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Original Article

Characteristics of non-carbapenemase producing carbapenem-resistant *Klebsiella pneumoniae* from a tertiary hospital in China

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Abstract

Introduction: The spread of carbapenem-resistant *Klebsiella pneumoniae* (CRKP) is a substantial severe global public health burden. Non-carbapenemase-producing CRKP (non-CP-CRKP) is increasingly recognized as the source of severe infections.

Methodology: We analyzed the genotypic, and phenotypic profiles of non-CP-CRKP strains with the whole-genome sequences isolated between 2017 and 2019 and the clinical characterization of non-CP-CRKP infection.

Results: A total of 91 CRKP strains were collected, of which 5 (5.49%) strains were non-CP-CRKP. Four strains were from male patients; three strains were isolated from the bile of patients who underwent biliary interventional surgery and four had a history of antibiotic exposure. Three strains were sequence type (ST)11, one was ST1, and one was ST5523. The non-CP-CRKP strains were insusceptible to ertapenem. Three strains were susceptible to amikacin. All the strains were susceptible to imipenem, meropenem, tigecycline, ceftazidime/avibatam and polymyxin B. The β-lactamases of non-CP-CRKP predominantly included *blaCTX-M*, *blaSHV*, and *blaTEM* subtypes. Two site mutations in *ompK36* (p.A217S and p.N218H) and four in *ompK37* (p.I70M, p.I128M, p.N230G, and m233_None234insQ) were detected accounting for carbapenem resistance. Plasmids IncFI and IncFII were found in most strains. Genes encoding aerobactin, yersiniabactin and allantoin utilization were not detected in several isolates, and all non-CP-CRKP strains did not carry *rmpA* gene.

Conclusions: Non-CP-CRKP infected patients had a history of previous antibiotic exposure or invasive procedures. Non-CP-CRKP strains were insusceptible to ertapenem. The mechanism of resistance includes β-lactamases production and the site mutations in *ompK36* and *ompK37*. Several virulence genes were not detected in non-CP-CRKP.

Key words: *Klebsiella pneumoniae*, ertapenem, resistance, virulence, epidemiology.

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Introduction

Carbapenem-resistant *Enterobacteriaceae* (CRE) infections are a serious healthcare issue and have been on the global priority list of the World Health Organization (WHO) for research and development of effective drugs [1]. CRE infection is highly endemic in China, with an annual incidence rate of 4.0 per 10,000 patients in 2015 calculated through a multicenter study that covered 25 tertiary hospitals in 14 provinces [2]. Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) causes more than 70% of these CRE infections [2]. According to the 2020 national surveillance in China, the resistance rate of *Klebsiella pneumoniae* to imipenem and meropenem was 21.5% and 22.4% [3]. The production of carbapenemase enzymes, including *Klebsiella pneumoniae* carbapenemase (KPC), metallo-β-lactamases (MBLs), Guiana extended-spectrum (GES) β-lactamase and OXA-like enzymes, is the primary mechanism underlying carbapenem resistance [4]. Other mechanisms of carbapenem resistance include overexpression of efflux pumps, mutation or downregulation of porins, and target modification and overproduction of extended-spectrum β-lactamases (ESBLs) and AmpC β-lactamases (AmpC) [4].

Non-carbapenemase-producing *Enterobacteriaceae* (NCPE) are the predominant CRE strains in some area [5]. In the CRACKLE-2 study, NCPE accounted for 19% of CRE infections and 30-day outcomes were similar between patients with CRE and NCPE infections [6]. Ertapenem is the most likely carbapenem antibiotic to be ineffective because of carbapenem non-susceptibility mechanisms. Ertapenem-resistant NCPE, which are susceptible to imipenem and meropenem, have a wider susceptibility profile than other CRE [7]. Higher mortality rates are associated with ertapenem-resistant *Enterobacteriaceae* infection than with ertapenem-sensitive *Enterobacteriaceae* infection [8].

Hypervirulent strains of *K. pneumoniae* (hvKp) have been prevalent for the past 30 years and some

hypervirulence associated genes have been identified including *rmpA*, *rmpA2*, *iroBCDN*, *iutA*, *iucABCD* and *ybt* [9,10]. ST11 hypervirulent carbapenem-resistant *K. pneumoniae* (hv-CRKP) strains were reported in China which may cause severe infections in healthy individuals and were highly resistant to antibiotics [11]. Subsequently, an increasing number of ST11 hv-CRKP strains have been discovered in different Chinese provinces [12]. The hv-CRKP evolved when drug-resistant strains acquired virulence genes or high-virulence strains acquired drug-resistant genes [13]. The analysis of the genomic sequence of *K. pneumoniae*, especially drug resistance and virulence related genes, have progressed due to the development of sequencing technology [14]. However, to our knowledge, there is lack of analysis of non-CP-CRKP genome structure, especially the virulence gene.

The aim of the present study was to obtain the comprehensive characteristic of non-CP-CRKP, by both collecting clinical data of patients and performing genome sequencing of non-CP-CRKP strains isolated from a tertiary care hospital in China from 2017 to 2019 to investigate their clinical history, antibiotics resistance profile, and molecular characteristics of resistant genes and virulence genes.

Methodology

Data collection

A retrospective epidemiologic surveillance study of carbapenem non-susceptible *K. pneumoniae* infection was conducted in our hospital from July 2017 to December 2019. A total of 91 CRKP were collected, and among them the 5 cases (5.49%) that were without carbapenemase production were included in this study. The clinical and epidemiologic data were collected by reviewing the medical records of 5 patients, including patient demographics, underlying medical conditions, location in the hospital, healthcare and antimicrobial therapy exposures during the prior year, clinical manifestations, specimen source, sample date, indwelling devices, treatment, and outcomes. The study was approved by the Institutional Review Board (No. 002).

Bacterial isolates and microbiological methods

Isolates identification and antibiotic susceptibility testing were carried out using an automated VITEK-2 compact system (Merieux, Lyon, France). The broth microdilution method was used to further confirm the susceptibility of CRKP strains to cefuroxime, piperacillin/tazobactam, ceftazidime/avibatam, aztreonam, amikacin, levofloxacin, meropenem, polymyxin B, and tigecycline. The susceptibility to ertapenem and imipenem were determined by the epsilometer test (E-test). The susceptibility interpretations are based on Clinical and Laboratory Standards Institute (CLSI) clinical breakpoints [15]. Carbapenem non-susceptible *K. pneumoniae* was defined according to the recommendation of CLSI as *K. pneumoniae* strains are non-susceptible to at least one carbapenem agent (ertapenem, imipenem, or meropenem) [16]. We defined carbapenem insusceptibility as minimum inhibitory concentration (MIC) > 1 μg/mL for meropenem or imipenem or MIC > 0.5 μg/mL for ertapenem. The *E. coli* American Type Culture Collection (ATCC) 25922 (negative control) and *K. pneumoniae* ATCC 700603 [positive extended spectrum ß-lactamase (ESBL) control] were used as quality control strains. Data were only included when the quality control test results were in acceptable ranges.

