Int J Clin Exp Med 2021;14(8):2189-2200
www.ijcem.com /ISSN:1940-5901/IJCEM0124025

Original Article

The prevalence and distribution characteristics of beta-lactamase resistant genes and virulence factors in *Klebsiella pneumoniae*

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Received October 13, 2020; Accepted May 31, 2021; Epub August 15, 2021; Published August 30, 2021

Abstract: Objective: To investigate the frequency of antibiotic resistance profiles and virulence factors of *Klebsiella pneumoniae* isolated from our hospital. Methods: A total of 54 clinical isolates of *Klebsiella. Pneumoniae* from August 2018 to June 2019 were collected. The minimal inhibitory concentration (MIC) of antibiotics was determined by broth microdilution method. The phenotypes were identified by mCIM, MHT and string test. The antibiotic resistance gene, virulence gene and multilocus sequence typing (MLST) were detected by PCR. Results: The majority of *Klebsiella pneumoniae* strains (83.3%) were MDR. PCR detection of β-lactamase genes showed the strains were positive for bla_{SHV} (96.3%), bla_{TEM} (64.8%), $bla_{CTX\text{-}M\text{-}1}$ group (40.7%), $bla_{CTX\text{-}M\text{-}2}$ group (53.7%), $bla_{CTX\text{-}M\text{-}8}$ group (74.1%), $bla_{CTX\text{-}M\text{-}9}$ group (38.9%) and $bla_{CTX\text{-}M\text{-}25}$ group (3.7%) ESBLs-encoding genes, bla_{KPC} (24.1%), bla_{NDM} (9.3%), bla_{VIM} (9.3%) carbapenemase genes, and bla_{DHA} (44.4%), bla_{EBC} (5.6%) AmpCs genes. All of the strains carried *mrkD* virulence genes, followed by *fimH* (88.9%), *ybt*S (51.9%), *rmpA* (20.4%), *entB* (9.3%), *allS* (7.4%), *aerobactin* (3.7%) genes and capsular serotype K1 (5.6%), K2 (3.7%), K5 (1.9%). MLST results showed that 32 STs were found in 54 isolates, and ST11 (14.8%) was the dominant sequence type. Two new STs were assigned as ST5214 and ST5215. Conclusion: In this study, *Klebsiella pneumoniae* carried high frequency and diversity of β-lactamase genes and virulence genes. These strains are resistant to a variety of antibiotics. We can strengthen the surveillance and control of *Klebsiella pneumoniae* with coexisting antibiotic resistance and virulence genes.

Keywords: *Klebsiella pneumoniae*, antimicrobial resistance, β-lactamase genes, virulence factors

Introduction

Klebsiella pneumoniae, a Gram-negative opportunistic pathogen, which is one of the most common pathogenic microorganisms found in hospitals and is responsible for community-acquired infection. It can cause serious infectious diseases, including pneumonia, liver abscess, meningitis, bloodstream infections and urinary tract infections [1]. Two different pathotypes of *K. pneumoniae* have been recognized: classic (cKP) and hypervirulent (hvKP) strains. In recent years, the majority of *K. pneumoniae* infections are caused by cKP. These strains persist in the hospital environment and cause infections in immunosuppressed patients [2]. cKP has a tendency to acquire antibiotic resistance. Due to overuse and pressure of antibiotic selection, *K. pneumoniae* continuously accumulates resistance genes by mutation and obtains mobile genetic elements such as plasmids, thus producing multidrug-resistant (MDR) strains [3].

MDR strains usually contain plasmid mediated β-lactamases with extended-spectrum hydrolytic activities [4], such as extended-spectrum β-lactamases (ESBLs). ESBLs are widely distributed amongst Enterobacteriaceae due to the overuse or abuse of antibiotics [5]. There are hundreds of varieties of ESBLs, among which TEM, SHV and CTX-M are the most common [6]. In addition, AmpC enzymes, also known as cephalosporinase, is one of the β-lactamases. AmpCs can hydrolyze penicillins, cephalosporins (except fourth-generation cephalosporins), cephamycins and β-lactamase inhibitors [7]. Nowadays, with the widespread use of car-

bapenems in recent years, carbapenemase-producing *Klebsiella pneumoniae* (CRKP) have increased rapidly. The surge in CRKP is mainly due to the emergence and spread of carbapenemases [8]. The common carbapenemases include KPC [9], NDM, VIM, IMP and OXA, which are widely disseminated worldwide [10].

In the mid-1980s and 1990s, hypervirulent *Klebsiella pneumoniae* (hvKP) was first described in Taiwan. HvKP was capable of causing community-acquired, invasive and metastatic infections, which may lead to serious and life-threatening infections in younger healthy hosts and diabetic patients [2, 11]. For the past 30 years, the number of hvKP infections has steadily increased in parts of Asia [12]. The pathogenicity of *K. pneumoniae* mainly arise from a variety of virulence factors, which assist in overcoming innate immunity of the host and maintain infection in patients [13, 14]. The main virulence factors that play an important role in pathogenicity are capsular polysaccharide, lipopolysaccharide, siderophores, and fimbriae [13]. HvKP have a hypermucoviscous phenotype, which are also highly associated with the genes encoding *rmpA* and capsular serotype K1/K2 [14-16].

The antibiotic resistant and hypervirulent isolates were non-overlapping for a long time. In recent years, the multidrug-resistant and hypervirulent (MDR-HV) K. pneumoniae strains are increasingly being reported, which make clinical infection complicated by devastating disseminated infections [17, 18]. MDR-HV *K. pneumoniae* are caused by two different mechanisms: (1) HvKP strains acquire antimicrobial resistance genes or plasmids. (2) The virulence plasmid transfer into classic MDR *K. pneumoniae* strains [17]. For example, serotype K1 ST23 ESBL-producing *K. pneumoniae* strains carrying transferable CTX-M-15 plasmids were found in Asia [19]. Carbapenem-resistant hypervirulent *K. pneumoniae* (CR-hvKP) were described sporadically in China [20]. *K. pneumoniae* infection has become an increasingly serious public health problem due to the existence of multidrug-resistant genes and virulence factors, causing the deterioration of nosocomial infection [21].

In this study, we analyzed the antibiotic resistance mechanisms and virulence factors of clinical *K. pneumoniae* collected recently from our hospital. We also investigated the distribution of antibiotic resistant and virulence genes, which enabled us to provide evidence for clinical and nosocomial infection control.

Materials and methods

Clinical isolates and data collection

A retrospective analysis was conducted in The Affiliated Chaohu Hospital of Anhui Medical University. From August 2018 to June 2019, 54 clinical strains of *K. pneumoniae* were collected from different clinical samples. All strains were identified by VITEK II compact system. The isolates were stored in BHI broth with 15% glycerol at -80°C. Ethical approval was granted by the Affiliated Chaohu Hospital of Anhui Medical University Ethics Committee (No. KYXM-201807-003). Informed consent of the patients was waived as this research was retrospective.

Antimicrobial susceptibility testing

According to the Clinical and Laboratory Standards Institute (CLSI), the antimicrobial susceptibility of all *K. pneumoniae* isolates was analyzed by the broth microdilution method. The minimal inhibitory concentration (MIC) of antibiotics was determined following the CLSI M100-S29 standard. *E. coli* ATCC 25922 was used as the quality control for all antimicrobial susceptibility tests.

