# Carbapenem-Resistant Klebsiella Pneumoniae in Southwest China: Molecular Characterizations and Risk Factors Caused by NDM and KPC Producers

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# Abstract

**Background:** *Klebsiella pneumoniae* is one of the most common *Enterobacteriaceae*. In recent years, carbapenem-resistant *Klebsiella pneumoniae* (CRKP) has become one of the most important carbapenem-resistant *Enterobacteriaceae*. CRKP are usually resistant to antibiotics. Up to this day, the emergence of carbapenemase-producing *K. pneumoniae* has been a challenge for treatment of clinical infection.

**Methods:** (i) 66 non-repetitive clinical CRKP isolates were identified by matrix-assisted laser analytical ionization time-of-flight mass spectrometer (MALDI-TOF-MS) and drug sensitivity analysis was performed by Vitek2 Compact. EDTA-synergy test and mCIM / eCIM test were used to detect drug-resistant phenotypes. (ii) Carbapenemase genes, extended-spectrum β-lactamase genes (ESBLs), cephalosporinase gene (AmpC), virulence genes, integron and resistance gene cassettes were amplified by PCR. (iii) Plasmid typing was performed by plasmid conjugation assay and PCR-based replicon typing (PBRT) method. (iv) The genetic environments of KPC-2 and NDM-1 were analyzed by using overlapping PCR. (v) MLST was used to analyze the molecular epidemiological characteristics of CRKP. (vi) Risk factors of CRKP infection by logistic regression model.

**Results:** Our study revealed that 42 of the 66 CRKP isolates obtained from patients were identified as $bla_{KPC-2}$, 24 $bla_{NDM-1}$-positive strains were identified (20 $bla_{NDM-1}$ and 4 $bla_{NDM-5}$), of which 18 were from the neonatal departments. And CRKP strains were ESBL (extended-spectrum β-lactamases) and AmpC enzymes producer, Notably, we found two CR-hvKp (carbapenem-resistant hypervirulent *klebsiella pneumoniae*) strains, which contains $bla_{KPC-2}$ gene and other resistant genes. Two of the 42 KPC-2-producing CRKP strains were positive for transconjugants, and the plasmid typing was the IncFII type. And two NDM-producing CRKP strains tested positive for transconjugants, which belonged to the IncX3 plasmid. Analysis of the genetic environment of these two genes has revealed that the highly conserved regions ($tnpA$-$tnpR$-IS$kpn8$-$bla_{KPC-2}$) and conserved regions ($bla_{NDM-1}$-$ble_{MBL}$-$trpF$-$tat$) are associated with the dissemination of KPC-2 and NDM-1. *Intl1* carrying drug resistance gene cassettes were widely distributed in CRKP. According to the MLST results, a total of 13 ST types were measured in 66 CRKP strains, ST11 and ST4495 were the main ST types, and the latter was the newly discovered ST type. Hematological disease, tracheal cannula and prior use of β-lactams and β-lactamase inhibitor combination were identified as independent risk factors for CRKP infections.

**Conclusion:** These findings manifested the need for intensive surveillance and precautions to monitor the further spread of KPC and NDM in southwest China.

# Introductin

*Klebsiella pneumoniae* (*K. pneumoniae*) is a common cause of opportunistic infections in hospitalized patients with antimicrobial resistance (1). it can often lead to aseptic site infections such as blood and other respiratory tract infections (2). Over the past decades, due to increasing prevalence of healthcare-associated infections caused by multidrug-resistant strains producing extended-spectrum β-lactamases and / or carbapenemases, *K. pneumoniae* has emerged as a major clinical and public health threat. A parallel phenomenon of severe community-acquired infections caused by 'hypervirulent' *K. pneumoniae* has also emerged owing to phenomenon of strains acquired virulence factors (1). Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) has become a particularly important problem worldwide due to the rapid increase in drug resistance and subsequent high mortality (3) (4). In China, the prevalence of CRKP infection has rapidly increased from 0.7% in 2004 to 13.4% in 2014 (5), and the mortality rate of CRKP infection was approximately 40−50% since poor prognosis.

The resistance mechanism of *Enterobacteriaceae* to carbapenem antibiotics is very complex. However, phenotypic drug resistance of carbapenems is usually caused by two major mechanisms: (i) β-lactamase activity-binding structural mutation and (ii) carbapenemases production (6). The former mechanism is combination of extended-spectrum β-lactamases (ESBLs) and AmpC enzyme with porin loss or reduced expression, resulting the decrease of outer membrane permeability and the change of penicillin binding protein in carbapenems (7, 8). As for later mechanism, carbapenemases are classified by molecular structure and belong to Ambler classification system A, B, D three β-lactamases, class A and D carbapenemases require serine hydrolysis of β-lactam, while class B metallo-β-lactamases (MBLs) require zinc at their hydrolysis active sites (6). In addition, KPCs is the most common class A gene in *Enterobacteriaceae* worldwide (9). KPC2 carbapenemases are widely disseminated, because its encoding gene $bla_{KPC-2}$ is usually located on the plasmid of bacteria, and the plasmid where it is located can often be widely transferred in different bacteria, especially in *Enterobacteriaceae*. Most KPC−2-producing strains belonged to clone group (CG)258, ST258 and ST11 were the dominant ST types. ST258 has spread worldwide since its emergence in the early 21st century, particularly in North America, Latin America and several European countries (10). However, in Asia, the dominant CRKP is ST11, which accounts for 60 % in China (11). NDM-1 carbapenemases in class B have been reported in China for the first time in 2013, and the NDM-1-producing clinical strains have spread rapidly in China, even causing explosive epidemic in some regions (12, 13).

Recently, not only the outbreaks of CRKP have become increasingly common in China (14), but also the prevalence of hvKp has created new challenges for the clinician. Genetic factors that confer high virulence to hvKp existing in a large virulence plasmid, perhaps an integrated binding element, therefore, we focus on predictive biomarkers located on virulence plasmid (e.g. *rmpA* and *iucA*) (15). Importantly, MDR-hvKp (multiple drug resistance hypervirulent *k. pneumoniae*) strains are gradually reported worldwide due to horizontal transfer of mobile genetic elements (16).

Therefore, the objectives of this study were the following: (i) To describe the prevalence of clinical CRKP isolates collected for approximately 4 years. (ii) To identify the antimicrobial resistance profiles, resistance genes, integrons, drug resistance gene cassettes, and virulence genes among these CRKP strains. (iii) To report plasmid analysis (iv) To detect genetic environment of KPC-2 and NDM-1. (v) To examine the clonal relatedness of these CRKP strains. (vi) To evaluate the risk factors CRKP infections in hospitalized patients.

# Materials And Methods

## Study Design and Data Collect.

During the 4 years study period (September 2016 to August 2019), this retrospective study was performed in the Affiliated Hospital of Southwest Medical University (Luzhou, China), where is a 3200-bed large teaching hospital with 43 wards and approximately 120,000 annual admissions. 565 clinical *K. pneumoniae* isolates were collected from this hospital (WHONET.2020). We selected 66 CRKP isolates which were from the patients for the first time and excluded duplicate isolates from the same patient. All CRKP isolates were confirmed by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS, Bruker Daltonics, Bremen, Germany).

