Infection and Drug Resistance

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ORIGINAL RESEARCH

Epidemiological Features and Impact of High Glucose Level on Virulence Gene Expression and Serum Resistance of *Klebsiella pneumoniae* Causing Liver Abscess in Diabetic Patients

Ling Tang*, Hui Wang*, Kangli Cao*, Yajuan Li, Tingting Li, Ying Huang, Yuanhong Xu

Department of Clinical Laboratory, First Affiliated Hospital of Anhui Medical University, Hefei, People's Republic of China

*These authors contributed equally to this work

Correspondence: Yuanhong Xu, Email xyhong1964@163.com

Purpose: *Klebsiella pneumoniae* (*K. pneumoniae*) is a Gram-negative bacterium that is predominantly associated with liver abscesses in global diabetic patients. High levels of glucose in the surrounding of *K. pneumonia* increase its pathogenicity including capsular polysaccharide (CPS) and fimbriae. Other important virulent factors include outer membrane protein A (ompA) and regulator mucoid phenotype A (rmpA). The objective of this investigation was to elucidate the effects of high glucose on *rmpA* and *ompA* gene expression and serum resistance of *K. pneumoniae* causing liver abscess.

Patients and Methods: The clinical history of 57 patients suffering from *K. pneumoniae*-caused liver abscesses (KLA) was acquired and their clinical and laboratory manifestations in the presence or absence of diabetes were analyzed. The antimicrobial susceptibility, serotypes, and virulence genes were tested. Clinical isolates of 3 serotype-K1 hypervirulent *K. pneumoniae* (hvKP) were used to detect the effect of exogenous high glucose on *rmpA, ompA*, and *clbB* genes expression, and bacterial serum resistance.

Results: KLA patients with diabetes showed higher C-reactive protein (CRP) compared to non-diabetic KLA patients. Furthermore, the diabetic group showed increased incidences of sepsis and invasive infections, and their length of hospital stay was also prolonged. Pre-incubation of *K. pneumoniae* in high glucose (0.5%) concentration up-regulated *rmpA, ompA*, and *clbB* genes expression. However, cAMP supplementation, which was inhibited by environmental glucose, reversed the increase of *rmpA* and *ompA* in a cAMP-dependent manner. Moreover, hvKP strains incubated in high glucose also exhibited enhanced protection from serum killing.

Conclusion: High glucose levels reflected by poor glycemic control has increased gene expression of *rmpA* and *ompA* in hvKP by the cAMP signaling pathway and enhanced its resistance to serum killing, thus providing a new and reasonable explanation for the high incidences of sepsis and invasive infections in KLA patients with diabetes.

Keywords: *Klebsiella pneumoniae*, liver abscess, diabetes, *rmpA*, *ompA*, serum resistance

Introduction

Klebsiella pneumoniae after colonization (in its virulent state) crosses the intestinal-mucosal barrier and targets the liver through the portal vein system, subsequently causing *K. pneumoniae*-caused liver abscesses (KLA). *K. pneumoniae* is bacteria that predominantly contributes to pyogenic liver abscesses worldwide.[1] KLA is generically cryptogenic and frequently becomes complicated because of invasive infection to other organs due to *K. pneumoniae* virulence factors, which include, capsular serotype, *rmpA*, and aerobactin.[2] Because of invasive systemic infections, the KLA mortality rate equates to 10% within 30 days of hospitalization.[3] Unfortunately, the dramatically increased multi-drug resistance (MDR) of hyper-virulent *K. pneumoniae* (hvKP), especially resistance to carbapenems and third-generation cephalosporins, presents a great challenge for clinical treatment.

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KLA is accompanied by many chronic diseases. Studies have shown that diabetes mellitus is a concomitant condition in approximately 29.3–44.3% of liver abscess cases.[4,5] Hyperglycemia weakens the immune barrier and increases the susceptibility to hvKP infection by inhibiting phagocytosis[6] and neutrophil extracellular traps (NETs) generation,[7] reducing macrophages' function,[8] and impairing the bacteriolysis of antibodies and complement.[9] Poor glycemic control, also affects the pathogenicity of *K. pneumoniae*. Lee et al found that high glucose levels stimulated the biosynthesis of polysaccharide and gene expression of hvKP's *cps*, thereby increasing phagocytosis resistance and development of invasive syndrome.[10] Exogenous glucose can also activate the production of *K. pneumoniae*'s type 3 fimbriae. These functions are under the control of global modulator cyclic AMP (cAMP) receptor protein (CRP) and cAMP-CRP signaling pathway.[11] However, it is unknown if the expressions of other known pathogenic factors, for instance, the *rmpA, ompA*, and colibactin are also affected.

