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ORIGINAL RESEARCH

Carbapenem-Resistant *Klebsiella aerogenes* Clinical Isolates from a Teaching Hospital in Southwestern China: Detailed Molecular Epidemiology, Resistance Determinants, Risk Factors and Clinical Outcomes

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Purpose: Little is known about the epidemiology and carbapenem-resistance determinants of carbapenem-resistant *K. aerogenes* (CRKA) isolated from a single medical center. The present study was initiated to characterize the molecular epidemiology and the carbapenem-resistance mechanisms of CRKA isolated during 2012–2018 from a teaching hospital in southwest China, and to investigate the risk factors and clinical outcomes of CRKA infections as well.

Methods: Pulsed-field gel electrophoresis (PFGE) was employed for epidemiological analysis. PCR amplification and DNA sequencing were used to examine the antibiotic-resistance determinants. Plasmids were extracted and characterized by PCR-based replicon typing and conjugation assays. In order to further investigate the risk factors and clinical outcomes of CRKA infections, a retrospective case–control study was also performed.

Results: PFGE analysis showed 32 different PFGE patterns among the 36 non-duplicated CRKA strains collected. Most of the isolates harbored multi-drug resistance (MDR) genes, including 2 (5.6%) carrying $bla_{\text{NDM-1}}$, 1 (2.8%) harboring $bla_{\text{KPC-2}}$, 13 (36.1%) carrying ESBL genes, 23 (63.9%) carrying ampC genes, 34 (94.4%) carrying quinolone resistance determinants (QRD) genes and 9 (25%) carrying aminoglycoside resistance determinants (ARD) genes. The outer membrane porins, OmpE35 and OmpE36, were, respectively, lost in 4 and 2 isolates. The efflux pump inhibition experiments were positive in 25 (69.4%) of the CRKA strains. Multivariate analysis indicated that hypo-albuminaemia, invasive procedures, and carbapenem exposure were independent risk factors for acquiring CRKA infections.

Conclusion: No clonality relationship was identified among most of the 36 CRKA isolates. The over-expression of ESBLs and AmpC coupled with the efflux pumps contributed to carbapenem resistance in *K. aerogenes*. Additionally, this is the first report of CRKA isolate co-harboring $bla_{\text{NDM-1}}$, $bla_{\text{CTX-M-15}}$, bla_{EBC}, bla_{ACC}, *acc (6')-Ib, armA, qnrD* and loss of OmpE36 in China. Hypo-albuminaemia, invasive procedures and carbapenem exposure were associated with acquisition of CRKA infections.

Keywords: case-control study, $bla_{\text{NDM-1}}$, $bla_{\text{KPC-2}}$, porins, efflux pumps

Introductions

With the increasing emergence of multi-drug-resistant (MDR) bacteria, carbapenems have been used as the last-line antibiotic for treating severe infections caused by gram-negatives and displayed strong activity against AmpC β-lactamase and/or

ESBL hyper-producing isolates.[1] Unfortunately, since the introduction of carbapenems into the clinical practice, nosocomial infections caused by carbapenem-resistant *Enterobacteriaceae* (CRE) have been increasing rapidly and posing serious challenges to the clinical management of infections caused by the gram-negatives.[2] To help direct research and development efforts toward the production of novel drugs, CRE was recently listed as one of the three critical-priority pathogens by the World Health Organization (WHO).[3]

Klebsiella aerogenes (*K. aerogenes*), formerly described as *Enterobacter aerogenes*, is a gram-negative, rod-shaped, facultative anaerobic bacteria belonging to the family *Enterobacteriaceae*.[4] It is an important nosocomial pathogen associated with a wide variety of infections including pneumonia, bacteremia, urinary tract and surgical site infections.[5] Most importantly, *K. aerogenes* exhibited MDR phenotype during various hospital outbreaks.[5,6]

Although *K. aerogenes* belongs to the family *Enterobacteriaceae*, its resistance mechanisms against carbapenems remain relatively unclear due to the limited amount of work performed to date in this field. With the successive and increasing isolation of CRKA in our center in recent years, and the limited amount of work performed to date in CRKA, more attention has been evoking concerning its molecular epidemiology and carbapenem-resistance mechanisms. The present study was initiated to characterize the molecular epidemiology and the carbapenem-resistance mechanisms of CRKA isolated during 2012–2018 from a teaching hospital in southwest China, and to investigate the risk factors and clinical outcomes of CRKA infections as well.

Previous studies showed that carbapenem resistance in *K. aerogenes* mainly arises from the over-expression of ESBLs or AmpC enzymes coupled with mutations affecting membrane permeability.[7] Carbapenemases, such as KPC, NDM, and OXA-48, have also been reported in clinical *K. aerogenes* isolates from different countries.[8–10] However, the risk factors, molecular epidemiology, and clinical outcomes pertaining to CRKA infections in a single medical center have not been systematically characterized.

