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ORIGINAL PAPER

# A Polyclonal Spread Emerged: Characteristics of Carbapenem-Resistant *Klebsiella pneumoniae* Isolates from the Intensive Care Unit in a Chinese Tertiary Hospital

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Abstract

Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) isolates often cause nosocomial infections with limited therapeutic options and spread rapidly worldwide. In this study, we revealed a polyclonal emergence of CRKP isolates from the intensive care unit in a Chinese tertiary hospital. We applied a series of methods including automated screening, antimicrobial susceptibility testing, the modified carbapenem inactivation method (mCIM), PCR amplification, DNA sequencing, and multilocus sequence typing (MLST) to characterize 30 non-duplicated CRKP isolates along with the collection of the related medical records. The results showed the polyclonal spread of CRKP isolates belonged to ST722, ST1446, ST111, ST896, ST290, and ST11. Among them, ST722 and ST1446 were two novel types of *K. pneumoniae*, and ST896 isolate harboring $bla_{KPC-2}$ was also found for the first time. Since the polyclonal spread of CRKP in the same ward is rare, the silent clonal evolution with the switching genotypes prompts us to stay alert for outbreaks caused by novel subclones.

K e y w o r d s: polyclonal spread, carbapenem-resistant *Klebsiella pneumoniae*, sequence type, intensive care unit, alert

## Introduction

*Klebsiella pneumoniae* is regarded as an opportunistic Gram-negative pathogen that can cause several infections such as pneumonia, urinary tract infections, and bloodstream infections (Magill et al. 2014). Due to the overuse of carbapenems for treating severe infections caused by extended-spectrum β-lactamases (ESBLs)-producing bacteria, carbapenem-resistant *K. pneumoniae* (CRKP) has rapidly increased globally in the past decade (Logan and Weinstein 2017). The expression of plasmid-mediated carbapenemases has been the primary mechanism of carbapenem resistance, and *K. pneumoniae* carbapenemase (KPC) is the most frequent type found in this species (Martin and Bachman 2018). Among these isolates, ST11 was the predominant clone responsible for disseminating the resistance gene $bla_{KPC-2}$ in China (Qi et al. 2011), whereas ST258 accounted for the large majority of KPC-producing *K. pneumoniae* in the world (Kitchel et al. 2009; Hammerum et al. 2010). Moreover, additional types of carbapenemases have also emerged in *K. pneumoniae* like NDM-1 and OXA-48 categorized as class B metallo-β-lactamase (MBL) and class D enzymes, respectively, which confer specific levels of resistance to carbapenems. Ever since NDM-1 was discovered in *K. pneumoniae* isolate collected from a Swedish patient who had been hospitalized in India in 2008 (Yong et al. 2009), twenty-four NDM variants have been identified. It poses a significant threat

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to public health and a severe challenge for clinical treatments (Wu et al. 2019).

ICU hospitalization itself has been considered as an independent risk factor for CRKP acquisition (Schwaber et al. 2008; Hussein et al. 2009; Debby et al. 2012). The estimated detection rate of CRKP in patients admitted to intensive care units increased by 75% in a 20-year surveillance study in China (Tian et al. 2019). The gastrointestinal carriage rate of CRKP among ICU patients could reach 39.0–74.5%. It can be recognized as a reservoir of CRKP for progression from colonization to infection and the potential route of transmission of carbapenem resistance genes (Bratu et al. 2005; Snitkin et al. 2012; Papadimitriou-Olivgeris et al. 2013). Additionally, ICU is often deemed the epicenter of nosocomial infections caused by multidrug-resistant organisms (MDRO) due to the burdens of the vulnerable populations of critically immunocompromised patients and multiple invasive procedures. Thus, the outcome of patients with CRKP infections is inferior, leading to higher mortality in the setting of ICU associated with limited therapeutic options (Vardakas et al. 2015).

Herein, we report an investigation of CRKP carriage and acquisition in the ICU to illustrate the clonal spread of CRKP isolates, their phenotypic and genotypic characteristics, and to track their evolutionary traits further.