The *K. pneumoniae* pathogenic factors help its survival and colonization outside the gastrointestinal tract. *RmpA* is present at the large virulence plasmid and promotes capsular production.[12] Outer membrane protein A (OmpA) is a unique protein, abundantly located in the outer membrane of Gram-negative bacteria, and it is highly conserved throughout its evolution.[13] Since *ompA* expression is under the control of two stress-stimulated ribonucleolytic mechanisms, many environmental triggers can modulate *ompA* expression, for example, acid challenge,[14] antimicrobial peptide,[15] cAMP,[16] and polyamines.[17] Moreover, in Gram-negative bacteria, ompA performs many functions such as toxicity, invasion, serum resistance, adhesion, and biofilm formation.[18]

Enhanced polysaccharide biosynthesis stimulated by glucose further increased the *K. pneumoniae* resistance to phagocytosis of neutrophils and destruction of blood leukocytes. However, it has not been studied whether *K. pneumoniae* cultured with high-glucose increases its resistance to serum killing. Serum comprises >30 complement system proteins, this system is associated with the host's innate immune response.[19] The structural integrity of the cellular envelope is crucial for serum survival. Capsule and OmpA have been implicated in serum resistance.[18,20]

In this investigation, the clinical manifestations associated with KLA patients in the presence or absence of diabetes and the association between glycemic control and the invasive syndrome were determined. Then, the present study aimed to explore the impact of high exogenous glucose levels on *rmpA, ompA*, and colibactin *clbB* gene expression and serum resistance of *K. pneumoniae* in in-vitro assays. It is expected that this will provide a new and reasonable explanation for the high incidences of sepsis and invasive infections in KLA patients with diabetes.

Materials and Methods

Study Population

This retrospective cohort investigation analyzed the KLA patients who were also diabetic and were enrolled at The First Affiliated Hospital of Anhui Medical University, from January 2020 to November 2022 in a tertiary medical center with a 3000-bed capacity. And was duly authorized by the human ethics council of First Affiliated Hospital of Anhui Medical University. The KLA patients were confirmed after the culture reports from the Department of Microbiology were reviewed and the diagnosis was based on the typical clinical symptoms, liver abscess cavity imaging examinations, and culture identification via *K. pneumoniae* blood isolate or puncture fluid samples. A total of 24 *K. pneumoniae* isolates were collected in non-diabetic group, including 14 strains from puncture fluid and 10 strains from blood. In diabetic group, 33 *K. pneumoniae* isolates were collected, including 23 strains from puncture fluid and 10 strains from blood. Diabetic patients were elaborated as those who were previously diagnosed with either type 2 or type 1 diabetes and/or those who were either taking insulin and/or oral hypoglycemic drugs. Participants' laboratory reports, sex, underlying diseases, age, clinical manifestations, and management were gathered. Sepsis-3.0 criteria were used for defining sepsis.[21] And the infection was deemed metastasized when the infection spread to a distant site by the same pathogen as the pyogenic liver abscess (*K. pneumoniae*).

Microbiologic Data

For recognizing all the isolates, Matrix-Assisted Laser Desorption/Ionization-Time of Flight mass spectrometry (MALDI-TOF MS, Vitek MS, bioMérieux, France) was utilized. In accordance with the 2019 Clinical and Laboratory Standards Institute's recommendations, 28 antibiotics were tested for their susceptibility via VITEK 2 (Card number: AST-GN13) system or Kirby-Bauer Disk Diffusion (Oxoid, UK) (CLSI) method. *K. pneumoniae* (ATCC700603) and *Escherichia coli* (ATCC25922) isolates were kept as standards to ensure quality control. Agar dilution assessment with the help of ceftazidime and cefotaxime combined with clavulanate was performed to confirm ESBL. Resistance to carbapenem (meropenem, imipenem, and ertapenem) was confirmed via disk diffusion protocol.

