


# Antimicrobial Resistance Patterns, Sequence Types, Virulence and Carbapenemase Genes of Carbapenem-Resistant *Klebsiella pneumoniae* Clinical Isolates from a Tertiary Care Teaching Hospital in Zunyi, China

Meijing Shen\*, Xianghao Chen\*, Jingyue He, Lin Xiong, Rengui Tian, Guangwu Yang, He Zha, Kaifeng Wu 

Department of Laboratory Medicine, the First People's Hospital of Zunyi (The Third Affiliated Hospital of Zunyi Medical University), Zunyi, People's Republic of China

\*These authors contributed equally to this work

Correspondence: Kaifeng Wu; He Zha, Department of Laboratory Medicine, the First People's Hospital of Zunyi (The Third Affiliated Hospital of Zunyi Medical University), Zunyi, People's Republic of China, Email [kiphoonwu@126.com](mailto:kiphoonwu@126.com); [zhahe666@126.com](mailto:zhahe666@126.com)

**Purpose:** Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) has seriously threatened public health worldwide. This study aimed to investigate the antimicrobial resistance patterns, sequence types (STs), virulence and carbapenemase genes of CRKP isolates from patients in Zunyi, China.

**Methods:** CRKP isolates were collected from the First People's Hospital of Zunyi between January 2018 and December 2020. Antimicrobial susceptibility was determined using a VITEK<sup>®</sup>2 analyzer and confirmed using either the broth dilution method, Kirby-Bauer method, or E-test assays. Carbapenemase production was examined using a modified carbapenem inactivation method. STs of the studied isolates were determined by multilocus sequence typing, and the presence of carbapenemase and virulence genes was examined using polymerase chain reaction assays.

**Results:** In total, 94 CRKP isolates were collected. All studied isolates produced carbapenemase, and the most common carbapenemase gene was New Delhi metallo- $\beta$ -lactamase (NDM; 72.3%), followed by *Klebsiella pneumoniae* carbapenemase (KPC; 24.5%), and Verona integron-encoded metallo- $\beta$ -lactamase (VIM; 3.2%). Of the studied isolates, 74.3% exhibited multidrug-resistant (MDR) phenotype, and 25.7% were either pandrug-resistant (PDR) or extensively drug-resistant (XDR) phenotypes. The most prevalent sequence type was ST2407 (37.2%), followed by ST76 (21.3%) and ST11 (11.7%). The NDM gene was present in 97.1% of ST2407 isolates and 90.0% of ST76 isolates, whereas the KPC gene was present in 90.9% of ST11 isolates. The majority of the isolates carried *wabG*, *uge*, and *fimH* virulence genes, with prevalence rates of 94.7%, 92.6%, and 94.7%, respectively.

**Conclusion:** This study describes NDM-producing ST2407 and ST76, as well as KPC-producing ST11, as the major clonal types of CRKP isolates in Zunyi, China. All CRKP isolates were resistant to multiple types of antibiotics, and the majority of isolates carried carbapenemase and virulence genes. Clonal spread of NDM-producing CRKP ST2407 and ST76, and KPC-producing CRKP ST11 should be strictly monitored.

**Keywords:** *Klebsiella pneumoniae*, antimicrobial resistance patterns, virulence genes, sequence types, carbapenemase genes

## Introduction

*Klebsiella pneumoniae* (*Kp*; *K. pneumoniae*) is an important hospital and community-acquired pathogen that primarily causes pneumonia, urinary tract infections, bloodstream-associated infections, meningitis, and pyogenic liver abscess.<sup>1–3</sup>

Carbapenem antibiotics represent the most important treatment for *Enterobacteriaceae*; however, with the widespread use of such antibiotics, carbapenem-resistant *K. pneumoniae* (CRKP) is increasingly prevalent worldwide.<sup>4,5</sup>

The effect of carbapenems can be abrogated by mechanisms including hydrolysis by carbapenemases, loss of outer membrane proteins and over-expression of efflux pumps.<sup>6</sup> In *K. pneumoniae*, resistance to carbapenems is mainly mediated by carbapenemases, which can hydrolyze carbapenem antibiotics. The most important carbapenemases are *K. pneumoniae* carbapenemase (KPC), metallo- $\beta$ -lactamases, such as the New Delhi metallo- $\beta$ -lactamase (NDM), Imipenemase metallo- $\beta$ -lactamase (IMP), Verona integron-encoded metallo- $\beta$ -lactamase (VIM), and Oxacillin hydrolyzing enzymes-48 (OXA-48).<sup>7–9</sup> Since the carbapenemase phenotype is closely related to the use of antimicrobial agents against *Kp* infection, it is important to continue detecting the genes that are responsible for carbapenem resistance in CRKP isolates.

Multidrug-resistant (MDR) bacterial pathogens have increased worldwide and are considered a public health threat.<sup>10</sup> Several recent investigations have reported the emergence of MDR bacterial pathogens from different origins that increase the necessity of the proper use of antibiotics.<sup>11–15</sup> Therefore, the routine application of antimicrobial susceptibility testing to detect the antibiotic of choice, as well as the screening of emerging MDR strains, is warranted.

The presence of virulence genes in *K. pneumoniae* is commonly associated with poor outcomes, including prolonged disease course and high mortality, in infected patients. It has been reported that virulence genes are associated with the pathogenicity of *Klebsiella pneumoniae*.<sup>16</sup> Indeed, virulence determinants encoded by *magA*, *rmpA*, *kfu*, *wabG*, *uge*, *fimH*, etc. have been found to play important roles in the pathogenesis of *Kp* infection.<sup>17</sup> For example, regulator of mucoid phenotype A (*rmpA*) is capable of activating the production of capsular polysaccharide.<sup>18</sup> Mucoviscosity-associated gene A (*magA*) was recognized as a capsule polymerase, which is associated with the occurrence of liver abscess during *Kp* infection.<sup>19</sup> Type 1 fimbrial adhesin (*fimH*) is an important mediator in attachment of *Kp* to the epithelial cells and hence facilitates colonization and invasion of the host.<sup>20</sup> *wabG* and *uge* genes which encode products contribute to the production of both capsular polysaccharide and lipopolysaccharide.<sup>21</sup> *Klebsiella* ferric iron uptake (*kfu*) is implicated in the uptake of iron from the host, which is associated with virulent hypermucoviscosity phenotype.<sup>22</sup> Shi et al described the high prevalence of *rmpA*, *kfu*, *fimH*, *wabG*, *iroN*, *iutA*, and *entB* virulence genes in *Kp* isolates.<sup>23</sup> Although Yang et al also reported the high prevalence of *uge* and *fimH* virulence genes,<sup>24</sup> the prevalence of *magA* and *rmpA* virulence genes was lower than that reported by Shi et al.<sup>23</sup> Thus, the frequency of virulence genes in CRKP isolates remains to be determined.