## Experimental

### Materials and Methods

**CRKP isolates and Antimicrobial Susceptibility Testing.** All the carbapenem-resistant *K. pneumoniae* strains were isolated from clinical specimens of the ICU patients in our hospital (Hwa Mei Hospital, University of Chinese Academy of Sciences, Ningbo, China) between October 2016 and March 2019. The identification of these isolates and antimicrobial susceptibility testing were done using the VITEK 2 Compact automated system (BioMérieux, Marcy l'Etoile, France). The routine antibiotic panel comprised ertapenem, amoxicillin/clavulanic acid, amikacin, aztreonam, ciprofloxacin, ceftriaxone, cefazolin, nitrofurantoin, cefepime, cefoxitin, gentamicin, imipenem, levofloxacin, trimethoprim/sulfamethoxazole, tobramycin, piperacillin/tazobactam, ampicillin, and tigecycline. The susceptibilities to ertapenem, imipenem, and tigecycline were confirmed by the disk diffusion method or E-test. According to manufacturer's instructions, *Enterobacter hormaechei* ATCC 700323 and *Escherichia coli* ATCC 25922 were used as controls for species identification and susceptibility testing, respectively. The isolates resistant to either ertapenem or imipenem were defined as CRKP isolates in this study. Antimicrobial susceptibility results were interpreted by the criteria of the Clinical and Laboratory Standards Institute (CLSI 2018). Patient clinical information was acquired from electronic medical records, and the Ethics Committee of our hospital approved the study.

**Detection of resistance determinants.** All the isolates studied were tested for the production of carbapenemases by the modified carbapenem inactivation method (mCIM) recommended by CLSI (CLSI 2018). The genes encoding carbapenemases were investigated by polymerase chain reaction (PCR) using a series of primers as previously reported (Queenan and Bush 2007; Nordmann et al. 2011). The amplification products were sent for DNA sequencing (Qingke Biotech, Hangzhou).

**Isolates genotyping.** CRKP isolates in the study were genotyped by multilocus sequence typing (MLST). Seven housekeeping genes including *gapA, infB, mdh, pgi, phoE, rpoB,* and *tonB* of *K. pneumoniae* were amplified and sequenced based on protocols as described (Diancourt et al. 2005). Sequence types (STs) were identified using the online database at the Pasteur Institute multilocus sequence typing website for *K. pneumoniae* (http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html). The evolutionary relationships between isolates were analyzed by the platform-independent Java software PHYLOVIZ using the goeBURST algorithm at a single-locus variant (SLV) level.

## Results

**Demographic and clinical characteristics of CRKP carriers.** A total of 30 CRKP strains were isolated from 26 patients in the intensive care unit (ICU), and the strains from different isolation sites of the same patient were included in this study. Among these CRKP isolates, 12 (40%) were isolated from sputum specimens, and the remaining isolates were obtained from other types of specimens including blood (three, 10%), wound (two, 6.7%), drainage fluid (four, 13.3%), urine or urinary catheter (seven, 23.3%), and bronchial perfusate (one, 3.3%). 69.2% (18) of the patients were male, and 76.9% (20) were over 60 years old.

All patients had undergone invasive procedures such as tracheal intubation and central venous catheterization. During the ICU admission, multiple antimicrobials were used for the treatment of various intercurrent infections. Among the 26 patients with CRKP acquisition, five died, six declined further therapy, and 15 were discharged from the hospital ward. The times from acquisition of CRKP to death for the five patients who died, listed in order, were four, 107, 29, 36, and 16 days, respectively. Other detailed records of patients and information on the bacteria were summarized in Table I.

Table I
The corresponding bacterial characteristics and medical records of patients with CRKP acquisition.