Detection of Capsular Serotypes and Virulent Genes

Columbia blood agar plates were prepared for inoculating the strains which were then incubated for 18 hours at 37°C in 5% CO2 chamber. *K. pneumoniae* isolates DNA was collected via a DNA extraction kit (Sangon Biotech, Shanghai, China) by following the instructions provided by the manufacturer. K1, K2, K5, K20, K54, and K57 capsular serotypes and virulence-associated genes, including *wcaG, magA, rmpA, alls, iucb, aerobactin, iroN, kfu, entB, irp1, clbA, clbB*, and *clbN*, were determined by PCR. 25μL reactions were prepared with 2× Spark Taq PCR Master Mix (Solely Bio, Shandong, China) (12.5μL), forward and reverse primer (10 pmol/μL primer stock) (both 1μL), genomic DNA (1μL), and water (9.5μL). PCR was run; for 4 min initial activation at 95°C, then 30s cycles 35 times at 94 °C, 60 °C for 30s, and 72 °C for 60s, and lastly, a 10 min final extension at 72 °C. 1% agarose gel electrophoresis was performed for separating PCR products. Table S1 enlists all the primers employed.

Quantitative RT-PCR

3 *K. pneumoniae* (serotype K1) strains isolated from the participants and classic *K. pneumoniae* ATCC700603 were considered the representing strains in the subsequent experiments. The three clinical isolates were named KP133, KP164 and KP188. Among these isolates, KP133 was isolated from the non-diabetic group and the remaining two were from the diabetic group. *K. pneumoniae* were cultured in glucose-free LB broth, 0.5% glucose LB broth, or 0.5% glucose containing 1mM cAMP LB broth, respectively. After incubated at 37°C for 6h, total RNA was acquired from exponential growth phase bacterial cells via the Bacterial RNA Kit (YEASEN, China) according to the developer's protocol. RNA was reverse-transcribed with the RT Master Mix (Takara, Japan) using random primers. qRT-PCR was carried out in a Light-Cycler 480 (Roche, Basel, Switzerland) instrument using SYBR Premix Ex TaqII (Takara, Japan). The cycling parameters included; 30s at 95°C, 5s of 40 cycles at 95°C, and 60°C for 20s. Relative gene expression was measured by the comparative threshold cycle $2^{-\Delta\Delta CT}$method. 23S rRNA was selected as an endogenous reference. All samples were analyzed thrice. The primer's sequences are listed in Table S1.

Serum Killing Assays

The bacterial susceptibility to human serum was assayed using a modified method by Yeh et al.[22] KP133, KP164 and KP188 strains were isolated from the participants and were considered the representing strains in the subsequent experiments. The serotypes and virulence-associated genes of the three clinical hvKP strains are presented in Table S2. Briefly, human serum from 8 healthy donors was incubated with LB broth cultured hvKP strains in the presence and absence of 0.5% glucose. Bacteria were diluted to 4×10^6 colony-forming units (c.f.u.)/mL in physiological saline. The bacterial suspensions (25μL) and the acquired nonimmune human serum (75μL) were dispensed in 96 well-plate, mixed, and incubated at 37 °C. Viability was determined immediately after 1h, 2h, and 3 h incubation. After mixing, samples were taken and serial dilutions were plated on MH agar, then incubated at 37°C for 18h for colony counts.

Statistical Analysis

SPSS (25.0 version) was utilized for assessing the statistics. Normally distributed continuous variables were demonstrated as the mean ± standard deviation and for their comparison, Student's *t*-test was applied. Categorical variable data were disclosed as n (%) and Fisher's exact test was performed for their comparative analysis. Statistical analysis was

performed using GraphPad Prism 6.01. All histograms related quantitative data were expressed as mean ± standard deviation (SD). Comparisons between two groups were made using an unpaired *t*-test. $P<0.05$ was considered significant.

Results

Comparison of Clinical Characteristics of KLA with Diabetes and without Diabetes

The 57 *K. pneumoniae* liver abscess diagnosed patient's demographic manifestations, underlying disorder, and complications associated with diabetes (n=33, 57.9%) and without diabetes (n=24, 42.1%) are summarized in Table 1. More than half of the patients were male (42/57, 73.7%) and the average age of the patients at diagnosis was 58.5±14.7 years (range 30 to 99). Fever and abdominal pain were the predominant manifestations in these two groups. Diabetes followed by hypertension (29.8%, 17/57) and biliary tract diseases (28.1%, 16/57) was the most common underlying disease. Poor glycemic control patients had the trend of higher sepsis rate (51.5% vs 20.8%, $P=0.028$) and invasive infections (45.5% vs 12.5%, $P=0.01$), and therefore longer hospital stays (23.3±7.4 vs 17.3±8.6, $P=0.01$) than those with controlled glycaemia. Laboratory investigations recorded on admission are also shown in Table 1. The levels of CRP were positively correlated with the degree of infection, and >100 mg/L CRP indicated a serious infection. In this research, the proportion of CRP>100 mg/L in the diabetic group was significantly higher than that in the non-diabetic group.