The present study was initiated: (i) to describe the prevalence of clinical CRKA isolates collected successively for approximately 6 years; (ii) to identify the carbapenem-resistance mechanisms and the clonal relatedness among CRKA strains; and (iii) to characterize the risk factors and clinical outcomes associated with the acquisition of CRKA infections.

Materials and Methods

Bacterial Strains

This retrospective study was performed in the First Affiliated Hospital of Chongqing Medical University, a 3200-bed tertiary hospital located in Southwest China. A total of 892 clinical *K. aerogenes* strains were isolated and identified from January 2012 to December 2018 by using the VITEK2 compact or VITEK MS (bioMerieux, Hazelwood, MO, United States) automated system at the department of laboratory medicine, among which 36 strains were resistant to at least one carbapenem on the basis of antimicrobial susceptibility testing results determined by the broth microdilution method, with the criteria of MIC of ≥2 mg/L for ertapenem, ≥4 mg/L for imipenem, and ≥4 mg/L for meropenem; and 5 strains were intermediate to at least one carbapenem, with the criteria of MIC of = 1 mg/L for ertapenem, = 2 mg/L for imipenem, and = 2 mg/L for meropenem (Supplementary Figure 1). Only the first isolate from each individual patient was included in this study.

Antimicrobial Susceptibility Testing and Activity of the Efflux Pumps

Initial antibiotic susceptibility testing was performed by using the VITEK2 compact (bioMerieux, Hazelwood, MO, USA) automated system. MICs of ertapenem (ETP), imipenem (IPM), and meropenem (MEM) were reassessed manually using the broth microdilution method and the results were categorized in accordance with the Clinical and Laboratory Standards Institute, M100-S28 (CLSI M100-S28) interpretive criteria. The activities of the efflux pumps were examined by comparing the MICs to carbapenems among resistant isolates in the presence and absence of carbonyl cyanide m-chlorophenylhydrazone (CCCP) as an efflux pump inhibitor. At least a two doubling dilution decrease in the resistance level was considered a positive result.[10] *K. aerogenes* ATCC13048 was used as the reference strain.

PFGE

The molecular epidemiology of all the CRKA strains was determined by pulsed-field gel electrophoresis (PFGE) after total chromosomal DNA digestion with *Xba*I in accordance with a previous report.[11] The genomic DNA restriction patterns of the isolates were analyzed and interpreted according to the previously proposed criteria.[12]

Detection of Antibiotic Resistance Genes

PCR analyses for the presence of carbapenemase-encoding genes (bla_{KPC}, bla_{VIM}, bla_{IMP}, bla_{NDM}, bla_{SME}, bla_{OXA-48}, bla_{OXA-23}-like, bla_{OXA-58}-like) were performed by using primers and conditions described previously.[13] In addition, ESBLs genes (bla_{CTXM}, bla_{TEM}, bla_{SHV}, bla_{OXA-1}), AmpC genes (bla_{EBC}, bla_{ACC}, bla_{ACT}, bla_{DHA}, bla_{MOX}), ARD genes (*acc(6')-Ib, armA, rmtB*), QRD genes (*qnrA, qnrB, qnrC, qnrS, qnrD, acc(6')-Ib-cr*), and porin genes (*ompE35* and *ompE36*) were also investigated by PCR with previously designed primers and conditions.[14] DNA sequencing with the BLAST program (http://www.ncbi.nlm.nih.gov/BLAST) was used to confirm the variants of the resistance genes.

Conjugation and Plasmid Replicon Typing

To confirm whether the carbapenemase genes were located on the plasmids, the carbapenemase-producing strains were collected and conjugation experiments were performed according to the previously described method.[15] All the carbapenemase-producing strains were served as the donors, while the *E. coli* EC600 was employed as the recipient strain. Potential transconjugants were isolated on Mueller-Hinton agar plates containing 8 mg/L ertapenem and 256 mg/L rifampicin. The transconjugants were tested for antimicrobial susceptibility by the VITEK2 compact system, and the presence of resistance determinants was confirmed by PCR. Additionally, all CRKA strains plasmids were determined by using the PCR-based replicon typing method as described previously.[16]

Risk Factors and Clinical Outcomes of CRKA Infections

We conducted a retrospective case–control study to explore the risk factors and clinical outcomes of patients infected with CRKA from 2012 to 2018 in Chongqing, China. All hospitalized patients with *K. aerogenes* infections were included. Patients with CRKA infections were included as cases. Controls were identified as patients with carbapenem-susceptible *K. aerogenes* (CSKA) infections with well-balanced demographic characteristics, pre-existing medical conditions, and immune-compromising comorbidities as compared with the cases. Clinical and epidemiological data, including the demographics, underlying diseases, the primary diagnosis at admission, invasive procedures prior to the isolation of CRKA, previous exposures of antibiotic within 3 months, and the clinical outcomes, were extracted from the patients' electronic medical records system and clinical microbiology laboratory database.

