0.313
Class A, AmpCs–, ESBLs+	33 (63.46)	19 (37.25)	32 (68.09)	0.008*	0.629	0.002*	0.004*
Class A, AmpCs–, ESBLs–	0 (0.00)	0 (0.00)	3 (6.38)	–	0.103	0.107	0.029*
Class B, AmpCs+, ESBLs+	0 (0.00)	0 (0.00)	0 (0.00)	–	–	–	–
Class B, AmpCs+, ESBLs–	0 (0.00)	0 (0.00)	0 (0.00)	–	–	–	–
Class B, AmpCs–, ESBLs+	4 (7.69)	1 (1.96)	3 (6.38)	0.363	1.000	0.347	0.437
Class B, AmpCs–, ESBLs–	0 (0.00)	0 (0.00)	0 (0.00)	–	–	–	–
Carbapenemases–							
AmpCs+	1 (1.92)	7 (13.73)	7 (14.89)	0.031	0.025	0.869	0.038*
ESBLs+	13 (25.00)	22 (43.14)	10 (21.28)	0.052	0.661	0.021	0.039*
AmpCs+, OMP loss	0 (0.00)	1 (1.96)	0 (0.00)	0.495	–	1.000	0.653
ESBLs+, OMP loss	0 (0.00)	1 (1.96)	0 (0.00)	0.495	–	1.000	0.653
AmpCs+, ESBLs+	1 (1.92)	7 (13.73)	7 (14.89)	0.031	0.025	0.869	0.055
AmpCs+, ESBLs+, OMP loss	0 (0.00)	1 (1.96)	0 (0.00)	0.495	–	1.000	0.653

Data are present as n (%). P value[a]: Chongqing *vs.* Nantong; P value[b]: Chongqing *vs.* Beijing; P value[c]: Nantong *vs.* Beijing; P value: Chongqing *vs.* Nantong *vs.* Beijing. –: not available. *P<0.05. OMP, outer membrane porin; ESBLs, extended-spectrum beta-lactamase genes; AmpCs, Class C carbapenemase genes.

separated CRKP stains in the three hospitals.

Production of carbapenemases is the most important mechanism of carbapenem resistance of *K. pneumoniae*. Only bla_{KPC-2} and bla_{NDM-1} were found in this study. The main carbapenemase gene in Chongqing and Nantong was bla_{KPC-2}, which was similar to previous studies (33,34). bla_{KPC-2} was also the major carbapenemase gene in Beijing, although previous research showed the reverse with predominance of bla_{NDM-1} in Beijing (35), for which the reason might be that wide dissemination of KPC-producing *K. pneumoniae* has already happened in China (12). Although the detection rate of bla_{NDM} was relatively low in China, it is predominant in India and Pakistan (36,37). Domestic travel or transportation of patients infected with carbapenemase-

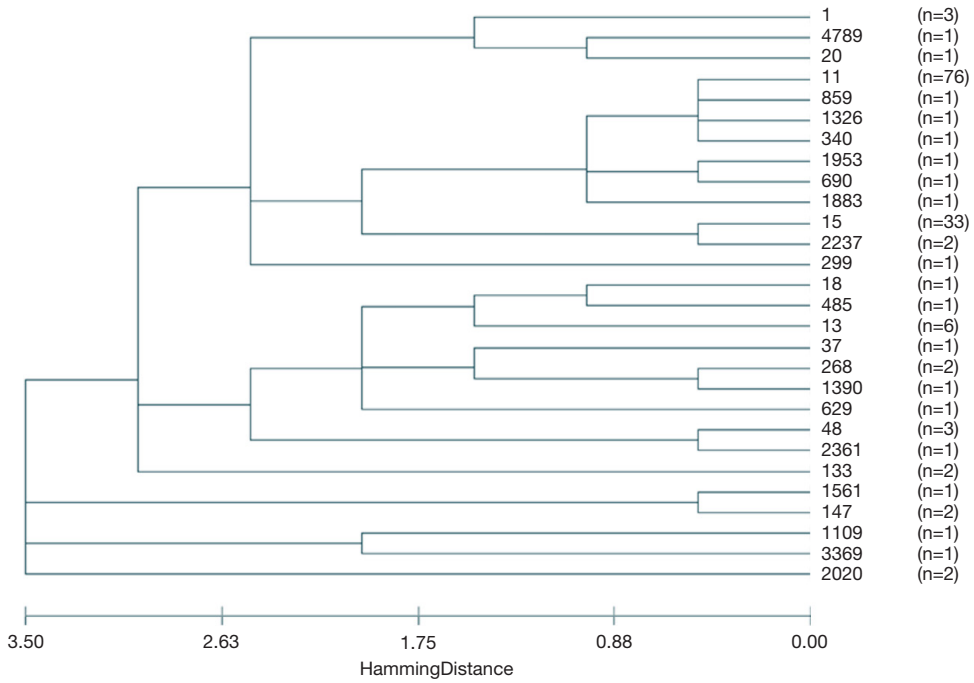

Figure 3 Phylogenetic tree of CRKP *ST11* as the main MLST type, followed by *ST15*. CRKP, carbapenem-resistant *Klebsiella pneumoniae*; MLST, multilocus sequence typing.

