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ORIGINAL RESEARCH

Genome Sequencing and Comparative Genomic Analysis of Multidrug-Resistant *Klebsiella pneumoniae* in a Neurological Intensive Care Unit

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Background: *Klebsiella pneumoniae* is an important cause of nosocomial infections and community-acquired pneumonia. However, the evolutionary convergence of multidrug resistance (MDR) and virulence factors undoubtedly increases the risk of infection and lethality of *K. pneumoniae*, especially in intensive care units. How to effectively prevent and correctly treat *K. pneumoniae* infections has become a significant challenge for healthcare professionals.

Objective: To assess multidrug-resistant *Klebsiella pneumoniae* (MDR-KP) resistance patterns in a Neurological ICU (NICU) and guide infection control strategies.

Methods: A total of 156 non-repetitive *K. pneumoniae* isolates from the NICU underwent strain identification and antimicrobial susceptibility testing. Six MDR-KP isolates were selected for whole genome sequencing (WGS) using high-throughput sequencing technologies, followed by comparative genomics and phylogenetic analysis.

Results: Antimicrobial susceptibility testing revealed that the 6 MDR-KP strains exhibited a resistance rate of 59% to 21 commonly used antibiotics, with seven antibiotics showing a resistance rate of 100%. The sequencing results provided basic genomic information such as genome size and GC content for the 6 MDR-KP strains. Six different sequence (ST)-capsular locus (KL) types were identified: ST11-KL47, ST11-KL64, ST23-KL1, ST25-KL2, ST412-KL57, and ST753-KL3. All strains carried multiple resistance genes and virulence factors. Among them (No. P14, P97) are carbapenem-resistant *K. pneumoniae* (CRKP) strains, which should attract our sufficient attention. Phylogenetic analysis showed that P116 was more closely related to the reference strain KP20, and P14 had the highest affinity to P97.

Conclusion: NICU *K. pneumoniae* could colonise patients and the ward air environment for a long time, suggesting that there may be a similar evolutionary or direct transmission relationship between strains. The ST11-KL47 and ST11-KL64 phenotypes were the dominant clone types of CRKP in China, suggesting that the ST11-type MDR-KP should be the focus of infection prevention and control in our hospital. Comparative genomics revealed homology and genetic variability of NICU *K. pneumoniae*.

Keywords: *Klebsiella pneumoniae*, whole genome sequencing, resistance genes, virulence factors, multidrug resistance, phylogenetic analysis

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Introduction

Klebsiella pneumoniae, a Gram-negative bacterium, is one of the most prominent species within the Klebsiella genus of the Enterobacteriaceae family. It is associated with a variety of infections, such as pneumonia, liver abscesses, sepsis, and urinary tract infections.[1] *K. pneumoniae* is typically classified into two types: classic *K. pneumoniae* (cKP) and hypervirulent

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K. pneumoniae (hvKP).[2] cKP is mainly composed of nosocomial infect-associated opportunistic bacteria and usually causes infection in immunocompromised patients with complications or preexisting traumatic procedures. HvKP, on the other hand, is considered a community-acquired bacteria, which generally shows high mucus viscosity through a string test and sensitivity to most antibiotics.[3–5] The virulence plasmids carried by *K. pneumoniae* can encode a variety of virulence genes, including capsular polysaccharide synthesis regulation genes (rmpA and rmpA2), iron acquisition system genes (iucA and iutA) and iron carrier genes. They can encode proteins with adhesion, invasion, anti phagocytosis and other functions, and provide support for the successful colonization of *K. pneumoniae* strains, the establishment of infection and the recognition of escaping host immune defence.[6,7] Not only do they have the ability to infect healthy individuals of any age, but they can also cause infected people to have multiple infection sites and/or the tendency of metastatic spread.[8,9] Given their high virulence, antibiotic resistance, and ability to evade host immune defences, these bacteria have been recognised as part of the "ESKAPE" group.[10,11] With the increasingly widespread use of antibiotics, the drug resistance rate and nosocomial infection rate of *K. pneumoniae* have increased year by year. According to the European Antimicrobial Resistance Surveillance report, resistance to third-generation cephalosporins among clinical isolates has reached 50%, with carbapenem resistance exceeding 25%.[12] In Zhejiang, China, the incidence of carbapenem-resistant *K. pneumoniae* (CRKP) infections in hospital settings has risen from 2.5% in 2008 to 15.8% in 2018, with even higher rates observed in intensive care units (ICU).[13] In response to the growing global challenge of antimicrobial resistance (AMR), the World Health Organization (WHO) updated its Early Warning of Bacterial Priority Pathogens List in 2024 (https://www.who.int). This update includes 24 pathogens across 15 antibiotic-resistant bacterial families, with CRKP included in the highest "Critical Group". In addition, hvKP has recently attracted a lot of attention, especially the carbapenem-resistant, highly virulent strains.[14] hvKP infections were first collected from a patient suffering from a liver abscess in Taiwan, China, in 1982, and since then have been seen in multiple locations around the globe, spanning Australia, the Americas, Europe, and Africa.[9] This indicates that *K. pneumoniae* has become a serious threat to human health. Therefore, rapid and accurate diagnosis with virulence markers such as iucA, iroB, peg-344, rmpA, and rmpA2, as well as efficient clinical management measures to curb the global spread of these strains, are becoming increasingly important.[15,16]

In recent years, bacterial molecular typing techniques have played an irreplaceable role in epidemiological investigations. As a result, it has been used since the 1970s and 1980s to monitor the spread of bacterial infections and outbreaks.[17,18] In recent years, the cost of whole genome sequencing (WGS) has decreased, and the technology has become more accessible and convenient, making it widely used in microbiological research.[19] WGS is not only an effective tool for elucidating bacterial transmission dynamics but also allows real-time analysis of resistance genes, providing technical support for in-depth studies of bacterial genomic characteristics.[20] This study aims to conduct a systematic analysis of the genomes of multidrug-resistant (MDR) *K. pneumoniae* (particularly CRKP) strains using WGS. The objective is to elucidate the relationship between clinical phenotypes and MDR-KP, as well as to investigate the distribution patterns of resistance genes and virulence factors. A comparative analysis of these data was conducted to trace the genetic evolutionary relationships of *K. pneumoniae* isolates from the Neurological Intensive Care Unit (NICU) of our hospital. This research provides a laboratory foundation for the prevention and control of *K. pneumoniae* transmission in clinical settings.

Materials and Methods

Clinical Data

Patients with infections during their stay in the NICU of Kailuan General Hospital in Tangshan City, China, from January 2020 to December 2024 were used as the study subjects, and the patients were mainly suffering from central nervous system diseases such as cerebral infarction and intracerebral haemorrhage. For patients diagnosed with infections (according to the "Infection Diagnosis Criteria" established by the Chinese Ministry of Health), corresponding specimens such as blood, urine, sputum, and cerebrospinal fluid were collected as per standard procedures. Environmental samples from the patient bed units (including air around the bed, pillows, blankets, bedside tables, etc.) were also collected using the LWC-1 microbial sampler. All collected specimens were subjected to microbiological examination. We selected 6 MDR-KP strains for whole genome sequencing from 156 non-duplicate *K. pneumoniae* strains isolated and identified (only the first isolate from the same site was counted). The 6 isolates were obtained from 6 different patients, three males and three females, with an average age of (76.0 ± 8.69) years old, ranging from 67 to 96 years old, five of whom were admitted for cerebral infarction and one for cerebral haemorrhage and all of whom had co-infections of the lungs during their hospital stay. Patients or guardians signed an informed consent form.

Bacterial Isolation and Identification

Bacterial strains obtained from specimens sent from infected patients in the NICU were smeared and Gram-stained to determine bacterial morphology and properties. According to the National Clinical Laboratory Practice (4th edition), Vitek-2Compact fully automatic bacterial identifier (bioMérieux, France) was applied for strain identification, and the resulting *K. pneumoniae* was stored in a special refrigerator at −80°C for spare use. Then, antimicrobial susceptibility testing was performed using the micro broth dilution method to determine the minimum inhibitory concentration (MIC) of the antimicrobial drugs. *Escherichia coli* ATCC25922, obtained from the Clinical Laboratory Center of the Ministry of Health, served as the quality control strain. Contaminating and colonizing strains were excluded from the analysis. Results were interpreted in accordance with the Clinical and Laboratory Standards Institute (CLSI) M100 guidelines.[21]

Selection of MDR-KP Sequencing Strains

In this study, the following criteria were developed based on multiple dimensions such as drug resistance characteristics, sample source, clinical background and time distribution, with the aim of selecting 6 representative MDR-KP strains from drug sensitivity tests for whole genome sequencing analysis:

(1) Resistance characteristics: Priority was given to CRKP strains resistant to carbapenem antibiotics; at the same time, different resistance patterns were covered, including strains with significant differences in susceptibility to three-generation cephalosporins, aminoglycosides and fluoroquinolones.
(2) Sample sources: Ensure that sputum (the main clinical sample), urine (representative of urinary tract infections) and air (risk of environmental colonisation) are covered.
(3) Clinical context: Include strains with different prognostic outcomes, emphasising strains from patients who have undergone invasive procedures and have multiple comorbidities.
(4) Temporal distribution: cover strains isolated between 2020 and 2024 to avoid temporal bias.

