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ORIGINAL RESEARCH

Poor Glycemic Control in Carbapenem-Resistant *Klebsiella pneumoniae* Infections: Impact on Epidemiological Features, Mortality Risks, and Polymyxin Resistance

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Purpose: This study aims to investigate the relationship between glycemic control and epidemiological characteristics of patients infected with carbapenem-resistant *Klebsiella pneumoniae* (CRKP), to identify mortality risk factors associated with CRKP infection, and to evaluate the impact of glucose on the resistance of CRKP to polymyxin and serum killing.

Patients and Methods: Clinical cases of 218 patients infected with CRKP were collected from a large tertiary public hospital in Anhui Province. We analyzed whether the glycemic control impacts the clinical and laboratory manifestations of infected patients. Logistic regression identified mortality risk factors. Antibiotic sensitivity, capsular serotypes, and virulence genes were tested of the strains. Three clinically isolated CRKP strains were used to investigate the effect of glucose on bacterial capsule synthesis and the impact on bacterial resistance to polymyxin and serum killing.

Results: Patients with poor glycemic control experienced more severe infections and had a higher likelihood of chronic kidney disease (CKD) and acute renal insufficiency compared to those with good glycemic control. They also exhibited an increased mortality rate. Logistic regression analysis identified age, glycosylated hemoglobin (HbA_{1c}) ≥7%, CKD, tumor, mechanical ventilation, and sepsis as independent risk factors for death associated with CRKP infection. A 0.5% (0.5 g/100mL) glucose environment can stimulate CRKP capsule synthesis, which is inhibitable by cyclic adenosine monophosphate (cAMP). Moreover, a high-glucose environment can enhance CRKP's resistance to polymyxin and serum killing.

Conclusion: A persistent hyperglycemic environment resulting from poor glycemic control may stimulate the synthesis of CRKP capsules, which could enhance the resistance of CRKP to polymyxin and serum killing, thereby further increasing the risk of patient mortality.

Keywords: carbapenem-resistant *Klebsiella pneumoniae*, poor glycemic control, polymyxin, serum resistance, capsule, diabetes

Introduction

Klebsiella pneumoniae (*K. pneumoniae*) is a Gram-negative bacillus and is currently one of the most common nosocomial pathogens worldwide. Based on its phenotypic and genotypic characteristics, it can be classified into classical *Klebsiella pneumoniae* (cKP) and hypervirulent *Klebsiella pneumoniae* (hvKP).[1] HvKP is more toxic and is a major cause of *K. pneumoniae*-related liver abscess (KLA).[2] Due to the widespread use of antibiotics, CRKP is increasing globally. Notably, in sequence typing (ST), ST11 is the predominant strain among CRKP in China, accounting for the vast majority of CRKP isolates.[3] The multidrug resistance and high mortality of CRKP pose significant challenges to clinical management.[4] Notably, CRKP infections are often more difficult to treat and cure in patients with diabetes.[5]

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Diabetes is a group of metabolic disorder diseases characterized by hyperglycemia.[6] Past studies have shown diabetes is mainly related to the occurrence of non-infectious metabolic diseases.[7] However, recent studies have shown that diabetes is also a key risk factor for the poor prognosis of infectious diseases.[8,9] Diabetes can be diagnosed by detecting the patient's HbA_{1c} level or blood glucose levels, including the fasting plasma glucose (FPG) value, the 2-hour glucose (2-h PG) value during a 75-g oral glucose tolerance test (OGTT), or random blood glucose levels. HbA_{1c} directly reflects the patient's average blood glucose level over nearly three months. Compared to blood glucose detection, HbA_{1c} offers several advantages, such as greater sensitivity and less variation during periods of stress, dietary changes, or illness.[6]

Studies have pointed out that diabetes increases the colonization and susceptibility of *K. pneumoniae*.[10] In patients with primary liver abscesses caused by hvKP, glycemic control significantly affect disease progression. Compared to non-diabetic infected patients, those with diabetes are more prone to metastatic infections, often have longer hospital stays, and are at a higher risk of death.[5,11,12] However, most of these previous studies focused on exploring the relationship between glycemic control and hvKP-related infections. Currently, there is insufficient discussion on the relationship between blood glucose levels and drug-resistant *K. pneumoniae* infections. Regarding whether blood glucose levels affect the susceptibility to drug-resistant *K. pneumoniae*, some studies suggest that patients with hyperglycemia are more likely to carry drug-resistant bacteria, particularly multidrug-resistant *K. pneumoniae*.[13–15] In this context, we need to further understand the impact of blood glucose levels on the epidemiological characteristics of patients infected with CRKP.

Capsular polysaccharide is one of the most important virulence factors of *K. pneumoniae*. Studies have shown that glucose can influence the synthesis of bacterial CPS, thereby affecting bacterial virulence. This process is regulated by the global regulator, the cyclic adenosine monophosphate (cAMP) receptor protein (CRP).[16] Additionally, it has been found that a hyperglycemic environment enhances bacterial resistance to neutrophil phagocytosis and leukocyte killing by stimulating the production of *K. pneumoniae* CPS and the expression of related genes, thereby further promoting the development of invasive syndromes.[17] In the process of controlling bacterial invasive infections, the clearance of pathogens by serum is also a crucial step.[1] However, few studies currently exist on how bacterial resistance to serum killing changes under hyperglycemic conditions.

Polymyxin is a cationic antimicrobial peptide that exerts bactericidal activity by disrupting the stability of the bacterial outer membrane, serving as a last line of defense in the treatment of CRKP. Among the polymyxin family, polymyxin B (PB) and polymyxin E are clinically used to treat drug-resistant Gram-negative bacilli due to their minimal nephrotoxicity. Unfortunately, polymyxin-resistant carbapenem-resistant *Klebsiella pneumonia* (PR-CRKP) has emerged[18] Both the CPS and lipopolysaccharide (LPS) are negatively charged outer membrane structures of *K. pneumoniae*, while the positively charged polymyxin exerts its effect primarily by binding to LPS. Lipopolysaccharide modification is the primary mechanism of polymyxin resistance in PR-CRKP. However, studies also suggest that the amount of capsule synthesis largely affects polymyxin resistance, as free capsules can neutralize polymyxin and decrease its binding to LPS.[19,20] *K. pneumoniae* with reduced capsule synthesis exhibits a lower survival rate in the presence of polymyxin,[21] while the addition of purified CPSs can enhance its resistance.[20] There are also studies pointing out that the hyperglycemic environment may affect polymyxin resistance by changing bacterial capsule expression. Fan et al found in alcohol-producing *K. pneumoniae* that glucose can enhance bacterial resistance to polymyxin by inducing capsule synthesis.[22] However, the specific effects of glucose on CRKP capsule synthesis and polymyxin resistance remain unclear.

