ORIGINAL RESEARCH

Rapid Identification of KL49 *Acinetobacter baumannii* Associated with Clinical Mortality

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Objective: We aimed to establish a tool for rapid identification of KL49 *Acinetobacter baumannii*.

Methods: Based on the capsular polysaccharide (CPS) synthesis genes database, we investigated the distribution of K locus type 49 (KL49) genes in other KL types and established a rapid identification method for KL49. We collected 61 clinical carbapenem-resistant *A. baumannii* (CRAB) strains, identified KL49 by *gtr100* detection, and used whole genome sequencing (WGS) for verification. A mouse pneumonia model was used to confirm the hypervirulence phenotype. We tested the presence of *gtr100* gene in 165 CRAB strains from three provinces in China and evaluated the correlation of *gtr100* carrying CRAB infection with mortality.

Results: The *gtr100* gene is the CPS synthesis gene found only in KL49. We screened out nine WGS-validated KL49 strains from 61 CRAB clinical strains using polymerase chain reaction (PCR) to detect the *gtr100* gene. The survival rates of KL49 strains were significantly lower than nonKL49 strains in a mouse pneumonia model. The survival rates of LAC-4 *gtr100* knockout strain decreased significantly. Analysis of phylogenetics showed the worldwide spread of KL49 *A. baumannii*. Infection of *gtr100* carrying CRAB is an independent risk for mortality (OR, 10.76; 95%CI: 3.08–37.55; *p*<0.001).

Conclusion: The hypervirulence phenotype of KL49 CRAB and the association with mortality highlight the urgent need for implementing control measures. The rapid identification assay has the potential to facilitate early medical intervention and worldwide surveillance.

Keywords: *Acinetobacter baumannii*, hypervirulent, K locus, Kaptive, mortality

Introduction

Acinetobacter baumannii is a gram-negative coccobacillus that can cause serious infections among critically ill patients, particularly in the intensive care unit (ICU) setting.[1] Worldwide, *A. baumannii* accounted for up to 20% of infections in ICUs.[2]

In general, *A. baumannii* has been regarded as a low-grade pathogen. Most laboratory strains and clinical strains do not cause severe infections in immunocompetent mice, inducing only a self-limiting pneumonia with very limited local bacterial replication and systemic dissemination, even when a large inoculum is used.[3,4] However, two studies have shown that some clinical carbapenem-resistant *A. baumannii* (CRAB) strains are lethal to immunocompetent mice and thus revealed the range of virulence in different strains of this pathogen[5,6] LAC-4 is a hypervirulent strain that killed 100% of mice within 48 h after being inoculated with 10^8 colony-forming units (CFU).[6] Subsequently, there have been some reports about the outbreak and epidemic of *A. baumannii* with enhanced virulence[7,8] However, based on multilocus sequence typing (MLST) genotyping, these hypervirulent strains are of different genotypes.[7–9]

Genomic analysis showed that these strains all shared a glycosylation gene cluster and type VI secretion system (T6SS), which was thought to be the potential virulence marker.[7–9] Notably, the glycosylation gene cluster shared by these hypervirulent strains was part of K locus 49 (KL49) CPS gene cluster, suggesting that the KL49 strain needs to be taken seriously.

In this study, we established a method for rapid identification of the KL49 strain and successfully screened out the hypervirulent strains from clinical strains. The reliability of the method was verified by whole genome sequencing (WGS) and animal experiments. Finally, clinical data analysis showed that KL49 *A. baumannii* infection is an independent risk for mortality.

Methods
K Loci Analysis

Capsular polysaccharide (CPS) synthesis gene cluster analysis was performed using *A. baumannii* K locus database.[10] All available *A. baumannii* genomes from NCBI (as of 2019-08-04) were downloaded. Kaptive (version 0.7.0) was used for KL type identification.[10]

Clinical Strains Collection and Phenotypic Characterization

Consecutive nonreplicate clinical strains of CRAB in the ICU of Shenzhen People's Hospital in 2017 (n=61) were used for WGS and in vivo assay in this study. Shenzhen People's Hospital is a medical center with 2500 beds in the Luohu district of Shenzhen, China. A VITEK-2 compact system (bioMérieux, Marcy-l'Étoile, France) was used to establish the strain identity and antimicrobial susceptibilities of the strains. All antimicrobial susceptibilities results were verified by broth microdilution method. The results were interpreted in accordance with the guidelines published by the Clinical and Laboratory Standards Institute (CLSI; document M100-S26).[11] The species identity of all strains was confirmed via matrix-assisted laser desorption/ionization mass spectrometry (bioMérieux, Marcy-l'Étoile, France).

