

Original Article

Analysis of the Type VI secretion system and microbiological characteristics of hypervirulent *Klebsiella pneumoniae* causing urinary tract infectionsFei-Fei Li¹, Jing-Jing Li¹, Yin Zhang¹, Zhi-Yu Wu¹, Yuan-Hong Xu¹¹ Department of Laboratory, The First Affiliated Hospital of Anhui Medical University, HeFei 230022, AnHui, China**Abstract**

Introduction: This study aimed to evaluate the Type VI secretion system (T6SS) and microbiological features in hypervirulent *Klebsiella pneumoniae* (hvKP) causing urinary tract infections (UTIs) in hospitalized adults.

Methodology: This retrospective, observational analysis encompassed 167 inpatients with UTIs caused by KP. The study investigated disease prevalence, antimicrobial susceptibility, gene carriage rates, and competition indices. Strains were classified as classic KP (cKP), T6SS-positive hvKP, or T6SS-negative hvKP, and compared for clinical traits, antimicrobial susceptibilities, and virulence gene carriage. Furthermore, the bacterial competition index of T6SS-positive hvKP strains was assessed through *in vitro* cultivation.

Results: Of 167 patients, 82 had hvKP and 85 had cKP. hvKP had higher rates of thrombosis, immunotherapy, hypoproteinemia, and longer hospital stays ($p < 0.05$). The 30-day mortality was 29.26% for hvKP vs. 12.94% for cKP ($p = 0.045$). hvKP showed highest resistance to cefuroxime (81.70%) and cKP to ampicillin/sulbactam (56.47%), with low resistance to tigecycline. The resistance to carbapenems (carbapenem-resistant KP, CR-KP) was significantly higher in hvKP compared to cKP (30.48% vs 16.47%, $p = 0.032$). *Aerobactin* and *iroB* differed between hvKP and cKP. T6SS-positive hvKP had higher rates of thrombosis and immunotherapy ($p < 0.05$). The 30-day mortality was 9.52% for T6SS-positive vs. 36.06% for T6SS-negative ($p = 0.043$). T6SS-positive hvKP strains exhibited lower resistance to carbapenems compared to T6SS-negative strains (9.52% vs 37.70%, $p = 0.015$). T6SS-positive strains had higher *aerobactin* and *iroB* positivity.

Conclusions: T6SS-positive hvKP exhibits lower antimicrobial resistance but stronger virulence, playing a major role in bacterial competition.

Key words: Hypervirulent *Klebsiella pneumoniae*; antimicrobial activity; bacterial competition; type vi secretion system; urinary tract infection.

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Introduction

Klebsiella pneumoniae (KP), a formidable opportunistic pathogen, has garnered significant recognition for its role in a diverse array of infectious diseases, including urinary tract infections (UTIs), bacteremia, and pneumonia [1,2]. The stratification of KP into classic *Klebsiella pneumoniae* (cKP) and hypervirulent *Klebsiella pneumoniae* (hvKP), based on their distinct virulence characteristics and pathogenic potential, provides a deeper understanding of this organism. hvKP, characterized by its enhanced invasiveness, poses a considerable threat to the dissemination of severe organ infections [3]. The propensity of hvKP to cause extensive tissue damage is a primary concern for clinicians, as it can rapidly progress to adverse patient outcomes [4]. The aggressive nature of hvKP in the community, attributed to its high virulence traits, underscores its formidable role in the ongoing battle against infectious diseases [5]. Clinically, UTIs caused by KP, particularly hvKP, exhibit severe manifestations that necessitate prompt and targeted therapeutic intervention [6,7]. These infections are frequently associated with a rapid

escalation to severe systemic inflammation, which can deteriorate into life-threatening conditions such as sepsis [7]. The epidemiological landscape is further complicated by the escalating prevalence of multidrug-resistant strains, which not only complicates treatment options but also underscores the pressing need for effective antimicrobial stewardship [8]. A comprehensive understanding of the unique attributes of hvKP, encompassing its virulence genes and resistance patterns, is paramount for the development of preventive strategies and therapeutic interventions aimed at mitigating the spread and impact of these infections.

In the intricate tapestry of infectious diseases, the clinical outcomes and prognoses of patients infected with hvKP are influenced by a myriad of factors. The chronic comorbidities inherent to the patients, the severity of the infection, and the progression of their conditions are intricately intertwined with the efficacy of recovery [9]. The scientific and rational design of antimicrobial treatment plans emerges as a pivotal determinant of clinical mortality rates. Furthermore, microbial factors, including drug resistance and

virulence traits, play a crucial role in shaping the clinical prognosis of KP infections [10-12]. Emerging research has shed light on the significance of the Type VI secretion system (T6SS), a factor present in over 25% of Gram-negative bacteria, including hvKP [13,14]. The T6SS exerts a direct impact on bacterial activities such as adhesion, colonization, and drug resistance, thereby influencing the clinical trajectory of infections [15]. As we delve deeper into the complexities of hvKP infections, understanding the intricate interplay between these diverse factors is imperative for the formulation of effective therapeutic strategies and the enhancement of patient outcomes.

Given the current state of advancing research in infectious diseases, it is imperative to address the extant knowledge gaps concerning the role of the T6SS in UTIs caused by hvKP. While prior studies have underscored the function of T6SS in a diverse array of pathogenic bacteria, there remains a scarcity of literature specifically focusing on the distribution of virulence genes, antimicrobial resistance patterns, and clinical characteristics of T6SS in hvKP associated with UTIs. The present study endeavors to bridge this gap in our understanding by conducting a comprehensive retrospective analysis of clinical data derived from patients with UTIs caused by hvKP. Through this meticulous exploration, we aim to elucidate the distinct characteristics of T6SS genes, along with their associated clinical and microbiological features in the context of UTIs. By shedding light on these critical aspects, we aspire to potentially inform and guide more targeted and efficacious clinical interventions for patients battling these formidable infections.