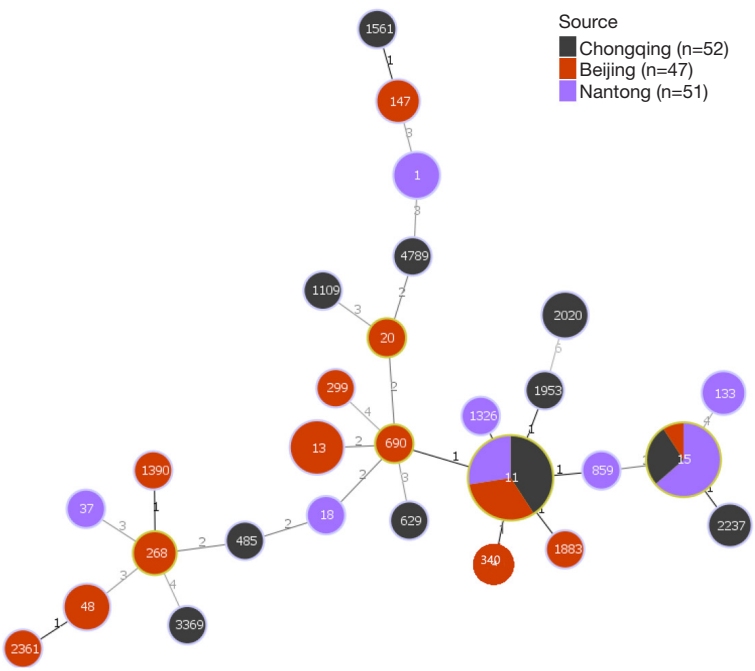

Figure 4 goeBURST full MLST. Numbers on solid lines between the connecting circles indicates the different alleles in the housekeeping genes. Node sizes vary linearly with the number of strains of a specific ST. MLST, multilocus sequence typing; ST, sequence typing.

producing *K. pneumoniae* might be the primary reason for its dissemination (12). A study in the United Kingdom verified that the epidemic of CRKP in Europe was driven by several carbapenemase-producing clonal lineages, so rapid dissemination could be also attributed to mobile resistance elements, and thus closer domestic and international communication increases the risk of CRKP transmission (38,39). Additionally, ESBL genes were prevalent in the CRKP strains and nearly all strains in this study harbored one or two ESBL genes (bla_{SHV}, bla_{TEM}, bla_{CTX-M}), and the prevalence rate was close to related reports in Chongqing, Jiangsu, and Beijing (35-37). As an important part of the outer membrane of bacteria, porin plays a key role in maintaining membrane permeability and regulating the entry and exit of substances. In this study, OMP genes (*ompK35/ompK36*) of CRKP were directly examined by PCR, and loss of porin existed in a small number of strains only in Chongqing and Nantong. Additionally, point and frameshift mutations were found in both *ompK35* and *ompK36* genes, which might result in abnormal expression of OMP, thus leading to drug resistance. Research has indicated that some strains producing no carbapenenase but lacking porin with ESBL- or AmpC-positive also showed resistance to imipenem, which seems to confirm the substitution mechanism of carbapenem resistance (40,41). Moreover, other mechanisms such as overexpression of the efflux pump and biofilm formation could also contribute to carbapenem resistance, which explains the result of antibiotic susceptibility that strains without resistance genes were also resistant to imipenem. Generally speaking, *K. pneumoniae* from the three ICUs developed resistance to carbapenem mainly by producing carbapenemases and other enzymes, which means more careful and appropriate antibiotic use should be considered. According to our experimental results the drug resistance mechanism of CRKP in the three hospitals tended to be similar.

The polyclonal spread of CRKP can be identified by MLST or pulsed field gel electrophoresis (PFGE), analyzing the major lineages of isolates could suggest whether local clonal expansions exist. ST11 (50.67%) and ST15 (22%) were the main genotypes in this study. ST11 is predominant in China and East Asia. ST15 differs from ST11 by only two alleles. ST11 *K. pneumoniae* possesses pathogenic potential and resistance to serum killing, so an outbreak would be difficult to control (42). The main STs in the three hospitals were slightly different, ST11 and ST15 were dominant in Nantong, while in Chongqing and Beijing only ST11 was the leading genotype, which proved the regional differentiation of major STs. There are few reports on ST15 *K. pneumoniae* in Nantong, however, a study revealed that ST15 was common in Shanghai, a city geographically close to Nantong, and the major STs might be related to interregional transmission (43). Among the 150 CRKP, bla_{KPC-2} carriers were mainly ST11. Moreover, some other STs also harbored bla_{KPC} or bla_{NDM}, which proved horizontal transmission of carbapenemase genes (44). Other less common STs were found in this study. Taken together, despite the discrepancy in rarer genotypes, the major types of the three hospitals were largely consistent.

Despite the three cities being geographically far apart, and the differing clinical characteristics of the patients with CRKP infection, the resistance profiles and resistance mechanism of CRKP essentially coincided with few differences. The bla_{KPC} resistance gene has faster and wider transmission. In order to better control the clinical prevalence of CRKP, its transmission mechanism is worthy of in-depth study.

In conclusion, our study indicated that under the premise of sufficient movement of people across the country, the dominant resistant strains of *K. pneumoniae* are widely distributed, which causes the prevalence of resistance strains in different cities to be consistent. Although the resistance mechanism of CRKP was not investigated thoroughly in this study and the sample size was small, it has provided some theoretical evidence for infection control and support for considered application of antibiotics.

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-4323/rc

Data Sharing Statement: Available at https://atm.amegroups.com/article/view/10.21037/atm-22-4323/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-4323/coif).

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 study conformed to the provisions of the Declaration of Helsinki (as revised in 2013). This clinical study was a retrospective study, and the Ethics Committee of the Second Affiliated Hospital of Chongqing Medical University waived the requirement of ethical approval and informed consent, and patients' information was processed anonymously and confidentially.

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