Previous studies have investigated the epidemiology and antimicrobial resistance patterns of CRKP isolates in some cities in China,<sup>6,25,26</sup> however, no detailed reports of antimicrobial resistance patterns and STs have been described in Zunyi, Guizhou Province, China. The present study aimed to investigate the antimicrobial resistance patterns, STs, and presence of virulence genes (*magA*, *rmpA*, *wabG*, *uge*, *fimH*, and *kfu*) and carbapenemase genes (KPC, NDM, OXA-48, IMP, and VIM) in CRKP isolates from patients at the First People's Hospital of Zunyi (the Third Affiliated Hospital of Zunyi Medical University) in Zunyi, Guizhou Province, China, between January 2018 and December 2020.

## Materials and Methods

### Isolation and Identification of CRKP

This study was conducted at the First People's Hospital of Zunyi (Third Affiliated Hospital of Zunyi Medical University), a university-affiliated teaching hospital in Zunyi, Guizhou Province, China. *K. pneumoniae* strains were isolated using standard microbial assays and identified using Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF-MS) (Bio-Mérieux, Marcy-l'Étoile, France). Between January 2018 and December 2020, 3387 *K. pneumoniae* isolates were identified and collected.

According to the Clinical and Laboratory Standards Institute (CLSI) M100-S31 criteria,<sup>27</sup> CRKP was defined when *K. pneumoniae* displayed a minimal inhibition concentration (MIC) for imipenem  $\geq 4$   $\mu\text{g/mL}$ , meropenem  $\geq 4$   $\mu\text{g/mL}$ , or ertapenem  $\geq 2$   $\mu\text{g/mL}$ . Based on these inclusion criteria, a total of 94 non-repeated clinical CRKP isolates were enrolled.

## String Test

As previously described,<sup>28</sup> all isolates were cultured at 37°C on blood agar plates (Autobio, Zhengzhou, China). The colony was gently touched and lifted using an inoculation loop. A positive test result was defined as a viscous string > 5 mm in length, which was observed visually.

## DNA Preparation of CRKP Isolates

Pure bacterial colonies were added to 800 µL distilled water in a 1.5 mL EP tube, mixed vigorously, boiled for 30 min to release genomic DNA and plasmids, and centrifuged at 12,000 rpm for 10 min. Afterwards, the supernatant was collected and used as template DNA.

## Antimicrobial Susceptibility Testing

Bacterial susceptibility to cephalosporins (cefazolin, cefuroxime, ceftriaxone, ceftazidime, and cefepime), monoamides (aztreonam), cephalomycins (cefotetan), carbapenems (imipenem, ertapenem, and meropenem), β-lactam combination agents (piperacillin/tazobactam, ampicillin/sulbactam, cefoperazone/sulbactam, and ceftazidime/avibactam), quinolones (ciprofloxacin and levofloxacin), aminoglycosides (gentamicin, tobramycin, and amikacin), glycyclcyclines (tigecycline), and sulfonamides (trimethoprim/sulfamethoxazole) was analyzed in this study. The broth dilution method was used to determine the minimum inhibitory concentration (MIC) of tigecycline (Yangtze River Pharmaceutical-Group Co., Ltd, Taizhou, China), and the MICs were interpreted using FDA (<http://www.fda.gov>) MIC breakpoint standards. The Kirby–Bauer method was used to determine the susceptibility to cefuroxime (Oxoid, Hampshire, UK), cefoperazone/sulbactam (Oxoid, Hampshire, UK), ceftazidime/avibactam (Pfizer Co., Ltd, New York, USA).<sup>27</sup> E-test assays were used to determine the MIC of imipenem (BIO-KONT, Wenzhou, China), meropenem (BIO-KONT, Wenzhou, China) and ertapenem (BIO-KONT, Wenzhou, China). The MIC values for other antibiotics were determined on a VITEK®2 Compact system (bioMérieux, Marcy l'Étoile, France) using the supporting antimicrobial susceptibility GN13 (bioMérieux, Marcy l'Étoile, France). *Escherichia coli* ATCC® 25922 was used for quality control in the antimicrobial susceptibility test. The antimicrobial susceptibility results were interpreted according to CLSI M100-S31.<sup>27</sup>

As previously mentioned,<sup>29</sup> CRKP isolates were classified as multidrug-resistant (MDR: non-susceptibility to more than one agent in three or more classes of antibiotics), extensively drug-resistant (XDR: non-susceptibility to one or more agents in all but one or two classes), or pandrug-resistant (PDR: non-susceptibility to representative agents of all classes).

## Detection of Carbapenemase Genes and Phenotypes

Polymerase chain reaction (PCR) testing was carried out to examine the presence of carbapenemase genes using a previously described method.<sup>30</sup> The carbapenemase genes KPC, NDM, OXA-48, IMP, and VIM were amplified with a T100 PCR Amplifier (Bio-Rad, Hercules, CA, USA), and the products were analyzed by gel electrophoresis and ultraviolet imaging. Primers were synthesized by Shenzhen Huada Gene Technology Company (Shenzhen, China) and primer sequences are displayed in [Supplementary Table 1](#). The PCR amplification parameters were pre-denaturation at 95 °C for 3 min, 95 °C for 15s, 55 °C for 30s, 72 °C for 1 min, 30 cycles, and extension at 72 °C for 5 min.

According to the (CLSI) M100-S31<sup>27</sup> and the protocol described by Tsai et al,<sup>31</sup> the modified carbapenem inactivation method (mCIM) and EDTA-carbapenem inactivation method (eCIM) were performed to determine carbapenemase phenotypes.