This study aims to explore the impact of glycemic control on the epidemiological characteristics of patients infected with CRKP and to analyze the risk factors for infection-related mortality during hospitalization. Additionally, this study will detect the effect of high-glucose environment on CRKP capsule production in vitro and further explore how CRKP resistance to polymyxin and serum killing changes under high glucose concentration.

Materials and Methods

Research Design

A single-center retrospective cohort study design was adopted. A total of 218 clinically isolated CRKP strains which were collected from a large tertiary grade A hospital in Anhui Province between January 2019 and June 2024 were analyzed. Duplicate strain isolates were excluded. Clinical information on patients was obtained through the hospital's

case system, and strain identification and antimicrobial susceptibility testing were performed in the microbiology laboratory. Ethical approval for the study was obtained from the hospital's institutional review board (approval numbers: KYLL20240200, LLSC20240703).

Data Collection

This study collected data on gender, age, specimen type, comorbidities, complications, invasive procedures, surgical history, clinical outcomes, and laboratory tests from the medical record system for patients. Notably, chronic obstructive pulmonary disease (COPD) encompasses chronic bronchitis and emphysema in this study. The laboratory tests conducted included assessments of HbA_{1c}, C-reactive protein (CRP), white blood cells (WBC), neutrophils (Neut), lymphocytes (Lymph), and platelets (PLT). Patients were categorized into two groups based on HbA_{1c} levels: those with HbA_{1c} less than 7% and those with HbA_{1c} 7% or greater.

Identification of Isolates and Drug Susceptibility Testing

All isolated strains were identified using the Microflex-LT/SH mass spectrometer (BRUKER, Germany). Use the VITEK 2 Compact automatic bacterial analyzer (with card numbers AST-N334 and AST-N335) from BioMérieux, France, or the Kirby-Bauer Disk Diffusion method from Oxoid, UK to detect the antimicrobial susceptibility of CRKP. Antimicrobial susceptibility results were analyzed in accordance with the guidelines of the Clinical and Laboratory Standards Institute (CLSI) issued in 2019. According to the guidelines of the CLSI, *Klebsiella pneumoniae* identified as resistant to any carbapenem antibiotic is confirmed as a CRKP strain.[23]

Capsular Serotype, MLST and Virulence Genotype Testing

According to the method of Sylvain Brisse and others,[24] bacteria were cultured on blood agar plates and isolated and purified. A single colony was picked and suspended in 200μL of PBS, heated at 94°C for 10 minutes, and then centrifuged at 7500×g for 5 minutes. The supernatant was aspirated to prepare a DNA template and stored at −20°C. Capsular serotyping of CRKP was performed using *wzi* gene sequencing. The PCR products were detected by 1% agarose gel electrophoresis. Once the sample quality was confirmed, it was sent to Sangon Biotech (Shanghai, China) for sequencing. The sequencing results were compared on the website (https://bigsdb.pasteur.fr/klebsiella/). Multilocus sequence typing (MLST) was employed to determine the ST of CRKP, following the *K. pneumoniae* MLST protocol published on the MLST website of the Institut Pasteur, France (http://bigsdb.pasteur.fr/). The virulence genes of CRKP, including *rmpA, rmpA2*, and *iucA*, were detected by PCR combined with agarose gel electrophoresis. The primers were synthesized according to relevant literature reports[25] (see Table 1 for details).

Glucuronic Acid Quantification

The hypervirulent *Klebsiella pneumoniae* strain ATCC43816 and three CRKP strains isolated from participants were selected as representative strains for subsequent experiments. The three clinically isolated strains, all belonging to ST11,

Table 1 The Primer Sequences Used in This Study

Primer Name	Primer Sequence (5'-3')	Product Size (bp)
wzi	Forward, GTGCCGCGAGCGCTT TCTATCTTGGTATTCC Reverse, GAGAGCCACTGGTTCCAGAATTACCGC	580
rmpA	Forward, TACATATGAAGGAGTAGTTAAT Reverse, GAGCCATCTTTCATCAAC	505
rmpA2	Forward, TGTGCAATAAGGATGTTACATTAGT Reverse, TTTGATGTGCACCATTTTTCA	609
iucA	Forward, ATAAGGCAGGCAATCCAG Reverse, CGCTTCACTTCTTTCACTGACAGG	239

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were named CRKP28, CRKP70, and CRKP214, respectively. Among them, CRKP28 and CRKP70 were from the group with HbA1c ≥ 7%, while CRKP214 was from the group with HbA1c < 7%. The main component (glucuronic acid) of the capsule of *K. pneumoniae* was extracted by the hot phenol water bath method. The research method is essentially similar to previously described methods.[24] The bacteria were centrifuged at high speed for 5–10 minutes, and the resulting bacterial pellet was resuspended in sterile water and incubated at 68°C for 2 minutes. Simultaneously, the bacterial suspension was continuously diluted and the concentration was determined. Then an equal volume of 75% phenol was added and incubation was continued for 30 minutes. After the sample was allowed to return to room temperature, chloroform was added, and the mixture was centrifuged to obtain the supernatant. CPS was then precipitated with anhydrous ethanol at −20°C. A standard curve was generated by the phenol sulfuric acid method. The concentrated sulfuric acid and 5% phenol solution were ice-bathed and mixed at a ratio of 5:1 as a chromogenic solution. The glucose standard solution and the samples to be tested were each reacted with the chromogenic solution at high temperature. The standard curve was drawn by measuring the OD490 value, and the capsule concentration was calculated.

Polymyxin B Time-Bactericidal Curve Assay and Serum Bactericidal Assay

To investigate the effect of glucose on *Klebsiella pneumoniae* polymyxin resistance, we selected polymyxin B (Sangon Biotech, Shanghai, China) as a representative polymyxin drug. Polymyxin B time-bactericidal curve experiments were conducted following the method described by Fan et al.[22] Briefly, the purified bacteria were incubated in Luria-Bertani (LB) medium with or without glucose, until logarithmic growth phase. The bacterial solution was then diluted into fresh medium to achieve a glucose concentration of 0.5% and a polymyxin concentration of 1 × MIC. The cultures were incubated in a constant temperature shaker at 37°C. At 0, 1, 2, 3, and 4 hours, equal volumes of bacterial suspensions were removed, serially diluted, and inoculated onto Mueller-Hinton (MH) plates. The plates were then incubated overnight for colony counting. To evaluate *Klebsiella pneumoniae* resistance to serum killing under hyperglycemic conditions, we collected sera from eight healthy males and eight healthy females at the clinic and mixed them to serve as bacterial media. The bacterial suspension, cultured with or without glucose, was diluted to a concentration of 5 x 10^5 CFU/mL. We then took 25μL of the diluted bacterial solution and 75μL of donor serum, mixed them, and co-incubated the mixture at 37°C. Bacterial survival was assessed consecutively at each hour for the first 3 hours.[26]

Data Analysis

Data were analyzed and figures were plotted using SPSS 26.0 and Graphpad Prism 10.0 software. Statistical comparisons were performed using the chi-square test, Fisher's exact test, or Mann–Whitney *U*-test, depending on the data type. Univariate and multivariate Logistic regression analyses were employed to identify risk factors. The *P* value less than 0.05 was used to determine whether there was a statistically significant difference between groups.