Whole Genome Sequencing, Assembly, and Annotation

Bacterial genomic DNA was extracted using a genomic extraction kit, and its purity and integrity were evaluated by agarose gel electrophoresis. DNA concentration was quantified using a Qubit fluorometer.[22] WGS was performed by combining Illumina second-generation and Oxford Nanopore Technology (ONT) third-generation sequencing techniques. Genomic DNA was extracted using the Gentra Puregene Yeast/Bact. Kit (Qiagen, Valencia, CA),[23] is recommended for Nanopore sequencing. Libraries were prepared using the Nanopore ligation sequencing kit. Illumina data underwent quality control with Fastp software, which filtered out sequences with adapter contamination, more than 10% N bases, or base quality scores (Q) ≤20 over 50% of the read length.[24] ONT sequencing data were utilized for initial genome assembly, which was subsequently refined with Illumina data to generate the final genomic sequence.[25]

The assembled genomes were analyzed using Kleborate v.2.3.1 (https://github.com/katholt/Kleborate) to determine multilocus sequence typing (MLST),[26] based on the seven housekeeping genes (gapA, infB, mdh, pgi, phoE, rpoB, and tonB).[27] K and O antigen serotypes were predicted from the wzi allele and Kaptive (https://github.com/katholt/kaptive).[28] Coding sequences (CDS) were predicted using Prodigal v2.6.3, ensuring completeness of the CDS. tRNA genes were identified with tRNAscan-SE v.1.3.1.[3] A chromosomal circular map for the 6 *K. pneumoniae* strains was generated using BRIG software.[29] Gene function annotation was performed by aligning predicted protein sequences with the COG and KEGG databases (https://www.genome.jp/kegg) using BLAST, selecting the best-matching proteins with ≥40% identity and ≥40% coverage for functional annotation.

Cluster analysis of the isolates was performed by generating a HeatMapDendrogram based on Euclidean distances between genes associated with antibiotic resistance and virulence factors, as calculated by Origin 2021 software. The pathogenicity and resistance profiles of *K. pneumoniae* strains were further explored by comparing amino acid sequences to the Comprehensive Antibiotic Research Database (CARD), Antibiotic Resistance Genes Database (ARDB), and the Virulence Factor Database (VFDB), using BLAST with criteria of identity >80% and e-value <0.00001. Genes were annotated based on the highest alignment scores.[30] Plasmid replicon types were identified with Staramr v.0.7.2 (https://github.com/phac-nml/staramr) and cross-referenced with the PlasmidFinder 2.1 database for confirmation.[31]

Comparative Genomics and Phylogenetic Analysis

A strain isolated from our hospital's NICU labelled KP20 (GenBank id: CP040354), was selected as the reference strain for comparative genomic analysis. KP20 is classified as an ST65-KL2 type *K. pneumoniae* strain, and its complete genome sequence is publicly available from NCBI (http://www.ncbi.nlm.nih.gov). We performed a synteny analysis between the 6 sample strains and KP20 using Mauve 2.3.1 software with default parameters to generate an XMFA file. Then, we visualised the XMFA file using Mauve (window20150226 build10) on a PC. MUMmer software was used to analyse the single nucleotide polymorphisms (SNP) sequence site differences and CDS mutations between the 6 *K. pneumoniae* and KP20 strains, using KP20 as the reference strain;[32] LASTZ software was used to obtain the preliminary results of the InDel and to compare the sequences of all isolates.[33] The pairwise average nucleotide identity (ANI) between isolates was calculated using FastANI software.[34] Additionally, genomic data from nine *K. pneumoniae* strains isolated from different regions of China were downloaded from the NCBI public database for comparative analysis. Phylogenetic tree construction was performed using KSNP3 v3.02 software based on whole-genome SNP, with the maximum likelihood method used to generate the final tree.[35]

Results

Clinical Characteristics and Antibiotic Susceptibility of *K. pneumoniae* Isolates

All 6 samples were isolated from the neurology NICU, with 3 males and 3 females. Four strains were obtained from sputum, one from urine, and one from an air sample collected beside the patient's bed. Of these, P14, P97, P116, and PA9 were hospital-acquired strains. The 6 patients were hospitalized in the NICU, with the majority having underlying conditions such as hypertension, diabetes, and coronary heart disease. Most of these patients had an acute onset of symptoms, severe illness, advanced age, and received surgery or invasive ventilation, catheter insertion and other invasive diagnosis and treatment operations, their own resistance and protection ability are poor, so they are the high-risk group of nosocomial infection (Table 1). Antibiotic susceptibility testing, conducted using MIC against 21 commonly used antibiotics, revealed that all isolates were resistant to ampicillin, ampicillin/sulbactam, ceftriaxone, cefazolin, piperacillin, and cefuroxime. Additionally, the P14 and P97 isolates exhibited resistance to imipenem and meropenem (Table 2).

Table 1 Clinical Characteristics of Patients with *K. pneumoniae* Infection

Isolates	P14	P65	P97	P113	P116	PA9
Gender	Man	Woman	Woman	Woman	Man	Man
Age	94	96	67	69	82	72
Main diagnosis	Cerebral infarction	Cerebral infarction	Cerebral infarction	Cerebral infarction	Cerebral infarction	Intracerebral haemorrhage
Basic disease	Coronary heart disease, Respiratory failure, Atrial fibrillation	Hypertension, Arrhythmia, Diabetes	Hypertension	Hypertension, Coronary heart disease	Hypertension, Diabetes, Liver cirrhosis	Old cerebral infarction, Hypertension, Renal Insufficiency, Arrhythmia
Infection source	Pneumonia, Urinary infection	Pneumonia	Pneumonia, Urinary infection	Pneumonia	Pneumonia	Pneumonia, Urinary infection

(*Continued*)

Table 1 (Continued).

Isolates	**P14**	**P65**	**P97**	**P113**	**P116**	**PA9**
Infection bacteria	*K. pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans*	*K. pneumoniae*	*K. pneumoniae*	*K. pneumoniae*	*K. pneumoniae, Haemophilus influenzae, Acinetobacter baumannii*	*K. pneumoniae, Escherichia coli*
Isolated specimen	Sputum	Urine	Sputum	Sputum	Sputum	Air
Coma	Yes	No	Yes	Yes	Yes	Yes
Operation	Yes	Yes	Yes	No	Yes	No
Invasive ventilation	Yes	No	No	No	No	Yes
Catheteriza-tion	Yes	Yes	Yes	Yes	Yes	Yes
Prognosis	Death	Survival	Survival	Survival	Survival	Death

Table 2 Results of Antibiotic Susceptibility Testing of 6 Strains of *K. pneumoniae*

Isolates	**P14**	**P65**	**P97**	**P113**	**P116**	**PA9**
AMP	R	R	R	R	R	R
SAM	R	R	R	R	R	R
AK	R	I	R	S	I	I
ATM	R	S	R	R	S	I
CIP	R	S	R	S	I	I
CTT	R	I	R	S	I	S
CRO	R	R	R	R	R	R
KZ	R	R	R	R	R	R
F	R	I	R	I	S	S
GM	R	R	R	S	I	S
IPM	R	S	R	S	S	S
LEV	R	R	R	S	R	S
MEM	R	S	R	S	S	S
PRL	R	R	R	R	R	R
CXM	R	R	R	R	R	R
CEF	R	R	R	R	R	R
SXT	S	R	R	S	S	S
CAZ	R	I	R	S	S	S
TOB	R	I	R	S	S	S
TZP	R	S	R	S	S	S
FEP	R	S	R	S	S	S

Notes: MIC break point: AMP (≤8, ≥32); SAM (≤8, ≥32); AK (≤16, ≥64); ATM (≤4, ≥16); CIP (≤0.25, ≥1); CTT (≤16, ≥64); CRO (≤1, ≥4); KZ (≤2, ≥8); F (≤16, ≥128); GM (≤4, ≥16); IPM (≤1, ≥4); LEV (≤0.5, ≥2); MEM (≤1, ≥4); PRL (≤8, ≥32); CXM (≤4, ≥32); CEF (≤4, ≥32); SXT (≤2, ≥4); CAZ (≤4, ≥16); TOB (≤4, ≥16); TZP (≤8, ≥32); FEP (≤2, ≥16).

Abbreviations: R, resistant; I, intermediate resistance; S, sensitive.

