Infection and Drug Resistance

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ORIGINAL RESEARCH

The Epidemiology, Virulence and Antimicrobial Resistance of Invasive *Klebsiella pneumoniae* at a Children's Medical Center in Eastern China

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Objective: This study investigated the epidemiology, virulence and drug resistance of invasive *Klebsiella pneumoniae* (*K. pneumoniae*) isolates at a children's medical center in eastern China in order to obtain epidemiologic, virulence, and antimicrobial resistance data that can guide for the selection and development of anti-infection treatments.

Methods: A total of 94 invasive *K. pneumoniae* strains were isolated from children between January 2016 and December 2020 at the Children's Hospital of Soochow University. The strains were identified by mass spectrometry. The Kirby–Bauer method and VITEK 2 Compact system were used to analyze the antimicrobial susceptibility. Polymerase chain reaction (PCR) and sequencing was performed to detect the capsular serotypes, virulence-associated genes, β-lactam antibiotic resistance genes and multilocus sequence typing.

Results: The PCR results showed that 87 strains (92.55%) of invasive *K. pneumoniae* were hypervirulent capsular serotypes, with K57 as the dominant capsular serotype (62.77%). All strains carried virulence-associated genes. Among them, 84 strains (89.36%) carried hypervirulence genes, with *iroB* (86.17%) being the predominant; meanwhile, other virulence genes, including *wabG* (100.00%), *mrkD* (98.94%), *ycfM* (96.81%), *fimH* (95.74%) and *Uge* (88.30%), were detected in most strains. All strains carried β-lactam antibiotic resistance genes; the main extended-spectrum β-lactamase gene was $bla_{\text{SHV-11}}$ (86.17%) and the major AmpC cephalosporinase genes were $bla_{\text{FOX-1}}$ (86.17%) and $bla_{\text{ACT-1}}$ (70.21%). Carbapenemase genes were detected in only a few isolates. Notably, 12 invasive *K. pneumoniae* isolates were identified as carbapenem-resistant and hypervirulent *K. pneumoniae* (CR-HVKP), and 14 other multidrug resistance (MDR) isolates were also detected.

Conclusion: The results of this study reveal the epidemiology, virulence and antimicrobial resistance of invasive *K. pneumoniae* in pediatric patients. Both CR-HVKP and MDR strains were identified, which should be of great concern to clinicians.

Keywords: *Klebsiella pneumoniae*, invasive infection, children, virulence factors, resistance genes

Introduction

Invasive bacterial infection caused by *Klebsiella pneumoniae* (*K. pneumoniae*) is one of the most common diseases in children.[1,2] The infection can involve the respiratory tract, blood, nervous system and surgical site, and lead to pneumonia, bacteremia/septicemia, meningitis and abscesses.[2] In severe clinical cases, it can also lead to multiple organ failure, or even death.[3]

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In recent years, infection with hypervirulent *K. pneumoniae* (HVKP) has been reported in many countries and has become a global public health concern.[4–6] The virulence of HVKP is associated with capsular polysaccharides, siderophores and other virulence factors.[7–9] Previous studies of HVKP mainly screened for aerobactin, which is encoded by *iucA*.[9] However, more hypervirulence genes such as *$_p$rmpA, $_r$mpA2, $_c$rmpA, peg-344, terB, iroB* and *irp2* have recently been identified.[7] These HVKP strains warrant closer attention as they have the ability to cause severe and life-threatening infections in healthy individuals through these virulence factors.[6]

The development of drug resistance by *K. pneumoniae* and the emergence of carbapenem-resistant *K. pneumoniae* (CRKP) caused by the misuse and overuse of antibiotics is also a major threat to public health.[10,11] The isolation rate of CRKP is higher among children than in adults and is increasing in China.[12,13] Meanwhile, the multidrug-resistant (MDR) is more likely to occur than CRKP,[14–16] and MDR pathogens can be transmitted to humans via the food chain or through direct contact with infected animals,[17–19] which should be of concern as well.

HVKP and CRKP were not traditionally overlapped.[13] However, cases of infection with carbapenem-resistant and hypervirulent *K. pneumoniae* (CR-HVKP) have recently been reported, which have a poor prognosis and are challenging to treat.[20–23] To date, there have been limited studies of CR-HVKP isolated from pediatric patients. On this basis, the present study investigated the virulence and antibiotic resistance characteristics of invasive *K. pneumoniae* at a children's medical center in eastern China.

Materials and Methods

Study Site

This study was conducted in Suzhou, a major city with a population of 12.7 million in the southeast area of Jiangsu Province in eastern China. The population of children aged under 15 years old was 1.7 million in 2020. The Children's Hospital of Soochow University (CHSU), located in the central area of Suzhou, is a children's medical center in

Table 1 Clinical Characteristics and Epidemiology of Invasive *K. pneumoniae* Infection at Children's Hospital of Soochow University from 2016 to 2020

	Bacteremia	Pneumonia	Meningitis	Total
	(n=84)	(n=8)	(n=2)	(n=94)
Gender, n (%)				
Male	51 (60.71)	6 (75.00)	2 (100.00)	59 (62.77)
Female	33 (39.29)	2 (25.00)	0 (0)	35 (37.23)
Age*				
Median	30 m	17 d	6 m	24 m
Range	3 d-168 m	7 d-180 m	2–10 m	3 d-180 m
Age*, n (%)				
<5 y	53 (63.10)	7 (87.50)	2 (100.00)	62 (65.96)
<1 m	14 (16.67)	5 (62.50)	0 (0)	19 (20.21)
1–5 m	14 (16.67)	0 (0)	1 (50.00)	15 (15.96)
6–11 m	5 (5.95)	0 (0)	1 (50.00)	6 (6.38)
12–59 m	20 (23.81)	2 (25.00)	0 (0)	22 (23.40)
5–18 y	31 (36.90)	1 (12.50)	0 (0)	32 (34.04)
Wards, n (%)				
Haematology	49 (58.33)	0 (0)	0 (0)	49 (52.13)
Neonatology	19 (22.62)	5 (62.50)	0 (0)	24 (25.53)
ICU	5 (5.95)	3 (37.50)	1 (50.00)	9 (9.57)
Infectious disease	3 (3.57)	0 (0)	0 (0)	3 (3.19)
Others	8 (9.52)	0 (0)	1 (50.00)	9 (9.57)

Notes: Others: including the departments of neurosurgery, cardiovasology, general surgery, gastroenterology, nephrology and emergency ward.
Abbreviations: *d, days; m, months; y, years; ICU, intensive care unit.