## Detection of Virulence Genes

The virulence genes *magA*, *rmpA*, *wabG*, *uge*, *fimH*, and *kfu* were analyzed according to available information.<sup>32</sup> Briefly, these virulence genes were amplified by PCR on a T100 PCR Amplifier (Bio-Rad, Hercules, CA, USA), and the products were analyzed by gel electrophoresis and ultraviolet imaging. Primers were synthesized by the Shenzhen Huada Gene Technology Company (Shenzhen, China). The sequences of the primers are listed in [Supplementary Table 2](#). The PCR amplification was performed under the conditions of pre-denaturation at 95 °C for 3 min, 95 °C for 15s, 55 °C for 30s, 72 °C for 1 min, 35 cycles, and extension at 72 °C for 5 min.

## Multilocus Sequence Typing (MLST)

MLST analysis was performed as previously described.<sup>33</sup> Briefly, according to the typing standards of the Institute Pasteur MLST and Whole Genome MLST database (<http://bigsdbs.pasteur.fr/klebsiella/>), seven housekeeping genes of *K. pneumoniae* (*gapA*, *infB*, *mdh*, *pgi*, *phoE*, *tonB*, *rpoB*) were amplified by PCR. The primers sequences are listed in [Supplementary Table 3](#). The PCR amplification parameters were pre-denaturation at 94 °C for 2 min, 35 cycles of 94 °C for 20s, 55 °C for 30s, 72 °C for 30s, and extension at 72 °C for 5 min. The amplified products were sent to Shanghai Bioengineering Co., Ltd for Sanger sequencing. The sequences of these housekeeping genes were uploaded to the MLST database for allele codes and STs of the isolates.

## Data Processing

The WHONET software (version 5.6; WHO, Geneva, Switzerland) was used to calculate the antimicrobial susceptibility rates of the CRKP isolates. Statistical analysis software (SPSS software version 26.0; IBM, Armonk, NK, USA) was used to analyze the significance of the differences between groups. The  $\chi^2$  test was used, and a *p* value less than 0.05 (*p* <0.05) was considered as statistically significant.

## Results

### Phenotypic Characteristics of the Recovered CRKP Isolates

The proportion of CRKP out of the total *Kp* clinical isolates was 1.4% (17/1214) in 2018, 2.3% (22/948) in 2019, and 4.5% (55/1225) in 2020, showing an increasing yearly trend. Based on their phenotypic characteristics, the CRKP isolates were divided into four typical phenotypes ([Supplementary Figure 1](#)). After an overnight growth on the blood plate, 74 (78.7%) isolates formed white, moist, and convex colonies; 10 (10.6%) isolates formed grey, moist, convex, mucous colonies, and colony fusion could be easily seen; 8 (8.5%) isolates formed grey, convex, and smooth colonies; and 2 (2.1%) isolates formed grey, moist, convex, and mucous colonies with a positive string test.

### General Information on the CRKP Clinical Isolates

[Table 1](#) displays that 75.5% (71/94) of the isolates were collected from children (≤14 years old), and 24.5% were collected from adults. Sputum (68.1%, 64/94), urine (13.8%, 13/94), and blood (5.3%, 5/94) were the main specimen types. CRKP isolates were mainly collected from pediatric and intensive care units (ICU).

**Table 1** Basic Information on CRKP Isolates in Zunyi, China

		Number of Isolates (n =94)	Percentage (%)
Age	Children (≤14 Years Old)	71	75.5
	Adults (≥15 years old)	23	24.5
Gender	Male	57	60.6
	Female	37	39.4
Specimen type	Sputum	64	68.1
	Urine	13	13.8
	Blood	5	5.3
	Ascites	4	4.3
	Secretion	4	4.3
	Throat swab	2	2.1
	Conduit	1	1.1
	Bronchoscopy lavage Solution	1	1.1
Department	Pediatric	55	58.5
	Children intensive care unit	16	17.0
	Adult intensive care unit	14	14.9
	Surgery	6	6.4
	Internal medicine	3	3.2

## In vitro Antimicrobial Susceptibility

To have an understanding on the antimicrobial resistance patterns, the antibiotic susceptibilities of all the strains were determined (Table 2). All isolates were resistant to cefazolin, cefuroxime, ceftriaxone, ceftazidime, imipenem, meropenem, ertapenem, ampicillin/sulbactam, piperacillin/tazobactam, and cefoperazone/sulbactam. Most CRKP isolates were resistant to cefepime (97.9%), aztreonam (71.3%), and cefotetan (75.5%). In comparison, CRKP isolates were less resistant to ciprofloxacin (23.4%), levofloxacin (17.0%), gentamicin (22.3%), tobramycin (16.0%), amikacin (14.9%), and trimethoprim/sulfamethoxazole (24.5%). The results showed that 21.3% of the isolates were resistant to tigecycline, and 76.6% were resistant to ceftazidime/avibactam.

The occurrence of MDR, XDR, and PDR in the recovered isolates was further analyzed. As shown in Table 3, 21.4% of the isolates were the XDR phenotype, and 4.3% of the isolates were the PDR phenotype. The antibiotic resistance genes carried by MDR and XDR isolates were statistically significant (Supplementary Table 4).

## Carbapenemase Phenotypes of the CRKP Isolates

To determine the carbapenemase phenotypes of the studied isolates, the modified carbapenem inactivation and EDTA-carbapenem inactivation assays were performed. The mCIM results demonstrated that all CRKP isolates produced carbapenemases. A representative image is shown in Figure 1A. The eCIM results revealed that 71 isolates (75.5%) produced metallo- $\beta$ -lactamase, whereas 23 isolates (24.5%) produced serine carbapenemase. A representative image is shown in Figure 1B.