Statistical Analysis

All analyses were performed using SPSS v.22.0 software (SPSS Inc., Chicago, IL, United States). Univariate analyses were performed separately for each of the variables. Categorical variables were compared using a chi-square test or Fisher's exact test as appropriate. Continuous variables were compared using Student's *t*-test (normally distributed variables) and Wilcoxon rank-sum test (non-normally distributed variables) as appropriate. The odds ratio (OR) and 95% confidence interval (CI) were calculated to evaluate the strength of any association. Variables with $P \leq 0.05$ on univariate analysis were evaluated as potential covariates in a stepwise multivariate logistic regression model. For all calculations, $P < 0.05$ was considered statistically significant.

Ethical Considerations

The data and samples analyzed in the present study were obtained in accordance with the standards and approved by the Chongqing Medical University Institutional Review Board and the Biomedical Ethics Committee. For this study, samples were collected at the microbiology laboratory of our hospital, with no contact with the patients. This study was retrospective and there was no patient identification performed during data collection. Therefore, the ethics committee determined that informed consent was not required.

Results

General Characteristics and Antimicrobial Susceptibility Profiles of 36 CRKA Isolates

During the 6-year period of the study, a total of 36 strains, which are resistant to at least one of the carbapenems tested (ertapenem, imipenem and meropenem), were isolated as CRKA. As shown in Table 1, these non-duplicated strains were mainly isolated from urine (10/36; 27.8%), followed by drainage-liquid (9/36; 25%), sputum (4/36; 11.1%), and bile (3/36; 8.3%). Among the 36 isolates, all were ertapenem-resistant, 72.2% (26/36) and 36.1% (13/36) of the isolates were resistant to imipenem and meropenem, respectively. In addition, CRKA isolates showed variable resistance rates to other antimicrobials: ceftazidime (35/36, 97.2%), ceftriaxone (32/36, 88.9%), cefepime (13/36, 36.1%), gentamicin (13/36, 36.1%), amikacin (5/36, 13.8%), tobramycin (9/36, 25.0%), ciprofloxacin (15/36,

Table 1 Distribution and Corresponding Carbapenem MIC Ranges for Strains with Different Resistance Determinants

Carbapenems Resistance Mechanisms	No. of Isolates	MIC Range(mg/L)		
		Imipenem	Meropenem	Ertapenem
Carbapenemase positive				
KPC-2+,efflux pump,loss of OMPs	1	128	128	256
NDM-1+,ESBL+,AmpC+,loss of OMPs,efflux pump	1	64	128	256
NDM-1+,ESBL+,AmpC+,efflux pump	1	64	128	256
Carbapenemase negative				
ESBL+,AmpC+,loss of OMPs, efflux pump	3	4	2–4	8–64
ESBL+,loss of OMPs, efflux pump	1	8	4	64
ESBL+,AmpC+,efflux pump	8	4–32	0.5–32	4–128
ESBL+,efflux pump	2	4–32	2–32	4–64
AmpC+,efflux pump	8	2–32	0.25–16	4–64
ESBL+,AmpC+	1	4	<0.25	8
ESBL+	1	<0.25	<0.25	2
AmpC+	6	0.25–4	0.25–2	2–4
NONE	3	0.5–2	0.25–1	2

Abbreviations: MIC, minimum inhibitory concentration; OMPs, outer membrane proteins.

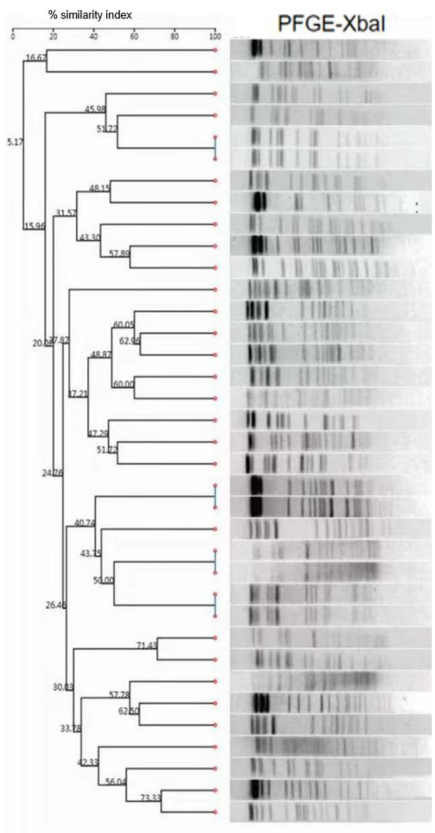
41.6%), levofloxacin (12/36, 33.3%). Notably, 55.5% (20/36) of the CRKA isolates were classified as MDR as they were resistant to three or more classes of antimicrobials.