β-lactamase identification and hypermucoviscousity

The phenotype positive isolates of carbapenemase production were detected by the modified carbapenem inactivation method (mCIM). AmpC production was carried out by modified Hodge test (MHT) for cefoxitin resistant isolates. The specific methods and results were determined according to CLSI. *E. coli* ATCC 25922, *K. pneumoniae* ATCC BAA-1705, *K. pneumoniae* ATCC BAA-1706, and *Aerobacter cloacae* 029M were used as quality controls.

The hypermucoviscous phenotype of the strain was tested by the string test following previously described methods [15]. The strains were inoculated onto blood agar plates and incubated at 37°C overnight. The string test was considered positive if it generated a viscous string >5 mm when a standard bacteriological loop was used to stretch the colony on agar plate.

Table 1. Sequences of primes used for detection of resistance genes

Resistance target	Sequence (5'-3'), F/R	Amplicon size (bp)	Tm (°C)	Reference
TEM	CATTTCCGTGTCGCCCTTATTC	800	56.2	[23]
	CGTTCATCCATAGTTGCCTGAC			
SHV	CACTCAAGGATGTATTGTG	885	52	[24]
	TTAGCGTTGCCAGTGCTCG			
CTX-M-1	ACAGCGATAACGTGGCGATG	216	58.3	[25]
	TCGCCCAATGCTTTACCCAG			
CTX-M-2	GCGACCTGGTTAACTACAATCC	351	56	[22]
	CGGTAGTATTGCCCTTAAGCC			
CTX-M-8	CGCTTTGCCATGTGCAGCACC	307	59	[26]
	GCTCAGTACGATCGAGCC			
CTX-M-9	GTGACAAAGAGAGTGCAACGG	857	58	[24]
	ATGATTCTCGCCGCTGAAGCC			
CTX-M-25	CACACGAATTGAATGTTCAG	924	50	[26]
	TCACTCCACATGGTGAGT			
KPC	CGTCTAGTTCTGCTGTCTTG	798	53	[25]
	CTTGTCATCCTTGTTAGGCG			
IMP	GGAATAGAGTGGCTTAAYTCTC	232	55	[27]
	GGTTTAAYAAAACAACCACC			
VIM	GATGGTGTTTGGTCGCATA	390	57	[27]
	CGAATGCGCAGCACCAG			
NDM	GGTTTGGCGATCTGGTTTTC	621	55	[27]
	CGGAATGGCTCATCACGATC			
OXA-48	GCGTGGTTAAGGATGAACAC	438	54	[27]
	CATCAAGTTCAACCCAACCG			
DHA	AACTTTCACAGGTGTGCTGGGTCCGTACGCATACTGGCTTTGC	405	60	[28]
MOX	GCTGCTCAAGGAGCACAGGATCACATTGACATAGGTGTGGTGC	520	58	[28]
FOX	AACATGGGGTATCAGGGAGATGCAAAGCGCGTAACCGGATTGG	190	58	[28]
EBC	TCGGTAAAGCCGATGTTGCGGCTTCCACTGCGGCTGCCAGTT	302	62	[28]

Detection of β-lactamase genes

In this study, DNA of *K. pneumoniae* was extracted by the boiling method. The common β-lactamase encoding genes were detected by polymerase chain reaction (PCR) and sequenced. The ESBL-encoding genes (bla_{SHV}, bla_{TEM}, $bla_{CTX\text{-}M\text{-}1}$ group, $bla_{CTX\text{-}M\text{-}2}$ group, $bla_{CTX\text{-}M\text{-}8}$, $bla_{CTX\text{-}M\text{-}9}$ group and $bla_{CTX\text{-}M\text{-}25}$ group), Carbapenemase-encoding genes (bla_{KPC}, bla_{IMP}, bla_{VIM}, bla_{NDM}, $bla_{OXA\text{-}48}$) and AmpC encoding genes (bla_{MOX}, bla_{CMY}, bla_{DHA}) were amplified by PCR with specific primers as previously described [22-28]. The primers and the length of expected PCR products are shown in **Table 1**. Amplified products were analyzed by electrophoresis in 1.2% agarose gel stained with ethidium bromide. Then the results were visualized under ultraviolet (UV) light.

Molecular detection of virulence-associated genes

Virulence-associated genes were tested by PCR as described previously, including encoding regulators of mucoid phenotype A (*rmpA*), type 1 (*fimH*) and type 3 (*mrkD*) adhesins, aerobactin, enterobactin (*entB*) and yersiniabactin (*ybtS*), associated with allantoin metabolism (*allS*), responsible for an iron uptake system (*kfu*) and capsular serotypes K1 and K2 K5, K20, K54 and K57 [15, 25, 29, 30]. The specific primers and the PCR products length are shown in **Table 2**.

Table 2. Sequences of primers used for detection of capsule serotypes and virulence genes

Gene	Primer sequence (5'-3'), F/R	Amplicon size (bp)	Tm (°C)	Reference
K1	GGTGCTCTTTACATCATTGC	1283	53	[29]
	GCAATGGCCATTTGCGTTAG			
K2	GACCCGATATTCATACTTGACAGAG	641	54	[29]
	CCTGAAGTAAAATCGTAAATAGATGGC			
K5	TGGTAGTGATGCTCGCGA	280	56	[29]
	CCTGAACCCACCCCAATC			
K20	CGGTGCTACAGTGCATCATT	741	58	[29]
	GTTATACGATGCTCAGTCGC			
K54	CATTAGCTCAGTGGTTGGCT	881	58	[29]
	GCTTGACAAACACCATAGCAG			
K57	CTCAGGGCTAGAAGTGTCAT	1037	56	[29]
	CACTAACCCAGAAAGTCGAG			
rmpA	ACTGGGCTACCTCTGCTTCA	535	54.1	[25]
	CTTGCATGAGCCATCTTTCA			
mrkD	CCACCAACTATTCCCTCGAA	226	54	[25]
	ATGGAACCCACATCGACATT			
fimH	TGCTGCTGGGCTGGTCGATG	550	61	[25]
	GGGAGGGTGACGGTGACATC			
Aerobactin	GCATAGGCGGATACGAACAT	556	54	[15]
	CACAGGGCAATTGCTTACCT			
ybtS	GACGGAAACAGCACGGTAAA	242	52	[30]
	GAGCATAATAAGGCGAAAGA			
entB	GTCAACTGGGCCTTTGAGCCGTC	400	60	[30]
	TATGGGCGTAAACGCCGGTGAT			
allS	CCGAAACATTACGCACCTTT	508	55	[15]
	ATCACGAAGAGCCAGGTCAC			
Kfu	GGCCTTTGTCCAGAGCTACG	638	59	[30]
	GGGTCTGGCGCAGAGTATGC			

Multilocus sequence typing (MLST)

MLST was performed in accordance with the protocol described on the *K. pneumoniae* MLST website (https://bigsdb.pasteur.fr/klebsiella/klebsiella.html). Seven housekeeping genes (*gapA*, *infB*, *mdh*, *pgi*, *phoE*, *rpoB*, and *tonB*) were amplified by PCR and subsequent sequencing. The sequencing results were uploaded to MLST online database to define the sequence type (ST).

