

Co-Production of NDM-I, CTX-M-9 Family and mcr-I in a *Klebsiella pneumoniae* ST4564 Strain in China

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Purpose: To identify novel sequence types 4564 (ST4564) carbapenem-resistant *Klebsiella pneumoniae* (CRKP). Characterizing the feature of the clinic, resistance, and virulence of a co-producing NDM-1 and CTX-M-9 family and mcr-1 ST4564 strain.

Methods: A novel ST4564 CRKP was collected from June 2018 to July 2018. We investigated its antimicrobial susceptibility by the microdilution method. Using the modified carbapenem inactivation method (mCIM) to screen phenotype of carbapenemases. Resistance mechanisms, virulence-associated genes, multilocus sequence typing (MLST), and capsular serotypes were characterized by polymerase chain reaction (PCR) and DNA sequencing. Next-generation sequencing (NGS) was carried out to determine the genetic features of carbapenem resistance and virulence.

Results: ST4564, co-carrying NDM-1, CTX-M-9 and mcr-1, was resistant to carbapenems, cephamycin, third- or fourth-generation cephalosporins, β -lactam combination agents, quinolones and tigecycline but remained susceptible to amikacin (AMK) and colistin (COL). Through the NGS analysis with the G+C content of 56.65%, multiple resistance and virulence genomes were detected. The genes encoding the β -lactams, aminoglycosides, quinolones, macrolides, sulfonamide, polysaccharide capsule, type-I fimbriae cluster, siderophore genes, transporter and pumps, T6SS and pullulanase secretion protein. goeBURST analysis showed that ST4564 belonged to the CC1571 and it was not related to the prevalent high-risk clones.

Conclusion: We first identified the novel ST4564 CRKP. Our finding suggested that the urgent need for infection control of the new clone to prevent it from becoming a high-risk clone of CRKP.

Keywords: *Klebsiella pneumoniae*, carbapenem resistant, ST4564, next-generation sequencing

Introduction

Klebsiella pneumoniae (KP), a gram-negative bacterium, is an opportunistic pathogen.¹ It is known to colonize the skin, respiratory and gastrointestinal tracts and could cause severe infections, including urinary tract infections, pneumonia, soft tissue infections, bacteremia, abscesses, and intra-abdominal infections. Carbapenems are considered to be an effective treatment for clinical infection caused by KP.² To make matters worse, the numbers of carbapenem-resistance *Klebsiella pneumoniae* (CRKP) strains have increased steadily. The infections caused by CRKP often lead to high morbidity and high mortality.

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CRKP strains are spreading as clones, especially the high-risk clones, which include but not limited to, ST11, ST15, ST16, ST23, ST25, ST101, ST147, ST307, ST258.^{3–5} First ST258 KP clone was reported in the United States and was disseminated around the world quickly.² After that, ST11, ST23, ST15, and ST101 clones were continuously discovered and further investigated. These clones could cause outbreak infections and lead to unimaginable outcomes. Furthermore, the dissemination of STs has geographical differences. ST258 has become the most prevalent clone of CRKP in North America and Europe.⁶ ST11 was frequently found in Asia, especially China, accounting for up to 60% of CRKP clones.⁷

Previous studies have highlighted the complex resistance mechanisms of CRKP and the transfer of resistance genes through epidemic plasmids in highly disseminating different clones.⁸ These factors could increase the difficulty in treatment.² The most main resistance mechanism in KP was the production of carbapenemases, including class A, class B, and class D. Among them, KPC-2 was the dominant carbapenemase in China.² Other resistance mechanisms were the expression of plasmid-mediated AmpC and extended-spectrum beta-lactamases (ESBLs). CTX-M-15- or CTX-M-14-producing ST11 or ST147 are high-risk multidrug-resistant KP (MDR) clones, which have spread globally.^{2,9} Recently, outbreaks of different STs with different resistance mechanisms have been reported in China.^{9–11} In this case, the identification of new clones' resistance mechanism was more important.

