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ORIGINAL RESEARCH

Molecular Epidemiology, Clinical Characteristics and Risk Factors for Bloodstream Infection of Multidrug-Resistant *Klebsiella pneumoniae* Infections in Pediatric Patients from Tianjin, China

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Purpose: The data on pediatrics with Multidrug-Resistant (MDR) *Klebsiella pneumoniae* infections are scarce. This study aims to investigate the molecular epidemiology of MDR *Klebsiella pneumoniae*, detect the mechanism of drug resistance, and determine the clinical risk factors for carbapenem-resistant *Klebsiella pneumonia* (CRKP) bloodstream infections (BSIs) in a children's hospital.
Methods: A total of 62 strains were collected from Tianjin Children's Hospital. Carba NP and polymerase chain reactions (PCR) were performed to detect MDR mechanisms. Multilocus sequence typing (MLST) was used for analyzing strain homology. Clinical data were collected and logistic regression was used for BSI risk factors.
Results: ST11 was the principal ST among the CRKP isolates clinically, accounting for 56.45% (35/62); there were also 57.14% (20/35) ST11 CRKP strains co-carrying bla_{NDM-5} and bla_{KPC-2}, which were resistant to most of the tested antibiotics, being susceptible only to cotrimoxazole and tigecycline. The clinical data showed that 72.73% (40/55) of children with CRKP infection had serious underlying diseases; 20.00% (11/55) patients developed BSIs with the potential to cause multiple organ failure, shock and death. The logistic regression showed that the risk of BSIs caused by CRKP strain infections in children with hematological malignancies after chemotherapy was 7 times that of other children (95%Cl: 1.298–45.415, *P*=0.025).
Conclusion: ST11 was the prevalent clone in our hospital. The emergence of ST11 CRKP co-carrying bla_{NDM-5} and bla_{KPC-2} should be a cause for alarm as they were resistant to most of the tested antibiotics. CRKP strain infections are mainly occurring in young immunocompromised patients and the chemotherapy for hematological malignancies is an independent risk factor for BSIs.
Keywords: carbapenem-resistant*Klebsiella pneumonia*, bla_{KPC-2}, bla_{NDM-5}, ST11, children, bloodstream infection

Introduction

Klebsiella pneumonia is a kind of gram-negative bacillus pathogen that causes a wide range of hospital- and community-acquired infections, mainly in immunocompromised individuals. The inappropriate use of antibiotics has attributed to the emergence of multidrug-resistant (MDR) *Klebsiella pneumoniae* worldwide.

In the decades since, MDR *Klebsiella pneumoniae* has spread globally and causes a variety of infections, such as pneumonia, urinary tract infections, surgical site or wound infections, and bloodstream infections (BSIs) associated with high morbidity and mortality.[1,2] Carbapenem has the characteristics of broadest antibacterial spectrum, which is always considered as the last resort for the treatment of the infections caused by multidrug-resistant strains.[3] However, with the

rapidly increasing numbers of carbapenem-resistant Enterobacterales (CRE), the Centers for Diseases Control and Prevention (CDC) classified them as an urgent threat to public health.[4,5] The World Health Organization released the Antimicrobial Resistance: Global Report on Surveillance 2014, which identified that carbapenem-resistant Klebsiella pneumonia (CRKP) infection is spreading worldwide.[6] Data relating to CRKP in the China Antimicrobial Resistance Surveillance Network (CHINET) report 2019 were analyzed: CRKP strains account for 72.4% of clinical CRE infections in China and the meropenem resistance rate in *Klebsiella pneumoniae* increased from 2.9% in 2005 to 26.8% in 2019.[7] Although the majority of patients infected with CRKP were adults, approximately 1–5% of CRE infections occurred in children. Alejandro Díaz et al considered that CRKP infections in children were increasing worldwide.[8]

Carbapenemase can be divided into the Ambler molecular class A (ie, KPC, GES), or D (ie, OXA-48-like) serine β-lactamases and B (ie, NDM, VIM, IMP, GIM, SPM, SIM, DIM) metallo-β-lactamases (MBLs).[9,10] Although the mechanisms of carbapenem resistance are heterogeneous, the production of carbapenemase which can hydrolyze carbapenems was considered to be more transmissible. This was because the spread of carbapenemase gene is frequently through horizontal transmission such as nosocomial infection rather than membrane impermeability or active drug efflux frequently through excessive antibiotic exposure.[11] Besides, the patients with carbapenemase-producing (CP)-CRE tend to be more associated with poorer, more outcomes as well as more virulent spread than non-CP-CRE.[12] The research conducted by Hovan et al showed that the hazard of death for CP-CRE at 30 days after bacteremia onset was 2.4 times that for non-CP-CRE bacteremia.[13]

BSIs mainly occur in intensive care units (ICUs) as well as being associated with high rates of morbidity and mortality.[14] Liu et al observed a 30-day all-cause mortality rate of 52.1% in patients with CRKP BSIs.[15] A systematic review reported that the mortality rate of CRKP BSIs ranged from 42% to 84%.[16] The study conducted by Yuanyuan Li showed that the proportion and mortality of CRKP BSIs has increased significantly, with the percentage of *Klebsiella pneumoniae* in BSIs increasing from 7 to 12% from 2014 to 2019.[17] Young people, who have immature immune systems, infected with CRKP tend to develop severe pneumonia and higher mortality, perhaps as a result of the limited antibiotic options remaining, which brought heavy burdens on families and children.[8] The risk factors for BSIs explored in adults are helpful to provide guidance for the prevention and reasonable treatment of hospital infections, which includes β-lactam/β-lactamase inhibitor combinations exposure, carbapenems exposure, solid organ transplantation, gastric tube indwelling, lower Hb, cholinesterase and so on.[18,19] However, the data of pediatrics with CRKP infection are scarce.