Characterization of the Molecular Epidemiology of the 36 CRKA Isolates

The characteristics of the molecular epidemiology of the 36 CRKA strains are displayed in Figure 1. All the 36 CRKA isolates were grouped into 32 different PFGE patterns and exhibited a high degree of genetic diversities. While 8 isolates were grouped into 4 different PFGE patterns, with two individual isolates showing almost the same typing, the left 28 isolates belonged to 28 different PFGE patterns. Most notably, the two isolates (No. 8 and No. 9) belonging to pattern 20 were shown to be isolated from the same ward during the same time period. Nevertheless, the two isolates belonging to patterns 5, 22 and 23, respectively, were isolated from different wards.

Analysis of Molecular Resistance Mechanisms

Of the 36 CRKA isolates, 3 (8.3%) were identified as carbapenemase-producers: 2 (5.6%) with $bla_{\text{NDM-1}}$, and 1 (2.8%) with $bla_{\text{KPC-2}}$. In addition, these CRKA isolates showed relatively higher expression rates for ESBL (13/36, 36.1%) and AmpC genes (23/36, 63.9%), with 25% of the strains being positive for both genes. The prevalence of ESBLs/AmpC genes in the 36 isolates was presented as follows: 14 (38.9%) produced TEM, 7 (19.4%) produced CTX-M-15, 2 (5.6%) produced SHV, 3 (8.3%) produced CTX-M-2 and 1 (2.8%) produced OXA-1; 21 (58.3%) produced EBC, 27 (75%) produced ACC and 22 (61.1%) produced ACT. The prevalence of QRD genes in the 36 CRKA strains was 34 out of 36 (94.4%), with 34 strains carrying qnrD, 9 carrying qnrS, 3 carrying qnrB, and 6 carrying aac(6′)-Ib-cr. The prevalence of ARD genes was 12 out of 36 (25%), and the genes aac (6′)-Ib, armA, and rmtB were detected in 12, 2, and 1 of the 36 isolates, respectively. The outer membrane porin genes *ompE35* and *ompE36* were lost in 4 and 2 isolates, respectively. Sequencing analysis of the *ompE35* and *ompE36* genes of these CRKA isolates confirmed no mutations or insertions. Moreover, the addition of the efflux pump inhibitor CCCP reduced the MICs to ertapenem by 2–4 log2 dilutions in 69.4% (25/36) of the CRKA isolates. On the other hand, we have also provided the clinical and genotypic characteristics of the 5 CIKA strains having borderline MIC values (i.e., MIC = 1 mg/L for ertapenem, 2 mg/L for imipenem, and 2 mg/L for meropenem) as in Supplementary Table 1. None of them produced carbapenemases at detectable levels as has been demonstrated by the negative results from both the simplified Carbapenem Inactivation Method (sCIM) and PCR screening of the carbapenemase genes including