Results

Clinical characteristics of patients infected with K. pneumoniae

A total of 54 non-repeated *K. pneumoniae* isoforms from a tertiary hospital located in the central region of China were collected. **Table 3** shows the clinical characteristics and the source of these samples among the 54 patients. All patients had nosocomial infection. Most of the patients (44/54, 81.5%) used invasive procedures and devices during hospitalization. The isolates were predominantly collected from sputum (32/54, 59.3%), and other sources including urine (10/54, 18.5%), blood (7/54, 13.0%), wound secretion (2/54, 3.7%), pus (1/54, 1.9%), bile (1/54, 1.9%), and ascitic fluid (1/54, 1.9%).

Antimicrobial susceptibility test and β-lactamase phenotype detection

All of the *K. pneumoniae* strains showed resistance towards AMP. The majority of the strains were resistant to CFZ (96.3%), CRO (88.9%), CTX (83.3%) and NIT (94.4%), respectively. The percentage of *K. pneumoniae* isolates towards other antibiotics was as follows: ATM (70.4%), GEN (68.5%), CAZ (66.7%), FEP (61.1%), TOB

Table 3. Clinical characteristics of patients with K. pneumoniae infections; n=54

Variable	n (%)
Age (years), mean ± SD	65.22±14.89
Sex	
male	42 (77.8)
female	12 (22.2)
Distribution of depart	
medical ward	20 (37.0)
surgical ward	24 (44.4)
intensive care unit	10 (18.5)
Infection source	
sputum	32 (59.3)
urine	10 (18.5)
blood	7 (13.0)
wound secretions	2 (3.7)
pus	1 (1.9)
bile	1 (1.9)
ascitic fluid	1 (1.9)
Comorbidity	
pneumonia	35 (64.8)
cerebral vascular disease	18 (33.3)
urinary tract infection	11 (20.4)
diabetes mellitus	9 (16.7)
coronary heart disease	6 (11.1)
malignancy	5 (9.3)
liver cirrhosis	2 (3.7)
Immunosuppression[a]	16 (29.6)
Invasive procedures and devices	
urethral catheter	38 (70.4)
nasogastric tube	32 (59.3)
tracheal aspiration	30 (55.6)
endotracheal intubation	18 (33.3)
tracheotomy	17 (31.5)
ventilator	17 (31.5)
surgical drainage	15 (27.8)
central venous catheter	9 (16.7)

[a]Immunosuppression was defined as meeting one of the following criteria: neutropenia, use of corticosteroids or receiving chemotherapy.

(59.3%), CIP (59.3%), SXT (53.7%), TZP (50.0%), LVX (50.0%), CTM (27.8%), MEM (27.8%), IMP (25.9%), ETP (25.9%) and AMK (18.5%).

There were 45 strains (83.3%) which showed resistance to more than three classes of antibiotics (β-lactam, cephalosporin, monobactam, aminoglycoside, qouinolone, sulfonamides, nitrofurans) and categorized as multidrug-resistant (MDR). Based on results of the mCIM method, 15 (27.8%) strains were carbapenemase producers. Thirty-three cefoxitin resistant strains were additionally screened with the MHT test set of which 26 (78.8%) showed positive.

Detection of antibiotic resistance genes

Among the 15 carbapenem-resistant *K. pneumoniae* strains, 13 (24.1%) carried bla_{KPC}. Five isolates harbored both bla_{KPC} and bla_{VIM} genes. Three isolates harbored both bla_{KPC} and bla_{NDM} genes. In addition, the *K. pneumoniae* isolates carried the bla_{SHV} (52, 96.3%), bla_{TEM} (35, 64.8%), $bla_{CTX\text{-}M\text{-}1}$ group (22, 40.7%), $bla_{CTX\text{-}M\text{-}2}$ group (29, 53.7%), $bla_{CTX\text{-}M\text{-}8}$ group (40, 74.1%), $bla_{CTX\text{-}M\text{-}9}$ group (21, 38.9%), and $bla_{CTX\text{-}M\text{-}25}$ group (2, 3.7%) ESBL-encoding genes. This was followed by bla_{DHA} (24, 44.4%), bla_{EBC} (3, 5.6%) AmpC-encoding genes. One isolate harbored both bla_{DHA} and bla_{EBC} genes (**Table 4**). The bla_{IMP}, $bla_{OXA\text{-}48}$, bla_{FOX} and bla_{MOX} genes were not detected.

Detection of hypermucoviscous phenotype and virulence genes

Further, String test was positive with 10 (18.5%) strains. PCR amplification results showed that only 6 strains of capsular serotype were detected, including K1 (3, 5.6%), K2 (2, 3.7%), and K5 (1, 1.9%). K20, K54 and K57 serotypes were not detected. For virulence genes *mrkD* were detected in all isolates. A total of 48 (88.9%) carried *fimH*, 28 (51.9%) carried *ybtS*, 11 (20.4%) harbored *rmpA*, 5 (9.3%) harbored *entB*, 4 (7.4%) contained *allS*, 2 (3.7%) contained *aerobactin* gene, and the *kfu* gene was not found in this study (**Table 4**).

MLST

Multilocus sequence typing analysis revealed 32 different STs among 54 *K. pneumoniae* isolates. Eight isolates belonged to ST11, followed by ST15 (4, 7.4%), ST307 (4, 7.4%), ST35 (3, 5.6%), ST147 (3, 5.6%), ST1326 (3, 5.6%), ST29 (2, 3.7%), ST340 (2, 3.7%), ST1887 (2, 3.7%), and single strains were identified in 21 other STs. Furthermore, the new STs were submitted to the Instiute Pasteur website and were accepted by the PubMLST database. These two new ST types were assigned as ST5214 and ST5215 (**Table 4**).

Table 4. Phenotypic, Resistance and Virulence genotypic characteristics of 54 *K. pneumoniae* Isolates