Genome Sequence Characterisation and Gene Function Annotation

Genome Characterisation

Sequencing and Assembly of the Genomes of 6 MDR-KP Isolates. The genome sizes ranged from 5.46 Mb to 5.89 Mb, with an average contig length between 841,067 bp and 2,728,214 bp. The GC content was approximately 57.19%, and the median N50 value was 5,343,315 bp. Annotation of the assembled genomes using RAST identified approximately 5252 CDS, 5368 protein-coding genes, and 86 tRNA. Based on computational typing and MLST, five distinct sequence types (STs) were identified among the isolates. The most common ST was ST11 (2/6), which aligns with previous studies identifying ST11 as the predominant KP MLST type in China. Capsular typing revealed that the 6 isolates exhibited distinct capsule serotypes. Among them, KL1 (P113), KL2 (P116), and KL57 (PA9) were considered highly virulent capsule serotypes of *K. pneumoniae*.[36,37] Additionally, five O-antigen loci were identified among the isolates: O1ab (2/6), O13, O2afg, O2a, and O3b (A summary of the genomic characteristics of the 6 *K. pneumoniae* isolates is presented in Table 3, and the circular genome maps of the 6 isolates are shown in Figure 1).

Functional Annotation of Genes

We annotated the genomes of the 6 MDR-KP isolates using the COG and KEGG databases. The COG-based annotation results (Figure 2) revealed that the number of functionally annotated genes was similar among the 6 isolates. Isolate P65 contained the highest number of genes (5435), while isolate P116 had the lowest (5092). The functional categories with the largest number of annotated genes were G-carbohydrate transport and metabolism, E-Amino acid transport and metabolism, and K-transcription. Additionally, genes related to mobile genetic elements, such as prophages and transposons, as well as those associated with cellular structures and defence mechanisms, were identified. These genes were categorized into 12 subcategories: RNA processing (AJ, 5.75%), energy (C, 5.46%), cellular processes (DN, 2.64%), metabolism (EFGHIPQ, 42.66%), DNA processing (KL, 12.16%), cell envelope (M, 5.75%), protein processing (O, 3.85%), regulation and cell signalling (T, 4.51%), membrane transport (U, 1.68%), stress responses, defence, and virulence (V, 2.60%), cell structure (WZ, 0.80%), and miscellaneous (RSX, 12.12%).

The KEGG annotation classification results (Figure 3) showed that the number of genes annotated across various functional pathways was generally similar among the 6 MDR-KP isolates. Isolate P65 had the highest number of annotated genes (6983), while isolate P14 had the lowest (6739). The pathways with the largest number of annotated genes were related to metabolism, particularly the Global map, as well as Carbohydrate metabolism, Amino acid metabolism, and Energy metabolism—key pathways essential for bacterial growth and reproduction. Specifically, the pathway with the most annotated genes was Metabolic pathways (ko01100) under the Global map category. Other pathways included ABC transporters (ko02010) in the Membrane transport category, the Two-component system (ko02020) under Signal transduction, and Quorum sensing (ko02024) within Cellular community-prokaryotes. In addition to basic metabolic pathways, the annotation results also covered viral information processing pathways, as well as pathways associated with human diseases, including infectious diseases, antibiotic resistance, and immune-related disorders.

Table 3 General Genomic Characteristics of *K. pneumoniae* Isolates

Isolates	P14	P65	P97	P113	P116	PA9
Genome size (bp)	5,466,136	5,643,257	5,887,469	5,711,232	5,547,887	5,456,429
Number of base (bp)	1,353,552,626	1,228,166,446	1,827,652,553	1,631,323,962	1,605,971,068	1,449,741,687
GC content (%)	57.41	57.07	56.99	57.13	57.25	57.31
ST	ST11	ST753	ST11	ST23	ST25	ST412
Capsule type	KL47	KL3	KL64	KL1	KL2	KL57
O type	O13	O2afg	O2a	O1ab	O1ab	O3b
Average contig length	1,366,534	1,128,651	841,067	1,903,744	1,386,971	2,728,214
N50	5,378,800	5,257,351	5,458,110	5,412,656	5,288,443	5,264,527
CDS	5170	5274	5607	5260	5120	5033
tRNA	85	87	87	86	87	86
Protein coding gene	5292	5398	5731	5382	5243	5159
Coverage (%)	95.68	95.41	98.91	98.24	98.21	95.80
Q>20(%)	99.06	99.06	98.34	98.20	98.20	99.08

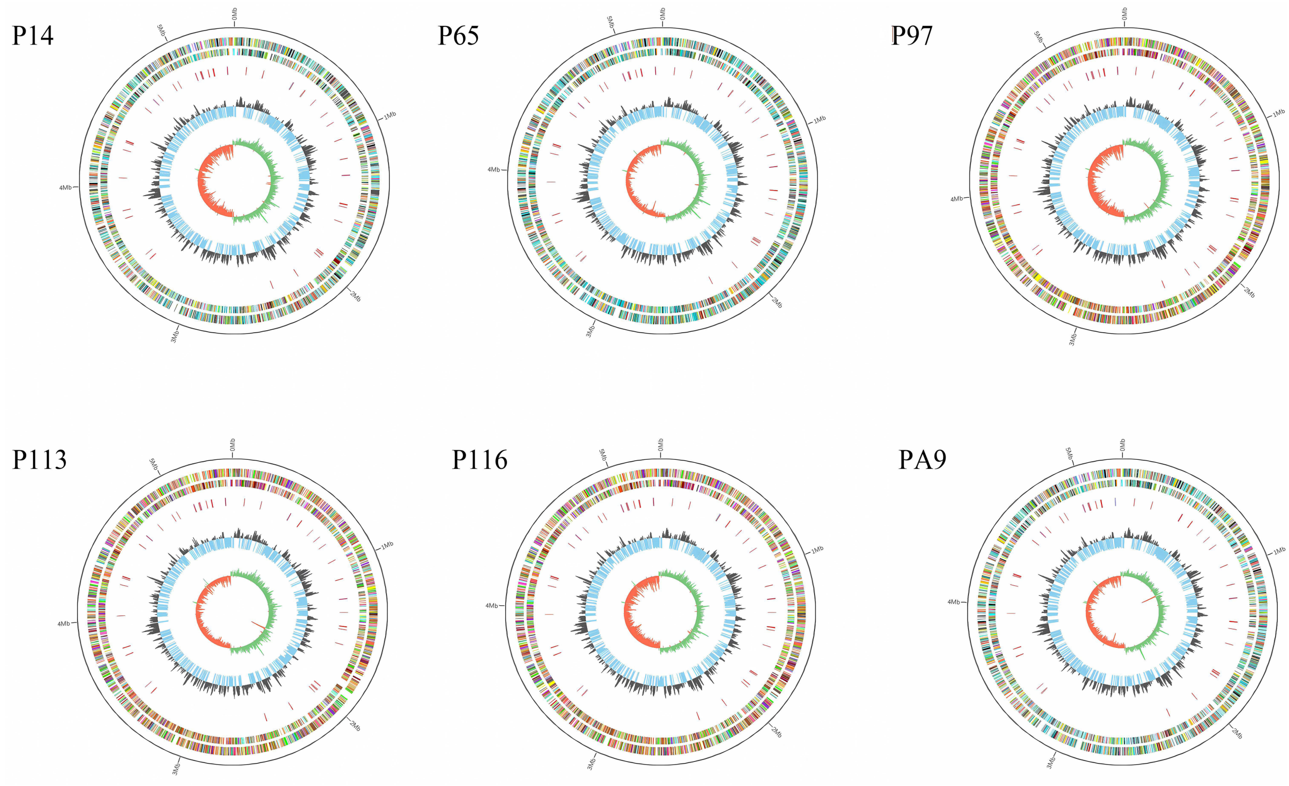

Figure 1 Genome sequence circle diagram of 6 *K. pneumoniae* isolates.

Resistance and Virulence Gene Profiles

We clustered related genes such as AMR and virulence factors. It can be observed from Figure 4 that the six MDR-KP isolates were clustered into two non-random groups. AMR had 6 subgroups, and virulence factors had five subgroups. The distribution of resistance genes in the two groups was relatively even, with P97 and P14 being CRKP strains and MLST typed as ST11, the dominant clone type in China. From the figure, it is obvious that the group of virulence factors P14, P65 and PA9 is significantly more than the other group, and the P14 strain contains all the virulence factors annotated.

