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The resistance mechanisms and risk factors of carbapenem-resistant Klebsiella pneumoniae to ceftazidime-avibactam

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Short Report

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Abstract

Objective To investigate the molecular epidemiological characteristics of carbapenem-resistant *Klebsiella pneumoniae*(CRKP),elucidate the resistance mechanisms to ceftazidime-avibactam (CZA) and identify the risk factors associated with CZA resistance. Clinical and microbiological data from 293 hospitalized patients with CRKP infections were retrospectively collected at the First Affiliated Hospital of Xi'an Jiaotong University from January 2024 to March 2025. Based on CZA susceptibility results, patients were divided into the CZA-sensitive CRKP group (n = 228) and the CZA-resistant CRKP group (n = 64). The colloidal gold detection method was used to identify five carbapenemase genes(*bla*KPC, *bla*NDM, *bla*VIM, *bla*IMP, and *bla*OXA)and their subtypes were determined through PCR amplification and sanger sequencing. The relative expression of the *bla*KPC gene was measured using real-time quantitative PCR (RT-qPCR). Multivariate logistic regression analysis was performed to identify risk factors for CZA-resistant CRKP infections.

Results The primary resistance mechanisms for CZA-resistant CRKP in our hospital are the production of metal enzymes, especially KPC-2 and NDM-1 co-producing strains. Some strains exhibit resistance to CZA due to blaKPC-2 mutations and increased gene expression. Multivariate logistic regression analysis revealed that renal replacement therapy (*OR* = 2.611, 95% *CI* 1.192–5.721) and prior CZA exposure (*OR* = 2.749, 95% *CI* 1.269 ~ 5.953) were independent risk factors for CZA-resistant CRKP infections.

Introduction

Klebsiella pneumoniae is recognized as an opportunistic pathogen that evolves into the highly concerning superbug carbapenem-resistant *Klebsiella pneumoniae* (CRKP) upon acquiring plasmids that harbor carbapenemase resistance genes[1]. The primary mechanisms conferring resistance in CRKP include the synthesis of carbapenemases, which encompass class A enzymes (KPC), class B enzymes (NDM, IMP), and class D enzymes (OXA-48), alongside the loss of porins OmpK35 and/or OmpK36[2]. According to the China Antimicrobial Drug Monitoring Network (CHINET)(http://www.chinets.com/), the resistance rate of *Klebsiella pneumoniae* to meropenem has surged from 2.9% in 2005 to 23.4% in 2024. The increasing prevalence of drug-resistant phenotypes and the dissemination of mobile genetic elements contribute to a continuous rise in CRKP cases globally, significantly limiting antibiotic treatment options and posing a major challenge for clinical management and infection control[3].

Ceftazidime-avibactam (CAZ/AVI) as a novel combination of ceftazidime and the β-lactamase inhibitor, has been approved by the Food and Drug Administration (FDA) for treatment of complicated intra-abdominal and urinary tract infections[4]. It can inhibit the activity of Ambler class A, class C and class D β-lactamases, however, it is ineffective against class B metallo-β-lactamases (MBL)[3, 5]. In recent years, CZA has demonstrated considerable efficacy in treating KPC-producing *Klebsiella pneumoniae* (KPC-Kp), leading to improved clinical survival outcomes. Nonetheless, the increased utilization of CZA has been paralleled by a rising incidence of CZA-resistant CRKP in various regions[3, 6]. In 2024, CHINET reported a resistance rate of 15.8% for CRKP against CZA(http://www.chinets.com/).Patients infected with CZA-

resistant CRKP had higher 28-day and in-hospital mortality rates[7].This emerging resistance has created new challenges for selecting effective therapies[8].

In order to facilitate the proactive monitoring of resistance to ceftazidime-avibactam and curb its dissemination, we systematically investigated the predominant resistance mechanisms, thereby establishing a theoretical framework to enhance clinical drug utilization and resistance management within our institution. Moreover, there exists a notable gap in the systematic analysis of risk factors pertinent to CZA-resistant CRKP infections. Consequently, this study amalgamates molecular biology testing and clinical epidemiological data to conduct a thorough analysis of the risk factors associated with CZA-resistant CRKP, thereby providing a scientific foundation for the early identification of high-risk patients.