This study aimed to assess the independent risk factor for BSIs of CRKP in children and characterize MDR *Klebsiella pneumoniae* clinical isolates, considering the following objects: 1) to determine the drug resistance, 2) to detect the carbapenemase genes, 3) to describe the STs of CRKP; epidemiology characteristics and drug susceptibility profiles are indispensable to appropriate and in-time antibiotic therapy, and critically important to the outcome.

Materials and Methods
Clinical Isolates and Antimicrobial Susceptibility Testing
A total of 62 clinical MDR *Klebsiella pneumoniae* strains (obtained from the guardian after informed consent) were collected from Tianjin Children's Hospital in 2019, and all strains were stored in −80°C. Ethical approval was given by the medical ethics committee of Tianjin Children's Hospital, with the following reference number: 2021-KY-05. The study complies with the Declaration of Helsinki. MDR *Klebsiella pneumoniae* species and antimicrobial susceptibility testing were performed by VITEK2 compact system. Results were interpreted according to the interpretive standards of Clinical and Laboratory Standards Institute.

Carba NP Test
Carba NP test was used for the detection of carbapenemase-producing MDR *Klebsiella pneumoniae* from clinical isolates through the visible change in color of phenol red, which is a kind of pH indicator. The protein of MDR *Klebsiella pneumoniae* strains was extracted with a B-PER™ Bacterial Protein Extraction Reagent (Thermo Scientific, USA). 100 μL extracted protein was added in A and B solution, respectively, and then Incubated at 37°C. The A solution contained 0.05% phenol red (pH=7.8±0.1), 6 mg/mL Imipenem (Solarbio, Beijing, China), and 0.1 mmol $ZnSO_4$. The

B solution contained 0.05% phenol red (pH=7.8±0.1). If the color of solution A changes from red to orange or yellow and the color of solution B is not changed, it can be regarded as positive.

Molecular Analysis of Carbapenem-Resistant Genes

DNA of MDR *Klebsiella pneumoniae* strains was extracted with a Rapid Bacterial Genomic DNA Isolation Kit (Sangon Biotech, Shanghai, China). Polymerase chain reactions (PCRs) were performed to detect the carbanemase resistant genes that include class A β-lactamases (bla_{KPC} and bla_{GES}), class B metallo-β-lactamases (bla_{IMP}, bla_{VIM}, bla_{NDM}, bla_{GIM}, bla_{DIM}, bla_{SIM}, and bla_{SPM}), and class D β-lactamases (bla_{OXA-48}). With reference to publications (bla_{SPM}, bla_{SIM}, bla_{NDM}, bla_{GIM} and bla_{DIM};[10] bla_{GES}, bla_{OXA-48}, bla_{IMP}, and bla_{KPC};[9] bla_{VIM}[20]), the primers and reaction of conditions are shown in Table 1. PCR products were sequenced to confirm and the obtained sequences were aligned using BLAST in GenBank.

Multilocus Sequence Typing Analysis (MLST)

Seven housekeeping genes-*gapA* (glyceraldehyde 3-phosphate dehydrogenase), *infB* (translation initiation factor 2), *mdh* (malate dehydrogenase), *pgi* (phosphoglucose isomerase), phoE (phosphorine E), *rpoB* (β-subunit of RNA polymerase), and *tonB* (periplasmic energy transducer), are internal fragments for MLST of *Klebsiella pneumoniae*, which were amplified and sequenced. The sequences were aligned in the Klebsiella PasteurMLST database (https://bigsdb.pasteur.fr/klebsiella/klebsiella.html) to obtain the unique allele of each housekeeping gene and the STs were determined by the combination alleles of 7 housekeeping genes. MLSTs were analyzed by PHYLOViZ on the website (https://online.phyloviz.net/index).