Antibiotic Resistance Genes

Using WGS, we identified 32 resistance genes in the six MDR-KP isolates. The detailed distribution is as follows:

1. Among 13 identified β-lactam resistance genes, the six isolates showed a scattered distribution. Many isolates carried extended-spectrum β-lactamase (ESBL) resistance genes such as $bla_{CTX\text{-}M\text{-}65}$, $bla_{TEM\text{-}1}$, $bla_{SHV\text{-}1}$, $bla_{SHV\text{-}11}$, etc. Notably, isolate P97 carried the highest number of β-lactam resistance genes, including the carbapenemase gene $bla_{KPC\text{-}2}$, which aligns with its antibiotic susceptibility results.
2. Among the 10 aminoglycoside resistance genes detected, acrD was found in all six isolates (100.00%), followed by AAC (6')-Isa in four isolates (66.67%) and adeR in three isolates (50.00%).
3. Three quinolone resistance genes were identified, with five isolates carrying both oqxA and oqxB (83.33%) and three isolates carrying QnrS1 (50.00%).
4. There were four resistance genes to folate pathway antagonists (sulfonamides, trimethoprim), only three isolates carried this gene, P97, and P65 carried two genes, and P116 carried only one sul2 gene.
5. All six isolates carried the chloramphenicol resistance gene cfrA (100.00%), and three isolates were found to carry the tetracycline resistance gene tet(A) (50.00%).

https://doi.org/10.2147/IDR.S528440

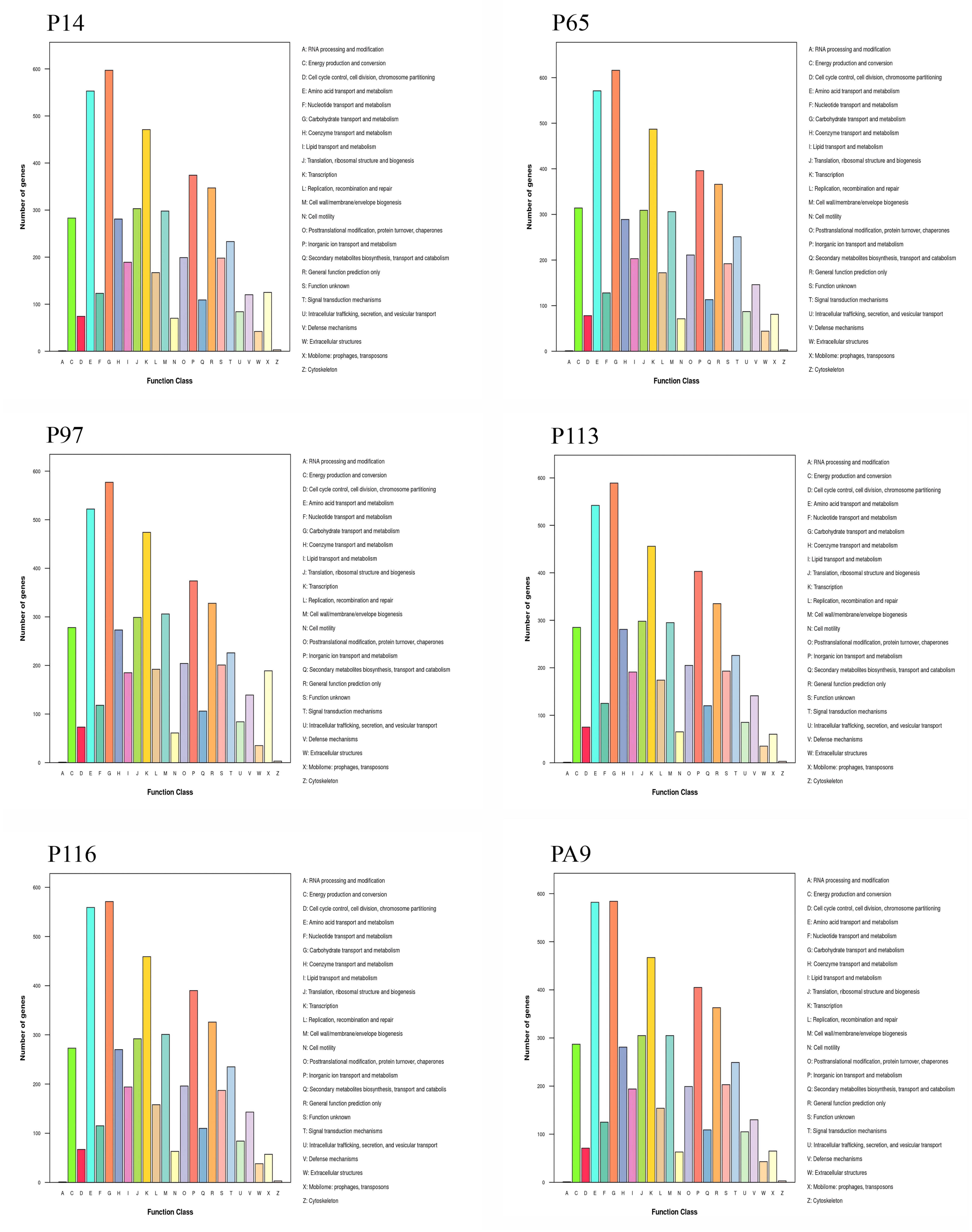

Figure 2 COG functional classification map of the 6 *K. pneumoniae* genomes of NICU strains.

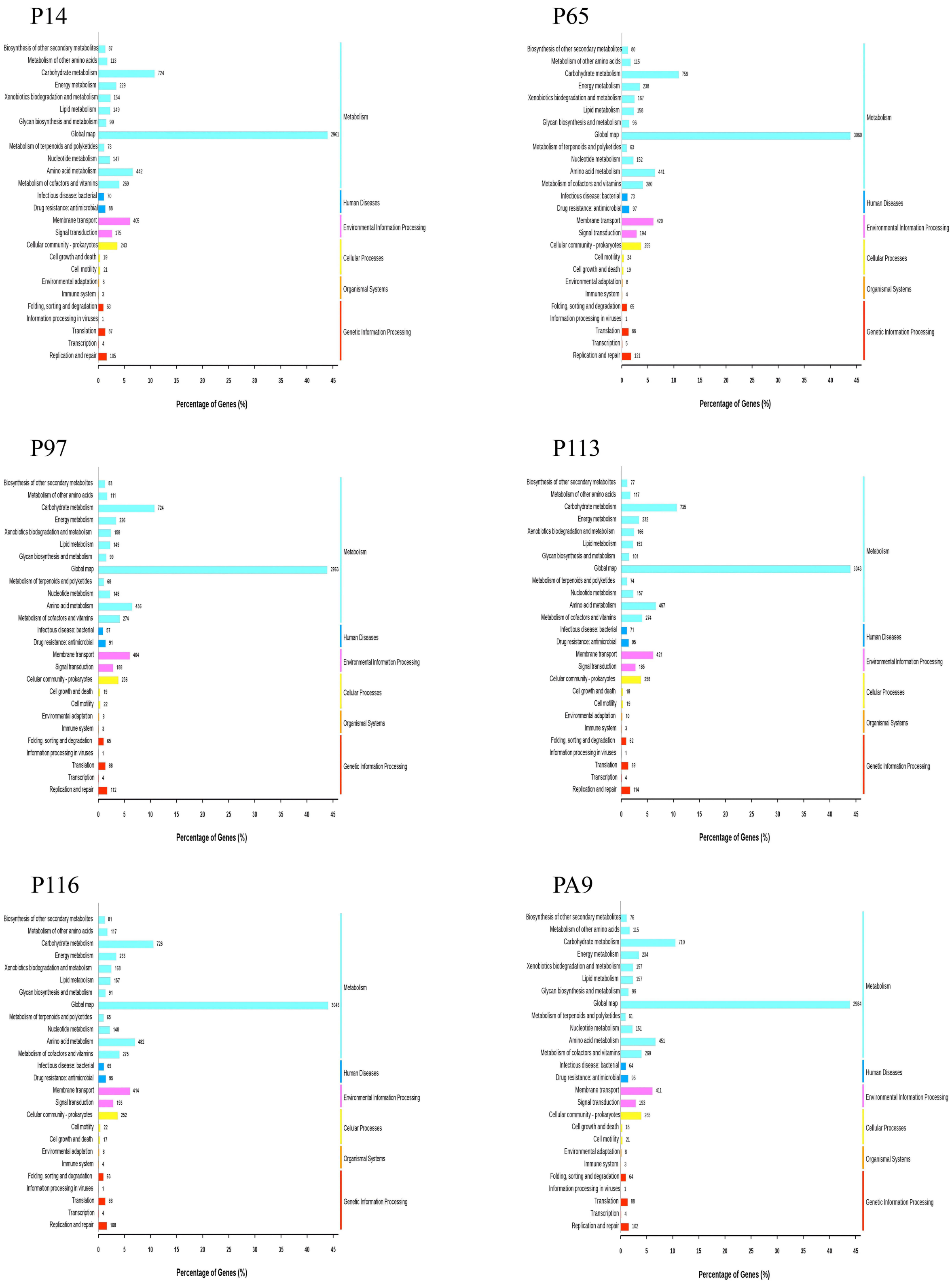

Figure 3 KEGG pathway classification map of the 6 *K. pneumoniae* genomes of NICU strains.

