

# Hybrid Sequencing-Based Genomic Analysis of *Klebsiella pneumoniae* from Urinary Tract Infections Among Inpatients at a Tertiary Hospital in Beijing

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**Background:** Urinary tract infection (UTI) associated with *Klebsiella pneumoniae* poses a serious threat for inpatients. This study aimed to describe the genomic characteristics of *K. pneumoniae* causing UTI in a tertiary-care hospital in Beijing, China.

**Methods:** A total of 20 *K. pneumoniae* strains collected from 2020 to 2021 were performed whole-genome sequencing. The Antibiotic susceptibility of 19 common antimicrobial agents was tested against all strains. The multi-locus sequence types (MLSTs) and serotypes were determined from the WGS data. *De novo* assemblies were used to identify resistance and virulence genes. The presence and characteristics of the plasmids were detected using hybrid assembly of long and short-read data.

**Results:** These *K. pneumoniae* strains were clustered into nine sequence types (STs) and twelve K-serotypes. All the carbapenem-resistant *K. pneumoniae* (CRKP) strains acquired carbapenemase *bla*<sub>KPC-2</sub> (n=7). Two CRKP strains exhibited increased resistance to Polymyxin B with MIC ≥ 4 mg/L due to insertion of an IS5-like sequence in the *mgrB* gene, and they were also involved in a transmission event in Intensive Care Unit. Long-read assemblies identified many plasmids co-carrying multiple replicons. Acquisition of a new IncM2\_1 type *bla*<sub>CTX-M-3</sub> positive plasmid was observed after transfer from ICU to neurovascular surgery by comparing the two strains collected from the same patient.

**Conclusion:** *K. pneumoniae* is a significant pathogen responsible for urinary tract infections. The ST11-KL47 strain, prevalent at our hospital, exhibits a combination of high drug resistance and hypervirulence. It is imperative to enhance ongoing genomic surveillance of urinary tract infection-causing pathogens.

**Keywords:** Antimicrobial resistance, *Klebsiella pneumoniae*, urinary tract infections

## Introduction

Urinary tract infection (UTI) commonly denotes a bacterial infection that originates in the region extending from the urethral opening to the perirenal fascia.<sup>1</sup> It is one of the most prevalent types of infections in hospital, with an estimated 150 million patients worldwide affected every year.<sup>2</sup> The diagnosis of urinary tract infection is usually based on symptoms and positive urine test or culture.<sup>3</sup> In China between 2006 and 2016, cases of urinary tract infections accounted for 11.65% of all healthcare-related infections,<sup>4</sup> and in the past three decades, there has been a notable surge in elderly women developing such infections.<sup>5</sup>

*K. pneumoniae* is a significant causative agent of UTI, which can result in high rates of illness and death.<sup>6,7</sup> Due to the high empiric use of antibiotics for the treatment of UTI, the antibiotic resistance of *K. pneumoniae* increased significantly worldwide.<sup>8</sup> In China, resistance rates to *K. pneumoniae* UTI isolates have risen for all tested antimicrobials, including carbapenems. The most severe cases were found in medical ICUs, where resistance rates have increased to 50–60% for

amikacin, ciprofloxacin, imipenem, and ertapenem.<sup>9</sup> Colistin is regarded as a last-resort agent to combat infections caused by multidrug-resistant bacteria, however reports of colistin-resistant *K. pneumoniae* are increasing in recent years.<sup>10</sup>

The isolation rate of *K. pneumoniae* in UTI exhibits regional variations, indicating a degree of geographical disparity. Over the period from 2009 to 2017, an extensive surveillance study at a Beijing hospital reported that *K. pneumoniae* accounted for 9.6% of all pathogens isolated from UTI cases,<sup>11</sup> positioning it as the second most prevalent Gram-negative bacterium just after *Escherichia coli*. Comparable findings were also documented in another hospital within Beijing, a notably elevated isolation rate of *K. pneumoniae* was observed among elderly patients admitted through the emergency department, reaching up to 21% prevalence over the period from 2009 to 2020.<sup>12</sup>

Genomic surveillance is increasingly pivotal in tracking and managing the spread of pathogenic bacteria. As yet, no genomic research on UTIs caused by *K. pneumoniae* within Beijing hospitals has been published to our knowledge. In this study, we aim to characterize the genomic traits of *K. pneumoniae* strains isolated from UTI cases at our facility. The hospital where this study was conducted has implemented robust infection control measures, yet understanding the genomic epidemiology of *K. pneumoniae* circulating in the local population is crucial for refining these efforts.

## Materials and Methods

### Setting

This study was conducted in The Third Medical Center of People's Liberation Army (PLA) General Hospital. It is a 900-bed tertiary general hospital with an estimated of 800 admissions per year in Beijing China.