Methodology

Study Design and Patients

This retrospective study included adult patients diagnosed with KP UTIs at the First Affiliated Hospital of Anhui Medical University between January 2022 and May 2023. A total of 167 patients were included, and stratified into the hvKP group and the non-hvKP group (classic KP, cKP group) based on virulence phenotypes. The patients were further divided into T6SS-positive and T6SS-negative groups based on the hypothesis that T6SS plays a significant role in the pathogenicity and drug resistance of KP. T6SS, a contractile nanomachine in Gram-negative bacteria, injects toxins into target cells. While extensively studied in other bacteria like *Escherichia coli* and *Pseudomonas aeruginosa*, limited research has focused on its role in KP, especially in UTIs. Therefore, the present study further investigated the differences in clinical characteristics, drug

resistance, genetic makeup, and bacterial competitiveness between T6SS-positive and T6SS-negative strains.

The study was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2013. Due to the retrospective nature of the study, the Ethics Committee of the First Affiliated Hospital of Anhui Medical University granted an exemption from ethical review procedures. All patient data were sourced from the hospital's database, and the Ethics Committee had previously granted an exemption for patient informed consent. All identifiable patient information was de-identified to ensure anonymity. The reporting of this study adheres to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines [16].

Inclusion Criteria: (1) **Diagnosis of KP UTI:** Patients who were hospitalized and confirmed to have KP UTI were eligible for inclusion. (2) **Positive Culture and Sensitivity Testing:** Only those with positive bacterial cultures and who underwent antimicrobial susceptibility testing were considered. **Diagnostic Criteria for UTI:** (1) **Quantitative Urine Culture:** A clean midstream urine culture yielding a bacterial count of $\geq 10^5$ CFU/mL for bacilli, $\geq 10^4$ CFU/mL for cocci, or a positive fungal culture. (2) **Clinical Symptoms:** Presence of symptoms such as urinary frequency, urgency, dysuria, systemic fever, or bacteremia. (3) **Pyuria:** Leukocytosis in the urine sediment with more than 5 white blood cells per high-power field. Patients meeting any of the above criteria were included in this study.

Exclusion Criteria: (1) **Age:** Patients under the age of 18 were excluded. (2) **Length of Hospital Stay:** Patients with a hospital stay of 24 hours or less were not considered. (3) **Incomplete Clinical Data:** Patients with incomplete clinical records were excluded from the analysis.

The String Test

The string test is a key method for determining the high virulence pathogenicity of bacterial strains [17]. This method involves inoculating a bacterial strain onto a solid agar plate and incubating it under controlled conditions. High-virulence strains are identified by the formation of a mucoid or stringy substance that can be physically pulled away from the agar surface, which results from the production of capsular polysaccharides and other extracellular matrix components. The test protocol is as follows: Adjust a small volume of bacterial suspension to a 0.5 McFarland standard and spread it uniformly on the surface of blood agar

medium. Incubate the inoculated plate at 37 °C for a specified period. After incubation, examine the plate for the presence of a stringy substance using a sterile loop or needle. Gently lift the substance from the agar surface and assess the length and viscosity of the string. A positive result is indicated by the formation of a string that is at least 5 millimeters in length, signifying a high-virulence phenotype.

Antimicrobial Susceptibility Testing (AST)

Antimicrobial susceptibility testing is a pivotal procedure in microbiology that assesses the sensitivity or resistance of bacterial strains to various antimicrobial agents. This test is crucial for guiding clinical decisions on the appropriate selection of antibiotics for effective treatment of infections. In this study, the MIC of each antimicrobial agent was interpreted according to the CLSI standards (2024). The method employed in this study for determining the antimicrobial susceptibility of the bacterial strains is the microbroth dilution method [18]. The procedure for AST is as follows.

Preparation of Bacterial Inoculum

A standardized suspension of the bacterial strain is prepared to match a specific turbidity, typically equivalent to a McFarland standard of 0.5, ensuring a consistent concentration of bacteria for the test.

Dilution of Antimicrobial Agents

A series of doubling dilutions of each antimicrobial agent is prepared in microbroth plates, creating a gradient of concentrations that spans a range from high to low potency.

Inoculation

The standardized bacterial suspension is added to each well of the microbroth plate containing the antimicrobial dilutions, ensuring that each well receives an equal amount of the bacterial suspension.

Incubation

The inoculated microbroth plates are incubated under conditions optimal for bacterial growth, allowing the bacteria to be exposed to the various concentrations of the antimicrobial agents.

Reading and Interpretation

After incubation, the plates are examined for bacterial growth. The lowest concentration of an antimicrobial agent that inhibits visible bacterial growth is recorded as the minimum inhibitory concentration (MIC).

Interpretation of Results

The results are interpreted based on the Clinical and Laboratory Standards Institute (CLSI) breakpoints. These breakpoints are critical thresholds that differentiate between susceptible, intermediate, and resistant categories for each antimicrobial agent.

Detection of Type VI Secretion System (T6SS) Genes and Virulence Genes

The detection of T6SS genes and virulence genes is a critical component in the study of bacterial pathogenicity. In this research, we employed the Polymerase Chain Reaction (PCR) amplification method to identify the presence of these genes. DNA extraction was performed using a commercial kit: Bacterial Genomic DNA Rapid Extraction Kit (100PREPS) from Sangon Biotech (Shanghai) Co., Ltd. The PCR reaction mixture, consisting of 25µL, was prepared using a SparkTaq PCR Master Mix reagent, with 12.5µL serving as the base, complemented by 1µL each of the forward and reverse primers, and 2µL of DNA template. This mixture was then diluted with distilled water to achieve the desired volume. Utilizing a BIO-RAD C1000 Touch thermal cycler, the PCR amplification was conducted under a precise temperature profile. The initial denaturation step was set at 94 °C for 3 minutes, followed by a series of cycles consisting of denaturation at 94 °C for 30 seconds, annealing at 55 °C for 20 seconds, and extension at 72 °C for 1 minute, with a total of 35 cycles. A final extension was performed at 72 °C for 5 minutes, after which the temperature was reduced to 4 °C to conclude the PCR process. The PCR products were subsequently subjected to electrophoresis on a 1.0% agarose gel at a voltage of 120V/300 mA for 40 minutes. The visualization of the DNA bands was achieved using a ChemiDoc™XRS + imaging system, which allowed us to assess the positivity of the genes in question. In this study, strains that exhibited positivity for the *icmF*, *vgrG*, and *hcp* genes were identified as T6SS-positive [19].