Table 1 Baseline Characteristics, Clinical Presentation, and Outcome of KLA Patients with Diabetes and without Diabetes

Characteristics	Diabetic-KLA (n=33)	Non-Diabetic-KLA (n=24)	*P* value
Male (%)	23(69.7)	19(79.2)	0.547
Age (years)	61.2±16.5	57.2±11.2	0.314
Fever>38°C (%)	32(97.0)	24(100)	1.000
Underlying conditions			
Hypertension (%)	12(36.4)	5(20.8)	0.251
Biliary tract diseases (%)	7(21.2)	9(37.5)	0.236
Tumors (%)	0(0)	3(12.5)	0.069
Complications			
Sepsis (%)	17(51.5)	5(20.8)	0.028*
Septic shock (%)	4(15.2)	1(4.17)	0.385
Pulmonary/renal/ subphrenic abscess (%)	15(45.5)	3(12.5)	0.01**
Death (%)	1(3.0)	0(0)	1.000
In-hospital days	23.3±7.4	17.3±8.6	0.01**
Laboratory findings			
Glucose (mmol/L)	16.1±6.6	6.8±1.7	< 0.0001****
HbA_{1C} (%)	10.2±2.0	5.9±0.3	< 0.0001****
WBC ($\times10^9$/L)	13.4±4.2	11.8±5.0	0.197
Neutrophils ($\times10^9$/L)	11.5±4.1	10.1±4.8	0.273
Lymphocytes ($\times10^9$/L)	1.0±0.4	1.0±0.5	0.845
Monocytes ($\times10^9$/L)	0.6±0.3	0.7±0.5	0.349
PLT ($\times10^9$/L)	195.7±104.8	205.2±109.2	0.750
CRP >100mg/L	33(100)	19(79.2)	0.01**
PCT (ng/mL)	19.0±32.8	12.2±19.7	0.385
ALT (U/L)	89.1±60.2	111.4±95.8	0.303
AST (U/L)	95.7±110.0	104.5±153.6	0.811
ALP (U/L)	225.3±127.8	187.1±77.6	0.223

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

Microbiological Characteristics and Antimicrobial Susceptibility

In addition to its natural ampicillin resistance, the isolated *K. pneumoniae* was highly sensitive to other antibiotics. A few strains were resistant to levofloxacin, ciprofloxacin, ampicillin/sulbactam, and cotrimoxazole, but there was no difference between the two groups (Table 2). Unfortunately, compared to the high antibiotic susceptibility of *K. pneumoniae* in the diabetic group, the non-diabetic group had two multi-drug resistant strains, including an extended-spectrum β-lactamase-producing *K. pneumoniae* (ESBL-KP) and a carbapenem-resistant *K. pneumoniae* (CR-KP) isolate.

Four serotypes were detected in *K. pneumonia* patients, these were K1 (57.9%, 33/57), K2 (19.3%, 11/57), K5 (1.8%, 1/57), and K57 (7%, 4/57), while K20 and K54 were not detected in any tested isolates (Table 3). Eight isolates (14.0%, 8/57) were not classified successfully (referred to as; K-non-typable isolates). The capsular serotypes prevalence among diabetic and non-diabetic isolates were similar. The expression of virulence genes including *wcaG, magA, rmpA, kfu, iro, entB, irp1, aero*, and *alls* showed no marked change between the diabetic and non-diabetic *K. pneumoniae* isolates. However, all diabetic isolates harbored *rmpA* and *irp1*, while 87.5% of non-diabetic isolates expressed these important virulence-associated genes (P=0.068). Colibactin encoded by the *pks* gene cluster (*clbA, clbB*, and *clbN*), damages DNA and enhances virulence.[23] In this research, the colibactin system markers *clbA, clbB*, and *clbN* were simultaneously identified in 42.1% (24/57) isolates, which were considered as pks^+ *K. pneumoniae*.[24] The positive rates of pks^+ *K. pneumoniae* among diabetic isolates (51.5%, 17/33) were higher than those among non-diabetic isolates (29.2%, 7/24). However, no notable difference (p=0.11) in *pks* expression between the two cohorts was noticed.