Methods

Study design and patients

We retrospectively collected clinical data from 293 hospitalized CRKP-infected patients at the First Affiliated Hospital of Xi'an Jiaotong University between January 2024 and March 2025. Inclusion criteria: (a) micro-

biologically confirmed CRKP bacteremia within 48 h of admission;(b) Inclusion of CZA susceptibility testing; (c) Complete clinical data. Exclusion criteria: (a) immunocompromised individuals; (b) age < 18 years;(c) patients exhibiting multiple positive cultures for the same pathogen during the same hospital stay, with each counted only once.The study protocol was approved by the Medical Ethics Review Committee of the First Affiliated Hospital of Xi'an Jiaotong University.

Clinical data collection

Date extracted from the hospital's electronic medical record system included their comorbidities,invasive procedures,laboratory results,microbiological profiles,prior antibiotic exposure and outcomes.The 293 CRKP infection patients were divided into CZA-resistant CRKP group and CZA-sensitive CRKP group based on CZA susceptibility results.

Strain identification and susceptibility testing

Isolates were identified by matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS) and antimicrobial susceptibility testing (AST) was performed using the VITEK-2 system (bioMérieux SA, Marcy l'Etoile, France).The minimum inhibitory concentration (MIC) of CZA was evaluated using E-test strips, with results interpreted according to Clinical and Laboratory Standards Institute(CLSI) guidelines.Polymyxins interpretation followed standards of the American Committee on

Antimicrobial Susceptibility Testing[9]. Tigecycline interpretation followed the standards of the U.S. Food and Drug Administration. Quality control strains (*Escherichia coli* ATCC 25922,ATCC35218 and *Pseudomonas aeruginosa* ATCC 27853) were included in all AST procedures to ensure methodological consistency.

Carbapenemase detection

- (1) The detection of carbapenemase production was executed utilizing the Dynamiker CGI test (Dynamiker Biotechnology, Tianjin, China), with colloidal gold immunoassay kits employed according to the manufacturer's instructions.(2)Carbapenemase Genes type (*bla*KPC, *bla*IMP, *bla*NDM, *bla*VIM and *bla*OXA-48)were detected by polymerase chain reaction (PCR) followed by Sanger sequencing (Qingke Biotechnology) and were compared with available sequences in GenBank.The primer sequences for PCR referenced from the literature[10].

Quantitative real-time PCR

Ten *Klebsiella pneumoniae* strains were analyzed through qualitative real-time PCR (qRT-PCR) to evaluate the relative expression of *bla*KPC, normalized against an internal housekeeping gene, rpoB. Total RNA was isolated from bacteria utilizing TRIzol reagent (Invitrogen China Limited, Beijing, China), with RNA subsequently reverse transcribed into complementary DNA (cDNA) using the ARScript 1st Strand cDNA synthesis kit (AccuRef Scientific, Xi'an, China). Quantitative Real-Time PCR was performed using HS Universal qPCR Master Mix (ACE Biotechnology) according to the manufacturer's instructions.Mean Ct values were used to calculate relative transcript levels by the 2^(−ΔΔCT) method.The qPCR primer sequences were qKPC-F (5'-CGGAACCTGCGGAGTGTATGG-3')and qKPC-R (5'-CGCTGTGCTTGTCATCCTTGTTA-3') for blaKPC gene, RPOB-F (5'-TGTAGAGCGTGCGGTGAAAGAG-3') and RPOB-R (5'-GGAAATCGGCTTGGCGTTGATC-3') for rpoB gene.

Statistical analysis

All analyses were performed in SPSS 26.0. continuous variables were presented as medians and interquartile ranges (IQR),the Student's t test and Mann-Whitney U test for continuous data.While categorical variables were reported as numbers and percentage (%) and evaluated with Chi-square test or Fisher's exact test. RT-qPCR data were analyzed using Welch's t-test.Potential risk factors were first screened by univariate analysis,variables with $P < 0.05$ were entered into a multivariate logistic regression to identify independent predictors. $P < 0.05$ was considered statistically significant.

Results

Specimen Sources and departmental distribution of CRKP Strains

The 64 CZA-resistant CRKP strains were mainly sourced from Sputum and bronchoalveolar lavage fluid(45.32%), they were primarily isolated from the Intensive Care Unit (ICU) (29.69%) and surgical ICU (34.38%).see Table 1.