WGS, Genomic Characterization and Phylogenetic Analysis

Bacteria from frozen stocks were cultured in Luria–Bertani (LB) broth overnight at 37°C with shaking (220 rpm). Overnight cultures were diluted 1/100 and re-cultured until the optical density at 600 nm (OD_{600}) was 0.6–0.8. Genomic DNA was extracted from each isolate using the SDS method.[12] The bacterial genomes were sequenced using Illumina HiSeq 2500 platform (Illumina, San Diego, CA, USA), and fastp was used to remove low-quality and low-complexity reads, and polyG/polyX tails.[13] The genomes were assembled with de novo SPAdes Genome Assembler (version 3.12.0).[14] Virulence factors in the strains were identified by scanning the genome contigs against VFDB databases using ABRicate (version 0.8.7). Parsnp (version 1.5.2) was used to align the core genome and call the variants (SNP).[15] Maximum likelihood tree was generated with RAxML v 7.0.4 using a GTRGAMMAI model.[16] Online tool iTOL was used to display, manipulate, and annotate phylogenetic tree.[17]

gtr100 Deletion and Complement Mutant Construction

The LAC-4 knockout strain was constructed using the suicide vector pCVD442. Briefly, the apramycin resistance (Apr) gene and the flanking sequences of *gtr100* was synthesized. The fragments were joined and cloned into the suicide plasmid pCVD442 to yield the plasmid pCVD442-Δ*gtr100*:Apr, which was then transformed into *Escherichia coli* β2155, and then positive clones were selected on LB agar containing ampicillin (50 μg/mL) and apramycin (50 μg/mL). Next, conjugation between the recipient LAC-4 and the donor β2155/pCVD442-Δ*gtr100*:Apr was performed to transfer the recombinant suicide plasmid pCVD442-Δ*gtr100*:Apr from β2155 to LAC-4. Apramycin- and sucrose-resistant colonies were selected and screened by PCR.

The *gtr100* complemented strain was constructed using the vector pWH1266. Briefly, the synthesized *gtr100* sequence was joined and cloned into the plasmid pWH1266 to yield the plasmid pWH1266:*gtr100* which was then transformed into *E. coli DH5a*. Positive clones were selected on LB agar containing ampicillin (50 μg/mL) and tetracycline (10 μg/mL). The isolated plasmid pWH1266:*gtr100* was electroporated into LAC-4:Δ*gtr* electrocompetent cells. The complemented LAC-4:Δ*gtr100*Ω*gtr100* strains were selected on LB agar with tetracycline (10 mg/mL) and confirmed by PCR.

Virulence Test in vivo

We used a pneumonia model of *A. baumannii* in mice to test the virulence of strains. Inbred C57BL/6 six to eight weeks old female mice under specificpathogen-free grade were purchased from Hunan SJA Laboratory Animal Co.,

Ltd (Hunan, China). The mice were intraperitoneally anesthetized with pentobarbital sodium (75 mg/kg) and inoculated with 20 μL (2.5×10^8, 5×10^7, 1×10^7, 0 CFU) of *A. baumannii* by noninvasive intratracheal instillation under direct vision. The survival of the mice was observed for seven days post infection.[5] All animal care and use protocols in this study were performed in accordance with the Regulations for the Administration of Affairs Concerning Experimental Animals approved by the State Council of People's Republic of China. All animal experiments in this study were approved by the Animal Ethical and Experimental Committee of the Army Military Medical University (Chongqing, Permit No. 2011–04) in accordance with their rules and regulations.

Clinical Data Collection and Analysis

CRAB clinical isolates (n=165) were collected from three provinces and used for univariable logistic regression analysis. We retrospectively used rapid identification tool of KL49 to test and collected demographic data, underlying diseases, sites of infection, comorbidities, invasive procedures, antibiotics usage and clinical characteristics of the corresponding patients. The PCR protocol for identifying the presence of *gtr100* was as follows: 20 μL reaction containing 1.0 μL of 2×SanTaq PCR Mix (with blue dye); 1.0 μL of bacteria culture (OD_{600}=0.6–0.8); 1.0 μL (10 μM) of forward primer, 1.0 μL (10 μM) of reverse primer; 7.0 μL of sterilized ddH$_2$O. Amplification conditions were: denaturation at 95°C for four minutes; 95°C for 15 seconds, 60°C for 30 seconds, 72°C for 30 seconds (35 cycles); final extension at 72°C for five minutes. Taq DNA polymerase but not high fidelity and hotstart DNA polymerase was recommended. The primary outcome was all-cause in-hospital mortality following the onset of *A. baumannii* infection. Logistic regression was used for outcome analysis. Variables with a *P*-value ≤0.05 in the univariable regression were selected for multivariable analysis.