We used a stepwise matching technique to identify appropriate control cases from patients infected with carbapenem-susceptible *Klebsiella pneumoniae* (CSKP) infection, in order to study the risk factors of CRKP infections. For each patient with CRKP infection, we selected a matched control patient from the pool of patients with CSKP infections. Patient matched to a case for the site of infection, gender, age ± 2 years and year of hospital admission.

## Antimicrobial Susceptibility Testing (AST) of CRKP Isolates.

The susceptibility of CRKP isolates to 16 antibiotics, including Cefepime, Cefotaxime, Cefazolin, Cefuroxime, Ceftazidime, Cefoxitin, Piperacillin/tazobactam, Ampicillin/sulbactam, Amikacin, Tobramycin, Gentamicin, Levofloxacin, Ciprofloxacin, Sulperazone, Compound sulfamethoxazole, Aztreonam, was tested by VITEK 2 Compact system (bioMérieux, Marcy l'Etoile, Lyon, France). The MICs of Meropenem, Imipenem, Ertapenem, Tigecycline and Polymyxin B were determined by broth dilution method, and the results were interpreted according to the standards of the Clinical and Laboratory Standards Institute (CLSI) 2020-M100 (17). *Pseudomonas aeruginosa* ATCC27853 was used as quality control strains (purchased from China National Health Inspection Center).

## Screening of CRKP and phenotypic detection of carbapenemase.

After overnight cultured, 0.5 McFarland bacterial suspension used to swab inoculate Mueller-Hinton ager plate (Haibo, Qingdao,China). When dry, 10-μg imipenem disk (OXOID, Thermo Fisher Scientific, Massachusetts, USA) and blank filter paper disk were placed 10 mm from each other, then a blank disk applied to 10 μL of 0.5 mol / L EDTA solution (Thermo Fisher Scientific, Massachusetts, USA), which concentration was about 1.5mg / mL. After 18-24h incubation, the EDTA-synergy positive presented that enlarged zone of inhibition (17) (18).

The CIM-test utilizes antibiotic susceptibility-testing disks as previously described (17) (19), 2-ml aliquots of Trypticase soy broth (TSB) (Haibo, Qingdao, China) were directly inoculated with a 1-μl loopful of *K. pneumoniae* colonies, a 2-mL tube of TSB supplemented with EDTA at a final concentration of 5mM. A 10-μg meropenem disk (OXOID, Thermo Fisher Scientific, Massachusetts, USA) was placed in each inoculated tube, which were incubated for 4 h (± 15 min), then meropenem disks placed on a Mueller-Hinton agar plate after lawn inoculation of 0.5 McFarland bacterial suspension of meropenem-susceptible *Escherichia coli* ATCC 25922, no carbapenemase activity will imply there will be a zone, whereas enzymatic inactivation will produce no zone. The mCIM is considered positive if the zone size is 6 to 15 mm or pinpoint colonies are present within a 16- to 18-mm zone (20). Only when the isolate is positive for mCIM, eCIM results are recorded. When the zone size increases by ≥ 5 mm compared to the zone size observed by mCIM, the test isolation of MBL production is positive, and if the area size increases by ≤ 4 mm, the test isolation of MBL is negative.

## The Detection of Resistance Gene, Virulence Gene and Integrase-associated Gene.

After the bacterial genomic DNA was extracted by bacterial DNA Kit (Tiangen, Beijing,China). This DNA was used as a template in polymerase chain reactions (PCR) to detect related genes. The PCR was performed to amplify carbapenemase genes ($bla_{KPC}$, $bla_{NDM}$, $bla_{VIM}$, $bla_{IMP-4}$ and $bla_{OXA-48}$), extended spectrumβ-lactamase genes ($bla_{TEM}$, $bla_{SHV}$, $bla_{CTX-M}$). plasmid-mediated AmpC genes ($bla_{MOX}$, $bla_{DHA}$, $bla_{ACC}$, $bla_{EBC}$, and $bla_{FOX}$) virulence genes (*rmpA*, *rmpA2*, *HI1B*, *iroN*, *iutA*, *magA*, *fimH*, *mrkD*, *entB*) (Table S1) and integrase-associated genes (*IntI*1, *IntI*2, *IntI*3, ISCR1VR) (Table S2) were also determined by using primers as described previously. The primers were shown in Table S1 and Table S2. PCR amplification system: ddH2O 10 μL, 5' and 3' primer 2 μL, 2×Taq MasterMix 11 μL (Innovagene, Changsha, China), DNA 2μL, totally 25 μL reaction volume. Reaction conditions: 95 °C denaturation for 5min, 95 °C 30s, annealing temperature 30s, 72 °C 30s with 30 cycles, and extension at 72 °C for 5min and the DNA samples collected were determined using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Massachusetts, USA); The reaction products were separated gel electrophoresis on a 1.5% agarose gel (Biorad, California, USA). Positive amplifications were subjected to Shanghai Jieli Biotechnology. The variable region of the class 1 Integron and the ISCR1 analyzed by BLAST (www.ncbi.nlm.nih.gov).

### Conjugation and plasmid analysis.

To evaluate whether the carbapenemase genes are located on the plasmid and assess whether these genes can be horizontal transferred. The receptor strain was sodium azide-resistant *E. coli* strain J53. Implant donor and recipient strains in Mueller-Hinton plate and incubated overnight at 37°C. Then, an appropriate amount of strains was inoculated in a glass tube containing 5 mL LB medium and cultured at 37°C for 16-18h. Subsequently, 400 μL donor strain and 200 μL recipient strain were added to the glass tube containing 800 μL LB broth medium and cultured at 37°C for 16−18 h. Transconjugants were selected using LB plates containing sodium azide (180 μg/mL) and imipenem (0.5 μg/mL), meanwhile, the LB plate was used as blank control (21). Confirmation that conjugation had taken place in *E. coli* J53 was carried out by MALDI-TOF MS system for the presence of resistance gene by PCR analysis. In addition, plasmid analysis was performed for strains with successful conjugation which was determined by PCR-based replicon typing as described previously (22).

### Genetic Environments of NDM-1-Carrying Plasmids and KPC-2-Carrying Plasmids.

To investigate the genetic environments of the NDM-1 and KPC-2, we use overlapping PCR and sequencing were applied to analyze. (23) (24). The PCR amplicon was sequenced, the DNA sequences obtained was spliced and compared to those in the NCBI gene bank database (25) (Table S3, S4).

### Molecular Epidemiological Study.

Multi-locus sequence typing (MLST) was performed using amplification of internal fragments of the seven housekeeping genes: *gapA, infB, mdh, pgi, phoE, rpoB, tonB.* Primer pairs designed according to MLST website (26)(www.pasteur.fr/mlst). Reaction conditions: 95 °C denaturation for 5min, 95 °C 30s, 55 °C 45s, 72 °C 30s with 30 cycles, and extension at 72 °C for 5min. The PCR products were analyzed by agarose gel electrophoresis (Biorad, California, USA) and the positive products were sequenced by Shanghai Jieli Biotechnology, the sequencing results were then submitted to *klebsiella pneumoniae* MLST data (https://bigsdb.pasteur.fr/klebsiella/klebsiella.html) for comparison.