Table 1
Specimen Sources and departmental distribution of CRKP Strains

Source	CZA-Susceptible Group(n = 228)		CZA-Resistant Group (n = 64)	
	Number of Isolates	Proportion(%)	Number of Isolates	Proportion(%)
Specimen types				
Sputum and Lavage Fluid	103	45.17	29	45.32
Whole blood	34	14.91	5	7.81
Urine	34	14.91	11	17.19
Drainage fluid	15	6.58	6	9.38
Bile	14	6.14	2	3.13
Rectal swab	8	3.51	4	6.25
Catheter	7	3.07	3	4.69
Ascites	6	2.63	3	4.69
Others	7	3.07	1	1.56
Departmental Distribution				
Critical Care Medicine	80	35.09	19	29.69
Surgical ICU	42	18.42	22	34.38
Hepatobiliary Surgery	20	8.77	3	4.69
LiverTransplantation Department	19	8.33	2	3.13
Hematology	10	4.39	-	-
Rehabilitation	10	4.39	-	-
Respiratory RCU	7	3.07	4	6.25
Cardiovascular Surgery	4	1.75	-	-
Neurology	5	2.19	-	-
Urology	4	1.75	1	1.56
Emergency Center	4	1.75	6	9.38
Department of Infectious Diseases	4	1.75	2	3.13
Nephrology	3	1.32	1	1.56

Source	CZA-Susceptible Group(n = 228)		CZA-Resistant Group (n = 64)	
	Number of Isolates	Proportion(%)	Number of Isolates	Proportion(%)
Specimen types				
Neurosurgery	4	1.75	2	3.13
Others	12	5.26	2	3.13

Table 2
Resistance of CZA-sensitive and CZA-resistant CRKP to common antibiotics

Antimicrobial agent	CZA-sensitive CRKP (n = 228)	CZA-resistant CRKP(n = 64)	χ^2	*P*
Ceftazidime	227 (99.56)	63 (98.44)	-	0.391
Cefepime	225 (98.68)	62 (96.88)	0.19	0.659
Cefotetan	220 (96.49)	60 (93.75)	0.38	0.535
Piperacillin/tazobactam	227 (99.56)	64 (100.00)	-	1.000
Cefoperazone/sulbactam	227 (99.56)	64 (100.00)	-	1.000
Ticarcillin/clavulanate	224 (98.25)	57 (89.063)	9.23	0.002
Aztreonam	227 (99.56)	60 (93.75)	6.87	0.009
Amikacin	191 (83.77)	44 (68.75)	7.18	0.007
Gentamicin	195 (85.53)	48 (75.00)	3.97	0.046
Tobramycin	195 (85.53)	56 (87.50)	0.16	0.688
Ciprofloxacin	227 (99.56)	61 (95.31)	-	0.034
Levofloxacin	226 (99.12)	57 (89.06)	13.73	< .001
Imipenem	227 (99.56)	57 (89.06)	16.92	< .001
Meropenem	228 (100.00)	60 (93.75)	-	0.002
Trimethoprim/sulfamethoxazole	182 (79.83)	42 (65.63)	5.64	0.018
Tigecycline	4 (1.75)	0 (0.00)	-	0.58
Colistin	62 (27.19)	28 (43.75)	6.43	0.011
Ceftazidime/avibactam	0 (0.00)	64 (100.00)	292.00	< .001

Table 3
Distribution of carbapenemase gene types detected by two methods in CZA-resistant CRKP strains

Carbapenemse genotype	Colloidal Gold		PCR	
	Number of Isolates	Proportion(%)	Number of Isolates	Proportion(%)
*bla*NDM + *bla*KPC	27	42.18	27	42.18
blaKPC-2 + *bla*NDM-1	-	-	26	40.62
*bla*KPC-2 + *bla*NDM-4	-	-	1	1.56
*bla*KPC	9	14.06	10	15.63
*bla*KPC-33	-	-	5	7.81
*bla*KPC-90	-	-	2	3.13
*bla*KPC-2	-	-	2	3.13
*bla*KPC-134	-	-	1	1.56
*bla*NDM	23	35.94	23	35.94
*bla*NDM-1	-	-	12	18.75
*bla*NDM-5	-	-	10	15.63
*bla*NDM-4	-	-	1	1.56
*bla*NDM + *bla*IMP	1	1.56	1	1.56
*bla*NDM-1 + *bla*IMP-4	-	-	1	1.56
Negative	4	6.25	3	4.69

Table 4
Comparison of clinical characteristics between patients with CZA-sensitive and CZA-resistant CRKP