Statistical Analysis

Statistics were analyzed by SPSS 19.0. Normally distributed data were expressed as mean±standard deviation. Chi-square test or Fisher's exact test were used for comparing categorical variables and unconditional logistic regression was

Table 1 Primers and Reaction Conditions Used for the Detection of Carbapenem Resistance Genes

Ambler Class	Primer[a]	Sequence (5'-3')[b]	Gene	Product Size	Annealing Temperature
A	KPC-F	CATTCAAGGGCTTTCTTGCTGC	bla_{KPC}	538bp	55°C
	KPC-R	ACGACGGCATAGTCATTTGC			
A	GES-F	AGTCGGCTAGACCGGAAAG	bla_{GES}	399bp	57°C
	GES-R	TTTGTCCGTGCTCAGGAT			
B	IMP-F	TTGACACTCCATTTACDG	bla_{IMP}	139bp	55°C
	IMP-R	GATYGAGAATTAAGCCACYCT			
B	VIM-F	GTTTGGTCGCATATCGCAAC	bla_{VIM}	389bp	54°C
	VIM-R	AATGCGCAGCACCAGGATAG			
B	NDM-F	GGTTTGGCGATCTGGTTTTC	bla_{NDM}	621bp	52°C
	NDM-R	CGGAATGGCTCATCACGATC			
B	GIM-F	TCGACACACCTTGGTCTGAA	bla_{GIM}	477bp	52°C
	GIM-R	AACTTCCAACTTTGCCATGC			
B	DIM-F	GCTTGTCTTCGCTTGCTAACG	bla_{DIM}	699bp	52°C
	DIM-R	CGTTCGGCTGGATTGATTTG			
B	SIM-F	TACAAGGGATTCGGCATCG	bla_{SIM}	570bp	52°C
	SIM-R	TAATGGCCTGTTCCCATGTG			
B	SPM-F	AAAATCTGGGTACGCAAACG	bla_{SPM}	271bp	52°C
	SPM-R	ACATTATCCGCTGGAACAGG			
D	OXA48-F	GCTTGATCGCCCTCGATT	bla_{OXA-48}	281bp	57°C
	OXA48-R	GATTTGCTCCGTGGCCGAAA			

Notes: [a]F: Forward Primer, R: Reverse Primer. [b]D = A or G or T; Y = C or T.

performed to analyse the risk factors for BSIs with CRKP. The *p*-value <0.05 is considered to be a statistically significant difference.

Results

Drug Resistance Detection of MDR *Klebsiella Pneumoniae*

The MDR *Klebsiella pneumoniae* clinical isolates were highly resistant to carbapenem antibiotics. The drug susceptibility results of the MDR *Klebsiella pneumoniae* strains are shown in Table 2. The resistance rates of ertapenem and meropenem were 96.72% (59/61) and the imipenem resistance rate was 93.44% (57/61). In addition, the penicillin, cephalosporin antibiotics, such as ampicillin, cefazolin, ceftriaxone resistance rates were 100.00% (61/61). The resistance rates of glycosides amikacin and tobramycin were 37.70% (23/61) and the resistance rate of gentamicin was 49.18% (30/61). Tigecycline is a new kind of glycine acyltetracycline antibiotic, whose resistance rate was only 8.20% (5/61).

Carbapenemase-Resistant Genes

The production of carbapenemase was the principal mechanism of MDR *Klebsiella pneumoniae* clinical isolates. The results of Carba NP test showed that carbapenemase-producing *Klebsiella pneumoniae* accounted for 90.32% (56/62). Then PCR screening revealed that bla_{KPC-2} belongs to the class A KPC-type β-lactamases, accounting for 51.61% (32/62). The class B metallo β-lactamases (MBLs) identified in isolate CRKP strains were mainly NDM, as well as bla_{NDM-5}, and accounted for 54.84% (34/62); bla_{NDM-1} accounted for 17.74% (11/62).

It is noteworthy that there were 20 ST11 strains co-carrying KPC-2 and NDM-5, and 12 ST11 CRKP strains carrying only bla_{KPC-2} resistance gene. bla_{NDM-5}-resistant genes were carried by ST20, ST35, ST6094, ST571, ST1565 and 57.14% (20/35) ST11. STs carrying bla_{NDM-1}-resistant genes were ST716, ST2715, ST5295, ST1569, and ST534.

MLST

ST11 was the principal ST among the CRKP isolates clinically, accounting for 56.45% (35/62), followed by ST20 8/62 (12.90%), ST716 (5/62, 8.06%), and ST1565, ST2715, ST571 3.23% (2/62), respectively. The remaining ST1569, ST193, ST347, ST35, ST5295, ST534, ST6094, ST65 each had only 1 strain. The ST type distribution is shown in Figure 1.