## Statistical analysis.

All analyses and graphs were performed using SPSS v.24.0 software (SPSS Inc., Chicago, USA) The chi-square test or Fisher's exact test was used to analyze categorical variables. Continuous variables were presented as means ± standard deviation (SD), and were evaluated by a more appropriate student's t-tests or Mann−Whitney U-test. Multivariable logistic regression analysis was performed to identify independent risk factors of CRKP infection. All biologically plausible variables with a value of $P < 0.1$ within univariate analysis were included in the following multiple logistic regression model. $P < 0.05$ was considered as statistically significant, and all probability values were two-tailed distribution.

## Results

### Bacterial Isolates.

In this study, a total of 66 consecutive non-duplicate CRKP isolates were identified from September 2016 to August 2019 in southwest China. The majority of patients were in neonatology department (27.3%, n = 18) and rehabilitation department (24.3%, n = 16), followed by department of hepatobiliary surgery (10.6%, n = 7), department of hematology (7.6%, n = 5), department of respiratory medicine (6.1%, n = 4), department of pediatrics (6.1%, n = 4),department of urology (4.5%, n = 3), department of neurosurgery (4.5%, n = 3), department of gastrointestinal surgery (3.0%, n = 2), cardiothoracic surgery department (3.0%, n = 2), intensive care unit (1.5%, n = 1) and digestive medicine (1.5%, n = 1)(Fig. 1A). These isolates were mainly cultured from sputum (34.8%, n = 23), urine (25.8%, n = 17) and blood (21.2%, n = 14), followed by secretion (6.1 %, n = 4), pleuroperitoneal fluids (6.1 %, n = 4), catheter tip (3 % n = 2), pus (3 % n = 2) (Fig. 1B). We conducted a matched case−control study and at a ratio of 1:1 as the control group, therefore, 132 patients were included in the final study cohort.

### Antimicrobial Susceptibility Testing (AST) and Carbapenem Resistance Phenotype.

As shown in Table 1, VITEK2-Compact results showed that 66 CRKP strains were multidrug resistance, the resistance rates to Cefepime, Imipenem, Piperacillin/tazobactam, Ceftriaxone, Ampicillin/sulbactam, Cefotaxime, Cefazolin, Ertapenem, Ceftazidime, Meropenem and Cefoxitin were all 100%. The resistance rates to Ciprofloxacin, Levofloxacin, Gentamicin, Tobramycin, Aztreonam, and Sulperazon were 80.3 %, 81.9 %, 83.3 %, 83.3 %, 87.9 %, and 95.5 %, respectively. In addition, the resistance rates of Amikacin and Cotrimoxazole were 54.5 % and 65.2 %, respectively. However, polymyxin B were effective for these strains.

The results of EDTA-synergy test, mCIM / eCIM test and drug susceptibility test were consistent. All 66 CRKPs showed positive mCIM test, namely carbapenemase. Among them, 24 strains showed eCIM positive, that is, Metallo Beta Lactamases (MBL) positive.

### Molecular Testing

Among the 66 carbapenemase producers: 42 (63.6%) isolates contained $bla_{KPC-2}$, 24 (36.4%) isolates possessed $bla_{NDM}$, in which, 20 strains were $bla_{NDM-1}$, 4 strains were $bla_{NDM-5}$. The results were consistent with EDTA-synergy test and mCIM/eCIM test. Notably, there were three isolates co-harbor $bla_{NDM}$ and $bla_{IMP}$ simultaneously. In addition to the production of carbapenemase, 100% (66/66) and 95.5% (63/66) of the CRKP isolates were positive for ESBL and AmpC genes, respectively, with 95.5% (63/66) of the strains positive for both. Otherwise, virulence genes were detected in all isolates, 54 (81.8 %) detected three different virulence genes. it was noteworthy that Kpn497 harbor 12 virulence genes and Kpn131 harbor 11 virulence genes (Table 2).

### Plasmid and Integrase-associated Analysis.

Transconjugants containing $bla_{KPC-2}$-carrying plasmids were successfully obtained from kpn406 and kpn490, whose plasmids belong to IncFII. Likewise, kpn473 and kpn475 containing $bla_{NDM}$-containing plasmids were positive for transconjugants and these plasmids belonged to IncX3.

Almost all isolates class 1 integrin-positive (84.8 %, n = 56), of which 46 strains were variable region positive, and no class 2 integrin and class 3 integrin strains were detected. ISCR1 positive was detected in 25 strains, whereas whose related drug-resistance genes were not. A total of 6 different types of variable region gene cassette arrays. The most common antibiotic resistance genes conferred resistance to aminoglycoside antibiotics [addA2 (40/46), aadA1 (1/46)]. Five trimethoprim-resistance genes [dfrA12 (6/46), dfrA27 (1/46)] conferred resistance to trimethoprim antibiotics; Furthermore, we also found OXA-10 gene (1/46) in the integron variable region, a gentamycin-resistance gene arr3 (1/46) and a rifampicin-resistance gene aacA4 (1/46). The different gene cassette arrays were ddfrA12，addA2，dfrA12-addA2，dfrA27-arr3，gcuF-dfrA12 and OXA-10-addA1-aacA4 (Table 2).

### Characterization of Genetic Environment of KPC-2 and NDM-1.

For KPC-2, the genetic structure compared with pKP048 (GenBank Accession No.FJ628167). 42 KPC-2-producing strains could be divided into three different types (A-C) based on the analysis of genetic structures, among which type C was the most principal (n = 32), followed by type A (n = 8), type B (n = 3). Type A harbored same structure as pKP048. In type B, Tn1721-tnpR and Tn1721-tnpA deletions downstream of ISKpn6-like. Compared with type B, type C lacks ISkpn6-ike, and its gene structure is tnpA-tnpR-ISkpn8-$bla_{KPC-2}$(Fig.2A).

For NDM-1, compared with pNDM-BJ01 (GenBank accession no. JQ001791), genetic structures surrounding the $bla_{NDM-1}$ gene demonstrated there are three types A, B and C. As for type A(n=12), a remnant of insertion sequence ISAba125 was present upstream of the $bla_{NDM-1}$ gene, and loss of downstream genes of tat. Compared with type A, the retention sequence ISAba125 was lacked in type B. In type C, the upstream of $bla_{NDM-1}$ was IS30, and the downstream of $bla_{NDM-1}$ was $ble_{MBL}$-trpF-tat-cutA-groES-groEL. (Fig.2B)

### Detection of ST types.

A total of 13 MLST was identified, which revealed that ST11 (56%, n=37) and ST4495 (19.7%, n=13) are the predominant ST types (Table 3).

### Risk Factors and Multivariate Analysis of CRKP Infection.

As shown in Table 4, we present the results of the univariable analysis of matched data regarding risk factors associated with CRKP infections. Compared with the CRKP group, statistically significant differences were observed for history of respiratory disease ($P$ = 0.034), renal disease ($P$ = 0.039) and hematological disease ($P$=0.02), need for tracheal cannula ($P$<0.001), prior use of β-lactams and β-lactamase inhibitor combination ($P$=0.009). Multivariate analysis for matched data revealed that history of hematological disease [odds ratio (OR) 2.568, 95% OR 1.106−5.964, $P$ =0.028], tracheal cannula [odds ratio (OR) 4.883, 95% OR 1.797−13.265, $P$ =0.002] and prior use of β-lactams and β-lactamase inhibitor combination [odds ratio (OR) 4.271, 95% OR 1.760−10.365, $P$ =0.001] were independent risk factors for CRKP infection (Table 4, 5).