Results

Clinical Characteristics of Patients With CRKP Infection in the Good and Poor Glycemic Control Groups

Among all CRKP-infected patients, 68.3% were male and 31.7% were female. The median age was 65 (50.0~77.25) years. The main underlying diseases were hypertension (43.1%) and diabetes mellitus (23.4%), followed by coronary artery disease (15.1%) and tumors (14.2%). Patients with poor glycemic control had a higher prevalence of comorbid CKD (16.0% vs 5.4%, $P<0.05$) and acute renal insufficiency (30.0% vs 15.5%, $P<0.05$) compared to those with good glycemic control. Among patients receiving deep vein catheterization, those with poor glycemic control had a higher likelihood of CRKP infection (78.0% vs 61.3%, $P=0.030$). Laboratory tests showed significantly higher levels of CRP, WBC, and NE in patients with poor glycemic control ($P<0.01$). Patients with poor glycemic control were more likely to develop sepsis subsequent to CRKP infection compared to those with good glycemic control (32.0% vs 23.8%, $P>0.05$). Furthermore, patients had a significantly higher mortality rate with poor glycemic control (48.0% vs 23.8%, $P=0.001$). (For detailed results, see Table 2).

Table 2 General Demographic and Clinical Characteristics of 218 CRKP Infected Patients

Characteristics	Total (n=218)	HbA_1c<7% (n=168)	HbA_1c≥7% (n=50)	*P* value
Male (%)	149 (68.3)	114 (67.9)	35 (70.0)	0.775
Age (years)	65 (50.0~77.3)	67 (51.3~78.8)	64 (53.0~75.0)	0.940
Specimen type				
Sputum (%)	107 (49.1)	83(49.4)	24 (48.0)	0.862
Blood (%)	35 (16.1)	26 (15.5)	9 (18.0)	0.670
Urine (%)	19 (8.7)	17 (10.1)	2 (4.0)	0.289
Underlying conditions				
COPD (%)	28 (12.8)	22 (13.1)	6 (12.0)	0.839
CHD (%)	33 (15.1)	23 (13.0)	10 (20.0)	0.275
CLD (%)	15 (6.9)	11 (6.5)	4 (8.0)	0.970
CKD (%)	17 (7.8)	9 (5.4)	8 (16.0)	0.031*
Tumors (%)	31 (14.2)	21 (12.5)	10 (20.0)	0.183
Diabetes (%)	62 (28.4)	28 (16.7)	34 (68.0)	0.000****
Hypertension (%)	94 (43.1)	70 (41.7)	24 (48.0)	0.427
Complications				
Sepsis (%)	56 (25.7)	40 (23.8)	16 (32.0)	0.245
Pneumonia (%)	130 (59.6)	100 (59.5)	30 (60.0)	0.952
UTI (%)	12 (5.5)	10 (6.0)	2 (4.0)	0.859
Acute hepatic insufficiency (%)	35 (16.1)	26 (15.5)	9 (18.0)	0.670
Acute renal insufficiency (%)	37 (17.0)	26 (15.5)	15 (30.0)	0.021*
Invasive procedures				
Mechanical ventilation (%)	132 (60.6)	99 (58.9)	33 (66.0)	0.369
FFB (%)	102 (46.8)	77 (45.8)	25 (50.0)	0.604
CVC (%)	45 (20.6)	33 (19.6)	12 (24.0)	0.504
Deep vein catheterization (%)	142 (65.1)	103 (61.3)	39 (78.0)	0.030*
Gastric tube insertion (%)	115 (52.8)	87 (51.8)	28 (56.0)	0.600
Urinary catheterization (%)	105 (48.2)	80 (47.6)	25 (50.0)	0.767
Glucocorticoid treatment (%)	72 (33.0)	53 (31.5)	19 (38.0)	0.394
Surgery (%)	70 (32.1)	53 (31.5)	17 (34.0)	0.744
ICU admission (%)	40 (18.3)	28 (16.7)	12 (24.0)	0.240
ICU length of stay (days)	8 (0~24.0)	6 (0~23.8)	11 (0~28.0)	0.296
Invasive ventilation duration (hours)	54 (0~278.0)	62.5 (0~264.8)	16 (0~312.0)	0.576
LOS (days)	37 (21.0~69.0)	38 (21.0~69.0)	36 (19.0~60.0)	0.582
Death (%)	64 (29.4)	40 (23.8)	24 (48.0)	0.001***
Laboratory findings				
CRP (mg/L)	64.8(24.7~135.8)	58.05 (18.2~118.1)	110.2 (47.4~192.9)	0.001***
WBC (10^9/L)	9.8 (6.6~13.1)	9.385 (6.1~12.7)	11.64 (8.1~16.4)	0.001***
Neutrophils (10^9/L)	7.81 (5.0~11.0)	7.13 (4.2~10.4)	9.57 (6.9~14.7)	0.003**
Lymphocytes (10^9/L)	0.98 (0.6~1.5)	1.015 (0.6~1.5)	0.85 (0.6~1.3)	0.103
PLT (10^9/L)	209 (133.0~282.0)	215.5 (135.3~288.3)	193 (107.0~250.0)	0.412

Notes: *, *P*<0.05, **, *P*<0.01, ***, *P*<0.001, ****, *P*<0.0001.

Abbreviations: COPD, chronic obstructive pulmonary disease; CHD, coronary heart disease; CLD, chronic liver disease; CKD, chronic kidney disease; UTI, urinary tract infection; FFB, flexible fiberoptic bronchoscopy; CVC, central venous catheter; LOS, Length of Stay.