Effect of Exogenous Glucose on the *rmpA, ompA, and clbB* Transcription of *K. pneumoniae*

The impact of exogenous high glucose level on the transcriptions of *K. pneumoniae* virulent genes was analyzed. With the help of classic *K. pneumoniae* ATCC700603 and 3 serotype-K1 hvKP clinical isolates, the effect of exogenous high glucose level on *rmpA, ompA*, and *clbB* genes expression were assessed. Virulence genes expression of the three isolates was shown in Table S2. The clinical isolates were cultured in LB broth (in the presence or absence of 0.5% glucose). The mRNA levels of *rmpA, ompA*, and *clbB* were quantified by qRT-PCR. As Figure 1B–D depicts, adding exogenous

Table 2 Antimicrobial Susceptibility of *K. pneumoniae* Isolates from KLA Patients with Diabetes and without Diabetes

Antimicrobial Susceptibility, n (%)	Diabetic-KLA (n=33)	Non-Diabetic-KLA (n=24)	*P* value
Ampicillin	0(0)	0(0)	-
Ampicillin/Sulbactam	31(93.9)	20(83.3)	0.227
Piperacillin-tazobactam	33(100)	22(91.7)	0.173
Cefazolin	33(100)	22(91.7)	0.173
Ceftriaxone	33(100)	22(91.7)	0.173
Ceftazidime	33(100)	22(91.7)	0.173
Cefepime	33(100)	22(91.7)	0.173
Aztreonam	33(100)	22(91.7)	0.173
Cefotetan	33(100)	22(91.7)	0.173
Meropenem	33(100)	23(95.8)	0.421
Imipenem	33(100)	23(95.8)	0.421
Levofloxacin	27(81.8)	19(79.2)	1.000
Ciprofloxacin	29(87.9)	22(91.7)	1.000
Amikacin	33(100)	24(100)	-
Trimethoprim-sulfamethoxazole	31(93.9)	21(87.5)	0.640
Gentamicin	33(100)	24(100)	-
Tobramycin	33(100)	24(100)	-
ESBL	0(0)	1(4.2)	0.421
CRKP	0(0)	1(4.2)	0.421

Table 3 Capsular Serotypes and Virulence Factors of *K. pneumoniae* Isolated from Patients with Diabetes and without Diabetes

Variable, n (%)	Diabetic-KLA (n=33)	Non-Diabetic-KLA (n=24)	*P* value
Capsular serotype			
K1	18 (54.5)	15(62.5)	0.596
K2	7(21.2)	4(16.7)	0.745
K5	1(3.0)	0(0)	1.000
K57	2(6.1)	2(8.3)	1.000
K-non-typable	5(15.2)	3(12.5)	1.000
Virulence genes			
rmpA	33(100)	21(87.5)	0.069
wcaG	22(66.7)	16(66.7)	1.000
magA	18(54.5)	11(45.8)	0.596
aero	31(93.9)	19(79.2)	0.119
kfu	15(45.5)	11(45.8)	1.000
iro	11(33.3)	8(33.3)	1.000
entB	33(100)	23(95.8)	0.421
irp1	33(100)	21(87.5)	0.069
alls	17(51.5)	12(50.0)	1.000
clbA+ clbB+ clbN	17(51.5)	7(29.2)	0.110

Note: K-non-typable, Non-K1/K2/K5/K20/K54/K57.

glucose (0.5%) in LB broth increased the mRNA of *rmpA, ompA*, and *clbB* genes expression. The intracellular cAMP levels were reduced in LB broth with glucose[25,26] and exogenous 1mM cAMP was added to the glucose-augmented broth to observe the mRNA levels of these genes. The exogenous cAMP addition suppressed the glucose effect on the transcription of *rmpA* and *ompA*, but did not affect *clbB* (Figure 1B–D). *K. pneumoniae* ATCC700603 did not express virulence factors *rmpA* and *clbB*. Our results showed high glucose levels also increased the transcription of *ompA* in *K. pneumoniae* ATCC700603, and this up-regulation was controlled by cAMP (Figure 1A). These results suggested that high glucose concentration up-regulated the transcription of *rmpA* and *ompA* in *K. pneumoniae* by the cAMP signaling pathway.