Strain	ST	Sting results	Antimicrobial resistance	Resistance genes			Virulence factors	
				Carbapenemase	ESBL	AmpC	Virulence genes	K serotype
Kp1	ST1119		AMP, CFZ, CRO, CTX, FEP, GEN, TOB, NIT		SHV, CTX-M-2, CTX-M-8, CTX-M-9		mrkD, fimH	
Kp2	ST307		AMP, CFZ, CAZ, CRO, CTX, FEP, ATM, GEN, TOB, CIP, LVX, SXT, NIT		TEM, SHV, CTX-M-1, CTX-M-8		mrkD, fimH	
Kp3	ST340		AMP, TZP, TOB, CIP, LVX, SXT, NIT		TEM, SHV, CTX-M-1, CTX-M-8		mrkD, fimH	
Kp4	ST45		AMP, CFZ, CAZ, CRO, CTX, NIT		TEM, SHV, CTX-M-1, CTX-M-2, CTX-M-9		mrkD, fimH, ybtS	
Kp5	ST5214		AMP, CFZ, CAZ, CRO, CTX, FEP, ATM, GEN, TOB, CIP, LVX, SXT, NIT		TEM, SHV, CTX-M-1, CTX-M-8	DHA	mrkD, fimH	K1
Kp6	ST3509	HM	AMP, CFZ, CTM, CAZ, CRO, CTX, ATM		TEM, SHV, CTX-M-1, CTX-M-9	DHA	mrkD, fimH, ybtS, rmpA, aerobactin	K2
Kp7	ST35		AMP, TZP, IMP, MEM, CFZ, CAZ, CRO, CTX, FEP, ATM, GEN, SXT, NIT	KPC	TEM, SHV, CTX-M-2, CTX-M-9	DHA	mrkD, fimH, ybtS, rmpA, aerobactin	
Kp8	ST378		AMP, CFZ, CAZ, CRO, CTX, FEP, ATM, GEN, TOB, CIP, LVX, SXT, NIT		TEM, SHV, CTX-M-1, CTX-M-2, CTX-M-9	DHA	mrkD, fimH, ybtS	
Kp9	ST15		AMP, CFZ, CTM, CAZ, CRO, CTX, FEP, ATM, GEN, TOB, SXT, NIT		TEM, CTX-M-1, CTX-M-8	DHA	mrkD, ybtS	
Kp10	ST307		AMP, CFZ, CAZ, CRO, CTX, FEP, ATM, GEN, TOB, CIP, SXT, NIT		TEM, SHV, CTX-M-1, CTX-M-8, CTX-M-9	DHA	mrkD, fimH	
Kp11	ST5215		AMP, CFZ, CRO, ATM, GEN, SXT, NIT		TEM, SHV, CTX-M-2, CTX-M-8, CTX-M-9	DHA	mrkD, fimH, allS	K1
Kp12	ST11		AMP, TZP, IMP, MEM, ETP, CFZ, CTM, CAZ, CRO, CTX, FEP, ATM, GEN, AMK, TOB, CIP, LVX, SXT, NIT	KPC	TEM, SHV, CTX-M-2, CTX-M-9	DHA	mrkD, fimH, ybtS	
Kp13	ST1922		AMP, CFZ, CRO, GEN, TOB, CIP, LVX, SXT, NIT		TEM, SHV, CTX-M-8		mrkD, fimH	
Kp14	ST1552		AMP, CFZ, CRO, CTX, FEP, GEN, TOB, SXT, NIT		SHV, CTX-M-2, CTX-M-8, CTX-M-9, CTX-M-25		mrkD, fimH	
Kp15	ST219		AMP, CFZ, CAZ, CRO, CTX, FEP, ATM, SXT, NIT		SHV, CTX-M-1, CTX-M-2, CTX-M-8		mrkD, fimH, ybtS	
Kp16	ST307		AMP, CFZ, CAZ, CRO, CTX, FEP, ATM, GEN, TOB, CIP, SXT, NIT		TEM, SHV, CTX-M-1, CTX-M-8		mrkD, ybtS	
Kp17	ST15		AMP, TZP, CFZ, CAZ, CRO, CTX, FEP, ATM, GEN, TOB, CIP, LVX, SXT, NIT		TEM, SHV, CTX-M-8	DHA	mrkD, fimH	
Kp18	ST828		AMP, CFZ, GEN, SXT, NIT		TEM, SHV		mrkD, fimH	
Kp19	ST35		AMP, TZP, MEM, ETP, CFZ, CTM, CAZ, CRO, CTX, FEP, ATM, GEN, TOB, CIP, LVX	NDM	TEM, SHV	DHA, EBC	mrkD, ybtS	
Kp20	ST15		AMP, TZP, CFZ, CAZ, CRO, CTX, FEP, ATM, GEN, TOB, CIP, LVX, SXT, NIT		TEM, SHV, CTX-M-1, CTX-M-8		mrkD, fimH	
Kp21	ST340		AMP, CFZ, CAZ, CRO, CTX, FEP, ATM, GEN, AMK, TOB, CIP, LVX, NIT		TEM, SHV, CTX-M-1, CTX-M-8		mrkD, fimH	
Kp22	ST1128		AMP, CFZ, CRO, CTX, ATM, GEN, TOB, CIP, LVX, NIT		TEM, SHV, CTX-M-2, CTX-M-9	DHA	mrkD, fimH	
Kp23	ST218	HM	AMP, TZP, IMP, MEM, ETP, CFZ, CAZ, CRO, CTX, ATM, NIT	KPC	TEM, SHV, CTX-M-1, CTX-M-2, CTX-M-9	DHA	mrkD, fimH, rmpA	
Kp24	ST37		AMP, TZP, CFZ, CTM, CAZ, CRO, CTX, ATM, NIT		SHV, CTX-M-2	DHA	mrkD, fimH	
Kp25	ST76		AMP, CFZ, CIP, LVX, SXT, NIT		SHV		mrkD, fimH, ybtS, rmpA	K5
Kp26	ST1326		AMP, TZP, IMP, MEM, ETP, CFZ, CAZ, CRO, CTX, FEP, ATM, TOB, CIP, LVX, SXT, NIT	KPC, VIM	TEM, SHV, CTX-M-1, CTX-M-2, CTX-M-8	DHA	mrkD, fimH, ybtS	
Kp27	ST307		AMP, TZP, CFZ, CAZ, CRO, CTX, FEP, ATM, GEN, TOB, CIP, LVX, SXT, NIT		TEM, SHV, CTX-M-1, CTX-M-8		mrkD, fimH, ybtS	
Kp28	ST1427		AMP, TZP, IMP, MEM, ETP, CFZ, CTM, CAZ, CRO, CTX, FEP, ATM, GEN, TOB, CIP, SXT, NIT	NDM	TEM, SHV, CTX-M-1, CTX-M-8, CTX-M-25	EBC	mrkD, fimH	