In vitro Competition Assay

In the realm of microbiology, the *in vitro* competition assay serves as a vital tool for gauging the competitive prowess of bacterial strains under controlled conditions. In this study, KP and *E. coli* strain MG1655 were subjected to an *in vitro* competition experiment to evaluate their relative competitive abilities. The experiment commenced with the overnight cultivation of both strains in Luria-Bertani (LB) broth at a constant temperature of 37 °C.

Following this, the optical density of each culture was standardized to an OD600 of 0.5, ensuring an equal starting point for both strains. Subsequently, the cultures were diluted 1:100 in 10 mL of fresh LB broth, creating a homogeneous bacterial suspension. The prepared bacterial suspensions were then mixed in a 1:1 ratio, simulating a competitive environment, and the mixture was agitated to ensure optimal aeration and uniform distribution of nutrients. This incubation continued for a duration of 24 hours at a maintained temperature of 37 °C, allowing ample time for the strains to compete for resources. To evaluate the competitive index (CI), the dilutions were uniformly spread in successive 10-fold increments onto blood agar plates and then incubated at a controlled constant temperature of 37 °C for 24 hours. This step was crucial in determining the ability of KP to outcompete the *E. coli* strain. A higher CI value indicates a greater competitive advantage of KP over *E. coli*. The obtained strains were counted individually for cell concentration using a hemocytometer (Neubauer chamber). The *in vitro* CI was determined based on the relative competitiveness of KP against *E. coli* strains. The CI

was calculated as the ratio of colony counts between the two strains multiplied by the ratio of their cell concentration counts.

Statistical Analysis

Statistical analysis was conducted using SPSS software version 24.0. For continuous data from the two groups, normality and homogeneity of variance were assessed. Data that were normally distributed are presented as the mean ± standard deviation (SD) and were analyzed using the Student's t-test. Data not conforming to a normal distribution are represented by the median and interquartile range and were evaluated using the Mann-Whitney U test. Mortality, a binary variable, was analyzed using binary logistic regression. Categorical variables were described using relative and absolute frequencies, and comparisons between qualitative variables were made using the Pearson chi-square test or the Fisher exact test, with all tests being two-tailed. A *p*-value of less than 0.05 was considered to indicate a statistically significant difference, thus conferring statistical significance.

Table 1. Clinical characterization of 167 patients with KP infection of urinary tract.

Index	hvKP group (n = 82)		<i>t/χ²/Z</i>	<i>p</i>	hvKP group (n = 82)	cKP group (n = 85)	<i>t/χ²/Z</i>	<i>p</i>
	T6SS positive group (n = 21)	T6SS negative group (n = 61)						
Age (years)	57.04 ± 17.06	58.70 ± 18.69	1.080	0.293	58.28±18.19	58.21±20.57	1.050	0.587
Gender (male/female)	11/10	29/32	0.146	0.702	40/42	40/45	0.050	0.824
Diseases (n, %)								
Diabetes	8 (38.095)	9 (14.754)	5.179	0.023	17 (20.732)	13 (15.294)	0.837	0.360
Hypertension	12 (57.143)	17 (27.869)	5.857	0.016	29 (35.366)	33 (38.824)	0.214	0.644
Hepatic insufficiency	3 (14.286)	2 (3.279)	1.663	0.197	5 (6.098)	3 (3.529)	0.172	0.679
Renal insufficiency	7 (33.333)	11 (18.033)	2.135	0.144	18 (21.951)	11 (12.941)	2.361	0.124
Lung diseases	11 (52.381)	22 (36.066)	1.729	0.189	33 (40.244)	25 (29.412)	2.160	0.142
Cerebrovascular disease	6 (28.571)	15 (24.590)	0.130	0.718	21 (25.610)	12 (14.118)	3.476	0.062
Tumors	1 (4.762)	7 (11.475)	0.219	0.640	8 (9.756)	6 (7.059)	0.395	0.530
Immunotherapy	5 (23.810)	3 (4.918)	6.332	0.012	8 (9.756)	1 (1.176)	4.460	0.035
Anemia	8 (38.095)	13 (21.311)	2.310	0.129	21 (25.610)	16 (18.824)	1.114	0.291
Thrombosis (lower limbs)	5 (23.810)	3 (4.918)	4.369	0.037	8 (9.756)	1 (1.176)	6.025	0.014
Urinary stones	2 (9.524)	12 (19.672)	0.533	0.466	14 (17.073)	20 (23.529)	1.073	0.300
Neurogenic bladder	1 (4.762)	6 (9.836)	0.070	0.791	7 (8.537)	8 (9.412)	0.039	0.843
Hydroperitoneum	2 (9.524)	7 (11.475)	0.000	> 0.99	9 (10.976)	5 (5.882)	0.825	0.364
Ureteral stenosis	0 (0.000)	3 (4.918)	0.131	0.718	3 (3.659)	3 (3.529)	1.000	0.642
Post-cystostomy	0 (0.000)	2 (3.279)	0.000	0.984	2 (2.439)	4 (4.706)	0.632	0.682
Nephropathy	5 (23.810)	4 (6.557)	3.157	0.076	9 (10.976)	7 (8.235)	0.362	0.548
Kidney transplantation	4 (19.048)	8 (13.115)	0.093	0.760	12 (14.634)	8 (9.412)	1.080	0.299
Renal failure	2 (9.524)	2 (3.279)	0.312	0.576	4 (4.878)	0 (0.000)	5.792	0.056
Baseline condition (n, %)								
Electrolyte disturbance	2 (9.524)	8 (13.115)	0.002	0.962	10 (12.195)	8 (9.412)	0.336	0.562
Neutrophil deficiency	0 (0.000)	1 (1.639)	0.000	> 0.99	1 (1.220)	0 (0.000)	1.429	0.491
Thrombocytopenia	1 (4.762)	1 (1.639)	0.000	> 0.99	2 (2.439)	4 (4.706)	0.632	0.682
Hypoproteinemia	5 (23.810)	8 (13.115)	1.339	0.247	13 (15.854)	5 (5.882)	4.315	0.038
Ureteral stent	1 (4.762)	4 (6.557)	0.000	> 0.99	5(6.098)	4 (4.706)	0.159	0.743
Intensive care unit	9 (42.857)	24 (39.344)	0.080	0.777	33 (40.244)	25 (29.412)	2.160	0.142
Days of hospitalization	32 (11, 44)	12 (6, 28)	2.594	0.009	16 (7, 33.75)	8 (6, 23)	2.112	0.035
Multidrug-resistant bacteria	9 (42.857)	50 (81.967)	11.84	0.001	59 (71.951)	35 (41.176)	3.132	0.038
Initial appropriate antimicrobial therapy	18 (85.714)	40 (65.574)	2.165	0.141	58 (70.732)	40 (47.059)	2.143	0.076
Outcome (n, %)								
Total mortality within 30 d	2 (9.524)	22 (36.066)	4.111	0.043	24 (29.268)	11 (12.941)	1.134	0.045