Likewise, types of capsule, fimbriae, lipopolysaccharide (LPS) and siderophores are important and well-characterized virulence factors in KP.¹² Virulence factors are associated with high pathogenicity and resulted in poor outcomes and shorten the process of diseases. *rmpA*, *rmpA2*, *magA* were often considered as the gene markers of *hyper-virulent Klebsiella pneumoniae* (hvKP). They could participate in the production of the capsule. In the part of studies, ST11, ST147, and ST101 clones were often related to hvKP and ST11 was the most predominant type.^{13,14}

Due to the relationship between the epidemic STs and the features of resistance or virulence was unclear. The aim of our study was to identify the novel sequences types of CRKP and characterize the features of genetics in it.

Materials and Methods

Bacteria Identification

From June 2018 to July 2018, one CRKP strain was isolated from the Second Hospital of Shanxi Medical

University. The confirmation of this CRKP isolate was conducted by the Vitek 2 instrument (BioMerieux Italia S.p.A) and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) (Bruker Daltonik, Bremen, Germany). This CRKP isolate was identified based on the non-susceptibility to carbapenems (imipenem, meropenem, or ertapenem), according to the Clinical and Laboratory Standards Institute (CLSI-2020) guidelines.^{15,16}

Susceptibility to Antibacterial Agents

One CRKP strain was tested for susceptibility by the broth microdilution method using cation-adjusted Mueller-Hinton broth. The susceptibility breakpoints were interpreted following the criteria of the CLSI-2020.¹⁵ Except for tigecycline and colistin, which were interpreted based on the European Committee for Antimicrobial Susceptibility Testing (EUCAST) criteria.¹⁷ (EUCAST-2021, v 11.0, <http://www.eucast.org>.) *Escherichia coli* ATCC 25922 was used as a control for antimicrobial susceptibility testing. We tested 17 common antimicrobial agents, which were listed as follows: meropenem (MEM), imipenem (IPM), ertapenem (ETP), ceftriaxone (CRO), ceftazidime (CAZ), cefotaxime (CTX), cefepime (FEP), aztreonam (ATM), cefoperazone/sulbactam (CSL), piperacillin/tazobactam (TZP), ciprofloxacin (CIP), levofloxacin (LEV), fosfomycin (FOS), chloramphenicol (CHL), amikacin (AMK), tigecycline (TGC) and colistin (COL).

Phenotypic Characterization

The modified carbapenem inactivation method (mCIM) was used for phenotypic detection of carbapenemases. The string test was used to detect hypermucoviscosity phenotype. When the length of the viscous string was ≥ 5 mm, the strain was defined as hypermucoviscosity *Klebsiella pneumoniae* (HMKP).⁹

PCR Amplifications and Sequencing

The presence of genes encoding CTX-M-type extended-spectrum β -lactamases (ESBLs), plasmid-borne AmpC β -lactamases, and carbapenemases were detected by polymerase chain reaction (PCR) as previously described.¹⁸ The positive PCR amplicons were visualized by agarose gel electrophoresis and sequenced using Sanger sequencing. The DNA sequences obtained were compared with those available in the NCBI GenBank database using BLAST searches. (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). Multiplex PCR for virulence genes including *iucA*, *iutA*, *rmpA*, *rmpA2* and *iroN* was performed

after phenotypic characterization. The HMKP isolates with positive results of virulence genes were defined as hvKP in our study.^{19–21} The capsular serotype of the KP isolate was determined by *wzi* gene sequencing and allele identification using Institute Pasteur, Paris, France, BIGS database. (<https://bigsd.b.pasteur.fr/Klebsiella>).