| Bacterial strain | Age | Isolation site | ST | Carbapen-emases | Underlying conditions | Invasive procedures | Antimicrobial treatment | The length of stay | Outcomes |
|---|---|---|---|---|---|---|---|---|---|
| 1025 | 61 | Wound | NA | NDM-5 | Septic shock, MODS, necrotizing fasciitis | Nasogastric tube, central venous catheter, surgical procedure | IMP, AMC, FEP, MXF, ISE | 23 days | Survived |
| 1029 | 81 | Wound | ST722 | NA | Pulmonary infection, hypertension, diabetes mellitus, | PICC catheter, mechanical ventilation heart disease, encephalorrhagia | IMP, TZP, SCF | 46 days | Stable, discharged |
| 1050 | 73 | Blood | ST11 | NDM-5 | Septic shock, biliary tract infection, COPD, MODS, pulmonary failure | Mechanical ventilation, urinary catheter, deep vein catheter | TZP, IMP, MEM, SCF, TGC | 55 days | Discontinuing treatment |
| 1051 | 89 | Sputum | ST11 | NDM-5 | Pulmonary infection, septic shock, pulmonary failure, hypertension, chronic renal failure, MDS, diabetes mellitus, hypertensive heart disease | Mechanical ventilation, urinary catheter, gastric tube | SCF, TGC | 20 dTays | Death |
| 1052 | 73 | Sputum | ST11 | NDM-5 | Septic shock, biliary tract infection, COPD, MODS, pulmonary failure | Mechanical ventilation, urinary catheter, deep vein catheter | TZP, IMP, MEM, SCF, TGC | 34 days | Discontinuing treatment |
| 1055 | 46 | Drainage fluid | ST1446 | NA | Pulmonary infection, pyothorax, septic shock, pulmonary failure, renal insufficiency, liver cirrhosis | Mechanical ventilation, urinary catheter, deep vein catheter, gastric tube | IMP, TZP, SCF | 38 days | Discontinuing treatment |
| 1062 | 79 | Bronchial perfusate | ST290 | NDM-5 | Pulmonary infection, chronic bronchitis, pulmonary failure, hypertension, cerebral infarction, pleural effusion | Mechanical ventilation, urinary catheter, deep vein catheter, gastric tube | SCF, AMC, FEP | 26 days | Survived |
| 1063 | 79 | Sputum | ST290 | NDM-5 | Pulmonary infection, chronic bronchitis, pulmonary failure, hypertension, cerebral infarction, pleural effusion | Mechanical ventilation, urinary catheter, deep vein catheter, gastric tube | SCF, AMC, FEP | 26 days | Survived |
| 1064 | 61 | Urine | ST290 | NDM-5 | Spinal cord injury, high falling injury, electric injury, pulmonary infection, pulmonary failure, fracture | Mechanical ventilation, urinary catheter, deep vein catheter, gastric tube | TZP, MEM, SCF | 83 days | Stable, discharged |
| 1076 | 42 | Sputum | ST290 | NDM-5 | Craniocerebral trauma, pulmonary contusion, hemorrhagic shock, deep venous thrombosis, renal failure | Mechanical ventilation, urinary catheter, deep vein catheter | TZP, AMC, FEP, ISE, MEM, SCF | 136 days | Death |
| 1102 | 79 | Sputum | ST290 | NDM-5 | Pulmonary infection, pulmonary failure, chronic bronchitis, parkinson | Mechanical ventilation, urinary catheter, deep vein catheter, gastric tube, surgical procedure | SCF, IMP, TZP | 123 days | Survived |
| 1165 | 76 | Drainage fluid | ST11 | KPC | Gastric perforation, peritonitis, fistulo of colon, pulmonary infection, liver cirrhosis | Mechanical ventilation, urinary catheter, deep vein catheter | TZP, IMP, AMC, FEP, TGC, SCF | 18 days | Discontinuing treatment |
| 1233 | 38 | Drainage fluid | ST290 | NA | Retroperitoneal abscess, acute necrotizing pancreatitis, hepatic insufficiency, hyperlipemia | Mechanical ventilation, urinary catheter, deep vein catheter, central venous catheter, drainage tube | TZP, LEV, IMP | 46 days | Discontinuing treatment |
| 1247 | 72 | Urinary catheter | ST11 | KPC | NMS, pulmonary infection, pulmonary failure, diabetes mellitus, hypertension, renal or hepatic insufficiency, hyperlipemia | Mechanical ventilation, urinary catheter, deep vein catheter | TZP | 27 days | Stable, discharged |
| 1762 | 57 | Sputum | ST11 | KPC | Septic shock, pulmonary encephalopathy, COPD, pulmonary failure, hypertension, fungal infection | Mechanical ventilation, urinary catheter, deep vein catheter | SCF, IMP, AMK, FEP | 45 days | Death |
| 1773 | 88 | Urinary catheter | ST11 | NA | Pulmonary infection, pulmonary failure, cerebral infarction, hypertension, diabetes mellitus, alzheimer disease | Mechanical ventilation, urinary catheter, gastric tube | TZP, IMP | 35 days | Stable, discharged |

Table I continued.

