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Epidemiological and molecular characteristics of carbapenem-resistant *Klebsiella pneumoniae* from pediatric patients in Henan, China

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Abstract

Purpose Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) is an emerging global threat, whereas its epidemiological characteristics in children are rarely reported. This study aims to analyze clinical and epidemiological characteristics of CRKP from children in Henan, China.

Methods CRKP strains were isolated from pediatric patients, and the antimicrobial susceptibility of CRKP was determined using broth microdilution methods. The epidemiological characteristics of CRKP, including specimen sources, clinical data, carbapenemase types, virulence factors, MLST and PBRT typing were analyzed.

Results In total, 108 CRKP isolates were isolated from specimens including sputum, blood and urine, mainly from preterm pediatric department and internal medical intensive care unit (ICU). Newborns and staying in the ICU were risk factors for crude mortality. 107 isolates exhibited a multi-drug resistant (MDR) phenotype, and one isolate was extensively drug-resistant (XDR). Bacterial susceptibility to colistin, tigecycline and trimethoprim/sulfamethoxazole was 98.10%, 78.50% and 91.43%, respectively. Carbapenemase *bla*_{KPC} (86.11%) was predominant, followed by *bla*_{NDM} (5.56%) and *bla*_{IMP} (2.78%). Two strains co-harbored *bla*_{KPC}-*bla*_{NDM}, one had *bla*_{KPC}-*bla*_{IMP} whereas three isolates did not carry any of the analyzed carbapenemase genes. All strains possessed *fimH*, and 98% of the isolates possessed *mrkD*. Hypervirulent factors *rmpA2* and *iucA* showed high positive rates (71.30% and 49.07%), with 48.15% of strains containing both genes. MLST analysis identified nine distinct sequence types (STs), with ST11 (82.41%) being the most common, followed by ST2154 (4.63%) and ST307 (3.70%). PBRT analysis revealed IncFII (85.19%) as the most prevalent plasmid.

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Conclusion In summary, this study reported the epidemiological features of CRKP in pediatric patients in Henan, China, highlighting the high prevalence of multi-drug-resistant and hypervirulent strains, and underscoring the significance of continuous surveillance.

Keywords *Klebsiella pneumoniae*, Antimicrobial resistance, Carbapenemases, Virulence factors, Pediatrics, Epidemiological features

Introduction

Klebsiella pneumoniae, a member of Enterobacterales, is traditionally considered an opportunistic pathogen capable of causing urinary tract infections, pneumonia and blood stream infections [1]. *K. pneumoniae* infections have spread rapidly in recent years and carbapenem-resistant *K. pneumoniae* (CRKP) has been classified by the WHO as a critical priority pathogen, emphasizing the urgent need to develop new antibiotics [2]. The prevalence of carbapenem resistant strains isolated from pediatric patients has dramatically increased worldwide in recent years. In China, the detection rate of CRKP in children was 2.2% in 2007 and increased to 25.4% in 2017 [3]. A multicenter study by Wang et al. examined 502 clinical CRKP infections and 489 colonized isolates from 71 hospitals in Argentina, Australia, Chile, China, Colombia, Lebanon, Singapore, and the United States [4]. The study found regional differences in the prevalence of CRKP, including variations in bacterial types, plasmid replicons and carbapenemase resistance genes. There was a significant difference in mortality rates between CRKP and carbapenem-susceptible *K. pneumoniae* (CSKP) infections, with a mortality rate of 42.1% for CRKP compared to 17.5% for CSKP [5]. Therapeutic options for children are limited, further increasing the mortality risk.

Carbapenemase production is the main cause of carbapenem resistance [6]. Differences in carbapenemase-producing *K. pneumoniae* isolated from children and adults have been observed. In China, CRKP isolated from adults predominantly produce *Klebsiella pneumoniae* carbapenemase (KPC), whereas New Delhi metallo- β -lactamases (NDM) are more common in children. The top three carbapenemases in children include KPC, NDM and oxacillinase (OXA)-48-like enzymes [7]. Phylogenetically, KPC-producing CRKP in China is mainly associated with ST11, whereas pediatric infections frequently involve ST278, ST15, and ST11 NDM-producing CRKP [8]. Therefore, ongoing molecular epidemiological surveillance of clinical CRKP isolates from children is essential for understanding the spread of drug-resistant strains and developing personalized treatment strategies and control measures.

Hypervirulent *K. pneumoniae* has raised concerns due to severe invasive infections such as pyogenic liver abscess, endophthalmitis and meningitis, which was first identified in Taiwan in the last century [9]. The coexistence of hypervirulence and carbapenem resistance in

K. pneumoniae has been reported worldwide [10]. Virulence factor genes, such as *rmpA/rmpA2* (regulators of mucoid phenotype), *mrkD* (encoding type 3 fimbriae), *fimH* (encoding type 1 fimbriae), and *iucA* (involved in aerobactin siderophore biosynthesis) serve as biomarkers for identifying hypervirulent strains [11]. The presence of *rmpA/rmpA2* with *iucA* indicates hypervirulence, which is often used for the identification of hypervirulent *K. pneumoniae* [12]. Hence, we used the coexistence of *rmpA/rmpA2* with *iucA* as diagnostic criteria for hypervirulent strains in this study. Antibiotic resistant genes (ARGs) and virulence factors are usually found on plasmids, facilitating the spread between hospitals and the community [13]. Therefore, identifying plasmid replication types is crucial for assessing the risk of drug resistance, and virulence acquisition and transmission.

The combination of compromised immune systems in children, the high prevalence of resistance, and the associated mortality of CRKP highlights the urgent need for effective control measures. To date, few studies have investigated the epidemiological features of CRKP strains isolated from pediatric patients. Here, we investigated the clinical characteristics of children with positive CRKP cultures, analyzed the drug resistance phenotype and the epidemiological features of CRKP isolates, including carbapenem resistance genes, plasmid replication types, virulence gene profiles, and MLST typing, to provide insights for CRKP prevention and control.

Materials and methods

Study population, bacteria isolation and identification

K. pneumoniae clinical isolates were consecutively collected after routine laboratory detection from January to December 2021 at Henan Children's Hospital, a large tertiary care teaching hospital in China. The specimen sources included sputum, blood, urine, bronchoalveolar lavage fluid (BALF), ascites, cerebrospinal fluid, joint fluid, hydrothorax and other specimens. All isolates were confirmed as *K. pneumoniae* using both Bruker Biotyper MALDI-TOF MS (Bruker Daltonik GmbH, Bremen, Germany) and 16 S rDNA sequencing analysis. The study complied with the Declaration of Helsinki and was approved by the Ethics Committee of Henan Children's Hospital (2022-H-K37).