Table 2 Results of Antimicrobial Susceptibility of MDR *Klebsiella pneumoniae*
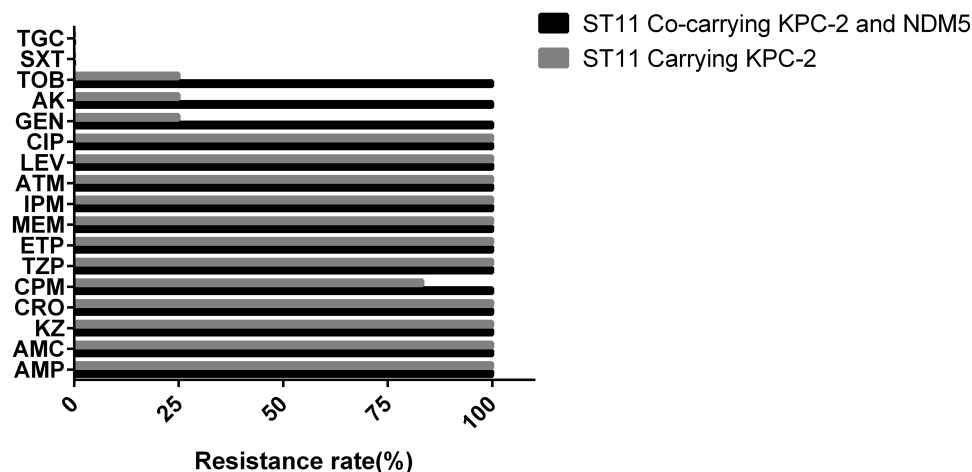
Type of Antibiotic	Antibiotic	Resistance Rate (%)	Mediation Rate (%)	Sensitivity Rate (%)
Penicillin	Ampicillin	100.00 (61/61)	0.00 (0/61)	0.00 (0/61)
	Amoxicillin/clavulanate	96.72 (59/61)	1.64 (1/61)	1.64 (1/61)
Cephalosporins	Cefazolin	100.00 (61/61)	0.00 (0/61)	0.00 (0/61)
	Ceftriaxone	100.00 (61/61)	0.00 (0/61)	0.00 (0/61)
	Cefepime	88.52 (54/61)	9.84 (6/61)	1.64 (1/61)
β-lactams/Penicillin	Piperacillin/tazobactam	96.72 (59/61)	1.64 (1/61)	1.64 (1/61)
Carbapenems	Ertapenem	96.72 (59/61)	0.00 (0/61)	3.28 (2/61)
	Meropenem	96.72 (59/61)	0.00 (0/61)	3.28 (2/61)
	Imipenem	93.44 (57/61)	3.28 (2/61)	3.28 (2/61)
Monoamides	Aztreonam	90.16 (55/61)	1.64 (1/61)	8.20 (5/61)
Quinolones	Levofloxacin	59.02 (36/61)	6.56 (4/61)	34.43 (21/61)
	Ciprofloxacin	63.93 (39/61)	11.48 (7/61)	24.59 (15/61)
Aminoglycosides	Gentamicin	49.18 (30/61)	0.00 (0/61)	50.82 (31/61)
	Amikacin	37.70 (23/61)	0.00 (0/61)	62.30 (38/61)
	Tobramycin	37.70 (23/61)	29.51 (18/61)	32.79 (20/61)
Sulfonamides	Cotrimoxazole	27.87 (17/61)	0.00 (0/61)	72.13 (44/61)
Glycylcyclines	Tigecycline	8.20 (5/61)	1.64 (1/61)	90.16 (55/61)
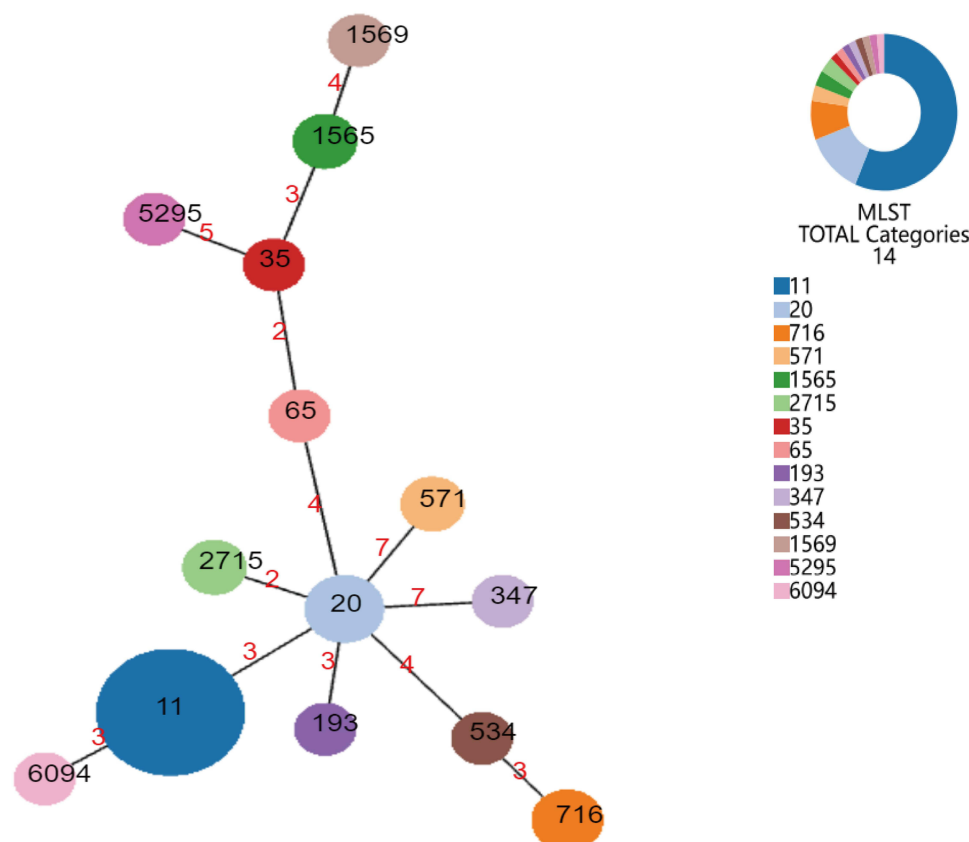
Figure 1 The minimum spanning tree constructed from the MLST results in this study.