| Bacterial strain | Age | Isolation site | ST | Carbapen-emases | Underlying conditions | Invasive procedures | Antimicrobial treatment | The length of stay | Outcomes |
|---|---|---|---|---|---|---|---|---|---|
| 1779 | 39 | Urinary catheter | ST111 | NA | MODS, pulmonary failure, traumatic shock, multiple fracture, sepsis, pulmonary infection, fungal infection | Mechanical ventilation, urinary catheter, deep vein catheter | IMP, SCF, ISE, MXF | 183 days | Survived |
| 1785 | 72 | Urinary catheter | ST11 | KPC | SCAP, pulmonary failure, urinary tract infection, diabetes mellitus, septic shock, fungal infection, pleural effusion | Mechanical ventilation, urinary catheter, deep vein catheter | IMP, SCF, TGC, AZM, MXF | 31 days | Discontinuing treatment |
| 1793 | 70 | Urinary catheter | NA | NA | Pulmonary infection, pulmonary failure, septic shock, fungal infection, cerebral infarction, hypertension, diabetes mellitus, gastrointestinal hemorrhage | Mechanical ventilation, urinary catheter, deep vein catheter, gastrointestinal tube | TZP, SCF, CIP, FEP | 40 days | Survived |
| 1932 | 75 | Sputum | ST11 | NA | Lung cancer, pulmonary infection, bronchiectasis, CHD, hypertension, diabetes mellitus | Mechanical ventilation, urinary catheter, deep vein catheter | TZP, SCF, IMP, TGC | 87 days | Survived |
| 1948 | 78 | Sputum | ST896 | KPC | Cerebral aneurysm, subarachnoid hemorrhage, intracranial infection, pulmonary infection, deep venous thrombosis, fungal infection | Mechanical ventilation, urinary catheter | TZP, MEM, LEV, ISE, IMP, PB | 63 days | Stable, discharged |
| 1975 | 85 | Sputum | ST11 | KPC | Pulmonary infection, pulmonary failure, cardiac failure, cerebral infaction, hypertension | Mechanical ventilation, urinary catheter, deep vein catheter, PICC catheter | IMP, TZP, TGC | 66 days | Stable, discharged |
| 1977 | 85 | Sputum | ST11 | KPC | COPD, pneumonia, pulmonary failure, pulmonary encephalopathy, cardiac failure, renal failure, pulmonary arterial hypertension | Mechanical ventilation, urinary catheter, deep vein catheter, PICC catheter | IMP, SCF, TGC, PB, CAZ/AVI | 46 days | Death |
| 1978 | 78 | Sputum | ST11 | KPC | Pulmonary infection, severe pneumonia, pulmonary failure, renal failure, hypertension, hepatic insufficiency, gastrointestinal hemorrhage, deep venous thrombosis | Mechanical ventilation, urinary catheter, deep vein catheter | SCF, MXF, MEM, IMP, TGC, AMK, PB, CAZ/AVI, MH | 51 days | Discontinuing treatment |
| 1982 | 70 | Sputum | ST11 | NDM-1, KPC | Tonsil carcinoma, hypertension, hyperlipemia, interstitial pneumonia, pulmonary failure, fungal infection | Mechanical ventilation, urinary catheter, deep vein catheter | IMP | 47 days | Stable, discharged |
| 1983 | 82 | Urinary catheter | ST11 | KPC | COPD, pulmonary failure, pulmonary encephalopathy, diabetes mellitus, hypertension, fracture, septic shock, gastrointestinal hemorrhage | Mechanical ventilation, urinary catheter, deep vein catheter, PICC catheter | TZP, IMP, SCF | 37 days | Death |
| 1984 | 66 | Drainage fluid | NA | NDM-1 | Peritonitis, septic shock, pulmonary failure, renal failure, hypertension, fungal infection, intestinal perforation | Mechanical ventilation, urinary catheter, deep vein catheter, PICC catheter, drainage tube | IMP, TGC, TZP, SCF | 43 days | Stable, discharged |
| 1987 | 89 | Sputum | ST11 | KPC | Severe pneumonia, pulmonary failure, CHD, hypertension, rectal cancer, myocardial infarction, fungal infection | Mechanical ventilation, urinary catheter, deep vein catheter | TZP, MEM, SCF, PB, TGC, CAZ/AVI | 22 days | Survived |
| 1990 | 85 | Blood | ST11 | KPC | COPD, pneumonia, pulmonary failure, pulmonary encephalopathy, cardiac failure, renal failure, pulmonary arterial hypertension | Mechanical ventilation, urinary catheter, deep vein catheter, PICC catheter | IMP, SCF, TGC, PB, CAZ/AVI | 46 days | Death |
| 1997 | 82 | Blood | ST11 | KPC | COPD, pulmonary failure, pulmonary encephalopathy, diabetes mellitus, hypertension, fracture, septic shock, gastrointestinal hemorrhage | Mechanical ventilation, urinary catheter, deep vein catheter, PICC catheter | TZP, IMP, SCF | 37 days | Death |