Sample ID	Source	Date	PFGE	Carbapenemase	ESBLs	AmpC	Porin loss	ARD	QRD	PBRT
KA-5	pus	08-2012	1	-	-	ACT EBC ACC	-	-	qnrD	-
KA-23	CSF	07-2012	2	-	-	ACT ACC	-	-	qnrD	-
KA-15	secretion	09-2012	3	-	-	-	-	-	qnrD、qnrS	-
KA-13	sputum	12-2012	4	-	-	ACT EBC ACC	-	-	qnrD、acc (6') -1b-cr	-
KA-17	bile	05-2016	5	-	-	ACT EBC ACC	-	-	qnrD	IncHI-1
KA-18	urine	07-2014	5	-	-	-	-	-	qnrD、qnrS	-
KA-11	sputum	01-2014	6	-	-	ACT EBC ACC	-	-	qnrD	-
KA-16	urine	09-2014	7	-	TEM	ACT EBC ACC	OmpE36	-	qnrD	IncHI-1
KA-10	abdominal fluid	10-2014	8	-	CTX-M-15 OXA-1	-	-	acc (6') -1b	qnrD	IncHI-1
KA-2	secretion	10-2015	9	-	TEM	ACT EBC ACC	-	-	qnrD	IncN
KA-19	blood	10-2014	10	-	CTX-M-2 TEM	EBC	OmpE35	-	qnrD、qnrS	IncHI-1
KA-30	blood	05-2014	11	-	-	ACT EBC ACC	-	-	qnrD	-
KA-32	bile	04-2015	12	-	-	ACT EBC ACC	-	-	qnrD	IncY
KA-27	urine	12-2015	13	-	CTX-M-2 SHV	ACC	-	-	qnrD、acc (6') -1b-cr	IncFIIAs
KA-29	urine	04-2016	14	-	-	EBC ACC	-	-	qnrD	-
KA-26	drainage-liquid	06-2016	15	-	CTX-M-15 TEM	EBC ACC	-	acc (6') -1b	qnrD、qnrS	IncFIIAs
KA-35	sputum	06-2016	16	-	-	EBC	-	-	qnrD	IncA/C
KA-36	urine	07-2016	17	-	-	ACT ACC	-	-	qnrD、qnrB	-
KA-28	urine	07-2016	18	-	-	ACT ACC	-	-	qnrD	-
KA-33	urine	09-2018	19	KPC-2	-	-	OmpE35	rmtB	qnrD、qnrS	Untypable
KA-8	drainage-liquid	02-2017	20	NDM-1	CTX-M-15 TEM	EBC ACC	-	acc (6') -1b、armA	qnrD	IncFIIAs
KA-9	drainage-liquid	06-2017	20	NDM-1	CTX-M-15 TEM	EBC ACC	OmpE36	acc (6') -1b、armA	qnrD	IncFIIAs
KA-34	urine	08-2017	21	-	CTX-M-15 TEM	ACT ACC	-	acc (6') -1b	qnrD、qnrB、acc (6') -1b-cr	IncY
KA-20	sputum	03-2018	22	-	TEM	-	OmpE35	acc (6') -1b	qnrD	IncA/C
KA-21	drainage-liquid	08-2017	22	-	-	ACT EBC ACC	-	-	qnrD	IncHI-1
KA-24	drainage-liquid	10-2017	23	-	CTX-M-15 TEM	ACT ACC	OmpE35	acc (6') -1b	-	IncY
KA-25	drainage-liquid	10-2018	23	-	CTX-M-2	ACT EBC ACC	-	-	qnrD、qnrB、acc (6') -1b-cr	IncHI-1
KA-12	drainage-liquid	09-2016	24	-	TEM	ACT EBC ACC	-	acc (6') -1b	qnrD、qnrS	IncFIC
KA-14	urine	05-2018	25	-	CTX-M-15 TEM	ACT EBC ACC	-	acc (6') -1b	qnrD、qnrS、acc (6') -1b-cr	IncHI-1
KA-22	blood	01-2017	26	-	TEM	-	-	-	qnrD、qnrS	IncHI-1
KA-1	drainage-liquid	06-2017	27	-	TEM	ACT EBC ACC	-	-	qnrD	IncHI-1
KA-3	sputum	11-2017	28	-	-	-	-	-	qnrD	IncHI-1
KA-4	abdominal-fluid	12-2017	29	-	TEM SHV	ACT EBC ACC	-	acc (6') -1b	qnrD、acc (6') -1b-cr	-
KA-6	sputum	05-2018	30	-	-	ACT ACC	-	-	-	-
KA-7	sputum	01-2018	31	-	-	ACT ACC	-	acc (6') -1b	qnrD、qnrS	IncHI-1
KA-31	drainage-liquid	07-2018	32	-	-	ACT EBC ACC	-	-	qnrD	-

Figure 1 PFGE-based dendrogram of CRKA strains. Sample ID, source of initial isolation, and demographic information are included along each PFGE lane.
Abbreviations: ESBLs, Extended-Spectrum Beta-Lactamases; QRD, quinolone resistance determinants; ARD, aminoglycoside resistance determinants; PBRT, PCR-based replicon type; "-", not detected.

bla$_{KPC}$, *bla*$_{VIM}$, *bla*$_{IMP}$, *bla*$_{NDM}$, *bla*$_{SME}$, *bla*$_{OXA-48}$, *bla*$_{OXA-23}$-like, and *bla*$_{OXA-58}$-like.

Conjugation and Plasmid Replicon Typing Analysis

Conjugation experiments showed that the transconjugants were obtained from two strains carrying *bla*$_{NDM-1}$ at frequencies of 2×10^{-5} per recipient cell, while no transconjugants were obtained from the *bla*$_{KPC-2}$-carrying strain. Susceptibility testings (Table 2) confirmed that the transconjugants exhibited resistance to the tested carbapenems and cephalosporins, and demonstrated significantly reduced susceptibilities to aminoglycosides, while remained susceptible to fluoroquinolones. PCR assays indicated that *bla*$_{NDM-1}$, *bla*$_{TEM}$, *bla*$_{CTX-M-15}$, *acc(6')-1b*, and *armA* were successfully transferred by conjugation (Table 2). On the other hand, PCR-based replicon typing revealed that the CRKA plasmids belonged to the groups IncHI-1 (n =11), IncFIIAs (n = 4), IncY (n = 3), IncA/C (n = 2), IncN (n = 1), and IncFIC (n = 1) (Table 2).