Differences in Drug Resistance Between Several STs

ST11 CRKP strains co-carrying bla_{NDM-5} and bla_{KPC-2} were resistant to most of the tested antibiotics, being susceptible only to cotrimoxazole and tigecycline. The resistance rates of ST11 strains co-carrying bla_{NDM-5} and bla_{KPC-2} to penicillins, cephalosporins, penicillins/β-lactams, carbapenems, monoamides, quinolones and aminoglycosides were 100.00% (20/20). Compared with ST11 co-carrying bla_{NDM-5} and bla_{KPC-2}, the gentamicin and amikacin resistance rates of ST11 strains carrying bla_{KPC-2} were only 25.00% (3/12), the tobramycin sensitivity rate was 58.33% (7/12), and the intermediate rate was 16.67% (2/12). The differences in drug resistance between ST11 co-carrying bla_{KPC-2} and bla_{NDM-5} and ST11 carrying bla_{KPC-2} only are shown in

Figure 2 Differences in antibiotic resistance between ST11 Co-carrying bla_{KPC-2} and bla_{NDM-5} and ST11 carrying bla_{KPC-2} only.
Abbreviations: AMP, Ampicillin; AMC, Amoxicillin/clavulanate; KZ, cefazolin; CRO, Ceftriaxone; CPM, cefepime; TZP, Piperacillin/tazobactam; ETP, Ertapenem; MEM, Meropenem; IPM, imipenem; ATM, Aztreonam; LEV, Levofloxacin; CIP, Ciprofloxacin; GEN, Gentamicin; AK, amikacin; TOB, Tobramycin; SXT, cotrimoxazole; TGC, tigecycline.

Figure 2. Besides, five tigecycline-resistant strains were isolated clinically, among which ST20 accounted for 60.00% (3/5); 42.86% (3/7) ST20 strains were resistant to tigecycline. The sensitivity rates of ST20 to gentamycin and amikacin were 100.00% (7/7) and the intermediate rate to tobramycin was 85.71% (6/7); the sensitivity rate was 14.29% (1/7). The characteristics of different STs varied.

Clinical Characteristics of Children Infected with CRKP

After excluding some patients from the analysis of clinical characteristics due to the incomplete clinical data offered, there were 55 patients infected with CRKP in children's hospital in 2019, and they were from the neonatology department (29.09%, (16/55), respiratory department (20.00%, 11/55), infectious diseases department (18.18%, 10/55), hematology department (14.55%, 8/55), pediatric intensive care unit (9.09%, 5/55), nephrology department (5.45%, 3/55), and gastroenterology department (3.64%, 2/55), respectively. The median age of hospitalized children infected with CRKP was 3 months (1, 9). Proportion of infants from 0–3 months was 52.73% (29/55), followed by young children from 3 to 12 months, accounting for 32.73% (18/55) of the total cases, as shown in Table 3. Younger patients were more likely to be infected with CRKP strains.

The clinical data showed that 72.73% (40/55) of children with CRKP infection had serious underlying diseases, such as leukemia, primary immunodeficiency disease, Hirschsprung's disease, malformations of the genitourinary system, congenital heart disease, spinal muscular atrophy and so on. Among them, 74.55% (41/55) of patients had respiratory symptoms and 69.09% (38/55) were diagnosed with pneumonia. The incidence of urinary tract infections was 30.91% (17/55). Besides, 20.00% (11/55) of patients developed BSIs, which had the potential to cause multiple organ failure, shock and death.

Risk Factors for BSIs in Children with CRKP

The BSI risk of patients treated with chemotherapy for hematological malignancies would increase significantly. The rate of BSIs in CRKP-infected children over 3 years was 66.67 (4/6), and only 13.79% (4/29) in those aged 0–3 months and 16.67% (3/18) in those aged 4–12 months. A significant statistical difference can be seen between the different age groups (χ^2=5.906, p<0.05), as shown in Table 3. Infection with CRKP strains was evident in seven hospitalized children with hematological malignancies, and 57.14% (4/7) had BSIs. Chi-square test revealed that there was a statistical difference between children who received chemotherapy for hematological malignancies and those who did not (χ^2=4.512, p<0.05). The logistic regression revealed that the risk of BSIs caused by CRKP in children with hematological malignancies after chemotherapy was 7 times that of other children (95%Cl: 1.298–45.415, p=0.025).

Table 3 Risk Factors for BSIs in Children with CRKP

	Number of CRKP Infected, n (%)	Number of BSIs, n (%)	Positive Rate of BSIs (%)	χ^2	p-value
Gender					
Male	35 (63.64)	7 (63.63)	20.00	<0.001	1.000
Female	20 (36.36)	4 (36.36)	20.00		
Age					
0–3 Months	29 (52.73)	4 (36.36)	13.79	5.906	0.015
4–12 Months	18 (32.73)	3 (16.72)	16.67		
1–3 Year	2 (3.64)	0 (0.00)	0.00		
>3 Year	6 (10.91)	4 (36.36)	66.67		
Premature	12 (21.82)	2 (18.18)	16.67	<0.001	1.000
Pneumonia	38 (69.09)	5 (45.45)	13.16	3.597	0.058
Urinary tract infection	17 (30.91)	1 (9.09)	5.88	3.636	0.057
Chemotherapy	7 (12.73)	4 (36.36)	57.14	4.512	0.034
Operation	15 (27.27)	4 (36.36)	26.67	0.143	0.705