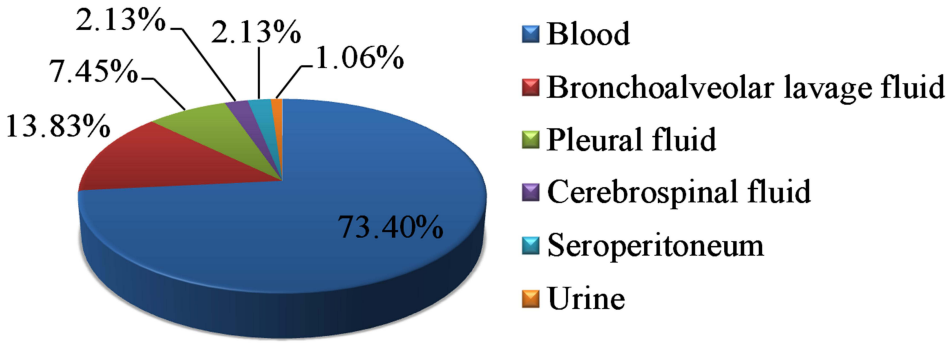

Figure 1 The first isolated samples of invasive *K. pneumoniae* strains in this study.

eastern China and the only provincial tertiary children's hospital in Jiangsu Province. CHSU has 1500 beds and serves >70,000 inpatients and >2 million outpatients annually. This study had no impact on patients and was approved by the Ethics Committee of CHSU (No. 2020CS099).

Bacterial Strains

Nonduplicated invasive *K. pneumoniae* isolates were collected at CHSU from January 1, 2016 to December 31, 2020. Invasive *K. pneumoniae* was defined as *K. pneumoniae* isolated from a normally sterile site, namely blood, cerebrospinal fluid (CSF), pleural fluid or joint fluid. All invasive *K. pneumoniae* strains were confirmed by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS, Bruker, Mannheim, Germany).[24]

Detection of Capsular Serotypes and Virulence-Associated Genes

The common hypervirulent capsular serotypes (K1, K2, K5, K20, K54 and K57), hypervirulence genes (*iucA*, $_{p}$*rmpA*, $_{p}$*rmpA2*, $_{c}$*rmpA*, *peg-344*, *terB*, *iroB* and *irp2*) and other virulence genes (*ybtS*, *mrkD*, *entB*, *kfu*, *allS*, *iutA*, k_2*A*, *wabG*, *Uge*, *fimH*, *wcaG*, *Kpn*, *ycfM*, *iroN*, *Hly* and *cnf-1*) of invasive *K. pneumoniae* isolates were screened by PCR as previously described.[7,13,25] The PCR products were analyzed by 1.5% agarose gel electrophoresis, and positive products were sequenced and aligned with sequences in GenBank using the Basic Local Alignment Search Tool (BLAST).

Antimicrobial Susceptibility Test

The antimicrobial susceptibility of invasive *K. pneumoniae* isolates was determined by the Kirby–

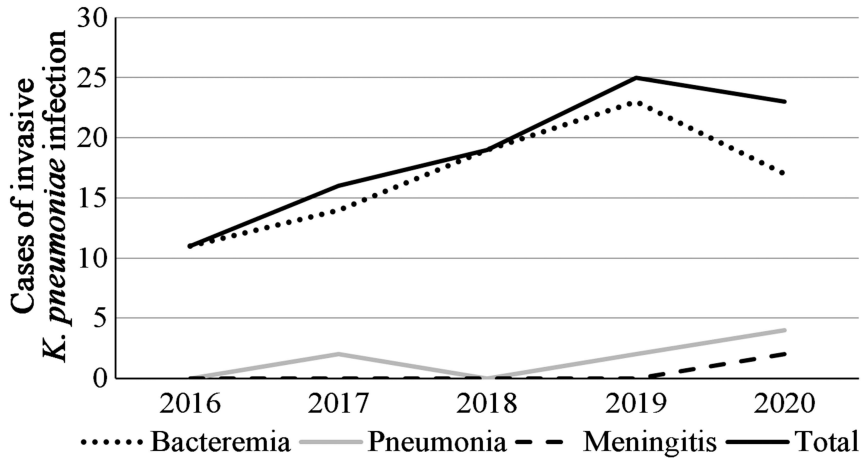

Figure 2 The annual trends of invasive *K. pneumoniae* cases by clinical diagnosis from 2016 to 2020 in this study.

Table 2 The Capsular Serotypes and Virulence-Associated Genes of Invasive *K. pneumoniae* Strains in This Study

	Total	CRHV-KP	non CRHV-KP	*P* value
	(n=94)	(n=12)	(n=82)	
Capsular serotypes, n (%)				
K1	7 (7.45)	0 (0)	7 (8.54)	0.5895
K2	11 (11.70)	2 (16.67)	9 (10.98)	0.9266
K5	4 (4.26)	0 (0)	4 (4.88)	1.0000
K20	0 (0)	0 (0)	0 (0)	1.0000
K54	6 (6.38)	2 (16.67)	4 (4.88)	0.1678
K57	59 (62.77)	8 (66.67)	51 (62.20)	0.9837
Others	7 (7.45)	0 (0)	7 (8.54)	0.5895
Hypervirulent genes, n (%)				
iucA	13 (13.83)	0 (0)	13 (15.85)	0.2992
$_p$rmpA	29 (30.85)	6 (50.00)	23 (28.05)	0.2289
$_p$rmpA2	2 (2.13)	0 (0)	2 (2.44)	1.0000
$_c$rmpA	6 (6.38)	0 (0)	6 (7.32)	1.0000
peg-344	32 (34.04)	1 (8.33)	31 (37.80)	0.0918
terB	0 (0)	0 (0)	0 (0)	1.0000
iroB	81 (86.17)	12 (100.00)	69 (84.15)	0.2992
irp2	0 (0)	0 (0)	0 (0)	1.0000
Other virulence genes, n (%)				
ytbS	11 (11.70)	3 (25.00)	8 (9.76)	0.2921
mrkD	93 (98.94)	12 (100.00)	81 (98.78)	1.0000
entB	20 (21.28)	3 (25.00)	17 (20.73)	0.9680
kfu	47 (50.00)	3 (25.00)	44 (53.66)	0.0637
allS	16 (17.02)	0	16 (19.51)	0.2046
iutA	2 (2.13)	0	2 (2.44)	1.0000
k2A	4 (4.26)	1 (8.33)	3 (3.66)	0.4264
wabG	94 (100.00)	12 (100.00)	82 (100.00)	1.0000
Uge	83 (88.30)	10 (83.33)	73 (89.02)	0.9266
fimH	90 (95.74)	12 (100.00)	78 (95.12)	1.0000
wcaG	23 (24.47)	2 (16.67)	21 (25.61)	0.7538
Kpn	50 (53.19)	9 (75.00)	41 (50.00)	0.1050
ycfM	91 (96.81)	12 (100.00)	79 (96.34)	1.0000
iroN	27 (28.72)	1 (8.33)	26 (31.71)	0.1836
Hly	0 (0)	0 (0)	0 (0)	1.0000
cnf-1	0 (0)	0 (0)	0 (0)	1.0000

Bauer (K-B) method and Vitek 2 Compact system (bioMérieux, Marcy-l'Etoile, France) according to the manufacturer's instructions. The results were analyzed according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI).[24,26]