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Microbial Capsular Serotyping, Virulence Gene Detection and Drug Susceptibility Test Results

Among the 218 collected CRKP strains, we detected the K64 capsular serotype with the highest current prevalence and three major virulence genes (*rmpA, rmpA2*, and *iucA*) of CRKP. As shown in Table 3, the carrying rates of the virulence genes were highest for *iucA* (68.8%), followed by *rmpA2* (45.4%). No significant differences were observed in the distribution of carrying rates for the three virulence genes or the K64 capsular serotype between the two groups. The susceptibility test results in Table 4 indicate that all clinically isolated bacteria exhibited broad resistance to carbapenems, penicillins, cephalosporins, cephamycin, and quinolones. Resistance rates to amikacin and compound sulfamethoxazole also exceeded 50%. In contrast, resistance rates to ceftazidime/avibactam, tigecycline, and polymyxin were relatively low, at 30.3%, 19.3%, and 4.6%, respectively. Studies have suggested a potential link between high-glucose environments and polymyxin resistance. Consistent with these findings, our study found that patients with poor glycemic control had a higher probability of infection with PR-CRKP than those with good glycemic control (8.0% vs 3.6%, $P > 0.05$). However, this difference did not reach statistical significance. This may be attributed to the relatively low overall detection rate of PR-CRKP, which resulted in a non-significant difference.

Table 3 The Capsular Serotypes and Virulence Factors of the Two Groups of CRKP Strains

Variable, n (%)	Total (n=218)	$HbA_1c<7\%$ (n=168)	$HbA_1c\geq7\%$ (n=50)	*P* value
Capsular serotype				
K64	104 (47.7%)	80 (47.6%)	24 (48.0%)	0.923
Virulence genes				
rmpA	53 (24.8%)	44 (26.2%)	9 (18.0%)	0.236
rmpA2	99 (45.4%)	78 (46.4%)	21 (42.0%)	0.581
iucA	150 (89.3%)	117 (69.6%)	33 (66.0%)	0.625

Table 4 The Antibiotic Resistance of 218 Clinical Isolates of CRKP Strains

Antibiotics, n (%)	Total (n=218)	$HbA_1c<7\%$ (n=168)	$HbA_1c\geq7\%$ (n=50)	*P* value
Amoxicillin/ Clavulanic Acid	214 (98.2)	164 (97.6)	50 (100)	0.576
Ticarcillin/ Clavulanic Acid	217 (99.5)	167 (99.4)	50 (100)	1.000
Cefoperazone/ Sulbactam	210 (96.3)	162 (96.4)	48 (96.0)	1.000
Piperacillin/ Tazobactam	218 (100)	168 (100)	50 (100)	–
Ceftazidime	216 (99.1)	166 (98.8)	50 (100)	1.000
Ceftriaxone	218 (100)	168 (100)	50 (100)	–
Cefepime	214 (98.2)	166 (98.8)	48 (96.0)	0.226
Cefoxitin	212 (97.2)	164 (97.6)	48 (96.0)	0.903
Cefuroxime	217 (99.5)	168 (100)	49 (98.0)	0.229
Aztreonam	209 (95.9)	160 (95.2)	49 (98.0)	0.648
Imipenem	199 (91.3)	154 (91.7)	45 (90.0)	0.935
Ertapenem	214 (98.2)	164 (97.6)	50 (100)	0.576
Meropenem	211 (96.8)	162 (96.4)	49 (98.0)	0.923
Amikacin	113 (51.8)	88 (52.4)	25 (50.0)	0.767
Levofloxacin	194 (89.0)	149 (88.7)	45 (90.0)	0.795
Trimethoprim/ Sulfamethoxazole	133 (61.0)	101 (60.1)	32 (64.0)	0.621
Ceftazidime/ Avibactam	66 (30.3)	53 (31.5)	13 (36.0)	0.367
Polymyxin B	10 (4.6)	6 (3.6)	4 (8.0)	0.353
Tigecycline	42 (19.3)	35 (20.8)	7 (14.0)	0.282

Analysis of Risk Factors for Death in Patients With CRKP Infection

Univariate Logistic regression analysis identified several independent risk factors for death in patients with CRKP infection, including age, HbA_{1c} ≥7%, CKD, tumor, glucocorticoid treatment, mechanical ventilation, flexible fiberoptic bronchoscopy (FFB), deep vein catheterization, gastric tube insertion, urinary catheterization, ICU admission, and sepsis. (The detailed results are presented in Table 5). After adjusting for confounding factors, multivariate Logistic regression analysis identified independent risk factors for death in patients with CRKP infection. These included age (OR=1.024, 95% CI=1.003–1.044, *P*=0.021), HbA_{1c} ≥7% (OR=2.417, 95% CI=1.155–5.055, *P*=0.019), CKD (OR=8.322, 95% CI=2.534–27.326, *P*< 0.001), tumor (OR = 4.562, 95% CI=1.811–11.492, *P*=0.001), mechanical ventilation (OR=4.586, 95% CI=2.000–10.517, *P*<0.001), and sepsis (OR=2.270, 95% CI=1.110–4.642, *P*=0.025). (See Figure 1).

The Influence of Glucose and cAMP on the Production of Capsule of CRKP

To evaluate the impact of a high-glucose environment on the capsule synthesis of *Klebsiella pneumoniae*, this study performed quantitative capsule assessments on the hypervirulent *Klebsiella pneumoniae* ATCC43816 and three clinically isolated CRKP strains (ST11). Initially, bacterial suspensions in the logarithmic growth phase were subjected to low-speed centrifugation (3000×g for 10 minutes). Figure 2A–D illustrates the state of the centrifuged suspensions of

Table 5 Univariate Logistic Regression Analysis of Mortality Risk Factors for CRKP Infection

Variables	OR (95% CI)	*P* value	Variables	OR (95% CI)	*P* value
Gender Male Female	1.000 (Reference) 0.878 (0.466~1.655)	0.688	Sepsis No Yes	1.000 (Reference) 2.543 (1.344~4.815)	0.004**
Age	1.020 (1.002~1.039)	0.027*	ICU admission No Yes	1.000 (Reference) 2.060 (1.013~4.190)	0.046*
HbA_1c <7% ≥7%	1.000 (Reference) 2.954 (1.529~5.708)	0.001***	Glucocorticoid treatment No Yes	1.000 (Reference) 2.139 (1.168~3.917)	0.014*
CHD No Yes	1.000 (Reference) 1.708 (0.791~3.685)	0.173	Mechanical ventilation No Yes	1.000 (Reference) 3.536	<0.001***
COPD No Yes	1.000 (Reference) 1.673 (0.735~3.805)	0.220	FFB No Yes	1.000 (Reference) 2.991 (1.627~5.499)	<0.001***
CLD No Yes	1.000 (Reference) 0.350 (0.077~1.597)	0.175	Deep vein catheterization No Yes	1.000 (Reference) 4.706 (2.172~10.199)	<0.001***
CKD No Yes	1.000 (Reference) 5.119 (1.804~14.528)	0.002**	Gastric tube insertion No Yes	1.000 (Reference) 2.842 (1.524~5.299)	0.001***
Hypertension No Yes	1.000 (Reference) 1.357 (0.755~2.438)	0.307	Urinary catheterization No Yes	1.000 (Reference) 3.038 (1.646~5.610)	<0.001***
Tumors No Yes	1.000 (Reference) 2.640 (1.215~5.738)	0.014*	Surgery No Yes	1.000 (Reference) 0.853 (0.453~1.605)	0.622

Notes: *, *P*<0.05, **, *P*<0.01, ***, *P*<0.001.