Results

By comparing the gene clusters of different KL in the Kaptive database, we found that only *gtr100* gene was specific to KL49, suggesting that the detection of *gtr100* has the potential to be used to identify KL49 (Table 1). We performed KL type analysis of all 3341 *A. baumannii* genomes retrieved from NCBI. Of the 80 genomes that contained the *gtr100* gene, 79 were identified as KL49 and one as KL52. Sixteen genomes without the *gtr100* gene

Table 1 Distribution of KL49 Capsular Polysaccharide (CPS) Synthesis Genes in Other KL Types

Gene Name	Proportion	Gene Name	Proportion
wzc	92/92	gtr100	1/92
wzb	92/92	wzx	92/92
wza	92/92	fnlA	13/92
gna	92/92	fnlB	13/92
lgaA	14/92	fnlC	13/92
lgaB	14/92	gtr20	7/92
lgaC	14/92	qnr1	3/92
lgaD	8/92	itrB2	7/92
lgaE	8/92	itrA3	35/92
lgaF	14/92	galU	92/92
elaA	2/92	ugd	92/92
elaB	2/92	gpi	92/92
elaC	2/92	gne1	72/92
wzy	89/92	pgm	92/92

were identified as KL49. However, 14 of these genomes showed "low" and "none" confidence levels called by Kaptive, probably due to the poor-quality sequence assembly (Table S1).

Nonrepetitive CRAB (n=61) strains collected from patients with confirmed *A. baumannii* infections were frozen and stored by the clinical microbiology laboratory at the Shenzhen People's Hospital. We then used forward primer (5′-TTGAGAAGCTAAATTATGGCTCG-3′) and reverse primer (5′-GATAGCACAGAAATCCATAAAGGAA-3′) to screen for the presence of *gtr100*, as a marker for KL49. Among them, the *gtr100* gene was identified in nine of the strains by PCR assay. WGS results confirmed that the nine strains were KL49 (Table 2). Virulence gene analysis showed that most strains shared the same profile except: *abaR* was missing in isolates 5121, 5122, 5123, 5127, 2096, 2092 and 2093; *bap* was missing in isolate 2096; and *hemO* was missing in isolates 20881 and 20883 (Figure S1).

To verify the virulence differences between KL49 and nonKL49 strains, two KL49 strains (5122 and 2092) and three nonKL49 strains (7152, 71517 and 20859) were randomly selected to test their virulence in the mouse model (Figure 1). ATCC17978 and LAC-4 was used as control. With an inoculum of 2.5×10^8 CFU, the survival with strains 5122, 2092 and LAC-4 was 0% at 48 h, and at seven days was 60% with ATCC17978, 50% with 7152, 71517 and 20859. With an inoculum of 5×10^7 CFU, survival was 0% with LAC-4 at 48 h; 10% with 5122 and 2092, 80% with ATCC17978 and 7152, 60% with 71517, and 90% with

Table 2 KL Types of 61 Clinical Carbapenem Resistant *Acinetobacter baumannii* Strains in This Study

Assembly	Strain	Best Match Locus	Match Confidence
GCA_007999645.1	1044	KL3	Very high
GCA_007999765.1	208210	KL77	Perfect
GCA_007999725.1	208211	KL77	Perfect
GCA_007999745.1	208212	KL77	Perfect
GCA_007999705.1	208213	KL77	Perfect
GCA_007999665.1	208214	KL77	Perfect
GCA_007999955.1	20821	KL77	Perfect
GCA_007999855.1	20822	KL77	Perfect
GCA_007999905.1	20823	KL77	Perfect
GCA_007999885.1	20824	KL77	Perfect
GCA_007999845.1	20825	KL77	Perfect
GCA_007999875.1	20826	KL77	Perfect
GCA_007999815.1	20827	KL77	Perfect
GCA_007999805.1	20828	KL77	Perfect
GCA_007999755.1	20829	KL77	Perfect
GCA_007999365.1	208510	KL3	High
GCA_007999525.1	20851	KL3	Very high
GCA_007999515.1	20852	KL3	Very high
GCA_007999505.1	20853	KL3	Very high
GCA_007999485.1	20854	KL3	Very high
GCA_007999465.1	20855	KL3	Very high
GCA_007999405.1	20856	KL3	Very high
GCA_007999395.1	20857	KL3	Very high
GCA_007999425.1	20858	KL3	Very high
GCA_007999375.1	20859	KL3	Very high
GCA_008000175.1	2087	KL3	High
GCA_007999985.1	20881	KL2	Very high
GCA_007999965.1	20882	KL2	Good
GCA_007999945.1	20883	KL2	Very high
GCA_007999625.1	2092	KL49	Very high
GCA_007999585.1	2093	KL77	Perfect
GCA_007999605.1	20941	KL3	Very high
GCA_007999635.1	20942	KL3	Very high
GCA_007999555.1	20943	KL3	Very high
GCA_008000185.1	2096	KL14	Low
GCA_008000165.1	5121	KL49	Very high
GCA_008000125.1	5122	KL49	Very high
GCA_008000095.1	5123	KL49	Very high
GCA_008000085.1	5124	KL49	Very high
GCA_008000065.1	5125	KL49	Good
GCA_008000075.1	5126	KL49	Very high
GCA_008000045.1	5127	KL49	Very high
GCA_008000025.1	5128	KL49	Very high
GCA_007999155.1	71510	KL3	Very high
GCA_007999145.1	71511	KL3	Very high
GCA_008000715.1	71512	KL3	Very high
GCA_007999045.1	71513	KL3	High
GCA_008000725.1	71514	KL3	High
GCA_007999055.1	71515	KL3	High

(*Continued*)

Table 2 (Continued).