Detection of β-Lactam Antibiotic and Colistin Resistance Genes

PCR was performed to detect carbapenemase genes (bla_{KPC}, bla_{OXA}, bla_{NDM}, bla_{VIM}, bla_{IMP}, bla_{AIM}, bla_{GIM}, bla_{SIM}, bla_{SPM}, bla_{BIC} and bla_{DIM}), extended-spectrum β-lactamase (ESBL) genes (bla_{TEM}, bla_{SHV}, bla_{CTX-M}, bla_{VEB} and bla_{PER}) and AmpC cephalosporinase genes (bla_{CMY}, bla_{ACT}, bla_{ACC}, bla_{DHA}, bla_{FOX}, bla_{MOX}, bla_{CIT} and bla_{EBC}).[13,27] Colistin resistance genes (*MCR-1* and *MCR-2*) were also examined in parallel.[28,29] The primers used to screen the abovementioned genes have been previously reported and the PCR products were analyzed as described above.

Multilocus Sequence Typing (MLST)

MLST was performed using the primers and protocol available from the Pasteur MLST website (https://bigsdb.

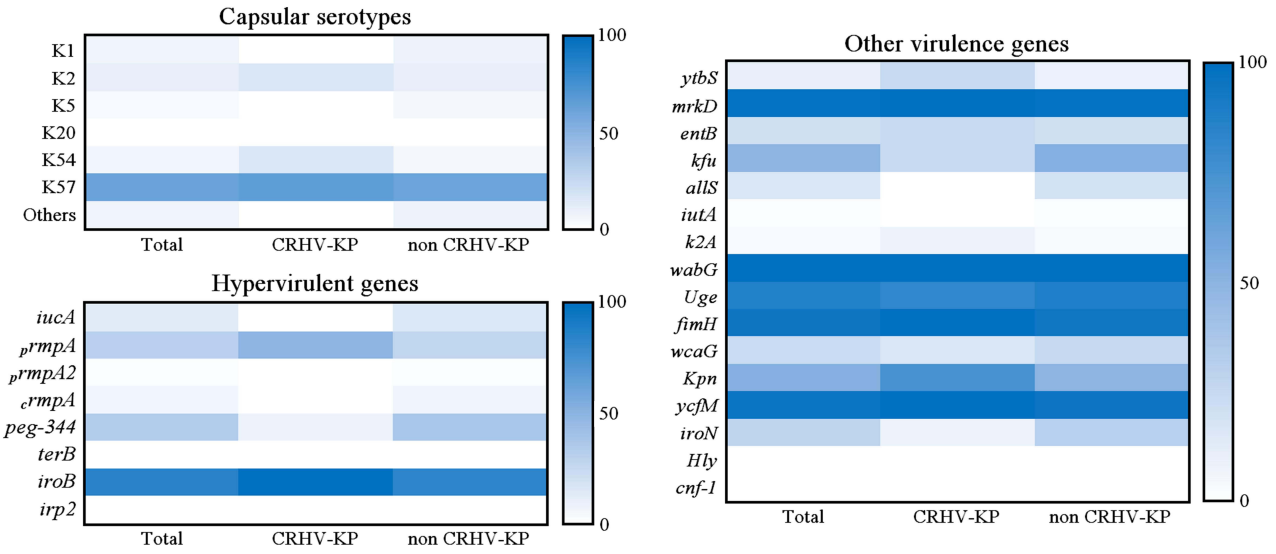

Figure 3 The heat-map of capsular serotypes and virulence-associated genes of invasive *K. pneumoniae* strains in this study.

pasteur.fr/klebsiella/primers_used.html). Seven housekeeping genes of invasive *K. pneumoniae* isolates, namely *rpoB, gapA, mdh, pgi, phoE, infB* and *tonB*, were analyzed and the sequence types (STs) were compared with those available in the Pasteur MLST database.

Data Analysis

The chi-squared test was used to analyze the data and $p<0.05$ was considered statistically significant. Antibiotic resistance data were analyzed with WHONET v5.6 software (WHO Collaborating Centre for Surveillance of Antimicrobial Resistance, Boston, MA, USA).

Results

Clinical Characteristics and Epidemiology of Invasive *K. pneumoniae* Infection

Over the 5-year study period, a total of 94 non-duplicated invasive *K. pneumoniae* strains were collected and identified at our hospital. The clinical characteristics of the study population are summarized in Table 1. Of the 94 children, 59 (62.77%) were male. The median age at the time of diagnosis was 24 months (range: 3 days-180 months); at the time of infection, 62 children (65.96%) were aged <5 years and 32 (34.04%) were aged ≥ 5 years. Most of the invasive *K. pneumoniae* strains were isolated from the department of hematology (52.13%) and neonatology (25.53%). The clinical syndromes recognized in children with invasive *K. pneumoniae* infection were bacteremia (89.36%, 84/94), pneumonia (8.51%, 8/94) and meningitis (2.13%, 2/94). Based on the first isolates, the primary sites of infection were blood (73.40%, 69/94), bronchoalveolar lavage fluid (13.83%, 13/94), pleural fluid (7.45%, 7/94), cerebrospinal fluid (2.13%, 2/94), seroperitoneum (2.13%, 2/94) and urine (1.06%, 1/94) (Figure 1). The annual trends of invasive *K. pneumoniae* cases by clinical diagnosis are shown in Figure 2. There was an increase in the overall number of cases between 2016 and 2019, primarily driven by an increase in *K. pneumoniae* bacteremia although there were also marginal increases in the number of cases of invasive *K. pneumoniae* pneumonia and meningitis. Additionally, 12 strains of invasive *K. pneumoniae* were CR-HVKP.

Distribution of Capsular Types and Virulence-Associated Genes

Previous studies have shown that some capsular serotypes of *K. pneumoniae* (eg, K1, K2, K5, K20, K54, and K57) contribute to hypervirulence.[13] In this study, common hypervirulent capsular serotypes were detected in 87 strains (92.55%) of invasive *K. pneumoniae*; the main capsular serotype was K57 (62.77%, 59/94) and the remaining ones were K2 (11.70%, 11/94), K1 (7.45%, 7/

Table 3 The Antimicrobial Resistance of Invasive *K. pneumoniae* Strains in This Study