**Table 2** Antimicrobial Susceptibility of 94 CRKP Isolates

Types of Antibiotics	Antimicrobial Agents	Resistance Rate (%)	Mediation Rate (%)	Sensitivity Rate (%)
Cephalosporins	CZO	100 (94/94)	0 (0/94)	0 (0/94)
	CXM	100 (94/94)	0 (0/94)	0 (0/94)
	CRO	100 (94/94)	0 (0/94)	0 (0/94)
	CAZ	100 (94/94)	0 (0/94)	0 (0/94)
	FEP	97.9 (92/94)	2.1 (2/94)	0 (0/94)
Monoamides	ATM	71.3 (67/94)	0 (0/94)	28.7 (27/94)
Cephalomycins	CTT	75.5 (71/94)	18.1 (17/94)	6.4 (6/94)
Carbapenems	IPM	100 (94/94)	0 (0/94)	0 (0/94)
	MEM	100 (94/94)	0 (0/94)	0 (0/94)
	ETP	100 (94/94)	0 (0/94)	0 (0/94)
$\beta$ -lactam combination agents	SAM	100 (94/94)	0 (0/94)	0 (0/94)
	TZP	100 (94/94)	0 (0/94)	0 (0/94)
	SCF	100 (94/94)	0 (0/94)	0 (0/94)
	CZA	76.6 (72/94)	0 (0/94)	23.4 (22/94)
Quinolones	CIP	23.4 (22/94)	8.5 (8/94)	68.1 (64/94)
	LEV	17 (16/94)	41.5 (39/94)	41.5 (39/94)
Aminoglycosides	CN	22.3 (21/94)	0 (0/94)	77.7 (73/94)
	TOB	16 (15/94)	0 (0/94)	84 (79/94)
	AK	14.9 (14/94)	0 (0/94)	85.1 (80/94)
Glycylcyclines	TGC	21.3 (20/94)	0 (0/94)	78.7 (74/94)
Sulfonamides	SXT	24.5 (23/94)	0 (0/94)	75.5 (71/94)

**Abbreviations:** CZO, cefazolin; CXM, cefuroxime; CRO, ceftriaxone; CAZ, ceftazidime; FEP, cefepime; ATM, aztreonam; CTT, cefotetan; IPM, imipenem; MEM, meropenem; ETP, ertapenem; SAM, ampicillin/sulbactam; TZP, piperacillin /tazobactam; SCF, cefoperazone /sulbactam; CZA, ceftazidime /avibactam; CIP, ciprofloxacin; LEV, levofloxacin; CN, gentamicin; TOB, tobramycin; AK, amikacin; TGC, tigecycline; SXT, trimethoprim / sulfamethoxazole.

**Table 3** The Occurrence of MDR, XDR and PDR Among the CRKP Isolates

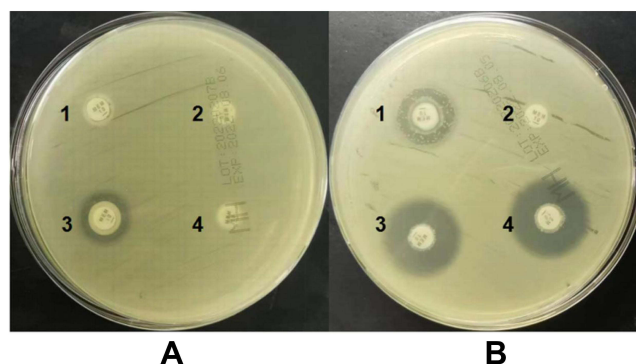
No. of Strains	%	Type of Resistance	Antibiotic Resistance Profiles <sup>a</sup>	Carbapenemase Genes (No.)
25	26.6	MDR	[1], [2], [3], [4], [5] <sup>b</sup>	NDM (21), KPC (4)
23	24.5	MDR	[1], [3], [4], [5]	NDM (23)
7	7.4	MDR	[1], [2], [3], [4], [5] <sup>b</sup> , [6]	NDM (6), KPC (1)
7	7.4	MDR	[1], [2], [3], [4], [5], [8]	NDM (6), VIM (1)
4	4.3	MDR	[1], [2], [3], [4], [5], [9]	NDM (3), VIM (1)
2	2.1	MDR	[1], [3], [4], [5], [8]	NDM (1)
1	1.1	MDR	[1], [3], [4], [5], [6]	NDM (2)
1	1.1	MDR	[1], [2], [4], [5] <sup>b</sup>	KPC (1)
12	12.8	XDR	[1], [2], [3], [4], [5] <sup>b</sup> , [6], [7] <sup>c</sup> , [9]	NDM (3), KPC (8), VIM (1)
3	3.2	XDR	[1], [2], [3], [4], [5] <sup>b</sup> , [6], [7]	KPC (3)
2	2.1	XDR	[1], [2], [3], [4], [5], [7] <sup>c</sup> , [9]	NDM (2)
1	1.1	XDR	[1], [2], [3], [4], [5] <sup>b</sup> , [6], [8], [9]	KPC (1)
1	1.1	XDR	[1], [2], [3], [4], [5], [6], [8]	NDM (1)
1	1.1	XDR	[1], [2], [3], [4], [5] <sup>b</sup> , [6], [9]	KPC (1)
4	4.3	PDR	[1], [2], [3], [4], [5] <sup>b</sup> , [6], [7], [8], [9]	KPC (4)

**Notes:** <sup>a</sup>Antibiotic resistance profiles: [1] Cephalosporins: CZO, CXM, CRO, CAZ, FEP; [2] Monoamides: ATM; [3] Cephalomycins: CTT; [4] Carbapenems: IPM, MEM, ETP; [5]  $\beta$ -lactam combination agents: SAM, TZP, SCF, CZA; [6] Quinolones: CIP, LEV; [7] Aminoglycosides: CN, TOB, AK; [8] Glycylcyclines: TGC; [9] Sulfonamides: SXT. <sup>b</sup>CZA can be sensitive. <sup>c</sup>TOB, AK can be sensitive.

**Abbreviations:** MDR, multidrug-resistant; XDR, extensively drug-resistant; PDR, pandrug-resistant; CZO, cefazolin; CXM, cefuroxime; CRO, ceftriaxone; CAZ, ceftazidime; FEP, cefepime; ATM, aztreonam; CTT, cefotetan; IPM, imipenem; MEM, meropenem; ETP, ertapenem; SAM, ampicillin/sulbactam; TZP, piperacillin/tazobactam; SCF, cefoperazone/sulbactam; CZA, ceftazidime/avibactam; CIP, ciprofloxacin; LEV, levofloxacin; CN, gentamicin; TOB, tobramycin; AK, amikacin; TGC, tigecycline; SXT, trimethoprim/sulfamethoxazole; NDM, New Delhi metallo- $\beta$ -lactamase; KPC, *Klebsiella pneumoniae* carbapenemase; VIM, Verona integron-encoded metallo- $\beta$ -lactamase.