Kp29	ST1887		AMP, TZP, IMP, MEM, ETP, CFZ, CTM, CAZ, CRO, CTX, ATM, GEN, AMK, TOB, CIP, NIT	KPC, NDM	TEM, SHV, CTX-M-8	DHA	mrkD, fimH, allS	
Kp30	ST1764	HM	AMP, TZP, IMP, MEM, ETP, CFZ, CAZ, CRO, CTX, FEP, ATM, NIT	KPC, NDM	TEM, SHV, CTX-M-8	DHA	mrkD, fimH, rmpA, allS	
Kp31	ST17		AMP, CFZ, CRO, CTX, GEN, TOB, NIT		TEM, SHV, CTX-M-2, CTX-M-8, CTX-M-9	DHA	mrkD, fimH	
Kp32	ST15		AMP, CFZ, CTM, NIT		SHV	EBC	mrkD, ybtS	
Kp33	ST11	HM	AMP, TZP, CFZ, CTM, CAZ, CRO, CTX, FEP, ATM, GEN, AMK, CIP, LVX, NIT		TEM, SHV, CTX-M-2, CTX-M-8, CTX-M-9	DHA	mrkD, fimH, rmpA, ybtS	
Kp34	ST3691		AMP, CFZ, CRO, GEN, TOB, NIT		SHV, CTX-M-8		mrkD, fimH, rmpA, ybtS	K2
Kp35	ST35		AMP, TZP, IMP, MEM, ETP, CFZ, CRO, CTX, ATM, GEN, SXT, NIT	KPC	SHV, CTX-M-2, CTX-M-8, CTX-M-9	DHA	mrkD, fimH, rmpA, ybtS	
Kp36	ST11	HM	AMP, TZP, CFZ, CTM, CAZ, CRO, CTX, FEP, ATM, GEN, AMK, CIP, LVX, NIT		SHV, CTX-M-2, CTX-M-8, CTX-M-9	DHA	mrkD, fimH, ybtS	
Kp37	ST11		AMP, TZP, IMP, MEM, ETP, CFZ, CTM, CAZ, CRO, CTX, FEP, ATM, GEN, AMK, TOB, CIP, LVX, SXT, NIT	KPC, VIM	TEM, SHV, CTX-M-8		mrkD, fimH, rmpA, ybtS	
Kp38	ST11	HM	AMP, TZP, CFZ, CAZ, CRO, CTX, FEP, ATM, GEN, CIP, LVX, NIT		SHV, CTX-M-2, CTX-M-8, CTX-M-9		mrkD, fimH, rmpA, ybtS	
Kp39	ST426	HM	AMP, CFZ, NIT				mrkD, fimH, rmpA	K1
Kp40	ST11	HM	AMP, TZP, CFZ, CTM, CAZ, CRO, CTX, FEP, ATM, GEN, AMK, TOB, CIP, LVX, NIT		SHV, CTX-M-2, CTX-M-8, CTX-M-9	DHA	mrkD, fimH, ybtS	
Kp41	ST11	HM	AMP, TZP, IMP, MEM, ETP, CFZ, CTM, CAZ, CRO, CTX, FEP, ATM, GEN, AMK, TOB, CIP, LVX, NIT	KPC, VIM	SHV, CTX-M-2, CTX-M-8, CTX-M-9	DHA	mrkD	
Kp42	ST722		AMP, GEN, CIP, LVX, SXT, NIT		TEM, SHV		mrkD, ybtS	
Kp43	ST716		AMP, CFZ, CRO, CTX, FEP, GEN, TOB, NIT		SHV, CTX-M-2, CTX-M-8, CTX-M-9		mrkD, fimH, ybtS	
Kp44	ST1887		AMP, TZP, IMP, MEM, ETP, CFZ, CTM, CAZ, CRO, CTX, ATM, GEN, AMK, TOB, CIP	KPC, NDM	SHV, CTX-M-8	DHA	mrkD, fimH, allS	
Kp45	ST11	HM	AMP, TZP, CFZ, CTM, CAZ, CRO, CTX, ATM, GEN, AMK, TOB, CIP, LVX, NIT		SHV, CTX-M-2, CTX-M-8	DHA	mrkD, fimH, ybtS	
Kp46	ST1326		AMP, TZP, IMP, MEM, ETP, CFZ, CAZ, CRO, CTX, FEP, ATM, TOB, CIP, LVX, NIT	KPC, VIM	TEM, SHV, CTX-M-1, CTX-M-2, CTX-M-8		mrkD, fimH, ybtS	
Kp47	ST29		AMP, CFZ, CRO, CTX, SXT, NIT		SHV, CTX-M-2, CTX-M-8, CTX-M-9		mrkD, fimH, ybtS	
Kp48	ST309		AMP, TZP, IMP, MEM, ETP, CFZ, CAZ, CRO, CTX, FEP, ATM, NIT	KPC	SHV, CTX-M-8		mrkD, fimH, entB	
Kp49	ST1407		AMP, CFZ, CAZ, CRO, CTX, FEP, ATM, SXT, NIT		TEM, SHV, CTX-M-1, CTX-M-2, CTX-M-8		mrkD, fimH, entB	
Kp50	ST29		AMP, CFZ, CRO, CTX, SXT, NIT		SHV, CTX-M-2, CTX-M-8, CTX-M-9		mrkD, fimH, ybtS, entB	
Kp51	ST1326		AMP, TZP, IMP, MEM, ETP, CFZ, CAZ, CRO, CTX, FEP, ATM, TOB, CIP, LVX, NIT	KPC, VIM	TEM, SHV, CTX-M-1, CTX-M-2, CTX-M-8		mrkD, fimH, ybtS, entB	
Kp52	ST147		AMP, CFZ, CRO, CTX, FEP, GEN, TOB, CIP, LVX, SXT, NIT		TEM, SHV, CTX-M-1, CTX-M-2, CTX-M-8		mrkD, fimH, entB	
Kp53	ST147		AMP, TZP, CFZ, CAZ, CRO, CTX, FEP, ATM, GEN, CIP, LVX, SXT, NIT		TEM, SHV, CTX-M-1, CTX-M-2, CTX-M-8		mrkD, fimH	
Kp54	ST147		AMP, TZP, CFZ, CAZ, CRO, CTX, FEP, ATM, GEN, TOB, CIP, LVX, SXT, NIT		TEM, SHV, CTX-M-1, CTX-M-2, CTX-M-8		mrkD, fimH, ybtS	

AMP: ampicillin, TZP: piperacillin/tazobactam, MEM: meropenem, IMP: imipenem, ETP: ertapenem, CFZ: cefazolin, CTM: cefotiam, CAZ: ceftazidime, CRO: ceftriaxone, CTX: cefotaxime, FEP: cefepime, ATM: aztreonam, GEN: gentamicin, AMK: amikacin, TOB: tobramycin, CIP: ciprofloxacin, LVX: levofloxacin, SXT: sulfamethoxazole-trimethoprim, NIT: nitrofurantoin, HM: hypermucoviscous phenotype.

Discussion

Klebsiella pneumoniae is considered to be the most clinically relevant pathogenic bacteria belonging to the Enterobacteriaceae family [13]. In recent years, the percentage of *K. pneumoniae* strains resistant to multiple antibiotics is increasing, which is a global public health problem [31]. *K. pneumoniae* infection becomes difficult to treat as a result of the emergence of antibiotic resistance. When a resistant strain is associated with a hvKp strain, it will make the situation worse [31].

In this study, the majority of *K. pneumoniae* are MDR strains, are resistant to a variety of antibiotics, including β-lactams, aminoglycosides, quinolones, and sulfonamides. MDR was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories [32]. *K. pneumoniae* as an invasive and virulent pathogen in Enterobacteriaceae carries several antibiotic resistance genes, including extended spectrum β-lactamases (ESBLs), AmpCs, and carbapenemases [33]. ESBLs-producing Enterobacteriaceae are widely prevalent in China [34]. Previous studies have shown that CTX-M was the predominant genotype in ESBLs [34, 35]. However, our PCR analysis of ESBLs demonstrated that bla_{SHV} (96.3%) was the most common gene. It can be relative to the use of antibiotics and genetic variations in causative strains. In our study, ESBLs had a high frequency and diversity of the ESBLs. Except one *K. pneumoniae*, all of these carried ESBL-encoding genes, and most (94.4%) of isolates co-produced two or more ESBL-encoding genes.

AmpC enzymes are β-lactamases encoded by resident chromosomal genes, but can also be mediated through plasmids in some species of Enterobacteriaceaes [7]. In our findings, we revealed that the genes producing DHA enzymes were more frequent in all the AmpCs-producing isolates. Previous studies have shown that the most common AmpC-encoding gene of *K. pneumoniae* is DHA in China [28].