DNA preparation, genome sequencing, and annotation

The genomic DNA of the strain of carbapenem non-susceptible *K. pneumoniae* without carbapenemase production was extracted using the bacterial genomic DNA extraction kit (Tiangen Biotech, Beijing, China). The sequencing of the strain was performed by Hisep2000 (llumina, San Diego, USA) and assembled with Unicycler v 0.5.0 [17]. Gene prediction for strains were conducted using the RAST server [18]. Multilocus sequence typing (MLST) was typed by MLST 2.0 provided with the Center for Genomic Epidemiology (CGE) [19]. The acquired antimicrobial resistance genes and chromosomal mutations mediating antimicrobial resistance were investigated by using ResFinder 4.1 from the website of the CGE (updated version: ResFinder software: 2020-10-21; ResFinder database: 2020-12-01; PointFinder software: 2020-10-21; PointFinder database: 2019-07-02) [20]. The plasmids were identified by PlasmidFinder 2.1 from CGE [updated version: Software version: 2.0.1 (2020-07-01); Database version: 2020-07-13] [21]. Putative virulence factors were predicted by the Virulence Factors Database (VFDB) [22]. The whole-genome single nucleotide polymorphism (SNP) tree was constructed using CSI Phylogeny [23] and labeled using iTOL software [24].

Genomes from sequence database

Genomic sequences available for *K. pneumoniae* were downloaded from the National Center for Biotechnology Information (NCBI) genome sequence repository [25]. The downloaded sequences comprised

11 whole-genome shotgun sequences available as scaffolds or contigs isolating from Zhejiang and Shanghai [NZ_JGYH01000001.1 (5422), NZ_LYNH01000039.1 (SKLX2467), NZ_LYWT01000001.1 (SKLX2848), NZ_NLFG01000001.1 (L86), NZ_NLFH01000001.1 (L9), NZ_PCFT01000063.1 (XPY193), NC_016845.1 (HS11286), NC_012731.1 (NTUH-K2044), NZ_MNLG01000049.1 (KP6), NZ_JNGV01000019.1 (NUFHKp), NZ_CP047160.1 (KP19-2029)].

Results

General clinical characteristics

Ninety-one CRKP strains were collected from July 2017 to December 2019, of which five strains (5.49%) were non-CP-CRKP. We identified three strains as sequence type (ST) 11, one as ST1, and one as ST5523. Four strains were isolated from male patients. Three strains were isolated from the bile of patients who underwent endoscopic retrograde cholangiopancreatography and stone removal procedures. The other two strains were isolated from the urine of patients who had a history of indwelling urethral catheterization. Four of the five patients had a history of pre-exposure to antibiotics (Table 1).

Antibiotic susceptibility

All strains that were isolated were resistant to $2^{nd}/3^{rd}/4^{th}$ generation cephalosporins, cefoxitin, amoxicillin/clavulanic acid, quinolones and ertapenem. The 19_469 and 19_839 strains were resistant to amikacin. All strains were sensitive to imipenem, meropenem, tigecycline, ceftazidime/avibactam and polymyxin B (Table 2).

Distribution of resistance genes

The main β-lactamases subtype genes expressed by the five strains included *blaCTX-M*, *blaSHV*, and *blaTEM* (Table 2). Four strains carried the tigecycline resistance gene *tet (A)*. The 18_674 strain carried the *acrR* gene which contained two tigecycline-resistant mutation sites, namely, p.M123* and p.E122K (Table 2). All strains carried the *fosA* gene.

Table 1. Demographic and clinical characteristics of the patients.

Items	Case 1	Case 2	Case 3	Case 4	Case 5
Isolate	18_674	19_340	19_469	19_832	19_839
Gender	F	M	M	M	M
Age (years)	83	67	59	77	71
Diagnosis	Choledocholithiasis Acute pancreatitis	Choledocholithiasis	Urinary tract infection Brown-Sequard syndrome	Residual stones after biliary surgery	Urinary tract infection Cerebral hemorrhage
Specimen	Bile	Bile	Urine	Bile	Urine
Previous hospitalization (within 1 year)	3	1	3	0	0
Hospital stay before isolated (days)	3	0	30	3	47
ICU admission	Yes	No	Yes	No	Yes
Use of systemic steroids	-	-	-	Methylprednisolone	-
Underlying disease	-	Hypertension	Thrombocytopenia	Diabetes mellitus, COPD	-
Cathetering	Nasogastric catheter, Urinary catheter, CVC, Tracheal cannula	Nasogastric catheter	Nasogastric catheter, Urinary catheter, CVC, Tracheal cannula	Nasogastric catheter, Non-invasive ventilation	Nasogastric catheter, Urinary catheter, CVC, Tracheal cannula
Gastroscopy	ERCP Otis sphincterotomy Biliary stent	ERCP	-	ERCP Otis sphincterotomy Biliary stent	-
Previous surgery	Cholecystectomy Left lateral hepatic lobectomy, Laparotomy for intestinal adhesions	-	Anterior cervical decompression and bone graft fusion and internal fixation Anterior cervical exploratory hematoma removal	Puncture drainage of subphrenic abscess	Right hematoma removal
Previous use of antibiotics					
Quinolones	-	-	Levofloxacin	-	-
$3^{rd}/4^{th}$ generation cephalosporins	Ceftriaxone Cefodizime Cefdinir	-	-	Ceftazidime	-
$1^{st}/2^{nd}$ generation cephalosporins	Cefotiam	-	-	Cefotiam	Cefuroxime
Oxacephem	-	Latamoxef	-	Latamoxef	-
β-lactamase inhibitor	Piperacillin/Tazobactam	-	-	-	-