MODS – Multiple organ dysfunction syndrome, COPD – Chronic obstructive pulmonary disease, MDS – Myelodysplastic syndrome, NMS – Neuroletic malignant syndrome, CHD – Coronary heart disease, IMP – Imipenem, AMC – Amoxicillin/clavulanic acid, FEP – cefepime, MXF – moxifloxacin, ISE – isepamicin, TZP – piperacillin/tazobactam, SCF – cefperazone/sulbactam – MEM, meropenem, TGC – tigecycline, LEV – levofloxacin, AMK – amikacin, CIP – ciprofloxacin, PB – polymyxin B, CAZ/AVI – ceftazidime/avibactam, MH – minocycline, ST – sequence type, NA – not available

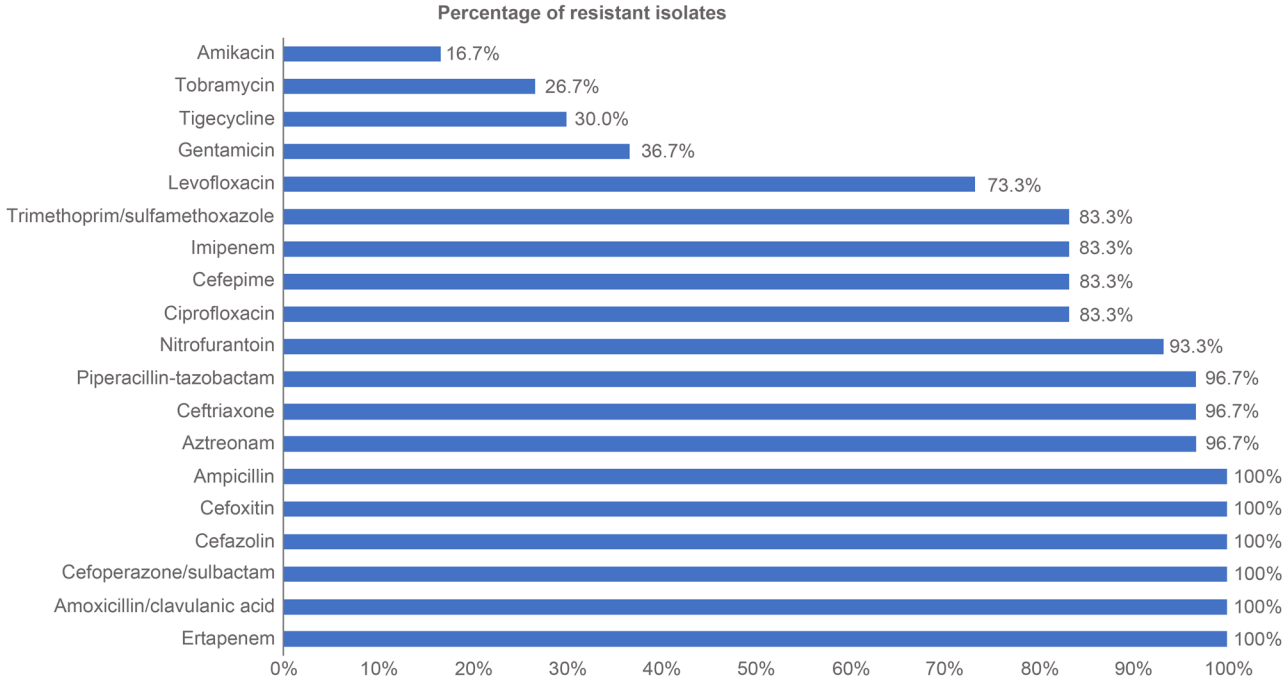


Fig. 1. Percentage of CRKP isolates resistant to a panel of antibiotics (30 isolates).