T6SS: type VI secretion system; hvKP: hypervirulent *Klebsiella pneumoniae*; cKP: classic *Klebsiella pneumoniae*.

Results

Clinical characterization of the included patients

In this study, among the 167 KP strains collected, 82 were identified as hvKP and 85 as cKP (Table 1). The hvKP group exhibited significantly higher rates of thrombosis, immunotherapy cases, hypoalbuminemia, and hospital stay duration compared to the cKP group ($p < 0.05$). Within the hvKP strains, 21 were positive for T6SS (25.61%), and 61 were negative (74.39%). The T6SS-positive group had significantly higher rates of thrombosis, immunotherapy cases, and hospital stay duration compared to the T6SS-negative group ($p < 0.05$). No statistically significant difference was observed in antibiotic use between the hvKP and cKP groups or between the T6SS-positive and T6SS-negative groups (all $p > 0.05$). The 30-day mortality rate was 29.26% in the hvKP group, which was statistically different from the 12.94% in the cKP group ($p = 0.045$). Within the hvKP group, the 30-day mortality rate for patients infected with T6SS-positive strains was 9.52%, while it was 36.06% for those infected with T6SS-negative strains, showing a statistically significant difference between the two groups ($p = 0.043$).

Drug susceptibility results of T6SS-positive and T6SS-negative isolates

The results of this study indicate that the highest resistance rate among the obtained hvKP strains was observed against cefuroxime (81.70%), with the lowest resistance noted against tigecycline (2.43%) (Table 2). For cKP strains, the highest resistance rate was

observed against ampicillin/sulbactam (56.47%), and again, the lowest resistance was noted against tigecycline (2.35%). Overall, hvKP strains exhibited higher resistance rates compared to cKP strains. Within the hvKP group, T6SS-positive strains demonstrated lower overall drug resistance than T6SS-negative strains, with statistically significant differences observed in drug susceptibility tests for all antibiotics except amikacin, sulfamethoxazole/trimethoprim, minocycline, tigecycline, cefotetan, ceftazidime, and ceftazidime/avibactam ($p < 0.05$). Notably, a statistically significant difference was observed in cefotetan resistance between the hvKP and cKP groups ($p < 0.05$), while no significant difference was found within the hvKP group ($p = 0.454$). Additionally, T6SS-positive hvKP strains exhibited lower resistance to carbapenems compared to T6SS-negative strains (9.52% vs 37.70%, $p = 0.015$).

Distribution of virulence genes

The distribution of virulence genes among hvKP and cKP strains is presented as follows (Table 3): significant differences were observed for *aerobactin* (25.60% vs 11.76%, $p = 0.021$) and *iroB* (23.17% vs 8.23%, $p = 0.008$). Within the hvKP group, the distribution of these genes between T6SS-positive and T6SS-negative strains also showed significant differences: *aerobactin* (52.38% vs 16.39%, $p = 0.001$) and *iroB* (47.62% vs 14.75%, $p = 0.002$). No significant differences were observed for *rmpA* and *rmpA2* among the various groups.

Table 2. Antimicrobial resistance rates in 167 patients with KP urinary tract infections.

Antibiotics	hvKP group (n = 82)				hvKP group (n = 82)	cKP group (n = 85)	χ^2	p
	T6SS positive group (n = 21)	T6SS negative group (n = 61)	χ^2	p				
<i>Aztreonam</i>	8 (38.09)	43 (70.49)	6.973	0.008	51 (62.19)	39 (45.88)	4.470	0.035
<i>Gentamycin</i>	6 (28.57)	36 (59.01)	5.795	0.016	42 (51.21)	28 (32.94)	5.727	0.017
<i>Tobramycin</i>	6 (28.57)	36 (59.01)	5.795	0.016	42 (51.21)	17 (20.00)	17.804	0.000
<i>Cotrimoxazole</i>	5 (23.81)	32 (52.45)	5.178	0.023	37 (45.12)	43 (50.58)	0.500	0.480
<i>Amikacin</i>	6 (28.57)	19 (31.14)	0.049	0.825	25 (30.48)	17 (20.00)	2.439	0.118
<i>Cefepime</i>	7 (33.33)	38 (62.29)	5.292	0.021	45 (54.87)	27 (31.76)	9.091	0.003
<i>Ceftriaxone</i>	8 (38.09)	46 (75.40)	9.673	0.002	54 (65.85)	43 (50.58)	3.995	0.046
<i>Cefotaxime</i>	8 (38.09)	47 (77.04)	10.733	0.001	55 (67.07)	21 (24.70)	30.210	0.000
<i>Cephazolin</i>	8 (38.09)	46 (75.40)	9.673	0.002	54 (65.85)	43 (50.58)	3.995	0.046
<i>Cefuroxime</i>	13 (61.90)	54 (88.52)	7.406	0.006	67 (81.70)	22 (25.88)	52.254	0.000
<i>Cefotetan</i>	7 (33.33)	26 (42.62)	0.561	0.454	33 (40.24)	13 (15.29)	13.018	0.000
<i>Ceftazidime</i>	8 (38.09)	38 (62.29)	3.715	0.054	46 (56.09)	36 (42.35)	3.155	0.076
<i>Ciprofloxacin</i>	8 (38.09)	49 (80.32)	13.148	< 0.001	57 (69.51)	46 (54.11)	4.185	0.041
<i>Levofloxacin</i>	8 (38.09)	45 (73.77)	8.698	0.003	53 (64.63)	41 (48.23)	4.562	0.033
<i>Imipenem</i>	2 (9.52)	24 (39.34)	5.112	0.024	26 (31.70)	13 (15.29)	6.281	0.012
<i>Meropenem</i>	2 (9.52)	23 (37.70)	4.600	0.032	25 (30.48)	4 (4.70)	19.333	0.000
<i>Ampicillin and Sulbactam</i>	9 (42.85)	50 (81.96)	11.840	0.001	59 (71.95)	48 (56.47)	4.727	0.030
<i>Minocycline</i>	3 (14.28)	19 (31.14)	1.485	0.223	22 (26.82)	20 (23.52)	0.241	0.623
<i>Tigecycline</i>	1 (1.63)	1 (1.63)	0.000	> 0.99	2 (2.43)	2 (2.35)	0.001	0.971
<i>Ceftazidime/Avibactam</i>	2 (9.52)	3 (4.91)	0.054	0.816	5 (6.09)	5 (5.88)	0.003	0.953
CR-KP	2 (9.52)	23 (37.70)	4.600	0.015	25 (30.48)	14 (16.47)	4.581	0.032