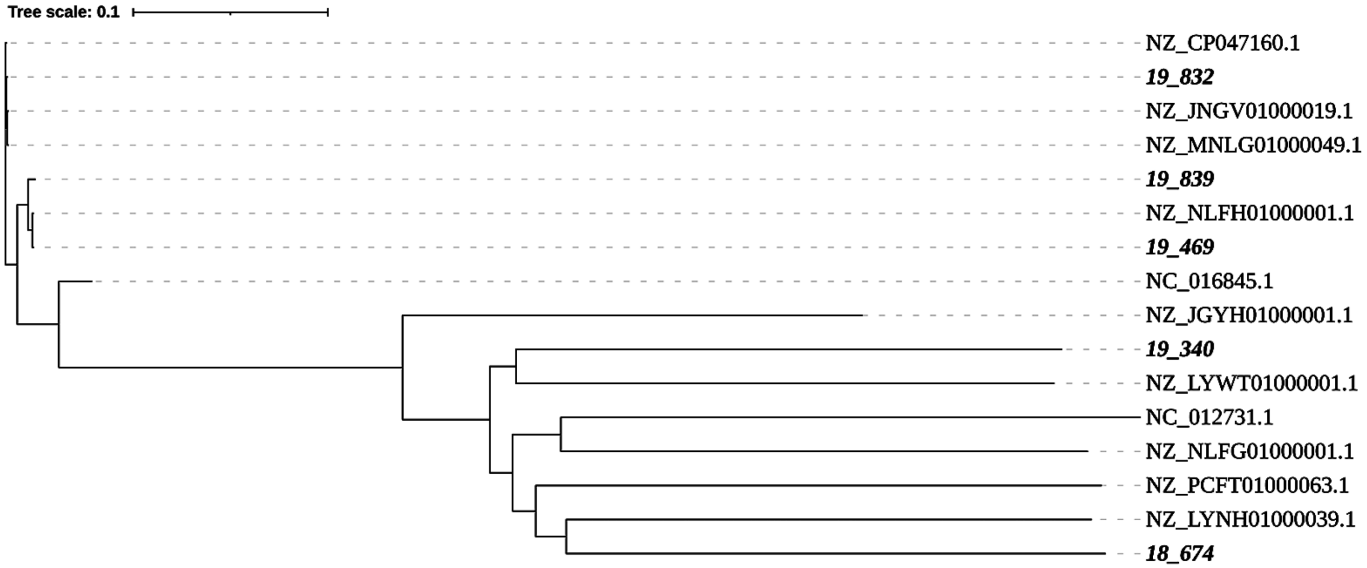
F: female; M: male; ICU: intensive care unit; COPD: chronic obstructive pulmonary disease; CVC: central venous catheter; ERCP: endoscopic retrograde cholangiopancreatography.

Table 2. The K-types, ST types, resistant genotypes, plasmid types and resistant phenotypes of the 5 *Klebsiella pneumoniae* isolates.

Strains	K types	STs	Presence of β-lactamase genes	Chromosomal mutations	Other resistant genes	Plasmid types	CXM	CAZ	CRO	FEP	AMC	TZP	CZA	ATM	FOX	AK	LEV	SXT	ETP	IMP	MEM	TGC
18_674	19	1	CTX-M-55, TEM	ompK36: p.A217S, p.N218H ompK37: p.I70M, p.I128M, p.N230G, m233_None234insQ	Mutations of Tigecycline resistance: p.M123*, p.E122K Fosfomycin: fosA	ColRNAI, IncFIB, IncFII	≥ 64	≥ 64	≥ 64	≥ 64	16	128/4	0.25/4	≥ 32	≥ 64	8	≥ 8	160	1	0.125	0.25	0.5
19_340	28	5523	CTX-M-3, SHV, TEM-1B	ompK36: p.A217S ompK37: p.I70M, p.I128M	Tigecycline: tet (A) Fosfomycin: fosA	ColpVC, IncFII, IncQ	≥ 64	≥ 64	≥ 64	≥ 64	≥ 32	32/4	1/4	≥ 32	≥ 64	≤ 2	≥ 8	≥ 320	4	0.5	0.5	2
19_469	64	11	CTX-M-65, LAP-2, SHV, TEM-1B	ompK37: p.I70M, p.N230G, p.I128M, p.N230G, m233_None234insQ	Tigecycline: tet (A) Fosfomycin: fosA	IncFII, IncHI1B, IncR, repB	≥ 64	≥ 64	≥ 64	≥ 32	≥ 32	≥ 256/4	2/4	≥ 32	≥ 64	≥ 128	≥ 8	≥ 320	2	0.125	0.5	4
19_832	47	11	SHV, TEM-1B	ompK37: p.I70M, p.I128M, p.N230G, m233_None234insQ	Tigecycline: tet (A) Fosfomycin: fosA	IncFIA, IncFII	≥ 64	≥ 64	≥ 64	≥ 32	≥ 32	≥ 256/4	8/4	16	≥ 64	≤ 1	≥ 8	≤ 20	2	0.25	1	0.5
19_839	21	11	CTX-M-65, LAP-2, SHV, TEM-1B	ompK37: p.I70M, p.I128M, p.N230G, m233_None234insQ	Tigecycline: tet (A) Fosfomycin: fosA	IncFIB, IncFII, IncR, repB	≥ 64	≥ 64	≥ 64	≥ 64	≥ 32	32/4	4/4	≥ 32	≥ 64	≥ 128	≥ 8	≥ 320	1	0.25	0.25	2

CXM: cefuroxime; CAZ: ceftazidime; CRO: ceftriaxone; FEP: cefepime; AMC: amoxicillin/clavulanic acid; TZP: piperacillin/tazobactam; CZA: ceftazidime/avibatam; ATM: aztreonam; FOX: cefoxitin; AK: amikacin; LEV: Levofloxacin; SXT: sulfamethoxazole; ETP: ertapenem; IMP: imipenem; MEM: meropenem; TGC: tigecycline; PB: polymyxin B.

Figure 1. Diverse genetic lineages of *Klebsiella pneumoniae*.



The network was constructed by using the CSI Phylogeny 1.4 [23]. The reference *K. pneumoniae* sequence is HS11286 (NC_016845.1).

Carbapenem-resistant antibiotic mutations

Collectively, six mutations associated with carbapenem resistance were detected. The p.A217S and p.N218H mutations were identified in the *ompK36* gene, whereas the p.I70M, p.I128M, p.N230G, and m233_None234insQ mutations were identified in the *ompK37* gene (Table 2). P.I70M and p.I128M mutations were detected in all five strains, and p.N230G and m233_None234insQ mutations were detected in four strains (Table 2). No mutations were observed in the *ompK35* gene. The *ompK36* deletion mutation was detected in the strains 19_469, 19_832, and 19_839 (Table 2).

Plasmids in non-CP-CRKP

The plasmids were mainly subtypes of IncFI and IncFII. IncFII (pHN7A8) was detected in four strains (Table 2).

Virulence genes of non-CP-CRKP

All strains contained genes encoding type I and III pili, and all strains, except 18_674, expressed the IV flagellum gene. The efflux pump gene (*acrAB*), iron uptake genes (*Ent siderophore*, *salmochelin*), and secretion system genes (*T6SS-I*, *T6SS-II*, and *T6SS-III*) were detected in all strains (Table 3). The strains 18_674 and 19_340 did not carry aerobactin-related genes, and 19_340 did not carry yersiniabactin-related genes. The allantoin utilization-related gene *allABCERS* was only detected in strain 18_674. All strains expressed the regulatory gene *rcsAB*, whereas none expressed the *rmpA* genes (Table 3).