The X-axis displays the percentage of the isolates resistant to a given antibiotic (Y-axis). Distribution of the MICs of antibiotics were as follows: Ertapenem (MIC ≥ 2 μg/ml, n = 30), Amoxicillin/clavulanic acid (MIC ≥ 32/16 μg/ml, n = 30), Cefoperazone (MIC ≥ 64 μg/ml, n = 30), Cefazolin (MIC ≥ 8 μg/ml, n = 30), Cefoxitin (MIC ≥ 32 μg/ml, n = 30), Ampicillin (MIC ≥ 32 μg/ml, n = 30), Aztreonam (MIC ≥ 16 μg/ml, n = 29), Ceftriaxone (MIC ≥ 4 μg/ml, n = 29), Piperacillin-tazobactam (MIC ≥ 128/4 μg/ml, n = 29), Nitrofurantoin (MIC ≥ 128 μg/ml, n = 28), Ciprofloxacin (MIC ≥ 1 μg/ml, n = 25), Cefepime (MIC ≥ 16 μg/ml, n = 25), Imipenem (MIC ≥ 4 μg/ml, n = 25), Trimethoprim/sulfamethoxazole (MIC ≥ 4/76 μg/ml, n = 25), Levofloxacin (MIC ≥ 2 μg/ml, n = 22), Gentamicin (MIC ≥ 16 μg/ml, n = 11), Tigecycline (MIC ≥ 8 μg/ml, n = 9), Tobramycin (MIC ≥ 16 μg/ml, n = 8), Amikacin (MIC ≥ 64 μg/ml, n = 5).

**Antimicrobial susceptibility.** The isolates in this study were resistant to nearly all clinically available antimicrobials; more than half of the isolates were only susceptible to one or two kinds of antimicrobials and were called extensive drug-resistant isolates. All isolates presented resistance to ertapenem, amoxicillin/clavulanic acid, cefoperazone/sulbactam cefazolin, cefoxitin, and ampicillin. The isolates were relatively susceptible to four antimicrobials: gentamicin, tigecycline, tobramycin, and amikacin, to which the resistance rates were 36.7, 30.0, 26.7, and 16.7%, respectively. The percentage of resistant isolates to each antibiotic was shown in Fig. 1.

**Profiling of resistance determinants.** The majority of 30 CRKP (n = 23, 76.7%) isolates were positive for the mCIM test. The results of PCR and DNA sequencing showed that nine isolates (30%) harbored the $bla_{\text{NDM-5}}$ gene, 12 isolates 40% harbored the $bla_{\text{KPC-2}}$ gene, and one isolate had the $bla_{\text{NDM-1}}$ gene. The coexistence of $bla_{\text{NDM-1}}$ and $bla_{\text{KPC-2}}$ in one isolate was also noticed. The carbapenemase-encoding genes were not detectable in seven isolates.

**Bacterial clonal relatedness.** Among 30 CRKP isolates, six sequence types (STs) were detected, namely ST722, ST1446, ST111, ST896, ST290, and ST11 as shown by MLST. ST290 and ST11 accounted for 20% (6/30) and 56.7% (17/30) of all isolates tested, whereas the other STs were sporadic. Three ST11 isolates carried $bla_{\text{NDM-5}}$, yet $bla_{\text{KPC-2}}$ was more likely to be identified among ST11 clones. By contrast, ST290 clones harbored only $bla_{\text{NDM-5}}$. Seven isolates that were negative for mCIM test belonged to diverse STs (ST722, ST1446, ST290, ST11, and ST111). Notably, two *K. pneumoniae* NDM-producers failed to be classified into any sequence types and there was the sole isolate that was negative for either mCIM or sequence typing. Figure 2 displays the annotated minimum spanning tree showing that ST1446, ST111, ST896, ST290, and ST11 clones belonged to different groups, except for ST722 listed in the sub-group of ST11 group.