Clinical data collection

Clinical data for the included patients were obtained from electronic medical records. The collected data included demographic characteristics, sample sources, clinical diagnoses, length of hospital stay, history of invasive procedures, and prognosis at discharge. Prognosis was classified as improved if symptoms remitted or disappeared, and exacerbated if the condition worsened, resulting in death. Laboratory data, including levels of white blood cells (WBC), neutrophils, lymphocytes and C-reactive protein (CRP), were also recorded.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing (AST) were performed using the BD Phoenix 100 System according to the Clinical and Laboratory Standards Institute (CLSI) protocol [14]. Tested antimicrobial agents included amoxicillin/clavulanic acid, cefuroxime, moxifloxacin, norfloxacin, tetracycline, cefepime, aztreonam, piperacillin/tazobactam, ceftazidime, cefoperazone/sulbactam, imipenem, meropenem, amikacin, tobramycin, ciprofloxacin, levofloxacin, minocycline, tigacycline, colistin, and trimethoprim/sulfamethoxazole. Interpretations of tigecycline and colistin susceptibilities were based on EUCAST ECOFFs (ECAST) [15], whereas other antimicrobial agents were interpreted according to the CLSI M100-S30 criteria [14]. *Escherichia coli* ATCC 25,922 was used as quality control strain for AST.

To characterize different patterns of resistance, isolates were classified as multi-drug resistant (MDR), extensively drug-resistant (XDR), or pandrug-resistant (PDR) [16]. MDR was defined as non-susceptibility to three or more different antimicrobial categories. XDR was defined as non-susceptibility to one or more agents in all but one or two antimicrobial classes. PDR was defined as non-susceptibility to representative agents of all tested antimicrobial classes [16].

Identification of carbapenemase genes

Genomic DNA from *K. pneumoniae* isolates was extracted using a crude genomic DNA method [17]. All CRKP strains were screened for the presence of common carbapenemase genes (*bla*_{KPC}, *bla*_{NDM}, *bla*_{OXA-48-like}, *bla*_{IMP} and *bla*_{VIM}) using multiplex touchdown polymerase chain reaction (PCR) [18]. The primer sequences are listed in the Table 1. PCR amplification was conducted as follows: denaturation at 94 °C for 30 s, 10 cycles of annealing at 65 °C (reduce 0.5 °C per cycle) for 30 s, and elongation at 72 °C for 30 s, followed by 94 °C for 30 s, 20 cycles of annealing at 60 °C for 30 s, and elongation at 72 °C for 30 s. The final extension was performed at 72 °C for 10 min for one cycle. PCR products were analyzed by agarose gel electrophoresis and compared to positive controls.

Multi-locus sequence typing (MLST)

Seven housekeeping genes were detected by PCR using primers as previously described (Table 1) [22]. The PCR products were analyzed by agarose gel electrophoresis, and positive amplicons were sequenced at Sangon (Shanghai, China). The sequences of housekeeping genes were uploaded to the Institute Pasteur MLST procedure webpage (<https://bigsdbs.pasteur.fr/klebsiella>, accessed on 1 August 2023) to determine the sequence type (ST) for each strain. Based on the MLST allele profile, the phylogenetic results were visualized using the PHYLOViZ online application (<https://online2.phyloviz.net/index>).

Detection of virulence factors

Virulence factors, including *rmpA*, *rmpA2*, *mrkD*, *fimH* and *iucA* were detected by PCR using primers as previously described (Table 1). The PCR products were detected using 1.5% agarose gel electrophoresis. Positive amplification products were sequenced, and the sequencing results were compared using BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>).

PCR-based replicon sequence typing

PCR-based replicon sequence typing (PBRT) was performed to classify plasmids into various incompatibility (Inc) groups, including IncI1, IncHI2, IncF (IncFIA, IncFIC, IncFII and IncFIIK), IncN and IncA/C. Both multiplex PCR and single PCR methods were conducted following previously described protocols [23, 24]. Five multiplex PCRs were designed in this study: I: HI1, HI2, I1-γ; II: X, L/M, N; III: FIA, FIB, W; IV: Y, P, FIC; V: A/C, T, FIIAS. Three simplex PCRs were also performed for FII, FIIK and I1. The amplicons were visualized on a 1.5% agarose gel.

Statistical analysis

IBM SPSS Statistics (version 21) was for statistical analysis. Descriptive statistics were used to summarize the epidemiologic and clinical characteristics of CRKP strains. A cohort study of CRKP infection was conducted using univariate and multivariate Cox proportional hazard regression models to investigate independent risk factors for crude mortality. Hazard ratio (HR), corresponding 95% confidence intervals (CIs), and *P*-values were calculated. Mortality rates for hypervirulent CRKP and classical CRKP infection were compared using the Kaplan–Meier method, with statistical analysis conducted via the log-rank test. A *P*-value < 0.05 was considered statistically significant. All tests were two-tailed.

Table 1 Primers used in this study

	Primer	Primer sequence (5'-3')	Size (bp)	Reference
Carbapenemase	KPC	F: CGTCTAGTTCTGCTGTCTTG R: CTTGTCATCCTTGTTAGGCG	798	[18]
	VIM	F: GATGGTGTGGTGCACATA R: CGAATGCGCAGCACCAG	390	[18]
	IMP	F: GGAATAGAGTGGCTTAAYTCTC* R: GGTTTAAAYAAAACAACCACC*	232	[18]
	NDM	F: GGTTTGGCGATCTGGTTTTC R: CGGAATGGCTCATCACGATC	621	[18]
	OXA-48	F: ACACCAAGCTTTAAGTGGGATG R: CCCGAAATGCTCCTATTACC	186	[18]
Virulence factor	<i>rmpA</i>	F: ATGGCCTAAAGCAGTTAACTG R: CTA AATACTTGGCAGAGCCA	560	[19]
	<i>rmpA2</i>	F: ATTTACTTTATGTGCAATAAGG R: CTAGGTATTTGATGTGCACCA	624	[19]
	<i>fimH</i>	F: ATGAACGCCTGGTCTTTTGC R: GCTGAACGCCTATCCCTGTC	688	[20]
	<i>mrkD</i>	F: CCACCAACTATCCCTCGAA R: ATGGAACCCACATCGACATT	240	[20]
	<i>iucA</i>	F: ATAAGGCAGGCAATCCAG R: TAACGGCGATAAACCTCG	2927	[21]
MLST	<i>rpoB</i>	F:GTTTTCCCAAGTCACGACGTTGTAGGCGAAATGGCWGAGAACCA R:TTGTGAGCGGATAACAATTCGAGTCTTCGAAGTTGTAACC	1075	[22]
	<i>gapA</i>	F: GTTTTCCCAAGTCACGACGTTGTATGAAATATGACTCCACTCACGG R:TTGTGAGCGGATAACAATTCCTTCAGAAGCGGCTTTGATGGCTT	662	[22]
	<i>mdh</i>	F: GTTTTCCCAAGTCACGACGTTGTACCCAACTCGCTTCAGGTTTCAG R: TTGTGAGCGGATAACAATTTCCCGTTTTTCCCCAGCAGCAG	756	[22]
	<i>pgi</i>	F: GTTTTCCCAAGTCACGACGTTGTAGAGAAAAACCTGCCTGACTGCTGGC R: TTGTGAGCGGATAACAATTTCCGCGCCACGCTTTATAGCGGTTAAT	566	[22]
	<i>phoE</i>	F: GTTTTCCCAAGTCACGACGTTGTAACTACCGCAACACCGACTTCTTCGG R: TTGTGAGCGGATAACAATTTCTGATCAGAAGTGGTAGGTGAT	602	[22]
	<i>infB</i>	F: GTTTTCCCAAGTCACGACGTTGTACTCGCTGCTGGACTATATTCG R: TTGTGAGCGGATAACAATTTCCGCTTCAGCTCAAGAACTTC	462	[22]
	<i>tonB</i>	F: GTTTTCCCAAGTCACGACGTTGTACTTTATACCTCGGTACATCAGGTT R: TTGTGAGCGGATAACAATTCATTCGCGGCTGRGCRGAGAG	539	[22]