T6SS: type VI secretion system; hvKP: hypervirulent *Klebsiella pneumoniae*; cKP: classic *Klebsiella pneumoniae*; CR-KP: carbapenem-resistant *Klebsiella pneumoniae*.

Table 3. Distribution of virulence genes in isolates of KP for urinary tract infections.

Virulence factor	hvKP group (n = 82)		χ^2	p	hvKP group (n = 82)	cKP group (n = 85)	χ^2	p
	T6SS positive group (n = 21)	T6SS negative group (n = 61)						
<i>rmpA</i>	13 (61.90)	29 (47.54)	1.290	0.256	42 (51.21)	38 (44.70)	0.710	0.400
<i>rmpA2</i>	12 (57.14)	26 (52.62)	1.325	0.250	38 (46.34)	37 (43.52)	0.133	0.715
<i>aerobactin</i>	11 (52.38)	10 (16.39)	10.621	0.001	21 (25.60)	10 (11.76)	5.292	0.021
<i>iroB</i>	10 (47.62)	9 (14.75)	9.478	0.002	19 (23.17)	7 (8.23)	7.083	0.008

T6SS: type VI secretion system; hvKP: hypervirulent *Klebsiella pneumoniae*; cKP: classic *Klebsiella pneumoniae*.

Competition index of T6SS-positive and T6SS-negative isolates

In this study, an *in vitro* competition assay was conducted to assess the competitive capabilities of the collected bacterial strains. Specifically, six strains of each type—T6SS-positive and T6SS-negative KP—were selected for the calculation of the CI. Notably, the T6SS-positive strains exhibited a significantly elevated average CI of 14.31, in contrast to the T6SS-negative strains, which had an average CI of 6.55. Further confirmation of the enhanced competitive ability of the T6SS-positive strains was provided by a t-test, which yielded a t-value of 10.427, indicating a statistically significant difference ($p < 0.05$). These findings suggest that T6SS-positive strains demonstrate greater competitiveness compared to their T6SS-negative counterparts during co-cultivation. For a detailed visual representation of these findings, refer to Figure 1.

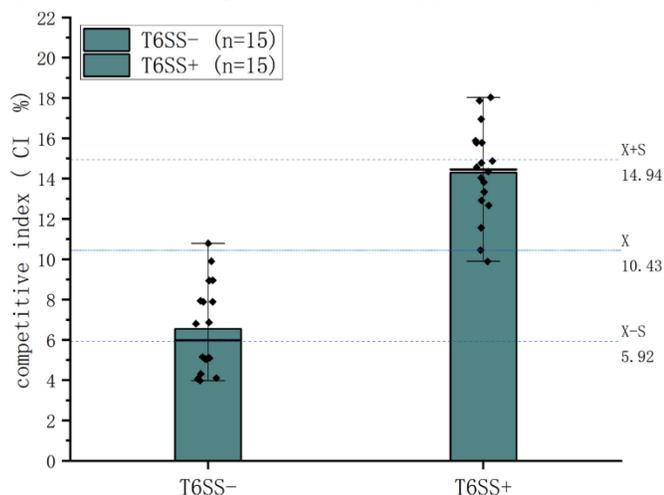
Discussion

hvKP, initially designated as hypermucoviscous *Klebsiella pneumoniae*, represents a highly virulent variant of KP that typically exhibits high invasiveness and pathogenicity, capable of causing bloodstream infections and other severe conditions. While case reports of KP-induced urinary tract infections are relatively scarce, in this study, the hvKP group

exhibited significantly higher rates of immunosuppression, thrombosis, and prolonged hospitalization compared to the cKP group. In terms of drug resistance, the overall resistance rates in the hvKP group were generally higher than those in the cKP group. Notably, this study encompassed multidrug-resistant, extensively drug-resistant, and even pandrug-resistant strains, many of which have become clinically concerning “superbugs.” Regarding virulence, the detection rates of *aerobactin* and *iroB* were higher in the hvKP group than in the cKP group. These findings suggest that hvKP poses a more severe threat to patient outcomes due to its enhanced virulence and resistance profiles, necessitating heightened clinical vigilance and targeted therapeutic strategies.