Next-Generation Sequencing (NGS)

The genomes of resistance and virulence were fully sequenced by Ion S5 plus (Thermo fisher) with 420 flows, which obtained read lengths ranging from 100 bp to 350 bp after read filtering. De novo assembly was performed using SPAdes genome assembler. The scaffold N50 (bp) was 169157 bp; the GC content was 56.65% and the assemble percentage of the whole genome was 97%. Antimicrobial resistance and virulence genes were screened and annotated using CARD and VFDB tools with 97% coverage and an identity threshold of 97%. All draft genome sequences were deposited into the NCBI Genome database and organized under BioProject PRJNA689622 and Bio-Samples number was SAMN17183116.

Multilocus Sequence Typing (MLST)

Multilocus sequence typing (MLST) was performed for this CRKP isolate by amplifying and sequencing seven conserved housekeeping genes (*gapA*, *infB*, *mdh*, *pgi*, *phoE*, *rpoB* and *tonB*). Alleles and sequence types (STs) are available on the MLST online database. (<http://bigsd.b.pasteur.fr/klebsiella/klebsiella.html>). One CRKP 102 strain containing alleles that did not match an existing sequence in the MLST database was submitted in the respective database for allele and ST assignments. Clonal complexes (CC) were determined to identify the molecular epidemiological relationships with the goeBURST 1.2.1 version software (<http://www.phyloviz.net/goeburst/>). ST profiles sharing 100% genetic identity in at least 6 of 7 MLST loci were grouped into a clonal complex (CC), named after its presumed ancestral genotype.

Results

Bacterial and Clinical Information

The first identified ST4564 CRKP102 strain was cultured from an alveolar lavage fluid sample from a 57-year-old male with no recent travel history. He was admitted on 6 June 2018 to the department of hematopathology, due to an unknown fever and lowest level of thrombocyte. Carbapenem antibiotic treatment, meropenem, had been

used as empirical therapy prior to hospitalization. Due to the lack of meropenem, he switched to imipenem for further treatment. Because of the poor effect of imipenem, linezolid was added to the treatment, and then ceftriaxone was used as de-escalation therapy after the fungus was cultured. Then, he received the combination treatment of tigecycline with ceftriaxone and cefoperazone/sulbactam, respectively, before the strain was isolated. Among these combination treatments, cefoperazone/sulbactam was used as de-escalation therapy, because of the improvement in the inflammatory response. On 10 July, the MDR CRKP was isolated, which was identified by clinical microbiologists. And he received meropenem therapy. However, this patient remained in long-term rehabilitation and has only been detected once CRKP. Furthermore, he has fever 5 times during the whole hospitalization (Figure 1). Eventually, this patient was discharged from the hospital.

Antimicrobial Sensitivity and Resistance Mechanism Results

ST4564 CRKP102 strain showed resistance to carbapenems, third- or fourth-generation cephalosporins, β -lactam combination agents and ciprofloxacin, levofloxacin but remained susceptible to amikacin, tigecycline and colistin (Table 1). CRKP102 was confirmed as a metallo- β -lactamase-producing strain with the mCIM test. CRKP102 subsequently was only positive for the *bla*_{NDM-1}, *bla*_{CTX-M-14,17} and *mcr-1* genes by PCR amplification and sequencing. Other resistance genes were not detected (Table 2).

Characterization of Virulence

CRKP102 isolate was positive for the string test and was negative for the *iucA*, *iutA*, *rmpA*, *iroN*, *rmpA2* genes by PCR and sequencing. By amplifying K1, K2, K47, K64 and *wzi* gene, none of the serotypes was detected in this study (Table 2).