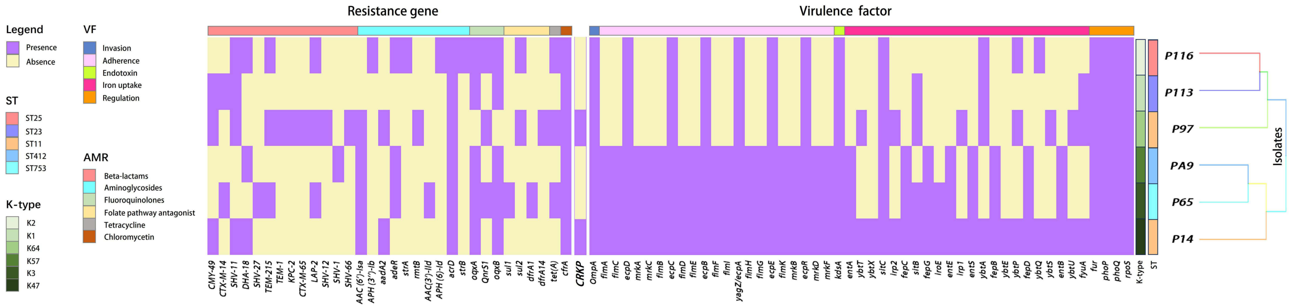

Figure 4 Heat map of drug resistance genes and virulence factors characteristics of 6 *K. pneumoniae* isolates in NICU.

In addition, our study examined point mutations associated with antibiotic resistance in the isolates. Notable mutations were identified in the OmpK35, OmpK36, GyrA, and parC genes. Mutations in the OmpK genes are known to impact β-lactam resistance in non-carbapenemase-producing strains, whereas mutations in parC and GyrA are linked to resistance to fluoroquinolones[6] (These findings are summarized in Table 4 and Figure 4).

Table 4 Resistance Genes of the 6 *K. pneumoniae* Isolates

Isolates	Antibiotics	Genes
P14*[a,b,c]	β-lactams	CMY-49, SHV-11
	Aminoglycosides	AAC(6') – Isa, acrD, aadA2
	Fluoroquinolones	oqxA, oqxB
	Folate pathway antagonist	—
	Tetracycline, chloramphenicol	cfrA
P65	β-lactams	CTX-M-14, DHA-18, SHV-27, TEM-215, LAP-2
	Aminoglycosides	AAC(6') – Isa, adeR, acrD, AAC (3') -IId
	Fluoroquinolones	oqxA, oqxB, QnrS1
	Folate pathway antagonist	sul1, dfrA1
	Tetracycline, chloramphenicol	tet (A), cfrA
P97*[a,b,c]	β-lactams	CMY-49, SHV-11, TEM-215, KPC-2, SHV-66, TEM-1, CTX-M-65, LAP-2, SHV-12
	Aminoglycosides	AAC(6') – Isa, rmtB, acrD, aadA2
	Fluoroquinolones	QnrS1
	Folate pathway antagonist	sul2, dfrA14
	Tetracycline, chloramphenicol	tet (A), cfrA
P113	β-lactams	CMY-49, SHV-11, CTX-M-14
	Aminoglycosides	acrD
	Fluoroquinolones	oqxA, oqxB
	Folate pathway antagonist	—
	Tetracycline, chloramphenicol	cfrA
P116	β-lactams	SHV-11, DHA-18, TEM-215, LAP-2
	Aminoglycosides	adeR, APH (3'')-Ib, APH (6)-Id, acrD, strA, strB
	Fluoroquinolones	oqxA, oqxB, QnrS1
	Folate pathway antagonist	sul2
	Tetracycline, chloramphenicol	tet (A), cfrA
PA9	β-lactams	DHA-18, SHV-1
	Aminoglycosides	AAC(6') – Isa, adeR, acrD
	Fluoroquinolones	oqxA, oqxB
	Folate pathway antagonist	—
	Tetracycline, chloramphenicol	cfrA

Notes: *Resistant to imipenem and meropenem; [a]OmpK35 gene deletion; [b]OmpK36 insertion inactivation; [c]GyrA, parC point mutations; OmpK36 porin was found in all *K. pneumoniae* isolates. —: negative.

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Virulence Genes

The genomic sequencing results of the six MDR-KP isolates were compared against the VFDB database, identifying 49 virulence genes grouped into five major categories: adhesion (21 genes, 42.9%), iron absorption (22 genes, 44.9%), regulation (4 genes, 8.2%), endotoxin (1 gene, 2.0%), and invasion (1 gene, 2.0%). The annotated virulence genes in *K. pneumoniae* are detailed as follows:

1. Adhesion virulence genes: Adhesion genes ecpB/C/D/E/R and yagZ/ecpA were detected in all six isolates (100.00%). Additionally, fimA/B/C/D/E/F/G/H/I/K and mrkA/B/C/D/F were identified in three isolates (50.00%).
2. Iron absorption virulence genes: The sitC gene was detected in all six isolates (100.00%), while sitB and ybtA were found in four isolates (66.67%). Three isolates (50.00%) carried genes including entA/B/E/S, fepB/C/D/G, fyuA, iroE, and ybtP. Furthermore, irp1, irp2, and ybtQ/S/T/U were present in two isolates (33.33%), and ybtE and ybtX were identified in one isolate (16.67%).
3. Regulation virulence genes: Regulatory genes fur, phoP, phoQ, and rpoS were detected in all six isolates (100.00%).
4. Endotoxin and invasion virulence genes: The endotoxin gene kdsA and the invasion gene OmpA were identified in all six isolates (100.00%) (Figure 4 and Supplementary Table 1).

The findings of this study highlight that in the whole genomes of *K. pneumoniae* strains isolated from the NICU of our hospital's Neurology Department, adhesion-related virulence factors include *E. coli* pilus ECP (ecpB/C/D/E/R, yagZ/ecpA), type 1 pili (fimA/B/C/D/E/F/G/H/I/K), and type 3 pili (mrkA/B/C/D/F). Iron absorption-related virulence factors include enterobactin (entA/B/E/S, fepB/C/D/G), salmonella (iroE), yersiniabactin (fyuA, irp1, irp2, ybtA/E/P/Q/S/T/U/X), and other iron transport proteins (sitB, sitC). Regulation virulence factors include fur, PhoPQ, and RpoS. Endotoxin and invasion-related virulence factors include LOS (lipooligosaccharide) and OmpA.

Plasmid Replicon Typing

Plasmid replicon typing revealed five types of plasmid replicons in the 6 MDR-KP isolates: IncFIB(K), IncFII(K), Col440II, ColRNAI, and repB_KLEB (Supplementary Table 1).

Comparative Genomic Analysis

To investigate the genetic variations of *K. pneumoniae* isolated from the NICU of our hospital's Neurology Department in recent years, a comparative genomic analysis was performed using KP20 as the reference genome. KP20 (GenBank accession number: CP040354) is an ST65-KL2 *K. pneumoniae* strain, originally isolated from the sputum of a patient with cerebral infarction in 2015 in our NICU. The ST65 clone type and KL2 serotype are widely recognized as highly virulent phenotypes.[38]

Genome Collinearity Analysis

Using Mauve software, collinearity analysis was performed on the whole genomes of the reference strain KP20 and six MDR-KP sequencing strains. The results showed that the overall sequences of all strains were relatively complete, and the sequence structures between genomes were also basically consistent. Regions with similar genome arrangement, namely Local Collinear Blocks (LCBs), are marked with coloured blocks in the figure. In addition, there are genome rearrangement events also such as inversion, translocation, and deletion within gene sequence segments similar to the strains (Figure 5).

SNP and InDel Analysis

SNP and InDel analyses were performed by mapping the sequencing reads of the 6 MDR-KP isolates to the KP20 reference genome. Compared with KP20, we found a very rich variety of SNP polymorphisms in 6 strains of *K. pneumoniae*. Specifically, 25,616 to 36,552 nucleotide variations were observed across the 6 strains relative to KP20. Approximately 85.7% of SNPs were located within CDS regions, of which 47.2% were synonymous mutations and 51.8% were nonsynonymous mutations. Among the strains, P116 exhibited the fewest variations and had the highest similarity to KP20 compared to the other five strains. The InDel mutations in the 6 *K. pneumoniae* strains are mostly located in non-