Phylogenetic network by SNPs

Phylogenetic network of the 16 *K. pneumoniae* genomes as determined on the basis of the concatenated alignment of the high quality SNPs is presented in Figure 1. A total of 11 sequences including the reference *K. pneumoniae* sequence (NC_016845.1) were taken from GenBank.

Discussion

Carbapenems possess the broadest spectrum of antibacterial activity and are the "last resort" antibiotics used to treat multidrug-resistant bacterial infection [26]. The prevalence of carbapenem-resistant organisms (CROs) is increasing and has become a public health concern because of limitations to antibiotic therapy [27]. The high prevalence of non-CP-CRKP strains in the Asia-Pacific region needs to be addressed [28]. Researchers isolated 41 CRKP strains in Texas between 2011 and 2019, of which 39% were non-CP-CRKP [5]. Three major mechanisms of carbapenem resistance in non-CP-CRKP are the overexpression of ESBLs or AmpC β-lactamase, resistance-nodulation-division efflux pumps, and decreased membrane permeability due to porin loss [4]. In our study, we isolated five non-CP-CRKP strains that were not susceptible to ertapenem, but were susceptible to imipenem and meropenem. Ertapenem is most likely hydrolyzed by β-lactamases in CRO isolates [7]. Insusceptibility to ertapenem is a sensitive initial screening tool for potential CRO isolates [29].

A retrospective study showed that intensive care unit stay, exposure to any antibiotic over 30 days, and prior central venous catheterization or mechanical ventilation were risk factors for ertapenem-resistant *Enterobacteriaceae* infection [30]. It was found that previous hospitalization and quinolone exposure were also risk factors for ertapenem resistance [8]. All five patients in this study had experienced the risk factors described in the previous studies [8,30]. Increased

Table 3. Virulence genes of *Klebsiella pneumoniae.*

Virulence factors	18_674	19_340	19_469	19_832	19_839
Type I fimbriae	*fim*	*fim*	*fim*	*fim*	*fim*
Type III fimbriae	*mrk*	*mrk*	*mrk*	*mrk*	*mrk*
Type IV pili	-	*pilW*	*pilU*	*pilW*	*pilU*
Efflux pump	*acrAB*	*acrAB*	*acrAB*	*acrAB*	*acrAB*
Aerobactin	-	-	*IucABCD, iutA*	*iutA*	*IucABCD, iutA*
Ent siderophore	*ent, fep, fes*	*ent, fep, fes*	*ent, fep, fes*	*ent, fep, fes*	*ent, fep, fes*
Salmochelin	*iroEN*	*iroEN*	*iroEN*	*iroEN*	*iroEN*
Yersiniabactin	*fyuA, irp1/2, ybt*	-	*fyuA, irp1/2, ybt*	*fyuA, irp1/2, ybt*	*fyuA, irp1, ybt*
Allantoin utilization	*all*	-	-	-	-
Regulation	*rcsAB*	*rcsAB*	*rcsAB*	*rcsAB*	*rcsAB*
T6SS-I	*tss , ompA, tle1*	*tss , ompA, tli1*	*tss , ompA, tli1*	*tss , ompA, tli1*	*tss , ompA, tli1*
T6SS-II	*clpV*	*clpV*	*clpV*	*clpV*	*clpV*
T6SS-III	*dotU, icmF, impAFGHJ, lysM, ompA, sciN, vgrG*	*dotU, impAFGHJ, lysM, ompA, sciN*	*dotU, impAFGHJ, ompA, sciN*	*dotU, impAFGHJ, ompA, sciN*	*dotU, impAFGHJ, ompA, sciN*

ertapenem exposure can lead to increased ertapenem resistance [31]. An in vitro study suggested that ertapenem exposure can lead to ertapenem resistance in ESBL-producing *E. coli* strains and that the combination of ESBL production and porin loss may cause ertapenem resistance [32].

Researchers analyzed 404 cases of ertapenem-insensitive non-carbapenemase-producing strains from the study for monitoring antimicrobial resistance trends (SMART) surveillance, including *E. coli* (n = 83), *K. pneumoniae* (n = 91), and *Enterobacter* species (n = 210). This study showed that the majority (> 84%) of these strains were sensitive to imipenem and amikacin and that the strains isolated from the hepatobiliary system displayed lower cefepime MICs than those isolated from the peritoneal space [33]. In our study, three of the five strains were sensitive to amikacin, and all strains were sensitive to imipenem, meropenem, tigecycline, ceftazidime/avibactam, and polymyxin, and resistant to cefepime. However, cefepime has not been approved for the treatment of non-CP-CRKP infections in our district. A multicenter, large-scale prospective study is needed to better understand the non-CP-CRKP burden and the mechanisms involved in drug resistance in these strains in mainland China.

The *blaCTX-M* and *blaSHV* genes were shown to be crucial for ESBL production in ertapenem-insensitive non-CP-CRKP in previous studies [7,34]. In contrast to previous studies, no *ampC* was detected in our study [7]. The spread of the β-lactamase gene is associated with the presence of the antibiotic resistance-associated plasmids IncFI, IncFII, and IncR in epidemic clones [35]. Similar to a previous study, our study demonstrated that the outer membrane proteins ompK36 and ompK37 had multiple carbapenem-resistant mutation sites, which was also one of the important drug resistance mechanisms [5].

The *aac(6')-Ib-cr* gene is one of the major determinants for plasmid-mediated quinolone resistance and was found in 89% of *K. pneumoniae* strains in a previous study [36]. Therefore, Muggeo *et al*. suggested that fluoroquinolones should not be used as alternative antibiotics for the treatment of ertapenem non-susceptible *K. pneumoniae* [36]. It has been suggested that the *qnrS* and *aac(6')-Ib-cr* genes are responsible for the underlying quinolone resistance in carbapenem-insensitive *K. pneumoniae* and *E. coli* [37]. The presence of the *qnrS1* and *aac(6')-Ib-cr* in our strains indicated that quinolone was not the appropriate choice for therapy.

The susceptibility rate of aminoglycosides, especially amikacin, to CRKP was previously high and considered in the combination therapy of CRKP [38]. According to the CHINET surveillance conducted in 2019, the susceptibility rate of *K. pneumoniae* to amikacin was 82% [39]. The presence of the mutant genes *aac(6')-Ib*, *aac(3)-II*, and *aph(3')-IIIa* is a major mechanism of aminoglycoside resistance in *K. pneumoniae* [40]. In our study, two strains expressed the *aac(6')-Ib*, *aac(3)-II* and *aph(3')-IIIa* genes and were still susceptible to amikacin. Two strains with high MIC to amikacin had the *aadA2b* mutant gene. This suggests that this gene may play a major role in drug resistance.

