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# Comprehensive genomic epidemiology and antimicrobial resistance profiles of clinical *Klebsiella pneumoniae* species complex isolates from a tertiary hospital in Wenzhou, China (2019–2021)

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## Abstract

**Background** The main issue of the *Klebsiella pneumoniae* species complex (KpSC) research in clinical settings is the accurate identification and differentiation of the closely related species within this complex. Moreover, the emergence and spread of carbapenem-resistant *K. pneumoniae* (CRKP) represent a significant public health threat due to limited treatment options and high mortality rates. Understanding the genetic basis of resistance and virulence is crucial for developing effective infection control strategies. In this work, the genomic epidemiology and antimicrobial resistance profile of KpSC isolates from Wenzhou, China, was investigated to fully understand the implications of the KpSC in clinical settings.

**Methods** We conducted a comprehensive analysis of 156 clinical KpSC isolates collected from a tertiary hospital in China over a three-year period (2019–2021). Antimicrobial susceptibility testing was performed according to CLSI standards. Whole-genome sequencing (WGS) and subsequent bioinformatic analyses were conducted to identify resistance genes, plasmid types, and virulence factors. Phylogenetic relationships were determined using maximum-likelihood analysis.

**Results** All CRKP isolates exhibited high levels of resistance to carbapenems, cephalosporins, and aminoglycosides. The most prevalent carbapenemase genes were $bla_{KPC-2}$ (100%), with significant associations between $bla_{KPC-2}$ and ST11. Phylogenetic analysis revealed considerable genetic diversity, with over 50 sequence types (STs) present. A subset of isolates harbored both resistance and hypervirulence genes, including *rmpA*, *rmpA2*, and siderophore systems, which were associated with potential higher pathogenesis.

**Conclusion** This study provides novel insights into the molecular epidemiology of KpSC in Wenzhou, China, highlighting the coexistence of antimicrobial resistance and virulence factors in clinical isolates. The findings

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underscore the importance of continuous genomic surveillance and targeted therapeutic strategies to combat KpSC infections. Our research fills critical gaps in the current understanding of KpSC epidemiology in China and offers valuable data for global comparative studies, contributing to the development of effective infection control measures. Genomic surveillance in China thus provides crucial insights for local risk mapping and informs necessary adaptions for implementation of control strategies.

**Keywords** *Klebsiella pneumoniae* species complex, Whole-genome sequencing, Antimicrobial resistance, Virulence factors, Phylogenetic analysis, China

## Introduction

*Klebsiella pneumoniae*, a prominent member of the *Enterobacteriaceae* family, has emerged as a critical nosocomial pathogen due to its capability to acquire resistance mechanisms, including carbapenem resistance, making infections difficult to treat [1]. However, species within the *Klebsiella pneumoniae* species complex (KpSC) are genetically very similar, which can lead to frequent misidentification in clinical laboratories that rely on traditional biochemical methods or even some molecular techniques [2]. Accurate species identification is crucial for understanding the epidemiology and pathogenicity of these organisms. The global increase in antimicrobial resistance (AMR) poses a significant threat to public health, particularly the rise of carbapenem-resistant *Enterobacteriaceae* (CRE) [3]. The spread of carbapenem-resistant *K. pneumoniae* (CRKP) is alarming as it leads to limited therapeutic options and higher mortality rates [4].

The rapid dissemination of CRKP is facilitated by various genetic elements, such as plasmids and transposons, which harbor antimicrobial resistance genes [5]. Whole-genome sequencing (WGS) has become an invaluable tool in understanding the genetic basis of resistance and tracking the epidemiology of CRKP outbreaks [6]. However, comprehensive studies involving large-scale genomic and phenotypic analyses of clinical KpSC isolates in China remain scarce. This gap in knowledge hampers the development of effective intervention strategies and limits our understanding of the local and global dissemination patterns of these highly resistant strains [7].

Understanding the resistance mechanisms and genetic diversity of *Klebsiella* isolates is crucial for developing effective infection control strategies and guiding empirical antibiotic therapy [8]. In this study, we analyzed 156 clinical KpSC isolates collected from a tertiary hospital in Wenzhou, China, over a three-year period (2019–2021). The isolates underwent antimicrobial susceptibility testing and whole-genome sequencing to determine their resistance profiles and genetic characteristics. This dataset represents one of a large collection of KpSC isolates from a single Chinese healthcare institution, providing a unique opportunity to study the evolution and spread of resistance in this high-risk setting. The findings will enhance our understanding of the genetic factors driving resistance and virulence in CRKP, offering valuable data for the development of targeted antimicrobial therapies and prevention measures [9]. Our study integrates phenotypic and genotypic data to offer a comprehensive view of KpSC in a Chinese tertiary hospital. Whole-genome sequencing and subsequent bioinformatic analyses were performed to identify resistance genes, mobile genetic elements, and clonal relationships among the isolates. By comparing our findings with existing public data, we aim to elucidate the local dynamics of KpSC transmission and resistance evolution, and to identify potential targets for therapeutic intervention.

## Materials and methods

### Bacterial isolates and identification

A total of 156 KpSC isolates were collected from clinical specimens in a tertiary hospital in Wenzhou, China, between January 2019 and December 2021. The species was identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/MS) (Bruker, Bremen, and Germany) and further confirmed by 16 S rRNA gene sequencing [10].

### Antimicrobial susceptibility test

The minimum inhibitory concentrations (MICs) of all strains were determined by in vitro antimicrobial susceptibility tests (ASTs). For aztreonam, imipenem, meropenem, ceftriaxone, cefotaxime, ceftazidime, levofloxacin, ciprofloxacin, amikacin, gentamicin, piperacillin/tazobactam, fosfomycin, chloramphenicol, cotrimoxazole, amoxicillin/clavulanate, cefepime, and florfenicol, the AST results were obtained using agar dilution method and in accordance with the standards of CLSI (https://clsi.org/). While the MICs of colistin and tigecycline were determined using the broth dilution method and interpreted according to EUCAST (http://www.eucast.org/) clinical breakpoints. *Escherichia coli* ATCC 25,922 and *Pseudomonas aeruginosa* ATCC 27,853 were used as controls.