The plasmid-encoded virulence factors, *rmpA* and its homologue *rmpA2*, play a crucial role in KP, exerting a direct impact on the regulation of capsular polysaccharide synthesis and modulating the transcriptional and synthetic activities of associated genes [20]. Previous investigations have demonstrated a strong association between *rmpA*-harboring strains and the hypermucoviscous phenotype exhibited by hvKP [21]. The lipopolysaccharide present in KP exhibits a specific capacity to attenuate inflammatory responses within the host organism. This mechanism directly influences the host’s capability to eliminate infected cells [22]. The hvKP strains are commonly associated with a range of virulence factors related to iron acquisition, including *enterobactin*, *yersiniabactin*, *salmochelins*, and *aerobactin*. Prior research has indicated that hvKP strains possess the ability to produce larger and more functionally active iron-chelating molecules in comparison to cKP strains. This enhanced capacity for iron sequestration may represent a crucial mechanism underlying the heightened virulence and pathogenicity observed in hvKP [23]. Russo and colleagues have provided compelling evidence that *aerobactin* plays a pivotal role in differentiating hvKP from cKP strains in their study [24]. Furthermore, Li and his team have uncovered that the concurrent detection of *aerobactin* and *rmpA*, coupled with the string test, holds substantial significance for enhancing the clinical detection

Figure 1. Comparison of competitive ability between T6SS positive and T6SS negative strain. T6SS: type VI secretion system.



efficiency of hvKP [25]. Despite these advancements, recent research has predominantly concentrated on the aforementioned virulence genes, while studies investigating the relationship between the T6SS and clinical infections remain relatively scant.

Drawing upon the existing literature, strains that test positive for *icmF*, *vgrG*, and *hcp* are designated as positive for the T6SS [26]. Based on this criterion, the results of our study indicate that the frequency of T6SS genes among the included hvKP strains causing UTIs is 25.61%. Our findings reveal that the prevalence of virulence genes is elevated in T6SS-positive strains compared to T6SS-negative strains, implying that T6SS-positive KP strains may exhibit heightened virulence and pathogenicity. Additionally, this study demonstrates that T6SS-positive hvKP strains associated with UTIs exhibit lower rates of antimicrobial resistance than T6SS-negative strains. However, the emergence of carbapenem-resistant and tigecycline-resistant hvKP remains a pressing concern. In clinical practice, carbapenems, renowned for their broad-spectrum antimicrobial activity, are frequently utilized for the treatment of severe KP infections. These drugs are regarded as potent due to their robust antimicrobial efficacy [27]. It is crucial to acknowledge that despite the widespread clinical utilization of carbapenems, the rate of resistance among KP remains persistently high [28,29]. This emphasizes the importance of adopting a scientific and rational approach to the clinical use of antimicrobial agents, adhering strictly to established guidelines, consensus statements, and principles for the prudent management of these drugs. Particularly during the initial treatment phase for patients diagnosed with KP-associated UTIs, clinicians often rely on their clinical experience to prescribe medications. However, the formulation of antimicrobial regimens for such patients should adhere to relevant treatment guidelines to ensure standardization and scientifically sound administration of antimicrobials [28]. Taking into account the patient's unique individual characteristics, treatment duration, dosage of interventions, and other pertinent factors is of paramount importance. The thoughtful selection and adjustment of antimicrobial agents, in conjunction with other intervention measures, are vital to mitigating the misuse of antimicrobials during hospital stays. This deliberate and measured approach not only enhances patient outcomes but also aligns with broader efforts to address antimicrobial resistance.

Previous studies have established a significant correlation between the presence of T6SS and resistance to meropenem, ciprofloxacin, and

levofloxacin [30]. However, no notable activity of T6SS has been detected when the strains are cultivated in conventional LB broth or M9 medium. Given the escalating severity of antimicrobial resistance in hospital-acquired infections, the relationship between the virulence of infecting strains and their level of drug resistance has emerged as a critical area of clinical concern [31,32]. When KP exhibits both heightened virulence and multidrug resistance, it presents substantial obstacles for the modification of antimicrobial treatment protocols and the advancement of novel therapeutic agents. Prior investigations have demonstrated that ICU admission, urinary catheterization, tracheostomy, and the use of antimicrobials prior to infection are independent risk factors for acquiring KP infections [33]. Similarly, advanced age and the presence of severe comorbidities are also identified as independent risk factors that contribute to an elevated mortality risk among patients infected with KP [34,35]. In this study, we evaluated the competitive prowess of KP against heterologous bacteria using *in vitro* competition assays. Our results indicated that T6SS-positive strains exhibited greater competitiveness compared to T6SS-negative strains during cocultivation. These observations suggest that a robust competitive capacity against heterologous bacteria may be intricately linked to KP's adaptability in colonization. We hypothesize that hvKP, leveraging the T6SS, may eliminate commensal bacteria within the human body, thereby establishing itself as the dominant species and precipitating infection. This is consistent with Hernandez *et al.*'s findings, who noted T6SS delivers toxic proteins via effectors, bolstering bacteria's competitive edge in multi-microbial settings and aiding colonization [26]. Hernandez *et al.* also highlighted T6SS's role in bacterial anti-eukaryotic effects [26]. This implies T6SS-positive hvKP may enhance host survival and colonization by altering the host cell environment, which could be another reason for their success in UTIs. These revelations underscore the pivotal role that T6SS may play in the pathogenesis of KP and emphasize the imperative for additional research to elucidate its mechanisms and clinical management implications.

Overall, T6SS-positive hvKP strains present a unique clinical challenge. Their high virulence, driven by the T6SS, which injects effector proteins into host cells or competing bacteria, can lead to severe infections by evading phagocytosis and modulating immune responses. Despite this virulence, their lower resistance profiles, particularly susceptibility to carbapenems, may initially respond to traditional

antibiotics like β -lactams and quinolones. However, in cases of mixed infections or when hvKP coexists with other resistant strains, empirical treatments might fail, necessitating precise susceptibility testing to confirm monomicrobial infections. There's also a risk that prolonged antibiotic pressure or hospital exposure could drive these strains to acquire resistance genes, potentially evolving into hypervirulent and highly resistant superbugs. This dual nature makes hvKP a “stealth threat” in empirical therapy, as it may be underestimated initially but could lead to explosive infections. Clinically, this necessitates a balanced approach—selecting targeted antibiotics based on susceptibility testing to avoid unnecessary broad-spectrum antibiotic use, while also exploring virulence-targeted therapies like T6SS inhibitors. Improved diagnostic tools for rapid identification of T6SS markers and enhanced infection control measures, especially in high-risk patients, are crucial for managing this pathogen effectively.