## Discussion

In 2017, WHO published a global priority pathogens list of antibiotic-resistant bacteria, in which CRE was ranked among the Priority 1 pathogens (WHO 2017). Carbapenem-resistant *K. pneumoniae*, which is the most common carbapenem-resistant *Enterobacteriaceae* (CRE), has already generated a worrisome crisis of epidemiological, clinical, and infection control issues worldwide (Bradford et al. 2004; Maltezou et al. 2009),

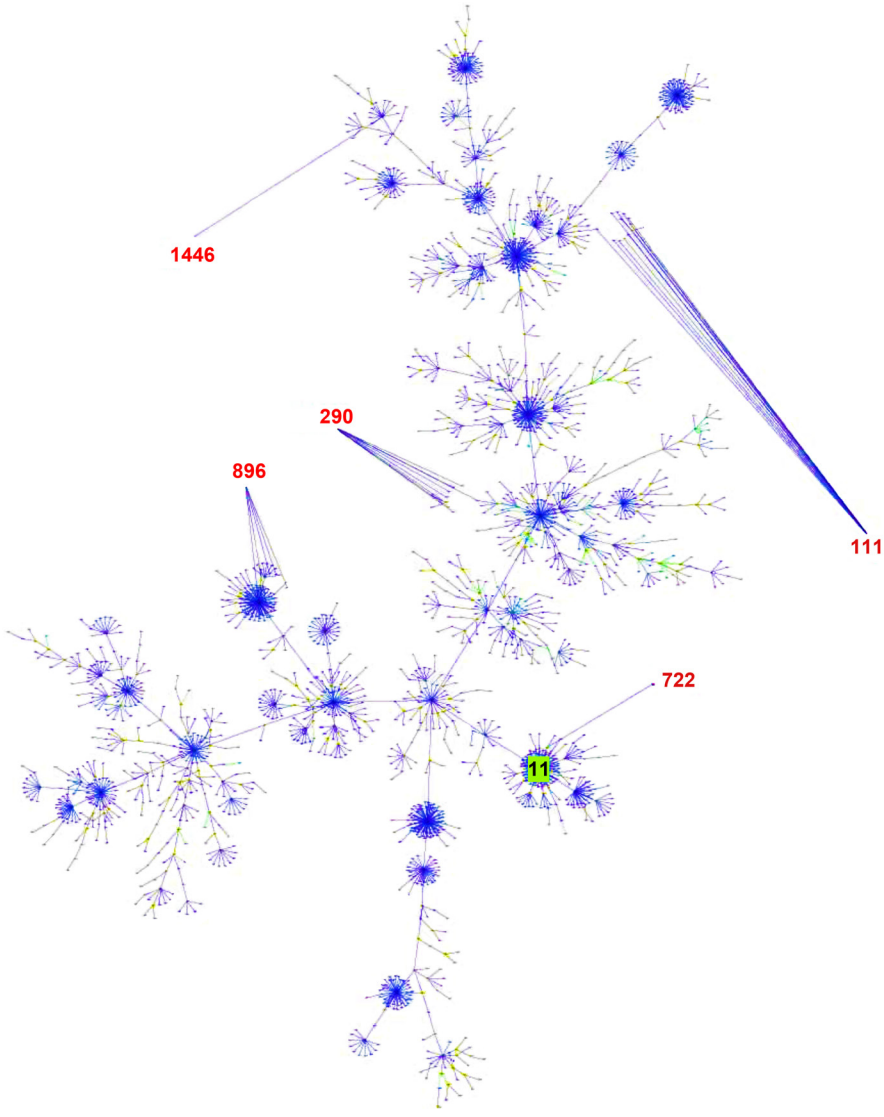


Fig. 2. The minimum spanning tree.

The relationships between different clones of six unique sequence types (ST290, ST11, ST722, ST1446, ST111, and ST896 emerging in the ICU. The graph was analyzed by PHYLOVIZ using the goeBURST algorithm at single-locus variant (SLV) level. ST11 node was colored in light green; other ST nodes were colored in dark green and manually dragged to the selected positions that were represented in red.

including the ICUs across China (Zhang et al. 2011; Yu et al. 2012; Yu et al. 2019).