### Whole genome sequencing and bioinformatic analysis

Total DNA of the 156 strains was generated using Gentra Puregene Yeast/Bact. Kit (Qiagen, Germany) following the manufacturer's protocol. Then the DNA

was sequenced using Illumina Novaseq 6000 (Illumina, San Diego, CA, USA) platform. The complete genomic sequences were assembled via Spades and annotated by Prokka [11]. The cleaned reads were assembled de novo using SPAdes [12]. The draft genomes were annotated using the Prokka pipeline, which predicts coding sequences, rRNAs, tRNAs, and other features [11]. The presence of antimicrobial resistance genes was determined using the ResFinder database [13], and plasmid replicon types were identified using the PlasmidFinder tool [14]. The multilocus sequence typing (MLST) analysis and detection of virulence genes were performed under the Institute Pasteur (http://bigsdb.web.pasteur.fr/klebsiella/klebsiella.html). The genetic environment surr ounding $bla_{KPC-2}$ genes was visualized with Easyfig 2.2.5 [15]. The presence of virulence factors was assessed using the Virulence Factor Database (VFDB) [16].

## Phylogenetic analysis

Core genome single-nucleotide polymorphism (cgSNP) analysis was performed using Snippy with *K. pneumoniae* HS11286 (CP003200.1), *K. variicola* LEMB11 (CP045783.1), and *K. quasipneumoniae* KqPF26 (CP065838.1) as the reference. The putative repetitive regions, mobile genetic elements (MGEs), and recombination events were filtered with Gubbins [17]. The maximum likelihood tree based on the core genome was constructed using FastTree and visualized by iTOL. Pairwise cgSNP differences between strains were calculated using snp-dists v0.4. Strains differing by ≤ eighteen cgSNPs were defined as a clonal group (CG) [18].

## Results

### Precise classification of 156 KpSC strains through Whole-Genome sequencing

Whole-genome sequencing (WGS) was performed on all 156 KpSC strains to achieve precise species identification and phylogenetic classification. The strains were initially identified as KpSC based on biochemical and phenotypic characteristics. However, WGS allowed for a more accurate taxonomic classification (Figs. 1 and 2). The WGS data revealed out of the 135 strains identified as *K. pneumoniae*, 7 strains initially identified as *K. variicola*, and 14 strains classified as *K. quasipneumoniae*.