### Sample Collection and Identification

The urine culture results of hospitalized patients in all departments in our hospital from January 2020 to December 2021 were reviewed retrospectively, all samples were collected as part of routine patient care and processed according to standard operating procedures as described previously.<sup>12</sup> In 2020, a total of 862 urine samples were collected with 228 (26.4%) yielding positive bacterial cultures, including 34 (14.9% of positives) identified as *Klebsiella species*. In 2021, 1929 urine samples were collected, with 371 (19.2%) showing positive results; among these, 45 (12.1% of positives) were identified as *Klebsiella species*.

Although a total of 79 strains were identified as *Klebsiella species* from urine samples collected between 2020 and 2021, only 21 of these strains were isolated from inpatients exhibiting symptoms of UTI and were subsequently subjected to whole-genome sequencing. One strain was subsequently excluded after being re-identified as *Klebsiella quasipneumoniae* based on the results of whole-genome sequencing, the remaining 20 strains confirmed as *K. pneumoniae* were subjected to further analysis.

The 20 confirmed *K. pneumoniae* strains were isolated from 16 inpatients across six different departments including Urology Surgery, Geriatric ward, Intensive care unit, Endocrinology ward, Neurovascular surgery and Obstetrics and gynecology ward. All *K. pneumoniae* strains used in this study were grown at 37°C for 18–24 h on Luria-Bertani (LB) agar plates.

### Antimicrobial Susceptibility Testing

Species identification and antimicrobial susceptibility were conducted using the VITEK2 compact system (BioMerieux, France) as described previously.<sup>13</sup> The AST-N334 card was used for antimicrobial susceptibility testing. The list of tested antimicrobial agents includes the following: Meropenem, Imipenem, Gentamicin, Amikacin, Levofloxacin, Ciprofloxacin, Nitrofurantoin, Ampicillin/Sulbactam, Piperacillin/clavulanic acid, Ticarcillin/Clavulanic acid, Cefoxitin, Cefepime, Ceftriaxone, Cefuroxime, Ceftazidime, Cefoperazone/Sulbactam, Minocycline, Trimethoprim/Sulfamethoxazole. The broth microdilution method was used to determine the minimum inhibitory concentration (MIC) values of Polymyxin B. Quality control was performed with both a colistin susceptible strain (*E. coli* ATCC 25922) and the colistin resistant (*E. coli* NCTC 13846). The susceptibility breakpoint was interpreted in accordance with the Clinical and Laboratory Standards Institute (CLSI-M100-2021).<sup>14</sup>

## Whole Genome Sequencing

Whole-genome sequencing was performed as described previously.<sup>13</sup> Briefly, genomic DNA was extracted from bacterial cultures using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA concentration and purity were measured using Qubit 4.0 (Thermo Fisher Scientific). Sequencing libraries were generated using NEB Next Ultra DNA Library Prep Kit for Illumina (New England Biolabs) following the manufacturer's recommendations. DNA libraries were constructed with 150-bp paired-end fragments and sequenced on the HiSeq 2500 sequencer at Novogene Company (Beijing, China). The nanopore sequencing library was prepared using the SQK-RAD004 rapid sequencing kit (Oxford Nanotechnology, UK) and sequenced on MinION Mk1B with R9.4 flowcell in our lab.

## Bioinformatics Analysis

Filtered reads were obtained by using the FastQC v0.11.8 software,<sup>15</sup> adapters and low-quality reads were removed and filtered out using Trimmomatic v0.39.<sup>16</sup> Assembly of sequencing reads was carried out with Unicycler v0.4.8<sup>17</sup> in the normal mode. The assembly was annotated by Prokka v1.12.<sup>18</sup>

MLST and capsular locus determination were carried out by analyzing each genome with Kleborate v2.2.0.<sup>19</sup> Unknown MLSTs were verified by stringMLST v0.6.3.<sup>20</sup> Novel alleles of ST profiles were submitted to BIGSdb.<sup>21</sup> Resistance genes, virulence genes, and plasmids were identified with Abricate v0.8 (accessible at <https://github.com/tseemann/abricate>) using resfinder, vfdb and plasmidfinder database respectively. Plasmid images were produced in CGView Comparison Tool v1.0.3.<sup>22</sup>

The *K. pneumoniae* strain 16HN-263 (NZ\_CP045263.1) was selected as the best-matching chromosomal reference by the genomic distance estimation tool Mash v2.3.<sup>23</sup> Single nucleotide polymorphisms (SNPs) were identified using Snippy v4.6.0 software (accessible at <https://github.com/tseemann/snippy>). The recombination regions were identified and excluded by using ClonalFrameML v1.12<sup>24</sup> with default parameters, except that the “output\_filtered” and “embranch” option were set to “true”. SNPs occurring outside the putative genomic recombination regions are identified and integrated with clinical data to infer transmission relationships among the collected strains. A maximum likelihood phylogenetic tree based on the non-recombination alignment was constructed using Raxml-ng v1.0.3<sup>25</sup> implementing with 1000 bootstrap replicates, GTR+I+G4 was selected as the best evolutionary model by using Modeltest-ng v0.1.7.<sup>26</sup> The phylogeny was midpoint rooted and visualized using Figtree v1.4.4 (accessible at <http://tree.bio.ed.ac.uk/software/figtree/>). All analyses were performed using default software parameters unless otherwise specified.