Antibiotics	Total	CRHV-KP	non CRHV-KP	*P* value
	(n=94)	(n=12)	(n=82)	
	R (n, %)	R (n, %)	R (n, %)	
Aminoglycosides				
Gentamicin	35 (37.23)	2 (16.67)	33 (40.24)	0.2083
Amikacin	2 (2.13)	2 (16.67)	0 (0)	0.0151
Tobramycin	28 (29.79)	2 (16.67)	26 (31.71)	0.4677
Carbapenems				
Meropenem	12 (12.77)	12 (100.00)	0 (0)	2.6496E-20
Imipenem	12 (12.77)	12 (100.00)	0 (0)	2.6496E-20
Ertapenem	12 (12.77)	12 (100.00)	0 (0)	2.6496E-20
Non-extended spectrum cephalosporins; 1st and 2nd generation cephalosporins				
Cefazolin	44 (46.81)	12 (100.00)	32 (39.02)	7.6944E-05
Cefuroxime	44 (46.81)	12 (100.00)	32 (39.02)	7.6944E-05
Extended-spectrum cephalosporins; 3rd and 4th generation cephalosporins				
Cefotaxime	36 (38.30)	12 (100.00)	24 (29.27)	1.1345E-05
Ceftriaxone	41 (43.62)	12 (100.00)	29 (35.37)	2.4768E-05
Ceftazidime	22 (23.40)	12 (100.00)	10 (12.20)	2.2286E-10
Cefepime	20 (21.28)	11 (91.67)	9 (10.98)	1.9556E-09
Ceftizoxime	25 (26.60)	12 (100.00)	13 (15.85)	6.1744E-09
Cephalosporins + β-lactamase inhibitors				
Cefoperazone/sulbactam	17 (18.09)	12 (100.00)	5 (6.10)	6.7846E-14
Cephamycins				
Cefoxitin	22 (23.40)	11 (91.67)	11 (13.41)	1.9690E-08
Cefotetan	13 (13.83)	10 (83.33)	3 (3.66)	2.2231E-12
Fluoroquinolones				
Ciprofloxacin	26 (27.66)	4 (33.33)	22 (26.83)	0.9006
Levofloxacin	4 (4.26)	2 (16.67)	2 (2.44)	0.0780
Monobactams				
Aztreonam	17 (18.09)	3 (25.00)	14 (17.07)	0.7911
Penicillins + β-lactamase inhibitors				
Ampicillin/sulbactam	46 (48.94)	12 (100.00)	34 (41.46)	0.0002
Piperacillin/tazobactam	12 (12.77)	10 (83.33)	2 (2.44)	1.5841E-13
Penicillins				
Piperacillin	18 (19.15)	10 (83.33)	8 (9.76)	1.5374E-08
Sulfonamides				
Aulfamethoxazole trimethoprim	42 (44.68)	8 (66.67)	34 (41.46)	0.1010
ESBL (+)	29 (30.85)	0 (0)	29 (35.37)	0.0321

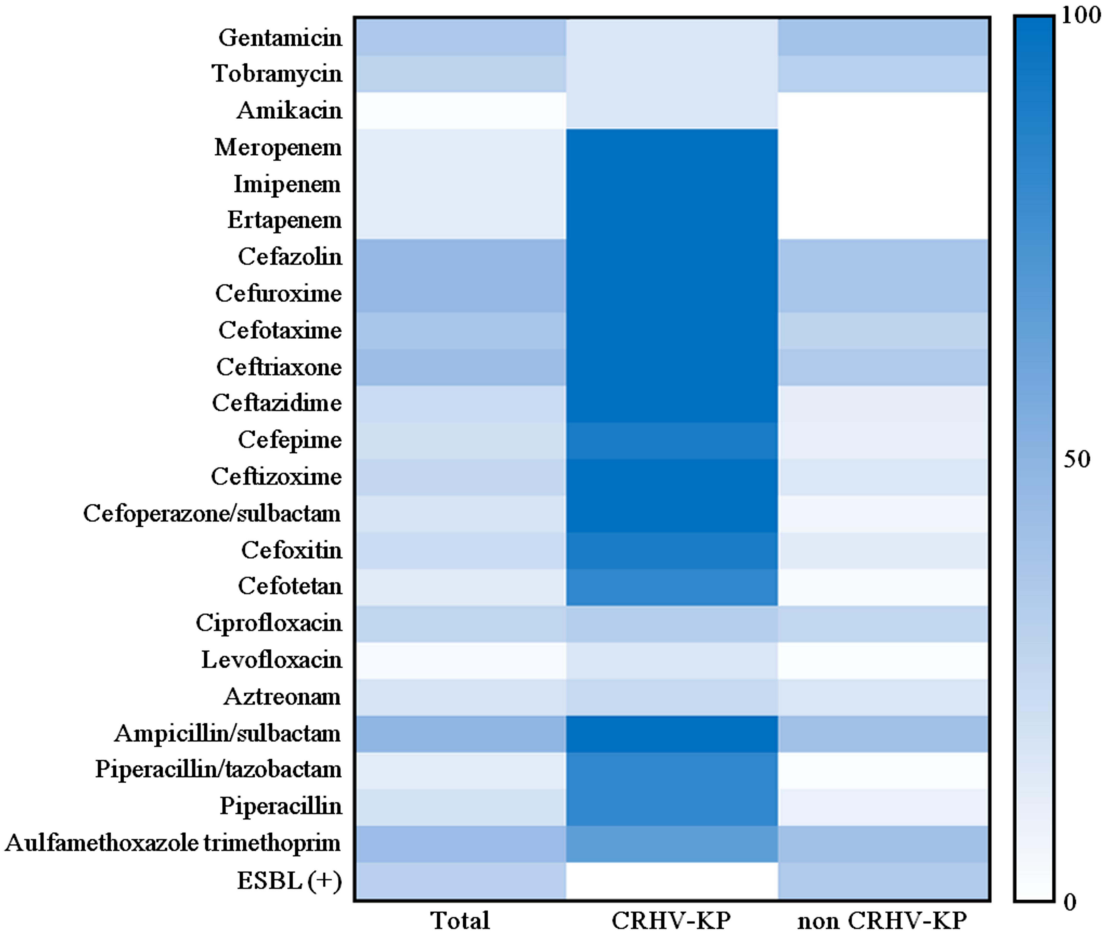

Figure 4 The heat-map of the different degrees of antimicrobial resistance of invasive *K. pneumoniae* strains in this study.

94), K54 (6.38%, 6/94), K5 (4.26%, 4/94) and others (7.45%, 7/94). The K20 capsular serotype was not detected (Table 2 and Figure 3). All invasive *K. pneumoniae* strains carried virulence-associated genes; among them, 84 (89.36%) carried hypervirulence genes, which were *iroB* (86.17%, 81/94), *peg-344* (34.04%, 32/94), $_{p}$*rmpA* (30.85%, 29/94), *iucA* (13.83%, 13/94), $_{c}$*rmpA* (6.38%, 6/94) and $_{p}$*rmpA2* (2.13%, 2/94). The other main virulence genes were *wabG* (100%, 94/94), *mrkD* (98.94%, 93/94), *ycfM* (96.81%, 91/94), *fimH* (95.74%, 90/94) and *Uge* (88.30%, 83/94) (Table 2 and Figure 3). There were no significant differences between CR-HVKP and non CR-HVKP strains in terms of capsular types and virulence-associated genes. These data suggest that the isolated invasive *K. pneumoniae* strains, including CR-HVKP and non CR-HVKP, all carried a high proportion of virulence genes that may contribute to disease.