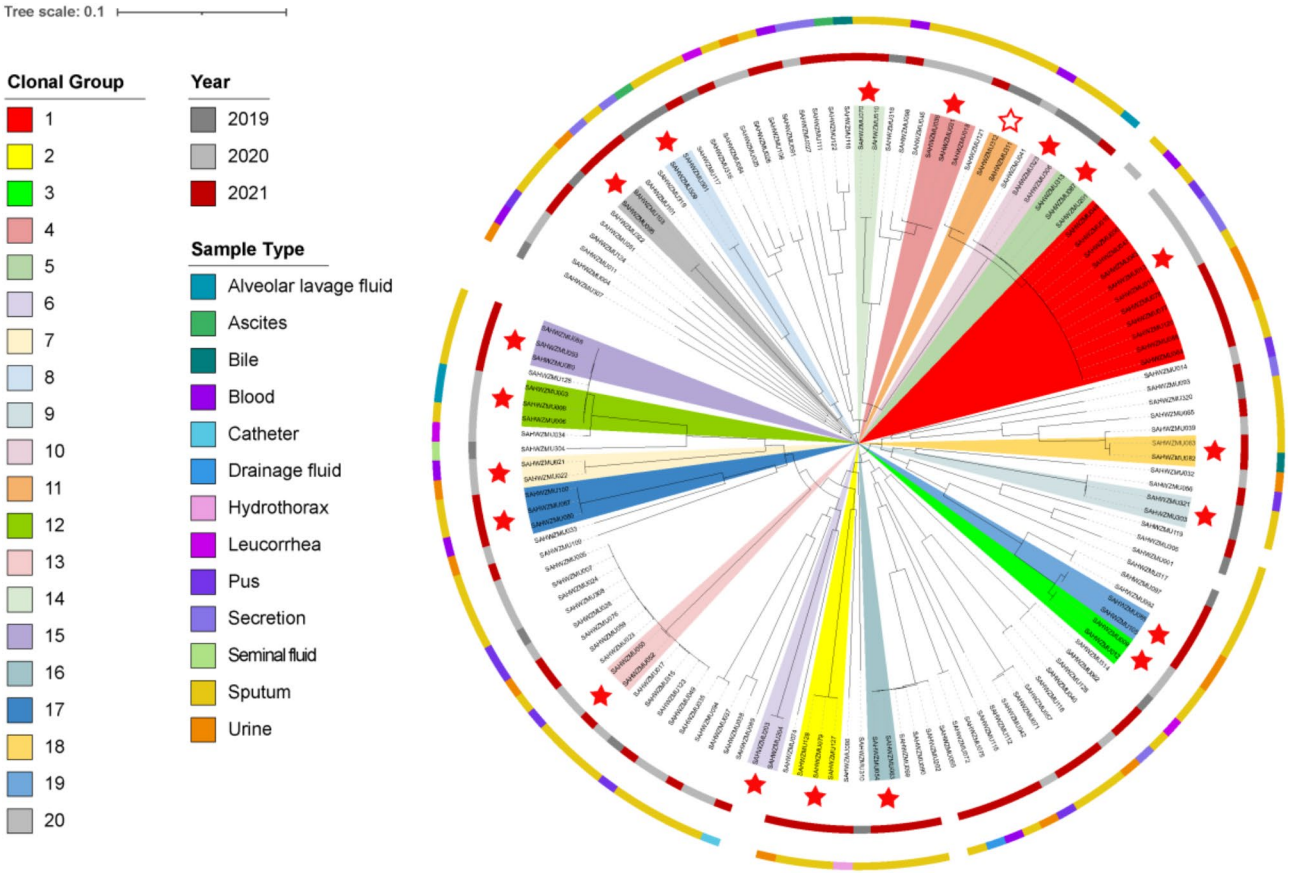


**Fig. 1** The cgSNP analysis and CG distribution of *K. pneumoniae* Isolates. Maximum likelihood phylogenetic tree of 135 *K. pneumoniae* strains based on the core genome. The different colors in branches indicate the clonal groups. Closely related genotypes (< 16 SNPs) are shaded in the same node, and clusters are numbered consecutively (1–20). Colorful rings denote the isolation years and sources. Solid stars represent the clonal transmission between different departments, and hollow stars represent the transmission within the same department

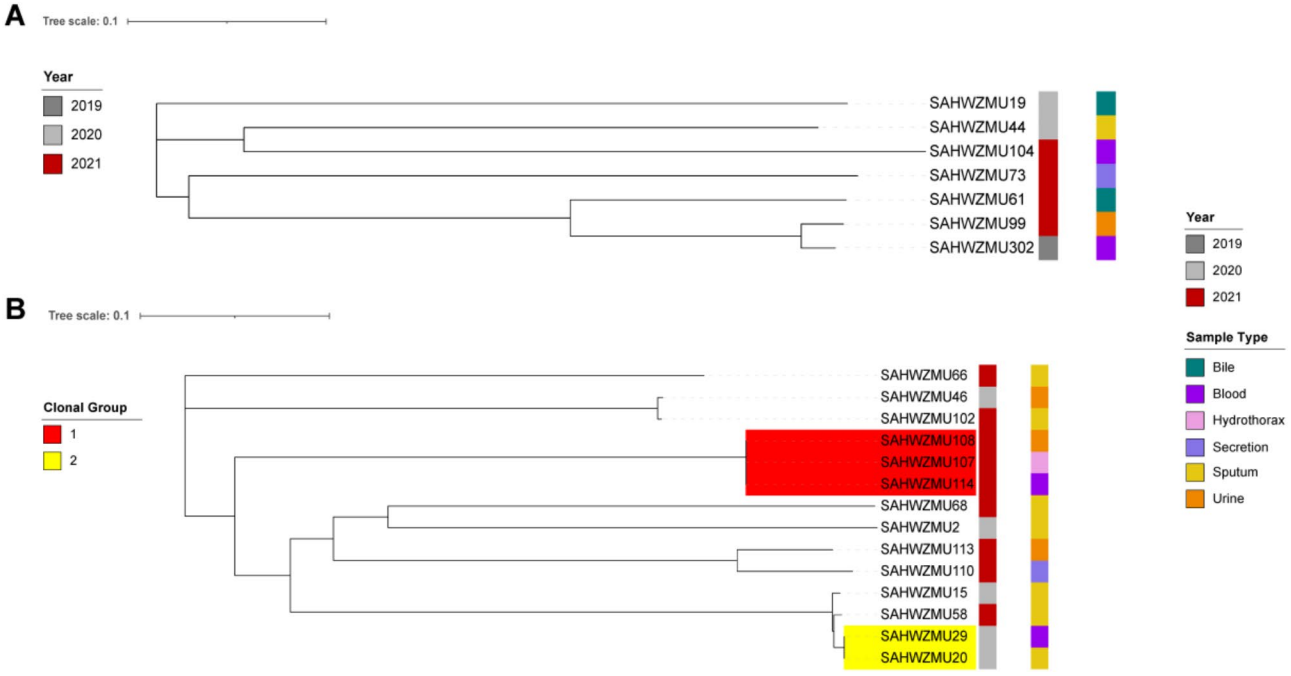


**Fig. 2** Maximum-likelihood phylogenetic tree of *K. variicola* and *K. quasipneumoniae* isolates

**Table 1** Statistical results of drug susceptibility of 135 *K. pneumoniae* strains

| Antimicrobials | Susceptible No./Percent (%) | Intermediate No./Percent (%) | Resistant No./Percent (%) |
|---|---|---|---|
| Aztreonam | 50/37.04 | 3/2.22 | 82/60.74 |
| Imipenem | 109/80.74 | 1/0.74 | 25/18.52 |
| Meropenem | 110/81.48 | 0/0.00 | 25/18.52 |
| Ceftriaxone | 80/59.26 | 4/2.96 | 51/37.77 |
| Cefotaxime | 80/59.26 | 3/2.22 | 52/38.52 |
| Ceftazidime | 85/62.96 | 3/2.22 | 47/34.81 |
| Levofloxacin | 87/64.44 | 14/10.37 | 34/25.19 |
| Ciprofloxacin | 77/57.04 | 6/4.44 | 52/38.52 |
| Amikacin | 118/87.41 | 0/0.00 | 17/12.59 |
| Gentamicin | 105/77.78 | 0/0.00 | 30/22.22 |
| Piperacillin/Tazobactam | 106/78.52 | 1/0.74 | 28/20.74 |
| Fosfomycin | 115/85.19 | 1/0.74 | 19/14.07 |
| Chloramphenicol | 93/68.89 | 0/0.00 | 42/31.11 |
| Cotrimoxazole | 95/70.37 | / | 40/29.63 |
| Amoxicillin/Clavulanate | 73/54.07 | 28/20.74 | 34/25.19 |
| Cefepime | 86/63.70 | 16/11.85 | 33/24.44 |
| Florfenicol | 41/30.37 | 71/52.59 | 23/17.04 |
| Colistin | / | 132/97.78 | 3/2.22 |
| Tigecycline | 135/100.00 | 0/0.00 | 0/0.00 |