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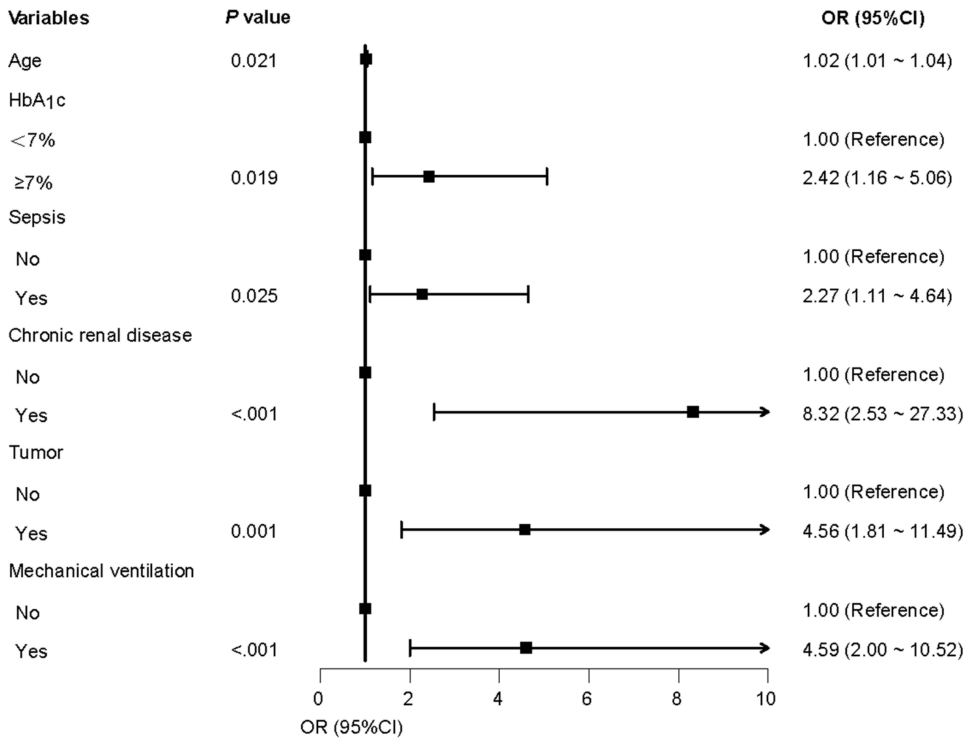

Figure 1 Multivariable Logistic regression analysis of mortality risk factors for CRKP infection.

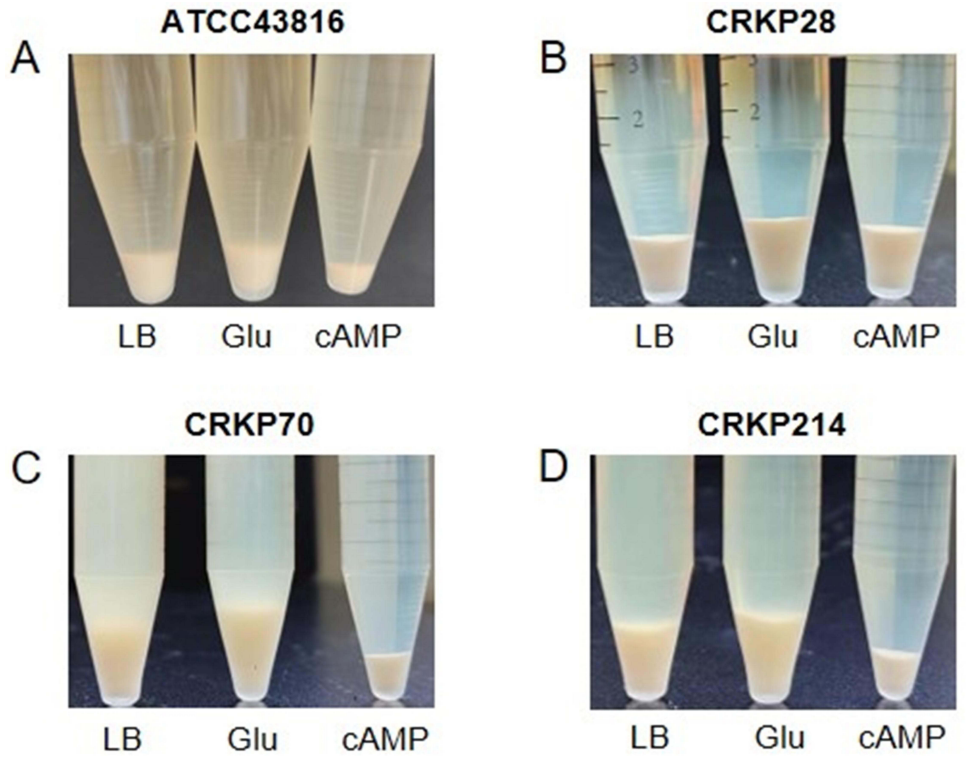

Figure 2 ATCC43816 (**A**), CRKP28 (**B**), CRKP70 (**C**), and CRKP214 (**D**) were separately cultured in LB broth, LB broth supplemented with 0.5% glucose (LB + 0.5% Glu), and LB broth with 0.5% glucose and 2 mm cyclic adenosine monophosphate (LB + 0.5% Glu + 2 mm cAMP) to the logarithmic phase of growth. Subsequently, the cultures were centrifuged at 3000 rpm for 10 minutes to obtain the bacterial pellets.

ATCC43816 and three clinically isolated CRKP strains. Compared to the suspensions cultured in LB medium alone, those co-incubated with glucose were more viscous and resistant to sedimentation at the bottom of the centrifuge tubes. The addition of cAMP facilitated the sedimentation of the suspensions. Subsequent quantitative polysaccharide extraction experiments demonstrated that exogenous glucose significantly enhanced capsular polysaccharide synthesis in ATCC43816 and the three clinical CRKP isolates ($P < 0.05$). Moreover, this increase in bacterial capsular polysaccharide synthesis was inhibited by cAMP (2 mm) (Figure 3A–D).