Assembly	Strain	Best Match Locus	Match Confidence
GCA_007999075.1	71516	KL3	High
GCA_008000225.1	71517	KL3	Very high
GCA_008000235.1	71518	KL3	High
GCA_007999345.1	7151	KL3	Very high
GCA_007999305.1	7152	KL3	Very high
GCA_007999275.1	7153	KL3	Very high
GCA_007999245.1	7154	KL3	Very high
GCA_007999295.1	7155	KL3	Very high
GCA_007999255.1	7156	KL3	Very high
GCA_007999225.1	7157	KL3	Very high
GCA_007999175.1	7158	KL3	Very high
GCA_007999165.1	7159	KL3	Very high

20859 at seven days. With an inoculum of 1×10^7 CFU, seven-day survival was 10% with LAC-4, 40% with 5122, 30% with 2092, 90% with 71517, 100% with ATCC17978 and 7152. The survival rates of KL49 strains (LAC-4, 5122 and 2092) were significantly lower than nonKL49 strains (7152, 71517, 20859 and ATCC17978) (Figure 1; $P<0.0001$ by log rank test). With the aim of identifying the role of *gtr100* in the virulence of KL49 *A. baumannii*, we constructed the *gtr100* knockout and complemented strain of LAC-4. As shown in Figure 1D, with an inoculum of 1×10^8 CFU, seven-day survival was 0% with LAC-4, 40% with *gtr100* knockout strain and 10% with complemented strain ($P=0.0121$ by log rank test). With an inoculum of 1×10^7 CFU, seven-day survival was 20% with LAC-4, 100% with *gtr100* knockout strain, and 40% with complemented strain ($P=0.0005$ by log rank test). The survival rates of *gtr100* knockout strain were significantly higher than LAC-4 (1×10^7 CFU, $P=0.0003$; 1×10^8 CFU, $P=0.0118$) and the complemented strain (1×10^7 CFU, $P=0.0040$ by log rank test).