Variable	CZA-Susceptible Group (n = 228)	CZA-Resistant Group (n = 64)	*P*
Age, M (Q_1, Q_3)	60.00 (51.00, 70.00)	64.00 (53.00, 71.50)	0.374
APACHE II, M (Q_1, Q_3)	6.50 (0.00, 17.25)	11.50 (0.00, 19.00)	0.059
SOFA, M (Q_1, Q_3)	1.50 (0.00, 7.00)	4.00 (0.00, 7.25)	0.066
Pre-CRKP infection hospital stay, M (Q_1, Q_3)	12.00 (4.00, 21.00)	21.00 (10.50, 34.75)	< .001
Total hospital stay, M (Q_1, Q_3)	24.50 (15.00, 36.25)	35.50 (24.75, 61.00)	< .001
Laboratory tests, M (Q_1, Q_3)			
White blood cell count	9.51 (5.63, 13.09)	8.99 (6.21, 12.52)	0.983
Platelets	115.00 (49.00, 226.50)	154.00(73.25,248.25)	0.057
Hemoglobin	89.50 (78.75, 101.00)	92.00 (83.00, 105.00)	0.164
Albumin	31.65 (28.68, 35.05)	31.85 (27.88, 34.83)	0.615
Procalcitonin	0.54 (0.18, 1.91)	0.74 (0.22, 4.10)	0.106
Gender, male	163 (71.49)	41 (64.06)	0.252
Comorbidities			
Hypertension	94 (41.23)	37 (57.81)	0.026
Diabetes mellitus	63 (27.63)	14 (21.88)	0.356
Malignant tumor	52 (22.81)	9 (14.06)	0.128
Respiratory disease	14 (6.14)	7 (10.94)	0.299
Renal disease	19 (8.33)	10 (15.63)	0.085
History of stroke	56 (24.56)	16 (25.00)	0.943
Chronic liver disease	34 (14.91)	14 (21.88)	0.184
Coronary heart disease	48 (21.05)	8 (12.50)	0.125
History of ICU admission	134 (58.77)	49 (76.56)	0.009
Septic shock	60 (26.32)	28 (43.75)	0.007
Previous invasive procedures			
Indwelling urinary catheter	150 (65.79)	56 (87.50)	< .001
Central venous catheter	177 (77.63)	58 (90.63)	0.02

Variable	CZA-Susceptible Group (n = 228)	CZA-Resistant Group (n = 64)	*P*
Mechanical ventilation	132 (57.90)	50 (78.13)	0.003
Tracheostomy/Intubation	117 (51.32)	43 (67.19)	0.024
Percutaneous drainage	116 (50.88)	32 (50.00)	0.901
Surgery	100 (43.86)	25 (39.06)	0.493
Renal replacement therapy	56 (24.56)	34 (53.13)	< .001
Nasogastric tube	137 (60.09)	50 (78.13)	0.008
Bronchoscopy	98 (42.98)	39 (60.94)	0.011
Co-infections			
Bloodstream infection	34 (14.91)	5 (7.81)	0.140
Pulmonary infection	103 (45.18)	29 (45.31)	0.984
Abdominal infection	16 (7.02)	10 (15.63)	0.033
Urinary tract infection	34 (14.91)	11 (17.19)	0.656
Other infections	45 (19.74)	8 (12.50)	0.184
Previous antimicrobial exposure			
β-Lactam/β-Lactamase inhibitor combination	144 (63.16)	47 (73.44)	0.127
Carbapenems	150 (65.79)	43 (67.19)	0.835
Quinolones	42 (18.42)	12 (18.75)	0.952
Aminoglycosides	8 (3.51)	4 (6.25)	0.535
Tigecycline	33 (14.47)	17 (26.56)	0.023
Polymyxins	41 (17.98)	25 (39.06)	< .001
Ceftazidime-avibactam	34 (14.91)	28 (43.75)	< .001
Glycopeptides	88 (38.60)	36 (56.25)	0.012
Linezolid	50 (21.93)	17 (26.56)	0.436
Poor prognosis	73 (32.02)	31 (48.44)	0.015

Table 5
Multivariate analysis of risk factors for ceftazidime–avibactam resistant carbapenem-resistant *Klebsiella pneumoniae* infection

Variables	*β*	*S.E*	*Z*	*P*	*OR* (95%*CI*)
Renal replacement therapy	0.960	0.400	2.398	0.016	2.611 (1.192 ~ 5.721)
Previous CZA exposure	1.011	0.394	2.565	0.010	2.749 (1.269 ~ 5.953)

Resistance of CRKP strains to common antibiotics

Compared with the CZA-susceptible group, the CZA-resistant CRKP group had lower resistance rates to piperacillin/tazobactam, aztreonam, amikacin, gentamicin, ciprofloxacin, levofloxacin, imipenem, and meropenem, but higher resistance to colistin (all $P < 0.05$). All other antibiotics showed no significant differences($P > 0.05$).