Tigecycline is recommended by guidelines for the treatment of CRKP [41]. Infection caused by tigecycline-insensitive *K. pneumoniae* leads to a high rate of mortality at 14 and 28 days post infection [41]. The 2019 CHINET surveillance report showed that tigecycline sensitivity of *K. pneumoniae* was 86.5% [39]. We detected the expression of the *tet(A)* gene in four strains and two *acrR* gene mutation sites in the 18_674 strain. These genetic factors are associated with tigecycline resistance. Despite this result, all five strains were sensitive to tigecycline. The reason for tigecycline sensitivity in these strains is unclear, as tigecycline exposure can increase the resistance rate of susceptible strains [42]. Overexpression of *acrAB* and/or *oqxAB* genes, together with the upregulation of the regulators *ramA* and/or *rarA*, can lead to tigecycline resistance [41]. Inhibition of *ramR* translation can also result in tigecycline resistance [42].

Recently, hvKP infections, especially carbapenem-resistant infections, have attracted considerable attention [11,43,44]. hvKP is a hypermucoviscous strain that lacks a genetic profile description [45]. Factors that are associated with virulence in *K. pneumoniae* include capsular lipopolysaccharides, siderophores, and pili. Genes involved in allantoin utilization, iron transport systems, efflux pumps, and a type VI secretion system have been identified as new virulence factors in *K. pneumoniae* [46]. It has been shown that the hypermucoviscosity of *K. pneumoniae* is associated with the presence of K1, K2, and *rmpA* genes and that hypervirulent *K. pneumoniae* strains cause invasive infections, including liver abscess, bloodstream infection, and sepsis [47]. It was demonstrated that *peg-344*, *iroB*, *iucA*, plasmid-borne *rmpA*, and *rmpA2* genes were associated with high virulence in *K. pneumoniae* [48]. *IucA* is a gene associated with hypervirulence and is highly prevalent in virulent CRKP [49]. *IucA* of the aerobactin pathway is a siderophore synthetase that acquires iron in iron-depleted environments such as in a human host [50].

We identified aerobactin gene deletions in strains 18_674, 19_340, and 19_832, but it is not clear whether these mutations would cause reduced virulence. We detected only the allantoin utilization gene (*allS*) in the 18_674 strain. The *allS* gene enhances the allantoin-utilizing capability of bacteria to compete for nitrogen sources [51]. An animal study in BALB/c mice demonstrated that *K. pneumoniae* liver isolate, which had an *allS* deletion mutation, showed a significant decrease in virulence in intragastric infection [51]. The prevalence of virulence factors varied in the isolates found in China and the United States with aerobactin present in 62% of CRKP in China and 1% in the United States. The percentage of colibactin in CRKP strains is 21% and 1% in the United States and China, respectively [52]. We did not detect colibactin in any of the five strains tested in this study. These differences may be because of the differences in the prevalence of strains in China and the United States, with ST11 being the dominant strain in China and ST258 being the dominant strain in the United States.

Conclusions

We isolated five non-CP-CRKP *K. pneumoniae* strains insusceptible to ertapenem. The genomic profile analysis revealed these strains carried β-lactamases and mutations in *ompK36* and *ompK37* genes accounting for ertapenem resistance. The patients infected with non-CP-CRKP had a history of antibiotic exposure and interventional operations. Some strains lacked the aerobactin, yersiniabactin and allantoin utilization-related genes and all non-CP-CRKP strains did not carry *rmpA* gene. Although non-CP-CRKP is not the main epidemic strain in our district, clinical staff should also raise awareness of non-CP-CRKP infection for better control of CRKP infection. Prospective, multicenter, large-scale studies should be conducted to better understand the prevalence of non-CP-CRKP.

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References

1. Tacconelli E, Carrara E, Savoldi A, Harbarth S, Mendelson M, Monnet DL, Pulcini C, Kahlmeter G, Kluytmans J, Carmeli Y, Ouellette M, Outterson K, Patel J, Cavaleri M, Cox EM, Houchens CR, Grayson ML, Hansen P, Singh N, Theuretzbacher U, Magrini N, Aboderin AO, Al-Abri SS, Awang Jalil N, Benzonana N, Bhattacharya S, Brink AJ, Burkert FR, Cars O, Cornaglia G, Dyar OJ, Friedrich AW, Gales AC, Gandra S, Giske CG, Goff DA, Goossens H, Gottlieb T, Guzman Blanco M, Hryniewicz W, Kattula D, Jinks T, Kanj SS, Kerr L, Kieny M-P, Kim YS, Kozlov RS, Labarca J, Laxminarayan R, Leder K, Leibovici L, Levy-Hara G, Littman J, Malhotra-Kumar S, Manchanda V, Moja L, Ndoye B, Pan A, Paterson DL, Paul M, Qiu H, Ramon-Pardo P, Rodríguez-Baño J, Sanguinetti M, Sengupta S, Sharland M, Si-Mehand M, Silver LL, Song W, Steinbakk M, Thomsen J, Thwaites GE, van der Meer JWM, Van Kinh N, Vega S, Villegas MV, Wechsler-Fördös A, Wertheim HFL, Wesangula E, Woodford N, Yilmaz FO, Zorzet A (2018) Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. Lancet Infect Dis 18: 318-327. doi: 10.1016/S1473-3099(17)30753-3.
2. Zhang YW, Wang Q, Yin YY, Chen HB, Jin LY, Gu B, Xie LY, Yang CX, Ma XB, Li HY, Li W, Zhang XQ, 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, Wang H (2018) Epidemiology of carbapenem-resistant Enterobacteriaceae infections: report from the China CRE Network. Antimicrob Agents Chemother 62: e01882-01817. doi: 10.1128/AAC.01882-17.
3. Hu FP, Guo Y, Zhu DM, Wang F, Jiang XF, Xu YC, Zhang XJ, Zhang ZX, Ji P, Xie Y, Kang M, Wang CQ, Wang A, Yuanhong X, Ying H, Ziyong S, Zhongju C, Yuxing N, Jingyong S, Yunzhuo C, Sufei T, Zhidong H, Jin L, Yunsong Y, Jie L, Bin S, Yan D, Sufang G, Lianhua W, Fengmei Z, Hong Z, Chun W, Yunjian H, Xiaoman A, Chao Z, Danhong S, Dawen G, Jinying Z, Hua Y, Xiangning H, Wen'en L, Yanming L, Yan J, Chunhong S, Xuesong X, Chao Y, Shanmei W, Yafei C, Lixia Z, Juan M, Shuping Z, Yan Z, Lei Z, Jinhua M, Fang D, Hongyan Z, Fangfang H, Han S, Wanqing Z, Wei J, Gang L, Jinsong W, Yuemei L, Jihong L, Jinju D, Jianbang K, Xiaobo M, Yanping Z, Ruyi G, Yan Z, Yunsheng C, Qing M, Shifu W, Xuefei H, Jilu S, Ruizhong W, Hua F, Bixia Y, Yong Z, Ping G, Kaizhen W, Yirong Z, Jiangshan L, Longfeng L, Hongqin G, Lin J, Wen H, Shunhong X, Jiao F, Rui D, Chunlei Y (2021) CHINET surveillance of bacterial resistance: results of 2020. Chin J Infect Chemother 21: 377-387. doi:10.16718/j.1009-7708.2021.04.001.
4. Suay-Garcia B, Perez-Gracia MT (2019) Present and future of carbapenem-resistant Enterobacteriaceae (CRE) infections. Antibiotics (Basel) 8: 122. doi: 10.3390/antibiotics8030122.
5. Black CA, So W, Dallas SS, Gawrys G, Benavides R, Aguilar S, Chen CJ, Shurko JF, Lee GC (2020) Predominance of non-carbapenemase producing carbapenem-resistant Enterobacterales in South Texas. Front Microbiol 11: 623574. doi: 10.3389/fmicb.2020.623574.
6. van Duin D, Arias CA, Komarow L, Chen L, Hanson BM, Weston G, Cober E, Garner OB, Jacob JT, Satlin MJ, Fries BC, Garcia-Diaz J, Doi Y, Dhar S, Kaye KS, Earley M, Hujer AM, Hujer KM, Domitrovic TN, Shropshire WC, Dinh A, Manca C, Luterbach CL, Wang M, Paterson DL, Banerjee R, Patel R, Evans S, Hill C, Arias R, Chambers HF, Fowler VG,