Eighty-seven KL49 assembly genomes were used for phylogenetic tree construction, including 78 retrieved from NCBI with a "perfect" or "very high" confidence level called by Kaptive and nine genomes in this study. In the phylogenetic tree, KL49 strains are divided into two distinct clades that are prevalent in Americas and Asia, respectively. However, two strains from China and Thailand were found in the Americas clade and one strain from the Czech Republic was found in the Asia clade, representing the worldwide spread of KL49, probably via human travel (Figure 2).
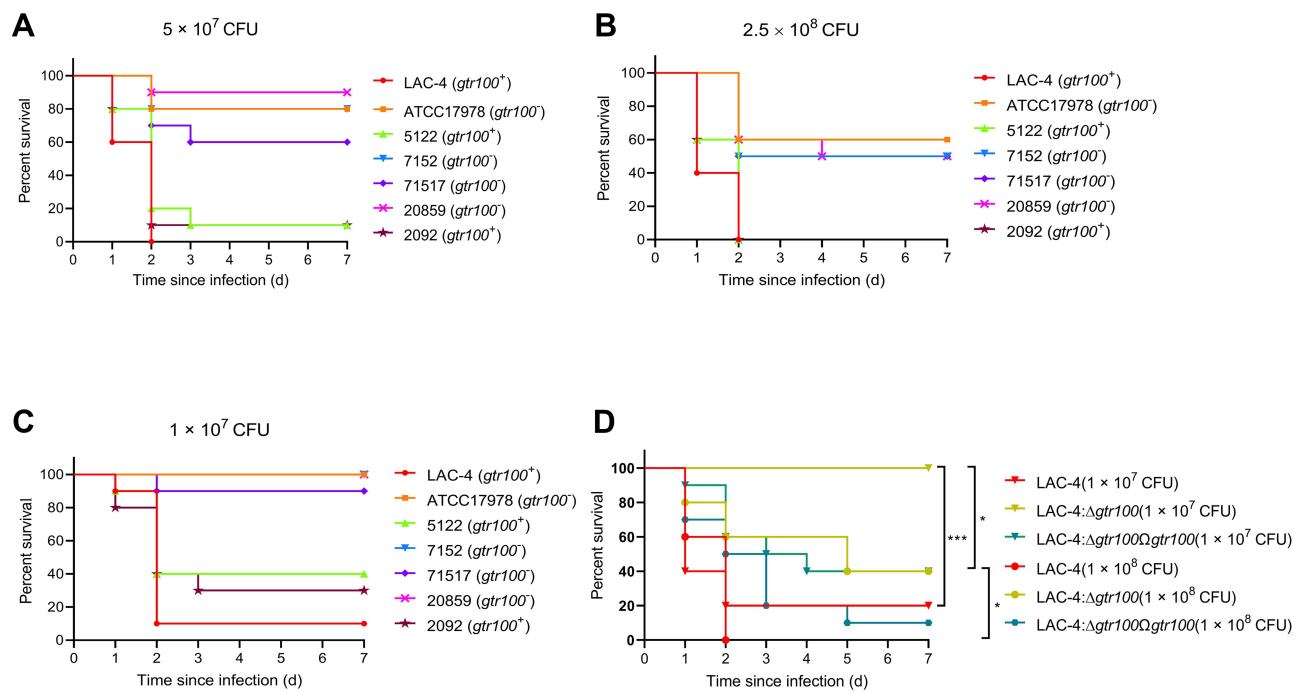
Figure 1 Virulence potential of *Acinetobacter baumannii* strains in a mouse infection model. (**A–D**) The effect of 2.5×10^8, 1×10^8, 5×10^7, 1×10^7 colony-forming units of each *A. baumannii* isolate (n=10; *P<0.05, **P<0.001, log-rank test) on survival was assessed in mouse. Strains 5122 and 2092 are two KL49 *A. baumannii* strains; 7152, 71517 and 20859 are KL3 *A. baumannii* strains. LAC-4 is a KL49 hypervirulent *A. baumannii* strain reported in a previous study. LAC-4:Δ*gtr100* is a *gtr100* knockout strain. LAC-4:Δ*gtr100*Ω*gtr100* is complemented strain. ATCC17978 is a KL3 *A. baumannii* strain.
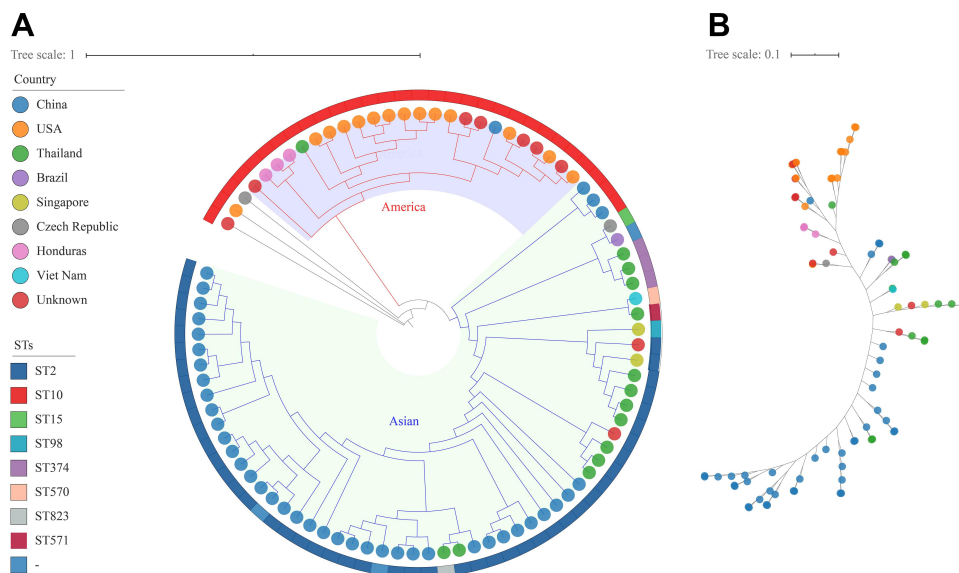

Figure 2 Worldwide spread of KL49 *Acinetobacter baumannii* strains. (**A**) A phylogenetic tree of the 78 KL49 strains retrieved from NCBI and nine KL49 strains in this study. LAC-4 was used as reference. The colors of the isolate tips represent the country of isolation. STs are shown in the outer ring. (**B**) An unrooted version of the tree shown in (**A**).

A total of 165 clinical CRAB strains were collected from three provinces of China. All-cause in-hospital mortality rate of 63.6% (21/33) in patients with *gtr100*$^+$ CRAB infection and 24.2% (32/132) in patients with *gtr100*$^-$ CRAB infection. In the univariable logistic regression analysis, the significant variables (*P*<0.05) associated with all-cause in-hospital mortality were coma, solid malignancy, mechanical ventilation, high level of urea nitrogen (>7.5 mmol/L), C-reactive protein (>10 mg/L), procalcitonin (>2 μg/L), sepsis, shock and

Table 3 Univariable Logistic Regression Analysis of the Factors Associated with Mortality