Carbapenemase genotype of 64 CZA-resistant CRKP Strains

Colloidal gold detection revealed that 60 of 64 CZA-resistant CRKP strains tested positive for carbapenemase genes. Among these, 27 strains exhibited co-production of KPC and NDM, 23 produced NDM exclusively, 1 strain co-produced NDM and IMP, 9 strains generated KPC alone, and 4 strains were negative for carbapenemases. PCR and sequencing confirmed carbapenemase genes in 61 isolates, yielding 98.4% concordance with the colloidal-gold assay. Notably, one isolate carrying blaKPC-134 was missed by the colloidal-gold assay.

Relative expression of blaKPC in KPC-KP

To assess whether blaKPC expression level drives CAZ-AVI resistance,10 CZA resistant and ATCC BAA-1705 strains were identified. The results showed that expression levels of *bla*KPC were significantly higher in 8 of the 10 KPC-KP strains compared with the control strain *Klebsiella pneumoniae* ATCC BAA-1705($p < 0.05$),while isolates KP-63 and KP-70 exhibited no elevation.

Risk factor for ceftazidime-avibactam resistant CRKP infections

Univariate analysis showed that patients in the CZA-resistant CRKP group had a higher proportion of hypertension, ICU admission history, septic shock, prior invasive procedures (such as indwelling catheters, central venous catheters, mechanical ventilation, tracheostomy/intubation, renal replacement therapy, nasogastric tubes, bronchoscopy), prior antibiotic exposure to tigecycline, polymyxins, ceftazidime-avibactam, and glycopeptide antibiotics, and poor prognosis compared to the CZA-sensitive CRKP group. Additionally, the length of hospitalization prior to CRKP infection and total length of hospitalization were longer in the CZA-resistant CRKP group. Variables with $P < 0.05$ in univariate analysis were included in multivariate analysis, which further revealed that renal replacement therapy

(*OR* = 2.611,95% *CI* 1.192–5.721) and prior exposure to ceftazidime-avibactam (*OR* = 2.749,95% *CI* 1.269 ~ 5.953) were independent risk factors for CZA-resistant CRKP infections.

Discussion

This study found that the 64 CZA-resistant CRKP strains mainly originated from respiratory specimens (45.32%), which is similar to the results of previous studies[11]. The strains were mainly distributed in the intensive care unit,where immunosuppressed patients and invasive procedures increase infection risk. Studies have shown that there is a significant correlation between ICU admission history and the incidence of CRKP infection[12].The closed ICU environment allows for easy spread of resistant bacteria among patients, highlighting the need for improved infection control and shorter ICU stays to prevent CRKP infections.

Compared with the CZA-susceptible CRKP patients, the CZA-resistant group showed increased polymyxin resistance, but the resistance rate to some β-lactams was actually decreased. This phenotype may be related to the production of KPC variant strains. Studies have shown that KPC variant strains are more likely to regain sensitivity to meropenem while exhibiting higher levels of CZA resistance [13]. we found that the imipenem MIC of most KPC variant strains was reduced (≤ 4μg/mL), while acquiring high-level CZA resistance (MIC ≥ 64μg/mL). Wild-type KPC-2 strains exhibited low CZA resistance (MIC < 64μg/mL), consistent with previous studies[13].

In this study,Genotyping of CZA-resistant CRKP by colloidal-gold assay and sequencing showed 98.44% concordance.One strain that produced only KPC enzyme was negative by colloidal gold, but identified as blaKPC-134 by sequencing. The colloidal-gold may produce false-negative results for KPC variants, which can lead to incorrect clinical decisions and treatment failure, Molecular detection technology can overcome this problem[14]. A study results showed that the positive percentages of three colloidal gold immunochromatographic methods for 16 KPC-2 variants were 87.5%, 87.5% and 68.8% compared with GeneXpert Carba-R[14].Negative colloidal gold immunochromatographic results that don't match resistance phenotypes should be retested with another kit or GeneXpert Carba-R for better accuracy. In this study, among the 64 CZA-resistant CRKP strains, 50 strains produced NDM, of which 42.18% were co-producing KPC and NDM, 35.94% were blaNDM, and in addition, one strain co-producing NDM and IMP metalloenzyme was found, indicating that the production of metalloenzyme, especially the co-production of KPC and NDM, is the main mechanism of CZA resistance in CRKP in this hospital. In addition to the production of metalloenzyme, KPC mutation is the main mechanism of high-level CZA resistance in this region, which is similar to the results of a study in Taiwan[15]. Studies have shown that the genomes carrying KPC and NDM are mainly reported in China (45.1%) and the United States (23.0%) [13]. with a 3.5-fold increase in strains from 2018 to 2022.common combinations include KPC-2 plus NDM-1 (53.6%), KPC-2 plus NDM-5 (18.5%), and KPC-3 plus NDM-1 (14.1%). It is worth noting that the co-production of KPC and NDM in *Klebsiella pneumoniae* seems to originate from high-risk clones positive for KPC worldwide, which then acquired plasmids carrying NDM. The emergence of multi-