Carbapenem was introduced into clinical practice in the late 1980s, and was used as the antibiotic of choice for the treatment of multidrug-resistant *K. pneumoniae* infections [36]. However, carbapenemase-producing strains have emerged. The infection caused by carbapenem-resistant Enterobacteriaceae (CRE) is widely distributed in the global community [10]. In our research, most of carbapenemase-producing *K. pneumoniae* strains carried bla_{KPC}, which was consistent with previous investigations [37], indicating that the KPC-producing strains were the dominant carbapenemase producers in various regions of China. Metallo-β-lactamases (MBLs) can hydrolyze almost all clinically available β-lactamases. VIM and NDM enzymes are the type of MBLs with the widest geographical distribution and bacterial host range [38]. The strains carrying bla_{NDM} and bla_{VIM} were five, respectively. In addition, three isolates co-produced bla_{KPC} and bla_{NDM} were found to have high antibiotic resistance and carry a variety of virulence genes. One study has reported that 24 strains of *K. pneumoniae* produced by bla_{KPC} and bla_{NDM} have a high diversity and frequency of resistance and virulence genes, which make the strains resistant to almost all antibiotics [39]. Therefore, strict inspection and management is required.

HvKP is associated with community-acquired invasive infections and is described as a virulent pathogen [12]. In hvKP strains, capsular polysaccharide is an important virulence factor. According to the composition of capsular polysaccharides, 78 capsular serotypes were identified in *K. pneumoniae* [40]. The important capsular serotypes were K1, K2, K5, K20, K54 and K57 [29]. In Asia, the isolates are mainly serotype K1 and K2 [41], which were highly pathogenic [25]. The hypermucoviscous phenotype has been associated with plasmid mediated *rmpA* gene, which encodes transcription regulators and can activate the biosynthesis of capsular polysaccharides [16, 42]. In our report, 20.4% of the isolates carried *rmpA* gene, 5.6% carried K1, 3.7% carried K2, and only one isolate carried K5. However, these were in disagreement with a previous study [43], which found that 64.3% of the isolates were K1 serotype, 19.6% were K2 serotype. The capsular serotypes of hvKP are associated with community-acquired liver abscesses [41], but there were only several samples of liver abscess in this study. The hvKP was usually associated with antibiotic-sensitivity [43], whereas a majority of strains in our research were MDR.

Fimbriae is an important virulence factor of *K. pneumoniae*, facilitating adherence to mucosal surfaces as well as to abiotic surfaces,

which may be a determining factor in the ability of a microorganism to overcome host defense and cause infection [2, 44]. In *K. pneumoniae*, *fimH* and *mrkD* genes encode type 1 and type 3 fimbriae, respectively, which are the primary adhesive structures [31]. Type 1 fimbriae is considered to enhance virulence through their ability to adhere to the mucosal or epithelial surface of the host; type 3 fimbriae also mediates adhesion to the host cell surface, and more importantly, strongly promotes the formation of biofilms [14]. In the current investigation, all strains carried the *mrkD* gene, and plenty of strains (88.9%) contained the *fimH* gene. This result was in agreement with many other research [25, 45] that state that these fimbriae adhesions are ubiquitous in *K. pneumoniae*.

Siderophores are low-molecular weight, high affinity iron chelating molecules that are crucial for the proliferation and virulence of bacteria [14, 46]. Compared with cKP strains, hvKP have the ability to produce iron-acquisition molecules with more quantity and more biological activity [47]. HvKP mainly includes four kinds of iron carriers: enterobactin (*entB*), salmochelin, yersiniabactin (*ybtS*), and aerobactin [30], and *kfu* is a gene encoding iron uptake system. Previous studies have shown that enterobactin systems, yersiniabactin or aerobactin systems may be the key factors to enhance the virulence of *K. pneumoniae* [48]. Yersiniabactin was important for the ability to maintain infection in a mammalian host, which can enhance the virulence of *K. pneumoniae* and also contribute to a more virulent phenotype [48]. Aerobactin was a major virulence factor for the increase of siderophore production in hvKP strains, and may also be a factor in enhancing the virulence of hvKP [49]. In this research, *ybtS* was another prevalent virulence gene, it was identified in 51.9% of the cases, followed by *entB* and *aerobactin* which were found in 9.3% and 3.7%, of all the strains, respectively, while *kfu* was not detected.

MLST results showed that ST11 was the dominating ST type. Three strains carrying bla_{KPC} belonged to ST11, and two of them were hypervirulent strains. ST11 has been proved to be the predominant clone of KPC-producing *K. pneumoniae* in Asia [50, 51]. A recent research study showed that the classic ST11 CRKP strains acquired a pLVPK-like virulence plasmid [52]. These ST11 CR-hvKP strains were demonstrated as being simultaneously hypervirulent, multidrug resistant, and highly transmissible. The prevalence of CR-hvKP infection and carriage of virulence plasmids among CRKP strains increased significantly from 2015 to 2017 in multiple regions of China [53]. This presents significant challenges for public health workers and effective control of infection. In this study, the two strains of CR-hvKP strains were ST35, ST218 while ST1764 was only one strain, respectively. It has been rarely reported that KPC-producing hvKP strains belong to these ST genotypes [54]. At present, *K. pneumoniae* with hypervirulent and carbapenem-resistant isolates is becoming an increasing concern in Chinese hospitals.

This study had some limitations. *K. pneumoniae* isolates were not abundant. The limited cases may preclude further significant results from the comparison of clinical features. Larger collections of isolates would be needed to obtain more meaningful results. In addition, our study was conducted at a single tertiary care teaching hospital and the isolates obtained herein might not be representative due to different patient populations in other hospitals and countries.

Conclusion

In conclusion, *K. pneumoniae* carried a high frequency and diversity of the resistance and virulence factors in this study. The majority of the MDR strains harbored virulence genes. In CPKP strains, bla_{KPC} were found to be the dominant carbapenemase encoding genes. In addition to the potential hospital-based spread of the high-risk ST11 CR-hvKP isolates, attention should also be paid to STs with less common and high degrees of antimicrobial resistance. Also, two new types ST5214 and ST5215 were found in this study. These strains with resistance and virulence factors create a serious threat to immunocompromised patients and even may infect healthy people. Therefore, we should take measures to prevent the spread of multidrug-resistant *K. pneumoniae* carrying virulence genes and strengthen its monitoring.

Acknowledgements

We thank the Institute Pasteur MLST/cgMLST curation team for importing novel alleles and profiles at http://bigsdb.pasteur.fr. This work was supported by “Teaching and Research

Project of Anhui Province (no. 2019jyxm1020)", "Scientific research project of Health Commission of Anhui Province (no. AHWJ2021a013)" and "Research Fund Project of Anhui Medical University (no. 2020xkj216)".

Disclosure of conflict of interest

None.

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References

[1] Caneiras C, Lito L, Melo-Cristino J and Duarte A. Community- and hospital-acquired klebsiella pneumoniae urinary tract infections in portugal: virulence and antibiotic resistance. Microorganisms 2019; 7: 138.

[2] Piperaki ET, Syrogiannopoulos GA, Tzouvelekis LS and Daikos GL. Klebsiella pneumoniae: virulence, biofilm and antimicrobial resistance. Pediatr Infect Dis J 2017; 36: 1002-1005.