## Discussion

In light of carbapenem-resistant *Klebsiella pneumoniae* being widely distributed in China, the increasing prevalence and global spread of these clinically resistant strains posed a serious threat to public health. At the same time, due to the acquisition of virulence plasmids, 'hypervirulent' *Klebsiella pneumoniae* was also emerged (1, 27). In this work, we have found some particularly noteworthy findings, first, our results revealed that the main mechanism of CRKP collected in this study could be attributed to carbapenemase producer. And, CRKP strains were ESBL and AmpC enzymes producer. $bla_{KPC-2}$ and $bla_{NDM-1}$ are two major carbapenemase genes in our study. Second, the result indicated almost CRKP strains carry virulence-associated genes (81.8%, entB+fimH+mrkD), otherwise, the genes peg-344, iroB, iucA, plasmid-borne rmpA gene ($_p$rmpA), and $_p$rmpA2 gene are regarded as regulator of hypervirulent phenotype. At present, in the study of CRKP it is found that it can be produced by hvKp through obtaining the plasmid encoding carbapenemases (15). Notably, we found two CR-hvKp strains, which contains $bla_{KPC-2}$ gene and other resistant genes. This conclusion proved that CR-hvKp began to appear and gradually became popular worldwide, with rapid spread (28). Third. The $bla_{NDM-1}$ gene has been rapidly prevalent worldwide and it often co-exists with other resistant genes in bacteria, leading to multidrug resistance, which further challenges the selection of clinical antibiotics (29). In this study, $bla_{NDM-1}$ and $bla_{IMP-4}$ genes co-exist in two strains, especially, we found a

strain carry $bla_{NDM-5}$ and $bla_{IMP-4}$ genes. The first reported drug-resistant bacteria carrying $bla_{IMP-4}$ and $bla_{NDM-1}$ simultaneously were *K. pneumoniae* isolated from 2012 (30). In recent years, strains harboring $bla_{NDM}$ gene and another type of carbapenemase genes have emerged worldwide (31) (32, 33). The location of these carbapenemase genes is variable, which may be located on chromosome genes and plasmids. Simultaneously, they harbor more resistant genes, making the possibility of horizontal transmission of resistant genes between strains greater, and even can produce higher resistance, which poses great challenges to clinical treatment (34).

By studying the molecular detection and genetic characteristics of CRKP. In China, *K. pneumoniae* ST11 were always associated with $bla_{KPC-2}$ (35). In the forty-two KPC-2-producing CRKP strains, there were 7 ST types, of which 35 belonged to ST11. The rest were ST318, ST467, ST147, ST405, ST23, ST17. In recent years, more and more studies have pointed out that mobile genetic elements may promote the transmission of $bla_{KPC-2}$ gene. Almost all $bla_{KPC-2}$ genes are located on plasmids, and resistance genes spread rapidly through plasmid horizontal transfer (36). Previous studies have reported KPC-2 located on diversity of plasmids IncN, IncL / M and IncR except IncFII. In our study, KPC-2 often binds to IncF plasmid, consistent with the results of this study (37). $bla_{KPC-2}$ is often located on Tn*4401* in European and American countries. The *tnpA* sequences of Tn*4401* and Tn*3* were about 22 % identical and 39 % similar, while the *tnpR* nucleotide sequences were completely different. In China, the immediate environment surrounding $bla_{KPC-2}$ in pKP048 was considered to result from the integration of a Tn*3*-based transposon and a partial Tn*4401* structure, with the ORFs ordered as follows: Tn*3*-*tnpA*, Tn*3*-*tnpR*, IS*Kpn8*, the $bla_{KPC-2}$ gene, the IS*Kpn6*-like element, Tn*1721*-*tnpR*, Tn*1721*-*tnpA* (38). Our results demonstrated that the genetic environment of KPC-2 was diversity, which can be divided into three types. Twenty-four NDM-producing strains were divided into 7 ST types. Interestingly, so it is considered to be the outbreak that 18 of the 24 NDM-producing strains were from the neonatal department. In recent years, the outbreak of NDM-producing CRKP has been mostly in the neonatal department, but ST types were different. First reported outbreak of NDM-1 *K. pneumoniae* ST76 and ST37 in Shanghai in 2016 (39). Subsequently, NDM-1-producing *K. pneumoniae* broke out in Yunnan, China, and all of them belonged to ST105, meanwhile, its $bla_{NDM-1}$ gene was located on IncFI plasmid. NDM-1 producing strains present in the hospital environment also posed a potential risk and the incubator water acted as a diffusion reservoir of NDM-1-producing bacteria (28). The outbreak of ST1419, ST234 and ST1412 followed (40, 41). In 2019, the outbreak of NDM-5-Producing *K. pneumoniae* in China began, and all strains belonged to ST337, carrying ESBLs resistant genes, all $bla_{NDM-5}$ genes were located on IncX3 plasmid (42). In our study, the 14 NDM-1-producing strains, 13 belonged to ST4495 (4-1-99-1-9-5-5), ST4495 is the newly discovered ST sequence type in this study. Among the 4 NDM-5-producing strains, Kpn494, Kpn499 and Kpn502 belonged to ST2407 (2-1-99-1-9-5-5), while Kpn504 belonged to ST11. It is worth noting that the main molecular sequence type of NDM-1 is ST4495(4-1-99-1-9-5-5) and the main molecular sequence type of NDM-5 is ST2407 (2-1-99-1-9-5-5), the difference between them was only *gapA* gene. The reasons for this phenomenon are speculated to following possibilities:(i) NDM-producing CRKP may undergo gene evolution and transformation in a short time, including NDM-1 evolution into its variant NDM-5, while housekeeping gene *gapA* changes. (ii) The NDM-5 is not evolved from NDM-1, but a new carbapenemase gene carried by the patient. It is necessary to further study its epidemiology to clarify the causes and mechanisms. Among CRKP in neonatal department, $bla_{NDM}$ gene is more common than $bla_{KPC}$, and its drug resistance is also significantly stronger than the latter. Due to the particularity of newborns, it is more restricted in the use of antibiotics compared with adults (43). The outbreak of NDM-producing CRKP poses greater challenges and threats treatment. Therefore, the emergence of NDM-producing CRKP in neonatal department should be paid enough attention. As one of the most significant metallo-β-lactamases, $bla_{NDM-1}$ has been rapidly spread worldwide recently. It was found that the rapid horizontal transmission of $bla_{NDM-1}$ was closely related to mobile genetic elements. $bla_{NDM-1}$ gene mostly located in transposon Tn*125*, the structure as following: IS*Aba125*-$bla_{NDM-1}$-$ble_{MBL}$-*trpF*-*dsbC*-*cutA*-*groES*-*groEL*-IS*CR27*-IS*Aba125*. In this study, we detected the surrounding environment of $bla_{NDM-1}$ gene and found that the surrounding environment of $bla_{NDM-1}$ gene was mainly composed of A, B and C three types. Our results demonstrated that the genetic environment of these types is partly similar to transposon Tn*125*.