The study acknowledges certain limitations. Firstly, due to its retrospective nature, the findings may be subject to inherent biases associated with non-experimental data analysis. Additionally, the study's conclusions are limited by the geographical and temporal scope of the data, which was collected from a single hospital over a specific period, potentially affecting the generalizability of the results. Furthermore, the sample size represents a significant constraint. The number of clinical isolates analyzed, despite being divided into two groups, may introduce some bias and limit the statistical robustness of the conclusions drawn. Moreover, no genomic analysis, such as whole-genome sequencing (WGS), was performed. This lack of genomic data limits the interpretation of clonal relationships and the contribution of mobile genetic elements to resistance and virulence. Lastly, while the study provides insights into the role of the T6SS in hvKP, further research is needed to elucidate the mechanisms by which T6SS influences virulence and antimicrobial resistance in clinical settings.

Conclusions

In summary, our study has demonstrated that hvKP strains positive for the T6SS exhibit reduced antimicrobial resistance while possessing enhanced virulence, thereby playing a pivotal role in bacterial competition. These findings underscore the crucial role of T6SS in the pathogenic potential of these strains and emphasize the necessity for a more profound understanding of their underlying mechanisms of

action. Such insights may inform the development of more effective clinical strategies to combat infections caused by these formidable pathogens.

Authors' contributions

Conceptualization: Yuan-hong Xu; Data curation: Fei-fei Li, Zhi-yu Wu; Investigation: Fei-fei Li, Yuan-hong Xu, Zhi-yu Wu, Yin Zhang; Methodology: Yuan-hong Xu, Yin Zhang; Project administration: Fei-fei Li; Supervision: Yuan-hong Xu; Visualization: Fei-fei Li, Zhi-yu Wu; Writing - original draft: Fei-fei Li; Writing - review & editing: Fei-fei Li, Yuan-hong Xu

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Conflict of interest

No conflict of interest is declared.

References

- Baughman RP (2009) The use of carbapenems in the treatment of serious infections. *J Intensive Care Med* 24: 230-241. doi: 10.1177/0885066609335660.
- Boattini M, Bianco G, Bastos P, Comini S, Corcione S, Almeida A, Costa C, De Rosa FG, Cavallo R (2024) Prevalence and mortality of ceftazidime/avibactam-resistant KPC-producing *Klebsiella pneumoniae* bloodstream infections (2018-2022). *Eur J Clin Microbiol Infect Dis* 43: 155-166. doi: 10.1007/s10096-023-04712-8.
- Brooks CA, Mahajan S, Beresford R, Damodaran O, Pope R (2022) Thoracic spondylodiscitis secondary to *Klebsiella oxytoca* urosepsis—a case report. *J Spine Surg* 8: 54-61. doi: 10.21037/jss-21-124.
- Chen L, Zhou Y, Wang S, Wu C, Zhou P, Wang B, Chen Z, Yu F (2023) Genomic analysis of carbapenem-resistant hypervirulent *Klebsiella pneumoniae* in a Chinese tertiary hospital. *Infect Drug Resist* 16: 6385-6394. doi: 10.2147/IDR.S425949.
- Chen Y, Ying S, Jiang L, Dong S, Dai J, Jin X, Yu W, Qiu Y (2022) A novel nomogram for predicting risk factors and outcomes in bloodstream infections caused by *Klebsiella pneumoniae*. *Infect Drug Resist* 15: 1317-1328. doi: 10.2147/IDR.S349236.
- Dai P, Hu D (2022) The making of hypervirulent *Klebsiella pneumoniae*. *J Clin Lab Anal* 36: e24743. doi: 10.1002/jcla.24743.
- Hernandez RE, Gallegos-Monterrosa R, Coulthurst SJ (2020) Type VI secretion system effector proteins: effective weapons for bacterial competitiveness. *Cell Microbiol* 22: e13241. doi: 10.1111/cmi.13241.
- Huang N, Jia H, Zhou B, Zhou C, Cao J, Liao W, Liu S, Wang L, Chen L, Chen L, Zhou T, Ye J (2022) Hypervirulent carbapenem-resistant *Klebsiella pneumoniae* causing highly