In this study, we have described the polyclonal spread of CRKP isolates of six distinct sequence types (ST290, ST11, ST722, ST1446, ST111 and ST896) in the same ward (ICU). Among these isolates, two novel clones of ST722 and ST1446 were found, and they did not produce carbapenemases since the negative results of the mCIM test were obtained. It inferred that other mechanisms of resistance might be relevant such as hyperproduction of ESBL enzymes, AmpC β-lactamases, or alteration of outer membrane porins as well as regulation of efflux systems (Kaczmarek et al. 2006; Bush and Jacoby 2010; Filgona et al. 2015). The minimum spanning tree demonstrated that ST722 clone probably shared the same ancestor with ST11 clone in the evolutionary process. As for ST111, it has long been identified among carbapenem-resistant, and ESBL-producing *K. pneumoniae* isolates, e.g., obtained from Riyadh (uz Zaman et al. 2014), South India (Kumar et al. 2018), New York (Diago-Navarro et al. 2014), and New Zealand (Lester et al. 2011). Further, the ST11 *K. pneumoniae*, one type of clone responsible for the outbreak of multi-drug carbapenem-resistant *K. pneumoniae* in Riyadh, carried the OXA-48 gene, suggesting that the acquisition of carbapenem-resistance genes by *K. pneumoniae* of different STs may contribute to the emergence of diverse CRKP clones. By contrast, only one ST896 CRKP isolate that was identified from Heilongjiang Province in China harbored the $bla_{\text{IMP-4}}$, $bla_{\text{SHV}}$, and $bla_{\text{TEM}}$ genes (Gong et al. 2018).

Therefore, this is also the first report on the observation of the $bla_{\text{KPC-2}}$-harboring ST896 CRKP clone in China.

ST290 CRKP isolates possessing the $bla_{NDM-5}$ gene were found disseminated in the ICU in this study. Interestingly, an outbreak of ST290 CRKP with blaNDM-5-positive took place in the same hospital's wound ward, as we have reported previously (Wang et al. 2019), raising speculation that intra-hospital transmission might be one of the contributory factors to this situation. However, in line with previous reports (Qi et al. 2011; Hu et al. 2016), ST11 was still the predominant type of CRKP clone, accounting for more than half of the isolates. Of note, three CRKP isolates of ST11 clone harbored $bla_{NDM-5}$ instead of $bla_{KPC-2}$, showing that the genotypic shift occurred in ST11 clone, which was probably attributed to the free movement of plasmid-borne genes responsible for carbapenem resistance among the clones within species as stated previously (Mathers et al. 2015). Moreover, the rise in the number of NDM-5-positive isolates of the ST11 clone generates the awareness of the propagation of the MBL genes in high-risk *K. pneumoniae* clones. ST11 clone could be a suitable vector for the rapid spread of carbapenem resistance mediated by genetic components such as plasmids, integrons, or transposons.

The ICU patients in this study suffered from at least three underlying diseases and underwent several surgical procedures causing damage of mucosal barriers, which could increase the risk of CRKP colonization and infection (Kofteridis et al. 2014). We found that 71.4% of patients aged over 80 years died, as did 85.7% of the patients who stayed in the ICU for more than one month, reflecting the challenges in managing the comorbidities of ICU patients. Additionally, the prolonged hospitalization of CRKP carriers may increase the frequency of patient-to-patient transmission of antimicrobial resistance. According to the medical records, local empirical antibiotic therapy could be administered in the ICU, e.g., cefepime combined with amoxicillin/clavulanic acid (Ji et al. 2015). Ceftazidime/avibactam, a relatively new salvage therapy against CRKP, was also used in some patients with different outcomes (those who survived, died, or for whom the treatment was discontinued). However, the resistance to these antibiotics has previously been revealed due to the MBL production, KPC-2 point mutation, and high KPC expression (Zhang et al. 2019). It indicates that the treatment with ceftazidime/avibactam against MBL-producing CRKP might fail if local variations in epidemiology and genomic evolution of antimicrobial resistance are not tracked. Hence, the focus on characteristics of CRKP in the ICU not only plays an essential role in guiding clinical practice for antibiotic use but also provides the recent information about the evolution of antimicrobial resistance and helps to assign urgently needed tactics for combating the spread of CRKP clones.

The limitations of this study are that we did not distinguish colonization from infection with CRKP, and the small number of isolates was insufficient to illustrate the prevalence of CRKP in the ICU comprehensively.

In conclusion, this is the first report on the polyclonal emergence of six unique STs (ST722, ST1446, ST111, ST896, ST290, and ST11) found in the same ward and on two ST722 and ST1446 clones as being the novel STs in China. Pathogens in the ICU evolve all the time due to intra- and inter-species interactions by the horizontal transfer of antibiotic resistance genes. The emergence of sporadic clones producing MBLs, e.g., producing NDM-5 CRKP isolates of ST290 and ST11 clones, is a warning signal of the genotypic switch in epidemic KPC-producing CRKP population. Therefore, valid interventions should be developed to avoid outbreaks caused by novel subclones.

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**Conflict of interest**

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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