Integrons are genetic elements that allow efficient capture and expression of exogenous genes. It plays a critical role in the dissemination of antibiotic resistance, particularly among gram-negative bacterial pathogens. In this study, almost all isolates carried *IntI1* gene, indicating that integron has become an important element of drug resistance in *K. pneumoniae*, and to some extent, it also makes the mechanism of drug resistance in *K. pneumoniae* more changeable and complex. There were six genes encoding drug resistance cassette structures in 46 strains with positive variable region, including *dfrA12, addA2, dfrA12-addA2, dfrA27-arr3, gcuF-dfrA12* and OXA-10-*addA1-aacA4.addA* was the most common resistance gene, followed by *dfrA*. The high detection rate of these two drug resistance genes may be related to the widespread use of the two antibiotics in clinic and environment, thus causing the prevalence of related drug resistance gene cassettes. In this study, the drug resistance gene cassette $bla_{OXA-10}$-*addA1-aacA4* was identified from a CRKP strain. OXA-10, as a class D carbapenemase, has weaker hydrolysis ability to carbapenem antibiotics. In recent years, many OXA-10-like enzymes have been found to be involved in bacterial resistance (44).

The main finding of our analysis is that hematological disease, tracheal cannula and exposure to β-lactams and β-lactamase inhibitor combination were independent risk factors associated with the development of CRKP infections. Most preview studies have demonstrated that the history of tracheal cannula was independent risk factor for CRKP infection (45-48). Meanwhile, exposure to antibiotic was also associated with CRKP infection, such as exposure to quinolones (49), β-lactams and β-lactamase inhibitor combination (50) and carbapenems (51), which was partly consistent with our study. Inappropriate treatment had the potential to increase the selection and transmission of CRKP. Notably, in the present study, hematological disease was rarely found to be a risk factor for CRKP infection. Possible explanation may be that part of the isolates in this study were from neonatology department, newborns could have poor functional status and they were susceptible to be infected. There are some

limitations in our study. First, we should acknowledge that the number of patients included in this study is relatively small, although this is a common problem in studies assessing risk factors for multidrug-resistant microbial infections (49). Secondly, the study was performed in a single-center setting, so that some important risk factors may be missed.

## Conclusion

this present study revealed the high prevalence of CRKP in a large teaching hospital in Southwest China. (i) 66 clinical isolates were multidrug resistant, and polymyxin B was effective. (ii) $bla_{KPC-2}$ and $bla_{NDM-1}$ are two major types of carbapenemase genes and coexist with other types of drug resistance genes, which are important reasons for CRKP resistance to carbapenem antibiotics. The outbreak of NDM-producing CRKP was also found in neonatal department of our hospital. (iii) Plasmids of CRKP and other types of removable genetic elements may be crucial in the generation and wide spread of drug resistance and virulence. (iv) The surrounding environment of KPC-2 and NDM-1 genes showed polymorphism. Analysis of the genetic environment of these two genes has revealed that the highly conserved regions ($tnpA$-$tnpR$-IS$kpn8$-$bla_{KPC-2}$) and conserved regions ($bla_{NDM-1}$-$ble_{MBL}$-$trpF$-$tat$) are associated with the dissemination of KPC-2 and NDM-1. $IntI1$ carrying drug resistance gene cassettes are widely distributed in CRKP. (v) The molecular cloning types of 66 CRKP strains were diverse, with 13 ST types. ST11 and ST4495 were the main ST types, and the latter was the newly discovered ST type. (vi) logistic regression model for matched data revealed that history of hematological disease [odds ratio (OR) 2.568, 95% CI 1.106-5.964, $P$ =0.028], tracheal cannula [OR 4.883, 95% CI 1.797-13.265, $P$ =0.002] and prior use of β-lactams and β-lactamase inhibitor combination [OR 4.271, 95% CI 1.760-10.365, $P$ =0.001] were independent risk factors for CRKP infection. In addition, our findings highlight an urgent need to develop effective measures to prevent and control the further spread of KPC-2 and NDM-1 in China.

## Abbreviations

ESBL: extended-spectrum β-lactamase;

MBL: metallo-β-lactamase;

hvKp: hypervirulent *klebsiella pneumoniae*;

CRKP: carbapenem-resistant *klebsiella pneumoniae*;

CSKP: carbapenem-susceptible *Klebsiella pneumoniae*;

CR-hvKp: carbapenem-resistant hypervirulent *klebsiella pneumoniae*;

MDR-hvKp: multiple drug resistance hypervirulent *klebsiella pneumoniae*;

ATCC: American Type Cell Cultures;

*bla*: Beta lactamase;

KPC: *Klebsiella pneumoniae* Carbapenemase;

NDM: New Delhi Metallo-β-lactamase;

OR: odds ratio;

CI: confidence interval

Int: Integron;

ISCR: Insertion sequence common region;

ORF:Open Reading Frame;

## Declarations

### Ethics approval and consent to participate

This study was approved by the Institutional Review Board of affiliated hospital of southwest medical university（KY2020043). Written informed consent were obtained from all participants.

### Consent for publication

Not applicable

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## Tables

Table 1 Susceptibility of 66 CKRP strains to antimicrobial agents.

| Antimicrobial agent | No. | %R | No. | %I | No. | %S |
|---|---|---|---|---|---|---|
| Amikacin | 36 | 54.5 | 0 | 0 | 30 | 45.5 |
| Cefepime | 66 | 100 | 0 | 0 | 0 | 0 |
| Imipenem | 66 | 100 | 0 | 0 | 0 | 0 |
| Piperacillin/tazobactam | 66 | 100 | 0 | 0 | 0 | 0 |
| Ceftriaxone | 66 | 100 | 0 | 0 | 0 | 0 |
| Ampicillin/sulbactam | 66 | 100 | 0 | 0 | 0 | 0 |
| Cefotaxime | 66 | 100 | 0 | 0 | 0 | 0 |
| Cefazolin | 66 | 100 | 0 | 0 | 0 | 0 |
| Cefuroxime | 66 | 100 | 0 | 0 | 0 | 0 |
| Ertapenem | 66 | 100 | 0 | 0 | 0 | 0 |
| Tobramycin | 55 | 83.3 | 4 | 6.1 | 7 | 10.6 |
| Sulperazon | 63 | 95.5 | 2 | 3.0 | 1 | 1.5 |
| Ceftazidime | 66 | 100 | 0 | 0 | 0 | 0 |
| Meropenem | 66 | 100 | 0 | 0 | 0 | 0 |
| Levofloxacin | 54 | 81.9 | 0 | 0 | 12 | 18.1 |
| Cefoxitin | 66 | 100 | 0 | 0 | 0 | 0 |
| Aztreonam | 58 | 87.9 | 0 | 0 | 8 | 12.1 |
| Ciprofloxacin | 53 | 80.3 | 0 | 0 | 13 | 19.7 |
| Gentamicin | 55 | 83.3 | 0 | 0 | 11 | 16.7 |
| Tigecycline | 1 | 1.5 | 0 | 0 | 65 | 98.5 |
| Compound sulfamethoxazole | 43 | 65.2 | 0 | 0 | 23 | 34.8 |

Table 2 Genotypes of the 66 CRKP strains.