Antimicrobial Susceptibility

The invasive *K. pneumoniae* strains exhibited different patterns of resistance to various antibiotics, but in general, they showed low resistance to aminoglycosides (amikacin and tobramycin), carbapenems (meropenem, imipenem and ertapenem), cephalosporins (ceftazidime, cefepime and ceftizoxime), cephalosporins+β-lactamase inhibitors (cefoperazone/sulbactam), cephamycins (cefoxitin and cefotetan), fluoroquinolones (ciprofloxacin and levofloxacin), monobactams (aztreonam), penicillins+β-lactamase inhibitors (piperacillin/tazobactam) and penicillins (piperacillin) (Table 3 and Figure 4). Notably, 29 strains (30.85%) of non CR-HVKP produced ESBLs. The resistance rates of CR-HVKP strains were much higher than those of non–CR-HVKP strains (Table 3 and Figure 4). Moreover, there were significant differences ($p<0.05$) between CR-HVKP and non CR-HVKP strains in terms of susceptibility to

Table 4 The β-Lactam Antibiotic and Colistin Resistance Genes of Invasive *K. pneumoniae* Strains in This Study

	Total	CRHV-KP	non CRHV-KP	*P* value
	(n=94)	(n=12)	(n=82)	
Carbapenemase genes, n (%)				
bla_{KPC-2}	10 (10.64)	0 (0)	10 (12.20)	0.4363
bla_{OXA-1}	5 (5.32)	1 (8.33)	4 (4.88)	0.5029
bla_{NDM-1}	18 (19.15)	1 (8.33)	17 (20.73)	0.5308
bla_{VIM-1}	13 (13.83)	2 (16.67)	11 (13.41)	0.8864
bla_{IMP}, bla_{AIM}, bla_{GIM}, bla_{SIM}, bla_{SPM}, bla_{BIC}, bla_{DIM}	0 (0)	0 (0)	0 (0)	1.0000
ESBL genes, n (%)				
bla_{TEM-1}	18 (19.15)	3 (25.00)	15 (18.29)	0.8738
bla_{SHV-11}	81 (86.17)	12 (100.00)	69 (84.15)	0.2992
$bla_{CTX-M-14}$	46 (48.94)	4 (33.33)	42 (51.22)	0.2470
bla_{VEB-1}	6 (6.38)	0 (0)	6 (7.32)	1.0000
bla_{PER}	0 (0)	0 (0)	0 (0)	1.0000
AmpC enzyme genes, n (%)				
bla_{ACT-1}	66 (70.21)	12 (100.00)	54 (65.85)	0.0377
bla_{DHA-1}	30 (31.91)	3 (25.00)	27 (32.93)	0.8269
bla_{FOX-1}	81 (86.17)	12 (100.00)	69 (84.15)	0.2992
bla_{CMY}, bla_{ACC}, bla_{MOX}, bla_{CIT}, bla_{EBC}	0 (0)	0 (0)	0 (0)	1.0000
Colistin resistance genes, n (%)				
MCR-1	0 (0)	0 (0)	0 (0)	1.0000
MCR-2	0 (0)	0 (0)	0 (0)	1.0000

aminoglycosides (amikacin), carbapenems, cephalosporins, cephalosporins+β-lactamase inhibitors, cephamycins, penicillins+β-lactamase inhibitors, and penicillins (Table 3 and Figure 4).

Distribution of β-Lactam Antibiotic and Colistin Resistance Genes

Carbapenemase, ESBL and AmpC cephalosporinase genes are the main β-lactam antibiotic resistance genes in *Enterobacteriaceae*.[13,27] In this study, all invasive *K. pneumoniae* strains carried β-lactam antibiotic resistance genes. The carbapenemase genes were bla_{NDM-1} (19.15%, 18/94), bla_{VIM-1}(13.83%, 13/94), bla_{KPC-2} (10.64%, 10/94) and bla_{OXA-1} (5.32%, 5/94); the ESBL genes were bla_{SHV-11} (86.17%, 81/94), $bla_{CTX-M-14}$ (48.94%, 46/94), bla_{TEM-1} (19.15%, 18/94) and bla_{VEB-1} (6.38%, 6/94); the AmpC cephalosporinase genes were bla_{FOX-1} (86.17%, 81/94), bla_{ACT-1} (70.21%, 66/94) and bla_{DHA-1} (31.91%, 30/94). Colistin resistance genes (*MCR-1* and *MCR-2*) were not detected (Table 4 and Figure 5). There were no significant differences between CR-HVKP and non CR-HVKP strains in terms of the rates of carbapenemase, ESBL and AmpC cephalosporinase genes (with the exception of bla_{ACT-1}). These data suggest that invasive *K. pneumoniae* strains including CR-HVKP and non CR-HVKP harbor a high proportion of β-lactam antibiotic resistance genes simultaneously.

Characteristics of CR-HVKP Strains

A total of 12 invasive *K. pneumoniae* strains were identified as CR-HVKP, which carried at least 3 β-lactam antibiotic resistance genes. Meanwhile, all 12 CR-HVKP strains carried hypervirulence and other virulence genes, and had hypervirulent capsular serotypes. Additionally, 7 distinct STs were observed among these CR-HVKP strains: ST29 (25.00%, 3/12), ST14 (16.67%, 2/12), ST17 (16.67%, 2/12), ST37 (16.67%, 2/12), ST45 (8.33%, 1/12), ST101 (8.33%, 1/12) and ST234 (8.33%, 1/12) (Table 5). The 12 CR-HVKP strains had low