Variables	Odds Ratio (95%CI)	P-value
Demographics		
Age (years)		
30–50	0.58 (0.20–1.66)	0.306
> 50	2.48 (0 0.89–6.94)	0.083
Gender (male)	0.77 (0.36–1.61)	0.481
Infection Site		
Bacteremia	1.22 (0.34–4.38)	0.755
Pulmonary infection	2.37 (0.91–6.16)	0.077
Urinary infection	1.23 (0.50–2.99)	0.652
Abdominal infection	1.28 (0.29–5.59)	0.739
Comorbidities		
Cerebral vascular disease	0.81 (0.42–1.56)	0.523
Hypertension	1.61 (0.80–3.24)	0.179
Diabetes	1.63 (0.83–3.21)	0.159
Coma	3.43 (1.62–7.23)	0.001
Solid malignancy	5.60 (1.84–17.10)	0.002
Invasive Procedures		
Mechanical ventilation	2.82 (1.25–6.37)	0.012
Central venous catheter	1.77 (0.87–3.58)	0.115
Foley catheter	2.14 (0 0.82–5.59)	0.122
Nasogastric tube	1.39 (0.64–3.06)	0.404
Clinical Characteristics		
Leukopenia (<4×10^9/L)	3.78 (0.87–16.48)	0.076
Anemia (<10 g/dL)	1.36 (0.66–2.82)	0.407
Platelet count (<50×10^9/L) (<50×10^9/L) (<50×10^9/L)	3.61 (1.21–10.76)	0.021
Aspartate aminotransferase (>60 IU/L)	1.44 (0.39–5.34)	0.584
Total bilirubin (>35 μmol/L)	1.24 (0.49–3.18)	0.648
Urea nitrogen (>7.5mmol/L)	3.19 (1.59–6.38)	0.001
C-reactive Protein (CRP)		
10–50 mg/L	0.38 (0.18–0.78)	0.008
>50 mg/L	4.41 (2.13–9.16)	< 0.001
Procalcitonin (PCT)		
0.5–2 ug/L	0.53 (0.26–1.08)	0.08
>2 ug/L	3.02 (1.53–5.95)	0.001
Duration of ICU (days)		
<7	1.56 (0.47–5.17)	0.465
7–30	1.18. (0.60–2.31)	0.627
>30	1.02 (0.48–2.19)	0.953
Antibiotic Treatment		
Cefoperazone/sulbactam	0.93 (0.42–2.04)	0.86
Piperacillin/tazobactam	1.34 (0.67–2.68)	0.41
Carbapenems	1.12 (0.18–2.21)	0.057

(*Continued*)

Table 3 (Continued).

Variables	Odds Ratio (95%CI)	P-value
Quinolone	1.08 (0.54–2.15)	0.826
Third generation cephalosporins	0.71 (0.34–1.45)	0.342
Aminoglycoside	1.82 (0.81–4.09)	0.149
Minocycline	1.14 (0.47–2.77)	0.767
Tigecycline	3.12 (1.46–6.69)	0.053
Colistin	1.06 (0.09–11.93)	0.964
Tigecycline + carbapenems	2.97 (0.64–13.76)	0.165
Colistin + carbapenems	2.13 (0.13–34.79)	0.594
Carbapenems + aminoglycoside	0.41 (0.05–3.61)	0.423
Proportion of XDR strains	0.79 (0.38–1.64)	0.526
Continuous renal replacement therapy	2.74 (0.79–9.39)	0.111
Sepsis	5.13 (2.01–13.07)	0.001
Shock	9.52 (4.48–20.20)	<0.001
Polymicrobial infection	1.18 (0.61–2.30)	0.622
gtr100$^+$	5.47 (2.42–12.33)	<0.001

gtr100$^+$ CRAB infection (Table 3). Multivariable logistic regression analysis showed that the significant independent predictors for mortality were solid malignancy (OR, 13.66; 95%CI: 3.03–61.47; P=0.001), urea nitrogen (>7.5 mmol/L) (OR, 4.03; 95%CI: 1.31–12.39; P=0.015), sepsis (OR, 6.19; 95%CI: 1.61–23.84; P=0.008), Shock (OR, 5.17; 95%CI: 1.79–14.88; P=0.002) and *gtr100*$^+$ CRAB infection (OR, 10.76; 95%CI: 3.08–37.55; P<0.001) (Table 4).

Discussion

The relationship between capsular type and bacterial virulence has been demonstrated in different bacteria. For *Klebsiella pneumoniae*, approximately 70% hypervirulent strains were of the K1 and K2.[18–20]

For *A. baumannii*, there have been many reports of KL49 strains being more virulent than others.[6–8] High virulence phenotype of KL49 may be explained by the existence of an unusual sugar legionaminic acid, which can be used to mimic the host cell surface and benefit to escape from host immune surveillance.[21] In this study, a glycosyltransferase encoding gene *gtr100* is highlighted because it is specifically present in the CPS gene cluster of KL49, and is regarded as a good candidate for rapid identification of KL49. Moreover, animal experiment proved that *gtr100* is crucial to the virulence of LAC-4, indicating that it has the potential to be a target of antivirulence.