Genetic Determinants of Resistance and Virulence

The next-generation sequencing analysis was performed on CRKP102 strain. It harbored multiple genes encoding resistance to β -lactams (*bla*_{CTX-M-14}, *bla*_{CTX-M-17}, *bla*_{NDM}, *bla*_{TEM}, *bla*_{LEN}), aminoglycosides [*aac* (3)-IIb, *aph* (6)-Id, *aph* (3')-Ia, *aadA6/aadA10*], quinolones (*qnrBs*), macrolides [*mphA*, *mrx*], and sulfonamide [*sulI*, *sul2*]. From this

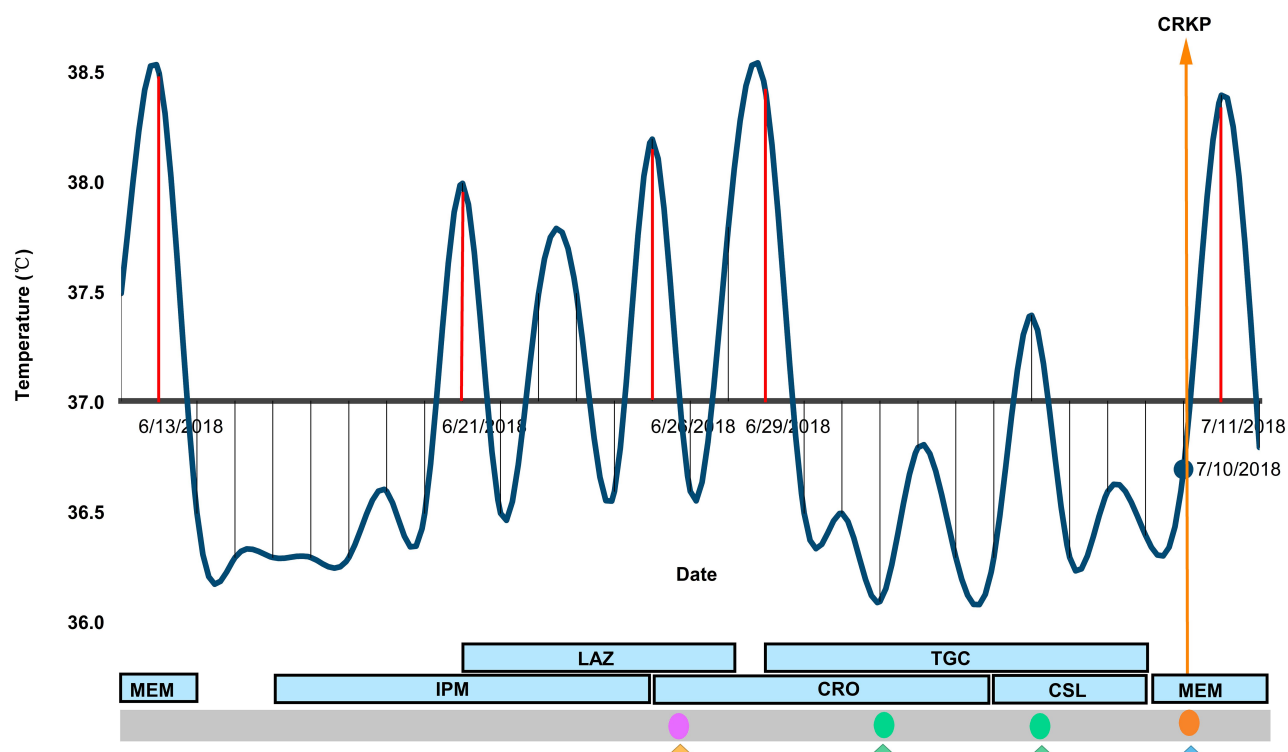


Figure 1 The distribution of temperature and medication. Blackline indicates the temperature of each day; the red line indicates the temperature $\geq 38^{\circ}\text{C}$; orange arrow line indicates the date of the isolation of carbapenem-resistant klebsiella pneumoniae strain; blue block indicated the medication during the hospitalization. Pink circle indicate isolated baumannii strains; green circles indicated baumannii and enterobacter cloacae strains; orange circle indicated carbapenem-resistant klebsiella pneumoniae strain. Triangles indicate types of samples (yellow denotes sputum, blue denotes alveolar lavage fluid, green denotes throat swab).