### Core Genome SNPs (cgSNPs) Analysis of *K. pneumoniae* Isolates

The core genome SNPs (cgSNPs) analysis was performed on 135 *K. pneumoniae* strains, and a maximum likelihood phylogenetic tree was constructed based on the core genome SNPs. The phylogenetic tree (Fig. 1) revealed a clear separation of the strains into multiple clonal groups (CGs). Each branch in the tree was color-coded to indicate different clonal groups. A total of 20 clonal groups were identified, with closely related genotypes (< 10 SNPs) grouped together and shaded within the same node. The presence of both solid and hollow stars on the tree indicates clonal transmission events. Solid stars represent clonal transmission between different departments, while hollow stars denote transmission within the same department. This suggests both inter-departmental and intra-departmental spread of specific *K. pneumoniae* strains. Certain clonal groups demonstrated significant clonal expansion and were isolated from multiple sources and years, indicating persistent and widespread distribution of these isolates. The cgSNP analysis highlighted the genetic diversity among the *K. pneumoniae* strains, with some clusters showing high genetic similarity, suggesting recent common ancestry or clonal expansion, whereas others were more genetically diverse, indicating longer evolutionary distances between them.

### Antimicrobial Susceptibility of *K. pneumoniae* Isolates

The antimicrobial susceptibility of 135 *K. pneumoniae* strains was tested against various antibiotics, and the results are summarized in Table 1. The highest susceptibility was observed for amikacin, with 87.41% (118/135) of the strains being susceptible and none showing intermediate resistance. Fosfomycin and imipenem followed, with susceptibility rates of 85.19% (115/135) and 80.74% (109/135) respectively. High susceptibility to amikacin

and fosfomycin despite prevalent aminoglycoside resistance genes, suggesting potential gene silencing or compensatory mechanisms (Fig. 3). Tigecycline showed complete susceptibility (100%), indicating it as the most effective antibiotic tested. Complete tigecycline susceptibility consistent with the absence of detected tet(X) variants. Conversely, aztreonam showed the lowest susceptibility rate, with only 37.04% (50/135) of the strains being susceptible and a significant 60.74% (82/135) being resistant. Critical cephalosporin resistance strongly correlates with $bla_{\text{CTX-M}}$ and $bla_{\text{SHV}}$ carriage, highlighting the need for combined diagnostics. Similarly, resistance rates were notably high for cefotaxime (38.52%) and ciprofloxacin (38.52%).

#### Antimicrobial Susceptibility of *K. variicola* Isolates

The antimicrobial susceptibility results for seven *K. variicola* strains are detailed in Table 2. This species showed high susceptibility across almost all antibiotics tested. Notably, all strains (100%) were susceptible to aztreonam, imipenem, meropenem, ceftriaxone, cefotaxime, ceftazidime, amikacin, gentamicin, piperacillin/tazobactam, and fosfomycin. However, slight intermediate resistance was observed for levofloxacin (14.29%) and ciprofloxacin (14.29%). Additionally, one strain (14.29%) showed resistance to florfenicol, while susceptibility to tigecycline was slightly lower at 85.71% (6/7).

#### Antimicrobial Susceptibility of *K. quasipneumoniae* Isolates

The antimicrobial susceptibility of 14 *K. quasipneumoniae* strains is presented in Table 3. The strains exhibited complete susceptibility (100%) to imipenem, meropenem, amikacin, fosfomycin, chloramphenicol, and tigecycline. High susceptibility rates were also observed for ceftazidime (78.57%), levofloxacin (92.86%), and ciprofloxacin (78.57%). However, resistance was noted in certain antibiotics, such as piperacillin/tazobactam, where all strains (100%) were resistant. Additionally, aztreonam, ceftriaxone, and cefotaxime each had a resistance rate of 28.57%. Interestingly, colistin showed a high intermediate resistance rate of 92.86% (13/14) and a low resistance rate (7.14%).

#### Statistical summary of drug susceptibility

The statistical results of the drug susceptibility testing are crucial for understanding the resistance patterns and guiding effective treatment strategies for infections caused by KpSC isolates. The detailed susceptibility percentages for each antimicrobial agent across the three KpSC species studied indicate a varied response, highlighting the necessity for precise antibiotic stewardship.

#### CgMLST analysis of 156 KpSC isolates

The core genome multilocus sequence typing (cgMLST) analysis was performed on 156 KpSC strains to elucidate their genetic relationships (Fig. 4). The clustering illustrates the presence of clonal groups among the 156 *Klebsiella* strains, suggesting possible clonal expansion or recent common ancestry. The MLST demonstrates the genetic diversity of the *Klebsiella* strains, with over 50 distinct cgMLST types observed. Certain cgMLST types showed a higher prevalence, indicating that some clonal types, such as ST11, ST23, and ST15, are more common within the studied population.