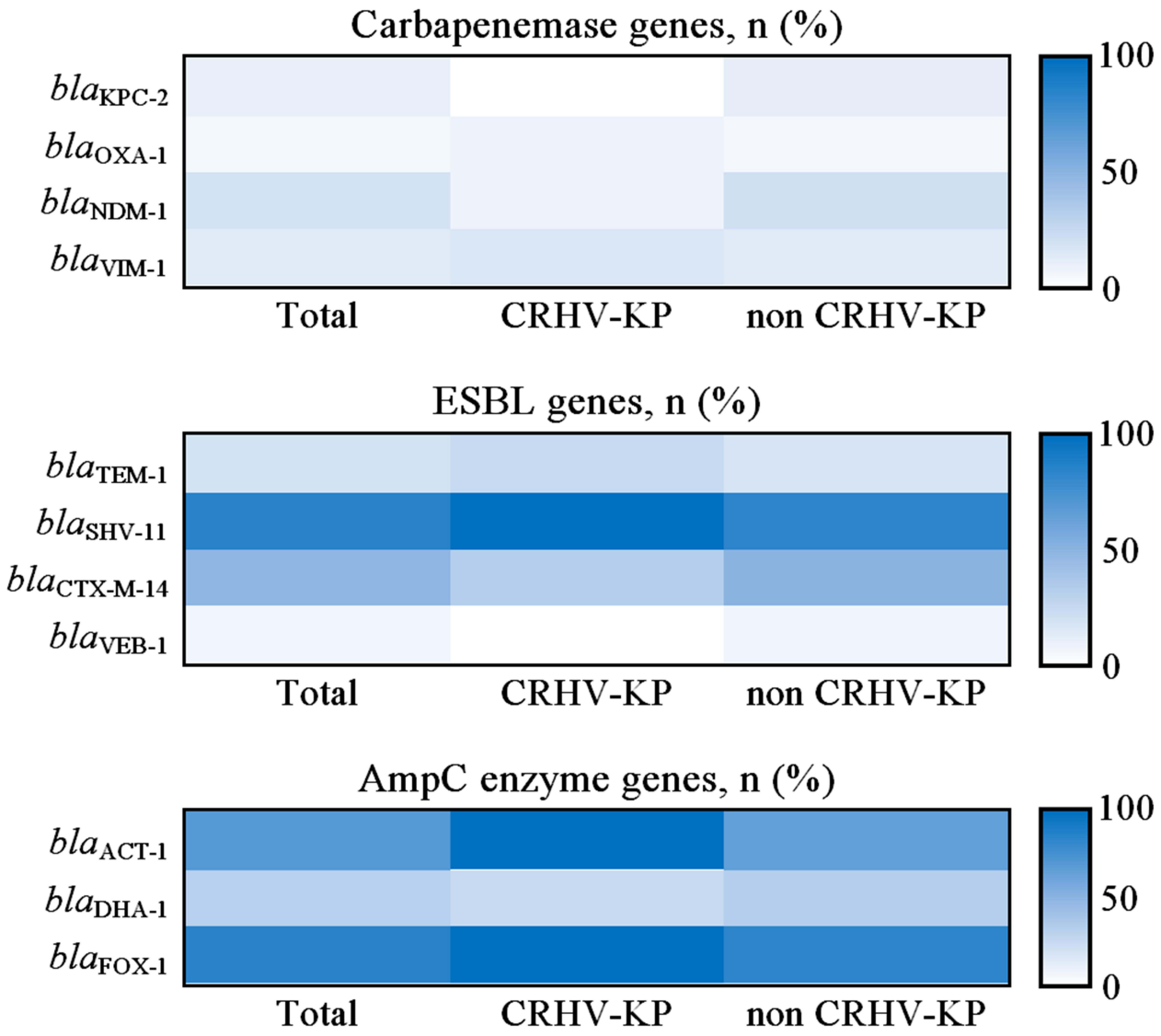

Figure 5 The heat-map of β-lactam antibiotic genes of invasive *K. pneumoniae* strains in this study.

resistance to gentamicin, amikacin, tobramycin, levofloxacin and aztreonam, but were resistant to most other antimicrobial drugs (Table 3 and Figure 4).

Correlation Between Phenotypic and Genotypic MDR Patterns in Invasive *K. pneumoniae*

Besides the 12 CR-HVKP strains, other MDR strains of invasive *K. pneumoniae* were also observed in this study. A total of 14 strains (14.89%) were MDR (defined as resistant to ≥ 1 agent in ≥ 3 antimicrobial classes[30]); these strains exhibited MDR to 7 antimicrobial classes (n=3), 6 antimicrobial classes (n=2), 5 antimicrobial classes (n=6), 4 antimicrobial classes (n=2), and 3 antimicrobial classes (n=1). Additionally, 8 of the strains harbored 5 β-lactam antibiotic resistance genes, 5 strains had 4 of these genes, and 1 strain had 3 of the genes (Table 6).

Discussion

K. pneumoniae is one of the most common pathogens responsible for nosocomial and community-acquired

Table 5 The Capsular Serotypes, Genotypes and MLST of CR-HVKP Strains Identified in This Study (n=12)

STs	Capsular Serotypes	Virulence Genes	β-Lactam Antibiotics Resistance Genes
ST14 (n=2)	K2	*$_p$rmpA, iroB, mrkD, kfu, K$_2$A, wabG, Uge, fimH, ycfM*	*bla*$_{SHV-11}$, *bla*$_{CTX-M-14}$, *bla*$_{ACT-1}$, *bla*$_{DHA-1}$, *bla*$_{FOX-1}$
	K2	*$_p$rmpA, iroB, mrkD, kfu, wabG, Uge, fimH, ycfM, iroN*	*bla*$_{SHV-11}$, *bla*$_{ACT-1}$, *bla*$_{DHA-1}$, *bla*$_{FOX-1}$
ST17 (n=2)	K57	*iroB, mrkD, wabG, Uge, fimH, Kpn, ycfM*	*bla*$_{SHV-11}$, *bla*$_{NDM-1}$, *bla*$_{VIM-1}$, *bla*$_{ACT-1}$, *bla*$_{FOX-1}$
	K57	*$_p$rmpA, iroB, mrkD, wabG, Uge, fimH, Kpn, ycfM*	*bla*$_{SHV-11}$, *bla*$_{ACT-1}$, *bla*$_{FOX-1}$
ST29 (n=3)	K57	*iroB, ytbS, mrkD, entB, wabG, Uge, fimH, Kpn, ycfM*	*bla*$_{TEM-1}$, *bla*$_{SHV-11}$, *bla*$_{OXA-1}$, *bla*$_{ACT-1}$, *bla*$_{FOX-1}$
	K54	*iroB, mrkD, entB, wabG, Uge, fimH, wcaG, Kpn, ycfM*	*bla*$_{TEM-1}$, *bla*$_{SHV-11}$, *bla*$_{ACT-1}$, *bla*$_{FOX-1}$
	K54	*$_p$rmpA, iroB, peg-344, ytbS, mrkD, wabG, Uge, fimH, wcaG, Kpn, ycfM*	*bla*$_{SHV-11}$, *bla*$_{ACT-1}$, *bla*$_{FOX-1}$
ST37 (n=2)	K57	*$_p$rmpA, iroB, mrkD, entB, wabG, Uge, fimH, Kpn, ycfM*	*bla*$_{SHV-11}$, *bla*$_{CTX-M-14}$, *bla*$_{VIM-1}$, *bla*$_{ACT-1}$, *bla*$_{FOX-1}$
	K57	*iroB, mrkD, wabG, fimH, Kpn, ycfM*	*bla*$_{SHV-11}$, *bla*$_{CTX-M-14}$, *bla*$_{ACT-1}$, *bla*$_{FOX-1}$
ST45 (n=1)	K57	*$_p$rmpA, iroB, ytbS, mrkD, wabG, Uge, fimH, Kpn, ycfM*	*bla*$_{TEM-1}$, *bla*$_{SHV-11}$, *bla*$_{ACT-1}$, *bla*$_{FOX-1}$
ST101 (n=1)	K57	*iroB, mrkD, kfu, wabG, Uge, fimH, ycfM*	*bla*$_{SHV-11}$, *bla*$_{ACT-1}$, *bla*$_{FOX-1}$
ST234 (n=1)	K57	*iroB, mrkD, wabG, fimH, Kpn, ycfM*	*bla*$_{SHV-11}$, *bla*$_{CTX-M-14}$, *bla*$_{ACT-1}$, *bla*$_{DHA-1}$, *bla*$_{FOX-1}$