Environmental Glucose Stimuli Increased *K. pneumoniae* Resistance to Serum Killing

High glucose levels could enhance *cps* genes expression of hvKP, which increases resistance to phagocytosis.[6] However, the literature suggesting whether *K. pneumoniae* cultured with high-glucose increases its resistance to serum killing is still missing. To evaluate the serum's sensitivity to the *K. pneumoniae* pathogenic effect, grown in high glucose, serum killing assays were performed. Bacterial survival in normal human serum was recorded at different time points. As shown in Figure 2A, the survival rate of *K. pneumoniae* ATCC700603 strain decreased over time. However, after co-cultured with serum for 2 and 3 hours, the survival rate of the strain cultured in LB with high glucose level (0.5%) was increased compared with that in LB broth. Compared with classic *K. pneumoniae* ATCC700603, clinical isolates (KP133, KP164 and KP188) expressed virulence factors and showed a hypermucoviscous phenotype. The rate of hvKP strains survival was also markedly enhanced when grown in LB with 0.5% glucose than that in LB broth lacked glucose (Figure 2B–D).

Discussion

Diabetes is a risk factor for infectious diseases, such as KLA, tuberculosis, melioidosis, and COVID-19.[27–30] Previous studies have shown that diabetes impairs the innate and adaptive immune system, impairing macrophages, T cells,

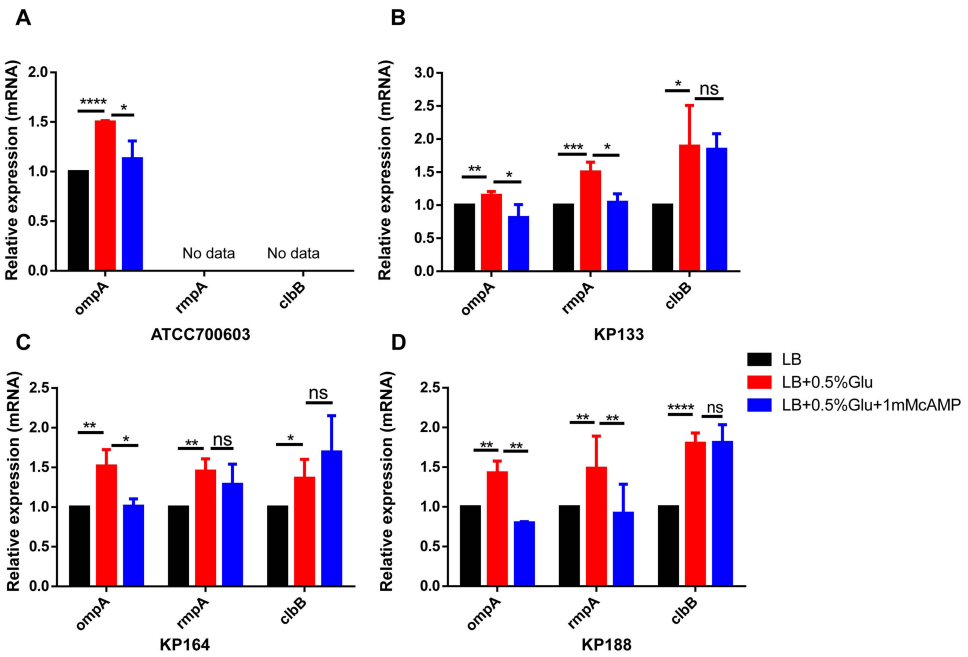

Figure 1 Exogenous glucose up-regulated *rmpA* and *ompA* transcription through the cAMP-signaling pathway. Quantitative reverse-transcription polymerase-chain-reaction (qRT-PCR) assays were performed to investigate the expression of *ompA, rmpA*, and *clbB* genes in *K. pneumoniae* ATCC700603 (**A**), clinical isolates KP133 (**B**), KP164 (**C**), and KP188 (**D**) cultured in just LB broth, 0.5% glucose LB broth, and 0.5% glucose containing 1mM cAMP LB broth at 37°C for 6h. **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001.

Abbreviation: n.s., not significant.