Risk Factors and Clinical Outcomes of CRKA Infections

The risk factors and outcomes of patients with CRKA infections are shown in Table 3. The univariate analysis indicated that hepatobiliary disease, gastrointestinal disease, hypo-albuminaemia, urinary tract infection, skin infection, drainage tube use, urinary catheter use, central venous catheter use, previous exposure to penicillins and carbapenems were significantly more frequent in patients with CRKA infections (P< 0.05). A multivariate logistic regression analysis showed that hypo-albuminaemia (OR [Odd ratio]: 3.57, 95% CI [Confidence Interval]: 1.04–-12.48, P=0.044), drainage tube use (OR: 5.78, 95% CI:1.81–18.47, P=0.003), urinary catheter use (OR: 3.32, 95% CI:1.03–10.68, P=0.044), and previous exposure to carbapenems (OR: 5.00, 95% CI: 1.29–19.40, P=0.020) were independent risk factors for the acquisition of CRKA infections. For clinical outcomes, we found significant differences in post-culture length of stay (19 days vs 9 days; P =0.003) and total length of hospital stay (52.5 days vs 24 days; P <0.001) between cases and controls. In addition, 4

Table 2 Antibiotic Resistance Genes and Susceptibility Profiles in Donor, *E. Coli* EC600, and Transconjugants

	Donor Strains		Recipient Strain	Transconjugants	
	KA8	**KA9**	**EC600**	**KA8-TC**	**KA9-TC**
Resistance genes	bla_{NDM-1}, bla_{TEM}, $bla_{CTX-M-15}$, *acc(6')-Ib, armA, qnrD,* bla_{EBC}, bla_{ACC}	bla_{NDM-1}, bla_{TEM}, $bla_{CTX-M-15}$, *acc(6')-Ib, armA, qnrD,* bla_{EBC}, bla_{ACC}	NA	bla_{NDM-1},bla_{TEM}, $bla_{CTX-M-15}$, *acc (6')-Ib, armA*	bla_{NDM-1}, bla_{TEM}, $bla_{CTX-M-15}$, *acc (6')-Ib, armA*
Plasmid replicon type	IncFIIAs	IncFIIAs	NA	IncFIIAs	IncFIIAs
Minimum inhibitory concentration (mg/mL)					
Imipenem	64	64	≤1	8	8
Meropenem	128	128	≤1	4	8
Ertapenem	256	256	≤0.5	8	16
Ceftriaxone	≥64	≥64	≤1	≥64	≥64
Ceftazidime	≥64	≥64	≤1	32	32
Cefepime	≥64	≥64	≤1	32	32
Ciprofloxacin	≥4	≥4	≤0.25	≤0.5	≤0.5
Levofloxacin	≥8	≥8	≤0.5	≤0.5	≤0.5
Gentamycin	≥16	≥16	≤1	4	4
Amikacin	≥64	≥64	≤1	16	8
Tobramycin	≥16	≥16	≤1	8	8

Abbreviations: KA, Klebsiella aerogenes; NA, not available; TC, transconjugant.

(11.1%) patients infected with CRKA and 5 (3.8%) patients infected with CSKA died during hospitalization, and no significant difference in in-hospital mortality rate was observed between the two groups.

Discussion

Despite the fact that *Enterobacter cloacae* is now the most frequently isolated *Enterobacter* sp. in clinical settings and the species expressing the widest panel of new β-lactamases or carbapenemases, *K. aerogenes* more easily causes septic shock in infected patients, is associated with higher mortality (39% of patients), shows greater virulence, and have a broad ability to acquire antibiotic resistance mechanisms.[4] Consequently, the issue of CRKA infection deserves particular attention. Our main findings of the present study were presented as follows:

First, previous studies indicated that the mechanisms underlying carbapenem resistance in *K. aerogenes* were carbapenemase-independent and were attributed to both AmpC β-lactamase over-expression and mutations affecting membrane permeabilities.[4] However, in this study, we have demonstrated that carbapenem resistance in *K. aerogenes* was mainly associated with the over-expression of ESBLs and/or AmpC enzymes coupled with the efflux pumps, highlighting that efflux pump inhibitors (EPIs) could be used as an antibiotic adjuvant in CRKA infections. Of note, while the prevalence of carbapenemases was particularly low (3/36, 8.33%) in CRKA isolates, those carbapenemase-producing strains, which also co-expressed other drug-resistance determinants, showed the highest MICs for multiple antibiotics. Notably, even though no specific carbapenem-resistance mechanisms were detected, ten isolates (ESBL only, n=1; AmpC only, n=6; none, n=3) showed MIC values of ≥2 mg/L for ertapenem (Table 1), and the possible carbapenem-resistance mechanism might be due to mutations in penicillin-binding proteins (PBPs). To the best of our knowledge, this is also the first report on CRKA isolate co-harboring bla_{NDM-1}, $bla_{CTX-M-15}$, bla_{EBC}, bla_{ACC}, a*cc (6)-Ib, armA, qnrD* with loss of *ompE36* in China.