Abbreviations: MEM, meropenem; IPM, imipenem; CRO ceftriaxone; CSL, cefoperazone/sulbactam; TGC, tigecycline; LAZ, linezolid.

finding, two variants of CTX-M-9 family were detected (Table 3).

Moreover, we also detected multiple virulence genes, such as: the genes associated with capsule, type-I fimbriae cluster, the siderophore genes (*iutA*, *fepD*, *iroE*, enterobactin genes), transporter and pumps (ABC transporter, RND efflux system (*acrAB*, *rcsAB*)), *T6SS* (the genes encoding pullulanase secretion protein). (Table 4)

Compared the resistance and virulence genome with the CARD and VFDB databases, CRKP102 strain had 7 *bla*_{LEN} genes, 7 *bla*_{NDM} genes, 5 *qnrB* genes, and the *galF* gene. These findings had a significant difference with the CARD and VFDB databases.

Molecular Typing Results

This strain had unique allelic profile (gapA_28, infB_24, mdh_21, pgi_76, phoE_146, rpoB_17, tonB_67), we submitted this ST profile in the respective database and this file was allocated 4564. This is the first time identified the ST4564. As determined by goeBURST analysis of MLST data (Figure 2), the novel sequence type 4564 was belonging to clonal cluster CC1571. Compared the allele of

ST4564 with high-risk clones (ST7, ST11, ST15, ST16, ST101, ST147, ST25, ST23, ST258, ST307, ST512, ST524, ST690, ST1764), we found that ST4564 was an independent clone and was not related to any of high-risk clones.

Discussion

In this study, we have first identified the novel sequence type 4564 (ST4564) in one CRKP strain, co-carrying NDM-, CTX-M, *mcr-1*, and used NGS to explore its resistance and virulence characteristics. In terms of causative pathogens, the emerging of CRKP poses a huge life-threatening to public health.⁶ Certain STs of KP clones are more likely to cause outbreak infections.² These clones should be timely identified, monitored and controlled. Thus, our finding enriches the available sequence types library of CRKP clones, and the molecular characteristics provide a strong microbiological basis for further studies of infections caused by CRKP clones.

Different clones have diverse resistance mechanisms. As we all know, KPC-2 enzyme is the most prevalent in China.²² NDM enzymes belong to Metallo- β -Lactamases

Table 1 Antimicrobial Susceptibility Testing Results of Carbapenem-Resistant *K. pneumoniae* Isolate (Mg/L)

Isolates	Carbapenem			Cephalosporins				Monobactams		β -Lactam/ β -Lactam inhibitor			Quinolones		Glycylcycline	Fosfomycin	Chloramphenicol	Aminoglycoside	Colistin
	MEM	IPM	ETP	CRO	CAZ	CTX	FEP	ATM	CSL	TZP	CIP	LEV	TGC	FOS	CHL	AMK	COL		
CRKP102	8	16	64	≥ 64	≥ 64	>256	256	8	>256	≥ 128	0.5	1	0.5	8	256	≤ 2	0.25		
ATCC 25922	≤ 0.25	≤ 1	≤ 0.5	≤ 1	≤ 1	≤ 1	≤ 1	≤ 1	≤ 0.25	≤ 4	≤ 0.25	≤ 0.25	≤ 0.25	2	2	≤ 2	0.125		

Abbreviations: MEM, meropenem; IPM, imipenem; ETP, ertapenem; CRO, ceftriaxone; CAZ, ceftazidime; CTX, cefotaxime; FEP, cefepime; ATM, aztreonam; CSL, ceftiofur/sulbactam; TZP, piperacillin/tazobactam; CIP, ciprofloxacin; LEV, levofloxacin; TGC, tigecycline; FOS, fosfomycin; CHL, chloramphenicol; AMK, amikacin; COL, colistin.