| N | Carbapenemase | ESBLs | AmpC | Int | Virulence gene | Gene Cassette | ISCR | MLST |
|---|---|---|---|---|---|---|---|---|
| 16 | KPC-2 | SHV+TEM+CTX-M | ACC | IntI1 | entB+fimH+mrkD | addA2 | ISCR1 | ST-11 |
| 26 | NDM-1 | SHV+CTX-M | DHA | | entB+mrkD | | ISCR1 | ST4495 |
| 27 | NDM-1 | SHV+CTX-M | DHA | | entB+fimH+mrkD | | ISCR1 | ST4495 |
| 30 | KPC-2 | SHV+TEM+CTX-M | ACC | IntI1 | entB+fimH+mrkD | addA2 | ISCR1 | ST-11 |
| 32 | NDM-1 | SHV+CTX-M | DHA | | entB+fimH+mrkD | | ISCR1 | ST4495 |
| 34 | NDM-1 | SHV+CTX-M | DHA | | entB+fimH+mrkD | | ISCR1 | ST4495 |
| 36 | NDM-1 | SHV+CTX-M | DHA | | entB+fimH+mrkD | | ISCR1 | ST4495 |
| 37 | NDM-1 | SHV+CTX-M | DHA | | entB+fimH+mrkD | | ISCR1 | ST4495 |
| 39 | NDM-1 | SHV | DHA | | entB+mrkD | | ISCR1 | ST4495 |
| 40 | NDM-1 | SHV | DHA+ACC | | entB+fimH | | ISCR1 | ST37 |
| 45 | KPC-2 | SHV+TEM+CTX-M | ACC | IntI1 | entB+fimH+mrkD | addA2 | | ST-11 |
| 46 | NDM-1 | SHV+CTX-M | DHA+ACC | | entB+fimH+mrkD | | ISCR1 | ST4495 |
| 49 | NDM-1 | SHV+CTX-M | DHA+ACC | | entB+fimH+mrkD | | ISCR1 | ST4495 |
| 51 | KPC-2 | SHV+CTX-M | ACC | | entB+fimH+mrkD | | | ST11 |
| 56 | NDM-1 | SHV+CTX-M | DHA+ACC | | entB+fimH+mrkD | | ISCR1 | ST4495 |
| 57 | NDM-1 | SHV+CTX-M | DHA+ACC | | entB+fimH+mrkD | | ISCR1 | ST4495 |
| 58 | NDM-1+IMP-4 | SHV+CTX-M | DHA+ACC | | entB+fimH | | | ST4495 |
| 101 | NDM-1 | SHV | ACC | | entB+fimH+mrkD | | | ST-37 |
| 131 | KPC-2 | SHV+TEM | ACC | IntI1 | entB+fimH+mrkD+ rmpA/rmpA2+ iucA+terB+aerobactin+HI1B+iroN+iutA | addA2 | ISCR1 | ST-11 |
| 210 | KPC-2 | SHV+TEM+CTX-M | ACC | IntI1 | entB+fimH+mrkD | addA2 | | ST-11 |
| 211 | KPC-2 | SHV+TEM+CTX-M | ACC | IntI1 | entB+fimH+mrkD | addA2 | | ST-11 |
| 214 | KPC-2 | SHV+TEM+CTX-M | ACC | IntI1 | entB+fimH+mrkD | addA2 | | ST-11 |
| 215 | KPC-2 | SHV+TEM+CTX-M | ACC | IntI1 | entB+fimH+mrkD | addA2 | | ST-11 |
| 221 | KPC-2 | SHV+TEM+CTX-M | ACC | IntI1 | entB+fimH+mrkD | addA2 | ISCR1 | ST-11 |
| 223 | KPC-2 | SHV+TEM+CTX-M | ACC | | entB+fimH+mrkD | dfrA12, addA2 | | ST-147 |
| 227 | KPC-2 | SHV+CTX-M | ACC | IntI1 | entB+fimH+mrkD | addA2 | | ST-11 |
| 228 | KPC-2 | SHV+CTX-M | ACC | | entB+fimH+mrkD | | | ST-11 |
| 230 | KPC-2 | SHV+TEM+CTX-M | ACC | IntI1 | entB+fimH+mrkD | addA2 | | ST-11 |

| 232 | KPC-2 | SHV+TEM+CTX-M | ACC | IntI1 | entB+fimH+mrkD | addA2 | | ST-11 |
|---|---|---|---|---|---|---|---|---|
| 233 | KPC-2 | SHV | ACC | IntI1 | entB+fimH+mrkD | dfrA12, addA2 | | ST-147 |
| 234 | KPC-2 | SHV+TEM+CTX-M | ACC | IntI1 | entB+fimH+mrkD | addA2 | | ST-11 |
| 241 | KPC-2 | SHV+TEM+CTX-M | ACC | IntI1 | entB+fimH+mrkD | addA2 | | ST-11 |
| 244 | KPC-2 | SHV | ACC | IntI1 | entB+fimH+mrkD | ddfrA12 | | ST-23 |
| 245 | KPC-2 | SHV+TEM+CTX-M | ACC | IntI1 | entB+fimH+mrkD | addA2 | | ST-11 |
| 251 | KPC-2 | SHV+TEM+CTX-M | ACC | IntI1 | entB+fimH+mrkD | addA2 | | ST-11 |
| 252 | KPC-2 | SHV+TEM+CTX-M | | IntI1 | entB+fimH+mrkD | addA2 | | ST-11 |
| 257 | KPC-2 | SHV+TEM+CTX-M | ACC | IntI1 | entB+fimH+mrkD | addA2 | | ST-11 |
| 258 | KPC-2 | SHV+TEM+CTX-M | ACC | IntI1 | entB+fimH+mrkD | addA2 | | ST-11 |
| 267 | NDM-1 | SHV | ACC | IntI1 | entB+fimH+mrkD | addA2 | ISCR1 | ST-11 |
| 271 | KPC-2 | SHV+TEM+CTX-M | ACC | | entB+fimH+mrkD | | | ST-11 |
| 285 | KPC-2 | SHV+TEM+CTX-M | ACC | IntI1 | entB+fimH+mrkD | addA2 | | ST-11 |
| 354 | KPC-2 | SHV+TEM+CTX-M | ACC | IntI1 | entB+fimH+mrkD | addA2 | | ST-11 |
| 406 | KPC-2 | SHV+TEM | DHA | IntI1 | fimH+mrkD | OXA-10, addA1, aacA4 | ISCR1 | ST-318 |
| 436 | KPC-2 | SHV+TEM | DHA | IntI1 | entB+fimH+mrkD | addA2 | | ST-17 |
| 440 | NDM-1 | SHV+TEM | ACC | IntI1 | mrkD | addA2 | | ST-307 |
| 445 | NDM-1 | SHV+TEM | ACC | | mrkD | | | ST-307 |
| 473 | NDM-1+IMP-4 | SHV+TEM | ACC | IntI1 | entB+fimH+mrkD | addA2 | ISCR1 | ST-152 |
| 475 | NDM-1 | SHV+TEM | ACC | IntI1 | fimH+mrkD | addA2 | ISCR1 | ST-15 |
| 478 | KPC-2 | SHV+TEM+CTX-M | ACC | IntI1 | entB+fimH+mrkD | addA2 | ISCR1 | ST-11 |
| 479 | KPC-2 | SHV+TEM+CTX-M | ACC | IntI1 | entB+fimH | addA2 | | ST-11 |
| 480 | KPC-2 | SHV+TEM+CTX-M | ACC | IntI1 | entB+fimH+mrkD | addA2 | | ST-11 |
| 483 | KPC-2 | SHV+TEM+CTX-M | ACC | IntI1 | entB+fimH+mrkD | addA2 | | ST-11 |
| 489 | KPC-2 | SHV+TEM+CTX-M | ACC | IntI1 | entB+fimH+mrkD | dfrA12, addA2 | | ST-11 |
| 490 | KPC-2 | SHV | ACC | IntI1 | entB+fimH+mrkD | dfrA27, arr3 | | ST-467 |
| 494 | NDM-5 | SHV+CTX-M | DHA | IntI1 | entB+fimH+mrkD | addA2 | ISCR1 | ST-2407 |
| 495 | KPC-2 | SHV+TEM+CTX-M | | IntI1 | entB+fimH+mrkD | ddfrA12 | ISCR1 | ST-11 |
| 496 | KPC-2 | SHV+TEM | ACC | IntI1 | entB+fimH+mrkD | gcuF, dfrA12 | | ST-405 |
| 497 | KPC-2 | SHV+TEM | | IntI1 | entB+fimH+mrkD+rmpA/rmpA2+ | addA2 | | ST-11 |