[3] Navon-Venezia S, Kondratyeva K and Carattoli A. Klebsiella pneumoniae: a major worldwide source and shuttle for antibiotic resistance. FEMS Microbiol Rev 2017; 41: 252-275.

[4] Kim D, Park BY, Choi MH, Yoon EJ, Lee H, Lee KJ, Park YS, Shin JH, Uh Y, Shin KS, Shin JH, Kim YA and Jeong SH. Antimicrobial resistance and virulence factors of Klebsiella pneumoniae affecting 30 day mortality in patients with bloodstream infection. J Antimicrob Chemother 2019; 74: 190-199.

[5] Peirano G, Sang JH, Pitondo-Silva A, Laupland KB and Pitout JD. Molecular epidemiology of extended-spectrum-beta-lactamase-producing Klebsiella pneumoniae over a 10 year period in Calgary, Canada. J Antimicrob Chemother 2012; 67: 1114-1120.

[6] Wyres KL and Holt KE. Klebsiella pneumoniae as a key trafficker of drug resistance genes from environmental to clinically important bacteria. Curr Opin Microbiol 2018; 45: 131-139.

[7] Meini S, Tascini C, Cei M, Sozio E and Rossolini GM. AmpC beta-lactamase-producing enterobacterales: what a clinician should know. Infection 2019; 47: 363-375.

[8] Iovleva A and Doi Y. Carbapenem-resistant enterobacteriaceae. Clin Lab Med 2017; 37: 303-315.

[9] Aires-de-Sousa M, Ortiz de la Rosa JM, Goncalves ML, Pereira AL, Nordmann P and Poirel L. Epidemiology of carbapenemase-producing klebsiella pneumoniae in a hospital, Portugal. Emerg Infect Dis 2019; 25: 1632-1638.

[10] Lutgring JD. Carbapenem-resistant enterobacteriaceae: an emerging bacterial threat. Semin Diagn Pathol 2019; 36: 182-186.

[11] Shon AS, Bajwa RP and Russo TA. Hypervirulent (hypermucoviscous) Klebsiella pneumoniae: a new and dangerous breed. Virulence 2013; 4: 107-118.

[12] Russo TA and Marr CM. Hypervirulent klebsiella pneumoniae. Clin Microbiol Rev 2019; 32: e00001-00019.

[13] Chen T, Dong G, Zhang S, Zhang X, Zhao Y, Cao J, Zhou T and Wu Q. Effects of iron on the growth, biofilm formation and virulence of Klebsiella pneumoniae causing liver abscess. BMC Microbiol 2020; 20: 36.

[14] Martin RM and Bachman MA. Colonization, infection, and the accessory genome of klebsiella pneumoniae. Front Cell Infect Microbiol 2018; 8: 4.

[15] Zhan L, Wang S, Guo Y, Jin Y, Duan J, Hao Z, Lv J, Qi X, Hu L, Chen L, Kreiswirth BN, Zhang R, Pan J, Wang L and Yu F. Outbreak by hypermucoviscous klebsiella pneumoniae ST11 isolates with carbapenem resistance in a tertiary hospital in China. Front Cell Infect Microbiol 2017; 7: 182.

[16] Harada S and Doi Y. Hypervirulent klebsiella pneumoniae: a call for consensus definition and international collaboration. J Clin Microbiol 2018; 56: e00959-00918.

[17] Lin YT, Cheng YH, Chuang C, Chou SH, Liu WH, Huang CH, Yang TC, Kreiswirth BN and Chen L. Molecular and clinical characterization of multidrug-resistant and hypervirulent klebsiella pneumoniae strains from liver abscess in Taiwan. Antimicrob Agents Chemother 2020; 64: e00174-20.

[18] Lee CR, Lee JH, Park KS, Jeon JH, Kim YB, Cha CJ, Jeong BC and Lee SH. Antimicrobial resistance of hypervirulent klebsiella pneumoniae: epidemiology, hypervirulence-associated determinants, and resistance mechanisms. Front Cell Infect Microbiol 2017; 7: 483.

[19] Cheong HS, Chung DR, Park M, Kim SH, Ko KS, Ha YE, Kang CI, Peck KR and Song JH. Emergence of an extended-spectrum beta-lactamase-producing serotype K1 Klebsiella pneumoniae ST23 strain from Asian countries. Epidemiol Infect 2017; 145: 990-994.

[20] Yao B, Xiao X, Wang F, Zhou L, Zhang X and Zhang J. Clinical and molecular characteristics of multi-clone carbapenem-resistant hypervirulent (hypermucoviscous) Klebsiella pneumoniae isolates in a tertiary hospital in Beijing, China. Int J Infect Dis 2015; 37: 107-112.

[21] Ranjbar R, Fatahian Kelishadrokhi A and Chehelgerdi M. Molecular characterization, serotypes and phenotypic and genotypic evalua-

tion of antibiotic resistance of the Klebsiella pneumoniae strains isolated from different types of hospital-acquired infections. Infect Drug Resist 2019; 12: 603-611.

[22] Dehshiri M, Khoramrooz SS, Zoladl M, Khosravani SA, Parhizgari N, Motazedian MH, Jahedi S and Sharifi A. The frequency of Klebsiella pneumonia encoding genes for CTX-M, TEM-1 and SHV-1 extended-spectrum beta lactamases enzymes isolated from urinary tract infection. Ann Clin Microbiol Antimicrob 2018; 17: 4.

[23] Dallenne C, Da Costa A, Decré D, Favier C and 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: 490-495.

[24] Briñas L, Moreno MA, Zarazaga M, Porrero C, Sáenz Y, García M, Dominguez L and Torres C. Detection of CMY-2, CTX-M-14, and SHV-12 β-lactamases in escherichia coli fecal-sample isolates from healthy chickens. Antimicrob Agents Chemother 2003; 47: 2056-2058.

[25] Ferreira RL, da Silva BCM, Rezende GS, Nakamura-Silva R, Pitondo-Silva A, Campanini EB, Brito MCA, da Silva EML, Freire CCM, da Cunha AF and Pranchevicius MDS. High prevalence of multidrug-resistant klebsiella pneumoniae harboring several virulence and β-lactamase encoding genes in a brazilian intensive care unit. Front Microbiol 2019; 9: 3198.

[26] Zhang Z, Zhai Y, Guo Y, Li D, Wang Z, Wang J, Chen Y, Wang Q and Gao Z. Characterization of unexpressed extended-spectrum beta-lactamase genes in antibiotic-sensitive klebsiella pneumoniae isolates. Microb Drug Resist 2018; 24: 799-806.

[27] Poirel L, Walsh TR, Cuvillier V and Nordmann P. Multiplex PCR for detection of acquired carbapenemase genes. Diagn Microbiol Infect Dis 2011; 70: 119-123.

[28] Liu Y, Yang Y, Chen Y and Xia Z. Antimicrobial resistance profiles and genotypes of extended-spectrum beta-lactamase- and AmpC beta-lactamase-producing Klebsiella pneumoniae isolated from dogs in Beijing, China. J Glob Antimicrob Resist 2017; 10: 219-222.

[29] Yan Q, Zhou M, Zou M and Liu WE. Hypervirulent Klebsiella pneumoniae induced ventilator-associated pneumonia in mechanically ventilated patients in China. Eur J Clin Microbiol Infect Dis 2016; 35: 387-396.