## Correlation Between Carbapenemase Phenotypes and Carbapenemase Genes

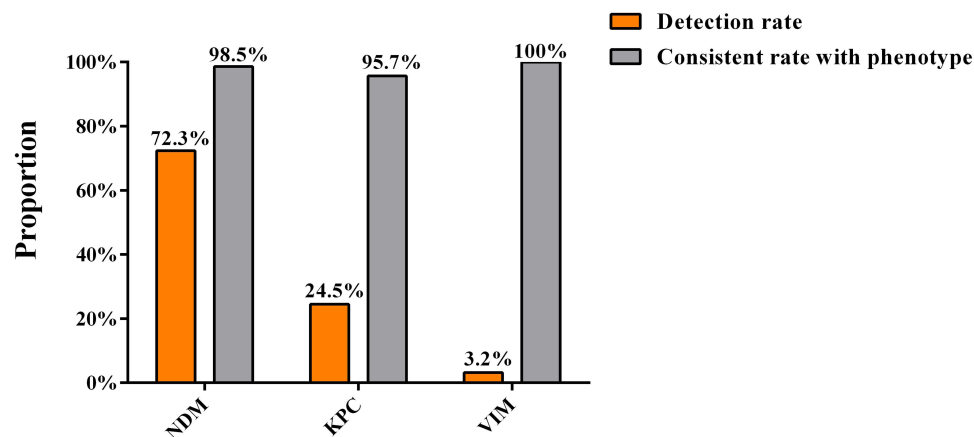
To determine the relationship between the carbapenemase genes and carbapenemase phenotypes, we examined the presence of carbapenemase genes in all the CRKP isolates. The results showed that NDM was detected in 68 isolates (72.3%, 68/94), with a coincidence rate of 98.5% between genotypes and phenotypes. KPC was detected in 23 isolates (24.5%, 23/94), and the coincidence rate between genotypes and phenotypes was 95.7%. VIM was detected in three isolates (3.2%), which completely coincided with the phenotype of the isolates (Figure 2). OXA-48 and IMP were not detected in any of these isolates.



**Figure 1** Representative images of mCIM and eCIM test results. (A) mCIM test results; (B) eCIM test results. 1: ATCC®BAA-1705™ (producing serine carbapenemase) as a positive control; 2: CRKP clinical isolates (producing serine carbapenemase); 3: ATCC®BAA-2146™ (producing metallo- $\beta$ -lactamase) as a positive control; 4: CRKP clinical isolates (producing metallo- $\beta$ -lactamase).

**Abbreviations:** mCIM, modified carbapenem inactivation method; eCIM, EDTA-carbapenem inactivation method.





**Figure 2** Prevalence of carbapenemase genes in CRKP isolates and the consistency between genotypes and phenotypes of the isolates.

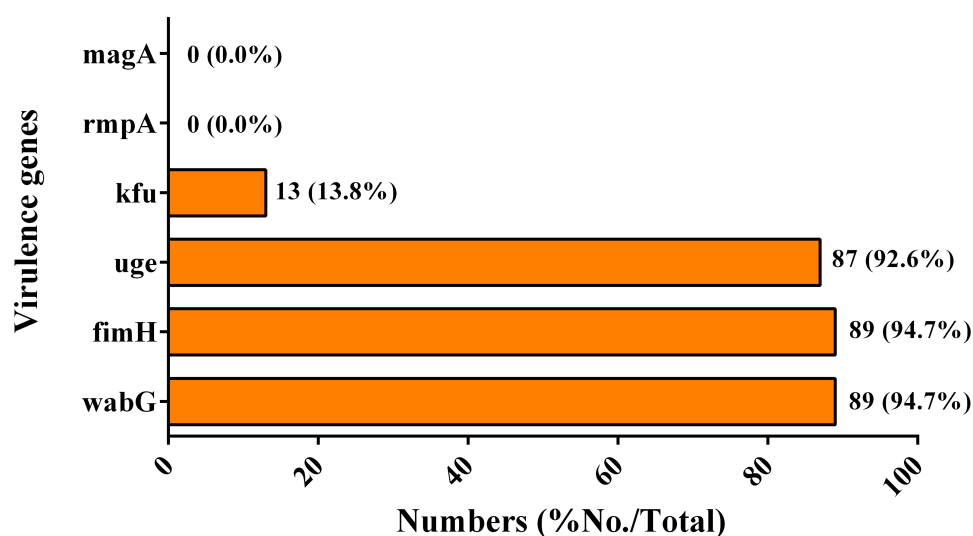
**Abbreviations:** NDM, New Delhi metallo-β-lactamase; KPC, *K. pneumoniae* carbapenemase; VIM, Verona integron-encoded metallo-β-lactamase.

## Prevalence of Virulence Genes

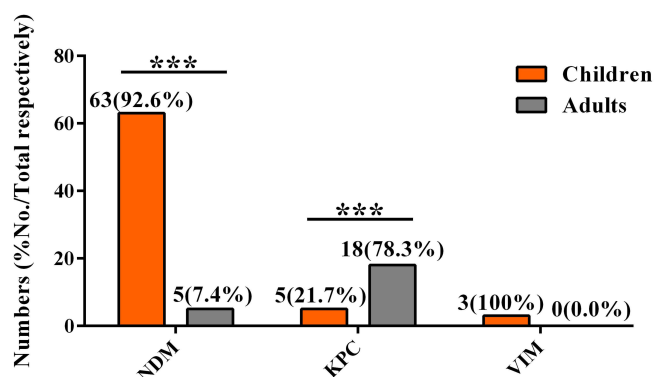
To have an understanding on the virulence determinants of the isolates, *wabG*, *fimH*, *uge*, *kfu*, *rmpA* and *magA* virulence genes were detected using PCR assays. Among the 94 CRKP isolates, 89 (94.7%), 89 (94.7%), 87 (92.6%), and 13 (13.8%) isolates carried the *wabG*, *fimH*, *uge*, and *kfu* virulence genes, respectively. *rmpA* and *magA* were not detected in the isolates. The results are shown in Figure 3.