## Statistical Analyses

Software R v4.1.1 and RStudio Desktop 2023.03.1 were used for statistical analysis. Categorical variables were expressed as percentages and analyzed by using  $\chi^2$ .  $p < 0.05$  was considered statistically significant.

## Ethical Statement

Due to the retrospective nature of the study, the need of informed consent was waived by the institutional ethics committees of The Third Medical Center of PLA General Hospital (Beijing, China). The study was also supervised by Chinese PLA Center for Disease Control and Prevention. We confirmed that all methods were performed in accordance with the relevant regulations and guidelines outlined in the Declaration of Helsinki.

## Data Availability

This Whole-Genome Shotgun project has been deposited at GenBank under accession numbers from SAMN27593772 to SAMN27593792, BioProject ID: PRJNA826970.

## Results

### General Features of the Patients and Strains

Following a retrospective examination of urine bacterial culture results from inpatients over the course of 2020 to 2021, a selection of 20 *K. pneumoniae* strains (Table 1) were identified as originating from inpatients presenting with symptoms of UTI. These strains were isolated from 16 inpatients across six different wards but mainly from the Urology Surgery (35.0%, 7/20), Geriatric ward (35.0%, 7/20) and Intensive Care Unit (15.0%, 3/20). One inpatient (Pa05) had three strains, two inpatients (Pa01 and Pa08) had two strains, and the other inpatients each had one strain. The mean age of these inpatients was 67.7 years (range, 22–92 years), except for the inpatient Pa01, all other inpatients were over 45 years old. Among the 16 inpatients, 11 (68.8%) inpatients were males, and 5 (31.2%) were females. Two (Pa05 and Pa08) of the 16 inpatients were admitted to the hospital twice and the average length of stays was 30.5 days for all the 18 hospitalization. Apart from 2 inpatients (Pa01 and Pa10), all others have used catheters during hospitalization.

**Table 1** Characteristics of the 16 Patients and 20 *K. Pneumoniae* Strains

Patient ID	Gender	Age	LOS	Strains	Sample Date	Ward	Use of Antimicrobials
Pa01	Male	22	63	BJ17-0106	2020.05.26	Intensive care unit	Cefoxitin, cefoperazone/sulbactam, levofloxacin, vancomycin, moxifloxacin, azithromycin
				BJ17-0107	2020.06.01	Neurovascular surgery	
Pa02	Male	92	152	BJ17-0100	2020.10.07	Intensive care unit	Loxceptazidime sodium, moxifloxacin, meropenem, levofloxacin, cefoperazone sodium, sulbactam sodium and voriconazole, meropenem +polymyxin+voriconazole combined
Pa03	Female	77	30	BJ17-0120	2020.11.21	Intensive care unit	Meropenem+moxifloxacin, Piperacillin sodium tazobactam sodium+ornidazole
Pa04	Female	90	21	BJ17-0113	2021.03.18	Geriatric ward	Cefoperazone/sulbactam, tigecycline, voriconazole, cefdinir
Pa05	Male	91	58	BJ17-0112	2021.03.19	Geriatric ward	Cefdinib, Sulphur, Ornidazole, Moxifloxacin, cefuroxime sodium, sulprost, fluconazole, Levofloxacin, voriconazole, cefoperazone/sulbactam
				BJ17-0115	2021.04.14	Geriatric ward	
			28	BJ17-0116	2021.12.30	Geriatric ward	
Pa06	Male	74	13	BJ17-0121	2021.04.21	Urology Surgery	Cefoxitin, etimicin
Pa07	Male	65	8	BJ17-0102	2021.05.19	Urology Surgery	Levofloxacin, gentamicin, metronidazole, moxifloxacin
Pa08	Female	68	22	BJ17-0111	2021.06.24	Urology Surgery	Meropenem, etimicin, moxifloxacin, imipenem
			9	BJ17-0103	2021.11.10	Urology Surgery	
Pa09	Male	76	32	BJ17-0109	2021.07.02	Geriatric ward	Moxifloxacin
Pa10	Female	47	56	BJ17-0110	2021.09.04	Urology Surgery	Cefoperazone/tazobactam, cefuroxime
Pa11	Male	58	12	BJ17-0118	2021.09.04	Urology Surgery	Omeprazole, cefdinir, meropenem, levofloxacin
Pa12	Female	71	8	BJ17-0117	2021.09.09	Obstetrics and gynecology ward	Furacilin
Pa13	Male	66	8	BJ17-0119	2021.11.27	Geriatric ward	Sulbactam and cefoperazone, cefoperazone sulbactam
Pa14	Male	69	4	BJ17-0108	2021.12.03	Geriatric ward	Not available
Pa15	Male	55	14	BJ17-0104	2021.12.09	Endocrinology ward	Piperacillin/tazobactam
Pa16	Male	62	11	BJ17-0114	2022.01.05	Urology Surgery	Levofloxacin, cefoperazone/sulbactam