[30] Fu L, Huang M, Zhang X, Yang X, Liu Y, Zhang L, Zhang Z, Wang G and Zhou Y. Frequency of virulence factors in high biofilm formation blaKPC-2 producing Klebsiella pneumoniae strains from hospitals. Microb Pathog 2018; 116: 168-172.

[31] Paczosa MK and Mecsas J. Klebsiella pneumoniae: going on the offense with a strong defense. Microbiol Mol Biol Rev 2016; 80: 629-661.

[32] Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT and Monnet DL. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 2012; 18: 268-281.

[33] Mbelle NM, Feldman C, Sekyere JO, Maningi NE, Modipane L and Essack SY. Pathogenomics and evolutionary epidemiology of multidrug resistant clinical klebsiella pneumoniae isolated from Pretoria, South Africa. Sci Rep 2020; 10: 1232.

[34] Quan J, Zhao D, Liu L, Chen Y, Zhou J, Jiang Y, Du X, Zhou Z, Akova M and Yu Y. High prevalence of ESBL-producing Escherichia coli and Klebsiella pneumoniae in community-onset bloodstream infections in China. J Antimicrob Chemother 2017; 72: 273-280.

[35] Zhang J, Zhou K, Zheng B, Zhao L, Shen P, Ji J, Wei Z, Li L, Zhou J and Xiao Y. High prevalence of ESBL-producing klebsiella pneumoniae causing community-onset infections in China. Front Microbiol 2016; 7: 1830.

[36] Wang X, Wang Y, Zhou Y, Li J, Yin W, Wang S, Zhang S, Shen J, Shen Z and Wang Y. Emergence of a novel mobile colistin resistance gene, mcr-8, in NDM-producing Klebsiella pneumoniae. Emerg Microbes Infect 2018; 7: 122.

[37] Zhang Y, Wang Q, Yin Y, Chen H, Jin L, Gu B, Xie L, Yang C, Ma X, Li H, Li W, Zhang X, Liao K, Man S, Wang S, Wen H, Li B, Guo Z, Tian J, Pei F, Liu L, Zhang L, Zou C, Hu T, Cai J, Yang H, Huang J, Jia X, Huang W, Cao B and Wang H. Epidemiology of carbapenem-resistant enterobacteriaceae infections: report from the China CRE network. Antimicrob Agents Chemother 2018; 62: e01882-01817.

[38] Khan AU, Maryam L and Zarrilli R. Structure, genetics and worldwide spread of new Delhi metallo-beta-lactamase (NDM): a threat to public health. BMC Microbiol 2017; 17: 101.

[39] Liu X, Zhang J, Li Y, Shen Q, Jiang W, Zhao K, He Y, Dai P, Nie Z, Xu X and Zhou Y. Diversity and frequency of resistance and virulence genes in bla KPC and bla NDM co-producing Klebsiella pneumoniae strains from China. Infect Drug Resist 2019; 12: 2819-2826.

[40] Pan YJ, Fang HC, Yang HC, Lin TL, Hsieh PF, Tsai FC, Keynan Y and Wang JT. Capsular polysaccharide synthesis regions in klebsiella pneumoniae Serotype K57 and a new capsular

serotype. J Clin Microbiol 2008; 46: 2231-2240.
[41] Bilal S, Volz MS, Fiedler T, Podschun R and Schneider T. Klebsiella pneumoniae-induced liver abscesses, Germany. Emerg Infect Dis 2014; 20: 1939-1940.
[42] Arena F, Henrici De Angelis L, D'Andrea MM, Cannatelli A, Fossati L, Di Pilato V, Giani T, Cavallo R and Rossolini GM. Infections caused by carbapenem-resistant Klebsiella pneumoniae with hypermucoviscous phenotype: a case report and literature review. Virulence 2017; 8: 1900-1908.
[43] Shi Q, Lan P, Huang D, Hua X, Jiang Y, Zhou J and Yu Y. Diversity of virulence level phenotype of hypervirulent Klebsiella pneumoniae from different sequence type lineage. BMC Microbiol 2018; 18: 94.
[44] Highsmith AK and Jarvis WR. Klebsiella pneumoniae: selected virulence factors that contribute to pathogenicity. Infect Control 1985; 6: 75-77.
[45] Ranjbar R, Memariani H, Sorouri R and Memariani M. Distribution of virulence genes and genotyping of CTX-M-15-producing Klebsiella pneumoniae isolated from patients with community-acquired urinary tract infection (CA-UTI). Microb Pathog 2016; 100: 244-249.
[46] Holden VI and Bachman MA. Diverging roles of bacterial siderophores during infection. Metallomics 2015; 7: 986-995.
[47] Russo TA, Shon AS, Beanan JM, Olson R, MacDonald U, Pomakov AO and Visitacion MP. Hypervirulent K. pneumoniae secretes more and more active iron-acquisition molecules than "classical" K. pneumoniae thereby enhancing its virulence. PLoS One 2011; 6: e26734.
[48] Lawlor MS, O'Connor C and Miller VL. Yersiniabactin is a virulence factor for Klebsiella pneumoniae during pulmonary infection. Infect Immun 2007; 75: 1463-1472.
[49] Russo TA, Olson R, Macdonald U, Metzger D, Maltese LM, Drake EJ and Gulick AM. Aerobactin mediates virulence and accounts for increased siderophore production under iron-limiting conditions by hypervirulent (hypermucoviscous) Klebsiella pneumoniae. Infect Immun 2014; 82: 2356-2367.
[50] Qi Y, Wei Z, Ji S, Du X, Shen P and Yu Y. ST11, the dominant clone of KPC-producing Klebsiella pneumoniae in China. J Antimicrob Chemother 2011; 66: 307-312.
[51] Zhao Y, Zhang S, Fang R, Wu Q, Li J, Zhang Y, Rocker A, Cao J, Lithgow T and Zhou T. Dynamic epidemiology and virulence characteristics of carbapenem-resistant klebsiella pneumoniae in Wenzhou, China from 2003 to 2016. Infect Drug Resist 2020; 13: 931-940.
[52] Gu D, Dong N, Zheng Z, Lin D, Huang M, Wang L, Chan EW, Shu L, Yu J, Zhang R and Chen S. A fatal outbreak of ST11 carbapenem-resistant hypervirulent Klebsiella pneumoniae in a Chinese hospital: a molecular epidemiological study. Lancet Infect Dis 2018; 18: 37-46.
[53] Zhang Y, Jin L, Ouyang P, Wang Q, Wang R, Wang J, Gao H, Wang X and Wang H; China Carbapenem-Resistant Enterobacteriaceae (CRE) Network. Evolution of hypervirulence in carbapenem-resistant Klebsiella pneumoniae in China: a multicentre, molecular epidemiological analysis. J Antimicrob Chemother 2020; 75: 327-336.
[54] Lin ZW, Zheng JX, Bai B, Xu GJ, Lin FJ, Chen Z, Sun X, Qu D, Yu ZJ and Deng QW. Characteristics of hypervirulent klebsiella pneumoniae: does low expression of rmpA contribute to the absence of hypervirulence? Front Microbiol 2020; 11: 436.