Glucose Enhances the Resistance of CRKP to Polymyxin and Serum Killing

This study explored the influence of elevated glucose concentration on the resistance of *Klebsiella pneumoniae* to polymyxin and serum killing through bactericidal curve experiments. Polymyxin showed bactericidal activity against the experimental strains, and bacterial counts at different time points indicated that the survival rate of bacteria decreased over time. As shown in Figure 4A–D, there was no significant difference in the bacterial growth curves between the presence and absence of 0.5% glucose. However, the survival rate of ATCC43816 and the three clinical CRKP isolates in polymyxin was significantly higher in the presence of 0.5% glucose than in its absence ($P < 0.001$). The serum bactericidal assay results indicated that the survival rate of ATCC43816 was enhanced when cultured in LB broth with 0.5% glucose compared to LB broth alone (Figure 5A). In contrast to the hypervirulent *Klebsiella pneumoniae* strain ATCC43816, the clinical strains (CRKP28, CRKP70, and CRKP214) showed weaker resistance to serum killing. However, the survival rates of the CRKP strains were significantly higher when grown in LB medium with 0.5% glucose compared to glucose-free medium (Figure 5B–D).

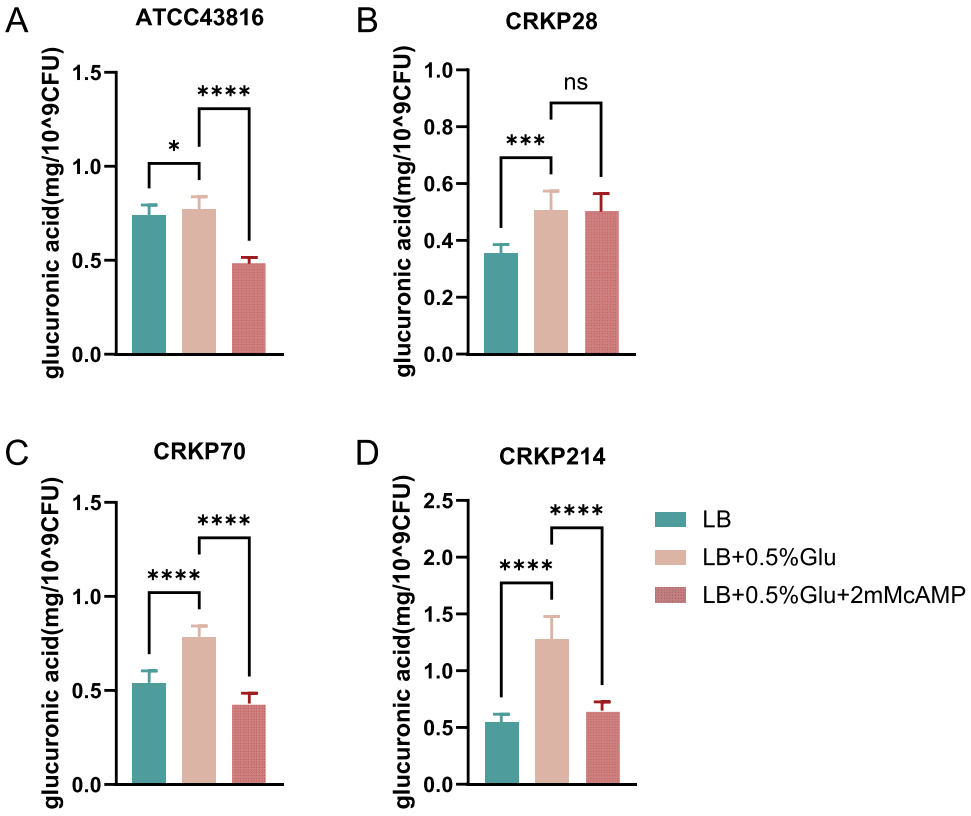

Figure 3 A high-glucose environment can stimulate the synthesis of CRKP capsules. ATCC43816 (**A**), clinical isolates CRKP28 (**B**), CRKP70 (**C**), and CRKP214 (**D**) were cultured in media with or without 0.5% glucose or with or without 2mM cAMP until they reached the logarithmic growth phase, after which the capsule polysaccharides of the bacteria were quantitatively extracted according to the hot phenol water bath method. *, $P<0.05$, ***, $P<0.001$, ****, $P<0.0001$.
Abbreviation: n.s., not significant.

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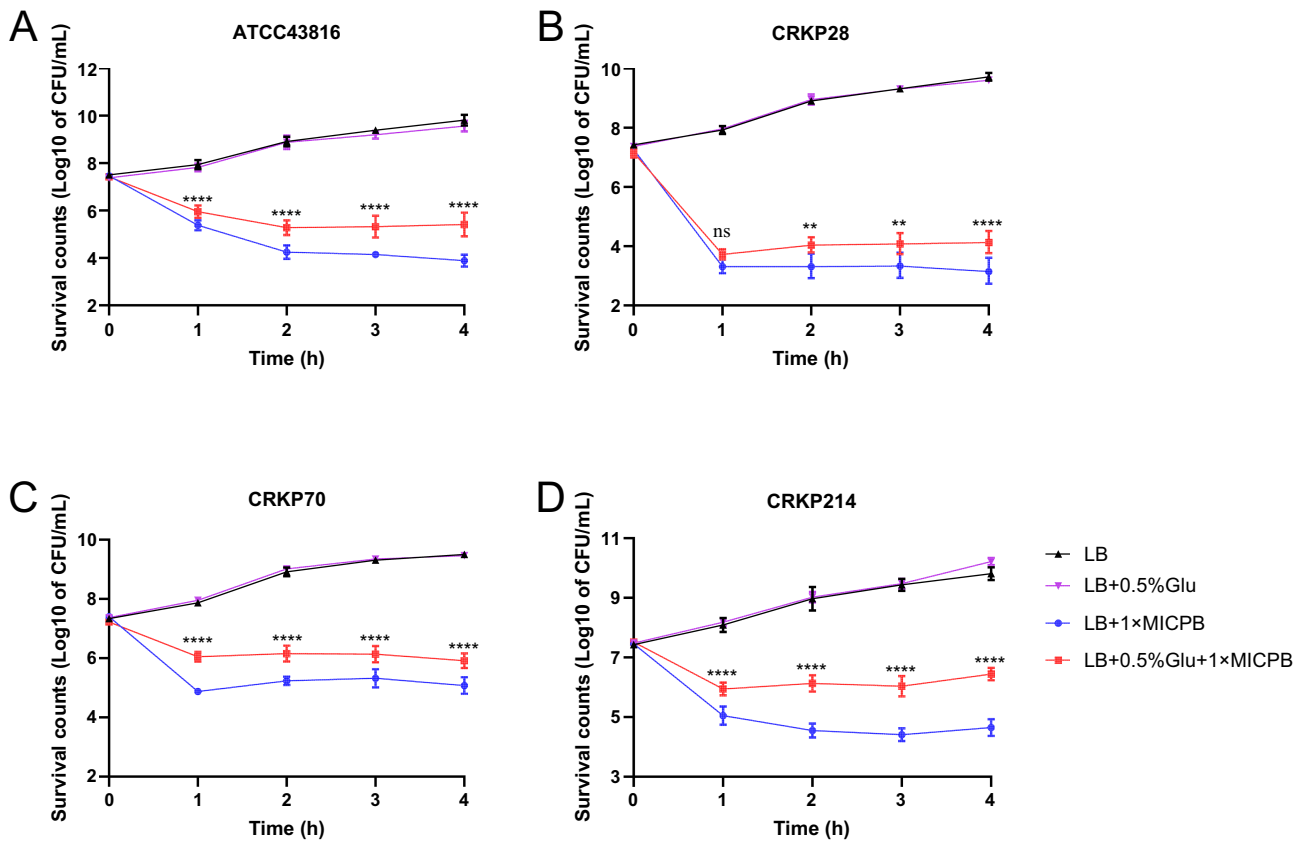