Kreiswirth BN, Bonomo RA (2020) Molecular and clinical epidemiology of carbapenem-resistant Enterobacterales in the USA (CRACKLE-2): a prospective cohort study. Lancet Infect Dis 20: 731-741. doi: 10.1016/S1473-3099(19)30755-8.
7. Jean SS, Hsueh PR, Group SA-P (2020) Antimicrobial susceptibilities of the ertapenem-non-susceptible non-carbapenemase-producing Enterobacterales isolates causing intra-abdominal infections in the Asia-Pacific region during 2008-2014: results from the study for monitoring the antimicrobial resistance trends (SMART). J Glob Antimicrob Resist 21: 91-98. doi: 10.1016/j.jgar.2019.10.004.
8. Teo J, Cai YY, Tang S, Lee W, Tan TY, Tan TT, Kwa AL (2012) Risk factors, molecular epidemiology and outcomes of ertapenem-resistant, carbapenem-susceptible Enterobacteriaceae: a case-case-control study. PLoS One 7: e34254. doi: 10.1371/journal.pone.0034254.
9. Yeh KM, Kurup A, Siu LK, Koh YL, Fung CP, Lin JC, Chen TL, Chang FY, Koh TH (2007) Capsular serotype K1 or K2, rather than magA and rmpA, is a major virulence determinant for *Klebsiella pneumoniae* liver abscess in Singapore and Taiwan. J Clin Microbiol 45: 466-471. doi: 10.1128/JCM.01150-06.
10. Holt KE, Wertheim H, Zadoks RN, Baker S, Whitehouse CA, Dance D, Jenney A, Connor TR, Hsu LY, Severin J, Brisse S, Cao H, Wilksch J, Gorrie C, Schultz MB, Edwards DJ, Nguyen KV, Nguyen TV, Dao TT, Mensink M, Minh VL, Nhu NT, Schultsz C, Kuntaman K, Newton PN, Moore CE, Strugnell RA, Thomson NR (2015) Genomic analysis of diversity, population structure, virulence, and antimicrobial resistance in *Klebsiella pneumoniae*, an urgent threat to public health. Proc Natl Acad Sci USA 112: E3574-3581. doi: 10.1073/pnas.1501049112.
11. Gu D, Dong N, Zheng Z, Lin D, Huang M, Wang L, Chan EW-C, Shu L, Yu J, Zhang R, Chen S (2018) A fatal outbreak of ST11 carbapenem-resistant hypervirulent *Klebsiella pneumoniae* in a Chinese hospital: a molecular epidemiological study. Lancet Infect Dis 18: 37-46. doi: 10.1016/S1473-3099(17)30489-9.
12. Liao WJ, Liu Y, Zhang W (2020) 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 23: 174-180. doi: 10.1016/j.jgar.2020.09.004.
13. Lan P, Jiang Y, Zhou JC, Yu YS (2021) A global perspective on the convergence of hypervirulence and carbapenem resistance in *Klebsiella pneumoniae*. J Glob Antimicrob Resist 25: 26-34. doi: 10.1016/j.jgar.2021.02.020.
14. Wyres KL, Lam MMC, Holt KE (2020) Population genomics of *Klebsiella pneumoniae*. Nat Rev Microbiol 18: 344-359. doi: 10.1038/s41579-019-0315-1.
15. Clinical and Laboratory Standards Institute (CLSI) (2019) Performance standards for antimicrobial susceptibility testing, M100 29th edition. Wayne, PA.
16. Centers for Disease Control and Prevention Facility (2015) Guidance for control of carbapenem-resistant Enterobacteriaceae (CRE) - November 2015 update - CRE toolkit. Available: https://www.cdc.gov/infectioncontrol/guidelines/pdf/cre/CRE-guidance-508.pdf. Accessed: 11 December 2022.
17. Wick RR, Judd LM, Gorrie CL, Holt KE (2017) Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput Biol 13: e1005595. doi: 10.1371/journal.pcbi.1005595.
18. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O (2008) The RAST server: rapid annotations using subsystems technology. BMC Genomics 9: 75. doi: 10.1186/1471-2164-9-75.
19. Larsen MV, Cosentino S, Rasmussen S, Friis C, Hasman H, Marvig RL, Jelsbak L, Sicheritz-Pontén T, Ussery DW, Aarestrup FM, Lund O (2012) Multilocus sequence typing of total-genome-sequenced bacteria. J Clin Microbiol 50: 1355-1361. doi: 10.1128/JCM.06094-11.
20. Bortolaia V, Kaas RS, Ruppe E, Roberts MC, Schwarz S, Cattoir V, Philippon A, Allesoe RL, Rebelo AR, Florensa AF, Fagelhauer L, Chakraborty T, Neumann B, Werner G, Bender JK, Stingl K, Nguyen M, Coppens J, Xavier BB, Malhotra-Kumar S, Westh H, Pinholt M, Anjum MF, Duggett NA, Kempf I, Nykäsenoja S, Olkkola S, Wieczorek K, Amaro A, Clemente L, Mossong J, Losch S, Ragimbeau C, Lund O, Aarestrup FM (2020) ResFinder 4.0 for predictions of phenotypes from genotypes. J Antimicrob Chemother 75: 3491-3500. doi: 10.1093/jac/dkaa345.
21. Carattoli A, Zankari E, García-Fernández A, Voldby Larsen M, Lund O, Villa L, Møller Aarestrup F, Hasman H (2014) In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. Antimicrob Agents Chemother 58: 3895-3903. doi: 10.1128/AAC.02412-14.
22. Liu B, Zheng D, Zhou S, Chen L, Yang J (2022) VFDB 2022: a general classification scheme for bacterial virulence factors. Nucleic Acids Res 50: D912-D917. doi: 10.1093/nar/gkab1107.
23. Kaas RS, Leekitcharoenphon P, Aarestrup FM, Lund O (2014) Solving the problem of comparing whole bacterial genomes across different sequencing platforms. PLoS One 9: e104984. doi: 10.1371/journal.pone.0104984.
24. Letunic I, Bork P (2021) Interactive tree of life (iTOL) v5: an online tool for phylogenetic tree display and annotation. Nucleic Acids Res 49: W293-w296. doi: 10.1093/nar/gkab301.
25. National Center for Biotechnology Information, National Library of Medicine (US) (1988) National Center for Biotechnology Information. Available: https://www.ncbi.nlm.nih.gov/. Accessed: 19 January 2021.
26. Doi Y (2019) Treatment options for carbapenem-resistant Gram-negative bacterial infections. Clin Infect Dis 69 Suppl 7: S565-S575. doi: 10.1093/cid/ciz830.
27. Bonomo RA, Burd EM, Conly J, Limbago BM, Poirel L, Segre JA, Westblade LF (2018) Carbapenemase-producing organisms: a global scourge. Clin Infect Dis 66: 1290-1297. doi: 10.1093/cid/cix893.
28. Su CF, Chuang C, Lin YT, Chan YJ, Lin JC, Lu PL, Huang CT, Wang JT, Chuang YC, Siu LK, Fung CP (2018) Treatment outcome of non-carbapenemase-producing carbapenem-resistant *Klebsiella pneumoniae* infections: a multicenter study in Taiwan. Eur J Clin Microbiol Infect Dis 37: 651-659. doi: 10.1007/s10096-017-3156-8.
29. McGettigan SE, Andreacchio K, Edelstein PH (2009) Specificity of ertapenem susceptibility screening for detection of *Klebsiella pneumoniae* carbapenemases. J Clin Microbiol 47: 785-786. doi: 10.1128/JCM.02143-08.
30. Hyle EP, Ferraro MJ, Silver M, Lee H, Hooper DC (2010) Ertapenem-resistant Enterobacteriaceae: risk factors for