#### Genomic features and resistance genes

Whole-genome sequencing and subsequent analysis revealed a diverse set of resistance genes among the KPC-carrying isolates (Fig. 3). These genes confer resistance to multiple classes of antibiotics, including beta-lactams, aminoglycosides, fluoroquinolones, tetracyclines, and sulfonamides. A significant proportion of the strains harbored beta-lactamase genes, including $bla_{\text{CTX-M}}$ (48/156, 30.8%), $bla_{\text{SHV}}$ (125/156, 80.1%), $bla_{\text{TEM}}$ (31/156, 19.9%), $bla_{\text{OXA}}$ (8/156, 5.1%), and $bla_{\text{KPC}}$ (25/156, 16.0%). Specifically, $bla_{\text{CTX-M-15}}$ (19/156, 12.2%) and $bla_{\text{SHV-27}}$ (5/156, 3.2%) were among the most frequently detected genes, suggesting their critical role in mediating resistance to beta-lactam antibiotics. Moreover, genes such as *aac(6')-Ib*, *aadA1*, *aadA2*, and *aph(3')-Ia* were prevalent, conferring resistance to aminoglycosides. The presence of these genes highlights the challenge in treating infections caused by these strains using aminoglycoside antibiotics. The *oqxAB* operon, conferring resistance to fluoroquinolones, was detected in numerous strains. Additionally, plasmid-mediated quinolone resistance (PMQR) genes like *qnrB19* and *qnrS1* were identified, indicating the potential for horizontal gene transfer and the spread of fluoroquinolone resistance. Tetracycline resistance genes *tet(A)* and *tet(D)* were identified, along with sulfonamide resistance genes *sul1*, *sul2*, and *sul3*. The presence of these genes suggests resistance to tetracyclines and sulfonamides, commonly used antibiotics in clinical settings. The analysis also identified resistance genes for other antibiotic classes, including chloramphenicol (*cmlA1*, *floR*) and fosfomycin (*fosA*, *fosA3*). This broad resistance profile underscores the adaptability of *Klebsiella* strains to various antimicrobial pressures.

#### Analysis of $bla_{\text{KPC-2}}$ Gene Environment in KPC-2 Positive *K. pneumoniae* Strains

The genetic context of the $bla_{\text{KPC-2}}$ gene was analyzed in 25 KPC-2 positive *K. pneumoniae* strains. The $bla_{\text{KPC-2}}$ gene environments were classified into five distinct groups (A-E) based on their surrounding genetic elements. These classifications provide insights into the

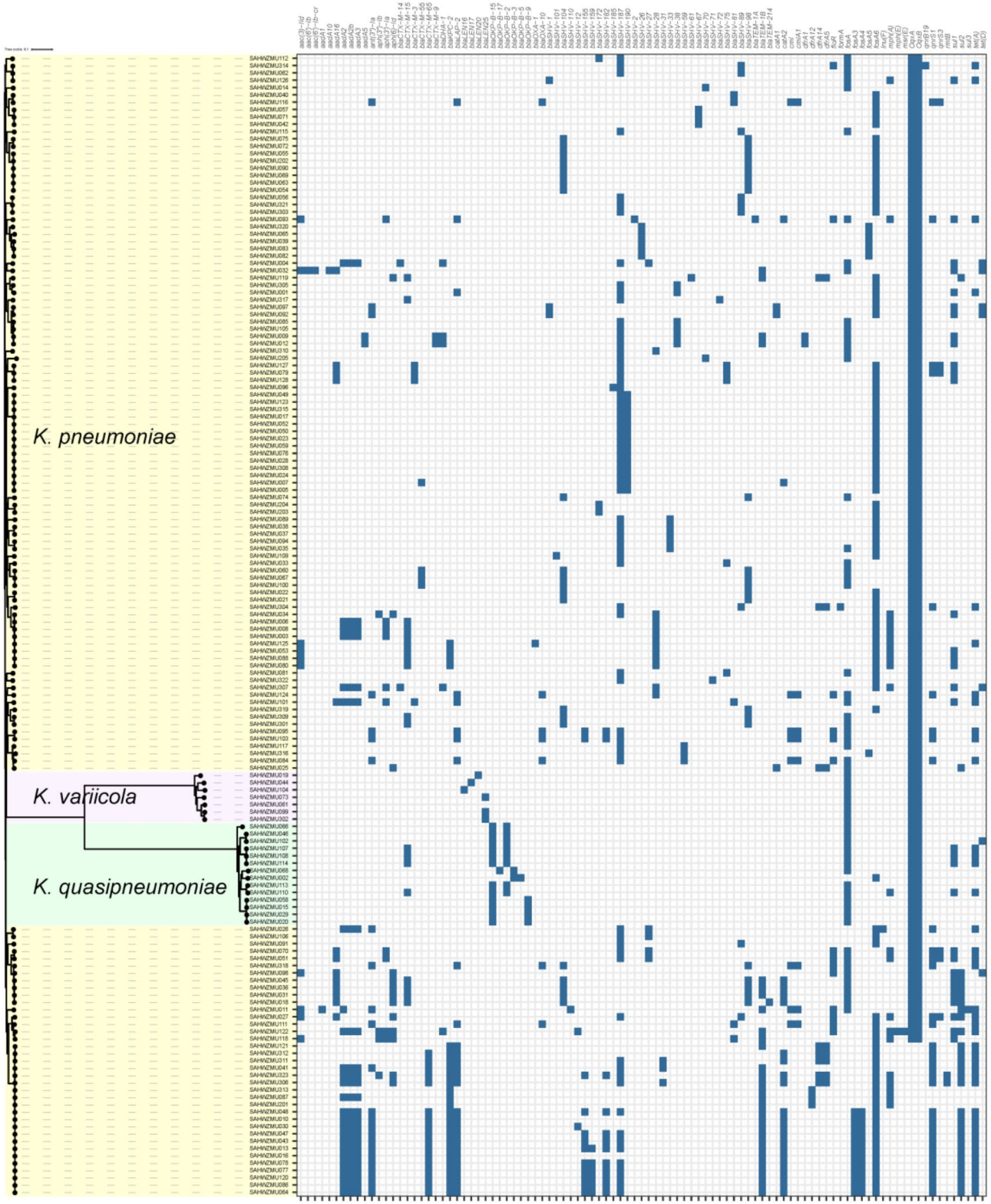


**Fig. 3** Prevalence of ARG Genes. Prevalence of antibiotic resistance genes virulence genes among the 156 KpSC isolates