Table 2 Distribution of Resistance Mechanism and Virulence Genes Harbored by ST4564 CRKP Strain

Isolate	mCIM/ eCIM	Carbapenemase				Ampc			ESBLs		MCR	string test	Virulence-Associated Genes				Serotypes	
		KPC	NDM	IMP	SIM	DHA	ACT	CMY	CTX-M-9,13-14,16-19, Toho-I	CTX-M-1,3,10-12,15			iucA	iutA	rmpA	rmpA2		iron
CRKP102	+/-	-	+	-	+	-	-	-	+	-	+	+	-	-	-	-	-	wzi

Notes: *Indicates the presence of the corresponding gene or positive for this test; - Indicates the absence of the corresponding gene or negative for this test.

Table 3 The Presence of Resistance Genome in ST4564

Antibiotics	Genetic Mechanisms of Resistance
β -Lactam	<i>bla</i> _{CTX-M-14} <i>bla</i> _{CTX-M-17} <i>bla</i> _{NDM-1} <i>bla</i> _{TEM} <i>bla</i> _{LEN}
Fluoroquinolone	<i>oqx</i> A <i>oqx</i> B <i>qnr</i> B13 <i>qnr</i> B3 <i>qnr</i> B30 <i>qnr</i> B5 <i>qnr</i> B6
Aminoglycoside	APH (3'')-Ib APH (6)-Id AAC (3)-IIb <i>aadA6/aadA10</i>
Sulfonamide	<i>sul</i> 1 <i>sul</i> 2
Macrolides	<i>mph</i> A <i>mr</i> x
Antibiotic efflux	<i>bae</i> R <i>cat</i> II <i>CRP</i>

(MBL), it has become the epidemic resistance mechanism, since it was first found.²¹ NDM-1 is the main carbapenemase-producing resistance in several regions, such as India, Pakistan and Sri Lanka.⁸ A report from a longitudinal large-scale CRE study in China

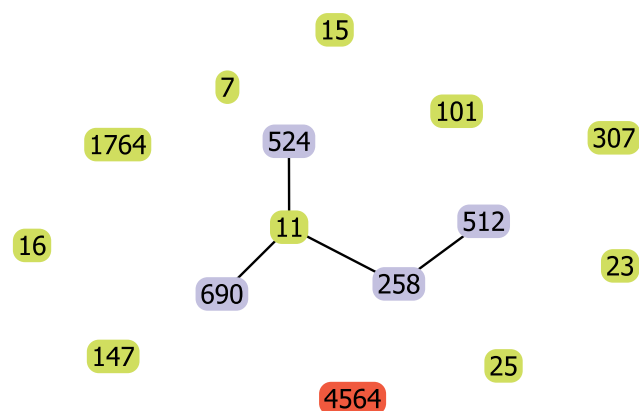


Figure 2 goeBurst-based population structure of ST4564 CRKP and high-risk STs. Blue circle indicated isolate STs; purple circle indicated one or two different loci with ST11, red circle indicated novel ST. Black lines indicated the relationship between them.

Table 4 The Presence of Virulence Genome in ST4564

Classes	Virulence Factors
Lipopolysaccharide	<i>ABC transporter</i>
Capsule	<i>phosphomannomutase</i> <i>cpsB</i> mannose-1-phosphate guanylyltransferase <i>uge</i> <i>Ga</i> IF
type-I fimbriae cluster	<i>fim</i> D
Iron acquisition systems	<i>iut</i> A <i>fep</i> D <i>iro</i> E <i>ent</i> F <i>ent</i> S <i>fep</i> A
Pumps	<i>RND efflux system</i> [<i>Acr</i> AB]
Transporters	<i>LuxR</i> family transcriptional regulator[<i>Rcs</i> AB (CVF856)] <i>acr</i> A
Pullulanase	<i>pu</i> 10 <i>pu</i> 1D <i>pu</i> 1E <i>pu</i> 1C <i>pu</i> 1F <i>pu</i> 1K <i>pu</i> 1L <i>pu</i> 1N

(2012–2016) showed that the isolated CRKP strains in Northwestern China produced a high level of NDM enzymes.²³ Moreover, one report from our local region showed that NDM-1 enzyme was the major resistance mechanism and NDM-1 enzyme was the predominant resistance mechanism.²¹ To date, more than 21 different NDM variants have been observed worldwide.^{24,25} These various resistance mechanisms of CRKP are attributed to the diversity of geographic location.