Results

Clinical characteristics and risk factors for CRKP infection mortality

A total of 108 CRKP strains were isolated, comprising of 56 boys (51.85%) and 52 girls, with a median patient age of 1.3 months (IQR 0.57–4.11) (Table 2). CRKP strains were obtained from various clinical specimens, including sterile fluids (29.63%, 32/108) and non-sterile fluids (70.37%, 76/108). The majority of samples were from sputum (58.33%, 63/108), with other sources consisting of venous blood (10.19%, 11/108), urine (9.26%, 10/108), BALF (8.33%, 9/108), ascites (4.63%, 5/108), cerebrospinal fluid (2.78%, 3/108), joint fluid (0.93%, 1/108), hydrothorax (0.93%, 1/108) and other specimens (1.85%, 2/108) (Fig. 1A).

Premature and dysplasia were common underlying conditions. CRKP strains originated from 13 different wards (Fig. 1B), with preterm pediatric department

(34/108) and internal medical intensive care unit (ICU) (22/108) being the top two sources, followed by the neonatal ICU (16.67%, 18/108), neonatal internal medicine department (8.33%, 9/108), surgical ICU (5.56%, 6/108) and neonatal surgery department (3.70%, 4/108). The renal rheumatology department ($n=3$), infant department ($n=3$) and pneumology department ($n=3$) each accounted for 2.78%. The urology surgery department ($n=2$) and cardio-thoracic surgery department ($n=2$) each accounted for 1.85%.

CRKP-positive children were hospitalized for an average of 44 days. Most patients were diagnosis as pneumonia and septicemia (sepsis) (Table 2). The symptoms included respiratory dysfunction, fever ($>37.5^{\circ}\text{C}$) and disturbance of consciousness. A total of 70 cases underwent mechanical ventilation (60.34%), and 37 cases (31.90%) underwent surgery. Analysis of clinical outcomes showed that 75% children recovered after treatment.

Table 2 Univariate and multivariate Cox analysis of CRKP infection mortality

	90-day survivors (n, %)	90-day nonsurvivors (n, %)	Univariate Cox		Multivariate Cox	
			HR (95% CI)	P	HR (95% CI)	P
Total	81 (100)	27 (100)	/	/		
Age (month)	Average	28.13	-2.796*	0.009		
	Newborn (≤ 28d)	39 (48.15)	0.300 (0.123–0.730)	0.008	0.444 (0.156–1.262)	0.128
	Nonnewborn	42 (51.85)	Reference	Reference		
Gender	Male	45 (55.56)	0.722 (0.327–1.594)	0.420		
	Female	36 (44.44)	Reference	Reference		
Hospital stays (days)	44.72	44.12	0.077*	0.939		
Main types of infection	Bloodstream infection	6 (7.41)	2.171 (0.799–5.896)	0.128		
	Respiratory infection	58 (71.60)	1.016 (0.423–2.438)	0.972		
	Urinary tract infection	9 (11.11)	0.835 (0.112–6.257)	0.861		
Separated department	ICU	30 (37.04)	2.309 (1.019–5.232)	0.045	1.456 (0.589–3.600)	0.416
Underlying condition	Premature	53 (65.43)	0.471 (0.206–1.076)	0.074		
	Pneumonia	33 (40.74)	1.173 (0.518–2.657)	0.702		
Diagnosis	Septicemia (sepsis)	19 (23.46)	0.942 (0.392–2.262)	0.893		
	NRDS	14 (17.28)	0.512 (0.172–1.526)	0.229		
	Fever	18 (22.22)	2.737 (1.223–6.125)	0.014	1.704 (0.701–4.142)	0.240
	Dyspnea	47 (58.02)	0.572 (0.258–1.267)	0.168		
Invasive manipulation	Cough	14 (17.28)	1.324 (0.296–5.911)	0.713		
	Operation	27 (33.33)	0.713 (0.305–1.665)	0.434		
	Mechanical ventilation	48 (59.26)	1.094 (0.400–2.993)	0.861		

*: Independent t-test was performed
Abbreviation ICU, intensive care unit; NRDS, neonatal respiratory distress syndrome

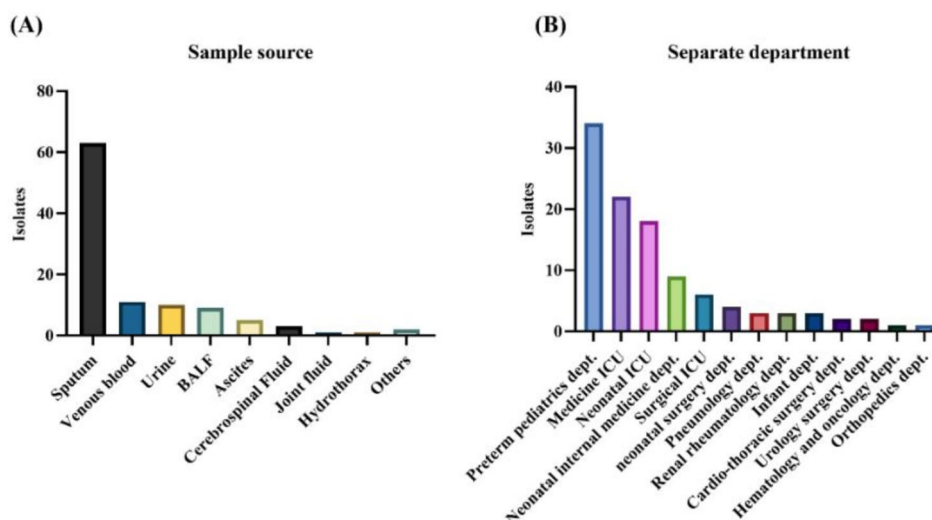


Fig. 1 CRKP strains isolated from children in Henan, China in 2021. Sample source (A) and separate department (B). Abbreviation: BALF: bronchoalveolar lavage fluid; dept.: department; ICU: intensive care unit