| No | Carbapenemase | ESBL | AmpC | Integron | Virulence genes | | Resistance gene | ISCR | ST |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | iucA+ terB+wcaG+ aerobactin+ HI1B+iroN+iutA | | | | |
| 498 | KPC-2 | SHV+CTX-M | ACC | Intl1 | entB+mrkD | | ddfrA12 | | ST-11 |
| 499 | NDM-5 | SHV+CTX-M | DHA | Intl1 | entB+fimH+mrkD | | addA2 | ISCR1 | ST-2407 |
| 500 | KPC-2 | SHV+TEM+CTX-M | ACC | | entB+fimH+mrkD | | | | ST-11 |
| 502 | NDM-5 | SHV+CTX-M | DHA | Intl1 | entB+fimH+mrkD | | addA2 | | ST2407 |
| 504 | NDM-5+IMP-4 | SHV+TEM+CTX-M | DHA | Intl1 | entB+fimH+mrkD | | addA2 | | ST-11 |
| 505 | KPC-2 | SHV+TEM+CTX-M | ACC | Intl1 | entB+fimH+mrkD | | addA2 | | ST-11 |
| 506 | NDM-1 | SHV+CTX-M | DHA | Intl1 | entB+fimH+mrkD | | addA2 | | ST4495 |
| 507 | KPC-2 | SHV+CTX-M | ACC | Intl1 | entB+fimH+mrkD | | addA2 | | ST-11 |

Table 3 The allelic profile and STs of 66 CRKP strains detected in this study.

| Number of isolates | ST | gapA | infB | mdh | pgi | phoE | ropB | tonB |
|---|---|---|---|---|---|---|---|---|
| 37 | 11 | 3 | 3 | 1 | 1 | 1 | 1 | 4 |
| 13 | 4495[a] | 4 | 1 | 99 | 1 | 9 | 5 | 5 |
| 3 | 2407 | 2 | 1 | 99 | 1 | 9 | 5 | 5 |
| 2 | 147 | 3 | 4 | 6 | 1 | 7 | 4 | 38 |
| 2 | 307 | 4 | 1 | 2 | 52 | 1 | 1 | 7 |
| 2 | 37 | 2 | 9 | 2 | 1 | 13 | 1 | 16 |
| 1 | 15 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 1 | 17 | 2 | 1 | 1 | 1 | 4 | 4 | 4 |
| 1 | 23 | 2 | 1 | 1 | 1 | 9 | 4 | 12 |
| 1 | 405 | 2 | 1 | 62 | 3 | 10 | 4 | 110 |
| 1 | 318 | 16 | 24 | 21 | 31 | 68 | 22 | 67 |
| 1 | 467 | 16 | 24 | 21 | 27 | 76 | 22 | 67 |
| 1 | 152 | 2 | 3 | 2 | 1 | 1 | 4 | 56 |

[a] Novel ST detected in this study.

Table 4. Univariable analysis of risk factors and outcomes associated with CRKP infections.

| Variable | CRKP (n=66) | CSKP (n=66) | P-value |
|---|---|---|---|
| Demographic, n (%) or IQR | | | |
| age (years) | 44.5 (0,60) | 46.5 (0.61) | 0.754 |
| sex (male) | 47 (71.2%) | 46 (69.7%) | 0.894 |
| length of hospital stay | 15 (7,27) | 25 (10,38) | 0.1 |
| admission to ICU | 14 (21.1%) | 7 (10.6%) | 0.096 |
| Co-morbidity, n (%) | | | |
| malignant disease | 7 (10.6%) | 15 (22.7%) | 0.062 |
| diabetes mellitus | 11 (16.7%) | 12 (18.2%) | 0.819 |
| hypertension | 16 (24.2%) | 16 (24.2%) | 1 |
| heart disease | 9 (13.6%) | 11 (16.7%) | 0.627 |
| hepatobiliary disease | 12 (18.2%) | 15 (22.7%) | 0.517 |
| respiratory disease | 45 (68.2%) | 33 (50%) | **0.034** |
| renal disease | 7 (10.6%) | 16 (24.2%) | **0.039** |
| urinary tract infection | 10 (15.2%) | 9 (13.6%) | 0.804 |
| craniocerebral disease | 15 (22.7%) | 9 (13.6%) | 0.176 |
| hematological disease | 32 (48.5%) | 19 (28.8%) | **0.02** |
| gastrointestinal disease | 8 (12.1%) | 5 (7.6%) | 0.381 |
| Invasive procedures and devices | | | |
| tracheal cannula | 24 (36.4%) | 7 (10.6%) | **<0.001** |
| central venous catheter | 5 (7.6%) | 12 (18.2%) | 0.069 |
| foreign material in the body | 14 (21.1%) | 7 (10.6%) | 0.096 |
| Surgical operations after admission | 9 (13.6%) | 16 (24.2%) | 0.12 |
| colostomy | 3 (4.5%) | 1 (1.5%) | 0.612 |
| gastrostomy | 2 (3%) | 0 | 0.476 |
| Prior antibiotic use, n (%). | | | |
| penicillins | 2 (3%) | 5 (7.6%) | 0.437 |
| first, second-generation cephalosporins | 7 (10.6%) | 15 (22.7%) | 0.21 |
| third, fourth-generation cephalosporins | 17 (25.6%) | 19 (28.8%) | 0.696 |
| aminoglycosides | 4 (6.1%) | 1 (1.5%) | 0.362 |
| quinolones | 20 (30.3%) | 21 (31.8%) | 0.851 |
| metronidazole | 0 | 1 (1.5%) | 1 |
| glycopeptides | 0 | 0 | NA |
| carbapenems | 30 (45.5%) | 32 (48.5%) | 0.727 |
| β-lactams and β-lactamase inhibitor combination | 40 (60.1%) | 25 (37.9%) | **0.009** |
| Clinical outcomes, n (%) | | | |
| patient outcome: mortality | 5 (7.6%) | 0 | 0.068 |

Note: Bold face indicates values that are significant (P < 0.05). CR, carbapenem-resistant; CS, carbapenem-susceptible; interquartile range; ICU, intensive care unit.