Furthermore, ST258 and ST512 were reported in KPC-producing *Klebsiella pneumoniae* strains. A report showed that *bla*_{KPC} is the common gene and its rapid dissemination has typically been caused by the clonal expansion of clonal complexes (CCs) 258 strains, including ST258 and ST512.²⁶ ST15 was reported in CTX-M-15-producing clones, and ST11 was reported in KP carried different carbapenemases.¹² In our study, ST4564 clone co-produced NDM-1 and CTX-M-9 variants and displayed MDR phenotype. It exhibited high MIC values against

carbapenems, cephalosporins, β -lactam, quinolones and CHL, whereas was susceptible to AMK, TGC and COL. This was in consistent with an epidemiology study from China, which showed that 66.9% of the KP was resistant to CIP.²⁷ At the same time, our results were in agreement with another study from Ohio.²⁸ NDM-producing CRKP strains had broad hydrolytic properties, especially for most β -lactam antibiotics, except for monobactams.⁸ However, MBL resistance is frequently associated with MDR, with MBL-producing isolates often co-expressing ESBLs, which inactivate monobactams.⁸ Our result was in line with this, CRKP102 ST4564 was a multi-drug resistance strain and was resistant to ATM. To in-depth studying the feature of its antimicrobial sensitivity, we compared the MIC values with other CRKP strains (three NDM-1-producing ST11 CRKP and one co-producing KPC-2 and CTX-M-14 ST11 CRKP clones). These strains were identified as CRKP strains from our archived clones' collection and were not included in this study. Interestingly, except the co-producing NDM-1, CTX-M-9 variants and *mcr-1* ST4564 clone had the lowest MICs of CIP and LEV, there were no obvious differences among them. However, due to the limited clones, the specific antimicrobial sensitivity of co-producing NDM-1, CTX-M-9 variants and *mcr-1* ST4564 CRKP102 clone remained unclear. Further researches are still needed. Moreover, the studies about CRKP co-carrying *bla*_{NDM-1} and *bla*_{CTX-M} were rare.

According to the NGS, a series of resistance genes were detected. ST4564 harbored antibiotic resistance genes to β -lactams, aminoglycosides, quinolones, macrolides, and sulfonamide. Also, we detected *aad* genes, multidrug resistance efflux pumps [*acrA*, *acrB*] genes, which were related to MDR phenotype.¹² This was consistent with our microbiological results. In our study, ST4564 harbored two variants of *bla*_{CTX-M}, which could confer resistance to third-generation cephalosporins as reported. Moreover, *bla*_{CTX-M} also could confer resistance to monobactams, on the contrary, our result was in disagreement with it. We also detected *oqxAB* gene, another report from China demonstrated that *oqxAB* gene was detected in all the KP strains.²⁸ *oqxAB* only can confer low-level resistance to quinolones; thus, the strain harbored *oqxAB* genes will still show susceptibility to quinolones.²⁹ Our result also supported it. The ST4564 clone was susceptible to quinolones. In addition to the aforementioned resistance genes, surprisingly, the *mcr-1* gene was first detected in our hospital. Regrettably, the NGS did not detect the *mcr-1* gene and other resistance genes associated with colistin

resistance. At the same time, CRKP102 was susceptible to colistin. It could be demonstrated that resistance phenotypes might involve complex resistance mechanisms and it still remains a challenge for genotypic detection and, consequently, phenotypic prediction.²² Consequently, more accurate sequencing technologies were needed. Furthermore, the acquisition of resistance profiles could make bacteria resistant to antibiotics frequently used within the medical therapies, thereby increasing the difficulty of clinical treatments.³⁰ Due to these multiple resistance profiles, we speculate that novel ST4564 is associated with multi-drug resistance. In light of the dire situation, early monitoring of CRKP infection is urgent.