## Prevalence of Carbapenemase Genes in Children and Adults

To determine the difference in the prevalence of carbapenemase genes between children and adults, the ratio of carbapenemase genes between the two populations was compared. Among the 68 NDM-positive isolates, 63 isolates (92.6%, 63/68) were from children, and 5 strains (7.4%, 5/68) were from adults. The KPC genes were present in 5 child isolates (21.7%, 5/23) and 18 adult isolates (78.3%, 18/23). The VIM gene was detected only in children. The prevalence of NDM-producing CRKP in children was significantly higher than that in adults. ( $P < 0.001$ ) (Figure 4).



**Figure 3** Prevalence of virulence genes in the CRKP isolates.

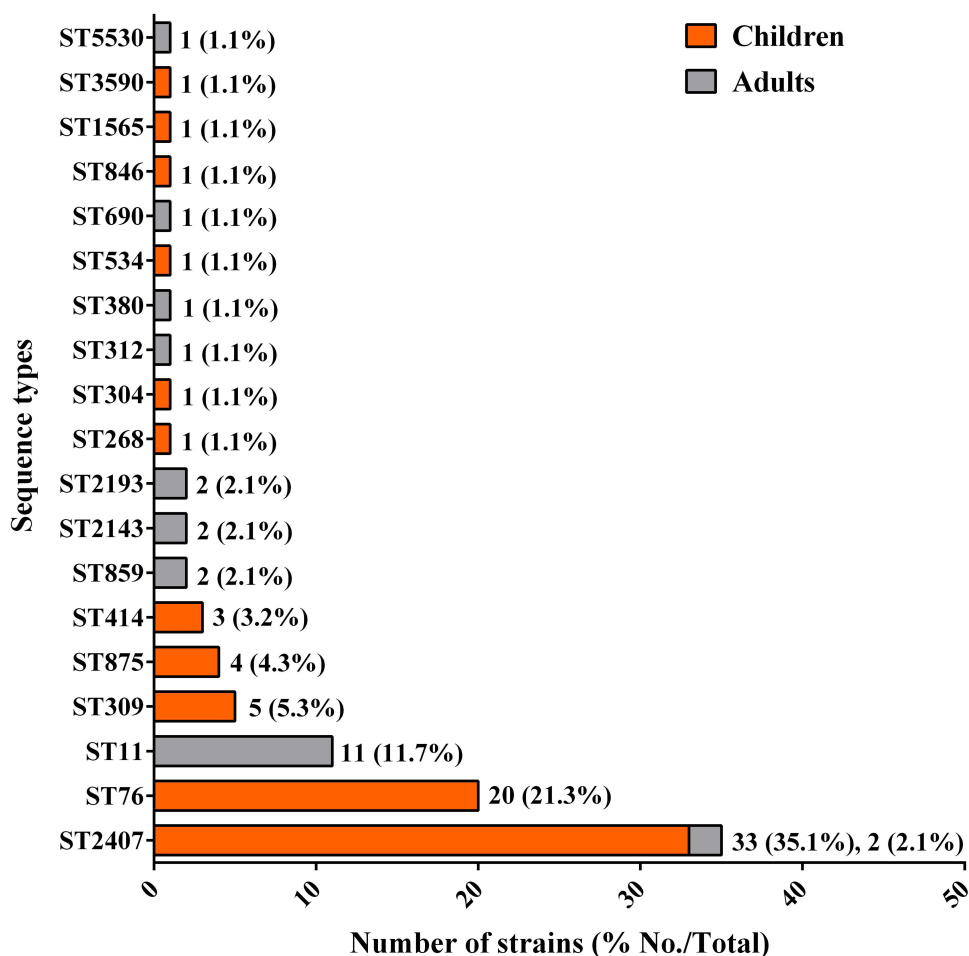


**Figure 4** Prevalence of carbapenemase genes in isolates from children and adults.

**Abbreviations:** NDM, New Delhi metallo-β-lactamase; KPC, *K. pneumoniae* carbapenemase; VIM, Verona integron-encoded metallo-β-lactamase. \*\*\**p* < 0.001.

## Sequence Types of the CRKP Isolates

Regarding the STs of the isolates, 94 CRKP isolates were subjected to MLST analysis. The 94 CRKP isolates belonged to 19 STs (Figure 5). Thirty-five isolates (37.2%, 35/94) belonged to ST2407, and most isolates (94.3%, 33/35) were from children. ST76 accounted for 21.3% (20/94) of the isolates (from children), whereas ST11 accounted for 11.7% (11/94) of the isolates (from adults).



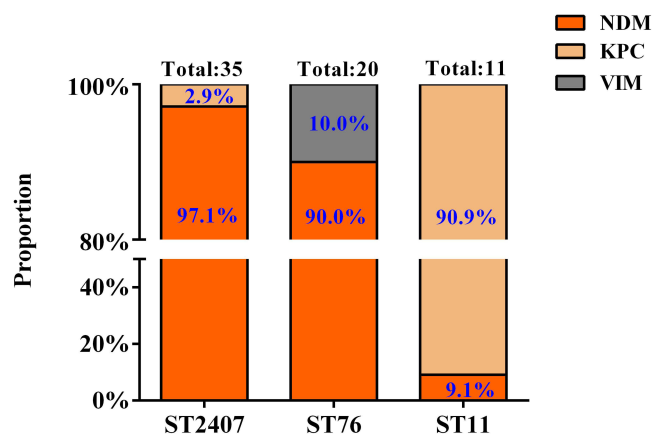
**Figure 5** Sequence types of the CRKP isolates.