Table 3 Laboratory examination results of CRKP-infection patients

		$\bar{x} \pm s$	Standard range
Laboratory examination	White blood cells ($10^9/L$)	10.72 ± 5.971	5.6–14.5
	Neutrophil counts ($10^9/L$)	6.16 ± 4.526	0.6–7.1
	Lymphocyte count ($10^9/L$)	3.29 ± 2.134	3.2–10.7
	Neutrophil percentage (%)	54.83 ± 18.75	7–51
	Lymphocyte percentage (%)	33.31 ± 16.96	34–81
	CRP (mg/L)	25.20 ± 49.83	0–10

Regarding risk factors for CRKP infection-related mortality, Cox regression analysis was performed (Table 2). In the univariate Cox proportional hazards regression analysis, being a newborn (HR 0.300; 95% CI 0.123–0.730; $P=0.008$), ICU admission (HR 2.309; 95% CI 1.019–5.232; $P=0.045$) and fever (HR 2.737; 95% CI 1.223–6.125; $P=0.014$) were associated with increased crude mortality. However, in the multivariate Cox analysis, none of these factors—being a newborn (HR 0.444; 95% CI 0.156–1.262; $P=0.128$), ICU admission (HR 1.456; 95% CI 0.589–3.600; $P=0.416$) and fever (HR 1.704; 95% CI 0.701–4.142; $P=0.240$)—showed statistical significance.

Medical laboratory indices were also analyzed (Table 3). Increased biomarkers included the average neutrophil percentage and CRP (54.83% vs. 51%, 25.20 vs. 10 mg/L); other biomarkers did not show significant differences compared to standard levels.

Antimicrobial susceptibilities of clinical CRKP isolates

The antimicrobial susceptibility profiles of CRKP strains were shown in Fig. 2. Of the 108 isolates, 107 exhibited a MDR phenotype, and one isolate was classified as XDR. There were no PDR isolates in this study. The isolates showed high levels of resistance to β -lactams/ β -lactamase inhibitor combinations. However, the

antibiotics commonly used in clinical treatment demonstrated excellent antibacterial activity against CRKP strains. Colistin and tigecycline susceptibilities were 98.10% and 78.50%, respectively, whereas trimethoprim/sulfamethoxazole showed potent activity against CRKP isolates (91.43%).

Carbapenemase genotypes of CRKP isolates

Carbapenemase production was the most prevalent antibiotic resistance mechanism in CRKP strains, with several carbapenemase types accounted for a significant proportion of clinical isolates. The distribution of carbapenemase genes was analyzed using multiplex PCR to detect the most common carbapenemase genes, including *bla*_{KPC}, *bla*_{NDM}, *bla*_{OXA-48-like}, *bla*_{IMP}, and *bla*_{VIM} (Fig. 3). Among the CRKP isolates, *bla*_{KPC} was the predominant carbapenemase gene (86.11%, 93/108), followed by *bla*_{NDM} (5.56%, 6/108) and *bla*_{IMP} (2.78%, 3/108). No *bla*_{VIM} and *bla*_{OXA-48} genes were detected. Three isolates did not harbor any of the carbapenemase genes analyzed in this study. Additionally, isolate CRKP 21,096 and CRKP21322 co-harbored *bla*_{KPC} and *bla*_{NDM}, whereas isolate CRKP 21,305 co-harbored *bla*_{KPC} and *bla*_{IMP}.