**Table 2** Statistical results of drug susceptibility of 7 *K. variicola* strains

| Antimicrobials | Susceptible No./Percent (%) | Intermediate No./Percent (%) | Resistant No./Percent (%) |
|---|---|---|---|
| Aztreonam | 7/100.00 | 0/0.00 | 0/0.00 |
| Imipenem | 7/100.00 | 0/0.00 | 0/0.00 |
| Meropenem | 7/100.00 | 0/0.00 | 0/0.00 |
| Ceftriaxone | 7/100.00 | 0/0.00 | 0/0.00 |
| Cefotaxime | 7/100.00 | 0/0.00 | 0/0.00 |
| Ceftazidime | 7/100.00 | 0/0.00 | 0/0.00 |
| Levofloxacin | 6/85.71 | 1/14.29 | 0/0.00 |
| Ciprofloxacin | 6/85.71 | 1/14.29 | 0/0.00 |
| Amikacin | 7/100.00 | 0/0.00 | 0/0.00 |
| Gentamicin | 7/100.00 | 0/0.00 | 0/0.00 |
| Piperacillin/Tazobactam | 7/100.00 | 0/0.00 | 0/0.00 |
| Fosfomycin | 7/100.00 | 0/0.00 | 0/0.00 |
| Chloramphenicol | 6/85.71 | 1/14.29 | 0/0.00 |
| Cotrimoxazole | 7/100.00 | / | 0/0.00 |
| Amoxicillin/Clavulanate | 7/100.00 | 0/0.00 | 0/0.00 |
| Cefepime | 7/100.00 | 0/0.00 | 0/0.00 |
| Florfenicol | 4/57.14 | 2/28.57 | 1/14.29 |
| Colistin | 7/100.00 | / | 0/0.00 |
| Tigecycline | 6/85.71 | 1/14.29 | 0/0.00 |

**Table 3** Statistical results of drug susceptibility of 14 *K. quasipneumoniae* strains

| Antimicrobials | Susceptible No./Percent (%) | Intermediate No./Percent (%) | Resistant No./Percent (%) |
|---|---|---|---|
| Aztreonam | 10/71.43 | 0/0.00 | 4/28.57 |
| Imipenem | 14/100.00 | 0/0.00 | 0/0.00 |
| Meropenem | 14/100.00 | 0/0.00 | 0/0.00 |
| Ceftriaxone | 10/71.43 | 0/0.00 | 4/28.57 |
| Cefotaxime | 10/71.43 | 0/0.00 | 4/28.57 |
| Ceftazidime | 11/78.57 | 2/14.29 | 1/7.14 |
| Levofloxacin | 13/92.86 | 1/7.14 | 0/0.00 |
| Ciprofloxacin | 11/78.57 | 0/0.00 | 3/21.43 |
| Amikacin | 14/100.00 | 0/0.00 | 0/0.00 |
| Gentamicin | 12/85.71 | 0/0.00 | 2/14.29 |
| Piperacillin/Tazobactam | 0/0.00 | 0/0.00 | 14/100.00 |
| Fosfomycin | 14/100.00 | 0/0.00 | 0/0.00 |
| Chloramphenicol | 14/100.00 | 0/0.00 | 0/0.00 |
| Cotrimoxazole | 10/71.43 | - | 4/28.57 |
| Amoxicillin/Clavulanate | 12/85.71 | 2/14.29 | 0/0.00 |
| Cefepime | 10/71.43 | 3/21.43 | 1/7.14 |
| Florfenicol | 9/64.29 | 5/35.71 | 0/0.00 |
| Colistin | - | 13/92.86 | 1/7.14 |
| Tigecycline | 14/100.00 | 0/0.00 | 0/0.00 |

potential mechanisms of gene transfer and stability within the bacterial genome. Class A: 2 isolates (SAHWZMU306, SAHWZMU323), Class B: 15 isolates (SAHWZMU010, SAHWZMU013, SAHWZMU030, SAHWZMU016, SAHWZMU041, SAHWZMU043, SAHWZMU047, SAHWZMU048, SAHWZMU064, SAHWZMU077, SAHWZMU078, SAHWZMU086, SAHWZMU120, SAHWZMU121, SAHWZMU125), Class C: 2 isolates (SAHWZMU311, SAHWZMU312), Class D: 3 isolates (SAHWZMU053, SAHWZMU080, SAHWZMU088), and Class E: 3 isolates (SAHWZMU087, SAHWZMU201, SAHWZMU313). Multiple insertion sequences such as IS*Kpn27*, IS*Ec38*, and IS*26* were identified flanking the $bla_{KPC-2}$ gene, suggesting their role in the mobilization and dissemination of the resistance gene (Fig. 5). Transposons like Tn*3* and Tn*As1* were present, which are known to facilitate the horizontal gene transfer of antibiotic resistance genes [19, 20]. The $bla_{SHV-12}$ gene, another beta-lactamase gene, was also identified in close proximity to $bla_{KPC-2}$ in some isolates, indicating co-localization of multiple resistance genes. The diverse genetic contexts of the $bla_{KPC-2}$ gene indicate multiple mechanisms for its spread, including insertion sequences and transposons. This underscores the complexity of controlling the dissemination of $bla_{KPC-2}$ mediated carbapenem resistance in clinical settings.

### Detection of virulence factors

The analysis of virulence genes (VIR) in 156 KpSC strains was conducted to identify the distribution and prevalence of various virulence factors (Fig. 6). The analysis revealed a diverse array of virulence genes among the *Klebsiella* strains, including genes associated with adhesion, iron acquisition, immune evasion, and secretion systems. Several fimbrial and non-fimbrial adhesion genes were detected, including *fimA* (16/156, 10.3%), *fimH* (152/156, 97.4%), and *mrkD* (141/156, 90.4%). These genes are crucial for the initial attachment of the bacteria to host tissues and the formation of biofilms, which protect the bacteria from the host immune response and antimicrobial treatments [21]. Iron acquisition genes, such as *entC* (131/156, 84.0%), *ybtQ* (54/156, 34.6%), and *iutA* (68/156, 43.6%), were prevalent among the strains. These genes enable the bacteria to obtain iron from the host, which is essential for their growth and pathogenicity [22]. The presence of multiple iron acquisition systems suggests a high potential for these strains to thrive in iron-limited environments within the host. Genes involved in the biosynthesis of the capsule (*wza* (40/156, 25.6%), *wzc* (21/156, 13.5%)) and LPS (*wbbM* (86/156, 55.1%)) were widely distributed among the strains. The capsule and LPS are critical for immune evasion, protecting the bacteria from phagocytosis and complement-mediated killing [23, 24]. The type VI secretion system (T6SS) genes (*tssF* (119/156, 76.3%), *tssG* (112/156, 71.8%)) were identified in several strains. T6SS plays a role in the secretion of virulence factors directly into host cells or competing bacteria, facilitating bacterial survival and virulence [25]. Of note, the *rmpA* and *rmpA2* genes, which are associated with hypermucoviscosity, were found in some