**Abbreviation:** LOS, length of stay.

## Antimicrobial Resistance Phenotype

The Antibiotic susceptibility of 19 common antimicrobial agents was tested against all strains ([Supplementary Table 1](#)). In terms of carbapenem antibiotics, 35.0% strains (7/20) exhibited resistance to both imipenem and meropenem with MICs  $\geq 16$  mg/L, including all the three strains isolated from Intensive Care Unit. In terms of Amino glycosides, 50.0% strains (10/20) exhibited resistance to Gentamicin, and 45.0% (9/20) strains were resistant to Amikacin. In terms of Quinolone antibiotics, 80.0% strains (16/20) were resistant to Levofloxacin, and 85.0% strains (17/20) were resistant to Ciprofloxacin. 75.0% strains (15/20) were resistant to Nitrofurantoin, which was widely used during urinary tract infection. In addition, two strains (10.0%, 2/20) isolated from Intensive Care Unit exhibited resistance to Polymyxin B with MIC  $\geq 4$  mg/L.

## Distribution of Multilocus Sequence Typing Types and Serotypes

All reads of the 20 *K. pneumoniae* strains were *de novo* assembled ([Supplementary Table 2](#)), the average of genomic size was 5.70 Mb, and the median of GC % content was 57.0%. The multi-locus sequence types (MLSTs) and serotypes were determined from the assemblies ([Table 2](#)). Twenty *K. pneumoniae* strains belonged to nine sequence types, including ST11(10), ST1(1), ST23(1), ST65(1), ST147(1), ST628(1), ST1076(1) and two novel MLST types. Three strains were identified as a novel sequence type ST6902, which was closest to the known ST147 with only one mutation in the *tonB-38* allele. One strain belonged to another novel sequence type ST6903, which had only one mutation in the *phoE-9* allele with ST6903. The analysis revealed there exists five O-serotypes and twelve K-serotypes, and the most prevalent serogroup of O and K locus was O101 (45.0%, 9/20) and KL47 (30.0%, 6/20), respectively.

## Resistance and Virulence Gene Profiles

*De novo* assemblies were screened for virulence ([Table 2](#)) and resistance genes ([Supplementary Table 3](#)). Seven ST11 strains carried the *bla*<sub>KPC-2</sub> gene (35.0%, 7/20), which was consistent with the antibiotic susceptibility test results of imipenem and meropenem ([Supplementary Table 1](#)). Thirteen strains (65.0%, 13/20) were CTX-M positive, one ST11 strain co-carried *bla*<sub>CTX-M-65</sub> and *bla*<sub>CTX-M-3</sub>, five ST11 strains carried *bla*<sub>CTX-M-65</sub>, while seven other ST strains carried *bla*<sub>CTX-M-14</sub> (4), *bla*<sub>CTX-M-27</sub> (2) and *bla*<sub>CTX-M-15</sub> (1). Two ST11 strains isolated from Intensive Care Unit had the MICs of Polymyxin B  $\geq 4$  mg/L, and genetic analysis further revealed an insertion of an IS5-like sequence in the *mgrB* gene, which were associated with colistin resistance.<sup>27</sup>

Seven ST11 strains harboured a set of virulence genes including aerobactin (*iuc1*), yerseniabactin (*ybt9*; ICEKp3) and *rmpA2*, six of them belonged to KL47 (85.7%, 6/7) and one belonged to KL64 (1/7, 14.3%). One strain belonged to ST65-KL2-O1/O2v2 harbored a different set of virulence genes including aerobactin (*iuc1*), yerseniabactin (*ybt17*; ICEKp10), *Cib3*, *Iro1* and *rmpA2*. Six ST11-KL47-OL101 strains, including the two strain with elevated resistance to Polymyxin B, co-harbored virulence genes (*iuc1*, *ybt9* and *rmpA2*) and carbapenemase gene (*bla*<sub>KPC-2</sub>).