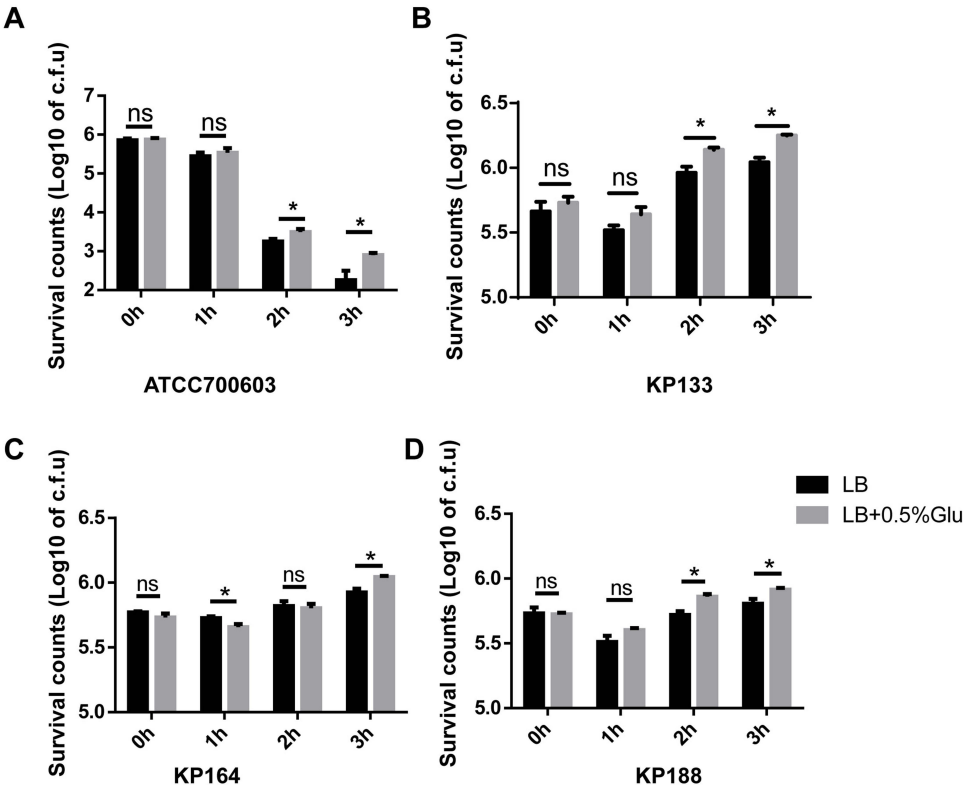

Figure 2 *K. pneumoniae* cultured with high concentration of glucose increased its resistance to serum killing. Human serum from 8 healthy donors was collected and incubated with *K. pneumoniae* ATCC700603 (**A**), clinical isolates KP133 (**B**), KP164 (**C**), and KP188 (**D**) that had been previously cultured in LB and LB with 0.5% glucose. Viability was determined immediately and after 1h, 2h, and 3 h of incubation. **P*<0.05.

Abbreviation: n.s., not significant.

neutrophils, and NK cells.[31] This immune dysfunction increases the risk of infection in diabetic patients. Lin et al found that diabetic control was crucial for the clinical KLA characteristics, especially when dealing with metastatic complications associated with KLA.[30] Consistent with the above study, we observed that substandard control of glucose in patients leads to a higher rate of invasive infections than in those with controlled glycaemia. The major abnormalities of KLA in the laboratory were increased CRP and liver dysfunction.[32,33] It was also demonstrated in this investigation that the levels of CRP in diabetic-KLA patients were higher, which suggested a more severe infection. Sepsis is a lethal organ dysfunction caused by a dysregulated host response to an infection. Septic-KLA patients had an increased risk of chronic metastatic complications and prolonged hospital stays.[33] The results from this research indicated that the incidence of sepsis was higher and the hospital stays were longer in KLA with diabetes.

In this study, the virulence genes and capsular serotypes were detected by PCR. 94.7% (55/57) *K. pneumoniae* expressed *rmpA* and at least one siderophore. Moreover, hvKP strains isolated from KLA patients were sensitive to a range of antibiotics. So, empirical antibiotic therapy for liver abscesses (including three generation of cephalosporin, carbapenems and piperacillin-tazobactam) was generally effective. However, compared to the high antibiotic susceptibility of *K. pneumoniae* in the diabetic group, the non-diabetic group had two multi-drug resistant strains, including an ESBL-KP and a CR-KP isolate. The CR-KP isolate belonged to K2 serotype and expressed siderophores virulence genes (*entB* and *irp1*). Both the two multi-drug resistant *K. pneumoniae* isolates recovered from patients with hepatic diseases and repeated hospitalizations. Therefore, it is also necessary to pay attention to the occurrence of KLA after liver surgery, especially caused by multi-drug resistant *K. pneumoniae*.