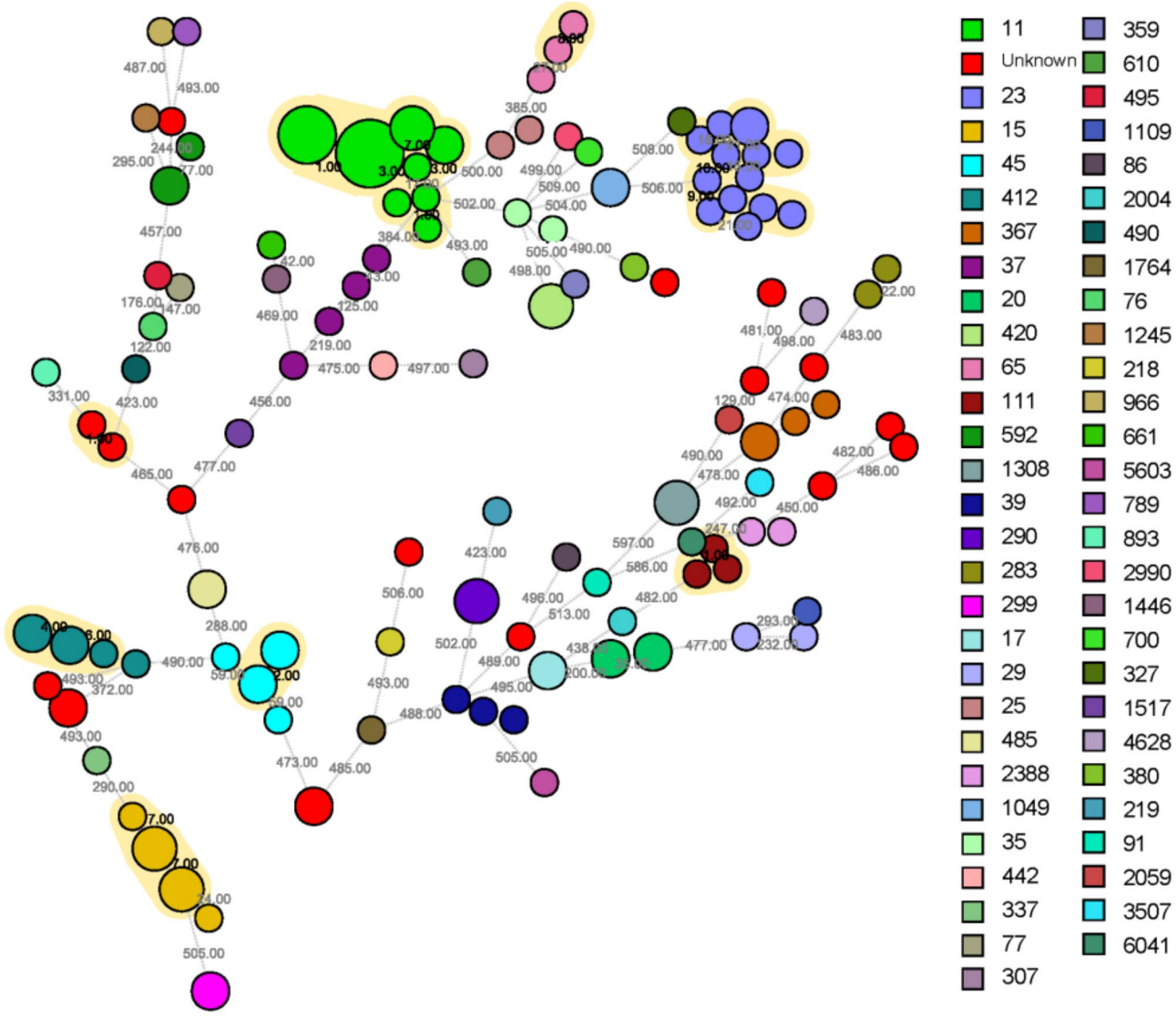


**Fig. 4** Minimum spanning tree constructed based on cgMLST allelic genes of 156 KpSC strains. Each circle depicts an allelic profile based on sequence analysis of cgMLST genes. The length of the connecting lines represents the number of target genes with different alleles, where smaller numbers (< 10) are indicated by values with a darker color. Each circle within the tree represents a cgMLST type, with diameters scaled to the number of isolates belonging to that type. Colors denote different MLST types. Closely related genotypes (< 10 alleles difference) are shaded in the same node

strains. Hypermucoid strains are more virulent and can cause severe infections, including liver abscesses and metastatic complications [26]. In general, the *rmpA* and *rmpA2* genes, were detected in 14% of the isolates. Additionally, siderophore systems, including aerobactin, salmochelin, and yersiniabactin, were present in 27%, 19%, and 22% of the isolates, respectively. The distribution of these virulence genes suggests that a subset of CRKP isolates possess enhanced virulence potential, which may complicate clinical outcomes [27]. The distribution of virulence genes varied among different clonal groups identified in the cgSNP analysis. Some clonal groups exhibited a higher prevalence of specific virulence genes, indicating the presence of particularly virulent strains within these groups.