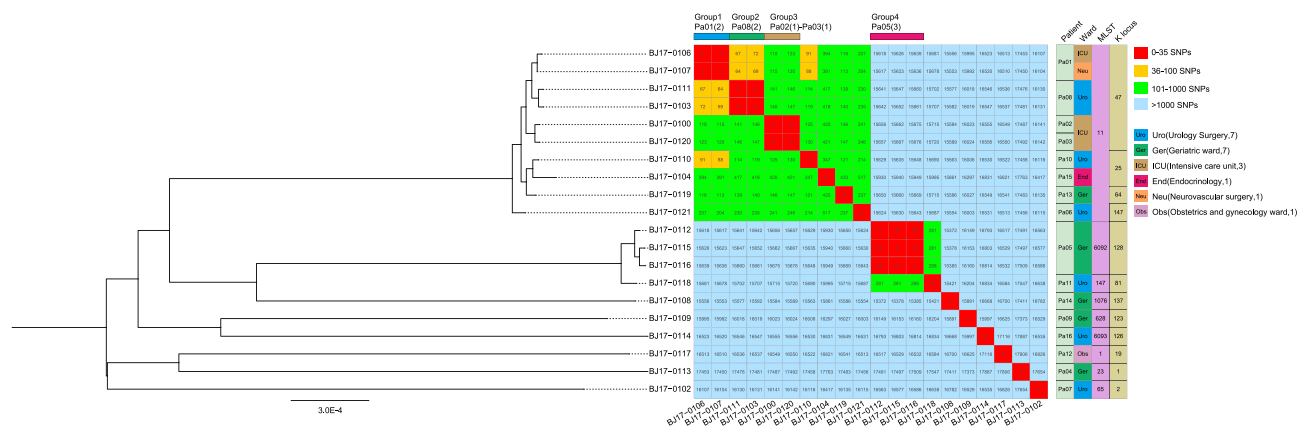
## Phylogenetic Analysis of Klebsiella Pneumoniae Strains

Reads mapping to *K. pneumoniae* reference genome NZ\_CP045263.1 showed an average of 92.5% coverage among all 20 genomes ([Supplementary Table 2](#)). A reference-based whole-genome alignment was constructed, from which 103,894 SNPs were identified. Putative recombination loci were further detected and removed, the final 4,289,862bp recombination-free alignment consisted of 49,483 variable SNP sites among the *K. pneumoniae* strains. The pairwise disparity between these strains ranges from 3 to 17,887 SNPs ([Figure 1](#)). This recombination-free SNP variation across the whole genome was used to construct a maximum likelihood phylogenetic tree. Interestingly, we found 4 groups of strains within which the pair-wise SNPs distances were obviously less than other strains. Three groups of strains were isolated from three patients, respectively, in addition, one group of strains were isolated from two different patients whom were both admitted in the Intensive Care Unit, suggesting a transmission event.

**Table 2** MLST, Serotypes, Virulence Genes, Replicon Types and Numbers of 20 *K. Pneumoniae* Genomes

Strains	MLST	K_ locus	O_ locus	Aerobactin	IUCA	IUCB	Iucc	Iucd	Iuta	Yersiniabactin	Rmpa2	Colibactin	Salmochelin	Replicon Types	Replicon Numbers
BJ17-0106	11	KL47	OL101	luc I	I	I	I	I	I	Ybt 9; ICEKp3	Rmpa2_8	-	-	3	4
BJ17-0107	11	KL47	OL101	luc I	I	I	I	I	I	Ybt 9; ICEKp3	Rmpa2_8	-	-	4	5
BJ17-0111	11	KL47	OL101	luc I	I	I	I	I	I	Ybt 9; ICEKp3	Rmpa2_8	-	-	3	4
BJ17-0103	11	KL47	OL101	luc I	I	I	I	I	I	Ybt 9; ICEKp3	Rmpa2_8	-	-	3	4
BJ17-0100	11	KL47	OL101	luc I	I	I	I	I	I	Ybt 9; ICEKp3	Rmpa2_6	-	-	5	6
BJ17-0120	11	KL47	OL101	luc I	I	I	I	I	I	Ybt 9; ICEKp3	Rmpa2_6	-	-	5	6
BJ17-0110	11	KL25	O5	-	-	-	-	-	-	Ybt 9; ICEKp3	-	-	-	2	2
BJ17-0104	11	KL25	O5	-	-	-	-	-	-	Ybt 9; ICEKp3	-	-	-	3	4
BJ17-0119	11	KL64	O1/O2v1	luc I	I	I	I	I	I	Ybt 9; ICEKp3	Rmpa2_3	-	-	5	6
BJ17-0121	11	KL147	O1/O2v2	-	-	-	-	-	-	-	-	-	-	4	5
BJ17-0112	6092	KL128	O3b	-	-	-	-	-	-	-	-	-	-	3	3
BJ17-0115	6092	KL128	O3b	-	-	-	-	-	-	-	-	-	-	3	3
BJ17-0116	6092	KL128	O3b	-	-	-	-	-	-	-	-	-	-	3	3
BJ17-0118	147	KL81	OL101	-	-	-	-	-	-	-	-	-	-	8	13
BJ17-0108	1076	KL137	OL101	-	-	-	-	-	-	-	-	-	-	1	1
BJ17-0109	628	KL123	O3b	-	-	-	-	-	-	-	-	-	-	2	2
BJ17-0114	6093	KL126	OL101	-	-	-	-	-	-	-	-	-	-	6	6
BJ17-0117	I	KL19	O1/O2v2	-	-	-	-	-	-	Ybt 14; ICEKp5	-	-	-	6	6
BJ17-0113	23	KL1	O1/O2v2	-	-	-	-	-	-	Ybt 1; ICEKp10	-	Clb 2	-	0	0
BJ17-0102	65	KL2	O1/O2v2	luc I	I	I	I	I	I	Ybt 17; ICEKp10	Rmpa2_3	Clb 3	iro I	2	2