Table 4 Multivariable Logistic Regression Analysis of the Factors Associated with Mortality

Variables	Odds Ratio (95%CI)	P-value
Coma	2.69 (0.87–8.33)	0.085
Solid malignancy	13.66 (3.03–61.47)	0.001
Mechanical ventilation	3.69 (1.01–13.61)	0.049
Urea nitrogen (>7.5mmol/L)	4.03 (1.31–12.39)	0.015
C-reactive protein (CRP) >50 mg/L	0.89 (0.26–3.11)	0.858
Procalcitionin (PCT) >2 ug/L	2.46 (0.83–7.26)	0.104
Sepsis	6.19 (1.61–23.84)	0.008
gtr100⁺	10.76 (3.08–37.55)	<0.001

Glycosyltransferases encoded by *gtr* genes are primarily responsible for the linkages between sugars in CPS. There are two *gtr* genes (*gtr20* and *gtr100*) located in KL49 locus. *gtr20* glycosyltransferase, also encoded in KL8, KL9, KL49, KL54, KL63, KL108 and KL109 (Table 1), forms the α-L-FucpNAc-(1→3)-D-GlcpNAc linkage.[22]

Previous study has confirmed that the structure of the polysaccharide isolated from LAC-4 was built of trisaccharide repeating units α-L-FucNAc-3-α-D-GlcNAc-8-α-Leg5,7Ac,[21] it is reasonable to speculate that *gtr100* probably forms the α-D-GlcNAc-8-α-Leg5,7Ac linkage, and this linkage may be closely related to the virulence of KL49 because the virulence of LAC-4 decreased significantly when *gtr100* was knocked out and *gtr20* was retained. These inferences need to be verified by further experiments.

Although both previous studies and this study have demonstrated that KL49 *A. baumannii* is more virulent than nonKL49, the clinical characteristics of KL49 infection remain unclear. In general, *A. baumannii* is an opportunistic human pathogen that predominantly infects critically ill patients.[1] However, due to the different virulence of different KL types, we have to regard them differently. Fatal outbreak of nosocomial infections caused by KL49 *A. baumannii* alerts the threat of this particular strain in clinic.[7] Clinical characteristics analysis showed that CRAB infections with strains carrying *gtr100* were the independent predictors for mortality, this is consistent with the conclusion that KL49 is a hypervirulent strain. Moreover, phylogenetic analysis suggests that KL49 *A. baumannii* has spread worldwide (Figure 2). Therefore, the development of a rapid identification assay without the need for WGS in this study should be very useful in advising the clinician to implement strict control measures and medical intervention earlier in order to avoid transmission and improve the prognosis of infection. Meanwhile, a rapid identification tool also makes the surveillance of these hypervirulent CRAB strains more convenient and faster.

The question raised by this study is how *gtr100* affects the virulence of KL49. Correlation of the genes found in the KL gene cluster and the CPS structure from the same isolate has successfully confirmed the functions of several genes or gene clusters involved in the bonds formed by the encoded glycosyltransferases.[23] In view of the important role of *gtr100* gene in virulence, further studies on its function are necessary.

Data Sharing Statement

Acknowledgments

Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

Funding

Disclosure

The authors report no conflicts of interest in this work.

References

1. Dijkshoorn L, Nemec A, Seifert H. An increasing threat in hospitals: multidrug-resistant *Acinetobacter baumannii*. *Nat Rev Microbiol*. 2007;5(12):939–951. doi:10.1038/nrmicro1789
2. Evans BA, Hamouda A, Amyes SG. The rise of carbapenem-resistant Acinetobacter baumannii. *Curr Pharm Des*. 2013;19(2):223–238. doi:10.2174/138161213804070285
3. van Faassen H, KuoLee R, Harris G, Zhao X, Conlan JW, Chen W. Neutrophils play an important role in host resistance to respiratory infection with *Acinetobacter baumannii* in mice. *Infect Immun*. 2007;75(12):5597–5608. doi:10.1128/IAI.00762-07

4. Knapp S, Wieland CW, Florquin S, et al. Differential roles of CD14 and toll-like receptors 4 and 2 in murine *Acinetobacter* pneumonia. *Am J Respir Crit Care Med*. 2006;173(1):122–129. doi:10.1164/rccm.20
   0505-730OC

5. Zeng X, Gu H, Cheng Y, et al. A lethal pneumonia model of *Acinetobacter baumannii*: an investigation in immunocompetent mice. *Clin Microbiol Infect*. 2019;25(4):516 e511–516 e514. doi:10.1016/j.cmi.2018.12.020