Figure 4 ATCC43816 and CRKP strains exhibited enhanced resistance to polymyxins under glucose-rich conditions. ATCC43816 (**A**) and CRKP strains CRKP28 (**B**), CRKP70 (**C**), and CRKP214 (**D**) were first pre-cultured in LB medium with and without 0.5% glucose, and then treated with polymyxin B at a concentration of 1×MIC in the presence and absence of 0.5% glucose. Viable bacterial counts were assessed hourly from 0 to 4 hours through serial dilution and plating. **, $P<0.01$, ****, $P<0.0001$.
Abbreviation: n.s., not significant.

Discussion

Diabetes is a disease of carbohydrate metabolism disorder. The primary pathogenic mechanism involves elevated blood glucose levels due to excessive endogenous glucose production and an impaired ability to utilize glucose effectively.[8] Studies have pointed out that patients with hyperglycemia are more prone to colonization and infection by *K. pneumoniae* and have a higher mortality rate.[5,12–14] In this study, we used HbA_{1c} levels of ≥7% as the criterion for grouping to investigate the correlation between glycemic control and the clinical features as well as pathogen drug resistance in patients infected with CRKP. Utilizing in vitro experiments, we assessed the impact of glucose on bacterial capsule synthesis and its underlying mechanisms. Additionally, we further explored the resistance changes of bacteria to polymyxin and serum killing under a high glucose environment.

We observed that patients with poor glycemic control typically exhibit a stronger inflammatory response upon infection. Patients with HbA1c ≥ 7% presented with elevated levels of CRP, WBC, and NE at the initial stage of infection ($P<0.01$), which is consistent with the findings of previous studies.[26] When HbA_{1c} is ≥7%, patients exhibit chronically elevated blood glucose levels. This sustained hyperglycemia can impair the function of white blood cells, including a reduction in phagocytic capacity. Consequently, the immune system's ability to eliminate pathogens is compromised, leading to chronically elevated inflammation in affected patients.[27] The heightened inflammatory response observed in patients with poor HbA_{1c} control may also be associated with dysregulated lipid metabolism. Previous studies have shown that hyperglycemia can interfere with the normal metabolism of lipids through multiple ways. Lipid substances can trigger the secretion of multiple cytokines and inflammatory mediators, thereby exacerbating the systemic inflammatory response.[28] Hyperglycemia is correlated with the absolute value of neutrophils. Patients with poor glycemic control exhibit higher neutrophil counts. This elevation may stem from the impairment of neutrophil extracellular trap