infections in children, which is associated with high morbidity and mortality.[1–3] In recent years, the increased prevalence of HVKP has become a serious global threat to public health.[4] HVKP can cause many types of invasive infection, such as severe pneumonia, bacteremia/septicemia and meningitis, which are often associated with severe symptoms and high mortality rates.[4–6] The capsular serotype of *K. pneumoniae* is one of the factors responsible for its hypervirulence, protecting the bacteria from phagocytosis. There are at least 78 capsular serotypes, with K1, K2, K5, K20, K54 and K57 being the most common hypervirulent serotypes.[8,31] In addition, *K. pneumoniae* also carries several genes that were shown to contribute to hypervirulence, such as *iucA* (involved in aerobactin siderophore biosynthesis), the plasmid-borne *rmpA* gene (*$_p$rmpA*), *$_p$rmpA2*, chromosomal gene *rmpA* (*$_c$rmpA*) (which regulates of the mucoid phenotype via increased capsule production), *peg-344* (a putative transporter), *terB* (involved in tellurite resistance), *iroB* (involved in salmochelin siderophore biosynthesis), and *irp2* (involved in yersiniabactin siderophore biosynthesis).[7] K1 and K2 are the most common hypervirulent serotypes of *K. pneumoniae*.[13,31,32] But in our study, K57 was the main capsular serotype of invasive *K. pneumoniae* strains, which may be explained by differences in serotype distribution according to geographic areas and across populations. Moreover, all invasive *K. pneumoniae* strains carried virulence-associated genes and the proportion with hypervirulence genes was significantly higher than that reported in previous studies, indicating that these factors play an important role in the pathogenicity of invasive *K. pneumoniae* in children.

The prevalence of CRKP is also a global public health problem and the clinical isolation rate of CRKP has been increasing in recent years.[10–13] In China, the rate of detection of CRKP in children increased from 3% to > 20% according to a surveillance program conducted from 2005 to 2017. Notably, this rate was higher than that recorded in adults.[11,13,22] CRKP is resistant to most antimicrobial drugs and there are currently limited therapeutic options available, making infections difficult to control and resulting in high mortality rates.[12,13] The main mechanism of CRKP resistance is the production of enzymes, such as carbapenemases, ESBLs and AmpC, that hydrolyze antimicrobial drugs.[13,27] *bla*$_{KPC-2}$ is the most common carbapenemase in adults in China,[13,33] while the predominant

Table 6 The Correlation Between Phenotypic and Genotypic Resistance Patterns Among the MDR *K. pneumoniae* Strains in This Study (n=14)

Strain Number	In-vitro Phenotypic Resistance	The Antimicrobial Resistance Genes
2	**Aminoglycosides**: Gentamicin	bla_{SHV-11}, bla_{ACT-1}, bla_{FOX-1},
	Non-extended spectrum cephalosporins; 1st and 2nd generation cephalosporins: Cefazolin, Cefuroxime	
	Extended-spectrum cephalosporins; 3rd and 4th generation cephalosporins: Ceftriaxone, Ceftizoxime	
	Monobactams: Aztreonam	
	Penicillins + β-lactamase inhibitors: Ampicillin/sulbactam	
	Penicillins: Piperacillin	
	Sulfonamides: Aulfamethoxazole trimethoprim	
6	**Non-extended spectrum cephalosporins; 1st and 2nd generation cephalosporins**: Cefazolin, Cefuroxime	bla_{TEM-1}, bla_{SHV-11}, bla_{OXA-1}, bla_{ACT-1}, bla_{FOX-1}
	Extended-spectrum cephalosporins; 3rd and 4th generation cephalosporins: Ceftriaxone, Ceftazidime, Cefepime, Ceftizoxime	
	Monobactams: Aztreonam	
	Penicillins + β-lactamase inhibitors: Ampicillin/sulbactam	
	Penicillins: Piperacillin	
	Sulfonamides: Aulfamethoxazole trimethoprim	
8	**Non-extended spectrum cephalosporins; 1st and 2nd generation cephalosporins**: Cefazolin, Cefuroxime	bla_{SHV-11}, bla_{OXA-1}, bla_{ACT-1}, bla_{FOX-1}
	Extended-spectrum cephalosporins; 3rd and 4th generation cephalosporins: Ceftriaxone, Ceftizoxime	
	Fluoroquinolones: Ciprofloxacin	
	Monobactams: Aztreonam	
	Penicillins + β-lactamase inhibitors: Ampicillin/sulbactam	
	Penicillins: Piperacillin	
	Sulfonamides: Aulfamethoxazole trimethoprim	
9	**Non-extended spectrum cephalosporins; 1st and 2nd generation cephalosporins**: Cefazolin, Cefuroxime	bla_{SHV-11}, $bla_{CTX-M-14}$, bla_{VIM-1}, bla_{ACT-1}, bla_{FOX-1}
	Extended-spectrum cephalosporins; 3rd and 4th generation cephalosporins: Ceftriaxone	
	Penicillins + β-lactamase inhibitors: Ampicillin/sulbactam	
	Penicillins: Piperacillin	
	Sulfonamides: Aulfamethoxazole trimethoprim	

(*Continued*)

Table 6 (Continued).