acquisition and outcomes. Infect Control Hosp Epidemiol 31: 1242-1249. doi: 10.1086/657138.
31. Lim CL-L, Lee W, Lee AL-C, Liew LT-T, Nah SC, Wan CN, Chlebicki MP, Kwa AL-H (2013) Evaluation of ertapenem use with impact assessment on extended-spectrum beta-lactamases (ESBL) production and Gram-negative resistance in Singapore General Hospital (SGH). BMC Infect Dis 13: 523. doi: 10.1186/1471-2334-13-523.
32. Tangden T, Adler M, Cars O, Sandegren L, Lowdin E (2013) Frequent emergence of porin-deficient subpopulations with reduced carbapenem susceptibility in ESBL-producing *Escherichia coli* during exposure to ertapenem in an in vitro pharmacokinetic model. J Antimicrob Chemother 68: 1319-1326. doi: 10.1093/jac/dkt044.
33. Jean SS, Lee WS, Hsueh PR, Group SA-P (2018) Ertapenem non-susceptibility and independent predictors of the carbapenemase production among the Enterobacteriaceae isolates causing intra-abdominal infections in the Asia-Pacific region: results from the study for monitoring antimicrobial resistance trends (SMART). Infect Drug Resist 11: 1881-1891. doi: 10.2147/IDR.S181085.
34. Hawser SP, Bouchillon SK, Lascols C, Hackel M, Hoban DJ, Badal RE, Woodford N, Livermore DM (2011) Susceptibility of *Klebsiella pneumoniae* isolates from intra-abdominal infections and molecular characterization of ertapenem-resistant isolates. Antimicrob Agents Chemother 55: 3917-3921. doi: 10.1128/AAC.00070-11.
35. Rodrigues C, Machado E, Ramos H, Peixe L, Novais A (2014) Expansion of ESBL-producing *Klebsiella pneumoniae* in hospitalized patients: a successful story of international clones (ST15, ST147, ST336) and epidemic plasmids (IncR, IncFIIK). Int J Med Microbiol 304: 1100-1108. doi: 10.1016/j.ijmm.2014.08.003.
36. Muggeo A, Guillard T, Klein F, Reffuveille F, Francois C, Babosan A, Bajolet O, Bertrand X, de Champs C, CarbaFrEst G (2018) Spread of Klebsiella pneumoniae ST395 non-susceptible to carbapenems and resistant to fluoroquinolones in North-Eastern France. J Glob Antimicrob Resist 13: 98-103. doi: 10.1016/j.jgar.2017.10.023.
37. Al-Agamy MH, Aljallal A, Radwan HH, Shibl AM (2018) Characterization of carbapenemases, ESBLs, and plasmid-mediated quinolone determinants in carbapenem-insensitive *Escherichia coli* and *Klebsiella pneumoniae* in Riyadh hospitals. J Infect Public Health 11: 64-68. doi: 10.1016/j.jiph.2017.03.010.
38. Shields RK, Clancy CJ, Press EG, Nguyen MH (2016) Aminoglycosides for treatment of bacteremia due to carbapenem-resistant *Klebsiella pneumoniae*. Antimicrob Agents Chemother 60: 3187-3192. doi: 10.1128/AAC.02638-15.
39. Hu F, Guo Y, Zhu DM, Wang F, Jiang XF, Xu YC, Zhang XJ, Zhang ZX, Ji P, Xie Y, Kang M, Wang CQ, Wang AM, Xu YH, Huang Y, Sun ZY, Chen ZJ, Ni YX, Sun JY, Chu YZ, Tian SF, Hu ZD, Li J, Yu YS, Lin J, Shan B, Du Y, Guo SF, Wei LH, Zou FM, Zhang H, Wang C, Hu YJ, Ai XM, Zhuo C, Su DH, Guo DW, Zhao JY, Yu H, Huang XN, Liu WE, Li YM, Jin Y, Shao CH, Xu XS, Yan C, Wang SM, Chu YF, Zhang LX, Ma J, Zhou SP, Zhou Y, Zhu L, Meng JH, Dong F, Zheng HY, Hu FF, Shen H, Zhou WQ, Jia W, Li G, Wu JS, Lu YM, Li JH, Duan JJ, Kang JB, Ma XB, Zheng YP, Guo RY, Zhu Y, Chen YS, Meng Q (2020) CHINET surveillance of bacterial resistance across tertiary hospitals in 2019. Chin J Infect Chemother 20: 233-243. doi: 10.16718/j.1009-7708.2020.03.001.
40. Nasiri G, Peymani A, Farivar TN, Hosseini P (2018) Molecular epidemiology of aminoglycoside resistance in clinical isolates of *Klebsiella pneumoniae* collected from Qazvin and Tehran provinces, Iran. Infect Genet Evol 64: 219-224. doi: 10.1016/j.meegid.2018.06.030.
41. Juan CH, Huang YW, Lin YT, Yang TC, Wang FD (2016) Risk factors, outcomes, and mechanisms of tigecycline-nonsusceptible *Klebsiella pneumoniae* bacteremia. Antimicrob Agents Chemother 60: 7357-7363. doi: 10.1128/AAC.01503-16.
42. Ye MP, Ding BX, Qian HL, Xu QQ, Jiang J, Huang JW, Ou HY, Hu FP, Wang MG (2017) In vivo development of tigecycline resistance in *Klebsiella pneumoniae* owing to deletion of the *ramR* ribosomal binding site. Int J Antimicrob Agents 50: 523-528. doi: 10.1016/j.ijantimicag.2017.04.024.
43. Chew KL, Lin RTP, Teo JWP (2017) *Klebsiella pneumoniae* in Singapore: hypervirulent infections and the carbapenemase threat. Front Cell Infect Microbiol 7: 515. doi: 10.3389/fcimb.2017.00515.
44. Karlsson M, Stanton RA, Ansari U, McAllister G, Chan MY, Sula E, Grass JE, Duffy N, Anacker ML, Witwer ML, Rasheed JK, Elkins CA, Halpin AL (2019) Identification of a carbapenemase-producing hypervirulent *Klebsiella pneumoniae* isolate in the United States. Antimicrob Agents Chemother 63: e00519-00519. doi: 10.1128/AAC.00519-19.
45. Zhu J, Wang T, Chen L, Du H (2021) Virulence factors in hypervirulent *Klebsiella pneumoniae*. Front Microbiol 12: 642484. doi: 10.3389/fmicb.2021.642484.
46. Martin RM, Bachman MA (2018) Colonization, infection, and the accessory genome of *Klebsiella pneumoniae*. Front Cell Infect Microbiol 8: 4. doi: 10.3389/fcimb.2018.00004.
47. Zhang YW, Zhao CJ, Wang Q, Wang XJ, Chen HB, Li HN, Zhang FF, Li SG, Wang RB, Wang H (2016) High prevalence of hypervirulent *Klebsiella pneumoniae* infection in China: geographic distribution, clinical characteristics, and antimicrobial resistance. Antimicrob Agents Chemother 60: 6115-6120. doi: 10.1128/AAC.01127-16.
48. Russo TA, MacDonald U (2020) The *Galleria mellonella* infection model does not accurately differentiate between hypervirulent and classical *Klebsiella pneumoniae*. mSphere 5: e00850-00819. doi: 10.1128/mSphere.00850-19.
49. Hu D, Li Y, Ren P, Tian D, Chen W, Fu P, Wang W, Li X, Jiang X (2021) Molecular epidemiology of hypervirulent carbapenemase-producing *Klebsiella pneumoniae*. Front Cell Infect Microbiol 11: 661218. doi: 10.3389/fcimb.2021.661218.
50. Hsieh PF, Lin TL, Lee CZ, Tsai SF, Wang JT (2008) Serum-induced iron-acquisition systems and TonB contribute to virulence in *Klebsiella pneumoniae* causing primary pyogenic liver abscess. J Infect Dis 197: 1717-1727. doi: 10.1086/588383.
51. Chou HC, Lee CZ, Ma LC, Fang CT, Chang SC, Wang JT (2004) Isolation of a chromosomal region of *Klebsiella pneumoniae* associated with allantoin metabolism and liver infection. Infect Immun 72: 3783-3792. doi: 10.1128/IAI.72.7.3783-3792.2004.
52. Wang MG, Earley M, Chen L, Hanson BM, Yu YS, Liu ZY, Salcedo S, Cober E, Li LJ, Kanj SS, Gao H, Munita JM, Ordoñez K, Weston G, Satlin MJ, Valderrama-Beltrán SL, Marimuthu K, Stryjewski ME, Komarow L, Luterbach C, Marshall SH, Rudin SD, Manca C, Paterson DL, Reyes J, Villegas MV, Evans S, Hill C, Arias R, Baum K, Fries BC, Doi