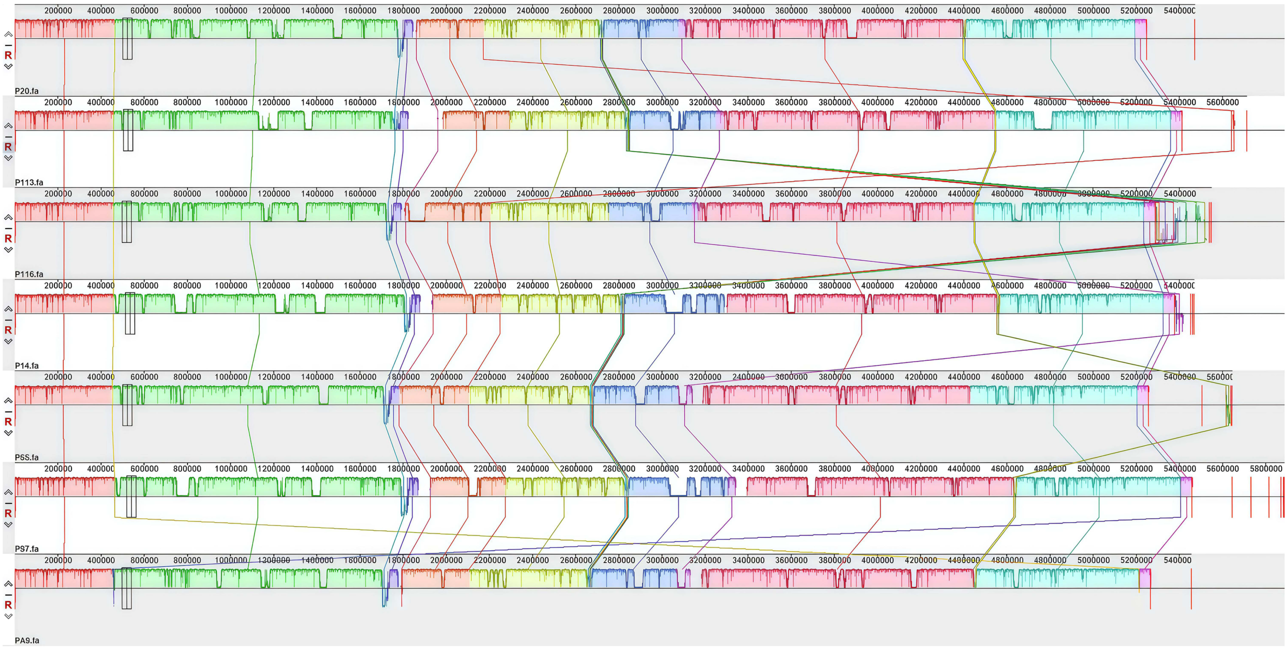

Figure 5 Global alignment of the genomes of 6 MDR-KP strains with the reference strain KP20.

coding regions outside of CDS, which may affect the regulation of gene expression (such as promoters, 5' and 3' untranslated regions, and other regulatory elements). However, functionally significant mutations, such as nonsynonymous SNPs and InDel mutations located in CDS regions, may lead to alterations in the genetic traits of the strains (Tables 5 and 6).

Table 5 *K. pneumoniae* SNP with KP20 as the Reference Sequence

Isolates	CDS								Intergenic	Total
	Start_Syn	Stop_Syn	Start_nonsyn	Stop_nonsyn	Premature_stop	Synonymous	Nonsynonymous	CDS_total		
P14	0	42	248	432	362	13,669	14,352	29,105	4991	34,096
P65	0	39	263	422	366	14,778	14,856	30,724	4882	35,606
P97	0	45	253	427	368	13,693	14,304	29,090	5276	34,366
P113	0	50	269	455	410	14,401	15,835	31,420	5102	36,522
P116	0	31	195	330	284	10,614	11,223	22,121	3495	25,616
PA9	0	32	231	413	361	13,864	14,624	29,525	4895	34,420

Abbreviations: Start_Syn, Start codon synonymous mutation; Stop_Syn, Stop codon synonymous mutation; Start_nonsyn, Start codon non-synonymous mutation; Stop_nonsyn, Stop codon non-synonymous mutation; Premature_stop, nonsense mutation; Synonymous, synonymous in coding region; Nonsynonymous, non-synonymous in coding region; Intergenic, SNPs in noncoding region.

Table 6 Detection of the Number of Polymorphism Sites in the *K. pneumoniae* InDel Gene Using KP20 as the Reference Sequence

Isolates	Insertion				Deletion				Total
	I_gene_start	I_gene_middle	I_gene_stop	I_total	D_gene_start	D_gene_middle	D_gene_stop	D_total	
P14	0	0	0	1	0	1	0	4	5
P65	0	0	0	1	0	2	0	6	7
P97	0	0	0	1	0	1	0	4	5
P113	0	0	0	4	0	0	0	13	17
P116	0	0	0	2	0	2	0	5	7
PA9	0	1	0	6	0	6	0	19	25

Abbreviations: I_gene_start, the insertion mutations located at start codon; I_gene_middle, the insertion mutations located at the middle of CDS; I_gene_stop, the insertion mutations located at stop codon; D_gene_start, the deletion mutations located at start codon; D_gene_middle, the deletion mutations located at the middle of CDS; D_gene_stop, the deletion mutations located at stop codon.

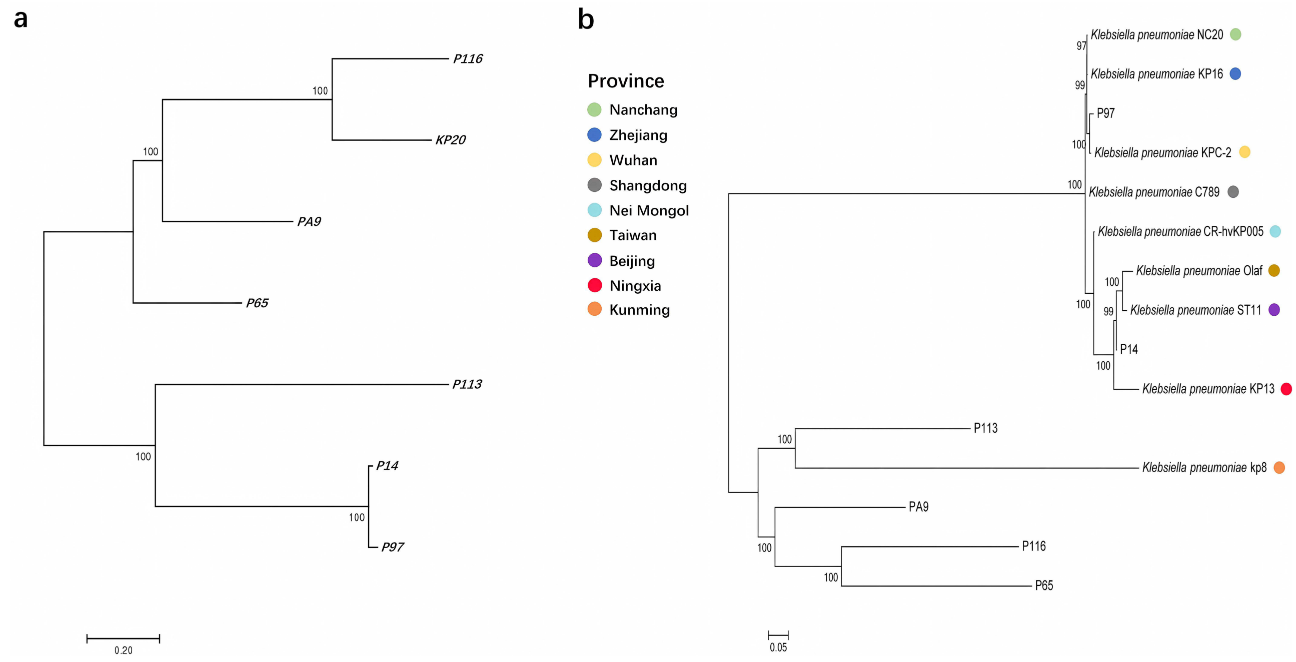

Figure 6 Phylogenetic trees (**a** and **b**) of *K. pneumoniae* strains.

Phylogenetic Tree

The phylogenetic tree was constructed among *K. pneumoniae* strains from NICU-infected patients, and the results of SNP analysis are Figure 6a. The results showed that P116, KP20, PA9, and P65 were on the same evolutionary branch, and P113, P14, and P97 were on the same evolutionary branch. P14 showed the highest similarity to P97, while P116 was relatively close to the reference strain KP20. FastANI analysis yielded genetic similarity among all the isolates, with P14 having the highest 99.9% similarity to P97, followed by P116 was 99.2% similar to KP20 (ANI threshold ≥96%) in agreement with the phylogenetic analysis.

The phylogenetic tree was constructed between 6 *K. pneumoniae* strains from NICU and 9 *K. pneumoniae* strains from different regions analysed by the NCBI registry, and the results are shown in Figure 6b. The results showed that the four *K. pneumoniae* strains (P113, PA9, P116, P65) isolated from our institution were on the same large evolutionary branch as kp8 isolated from Kunming, China, and P113 was relatively close to kp8. On the other large evolutionary branch, P97 had the highest similarity to KPC-2 isolated from Wuhan, China, while P14 was on the same sub-branch as ST11 isolated from Beijing and Olaf from Taiwan, China.