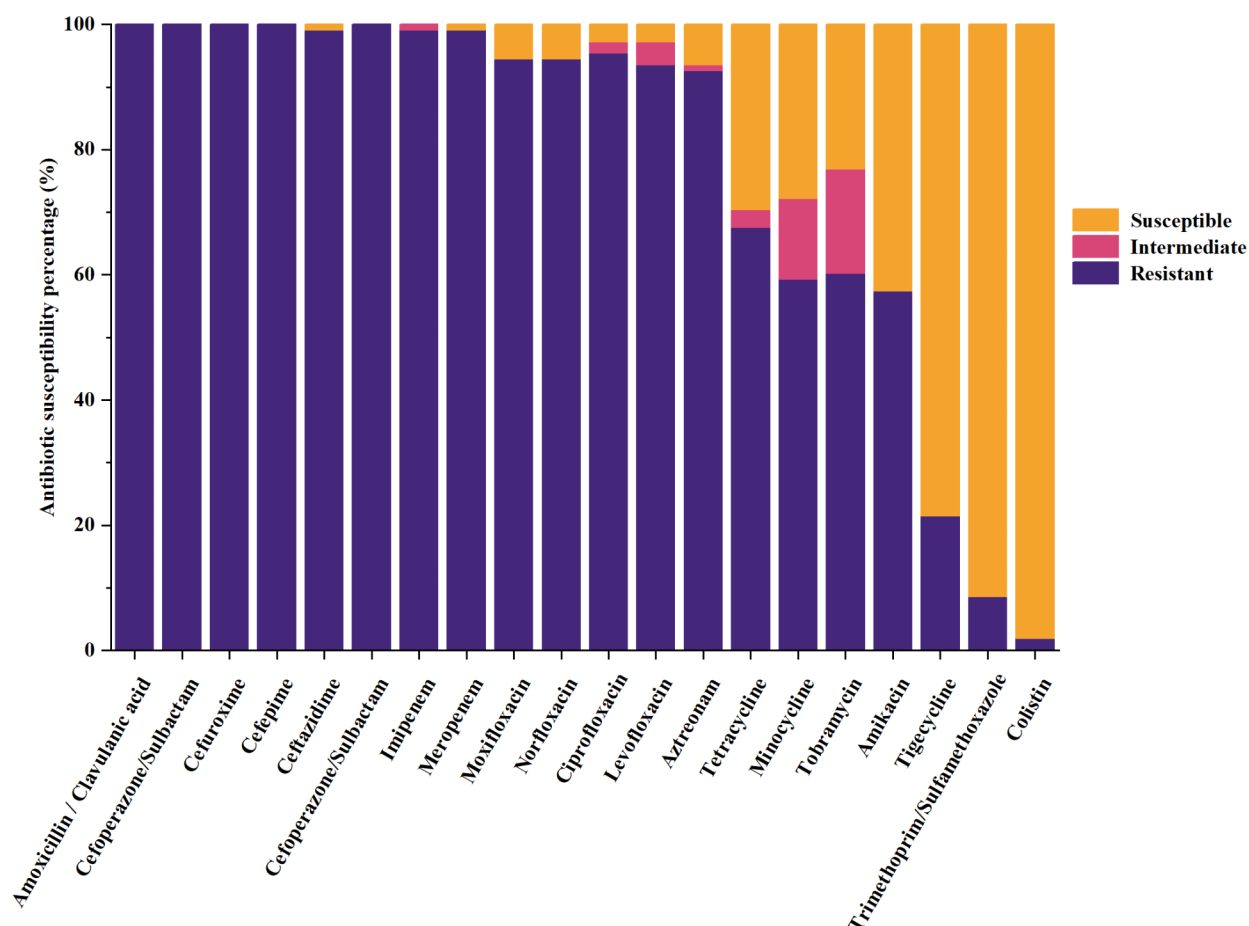


Fig. 2 Antimicrobial susceptibility testing results of clinical CRKP Isolates

Virulence genes of CRKP isolates

Bacterial virulence characteristics are closely related to clinical manifestations, thus common virulence genes were tested (Fig. 3). The coexistence of *rmpA/rmpA2* with *iucA* was identified as an indicator of hypervirulent CRKP in our analysis. As shown in the heatmap, all strains possessed *fimH*, and 98% of isolates harbored *mrkD*. The high virulence factor *rmpA* was not detected, but *rmpA2* was present in 77 isolates (71.30%). Moreover, the prevalence of the virulence factor *iucA* was 53 (49.07%), and strains containing both *rmpA2* and *iucA* accounted for 48.15% (52/108).

The children infected with hypervirulent CRKP (52) and classical CRKP (56) were divided into two subgroups to investigate their survival rate using a Kaplan-Meier curve (Fig. 4). The survival rate of the hypervirulent CRKP-infected subgroup was higher than that of the classical CRKP-infected subgroup, but the difference was not statistically significant ($P=0.631$, log-rank test).

MLST analysis of CRKP isolates

To explore the genetic composition of the CRKP strains, MLST was conducted to analyze the STs. Seven house-keeping genes were amplified and sequenced, and the sequences were uploaded to the Institute Pasteur MLST database to obtain their ST. Nine distinct STs were identified among the 108 CRKP isolates. Figure 5 shows the minimum spanning tree based on MLST results. The most prevalent type was ST11 (82.41%, 89/108), followed by ST2154 (4.63%, 5/108) and ST307 (3.70%, 4/108). ST2237 ($n=1$), ST29 ($n=1$), ST37 ($n=1$), ST45 ($n=1$), ST340 ($n=1$) and ST1640 ($n=1$) each accounted for 0.93%. In combination with the carbapenemase gene analysis results, ST11-KPC CRKP was the most prevalent type. Notably, all ST307 CRKP strains carried *bla*_{NDM}, whereas four ST2154 strains harbored *bla*_{IMP}.

PBRT typing of CRKP isolates

Since the transmission of virulence genes and resistance genes depends mainly on plasmids, plasmid typing was conducted using PCR-based replicon sequence typing.

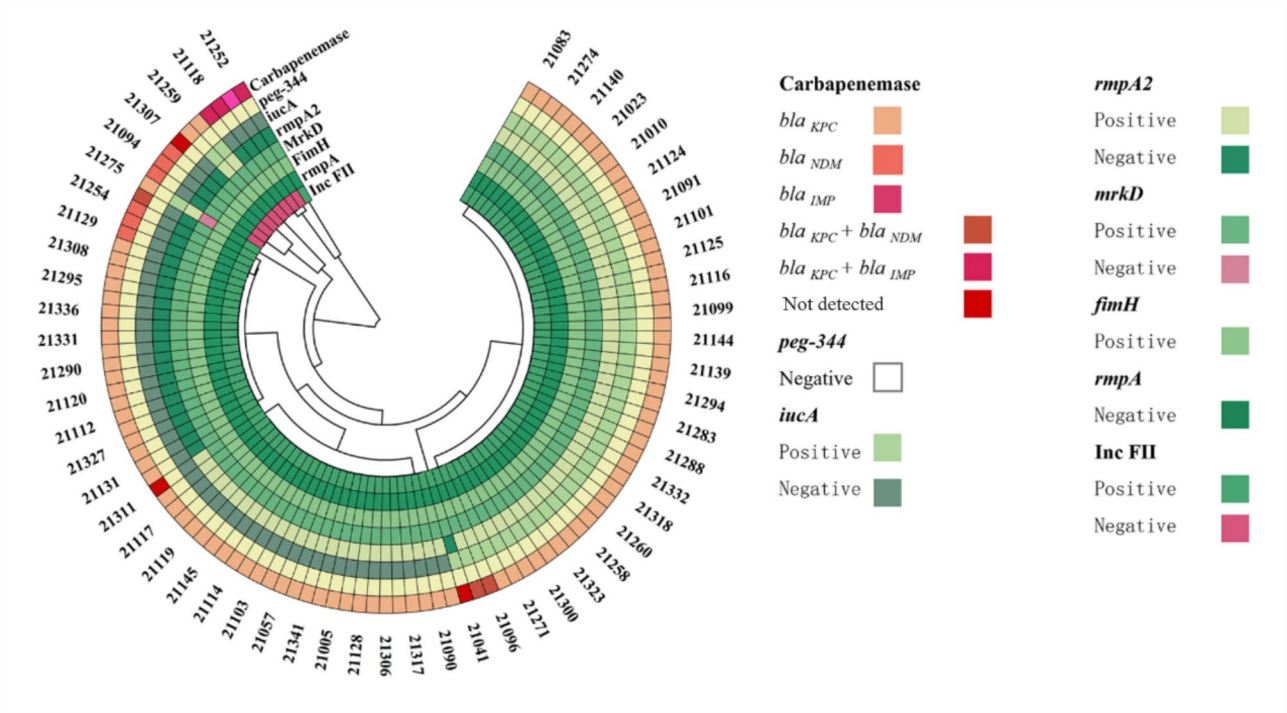


Fig. 3 Phenotypic and core genes for all carbapenem-resistant *Klebsiella pneumoniae* strains