Discussion

In the last few years, the rapid spread of MDR *Klebsiella pneumoniae* worldwide is particularly worrying because it is the most important threat to public health and presents great trouble to clinical treatment.[21–23] In 2019, a total of 62 non-replicated clinical MDR *Klebsiella pneumoniae* strains were isolated from clinic and the detection of drug resistance showed that MDR *Klebsiella pneumoniae* strains are highly resistant to antibiotics widely used in clinic, such as penicillin, cephalosporin, monoamine, etc. It should be also noted that the resistance rate of ertapenem and meropenem was 96.72% (59/61) and the imipenem resistance rate was 93.44% (57/61). The high resistance rates of carbapenems requires special concern, since these agents are always considered as the last line of effective therapy available for the treatment of infections caused by MDR *Klebsiella pneumoniae*.[24]

Resistance to carbapenems in *Klebsiella pneumoniae* is linked to different mechanisms. The results of Carba NP test and PCR screening revealed that the occurrence of bla_{NDM} and bla_{KPC} carbapenemase genes was the important cause of carbapenems resistance. MDR *Klebsiella pneumoniae* isolates harbour these enzymes are capable of breaking down a broad spectrum of beta-lactams. Several researches confirmed CRKP was a looming threat in clinical settings, causing much higher mortality than CSKP. The study conducted by Xu et al suggested that patients infected with CRKP have higher mortality than those infected with CSKP.[16] According to a matched case-control in Eastern China, resistance to carbapenem was associated with higher mortality (35.1%).[25] In New York (USA), patients infected with CRKP had an in-hospital mortality rate of 48%, which was significantly higher than those infected with CSKP.[26]

In our study, the MLST of *Klebsiella pneumoniae* with carbapenemases includes ST11, ST20, ST716, ST1565, ST2715 and ST571, etc, implying the genetic diversity of CRKP. Besides, our findings in MLST of CRKP clinical isolates clearly indicate that ST11 strains producing KPC, which is closely related to ST258, were prevalent in Tianjin Children's Hospital, consistent with several relevant reports about the molecular epidemiology of CRKP in China.[27–29] It should be noted that our results showed that 57.14% (20/35) of ST11 carbapenemases in *Klebsiella pneumoniae* isolates clinically co-harbored bla_{KPC-2} and bla_{NDM-5}, which were resistant to most of the tested antibiotics, being susceptible only to cotrimoxazole and tigecycline. Co-occurrence of bla_{NDM-5} and bla_{KPC-2} in ST11 carbapenem-resistant *Klebsiella pneumoniae* had been reported by Hu et al. They revealed that the genetic characterization of *Klebsiella pneumoniae* co-harbouring bla_{NDM-5} and bla_{KPC-2} was not only 25 resistance genes and a large number of virulence factors, but also various mobile genetic elements (MGEs) such as plasmids and genomic islands.[30] Accordingly, we considered the transmission model of the CRKP in the hospital as complex and the ST11 carbapenemases in *Klebsiella pneumoniae* co-harboring bla_{KPC-2} and bla_{NDM-5} was partly due to the spread of MGEs or clonal expansion and partly acquired through exogenous exposure during hospitalization. The emergence of CRKP co-carrying bla_{NDM-5} and bla_{KPC-2} in pediatrics is alarming and should be treated according to strict standard guidelines.

Several studies had confirmed that the history of intensive care unit (ICU) hospitalization was an independent risk factor for MDR *Klebsiella pneumoniae* strain infection.[14,31–33] However, data in our study showed that 29.09% (16/55) of MDR *Klebsiella pneumoniae* strains were collected from the neonatology department, while the pediatric intensive care unit (PICU) accounted for 9.09% (5/55), implying that newborns and children have a high risk of MDR *Klebsiella pneumoniae* infection owing to their immature immune system. The results in our study confirmed that infections were mainly occurring in young immunocompromised patients with severe chronic comorbidities.

BSIs are associated with high mortality and morbidity. The data from a systematic literature review conducted by McNamara et al showed that the pooled risk ratio of death at one year was significantly higher for patients with BSI when compared to the matched cohort (RR 4.04 [95% CI 1.84–8.87]).[34] More attention should be focused on assessing the independent risk factors for BSIs for those children facing fewer therapeutic options. Our findings on the incidence of BSIs were 20.00% (11/55) in CRKP clinical isolates, and logistic regression revealed that the risk of BSIs caused by CRKP strain infections in children with hematological malignancies after chemotherapy was 7 times that of other children (95%Cl: 1.298–45.415, *p*=0.025). These findings may contribute to the implementation of appropriate BSI controls in young patients.