**Figure 1** Phylogenetic analysis of *Klebsiella pneumoniae* strains A maximum-likelihood phylogenetic tree based on the recombination-free alignment of 20 *Klebsiella pneumoniae* strains was constructed, the tree was mid-point rooted. The single-nucleotide polymorphisms (SNPs) numbers between each *K. pneumoniae* strain, 4 groups of strains (red) were observed within which the pair-wise SNPs distances were less than 35.

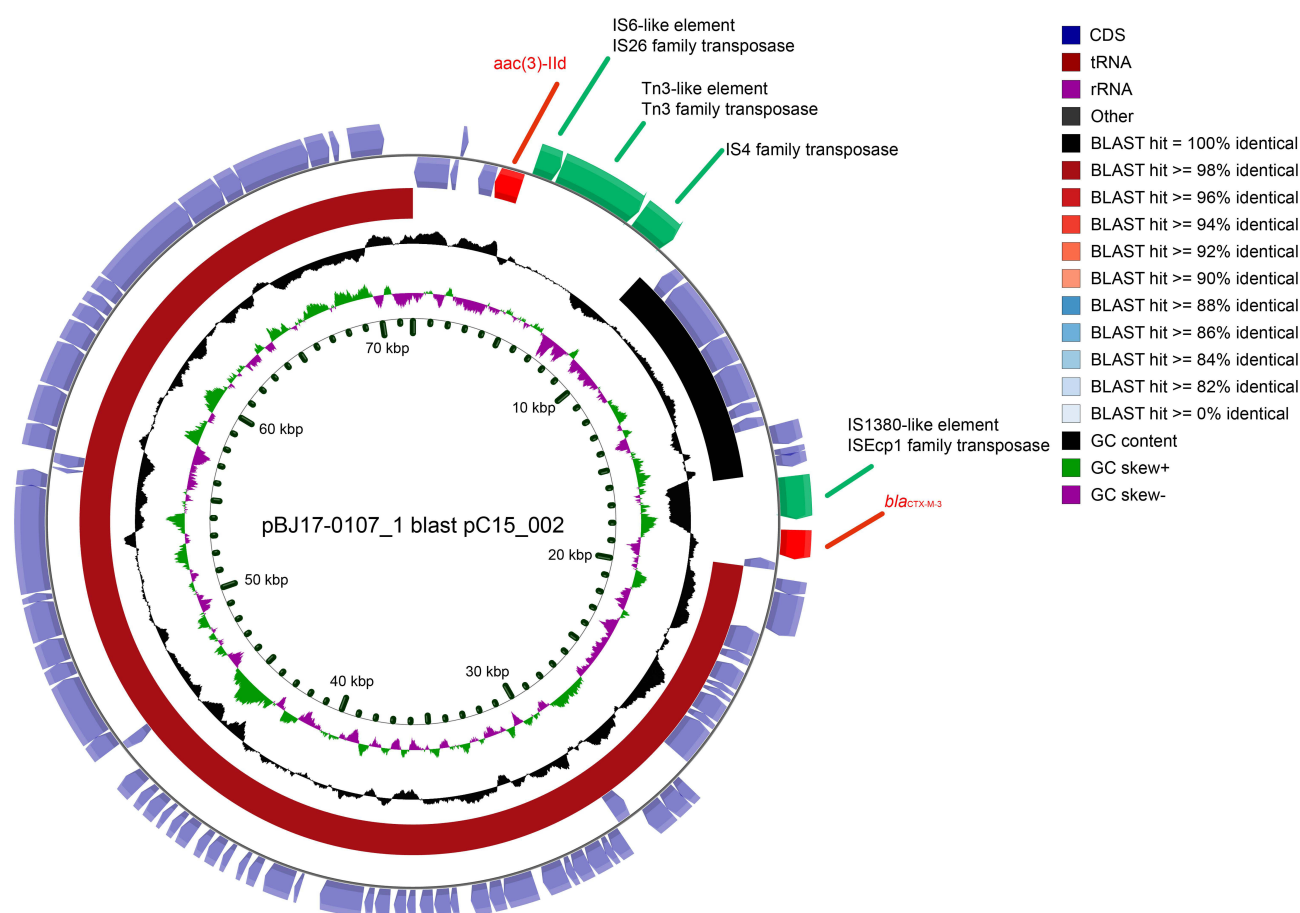
## Plasmid Carriage Profile

The presence and characteristics of the plasmids were detected using hybrid assembly of long and short-read data. With exception of the ST23-KL1 strain BJ17-0113, all other *K. pneumoniae* strains have at least one plasmid replicon. An average of  $4.3 \pm 2.7$  plasmid replicons per strain was observed. Among all the strains, 11 ColRNAI\_1 and 9 IncFII (pHN7A8)\_1 plasmid replicons were identified, and 90.9% (10/11) and 100% (9/9) of which were found in ST11 strains, respectively. One ST147-KL81 strain BJ17-0118 had the largest plasmid carriage with 8 different plasmid replicons. Long-read assemblies revealed that multiple replicons coexist in the same plasmid. For example, IncR\_1 and IncFII (pHN7A8)\_1 plasmid replicons were located within the same plasmid sequence in three strains. Compared with strain BJ17-0106 (Figure 2), BJ17-0107 isolated from the same patient Pa01 had acquired a IncM2\_1 plasmid (pBJ17-0107\_1). However, no difference in the antimicrobial resistance phenotype were observed between strains BJ17-0106 and BJ17-0107. The plasmid pBJ17-0107\_1 had high similarity (95.1%) to *Enterobacter hormaechei* strain C15 plasmid pC15\_002 (NZ\_CP042490.1) from Australia, but had acquired two additional resistance genes (*aac* (3)-IId and *bla*<sub>CTX-M-3</sub>) mediated by *ISEcp1* and *IS26* family transposases (Figure 2).