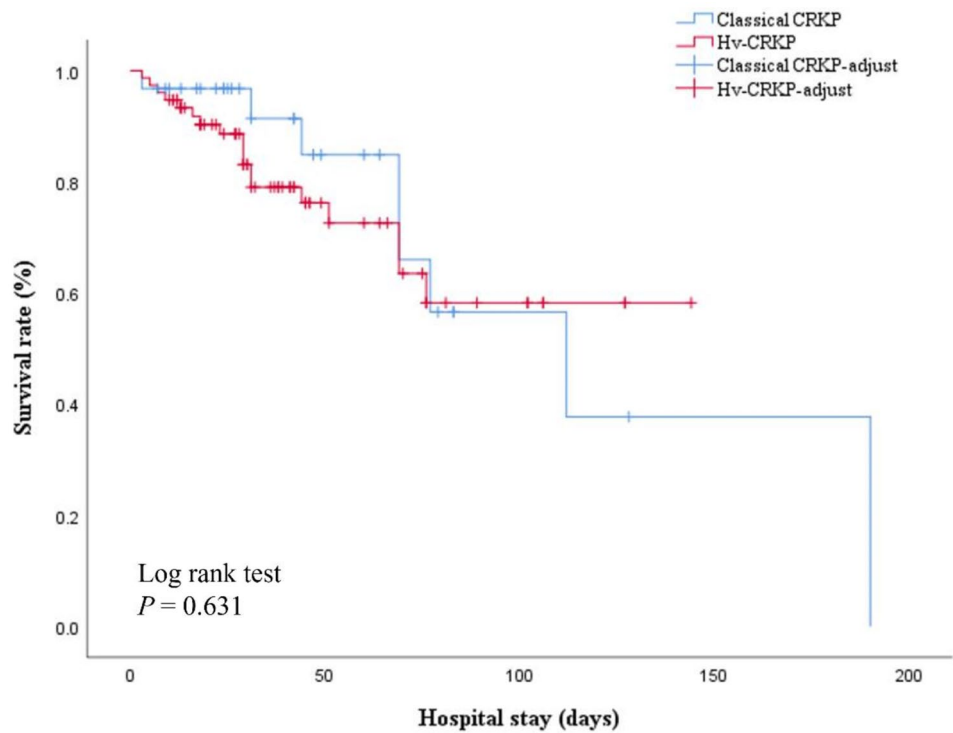


Fig. 4 The Kaplan-Meier curve survival of hospital stay in children with hypervirulent CRKP and classical CRKP infections

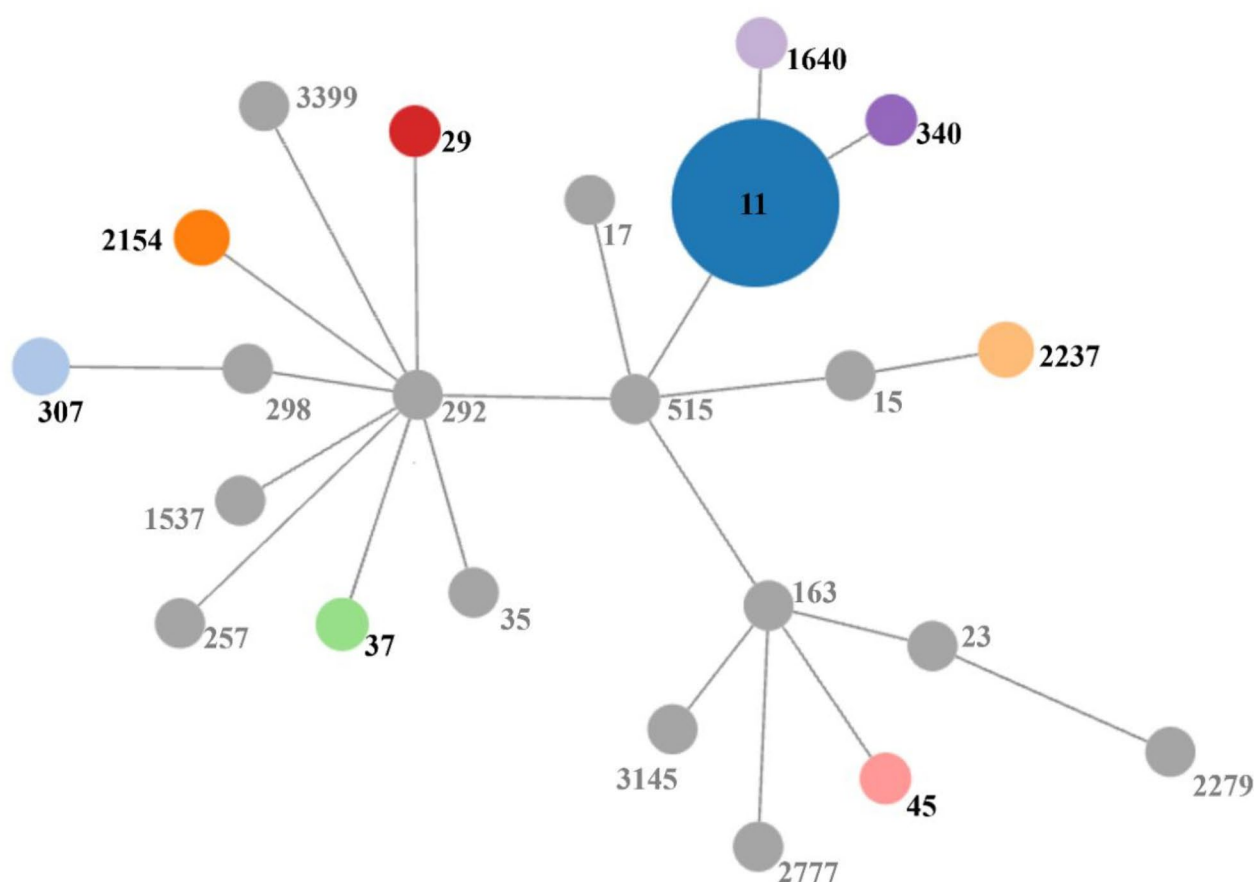


Fig. 5 Minimum spanning tree of MLST results of carbapenem-resistant *Klebsiella pneumoniae* (CRKP). Each node represents an identical sequence type. Circle diameter based on the number of isolates. Colorful nodes represent CRKP ST types included in this study. Grey nodes represent correlative *K. pneumoniae* ST types from NCBI database

The distribution of plasmid replicons showed that 85.19% (92/108) of the isolates possessed the IncFII plasmid. Interestingly, we found one strain that harbored both Inc FII and FIB plasmids, whereas another strain contained four plasmid replicons, consisting of Inc FII, I1, FIA and FIB.

Discussion

Antimicrobial resistance remains a global concern, particularly regarding infections caused by carbapenem-resistant bacteria. Increased prevalence and resistance rates of carbapenem-resistant strains in pediatric populations are alarming. Previous studies have identified various mechanisms underlying carbapenem resistance, including carbapenemase production, structural variations in penicillin binding proteins, changes in membrane permeability, and the alteration of biofilm [25]. Globally, the production of carbapenemase is the leading cause of carbapenem resistance, including in China [26]. However, the distribution of carbapenem-resistant strains and carbapenemase genes varies geographically. Research on

pediatric CRKP infections is more limited compared to adults, with few reports available from Henan. Therefore, we systematically analyzed the molecular and clinical features of CRKP infections in Henan, China, to provide insights for the epidemiological monitor and control of CRKP infections in children.