6. Harris G, Kuo Lee R, Lam CK, et al. A mouse model of *Acinetobacter baumannii*-associated pneumonia using a clinically isolated hypervirulent strain. *Antimicrob Agents Chemother*. 2013;57(8):3601–3613. doi:10.1128/AAC.00944-13

7. Jones CL, Clancy M, Honnold C, et al. Fatal outbreak of an emerging clone of extensively drug-resistant *Acinetobacter baumannii* with enhanced virulence. *Clin Infect Dis*. 2015;61(2):145–154. doi:10.1093/cid/civ225

8. Zhou K, Tang X, Wang L, et al. An emerging clone (ST457) of Acinetobacter baumannii clonal complex 92 with enhanced virulence and increasing endemicity in South China. *Clin Infect Dis*. 2018;67 (suppl_2):S179–S188. doi:10.1093/cid/ciy691

9. Ou HY, Kuang SN, He X, et al. Complete genome sequence of hypervirulent and outbreak-associated *Acinetobacter baumannii* strain LAC-4: epidemiology, resistance genetic determinants and potential virulence factors. *Sci Rep*. 2015;5:8643.

10. Wyres KL, Cahill SM, Holt KE, Hall RM, Kenyon JJ. Identification of *Acinetobacter baumannii* loci for capsular polysaccharide (KL) and lipooligosaccharide outer core (OCL) synthesis in genome assemblies using curated reference databases compatible with *Kaptive*. *Microb Genom*. 2020;6(3).

11. Clinical, Institute LS. *Performance Standards for Antimicrobial Susceptibility Testing*. Wayne, PA: Clinical and Laboratory Standards Institute; 2017.

12. Chen WP, Kuo TT. A simple and rapid method for the preparation of gram-negative bacterial genomic DNA. *Nucleic Acids Res*. 1993;21 (9):2260. doi:10.1093/nar/21.9.2260

13. Chen S, Zhou Y, Chen Y, Gu J. fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics*. 2018;34(17):i884–i890. doi:10.1093/bioinformatics/bty560

14. Antipov D, Korobeynikov A, McLean JS, Pevzner PA. hybridSPAdes: an algorithm for hybrid assembly of short and long reads. *Bioinformatics*. 2016;32(7):1009–1015. doi:10.1093/bioinformatics/btv688

15. Treangen TJ, Ondov BD, Koren S, Phillippy AM. The Harvest suite for rapid core-genome alignment and visualization of thousands of intraspecific microbial genomes. *Genome Biol*. 2014;15(11):524. doi:10.1186/s13059-014-0524-x

16. Stamatakis A. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*. 2006;22(21):2688–2690. doi:10.1093/bioinformatics/btl446

17. Letunic I, Bork P. Interactive Tree Of Life (iTOL) v4: recent updates and new developments. *Nucleic Acids Res*. 2019;47(W1):W256–W259. doi:10.1093/nar/gkz239

18. Russo TA, Luke NR, Beanan JM, et al. The K1 capsular polysaccharide of *Acinetobacter baumannii* strain 307-0294 is a major virulence factor. *Infect Immun*. 2010;78(9):3993–4000. doi:10.1128/IAI.00366-10

19. Yu W-L, Ko W-C, Cheng K-C, Lee -C-C, Lai -C-C, Chuang Y-C. Comparison of prevalence of virulence factors for *Klebsiella pneumoniae* liver abscesses between isolates with capsular K1/K2 and non-K1/K2 serotypes. *Diagn Microbiol Infect Dis*. 2008;62(1):1–6. doi:10.1016/j.diagmicrobio.2008.04.007

20. Fang C-T, Lai S-Y, Yi W-C, Hsueh P-R, Liu K-L, Chang S-C. Klebsiella pneumoniae genotype K1: an emerging pathogen that causes septic ocular or central nervous system complications from pyogenic liver abscess. *Clin Infect Dis*. 2007;45(3):284–293. doi:10.1086/519262

21. Vinogradov E, Maclean L, Xu HH, Chen W. The structure of the polysaccharide isolated from *Acinetobacter baumannii* strain LAC-4. *Carbohydr Res*. 2014;390:42–45. doi:10.1016/j.carres.2014.03.001

22. Arbatsky NP, Kenyon JJ, Kasimova AA, et al. K units of the K8 and K54 capsular polysaccharides produced by Acinetobacter baumannii BAL 097 and RCH52 have the same structure but contain different di-N-acyl derivatives of legionaminic acid and are linked differently. *Carbohydr Res*. 2019;483:107745. doi:10.1016/j.carres.2019.107745

23. Kasimova AA, Kenyon JJ, Arbatsky NP, et al. *Acinetobacter baumannii* K20 and K21 capsular polysaccharide structures establish roles for UDP-glucose dehydrogenase Ugd2, pyruvyl transferase Ptr2 and two glycosyltransferases. *Glycobiology*. 2018;28(11):876–884.