**Abbreviation:** ST, sequence type.



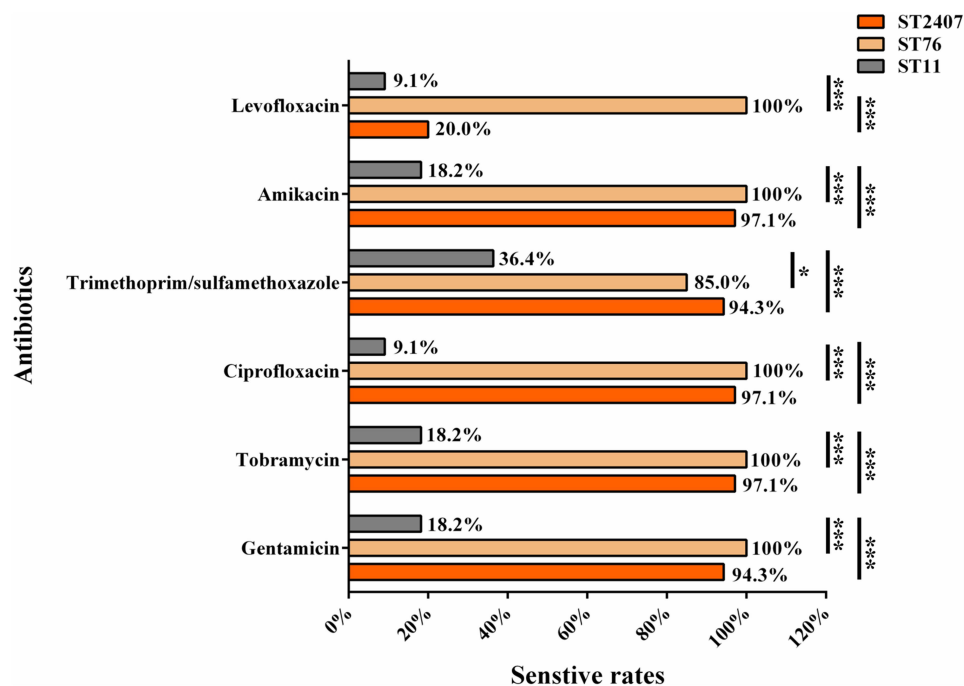
## Carbapenemase Gene Characteristics and Antimicrobial Susceptibilities of the Top Three STs

As demonstrated in Figure 6, the majority of ST2407 clones (34/35, 97.1%) and ST76 clones (18/20, 90.0%) carried the NDM gene, whereas the majority of ST11 clones (10/11, 90.9%) carried the KPC gene. Figure 7 displays the antimicrobial susceptibilities of the top three STs. ST2407, ST76, and ST11 clones were completely resistant to cefazolin, cefuroxime, ceftriaxone, ceftazidime, cefotetan, cefepime, ampicillin/sulbactam, piperacillin/tazobactam, cefoperazone/sulbactam, imipenem, meropenem, and ertapenem. Most ST2407 clones were sensitive to tobramycin, ciprofloxacin, amikacin, gentamicin, and trimethoprim/sulfamethoxazole, whereas only 20.0% of ST2407 clones were sensitive to levofloxacin. The ST76 clones were completely sensitive to tobramycin, ciprofloxacin, amikacin, gentamicin, and levofloxacin, while 85.0% of the isolates were sensitive to trimethoprim/sulfamethoxazole. Only 18.2% of ST11 isolates were sensitive to gentamicin, tobramycin, and



**Figure 6** The proportion of carbapenemase genes in the top three CRKP STs.

**Abbreviations:** NDM, New Delhi metallo- $\beta$ -lactamase; KPC, *K. pneumoniae* carbapenemase; VIM, Verona integron-encoded metallo- $\beta$ -lactamase; ST, sequence type.



**Figure 7** Antimicrobial susceptibility of the top three STs. \* $P < 0.05$ , \*\*\* $P < 0.001$ .

**Abbreviation:** ST, sequence type.

amikacin; 9.1% of the isolates were sensitive to ciprofloxacin and levofloxacin; and 36.4% of the isolates were sensitive to trimethoprim/sulfamethoxazole.

## Discussion

This study described that all CRKP isolates were completely resistant to cephalosporins, but more than 70% isolates maintained sensitivity to antimicrobial agents, including gentamicin, tigecycline, tobramycin, and amikacin. All CRKP isolates carried carbapenemase genes, most frequently the NDM gene. The studied isolates were found to have 19 different STs and ST2407, ST76, and ST11 were the leading STs, suggesting their diversity in the CRKP Zunyi isolates. This study strengthened our knowledge on the molecular epidemiology and antimicrobial susceptibility of CRKP isolates in China, which will aid in the clinical treatment and control of CRKP infection.

The prevalence of CRKP in Zunyi increased with year from 2018 to 2020, but the proportion was at relatively low level of no more than 5%. According to the 2021 China Antimicrobial Resistance Surveillance (<http://www.carss.cn/>) report, the proportion of CRKP increased from 3.0% in 2005 to 27.1% in 2021, and a particularly high CRKP proportion (more than 50%) was found in Zhejiang Province and other regions.<sup>34</sup> Compared to the situation in other countries and most cities in China,<sup>34–36</sup> the management of infections due to CRKP remains under control in this region.

The studied CRKP isolates were highly resistant to some of commonly used antibiotics, especially  $\beta$ -lactam antibiotics with resistance rates to most antibiotics of more than 97.0%. Ceftazidime/avibactam has been mainly used to treat severe infections caused by carbapenem-resistant *Enterobacteriaceae*, which produce KPC or OXA-48-like carbapenemases and are ineffective against metallo- $\beta$ -lactamase producers.<sup>37,38</sup> As previously reported,<sup>39–41</sup> the present study revealed that the majority of the KPC-producing isolates (95.7%) were sensitive to ceftazidime/avibactam, while metallo- $\beta$ -lactamase isolates were completely resistant to ceftazidime/avibactam.

The CLSI recommends applying the modified carbapenem inactivation method and EDTA-carbapenem inactivation method as a reliable way to detect carbapenem resistance phenotypes in *Enterobacteriaceae*.<sup>27</sup> Consistent with the results of Tsai et al,<sup>31</sup> our results depicted that the presence of carbapenemase genes of NDM, VIM, and KPC correlated well with the phenotypes of the isolates, indicating the potential of detecting the carbapenemase resistance phenotype to predict the carbapenemase gene from CRKP isolates.

Resistance to carbapenems in *Kp* is associated with various mechanisms. In China, it has been reported that the presence of KPC is responsible for carbapenem resistance in adult and pediatric isolates.<sup>25,42</sup> The present work demonstrated that the NDM and KPC genes detected in CRKP were mainly isolated in children and adults, respectively. Although OXA-48 is a prevalent carbapenemase type associated with *Kp* in some European countries, such as France and Turkey,<sup>43,44</sup> it is seldom detected in *Kp* isolates in China,<sup>45,46</sup> and the present study further support the observation that OXA-48 is not currently the primary carbapenemase types associated with *Kp* in China.