Conclusion

In this study, we hold the view that ST11 is the prevalent clone in our hospital and the emergence of ST11 CRKP strains co-carrying bla_{NDM-5} and bla_{KPC-2} should be alarming because it is resistant to most of the tested antibiotics and might spread through horizontal transmission including MGEs, clonal expansion and exogenous exposure during hospitalization. Although risk factors for BSIs have been reported, the majority of research has focused on adults. We proposed that newborns and infants with a high risk of developing MDR *Klebsiella pneumoniae* infection and receiving chemotherapy for hematological malignancies is an independent risk factor for BSIs. There are also some limits in this study. First, the hypervirulent genes of clinical isolates were not discussed in this article, while the mechanisms of carbapenem resistance are complicated. Second, it is a single-center retrospective study and the patients and MDR *Klebsiella pneumoniae* isolates are limited. In the future, more study is needed to explore MDR in children.

Acknowledgments

Disclosure

The authors declare no conflicts of interest in this work.

References

1. Mazzariol A, Bazaj A, Cornaglia G. Multi-drug-resistant Gram-negative bacteria causing urinary tract infections: a review. *J Chemother*. 2017;29 (sup1):2–9. doi:10.1080/1120009X.2017.1380395
2. De Rosa FG, Corcione S, Cavallo R, Di Perri G, Bassetti M. Critical issues for Klebsiella pneumoniae KPC-carbapenemase producing K. pneumoniae infections: a critical agenda. *Future Microbiol*. 2015;10(2):283–294. doi:10.2217/fmb.14.121
3. Tzouvelekis LS, Markogiannakis A, Psichogiou M, Tassios PT, Daikos GL. Carbapenemases in Klebsiella pneumoniae and other Enterobacteriaceae: an evolving crisis of global dimensions. *Clin Microbiol Rev*. 2012;25(4):682–707. doi:10.1128/CMR.05035-11
4. Gandra S, Burnham CD. Carbapenem-resistant Enterobacterales in the USA. *Lancet Infect Dis*. 2020;20(6):637–639.
5. Zowawi HM, Forde BM, Alfaresi M, et al. Stepwise evolution of pandrug-resistance in Klebsiella pneumoniae. *Sci Rep*. 2015;5:15082.
6. World Health Organization. *Antimicrobial Resistance: Global Report on Surveillance 2014*. Geneva, Switzerland: World Health Organization; 2014.
7. Fupin Guo YG, Zhu D, Wang F, Jiang X. Surveillance of bacterial resistance in CHINET tertiary hospitals in 2019. *Chin J Infect Chemother*. 2020;20(3):233–243.
8. Diaz A, Ortiz DC, Trujillo M, Garces C, Jaimes F, Restrepo AV. Clinical characteristics of carbapenem-resistant Klebsiella pneumoniae infections in ill and colonized children in Colombia. *Pediatr Infect Dis J*. 2016;35(3):237–241.
9. Dallenne C, Da Costa A, Decre D, Favier C, Arlet G. Development of a set of multiplex PCR assays for the detection of genes encoding important beta-lactamases in Enterobacteriaceae. *J Antimicrob Chemother*. 2010;65(3):490–495.
10. Poirel L, Walsh TR, Cuvillier V, Nordmann P. Multiplex PCR for detection of acquired carbapenemase genes. *Diagn Microbiol Infect Dis*. 2011;70 (1):119–123. doi:10.1016/j.diagmicrobio.2010.12.002
11. Goodman KE, Simner PJ, Tamma PD, Milstone AM. Infection control implications of heterogeneous resistance mechanisms in carbapenem-resistant Enterobacteriaceae (CRE). *Expert Rev Anti Infect Ther*. 2016;14(1):95–108. doi:10.1586/14787210.2016.1106940
12. Tamma PD, Goodman KE, Harris AD, et al. Comparing the outcomes of patients with carbapenemase-producing and non-carbapenemase-producing carbapenem-resistant Enterobacteriaceae Bacteremia. *Clin Infect Dis*. 2017;64(3):257–264. doi:10.1093/cid/ciw741
13. Hovan MR, Narayanan N, Cedarbaum V, Bhowmick T, Kirn TJ. Comparing mortality in patients with carbapenemase-producing carbapenem resistant Enterobacterales and non-carbapenemase-producing carbapenem resistant Enterobacterales bacteremia. *Diagn Microbiol Infect Dis*. 2021;101(4):115505. doi:10.1016/j.diagmicrobio.2021.115505
14. Ara-Montojo MF, Escosa-Garcia L, Alguacil-Guillen M, et al. Predictors of mortality and clinical characteristics among carbapenem-resistant or carbapenemase-producing Enterobacteriaceae bloodstream infections in Spanish children. *J Antimicrob Chemother*. 2021;76(1):220–225. doi:10.1093/jac/dkaa397
15. Liu KS, Tong YS, Lee MT, Lin HY, Lu MC. Risk factors of 30-day all-cause mortality in patients with carbapenem-resistant Klebsiella pneumoniae bloodstream infection. *J Pers Med*. 2021;11(7). doi:10.3390/jpm11070616
16. Xu L, Sun X, Ma X. Systematic review and meta-analysis of mortality of patients infected with carbapenem-resistant Klebsiella pneumoniae. *Ann Clin Microbiol Antimicrob*. 2017;16(1):18. doi:10.1186/s12941-017-0191-3
17. Li Y, Li J, Hu T, et al. Five-year change of prevalence and risk factors for infection and mortality of carbapenem-resistant Klebsiella pneumoniae bloodstream infection in a tertiary hospital in North China. *Antimicrob Resist Infect Control*. 2020;9(1):79. doi:10.1186/s13756-020-00728-3
18. Cao Z, Yue C, Kong Q, Liu Y, Li J. Risk factors for a hospital-acquired carbapenem-resistant Klebsiella pneumoniae bloodstream infection: a five-year retrospective study. *Infect Drug Resist*. 2022;15:641–654. doi:10.2147/IDR.S342103