Discussion

The 2024 CHINET China Antimicrobial Surveillance Network report (https://www.chinets.com) indicated that, of 458,271 clinical isolates collected from participating hospitals, approximately 65.7%–73.0% were Gram-negative bacteria. Among these, *K. pneumoniae* represented 13.9% of the isolates, ranking as the second most common clinical isolate. The resistance rates of *K. pneumoniae* to imipenem and meropenem increased significantly from 3.0% and 2.9% in 2005 to 22.6% and 23.4% in 2024, respectively, an over seven-fold increase. Despite a recent slight decline, the resistance trend remains a serious concern. Moreover, as one of the major ESKAPE pathogens causing infections in neurocritical care patients, *K. pneumoniae* can not only colonise the respiratory, gastrointestinal and urinary systems of patients for a long period, but also spread the infection through the ward environment, which makes the clinical treatment more difficult, and seriously affects the healing process and the quality of life, especially for immunodeficient patients.[39–41] Furthermore, in an Egyptian study on the One Health pathogen,[42] *K. pneumoniae* was found to be present not only in the human healthcare system but also in a wide range of poultry, animals, living environments and food. This not only raises concerns about the risk of transmitting drug resistance between humans and animals but also represents a serious test of traditional pathogen detection methods.[43,44] Bacterial molecular typing has become increasingly vital for the identification, classification, and tracing of pathogens. With the decreasing time and cost of WGS, this technology offers a novel approach to monitoring outbreaks, transmission pathways, and genomic characteristics of MDR-KP clinical isolates.[19,45] In this study, leveraging WGS, we investigated key issues regarding the resistance to commonly used antibiotics, genomic features, resistance genes, and virulence factors of MDR-KP strains isolated from the NICU at Kailuan General Hospital in Tangshan, China.

To gain a comprehensive understanding of the genomic characteristics of MDR-KP, we selected 6 MDR-KP strains for sequencing and comparative genomics analysis from 156 non-repetitive *K. pneumoniae* isolates obtained from the NICU of our hospital. The identification of various sequence types (STs) and capsular types (K-types) revealed significant genetic diversity among these clinical isolates (Table 3). Among them, ST11 (33.3%, 2/6) was the predominant type, which is also the most common clone of CRKP in hospitals in China.[39,46] Notably, the ST11 strains we isolated (P14, P97) were both CRKP. It has been reported that ST11, along with the globally prevalent ST258, belongs to the clonal complex CG258, and ST11 is a single-site variant of ST258 (housekeeping gene tonB).[47] There is a very close relationship between the two, and studies have shown that both ST11 and ST258 strains carry the integrative conjugative element ICEKp258.1 on their chromosomes, which can harbour the type IV secretion system and facilitate the spread of mobile genetic elements such as plasmids, genomic islands, and bacteriophages, thereby contributing to the global dissemination of CG258, including ST11.[48] ST11 *K. pneumoniae* is one of the key opportunistic pathogens causing hospital-acquired infections.[49] Research has shown that ST11 CRKP infections predominantly occur in critically ill patients with multiple underlying diseases, immunocompromised conditions, and those who have undergone invasive procedures, particularly in ICU and NICU, and can colonize anywhere it can reach, such as blood and surfaces of invasive medical devices.[50] This suggests that infection control departments should focus on this clonal type of bacteria, and in the future, in addition to the daily monitoring of clinical isolates, it should also carry out regular screening of storage environments that may have the risk of colonisation, and eliminate the source of infection immediately when it is found to cut off the transmission pathway, to reduce the risk of nosocomial infection and transmission of CRKP. Furthermore, we also identified ST23, ST25, ST412, as well as the ST753 clone strain that has not been reported in other regions of China, indicating the genetic polymorphism of clinical *K. pneumoniae* isolates in our city, these bacteria have been confirmed to carry both antibiotic resistance and virulence genes, and there is potential for rapid transmission between different clones.[51–53] Despite the limited number of cases, the intensive care unit has all the favourable conditions for high virulence and convergence of carbapenem resistance, so the urgent need we must take immediate control measures to prevent its spread in clinical environments. Capsules are a crucial virulence factor of *K. pneumoniae*. In our hospital, we identified 6 K-types in *K. pneumoniae* isolates: KL47, KL3, KL64, KL1, KL2, and KL57. KL1 and KL2 are considered the most common serotypes of hvKP in the traditional sense,[54] but in recent years, virulence genes have been transferred from KL1 and KL2 to other clones, leading to the diversity of the hvKP lineage, especially KL64 and KL47, which have become the most prevalent capsule types in China.[51,55] The main K-types of ST11 CRKP are KL47 and KL64,[47] which is consistent with the results of this study. Studies have shown that ST11-KL64 CRKP can acquire a pK2044-like virulence plasmid from ST23-KL1, leading to the presence of a 2698 bp sequence deletion in all ST11-KL64 strains. This acquisition enhances the pathogenicity and survival rate of ST11-KL64, making it a carbapenem-resistant hypervirulent *K. pneumoniae* (CR-hvKP).[56] Notably, it has been reported that ST11-KL64 hv-CRKP (CRKP acquiring hypervirulence) is gradually replacing the ST11-KL47 subtype and shows stronger pathogenic potential.[57,58] Compared to other CR-hvKP (hvKP acquiring carbapenem resistance) strains, ST11-KL64 hv-CRKP is more adaptable in the hospital environment and has become the predominant hv-CRKP type in the nosocomial setting.[59] Therefore, especially for critically ill patients, timely detection of changes in resistance and virulence of this strain is warranted. In addition, regarding the *K. pneumoniae* serotype KL57, research has found that its phenotype is mostly ST412. There are reports that ST412-KL57 *K. pneumoniae* is closely related to liver abscess due to its special capsule acetylation, and it can also acquire virulence and resistance plasmids through horizontal gene transfer (HGT) and other methods, posing a huge threat to clinical practice.[52,60] The evolutionary convergence of high virulence and carbapenem resistance in *K. pneumoniae*, as well as its global spread, has raised alarm bells for public health safety, warranting serious attention.

In this study, the WGS analysis of resistance genes revealed that the 6 MDR-KP strains isolated from our hospital's NICU carry 32 antibiotic resistance genes, which confer a multidrug-resistant phenotype to *K. pneumoniae*. Moreover, we found a strong correlation between the predicted AMR based on resistance gene analysis and the results from antibiotic susceptibility testing (Figure 4, Tables 2 and 4). Notably, among the 6 MDR-KP strains, P14 and P97 were identified as CRKP strains (resistant to imipenem and meropenem). However, the carbapenemase gene bla_{KPC-2} was only detected in P97, while other major carbapenem resistance genes, such as $bla_{NDM,}$[61] $bla_{IMP,}$[62] $bla_{VIM,}$[63] and $bla_{OXA,}$[64] were not detected in either P14 or P97. The resistance to carbapenems in these strains may be associated with the deletion or mutation of the outer membrane protein genes OmpK35 and OmpK36 in P97 and P14.[65,66] Studies have shown that outer membrane proteins interact with β-lactamase genes and are potential risk factors for carbapenem resistance in *K. pneumoniae*.[67] Reports from Tunisia also suggest that mutations in the outer membrane proteins OmpK35 and OmpK36 in strains expressing ESBL or AmpC-type β-lactamases contribute to carbapenem resistance. Sequencing of the corresponding outer membrane protein genes revealed that amino acid insertions or deletions, along with mutations in the promoter region, are key factors driving resistance.[68] Similarly, Lee et al[69] in a study of 54 strains that did not produce the carbapenemase CRKP found expression of the AmpC-type β-lactamase gene, bla_{DHA}, as well as the other non-carbapenemase β-lactamase genes, bla_{TEM}, bla_{SHV}, bla_{CTX-M}, and bla_{OXA-1}, in 46.3% of the pore-protein-deficient strains. In addition, a variety of other antibiotic resistance genes were detected, including β-lactam resistance genes (SHV, TEM, CTX-M, etc)., aminoglycoside resistance genes (AAC (6')-Isa, acrD, aadA2, etc)., fluoroquinolone resistance genes (oqxA, oqxB, etc)., sulfonamide resistance genes (sul1, sul2), trimethoprim resistance genes (dfrA1, dfrA14), as well as tetracycline and chloramphenicol resistance genes (tet(A), cfrA). All the strains carried β-lactam, aminoglycoside, and fluoroquinolone resistance genes, which may lead to the failure of clinical treatment of a variety of antibiotics, and the carrying of ESBLs resistance gene SHV is the main reason for *K. pneumoniae's* natural resistance to ampicillin.[70] In addition, although WGS detected folate pathway antagonists, tetracycline, and chloramphenicol resistance genes, it did not carry out drug sensitivity tests on them, which could challenge the correct use of clinical antibiotics. Therefore, clinical laboratories need to establish more comprehensive, unified and perfect detection standards in order to find more effective anti-infection treatment methods. Regarding virulence factors, we identified 49 genes related to adhesion, iron absorption, regulation, endotoxin and invasion (Figure 4 and Supplementary Table 1). We found that three of the 6 *K. pneumoniae* (P14, P65, PA9) harbored genes encoding type I pili (fimA/B/C/D/E/F/G/H/I/K), type III pili (mrkA/B/C/D/F), enterobactin (entA/B/E/S, fepB/C/D/G), and salmonella (iroE), with P14 also carrying all detectable yersiniabactin genes (ybtA/E/P/Q/S/T/U/X, fyuA, irp1/2). Showed that P14 has both stronger resistance and virulence compared to the other strains, indicating variability in pathogenicity among the 6 *K. pneumoniae* strains. Type I pili is an important factor leading to the interaction between *K. pneumoniae* and host cells. It participates in the formation of intracellular biological communities and is the premise of further bacterial infection; The expression of type III pili can promote biofilm formation and help bacteria build defence barriers.[71,72] Virulence factors of iron absorption genes include enterobactin, salmonella, yersiniabactin and aerobactin (not detected). Studies have found that iron is an essential element for *K. pneumoniae* to survive and reproduce, but during infection, *K. pneumoniae* cannot directly acquire iron from the host, which needs to be obtained by secreting iron carriers.[73] Enterobactin has a high affinity for iron and promotes bacterial growth by binding iron in plasma transferrin,[74] while yersiniabactin activates the outer membrane protein fyuA under iron-depleted conditions to facilitate biofilm formation in *K. pneumoniae*.[75] In addition, genes encoding *E. coli* pili, regulatory, endotoxin, and invasive virulence factors were detected in all strains, which further enhanced *K. pneumoniae* adhesion to host cells and immune regulation associated with inflammatory responses, complement activation inhibition, and phagocytosis, among others.[76] The host inflammatory response resulting from MDR-KP infection is mainly characterised by a biphasic disorder of early hyperinflammation and late immunosuppression. Clinical studies have shown that serum levels of pro-inflammatory factors such as TNF-α, IL-6 and IL-1β in patients with MDR-KP bloodstream infection are significantly elevated in the early stage of infection, but this elevation is often accompanied by an abnormal up-regulation of the anti-inflammatory factor IL-10, which leads to a rapid shift of inflammatory response to an inhibitory state, a dysregulation that is closely associated with poor patient prognosis.[77,78] It is worth noting that the dynamic changes of inflammatory markers in patients with MDR-KP infection are significantly different from those with sensitive strain infection. Procalcitonin (PCT), as an important biomarker of bacterial infection, often shows a sustained high level or slow decline in MDR-KP infection, reflecting the delay in pathogen clearance.[79] Studies have shown that the PCT decline rate of patients with MDR-KP infection is significantly slower than that of patients with sensitive