Second, we performed conjugation experiments on carbapenemase-producing strains, and found that all the bla_{NDM-1} plasmids could be successfully transferred by conjugation. But the repeated transfer of bla_{KPC-2} was unsuccessful. The most plausible explanation of this result is that bla_{NDM-1} was carried on self-transmissible plasmids, while bla_{KPC-2} was located on non-self-transmissible plasmids or on the chromosome. Previous studies have shown the emergence of bla_{NDM-1} in China,

Table 3 Univariate and Multivariate Analyses of Risk Factors and Outcomes for Patients Infected with CRKA Isolates Compared with CSKA Isolates

Variable	CRKA	CSKA	Univariate Analysis		Multivariate Analysis	
	(N=36)	(N=131)	OR (95% CI)	P-value	OR (95% CI)	P-value
Demographics, n (%)						
Elderly (≥ 60 years)	17(47.2%)	68(51.9%)	0.829(0.396–1.735)	0.618		
Male gender	26(77.2%)	88(67.2%)	1.270(0.562–2.871)	0.564		
Surgical units	17(47.2%)	62(47.3%)	0.996(0.476–2.871)	0.991		
Transfer from another hospital	14(38.9%)	51(38.9%)	0.998(0.468–2.127)	0.996		
Admission to ICU	13(36.1%)	42(32.1%)	1.198(0.553–2.594)	0.647		
Comorbid conditions, n (%)						
Hypertension	9(25%)	36(27.5%)	0.880(0.277–2.050)	0.766		
Malignant disease	11(30.6%)	37(28.2%)	1.118(0.500–2.500)	0.786		
Cardiovascular disease	6(16.7%)	17(13.0%)	1.341(0.487–3.697)	0.504		
Chronic kidney disease	6(16.7%)	23(17.6%)	0.939(0.351–2.516)	0.901		
Hepatobiliary disease	15(41.7%)	26(19.8%)	2.885(1.31–6.35)	**0.007**		
Gastrointestinal disease	14(38.9%)	28(21.4%)	2.341(1.063–5.156)	**0.032**		
Respiratory diseases	15(41.7%)	39(29.8%)	1.69(0.79–3.61)	0.177		
Neurological disease	4(11.1%)	25(19.1%)	0.53(0.172–1.636)	0.263		
Endocrine, metabolic disease	2(5.6%)	2(1.5%)	3.794(0.516–27.924)	0.203		
Vascular, hematological disease	10(27.8%)	20(15.3%)	2.135(0.893–5.10)	0.083		
Hypoalbuminaemia	29(80.6%)	61(46.6%)	4.754(1.945–11.622)	**<0.001**	3.569(1.036–12.481)	**0.044**
Urinary tract infection	9(25%)	15(11.5%)	2.578(1.021–6.510)	**0.04**		
skin infection	8(22.2%)	10(7.6%)	3.457(1.252–9.554)	**0.028**		
Respiratory infection	16(44.4%)	41(31.3%)	1.756(0.826–3.733)	0.141		
Invasive procedures within prior 4 weeks, n (%)						
Surgery in the past 6 months	19(52.8%)	53(40.5%)	1.645(0.784–3.453)	0.186		
Receipt of total parenteral nutrition	0(0%)	1(0.8%)	0.783(0.723–0.848)	1		
Mechanical ventilation	10(27.8%)	30(22.9%)	1.295(0.562–2.986)	0.544		
Bladder irrigation	4(11.1%)	4(3.1%)	3.969(0.941–16.736)	0.067		
Drainage tube	25(69.4%)	44(33.6%)	4.494(2.026–9.966)	**<0.001**	5.777(1.807–18.468)	**0.003**
Urinary catheter	27(75.0%)	51(38.9%)	4.706(2.048–10.815)	**<0.001**	3.321(1.032–10.684)	**0.044**
Tracheal cannula	16(44.4%)	42(32.1%)	1.695(0.798–3.590)	0.167		
Nasal catheter	0(0%)	7(5.3%)	0.775(0.713–0.842)	0.348		
Central venous catheter	10(27.8%)	10(7.6%)	4.654(1.758–12.320)	**0.003**		
Antimicrobial exposure within 3 months, n (%)						
Penicillins	14(38.9%)	23(17.6%)	2.988(1.33–6.698)	**0.006**		
Cephalosporins	23(63.9%)	64(48.9%)	1.852(0.865–3.966)	0.11		
Carbapenems	13(36.1%)	18(13.7%)	3.55(1.53–8.24)	**0.002**	5.007(1.292–19.406)	**0.02**
Fluoroquinolones	5(13.9%)	4(3.1%)	5.121(1.298–20.196)	**0.023**		
Aminoglycosides	3(8.3%)	2(1.5%)	5.864(0.941–36.538)	0.068		
Glycopeptides	7(19.4%)	11(8.4%)	2.633(0.929–7.382)	0.071		
Clinical outcomes n (%)						
In-hospital mortality	4(11.1%)	5(3.8%)	3.15(0.80–12.41)	0.102		
Functional status deterioration	6(16.7%)	9(25%)	2.711(0.896–8.206)	0.095		
Post-culture length of stay, median (IQR), days	19(6.75–30)	9(4–18)	NA	**0.003**		
Total length of hospital stay, median (IQR), days	52.5 (31.25–101.75)	24(14–42.5)	NA	**<0.001**	1.008(1.001–1.014)	**0.016**

Notes: Data are expressed as n (%) of patients for categorical variables and median (IQR) for continuous variables as appropriate. Bold face indicates values that are significant (P<0.05).