- fatal meningitis in southeastern China. *Front Public Health* 10: 991306. doi: 10.3389/fpubh.2022.991306.
9. Hyun M, Lee JY, Lim KR, Kim HA (2024) Clinical characteristics of uncomplicated acute pyelonephritis caused by *Escherichia coli* and *Klebsiella pneumoniae*. *Infect Dis Ther* 13: 581-595. doi: 10.1007/s40121-024-00940-3.
 10. Jiang X, Li H, Ma J, Li H, Ma X, Tang Y, Li J, Chi X, Deng Y, Zeng S, Liu Z (2024) Role of Type VI secretion system in pathogenic remodeling of host gut microbiota during *Aeromonas veronii* infection. *ISME J* 18: wrac053. doi: 10.1093/ismej/wrac053.
 11. Karampatakis T, Tsergouli K, Behzadi P (2023) Carbapenem-resistant *Klebsiella pneumoniae*: virulence factors, molecular epidemiology, and latest updates in treatment options. *Antibiotics* 12: 234. doi: 10.3390/antibiotics12020234.
 12. Kocsis B (2023) Hypervirulent *Klebsiella pneumoniae*: an update on epidemiology, detection and antibiotic resistance. *Acta Microbiol Immunol Hung* 70: 278-287. doi: 10.1556/030.2023.02186.
 13. Li G, Shi J, Zhao Y, Xie Y, Tang Y, Jiang X, Lu Y (2020) Identification of hypervirulent *Klebsiella pneumoniae* isolates using the string test in combination with *Galleria mellonella* infectivity. *Eur J Clin Microbiol Infect Dis* 39: 1673-1679. doi: 10.1007/s10096-020-03890-z.
 14. Li J, Ren J, Wang W, Wang G, Gu G, Wu X, Wang Y, Huang M, Li J (2018) Risk factors and clinical outcomes of hypervirulent *Klebsiella pneumoniae*-induced bloodstream infections. *Eur J Clin Microbiol Infect Dis* 37: 679-689. doi: 10.1007/s10096-017-3160-z.
 15. Mazza E, Prosperi M, Panzeri MF, Limuti R, Nichelatti M, De Gasperi A (2017) Carbapenem-resistant *Klebsiella pneumoniae* infections early after liver transplantation: a single-center experience. *Transplant Proc* 49: 677-681. doi: 10.1016/j.transproceed.2017.02.028.
 16. Mehrad B, Clark NM, Zhanel GG, Lynch JP 3rd (2015) Antimicrobial resistance in hospital-acquired Gram-negative bacterial infections. *Chest* 147: 1413-1421. doi: 10.1378/chest.14-2171.
 17. Merciecca T, Bornes S, Nakusi L, Theil S, Rendueles O, Forestier C, Miquel S (2022) Role of *Klebsiella pneumoniae* Type VI secretion system (T6SS) in long-term gastrointestinal colonization. *Sci Rep* 12: 16968. doi: 10.1038/s41598-022-21396-w.
 18. Mohamed NA, Alrawy MH, Makbol RM, Mohamed AM, Hemdan SB, Shafik NS (2023) Type VI secretion system (T6SS) in *Klebsiella pneumoniae*, relation to antibiotic resistance and biofilm formation. *Iran J Microbiol* 15: 601-608. doi: 10.18502/ijm.v15i5.13865.
 19. Nguyen M, Joshi SG (2021) Carbapenem resistance in *Acinetobacter baumannii*, and their importance in hospital-acquired infections: a scientific review. *J Appl Microbiol* 131: 2715-2738. doi: 10.1111/jam.15130.
 20. Russo A, Fusco P, Morrone HL, Treccarichi EM, Torti C (2023) New advances in management and treatment of multidrug-resistant *Klebsiella pneumoniae*. *Expert Rev Anti Infect Ther* 21: 41-55. doi: 10.1080/14787210.2023.2151435.
 21. Russo TA, Marr CM (2019) Hypervirulent *Klebsiella pneumoniae*. *Clin Microbiol Rev* 32. doi: 10.1128/CMR.00001-19.
 22. Russo TA, Olson R, Fang CT, Stoesser N, Miller M, MacDonald U, Hutson A, Barker JH, La Hoz RM, Johnson JR (2018) Identification of biomarkers for differentiation of hypervirulent *Klebsiella pneumoniae* from classical *K. pneumoniae*. *J Clin Microbiol* 56. doi: 10.1128/JCM.00776-18.
 23. Singh RP, Kumari K (2023) Bacterial type VI secretion system (T6SS): an evolved molecular weapon with diverse functionality. *Biotechnol Lett* 45: 309-331. doi: 10.1007/s10529-023-03354-2.
 24. Tan TY, Ong M, Cheng Y, Ng LSY (2019) Hypermucoviscosity, rmpA, and aerobactin are associated with community-acquired *Klebsiella pneumoniae* bacteremic isolates causing liver abscess in Singapore. *J Microbiol Immunol Infect* 52: 30-34. doi: 10.1016/j.jmii.2017.07.003.
 25. Taraghian A, Nasr Esfahani B, Moghim S, Fazeli H (2020) Characterization of hypervirulent extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* among urinary tract infections: the first report from Iran. *Infect Drug Resist* 13: 3103-3111. doi: 10.2147/IDR.S264440.
 26. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP (2008) The strengthening the reporting of observational studies in epidemiology (STROBE) statement: guidelines for reporting observational studies. *J Clin Epidemiol* 61: 344-349. doi: 10.1016/j.jclinepi.2007.11.008.
 27. Wang H, Guo Y, Liu Z, Chang Z (2023) The Type VI secretion system contributes to the invasiveness of liver abscess caused by *Klebsiella pneumoniae*. *J Infect Dis* 228: 1127-1136. doi: 10.1093/infdis/jiad166.
 28. Wang X, Bi C, Xin X, Zhang M, Fu H, Lan L, Wang M, Yan Z (2023) Pyroptosis, apoptosis, and autophagy are involved in infection induced by two clinical *Klebsiella pneumoniae* isolates with different virulence. *Front Cell Infect Microbiol* 13: 1165609. doi: 10.3389/fcimb.2023.1165609.
 29. Xu L, Sun X, Ma X (2017) Systematic review and meta-analysis of mortality of patients infected with carbapenem-resistant *Klebsiella pneumoniae*. *Ann Clin Microbiol Antimicrob* 16: 18. doi: 10.1186/s12941-017-0191-3.
 30. Xu Q, Xie M, Yang X, Liu X, Ye L, Chen K, Chan EW, Chen S (2024) Conjugative transmission of virulence plasmid in *Klebsiella pneumoniae* mediated by a novel IncN-like plasmid. *Microbiol Res* 289: 127896. doi: 10.1016/j.micres.2024.127896.
 31. Yang X, Dong N, Chan EW, Zhang R, Chen S (2021) Carbapenem resistance-encoding and virulence-encoding conjugative plasmids in *Klebsiella pneumoniae*. *Trends Microbiol* 29: 65-83. doi: 10.1016/j.tim.2020.04.012.
 32. Yang X, Sun Q, Li J, Jiang Y, Li Y, Lin J, Chen K, Chan EW, Zhang R, Chen S (2022) Molecular epidemiology of carbapenem-resistant hypervirulent *Klebsiella pneumoniae* in China. *Emerg Microbes Infect* 11: 841-849. doi: 10.1080/22221751.2022.2049458.
 33. Yu WL, Lee MF, Chen CC, Tang HJ, Ho CH, Chuang YC (2017) Impacts of hypervirulence determinants on clinical features and outcomes of bacteremia caused by extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae*. *Microb Drug Resist* 23: 376-383. doi: 10.1089/mdr.2016.0018.
 34. Zhang W, Wang Y, Wang K, Li J, Liu J, Li S, Song L, Liao C, Yang X, Li P, Liu X (2024) Hybrid sequencing-based genomic analysis of *Klebsiella pneumoniae* from urinary tract infections among inpatients at a tertiary hospital in Beijing. *Infect Drug Resist* 17: 1447-1457. doi: 10.2147/IDR.S448253.
 35. Zhou Y, Wu C, Wang B, Xu Y, Zhao H, Guo Y, Wu X, Yu J, Rao L, Wang X, Yu F (2023) Characterization difference of typical KL1, KL2 and ST11-KL64 hypervirulent and carbapenem-resistant *Klebsiella pneumoniae*. *Drug Resist Updat* 67: 100918. doi: 10.1016/j.drug.2023.100918.