replicon plasmids carrying KPC and NDM is an important mechanism for pan-β-lactam resistance[16]. The emergence of KPC-NDM-CRKP leads to higher levels of antimicrobial resistance and extremely limited treatment options, which may further lead to increased mortality[17]. Therefore, clinical laboratories should actively carry out enzyme typing and genotyping detection. For strains producing double enzymes, clinicians can give combination therapy (ceftazidime-avibactam combined with aztreonam) based on enzyme typing and genotyping results to prevent the spread and prevalence of this resistant bacteria in medical institutions.

Since 2019, the incidence of new KPC gene subtypes has increased significantly. According to the NCBI database, more than 200 *bla*KPC gene variants have been found worldwide, half of which may have CZA resistance[18]. In addition, the spread of these *bla*KPC genes is mainly mediated by plasmids or small genetic elements on them, especially the Tn4401 transposon[8]. *Klebsiella pneumoniae* bearing with KPC variant often mislead clinical anti-infection treatment because of their unique antimicrobial susceptibility profile and the tendency of conventional carbapenemase assays to give false negative results. Therefore, timely identification of KPC variants and effective anti-infective therapy are key to saving infected patients[19].In this study, sequencing identified 10 KPC-only producers, eight of which carried variants: five blaKPC-33, two blaKPC-90, and one blaKPC-134. Although these three alleles have previously been reported in Beijing[20], Shanghai[21], Fujian[22], and Sichuan[19], but this is their first detection in Shaanxi.In addition, two isolates harboring wild-type blaKPC-2 were found. Studies have reported that wild-type KPC strains resistant to CZA is related to the high expression of blaKPC genes and the loss of outer membrane porins[23]. RT-qPCR revealed that *bla*KPC-2 expression in both isolates was significantly elevated compared with control strain *Klebsiella pneumoniae* ATCC BAA-1705, likely accounting for their CZA resistance. However, whether there is a combination of membrane porin loss and overexpression of efflux pumps needs further study. Although meropenem-vaborbactam is considered a salvage treatment for CZA-resistant KPC variant-producing *Klebsiella pneumoniae*[24]. However, this new agent remains unavailable in many regions, especially resource-limited settings,Consequently, evaluating alternative regimens such as ceftazidime–avibactam plus imipenem is urgently needed[3, 21].

Most studies mainly focus on the resistance mechanisms of CZA-resistant CRKP, with few on risk factors. The study showed that renal replacement therapy and previous CZA exposure as independent risk factors for CZA-resistant CRKP infection. Renal-replacement therapy promotes CRKP colonization and linadequate drug exposure, thereby increasing the risk of CAZ-AVI-resistant infection.[7]. In addition, it was also found that the proportion of previous CZA exposure in the CZA-resistant CRKP group was significantly higher than that in the CZA-susceptible CRKP group. Previous studies have shown that CZA treatment may be an independent risk factor for inducing mutations of blaKPC[25].New KPC mutations are usually associated with clinical use of CZA for 9–19 days[20, 25–26]. In this study, we found that KPC mutations were closely related to CZA treatment. Among the eight patients with KPC-mutant CRKP, six

had received CZA for a median of 11 days before resistance was detected, whereas the remaining two developed resistance without any CZA exposure, confirming previous studies[27].

Strengthen and limitation

Our study has several limitations. Firstly, the single-center retrospective study design may introduce selection bias. Secondly, molecular mechanisms such as porin loss and overexpression of efflux pumps were not thoroughly explored. Moreover, the limited sample size restricted the detection of rare resistance subtypes.

Conclusions

This study demonstrates that CZA-resistant CRKP in our hospital is driven primarily by metalloenzyme production—especially KPC-2/NDM-1 co-producers—while KPC mutations and over-expression are additional key mechanisms. Renal replacement therapy and a history of previous CZA exposure are identified as independent risk factors for CZA-resistant CRKP.

Declarations

Ethics approval

This retrospective study was conducted in accordance with the Declaration of Helsinki and was approved by the Medical Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University, which waived pan-informed consent.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Author Contribution

Xiaqin He and Meng Liu carried out the medical records database search, statistical analysis, and drafted the manuscript.Xiaoqian Wang, Sijia Li,Yi Zhang Zhe Liu and Xiaoqin Wang participated in the

design and coordination of the study and helped to draft the manuscript. All authors read and approved the final manuscript.