Abbreviations: CRKA, carbapenems-resistant K. aerogenes; CSKA, carbapenems-sensitive K. aerogenes; ICU, intensive care unit; OR, odds ratio; CI, confidence interval; IQR, interquartile range; NA, not available.

with most of them being carried on the IncFIIAs-type plasmids.[17] In this study, plasmids from all the bla_{NDM-1}-carrying strains (including those in the donors and the transconjugants) also belonged to the plasmid replicon type IncFIIAs. In addition, the plasmids containing bla_{KPC-2} were un-typable. It is worth noting that multiple resistance genes were found to be co-expressed in the same self-transmissible plasmids, which might have allowed the accumulation and spread of multiple drug-resistance determinants.

Third, no clonality relationship was identified among most of the CRKA isolates. Therefore, the prevalence of these CRKA isolates could not be attributed to the spread of clonal dissemination. Interestingly, the two bla_{NDM-1}-carrying isolates belonging to the PFGE pattern 20 were isolated from the same ICU ward in October 2014. This local spread from patient to patient appears to be caused by inadequate attention to infection control measures, especially handwashing.

Fourth, we initially performed an epidemiological investigation to assess the clinical predictors and outcomes for CRKA infections. In this retrospective case–control study, hypo-albuminaemia, drainage tube use, urinary catheter use, and previous carbapenem exposure were demonstrated to be independently associated with CRKA infections. One possible explanation for hypo-albuminaemia as an independent risk factor may be due to the hosts' poor immunity and functional status. Moreover, the invasive medical procedures might have destroyed their natural barrier functions, promoted the formation of microbial biofilms, and possibly led to catheter-related infections, thus increasing mortality in these patients.[18] Given that invasive procedures such as the use of drainage tube and urinary catheter were potential risk factors, infection control measures preventing the microbial colonization of the insertion sites are necessary. In addition, our study identified carbapenem exposure as being associated with CRKA infections for case patients, in agreement with previous reports on the assessment of CRE.[19] One possibility is that inappropriate antibiotic therapy may disrupt the gastrointestinal microflora and eradicate susceptible competing strains, thus elevating the incidences of CRE infections.[20] Considering that patients receiving carbapenem treatment may be exposed to further risk for CRKA isolation, it is crucial to maintain an effective administration of antimicrobial agents to avoid their further spread. Many outbreaks of CRE have been described in ICUs, where there were more patients with severe underlying diseases, thus conferring a greater possibility of contaminating life support equipment. Moreover, use of broad-spectrum antibiotics can further place patients at higher risk for poor outcomes.[21,22] However, admission to ICU was not demonstrated to be a significant risk factor. This could be explained by the limited cases in our study. For the clinical outcomes, our results demonstrated a significant difference in total length of stay between case and control group, probably due to the fact that the patients infected with CRKA were generally at a higher risk of poorer outcomes than those with CSKA infections. Unexpectedly, no statistical significance was observed in in-hospital mortality, probably due to the small size of CRKA cases in our study.

Our study had several limitations. First, we did not perform real-time reverse transcription PCR to determine the levels of expression of outer membrane porins and efflux pumps in mRNA levels. Second, the significance of specific sequence types (STs) of CRKA remains unknown, and MLST is still needed for understanding the characteristics of each CRKA isolate. Finally, this was a retrospective single-center case–control study with a relatively small sample size, and our results might not be applicable to other settings.

In conclusion, this study provided the first comprehensive report of molecular epidemiology, carbapenem-resistance mechanisms, and risk factors for CRKA infections over a six-year period in one medical center. Our data showed that the production of ESBL and/or AmpC enzymes coupled with the efflux pumps was the main carbapenem-resistance mechanism for CRKA. As far as we know, this is the first report on MDR *K. aerogenes* isolate co-harboring bla_{NDM-1}, $bla_{CTX-M-15}$, bla_{EBC}, bla_{ACC}, *acc (6')-Ib, armA, qnrD* with loss of OmpE36 in China. In addition, our findings showed that hypo-albuminaemia, invasive procedures such as drainage tube and urinary catheter use, and previous carbapenem exposure could be independently associated with the CRKA infections. Therefore, appropriate antimicrobial therapy and strict infection control measures are critical to reduce the frequency of CRKA infections.

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Author Contributions

All authors contributed to data analysis, drafting and revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest.

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