K. pneumoniae infection even after receiving appropriate antibiotic treatment, which is consistent with the characteristics of MDR-KP resistance to multiple antibiotics.[80] However, the interplay of mechanisms such as podoplanes (such as hindering the deposition of complement C3b and other components on the surface of bacteria and scavenging ROS), LPS modification and biofilm synergistic escape as well as active regulation of MDR-KP to inhibit the formation of the host microenvironment can enable MDR-KP to effectively evade host immunosurveillance and clearance, which is the key factor for its strong pathogenicity and difficult treatment.[81–83] However, no other classical virulence factor genes (such as clb, iuc, rmpA, and rmpA2) except ybt and iro were found in all isolates. These genes are involved in the regulation of iron siderophore and high mucus phenotype, which are the signs of high virulence strains.[84] The latest study reported a rare case of ST14 type MDR-hvKP. Although this strain lacks the high mucus phenotype regulating gene rmpA/A2, it still presents high mucus characteristics and carries the siderophore genes iroE, iroN (salmochelin) and iutA (aerobactin).[85] Coincidentally, Indian scholars have also recently found a clinical isolate, which shows significantly high mucus viscosity in the absence of known mucus-regulating factors (rmpA and rmpD) and does not carry the siderophore genes (aerobactin and salmochelin).[86] Therefore, although the 6 MDR-KP strains did not carry typical virulence determinants. However, the misgivings that isolates without any high virulence marker genes exhibit a high virulence phenotype should attract our sufficient attention.

Through comparative genomic analysis of MDR-KP, this study elucidated the genetic structural characteristics and evolutionary relationships among six MDR-KP strains. The collinearity map (Figure 5) demonstrated that the overall genome structure of all strains was highly conserved, with varying degrees of homologous DNA sharing between strains. However, local genomic rearrangements, such as inversions and translocations, were observed along their independent evolutionary trajectories, potentially attributable to insertions of mobile genetic elements or genome recombination under adaptive selective pressures.[87] Studies indicate that SNP are DNA sequence polymorphisms caused by single nucleotide variations at the genomic level, and their nonsynonymous mutation in the CDS region is the main reason for the change in bacterial biological characteristics.[88] InDel refers to insertions or deletions of nucleotide fragments of varying sizes at the same genomic site between different individuals, which is the phenomenon of gap generated by homologous sequence alignment.[89] SNP analysis identified substantial nucleotide disparities between the six MDR-KP strains and KP20, likely resulting from adaptive mutations under antibiotic pressure or community-acquired genetic divergence. Notably, SNP within coding regions accounted for approximately 85.7% of total nucleotide variations, with a higher proportion of nonsynonymous mutations than synonymous mutations, highlighting the role of SNP in modulating gene function and altering bacterial phenotypes. In contrast to SNP, InDel mutations in the six MDR-KP strains predominantly occurred in non-coding regions, which may affect gene expression regulation. Notably, strain P116 exhibited the lowest SNP count (25,616) compared to the other five MDR-KP strains, while P14 and P97 showed nearly identical CDS mutation numbers (differing by only 15). Phylogenetic tree analysis (Figure 6a) clustered the six MDR-KP strains and KP20 into two major branches. Its results further show that P116 was genetically closest to KP20, while P14 was most closely related to P97. FastANI analysis further supported these findings, showing a 99.2% genetic similarity between P116 and KP20 and a 99.9% similarity between P14 and P97, suggesting a potential evolutionary or direct transmission relationship between these strains.[3,90] Furthermore, in comparison with *K. pneumoniae* isolated from different regions in China, it was found (Figure 6b) that P14 is in the same branch as ST11 (SAMN10527392) isolated from Beijing, while P97 showed high similarity to KPC-2 from Wuhan (SAMN19312573). This means that the hospital transmission of *K. pneumoniae* strains may be promoted through patient referral, external training and learning by healthcare workers, and the same ST type between strains.[48,91] These findings emphasize the necessity of establishing comprehensive molecular epidemiological monitoring to elucidate the origins of interspecies evolution.

Conclusion

In conclusion, the MDR-KP strains isolated from our hospital's NICU exhibit resistance to multiple antibiotics, which is closely associated with the generation of resistance genes and the carriage of virulence factors. WGS revealed that two of the 6 MDR-KP strains are CRKP strains, and their ST11-KL47 and ST11-KL64 phenotypes are the mainstream types of CRKP in China, suggesting that ST11-type *K. pneumoniae* should be a key focus for infection prevention and control in our hospital. Furthermore, NICU is easy to cause long-term colonization and infection outbreaks of MDR-KP due to the particularity of its patients, so it is extremely necessary to develop scientific disinfection and sterilization and sterilization methods, rapid epidemiological investigation, and strict and standardized antibiotic use system. Secondly, the functional annotation and comparative genomic analysis of the strains revealed, and the structural and functional differences, as well as genetic variability of *K. pneumoniae*, were revealed, providing a molecular basis for further research on the functional metabolism and genetic evolution trajectory of MDR-KP. WGS is an effective strategy for monitoring hospital-acquired infections, and our results emphasize the importance of implementing WGS in the NICU to prevent MDR-KP outbreaks. In addition, although we provided convincing clinical and WGS data, this study is a single-centre investigation with limited sample size and public data, lacking comprehensive epidemiological studies. At the same time further multi-centre, integrative research is needed for a more reliable analysis.

Data Sharing Statement

The datasets used and/or analyzed during the present study are available from the corresponding author upon reasonable request.

Ethics Approval and Consent to Participate

This study was reviewed and approved by the Ethics Committee of Kailuan General Hospital, Tangshan City, Hebei Province, China (Protocol No. 2020018). By the World Medical Association (Declaration of Helsinki), all patients were informed of the study's purpose, and a signed consent form was obtained before sample collection. Our single-center study used only one ethics committee and the content and procedures were based on the single-center research. This study was a retrospective analysis of laboratory sequencing data only, with no personal privacy or commercial interests involved. All clinical samples were part of routine hospital laboratory procedures with no additional burden on patients. Meanwhile, strict confidentiality was maintained for all patient information, and no intervention was performed on patients. During data review, patients' clinical samples will be identified by research identification numbers rather than names. Any information that could identify patients will not be disclosed to members outside the research team. During the publication of the paper, either the patient or the guardian reviewed the manuscript and consented to the publication of their information.

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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

All authors report no conflicts of interest in this work.

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