Apart from resistance characteristics, we found multiple virulence factors to evaluate the pathogenicity of ST4564. It was positive for the string test while negative for the PCR test of typical virulence genes. Interestingly, ST4564 was not detected *rmpA* or *rmpA2* genes, which were related to hypermucoviscous.¹² The presence in ST4564 strain of type-I fimbriae, encoded by the *fimD* and *fimH*, seems to be linked to down-regulation in biofilm cells, while these genes did not affect biofilm formation.³¹ This finding could state that the ST4564 clone was positive for string test without type III fimbriae because type-III fimbriae genes were expressed during the CRKP colonization.³² Another feature of the virulence factor was capsular serotypes. In ST4564, we found *rcsAB*. By regulating the capsule synthesis A and B genes (*rcsA*, *rcsB*), capsule production could be enhanced.³¹ To date, nearly 78 KP capsular serotypes were found. Among them, K1 and K2 capsular serotypes were most notorious and they were always associated with hvKP.⁶ However, none of the capsular serotypes was detected in ST4564. From other researches, the CRKP clones of the same capsular type with different STs were reported, such as: K1 was reported in ST23 clones, K2 was reported in ST11 clones, K149 was reported in ST307 clones; K47/K64 with ST11.^{6,12} Thus, virulence factors did not always singularly determine the hvKP clone, which required further investigation and identification. In addition, it is well known that antibiotic resistance genes and virulence genes could undergo the horizontal gene transfer in highly disseminating different clones.^{8,16,33} Thus, it is necessary to further investigate the epidemic resistance and virulence plasmids and other possible mobile genetic elements.

Up to now, more than 5000 sequence types have been discovered. To our knowledge, the dissemination of CRKP is mostly clonal, and the type of clones is geographically

specific.⁶ ST258, ST11, ST521, and ST101 are detected worldwide and considered as the main high-risk and epidemic clones.^{2,13} ST11 is the predominant type in China.⁹ Compared the loci of housekeeping genes of ST4564 with ST11 and ST258, we found that the new clone was not related to any of them. Previous studies showed that KPC-producing KP strains were belonging to the CC258, which was the dominant clonal complex.²² In our study, ST4564 was belonging to CC1571. This was not associated with the high epidemic clonal complexes, such as CC258.

There were several limitations in this study. First, this study was explored at a single hospital. Because of that, our finding suggested that the infection control measures for CRKP infections in this region are urgently needed. Second, the total number of CRKP strain was small. The genetic characteristic of new clones was still unknown. Thus, the timely infection controls for STs, especially for new ST, were more important. Third, unfortunately, due to the NGS data were based on short-read sequencing, the specific information of plasmid content and whether resistance genes are chromosomally located or not was unclear. Thus, further experiments such as conjugation and transformation test need to be performed.

In conclusion, our study was the first research in-depth conducted MLST study of CRKP clinical clones in our local hospital. Our study showed that analysing the genetic features of a novel sequence type, ST4564, in relation to their resistance and pathogenicity may be helpful for outbreak surveillance in hospital settings. Our findings suggest the urgent need for infection control of the new clone to prevent it from becoming a high-risk clone of CRKP. Furthermore, co-producing NDM-1, CTX-M-9 variants and *mcr-1* ST4564 clone should take more attention to its resistance.

Ethical Approval

This study was reviewed and approved by the research ethics committee of the Second Hospital of Shanxi Medical University (2019 YX-181). The data of patients' clinical variables were collected from their medical records and did not contain name, address, or other personal information. The patients' written informed consent was exempt. This study was also in line with the guidelines outlined in the Declaration of Helsinki.

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Disclosure

The authors report no conflicts of interest in this work.

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