Data Availability

All data generated or analyzed in the study are included in the article and further inquiries can be directly contacted with the corresponding author Xiaoqin Wang via e-mail.

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Figures

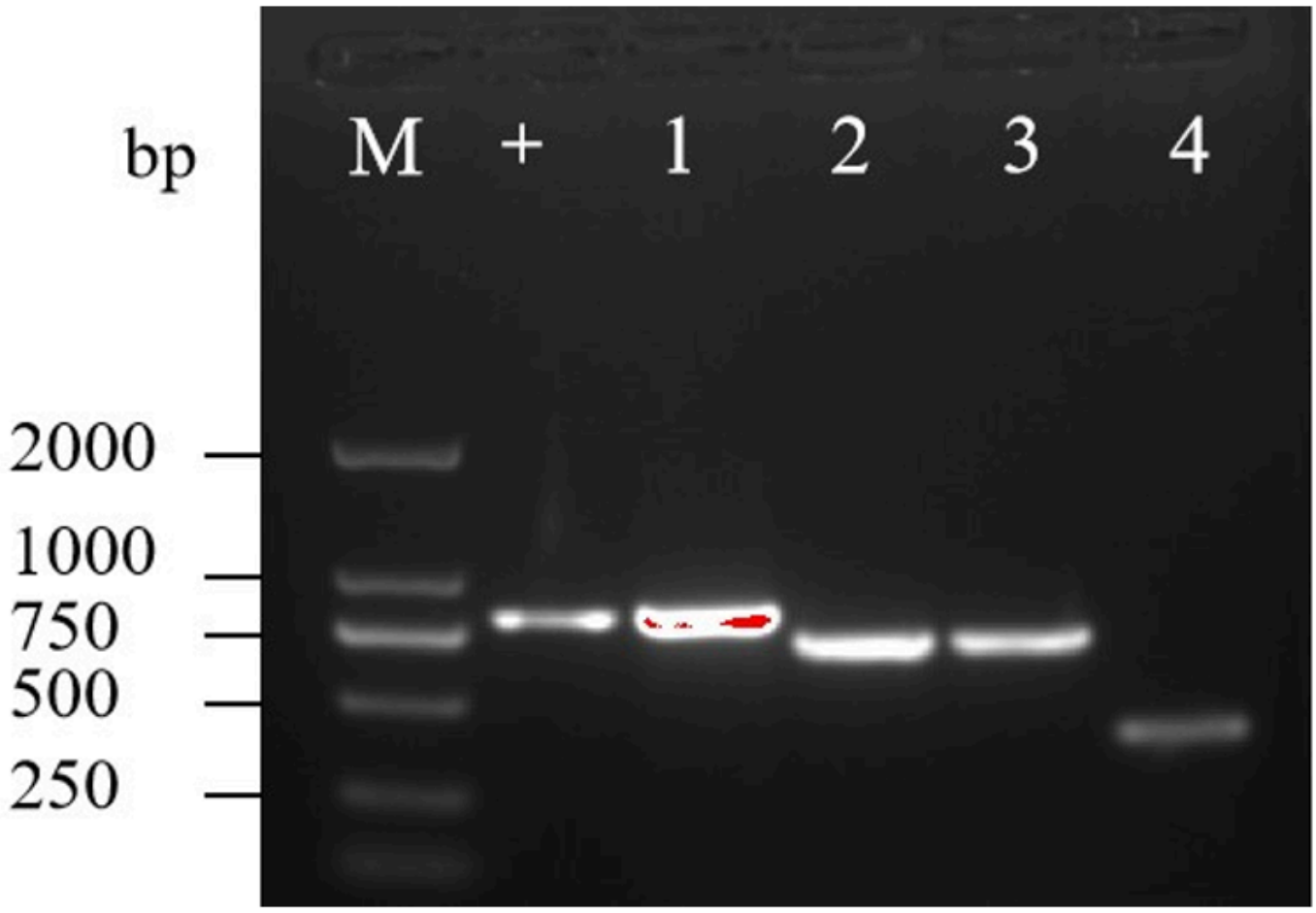

Figure 1

Detection of Carbapenemase genes by agarose gel electrophoresis. Lane M shows a 2000 bp molecular strand DNA ladder, + is the positive control for *bla*KPC, *bla*KPC gene; 1 Lanes show positive specimens (811bp), *bla*NDM gene; 2 and 3 Lanes show positive specimens (704bp),*bla*VIM gene; 4 Lanes show positive specimens (383bp).