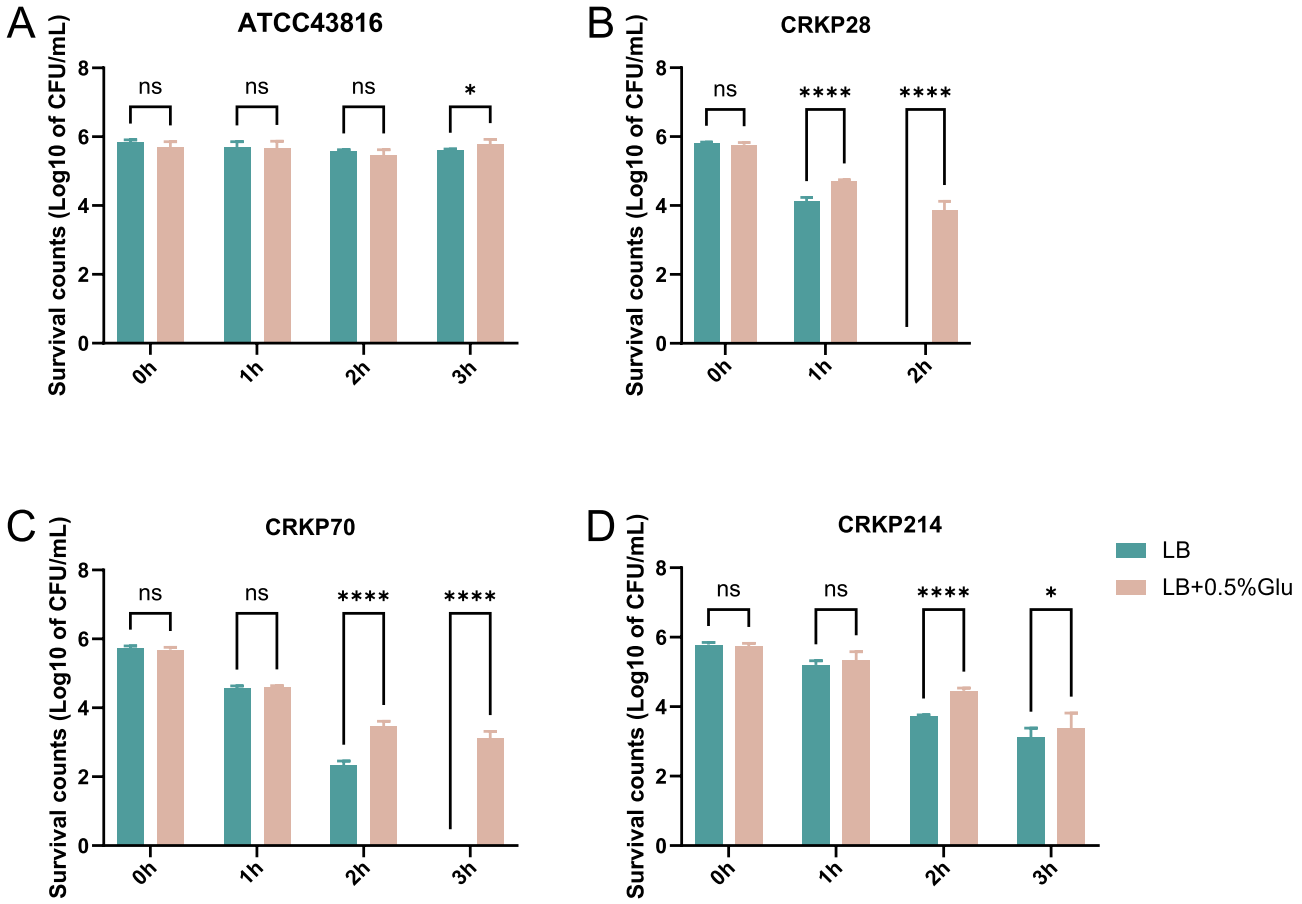

Figure 5 *Klebsiella pneumoniae* strains cultured under high-glucose conditions exhibit enhanced resistance to serum killing. Serum samples from 16 healthy individuals were incubated with strains ATCC43816 (**A**), CRKP28 (**B**), CRKP70 (**C**), and CRKP214 (**D**), which had been pre-cultured in LB broth with and without the addition of 0.5% glucose. The incubation lasted for 3 hours, with bacterial survival rates determined every hour. *, $P<0.05$, ****, $P<0.0001$.
Abbreviation: n.s., not significant.

(NET) pathogen-killing functions in patients with chronic hyperglycemia, leading to a pronounced increase in neutrophils upon pathogen infection.[29]

In recent years, *K. pneumoniae* strains with high virulence and carbapenem resistance have become widely prevalent,[2] posing significant challenges to clinical treatment. Even worse, studies have indicated that diabetic patients are more likely to be infected by these superbugs.[30] Among these bacteria, the CRKP-K64 is predominant and has been confirmed to possess virulence equivalent to that of the K1 and K2 capsular serotypes of *K. pneumoniae*.[31] The presence of *rmpA, rmpA2* and *iucA* genes is commonly used to assess the virulence level of these bacteria.[32] Our study revealed no significant differences in the detection rates of CRKP-K64 and major virulence genes (*rmpA, rmpA2, iucA*) between the groups with poor and good glycemic control. Consequently, we conclude that hyperglycemia does not appear to influence susceptibility to CRKP-K64.

Our study shows that the mortality rate of patients infected with CRKP is relatively high, at 29.4%. Therefore, we tried to find risk factors associated with mortality in these patients. In this study, after adjusting for potential confounders, multivariate regression analysis identified independent risk factors for mortality in CRKP-infected patients, which include age, HbA_{1c} ≥7%, CKD, tumor, sepsis, and mechanical ventilation. Traditional risk factors such as underlying diseases and invasive treatments are predictable and understandable, which is basically consistent with previous studies.[6] It should still be noted that regarding the relationship between blood glucose level and the mortality rate of *K. pneumoniae* infection, studies have shown that in patients with KLA, the glycemic control level is not related to the patient's mortality rate.[11] However, our study indicates that among patients with CRKP infection, those with HbA_{1c} ≥7% have a 2.417-fold higher risk of mortality compared to those with HbA_{1c} <7% (OR=2.417,95% CI=1.155–5.055,

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P=0.019). Therefore, increased vigilance is warranted for patients with poor glycemic control who are infected with CRKP. In addition to the heightened mortality rate from infections attributable to hyperglycemia-induced impairment of the immune system, chronic complications arising from long-term hyperglycemia, such as chronic kidney disease, may also contribute to increased patient mortality (16% vs 5.4%, $P<0.05$). In the advanced stages of CKD, renal dysfunction ensues, often progressing to end-stage renal disease (ESRD). With severely impaired renal function, patients often rely on dialysis or transplantation as treatment options, which increases the risk of death associated with CKD.[33]

Literature indicates that glucose stimulates CPS production in *K. pneumoniae*, thereby modulating bacterial pathogenicity.[22] In this study, 0.5% glucose was used to simulate the hyperglycemic environment in the human body.[26] The results showed that 0.5% glucose significantly enhanced the production of CRKP capsules. The capsules afford the bacteria protection within the host, including enhancing the resistance of bacteria to phagocytosis and serum killing,[34,35] and inhibiting airway epithelial cells from expressing β-defensins to promote bacterial colonization in the lungs.[36] Thus, we hypothesize that in vivo, a chronic hyperglycemic environment may stimulate the production of CPS, leading to enhanced bacterial invasiveness and virulence. This also provides another plausible explanation for our finding that patients with hyperglycemic infections have a higher mortality rate.

Although polymyxin is the antibiotic of last resort for the treatment of CRKP infections,[18] the emergence of PR-CRKP has become a concern, and hyperglycemia may exacerbate the development of this resistance. Prior research has documented that glucose increases the resistance of Bacillus subtilis and alcohol-producing *K. pneumoniae* to polymyxin. The mechanisms include maintaining intracellular ATP content and producing a higher amount of CPS.[22,37] In this context, our study confirmed that high-glucose environment increases capsule production in CRKP and enhances the bacteria's resistance to polymyxin as well as its serum bactericidal resistance. By monitoring the bacterial growth curve, we found that the presence of glucose hardly affects the growth rate of bacteria. Consequently, the enhanced resistance of bacteria to polymyxin and serum killing in a glucose-rich environment is not related to the bacterial proliferation rate. Exogenous cAMP can inhibit the stimulatory effect of glucose on capsular polysaccharide synthesis but does not suppress the bacteria's resistance to polymyxin. This may be due to the fact that capsular production does not entirely dominate the emergence of polymyxin resistance. Therefore, merely reducing capsular synthesis may not completely reverse the enhancing effect of a high-glucose environment on bacterial polymyxin resistance.

Conclusion

In this study, we found that long-term poor glycemic control is associated with more severe infections and significantly increases the risk of death in patients infected with CRKP. Our research also shows that high-glucose environment can enhance the resistance of CRKP to polymyxin and serum killing. Therefore, implementing strict blood glucose management during hospitalization is of crucial importance for improving the prognosis of patients infected with CRKP and effectively curbing the evolution and spread of bacterial drug resistance.

Ethics Approval and Informed Consent

This retrospective cohort study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Anhui Medical University, with the approval number KYLL20240200. The CRKP strains were derived from previously isolated clinical samples. The study posed no adverse effects or risks to participants, and the committee waived the requirement for informed consent.

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Disclosure

The authors report no conflicts of interest in this work.

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