## Discussion

In this study, we focused on Urinary tract infection caused by *K. pneumoniae* among inpatients at a tertiary hospital in Beijing and performed a high-resolution genomic analysis using short- and long-read sequencing. Traditionally, UTIs have been regarded as a female-specific disease due to anatomical factors such as shorter urethral length, reduced distance from the anus to the urethral meatus, and the permissive nature of the vaginal and perineal environments for microbial colonization.<sup>28</sup> We found that there were more than twice as many male patients ( $n=11$ ) as female patients ( $n=5$ ), in addition, the average age was nearly 70 years old, indicating that elderly men are also at high risk of UTI. We found that 87.5% (14/16) patients had used catheters during hospitalization, the use of indwelling urinary catheters is widely recognized as a major predisposing factor for urinary tract infection,<sup>29</sup> healthy monitoring of these patients should be strengthened, especially regarding the insertion, maintenance, and timely removal of urinary catheters.

We identified that 35% strains were resistant to imipenem and meropenem, all of which belonged to ST11<sup>30</sup> and carried the *bla*<sub>KPC-2</sub> gene. Whereas in a study conducted in Portuguese, most carbapenem-resistant strains detected were found to harbor the *bla*<sub>KPC-3</sub> gene within the ST14 lineage highlighting the geographical differences.<sup>31</sup> Two carbapenem-resistant strains isolated from ICU showed increased MICs to Polymyxin B due to insertion of an IS5-like sequence in the *mgrB* gene which encoding a negative regulator of the PhoQ/PhoP signaling system.<sup>27</sup> Polymyxin B is one of the few agents that retain activity against Carbapenem-resistant *K. pneumoniae*,<sup>32</sup> raising the great concern of more strict infection control in ICU.



**Figure 2** CGView Comparison between two plasmids. The newly acquired plasmid from BJ17-0107 (outer ring) had high similarity to a previously plasmid pC15\_002 (inner ring) from Australia. Arrows are proportional to the lengths of the genes and oriented in the direction of transcription, two resistance genes were marked as red, four transposase were marked as yellow, other genes were marked blue. Tracks shown are (from inner to outer): GC skew (G-C/G+ (C)), G+C content.