19. Chen Y, Ying S, Jiang L, et al. A novel nomogram for predicting risk factors and outcomes in bloodstream infections caused by Klebsiella pneumoniae. *Infect Drug Resist*. 2022;15:1317–1328. doi:10.2147/IDR.S349236

20. Doyle D, Peirano G, Lascols C, Lloyd T, Church DL, Pitout JD. Laboratory detection of Enterobacteriaceae that produce carbapenemases. *J Clin Microbiol*. 2012;50(12):3877–3880. doi:10.1128/JCM.02117-12

21. Navon-Venezia S, Kondratyeva K, Carattoli A. Klebsiella pneumoniae: a major worldwide source and shuttle for antibiotic resistance. *FEMS Microbiol Rev*. 2017;41(3):252–275. doi:10.1093/femsre/fux013

22. Lee CR, Lee JH, Park KS, Kim YB, Jeong BC, Lee SH. Global dissemination of carbapenemase-producing Klebsiella pneumoniae: epidemiology, genetic context, treatment options, and detection methods. *Front Microbiol*. 2016;7:895. doi:10.3389/fmicb.2016.00895

23. Pitout JD, Nordmann P, Poirel L. Carbapenemase-producing Klebsiella pneumoniae, a key pathogen set for global nosocomial dominance. *Antimicrob Agents Chemother*. 2015;59(10):5873–5884. doi:10.1128/AAC.01019-15

24. Nordmann P, Dortet L, Poirel L. Carbapenem resistance in Enterobacteriaceae: here is the storm! *Trends Mol Med*. 2012;18(5):263–272. doi:10.1016/j.molmed.2012.03.003

25. Zuo Y, Zhao D, Song G, Li J, Xu Y, Wang Z. Risk factors, molecular epidemiology, and outcomes of carbapenem-resistant Klebsiella pneumoniae infection for hospital-acquired pneumonia: a matched case-control study in Eastern China during 2015–2017. *Microb Drug Resist*. 2021;27(2):204–211. doi:10.1089/mdr.2020.0162

26. Patel G, Huprikar S, Factor SH, Jenkins SG, Calfee DP. Outcomes of carbapenem-resistant Klebsiella pneumoniae infection and the impact of antimicrobial and adjunctive therapies. *Infect Control Hosp Epidemiol*. 2008;29(12):1099–1106. doi:10.1086/592412

27. Gu D, Dong N, Zheng Z, et al. A fatal outbreak of ST11 carbapenem-resistant hypervirulent Klebsiella pneumoniae in a Chinese hospital: a molecular epidemiological study. *Lancet Infect Dis*. 2018;18(1):37–46. doi:10.1016/S1473-3099(17)30489-9

28. Zhang M, Li J, Lu Y, et al. Expanding of ST11 carbapenemase-producing Klebsiella pneumoniae subclones in a Chinese Hospital, Shenzhen, China. *Infect Drug Resist*. 2021;14:1415–1422. doi:10.2147/IDR.S299478

29. Liao W, Liu Y, Zhang W. Virulence evolution, molecular mechanisms of resistance and prevalence of ST11 carbapenem-resistant Klebsiella pneumoniae in China: a review over the last 10 years. *J Glob Antimicrob Resist*. 2020;23:174–180. doi:10.1016/j.jgar.2020.09.004

30. Hu R, Li Q, Zhang F, Ding M, Liu J, Zhou Y. Characterisation of blaNDM-5 and blaKPC-2 co-occurrence in K64-ST11 carbapenem-resistant Klebsiella pneumoniae. *J Glob Antimicrob Resist*. 2021;27:63–66.

31. Qin X, Wu S, Hao M, et al. The colonization of carbapenem-resistant Klebsiella pneumoniae: epidemiology, resistance mechanisms, and risk factors in patients admitted to intensive care units in China. *J Infect Dis*. 2020;221(Suppl 2):S206–S214.

32. Kofteridis DP, Valachis A, Dimopoulou D, et al. Risk factors for carbapenem-resistant Klebsiella pneumoniae infection/colonization: a case-case-control study. *J Infect Chemother*. 2014;20(5):293–297.

33. Candevir Ulu A, Kurtaran B, Inal AS, et al. Risk factors of carbapenem-resistant Klebsiella pneumoniae infection: a serious threat in ICUs. *Med Sci Monit*. 2015;21:219–224.

34. McNamara JF, Righi E, Wright H, Hartel GF, Harris PNA, Paterson DL. Long-term morbidity and mortality following bloodstream infection: a systematic literature review. *J Infect*. 2018;77(1):1–8.