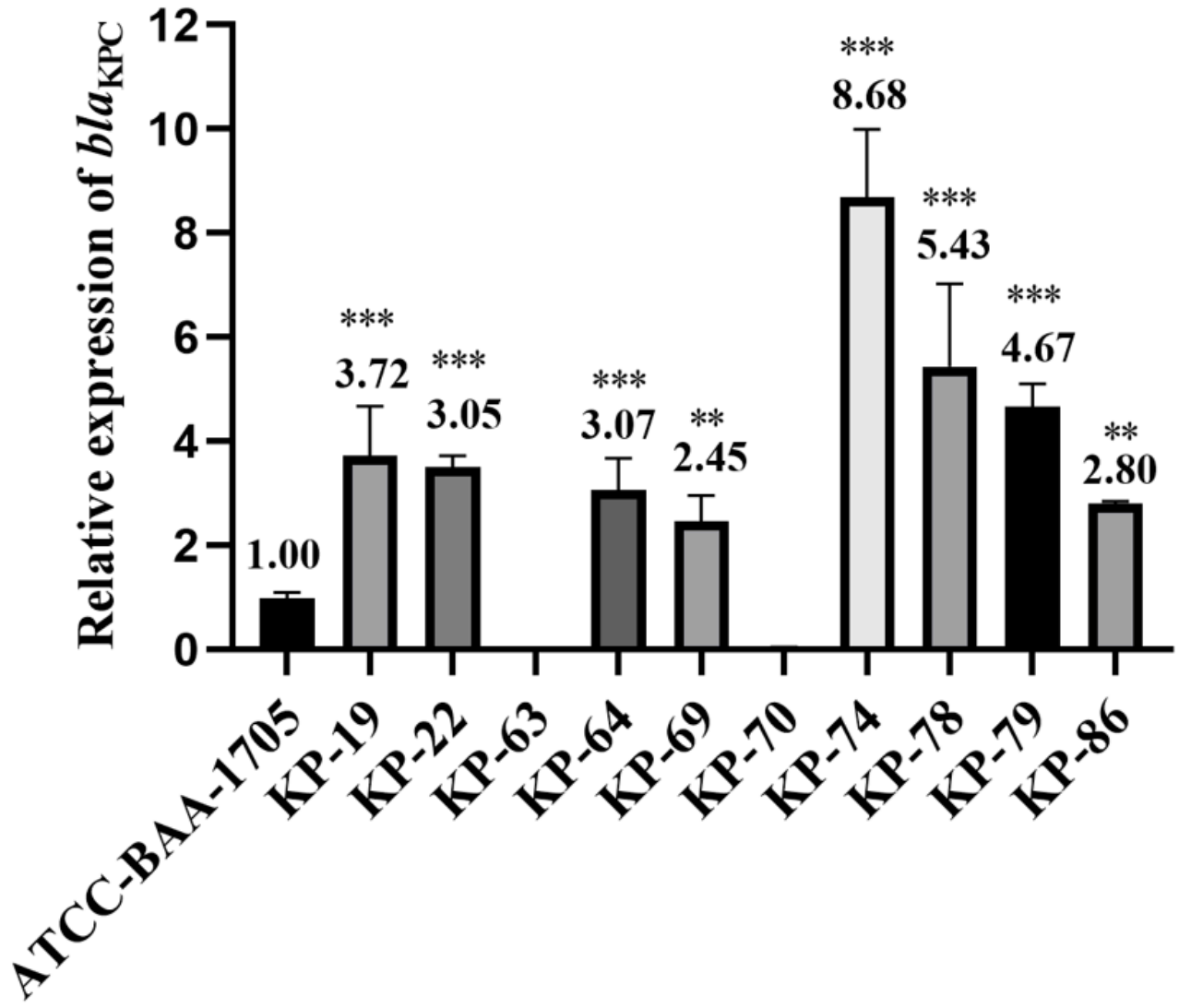

Figure 2

Relative expression of *bla*KPC in CZA-resistant CRKP.The relative expression levels of *bla*KPC were significantly higher in isolates KP-19, KP-22, KP-64, KP-69,KP-74,KP78,KP79 and KP86 compared with the control strain *Klebsiella pneumoniae* ATCC BAA-1705, *bla*KPC-33;isolates KP-19, KP-64, KP-69, KP-74 and KP-78 was positive,*bla*KPC-90;isolates KP-70 and KP-79 was positive, *bla*KPC-134; isolates KP-63 was postive;*bla*KPC-2;isolates KP-22 and KP-86 was postive.