Possible sources of CRKP infections in children may include prolonged stays in healthcare settings; use of invasive medical devices such as ventilators, urinary catheters, and intravenous catheters; extended use of broad-spectrum antibiotics; exposure to contaminated environmental surfaces; close contact with individuals harboring CRKP; and infections resulting from colonization by CRKP strains [27, 28]. Extensive use of carbapenem has led to the increase of CRKP strains, and plasmid mediated antibiotic resistance gene transmission may exacerbate this issue. Therefore, it is essential for healthcare providers and caregivers to remain vigilant about infection control measures and antimicrobial stewardship practices to prevent the spread of CRKP in pediatric populations. Younger age is a risk factor for infection

and poor outcomes, as is staying in the ICU. ICU patients often have more severe complications and may be treated with broad-spectrum antibiotics or longer durations of antibiotics use, along with more invasive procedures, all of which could contribute to poor outcomes [29]. Newborn children are particularly susceptible to infections and adverse outcomes due to an immature immune system, especially if they are preterm and have underlying conditions [30]. Additionally, the use of mechanical ventilation and surgical procedures is closely associated with increased antibiotic use among immunocompromised patients [31]. The higher isolation rate of CRKP strains in preterm pediatric wards, internal medical ICUs, and neonatal ICUs warrants special attention, which may be linked to the high proportion of patients transferred to these departments.

The antibiotic susceptibility results indicated that all CRKP strains were MDR strains and two strains were XDR. These strains exhibited highly resistance to cephalosporins, aminoglycosides, and fluoroquinolones, while showing relative susceptibility to colistin and tigecycline. Considering the susceptibility of antibiotics when designing monotherapy or combination therapy regimens is recommended. However, there are limited therapeutic options of antibiotics in children due to side effects. Tigecycline is not recommended for patients under 8-years-old due to dental staining; whereas fluoroquinolones are only permitted for children under 18 years old in China when no other effective drugs are available for severe clinical infection [32]. According to our data, colistin, tigecycline and trimethoprim/sulfamethoxazole showed potent activity against CRKP isolates in our region. Therefore, pediatric treatment decisions should be based on bacterial minimum inhibitory concentrations (MICs), carbapenemase types and illness severity to achieve individualized therapy.

Carbapenemase production is the primary mechanism for carbapenem-resistance in CRKP; however, the enzyme types vary across regions and populations. Our analysis showed an increased prevalence of KPC-producing isolates, with *bla*_{NDM} also detected, albeit at a lower frequency (86.11% vs. 5.56%), which differs from previous reports. In China, *bla*_{NDM} is the predominant carbapenemase in pediatric CRKP isolates, whereas *bla*_{KPC} is more common in adults [33]. A higher proportion of KPC-producing CRKP isolates (87.2%) was also reported in pediatric patients in Nanjing, China [34]. The prevalence of carbapenemase shifted over time, with *bla*_{NDM-1} being predominant in 2017 and 2018, whereas *bla*_{KPC-2} became more dominant in 2019, being the predominant gene from 2020 to 2021 in the neonatal CRKP isolates from Shanghai, China [35]. Similarly, Zhou et al. reported that the *bla*_{NDM-5} gene was the predominant resistance gene (58.1%, 18/31) detected in pediatric CRKP strains

from Xuzhou, China [5]. The *bla*_{KPC} gene was also the most frequent resistance gene detected in CRKP strains from pediatric patients in Southern Brazil [36]. In contrast, Fisher et al. conducted an analysis of CRE characteristics in children across 18 U.S Health Care System Study Sites and reported a lower percentage of carbapenemase genes (29%, 14/48), nine of which was *bla*_{KPC} [37]. A systematic review of the epidemiology of carbapenem-resistant Enterobacterales in Africa indicated that NDM (43.1%) and OXA-48-like (42.9%) carbapenemases were the most frequently detected [38]. Han et al. [7] reported that *bla*_{OXA-48-like} could become the “third popular” carbapenemase after KPC and NDM in China due to its presence on mobile genetic elements and rapid transmission. However, no *bla*_{OXA-48-like} or *bla*_{VIM} genes were detected in our analysis.

Three isolates contained *bla*_{IMP}, while two isolates co-expressed *bla*_{KPC} and *bla*_{NDM}, and one isolate CRKP 21,305 co-expressed *bla*_{KPC} and *bla*_{IMP}. Although the KPC enzyme can hydrolyze monobactam antibiotics, certain β -lactamase inhibitors, such as avibactam, inhibit it. However, avibactam is ineffective against metallo- β -lactamase (MBL) producers (e.g., *bla*_{IMP} and *bla*_{NDM}). Consequently, the co-expression of various carbapenemase classes, including *bla*_{KPC} and *bla*_{NDM}, may result in elevated levels of resistance to carbapenems and other antimicrobials [39]. This phenomenon carries significant clinical implications regarding treatment efficacy and patient outcomes, particularly in pediatric populations. Zheng et al. noted that *bla*_{IMP}-positive CRKP strains were less commonly reported and the reported strains belonged to different ST strains [40], whereas in our analysis four *bla*_{IMP} CRKP strains were ST2154 strains, warranting further investigation. Additionally, three isolates did not contain the carbapenemase genes analyzed in this study, suggesting other resistance mechanisms. Non-carbapenemase-producing CRKP may acquire carbapenem resistance through a combination of non-carbapenemase β -lactamases and potential chromosomal mutations, including missense mutations, the loss of porins such as OmpK35/36, or frameshift missense mutations in efflux pump systems like the cation efflux system protein CusF and nickel/cobalt efflux protein RcnA [12, 41, 42]. This underscores the significance of whole-genome sequencing in elucidating the mechanisms underlying resistance.

K. pneumoniae is generally classified into classic and hypervirulent *K. pneumoniae*. The classic type is prevalent in hospital, often carrying antibiotic resistant plasmids and lacking an excessive capsule. The hypervirulent type is commonly found in the community, carrying high virulence plasmids [9, 43]. Capsule, lipopolysaccharide, fimbriae, and siderophores are four well characterized virulence factors [44]. Hence, we analyzed the prevalence of four main virulence factor genes in CRKP isolates to

explore the composition of virulence genes. Type 1 fimbriae (*fimH*), commonly expressed during urinary tract infections, facilitate the invasion of bladder cells, whereas Type 3 fimbriae (*mrkD*) contributes to biofilm formation and evasion of the host immune response [45]. Our study revealed that almost all strains contained *fimH* and *mrkD* genes, suggesting that these genes may be insufficient in distinguishing high virulence. A lower proportion of *mrkD* (51.3%) and *fimH* (30.7%) has been reported for nosocomial *K. pneumoniae* strains [45]. In other studies, the positive rates of *fimH* and *mrkD* genes varied from 80 to 100% [46]. These results indicate that virulome patterns in *K. pneumoniae* can differ between strains, and their roles in pathogenicity and their clinical significance warrants further study.