Diabetes is an important risk factor for KLA.[34] Hyperglycaemia dysregulates the neutrophil phagocytosis of *K. pneumoniae* capsule serotypes K1 and K2.[6] While, high glucose levels also affect *K. pneumoniae* pathogenicity, including enhancement of CPS and type 3 fimbriae.[10,11] The glucose levels used in our experiments is supposed to be similar to the human bloodstream concentrations. Literature suggests that healthy control would have 0.1% glucose blood concentration, while diabetic one would have 0.2–0.5% glucose blood concentrations.[10] Our study showed that addition of exogenous glucose (0.5%) in LB broth enhanced mRNA of *ompA* and *rmpA* gene expression. *RmpA* is an important virulent factor in hvKP isolate, which could stimulate capsular production, resulting in the formation of hypermucoviscous phenotype.[12] In our study, 87.5% (21/24) of *K. pneumoniae* isolated from KLA without diabetes harbored the *rmpA* gene, while 100% of *K. pneumoniae* in KLA with diabetes were *rmpA*-positive. Furthermore, high glucose increased the mRNA of *rmpA* by regulating the cAMP-CRP signal pathway. As *rmpA* activates *K. pneumoniae's* CPS biosynthesis, the results indicated that this is done not only by the direct *cps* genes regulation but also probably by increasing *rmpA* gene expression.

OmpA is an abundant predominant outer membrane protein of Enterobacteriaceae[13] and is under the influence of many environmental stimuli. Gibert et al revealed that glucose augmented culture medium reduced the transcription of the *ompA* gene in *Escherichia coli*.[16] Increased glucose inhibits intracellular second messenger cAMP production and inactivates the cAMP-CRP signaling pathway.[25,26] Liu et al demonstrated that *ompA* increased in protein levels in the *crp* deletion *K. pneumoniae* by proteomics analysis. However, there was no significant difference in the level of transcription in the high-glucose environment.[35] Therefore, the regulation of *ompA* by high glucose is still controversial. This investigation revealed that high glucose enhanced the mRNA of *ompA* and the addition of exogenous cAMP suppressed its enhancement.

Resistance to serum killing is associated with *K. pneumoniae* hyper-virulence. The CPS, O-antigen of LPS, and outer membrane proteins are important pathogenic factors that protect *K. pneumoniae* from being killed by the serum.[19] *K. pneumoniae* was able to prevent complement activation by modifying sugar structures.[36] A thick outside polysaccharide layer creates a physical barrier in the bacterium that inhibits the penetration of the membrane attack complex (MAC), thereby preventing bacterial lysis.[37] Apart from the capsular polysaccharides and LPS structures, *K. pneumoniae* also utilizes outer membrane proteins to escape from the complement system detection.[38] OmpA directly restricts the complement cascade to mediate serum resistance.[39] Our results indicate that pre-incubation of *K. pneumoniae* strains in media containing high glucose enhanced *rmpA* and *ompA* gene expression, which might lead to increased bacterial resistance to serum killing.

Conclusion

In summary, KLA patients with diabetes showed a higher incidences of sepsis and invasive infections, and their length of hospital stay was also prolonged than those without diabetes. But there was no significant differences in antibiotic susceptibility and virulence genes between the two groups. Furthermore, high glucose levels increased the transcription of *rmpA* and *ompA* in hvKP by the cAMP signaling pathway and enhanced the resistance to serum killing, thus providing a new and reasonable explanation for the high incidence of sepsis and invasive infections in KLA patients with diabetes.

Data Sharing Statement

All primers used in this article and virulence genes expression of the three clinical isolates are available in Supplementary Materials. Readers can contact the corresponding author if they want access to additional materials.

Ethics Approval and Consent to Participate

This study complies with the guidelines for human studies and is in accordance with the Declaration of Helsinki. All clinical data of the patients were collected in accordance with the Local Research Ethics Committee of the First Affiliated Hospital of Anhui Medical University (Quick-PJ 2023-01-16). The need for written informed consent was waived because the samples were routinely collected and patients' anonymous information was provided by the microbiology hospital laboratory. This study completely followed the principles outlined in the Declaration of Helsinki.

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Disclosure

The authors have no conflicts of interest to declare for this work.

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