In the United States and Europe, ST258 is the dominant sequence that hosts KPC enzymes.<sup>43</sup> In most cities of China, it was the ST11, a clone closely related to ST258, which was responsible for most *K. pneumoniae* infections.<sup>47–50</sup> In this study, neither ST258 nor ST11 was the most prevalent clone in Zunyi. This study revealed that ST2407, isolated mainly from children, was the most prevalent CRKP isolate in this region. For clones circulating in children, a study in Shenzhen found that ST20 was the dominant clone, which carries the KPC enzymes.<sup>51</sup> In Jiangsu Province, and Wuhan, China, the main antimicrobial resistance gene in pediatric patients carried by CRKP was KPC2, and the prevalent strains were ST11 with high virulence and antimicrobial resistance.<sup>6,25</sup> Another work in Shenzhen demonstrated that ST307 was the most dominant genotype, and NDM represented the main resistance mechanism.<sup>52</sup> On the other hand, most of the isolates belonged to ST2407, which produces NDM enzymes, as revealed in this work. We highlight the diversity of CRKP STs circulating worldwide and the importance of monitoring ST2407 in *K. pneumoniae* infection.

An important finding of the present work is the high presence of *wabG*, *uge*, and *fimH*, but the extremely low presence of *magA* and *rmpA* among the strains under study. In this study, it was found that more than 90% CRKP Zunyi isolates carried the *uge*, *fimH*, and *wabG* virulence genes. In agreement with the results reported by Yang et al,<sup>24</sup> the prevalence of *magA* and *rmpA* genes was not as high as that reported by Shi et al who described a relatively high prevalence of *magA* and *rmpA* virulence genes in both hypervirulent and moderate-virulent *Kp* isolates.<sup>23</sup> Association between the virulence level of *Kp* and the presence of these virulence-associated genes has been investigated; however,

except for *magA* and *allS*, the presence of other genes showed no connection with bacterial virulence.<sup>23</sup> Although these virulence genes play important roles in the pathogenesis of *Kp* infection, the absence of some virulence genes, such as *magA* and *rpmA*, may suggest the existence of redundant molecules implicated in the pathogenesis of *Kp* infection. In addition, the discrepancy in the presence of virulence genes suggests that it may not be a good choice to evaluate the virulence of the *Kp* isolates merely by examining the presence of virulence-associated genes.

In agreement with other studies,<sup>53</sup> we found that ST11 demonstrated a higher rate of resistance to levofloxacin, tobramycin, ciprofloxacin, amikacin, gentamicin, and trimethoprim/sulfamethoxazole. Other studies have also highlighted a higher risk of ST11 than non-ST11 in patients in the ICU.<sup>54,55</sup> Thus, although ST11 was not the most prevalent sequence in Zunyi, the emergence of ST11 warrants careful monitoring. Su et al revealed that ST76 carrying the KPC gene was the main CRKP sequence type in the intensive care and neurosurgery units in the hospital.<sup>56</sup> Similar results were reported in the eastern region of Heilongjiang Province, China. Contrary to their results, the present study showed that ST76 circulating in Zunyi mainly hosts NDM enzymes. This suggests the existence of different antimicrobial resistance mechanisms for certain STs.

This study had some limitations. First, the number of isolates collected was relatively small; hence, further isolates are needed to gain a comprehensive insight into the prevalence of CRKP isolates. Second, the subtypes of carbapenemase genes in NDM, VIM, and KPC were not determined.

## Conclusion

The present work depicts the prevalence of NDM-producing CRKP ST2407 and ST76, as well as KPC-producing ST11, as the major clonal types in Zunyi, Guizhou Province, China. All CRKP isolates were resistant to multiple types of antibiotics, and the majority of isolates carried carbapenemase and virulence genes. Clonal spread of NDM-producing CRKP ST2407 and ST76, and KPC-producing CRKP ST11 should be strictly monitored.

## Abbreviations

CRKP, carbapenem-resistant *Klebsiella pneumoniae*; *Kp*, *Klebsiella pneumoniae*; KPC, *Klebsiella pneumoniae* carbapenemase; NDM, New Delhi metallo- $\beta$ -lactamase; VIM, Verona integron-encoded metallo- $\beta$ -lactamase; IMP, Imipenemase metallo- $\beta$ -lactamase; OXA-48, Oxacillin hydrolyzing enzymes-48; mCIM, modified carbapenem inactivation method; eCIM, EDTA-carbapenem inactivation method; MLST, multilocus sequence typing. MDR, multidrug-resistant; XDR, extensively drug-resistant; PDR, pandrug-resistant; CZO, cefazolin; CXM, cefuroxime; CRO, ceftriaxone; CAZ, ceftazidime; FEP, cefepime; ATM, aztreonam; CTT, cefotetan; IPM, imipenem; MEM, meropenem; ETP, ertapenem; SAM, ampicillin/sulbactam; TZP, piperacillin/tazobactam; SCF, cefoperazone/sulbactam; CZA, ceftazidime/avibactam; CIP, ciprofloxacin; LEV, levofloxacin; CN, gentamicin; TOB, tobramycin; AK, amikacin; TGC, tigecycline; SXT, trimethoprim/sulfamethoxazole.

## Ethics Statement

This study was approved by the Ethics Committee of the First People's Hospital of Zunyi (No. 2020-068). The need for written informed consent was waived because the samples were routinely collected and patients' anonymous information was provided by the microbiology hospital laboratory. This study completely followed the principles outlined in the Declaration of Helsinki.

## Funding

This project was supported by the Guizhou High-level (BAI) Innovative Talents Project (QIANKehe Platform & Talents-GCC[2022]042-1); National Natural Science Foundation of China (No.81760475); Innovation Group Project provided by the Guizhou Provincial Department of Education (QianJiaoheKYzi [2021]019); Key Discipline Project of Clinical Laboratory Diagnostics funded by Guizhou Provincial Health Commission (QianWeijianhan[2021]160); Key Discipline Project of Clinical Laboratory Diagnostics funded by Zunyi Municipal Health Bureau (2022-1444); and Zunyi United Science and Technology Fund Project (Zunyi Kehe HZzi [2019]147, [2019]173 & [2022] 97).

## Disclosure

The authors report no conflicts of interest in this work.

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