At present, there is no standardized definition for hypervirulent *K. pneumoniae* strains. Identification of hypervirulence through biomarker detection is a commonly used method [11]. Aerobactin is the primary siderophore produced by hvKP and plays a crucial role in enhancing virulence both in vitro and in vivo. The genes encoding aerobactin, particularly *iucA*, serve as important markers for the identification of hvKP. Furthermore, *rmpA*- or *rmpA2*-mediated capsule overproduction also contributes to the hypervirulent phenotype by enhancing resistance to complement-mediated bactericidal activity and inhibiting phagocytosis. Consequently, a combination of markers- *rmpA* or *rmpA2* and/or *iucA*- has been proposed to define hypervirulence. Hence, we identified co-existence of *rmpA/rmpA2* with *iucA* as indicators of hypervirulent biomarkers [12, 47]. We found that 71.30% isolates carried *rmpA2* and 49.07% had *iucA*, with co-existing strains of *rmpA2* and *iucA* accounting for 48.15%. Du et al. [48] found that hypervirulent *K. pneumoniae* producing KPC-2 increased significantly from 2019 to 2020 (5.3% vs. 67.6%). Hu et al. reported a 40.6% prevalence of hypervirulent strains among 1,017 CRKPs collected from 40 hospitals across China between 2016 and 2020 [12]. These results suggested that transmission of strains exhibiting both virulence and carbapenem resistance warrants close monitoring. There was no significant difference in the survival rates between patients infected with hypervirulent CRKP or those infected with classical CRKP. Similarly, Kim et al. reported that hypermucoviscous *K. pneumoniae* was not associated with worse clinical outcomes [49]. The lack of consistent survival differences may be influenced by varying criteria used to identify hypervirulent strains, underscoring the need for standardization and effective identification methods. To prevent nosocomial transmission of CRKP, comprehensive measures have been implemented. These measures primarily include implementing active surveillance for CRKP colonization and infections, placing infected or colonized patients under contact precautions

such as the use of gloves and gowns by healthcare workers during patient care, strengthening infection control measures (e.g., hand hygiene and environmental cleaning), and employing strategies to optimize antibiotic stewardship [50]. To examine the genetic composition of CRKP strains, MLST was conducted to identify STs. Nine distinct STs were identified, with ST11 being the most prevalent, followed by ST2154 and ST307. KPC was considered as a predominant carbapenemase in ST11 CRKP. This finding is consistent with Fu et al., who reported ST11 as the most common type in KPC-producing CRKP [8], whereas other common STs were rarely detected in our study. Similarly, Tao et al. reported that ST11 (75/86) was the most common CRKP sequence type isolated from Nanjing Children's Hospital in Jiangsu, China [34]. The most prevalent STs were ST11 (8.06%), ST37 (8.06%) and ST76 (8.06%) among 62 CRKP isolates colonization in pediatric inpatients hospitalized in Guangzhou, China [51]. ST11, accounting for 56.45% (35/62) and 82.5% (170/231), was the principal ST among the CRKP isolates causing bloodstream infections in pediatric patients from Tianjin [52] and Nanjing, China [53]. In a children's hospital in Shanghai, China, the predominant sequence type of neonatal CRKP infections shifted from ST278 during 2017 and 2018 to ST11 in 2020 and 2021 [35]. ST2735 was the predominant ST type in pediatric CRKP strains from Xuzhou, China, representing 19.4% (6/31) of cases [5]. ST11 accounted for 40% (10/25) of cases among the CRKP strains collected from pediatric patients in Southern Brazil [36]. A systematic review of the epidemiology of carbapenem-resistant Enterobacterales in Africa indicated that ST147 was the most commonly reported CRKP ST, followed by ST101 [38]. As no relevant literature was found in PubMed, the ST2154 strain identified in this study appears to be a novel ST. All ST307 CRKP strains carried the *bla*_{NDM} gene. The diversity of dominant strains across different geographical regions was observed, with ST11 consistently emerging as the principal ST among the pediatric CRKP isolates in China.

Plasmids play a crucial role in the transmission of genes contributing to antibiotic resistance and virulence. Plasmid replicant groups were widely distributed in CRKP and were associated with different carbapenemase. IncF, A/C, and X were the most prevalent compared to other Inc groups, and IncF has been reported worldwide [54]. In this study, plasmid classification revealed a high incidence of IncFII (85.19%), corresponding with a previous report [55]. These results revealed that prevalence of IncFII in children was comparable to that in adults. Notably, one strain not only harbored IncFII, but also IncI1, IncFIA and IncFIB. Thus, continuous monitoring of plasmid profiles in both adults and children, especially for IncFII, is essential to control the spread of virulent and AMR plasmids.

A limitation of our study is that the strains were collected from patients admitted to one large hospital in Henan, even though a high proportion of patients were transferred from various regions of Henan. These referrals may affect the generalizability of the study population. To improve the understanding of epidemiological trends, longitudinal and multicenter studies with larger sample sizes should be conducted to monitor CRKP trends over time and to identify risk factors contributing to the clinical outcomes. In addition, while we focused on commonly analyzed antibiotic resistance and virulence genes, whole genomic analysis should be applied in the future to uncover the genetic basis of the strains, especially for strains where no common carbapenemase genes were detected. It would also be valuable to investigate the potential resistance mechanisms against colistin and tigecycline in CRKP isolates.

Conclusion

In summary, our study highlights the presence of MDR CRKP strains in patients admitted to a large pediatric hospital in Henan, China. Regarding risk factors for mortality of CRKP infection, being a newborn, ICU admission, and fever were associated with the crude mortality. KPC production was the predominant mechanism of carbapenem resistance in Henan pediatric patients, which was different from the NDM-dominated carbapenem-resistant mechanism among children. The ST11 clone and IncFII plasmids showed high prevalence. Additionally, a novel ST2154 CRKP strain was identified, with four of these strains carrying the *bla*_{IMP} gene. In addition, a significant proportion of strains exhibited both hypervirulence and MDR traits, which were identified using commonly applied hypervirulent markers. This study highlights the unique epidemiological, antibiotic resistance and virulence patterns of CRKP strains in Henan pediatric patients, emphasizing the significance of effective and continuous surveillance on the epidemiology characteristics of CRKP among children within the region in order to provide insight for the management and control of this pathogen.

Author contributions

JYM: Conceptualization, Methodology, Writing-original draft; KJG: Investigation, Resources; MCL: Methodology, Formal analysis; JJZ: Methodology, Resources; XRS: Methodology, Supervision; YDZ: Resources; ZDY: Data curation; ZYY: Writing-review & editing; WLC: Writing-review & editing; WCZ: Data curation; ADS: Supervision; JMY: Resources, Supervision; HQS: Formal analysis, Supervision, Writing-review & editing; LFL: Supervision, Conceptualization, Writing-review & editing. All authors have revised the manuscript critically and approved the submission.

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Data availability

Data are provided within the manuscript. The raw datasets used in the current study are available from the corresponding author on request.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Henan Children's Hospital (2022-H-K37). Informed consent was exempt.

Competing interests

The authors declare no competing interests.

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