Strain Number	In-vitro Phenotypic Resistance	The Antimicrobial Resistance Genes
15	**Aminoglycosides**: Gentamicin, Tobramycin	bla_{SHV-11}, $bla_{CTX-M-14}$, bla_{ACT-1}, bla_{DHA-1}, bla_{FOX-1}
	Non-extended spectrum cephalosporins; 1st and 2nd generation cephalosporins: Cefazolin, Cefuroxime	
	Extended-spectrum cephalosporins; 3rd and 4th generation cephalosporins: Ceftriaxone, Ceftazidime, Cefepime	
	Monobactams: Aztreonam	
	Penicillins + β-lactamase inhibitors: Ampicillin/sulbactam	
	Penicillins: Piperacillin	
	Sulfonamides: Aulfamethoxazole trimethoprim	
16	**Non-extended spectrum cephalosporins; 1st and 2nd generation cephalosporins**: Cefazolin, Cefuroxime	bla_{SHV-11}, $bla_{CTX-M-14}$, bla_{ACT-1}, bla_{DHA-1}, bla_{FOX-1}
	Extended-spectrum cephalosporins; 3rd and 4th generation cephalosporins: Ceftriaxone, Ceftizoxime	
	Cephamycins: Cefoxitin	
	Penicillins + β-lactamase inhibitors: Ampicillin/sulbactam	
	Penicillins: Piperacillin	
22	**Non-extended spectrum cephalosporins; 1st and 2nd generation cephalosporins**: Cefazolin, Cefuroxime	bla_{TEM-1}, bla_{SHV-11}, bla_{VIM-1}, bla_{ACT-1}, bla_{FOX-1}
	Extended-spectrum cephalosporins; 3rd and 4th generation cephalosporins: Cefotaxime, Ceftriaxone	
	Penicillins + β-lactamase inhibitors: Ampicillin/sulbactam	
24	**Aminoglycosides**: Gentamicin	bla_{TEM-1}, bla_{SHV-11}, bla_{ACT-1}, bla_{FOX-1}
	Non-extended spectrum cephalosporins; 1st and 2nd generation cephalosporins: Cefazolin, Cefuroxime	
	Extended-spectrum cephalosporins; 3rd and 4th generation cephalosporins: Cefotaxime, Ceftriaxone	
	Penicillins + β-lactamase inhibitors: Ampicillin/sulbactam	
	Sulfonamides: Aulfamethoxazole trimethoprim	
37	**Non-extended spectrum cephalosporins; 1st and 2nd generation cephalosporins**: Cefazolin, Cefuroxime	bla_{TEM-1}, bla_{SHV-11}, bla_{ACT-1}, bla_{FOX-1}
	Extended-spectrum cephalosporins; 3rd and 4th generation cephalosporins: Cefotaxime, Ceftriaxone	
	Penicillins + β-lactamase inhibitors: Ampicillin/sulbactam	
	Sulfonamides: Aulfamethoxazole trimethoprim	

(*Continued*)

Table 6 (Continued).

Strain Number	In-vitro Phenotypic Resistance	The Antimicrobial Resistance Genes
48	**Aminoglycosides**: Gentamicin	bla_{TEM-1}, bla_{SHV-11}, bla_{ACT-1}, bla_{FOX-1}
	Non-extended spectrum cephalosporins; 1st and 2nd generation cephalosporins: Cefazolin, Cefuroxime	
	Extended-spectrum cephalosporins; 3rd and 4th generation cephalosporins: Cefotaxime, Ceftriaxone	
	Penicillins + β-lactamase inhibitors: Ampicillin/sulbactam	
	Sulfonamides: Aulfamethoxazole trimethoprim	
65	**Non-extended spectrum cephalosporins; 1st and 2nd generation cephalosporins**: Cefazolin, Cefuroxime	bla_{SHV-11}, bla_{NDM-1}, bla_{VIM-1}, bla_{VEB-1}, bla_{FOX-1}
	Extended-spectrum cephalosporins; 3rd and 4th generation cephalosporins: Cefotaxime, Ceftriaxone	
	Monobactams: Aztreonam	
	Penicillins + β-lactamase inhibitors: Ampicillin/sulbactam	
	Sulfonamides: Aulfamethoxazole trimethoprim	
73	**Non-extended spectrum cephalosporins; 1st and 2nd generation cephalosporins**: Cefazolin, Cefuroxime	bla_{SHV-11}, $bla_{CTX-M-14}$, bla_{DHA-1}, bla_{FOX-1}
	Extended-spectrum cephalosporins; 3rd and 4th generation cephalosporins: Cefotaxime, Ceftriaxone	
	Cephamycins: Cefoxitin	
	Penicillins + β-lactamase inhibitors: Ampicillin/sulbactam	
	Sulfonamides: Aulfamethoxazole trimethoprim	
79	**Non-extended spectrum cephalosporins; 1st and 2nd generation cephalosporins**: Cefazolin, Cefuroxime	bla_{SHV-11}, bla_{NDM-1}, bla_{VIM-1}, bla_{VEB-1}, bla_{FOX-1}
	Extended-spectrum cephalosporins; 3rd and 4th generation cephalosporins: Cefotaxime, Ceftriaxone, Ceftizoxime	
	Monobactams: Aztreonam	
	Sulfonamides: Aulfamethoxazole trimethoprim	
94	**Aminoglycosides**: Gentamicin	bla_{TEM-1}, bla_{SHV-11}, bla_{KPC-2}, bla_{ACT-1}, bla_{FOX-1}
	Non-extended spectrum cephalosporins; 1st and 2nd generation cephalosporins: Cefazolin, Cefuroxime	
	Extended-spectrum cephalosporins; 3rd and 4th generation cephalosporins: Cefotaxime, Ceftriaxone	
	Fluoroquinolones: Ciprofloxacin	
	Monobactams: Aztreonam	
	Penicillins + β-lactamase inhibitors: Ampicillin/sulbactam	

carbapenemases in children were *bla*$_{IMP-4}$, *bla*$_{NDM-1}$ and *bla*$_{OXA-232}$ in different periods.[13,34] In our study, carbapenemase genes were detected in only a few invasive *K. pneumoniae* strains; however, most strains harbored ESBL and AmpC enzyme genes, mainly *bla*$_{SHV-11}$, *bla*$_{FOX-1}$ and *bla*$_{ACT-1}$. This diverges from previous findings that *bla*$_{TEM-1}$ was the main ESBL gene while the proportion of AmpC genes was low.[27,35] An evaluation of drug susceptibility showed that the 94 invasive *K. pneumoniae* strains identified in this study had low drug resistance rates but high carriage rates of ESBL and AmpC enzyme genes. Moreover, we found 14 MDR *K. pneumoniae* strains that carried at least 3 β-lactam antibiotic resistance genes, representing potential threats. Therefore, the drug resistance of invasive *K. pneumoniae* in children warrants further attention.

In this study, 12 CR-HVKP strains were identified belonging to 7 STs. ST29 was the most common ST in these strains, whereas ST11 was previously reported as the predominant ST among CRKP isolated from both adults and children in China.[27,36] The predominant ST of CRKP and CR-HVKP may differ. However, there have been limited studies of CR-HVKP isolated from pediatric patients and further investigations are required. CR-HVKP has high pathogenicity in children and is resistant to most antimicrobial drugs, which presents a challenge for the clinical management of infections. More research is needed to develop strategies to overcome these challenges.

Conclusions

In summary, our findings reveal the epidemiology, virulence, and antimicrobial resistance of invasive *K. pneumoniae* at a children's medical center in eastern China. Both CR-HVKP and MDR strains were identified. HVKP and CRKP were found to overlap in pediatric patients; their accurate identification can aid diagnosis and effective treatment.

Ethical Approval

The authors are accountable for all aspects of the work and ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was approved by the Ethics Committee of Children's Hospital of Soochow University (No. 2020CS099). The investigation was conducted in accordance with the guidelines of the Declaration of Helsinki.

This was a retrospective study, and the clinical isolates were collected and stored during routine diagnostic laboratory examination. The Review Board exempted the study from the requirement informed consent as it was focused on the bacteria and did not have an impact on the patients.

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Disclosure

The authors report no conflicts of interest in this work.

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