Table 5. Multivariate analysis of risk factors for CRKP infections.

| Factors | Odds ratio | 95%, Confidence interval | P-value |
|---|---|---|---|
| hematological disease | 2.568 | (1.106,5.568) | 0.028 |
| tracheal cannula | 4.883 | (1.797,13.265) | 0.002 |
| prior β-lactams and β-lactamase inhibitor combination use | 4.271 | (1.760,10.365) | 0.001 |

# Figures



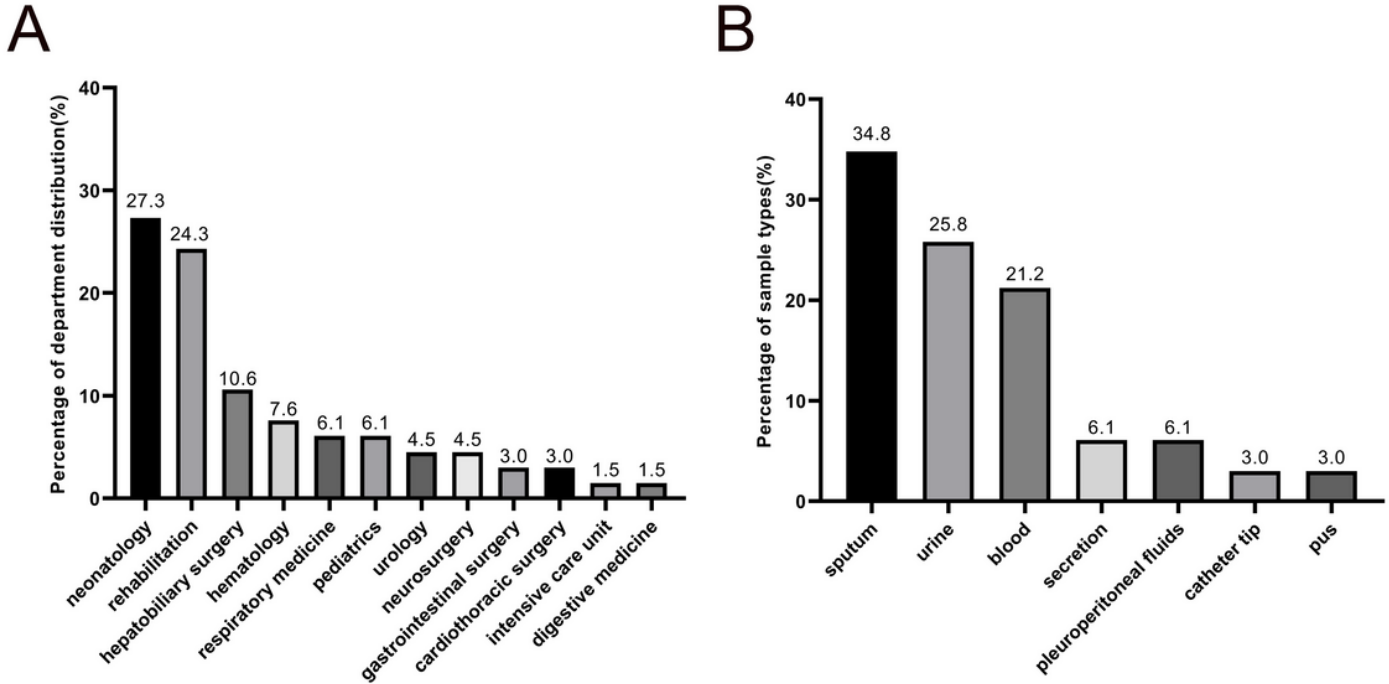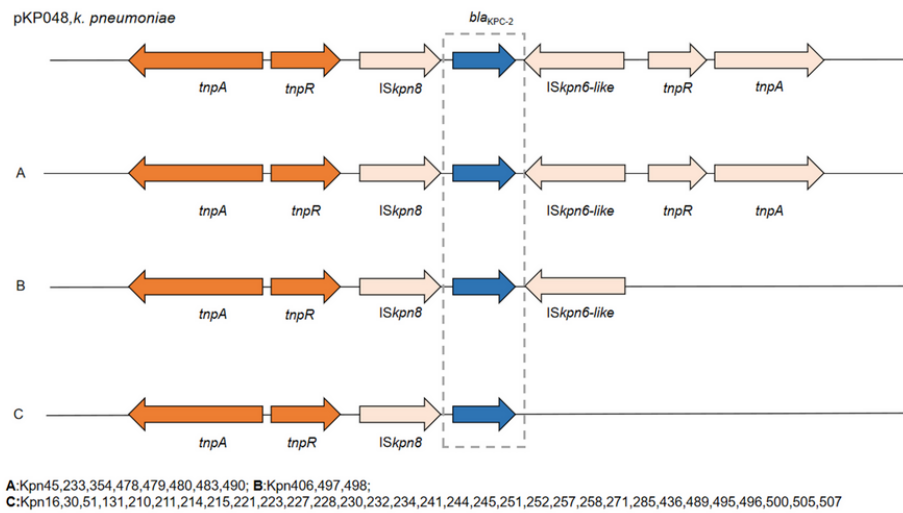## Figure 1

(A) Departments distribution of 66 carbapenem-resistant k. pneumoniae; (B) Sample types of 66 carbapenem-resistant k. pneumoniae.
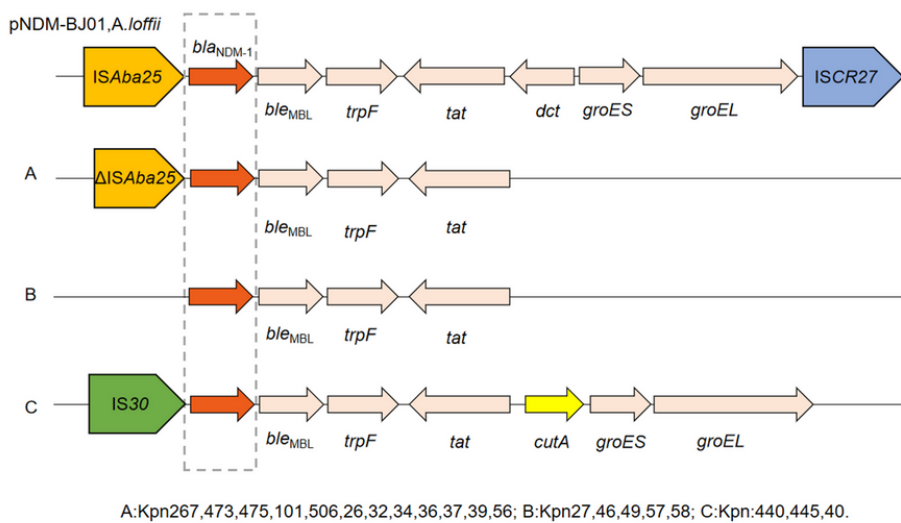
A:Kpn45,233,354,478,479,480,483,490; B:Kpn406,497,498;
C:Kpn16,30,51,131,210,211,214,215,221,223,227,228,230,232,234,241,244,245,251,252,257,258,271,285,436,489,495,496,500,505,507



A:Kpn267,473,475,101,506,26,32,34,36,37,39,56; B:Kpn27,46,49,57,58; C:Kpn:440,445,40.

## Figure 2

Comparison of the genetic elements surrounding the blaKpc_2 and blaNDm_i genes identified in this study.Reference sequences: kipneumoniae pKPO48 (GenBank Accession No.FJ628167) and A. lwoffii pNDM-BJ01, GenBank accession no. JQ001791).

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplement.docx