Y, Patel R, Kreiswirth BN, Bonomo RA, Chambers HF, Fowler VG, Arias CA, van Duin D, Abbo LM, Anderson DJ, Arias R, Arias CA, Baum K, Bonomo RA, Chambers HF, Chen L, Chew KL, Cober E, Cross HR, De PP, Desai S, Dhar S, Di Castelnuovo V, Diaz L, Dinh AQ, Doi Y, Earley M, Eilertson B, Evans B, Evans S, Fowler Jr VG, Fries BC, Gao H, Garcia-Diaz J, Garner OB, Greenwood-Quaintance K, Hanson B, Herc E, Hill C, Jacob JT, Jiang J, Kalayjian RC, Kanj SS, Kaye KS, Kim A, Komarow L, Kreiswirth BN, Lauterbach C, Li L, Liu Z, Manca C, Marimuthu K, Marshall SH, McCarty T, Munita J, Ng OT, Oñate Gutierrez JM, Ordoñez K, Patel R, Paterson DL, Peleg A, Reyes J, Rudin SD, Salata RA, Salcedo S, Satlin MJ, Schmidt-Malan S, Smitasin N, Spencer M, Stryjewski M, Su J, Tambyah PA, Valderrama S, van Duin D, Villegas Botero MV, Wang M, Waters M, Weston G, Wong D, Wortmann G, Yang Y, Yu Y, Zhang F (2021) Clinical outcomes and bacterial characteristics of carbapenem-resistant *Klebsiella pneumoniae* complex among patients from different global regions (CRACKLE-2): a prospective, multicentre, cohort study. Lancet Infect Dis 22: 401-412. doi: 10.1016/S1473-3099(21)00399-6.s

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