A total of 45% (9/20) of the strains were identified as hypervirulent *K. pneumoniae*, based on the presence of virulence genes, which was significant higher ( $p < 0.01$ ) than that found in a similar study in Changsha, China, which report a rate 24.8% (30/121).<sup>33</sup> Although hypervirulent strains in both hospitals predominantly belonged to ST11, with prevalence rates of 77.8% and 56.7% respectively, there was a clear disparity in the distribution of K serotypes. Among our 9 hypervirulent strains, 3 were classified as K1, K2, and K64 respectively, while the remaining 6 strains were all belong to the K47 serotype (66.7%, 6/9). In contrast, at the hospital in Changsha, a notably different pattern emerged where 17 strains were identified as K64 (56.7%), 5 were K2, 1 was K1, and 7 other K serotypes strains, no K47 strains were identified. There is mounting evidence suggesting that KL64 has evolved from KL47 via genomic recombination events.<sup>34</sup> The current study will provide a important basis for an in-depth examination of the adaptive genomic changes that take place during the progression from KL47 to KL64.

The utilization of WGS data permitted us to gain further insight into potential transmissions of these strains. In all six wards, two or more patients were found in three specific wards: the Intensive Care Unit (ICU), Geriatrics ward and Urology Surgery. Notably, only found within the ICU, two patients (Pa01 and Pa02) carried strains of the same sequence type (ST) and serotype, with a difference of 5 single nucleotide polymorphism (SNP) sites between the strains isolated from two distinct patients. The overlapping admission and hospitalization date strongly supported a transmission event or a shared source of infection between these two patients in ICU. However, in the Geriatrics ward, where 5 patients (Pa04, Pa05, Pa09, Pa13 and Pa14) were collected, and the Urology surgery with 6 patients (Pa06, Pa07, Pa08, Pa10, Pa11 and Pa16), the STs, serotypes, and genomic SNP variations between strains from different patients showed significant diversity, indicating that their sources of infection were likely distinct from one



another. Therefore, we inferred that the ICU was potentially one of the critical areas for nosocomial transmission within the hospital, and hence, heightened infection control measures are warranted in this unit to mitigate the risk of cross-infection and outbreaks.

The hybrid assembly of long and short-read data helped to found a plasmid acquisition event during the within-host evolution of UTI.<sup>35</sup> Two strains isolated from the same patient with six days apart showed only 3 SNP differences; nevertheless, the plasmid carriage was different between them. The latter isolated strain had acquired a new IncM2\_1 *aac* (3)-*IId* and *bla*<sub>CTX-M-3</sub> positive (71,730 bp) plasmid after transfer to Neurovascular surgery from ICU, which highly suggesting the dynamics of plasmid in *K. pneumoniae* and the important influence of environment.<sup>36–38</sup> Most of ST11 strains co-harbored aerobactin (*iucI*), yerseniabactin (*ybt*) and *rmpA2* genes and were KPC-2 positive, showing both carbapenem-resistant and hypervirulent, which are anticipated to pose a significant threat to human health. Future research should concentrate on devising intervention strategies to prevent the dissemination and transmission of *K. pneumoniae* ST11 strains in healthcare settings.<sup>39</sup>

Our study has a main limitation; specifically, the number of isolates and patients is limited. Our objective was to characterize the genomic features and possible transmission of *K. pneumoniae* causing UTI in a tertiary-care hospital. Therefore, among the 79 *Klebsiella species* strains collected, we only selected the 20 strains from 16 patients who presented with urinary tract infection symptoms. Measures were implemented to control nosocomial infection of multidrug-resistant bacteria in the hospital. The limited isolation of *K. pneumoniae* strains over a two-year period indicates the efficacy of these measures in preventing and controlling nosocomial infections.

## Conclusion

*K. pneumoniae* is a notable causative agent of urinary tract infections (UTIs), with the ST11-KL47 lineage being notably prevalent at our hospital, characterized by a worrying dual phenotype of heightened drug resistance and hypervirulence. Strengthening the ongoing genomic surveillance of pathogens responsible for UTIs is of utmost importance to effectively monitor and manage the emergence and spread of such multi-drug resistant and highly virulent strains.

## Data Confidentiality Statement

In this study, patient data confidentiality was stringently upheld. Due to the retrospective nature of the research, the institutional ethics committees of the Third Medical Center, PLA General Hospital (Beijing, China) waived the requirement for informed consent from individual patients. All methods were conducted in compliance with the relevant regulations and guidelines outlined in the Declaration of Helsinki. We have implemented strict protocols to protect patient confidentiality throughout the process, including during the analysis and dissemination of the genomic data obtained from the bacterial isolates causing urinary tract infections in the studied population.

## Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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## Disclosure

The authors declare no conflicts of interest in this work.

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