## Discussion

This study provides a comprehensive analysis of 156 clinical KpSC isolates collected from a tertiary hospital in China over a three-year period (2019–2021). Our findings revealed a prevalent of carbapenemase genes, extensive antimicrobial resistance, and considerable genetic diversity among the KpSC isolates. The identification of both resistance and virulence genes highlights the complex nature of CRKP and its potential to cause severe infections with limited treatment options [9, 28, 29].

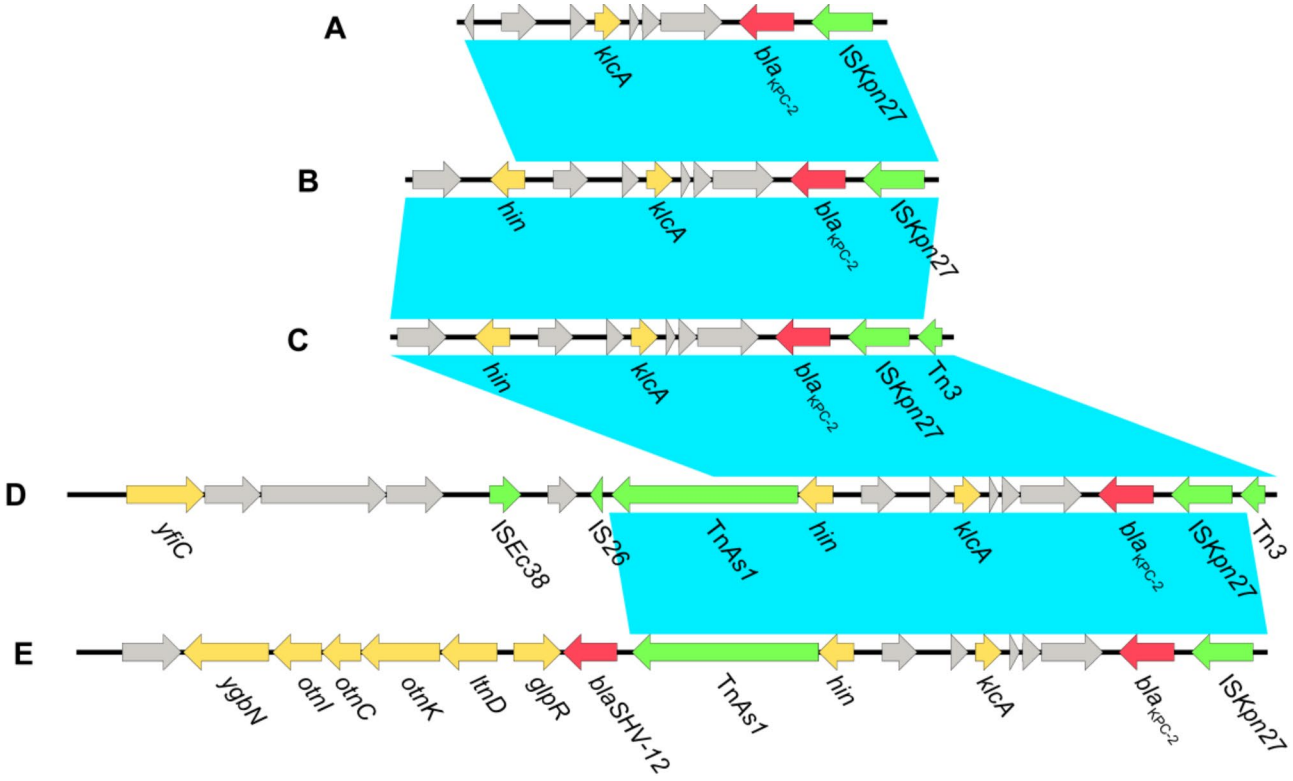


**Fig. 5** Genetic environment of $bla_{KPC-2}$ in *K. pneumoniae* isolates created by Easyfig. Red arrows indicate resistance genes, green arrows indicate mobile genetic elements, gray arrows indicate hypothetical proteins, and yellow arrows indicate genes encoding other functional proteins. Blue shading indicates regions of high homology

The high prevalence of $bla_{KPC-2}$ genes among the CRKP isolates aligns with previous reports from China and other parts of Asia, indicating regional dissemination patterns of these resistance determinants [30, 31]. The presence of multiple sequence types, particularly ST11, ST15, and ST23, underscores the genetic diversity of KpSC and suggests multiple introductions of different clonal lineages into the hospital setting. This diversity poses a significant challenge for infection control and emphasizes the need for continuous surveillance and genomic monitoring [6, 32].

The detection of virulence genes such as *rmpA*, *rmpA2*, and various siderophore systems in a subset of CRKP isolates is concerning, as these factors are associated with hypervirulence and increased pathogenicity [33]. The coexistence of resistance and virulence genes in some isolates suggests that these strains have the potential to cause severe and difficult-to-treat infections. Our analysis revealed that infections caused by these hypervirulent and multidrug-resistant strains were associated with higher mortality rates, highlighting the critical need for effective therapeutic strategies and robust infection control measures [29].

This comprehensive virulence gene analysis underscores the pathogenic potential of KpSC strains, highlighting their ability to adhere to host tissues, evade the immune system, and acquire essential nutrients. The presence of multiple virulence factors in these strains necessitates stringent infection control measures and continuous surveillance to prevent and manage outbreaks effectively.

Comparative genomic analysis with global CRKP isolates showed significant similarities in resistance gene profiles, particularly among ST11 isolates. This finding suggests that ST11 has become a dominant clonal lineage in both local (Wenzhou, China) and global contexts, contributing to the widespread dissemination of carbapenem resistance [31]. However, the presence of unique genetic markers in our isolates indicates local evolutionary adaptations, which could inform targeted interventions and treatment strategies.

The cgSNP analysis provided valuable insights into the genetic relatedness and epidemiological characteristics of the KpSC strains, emphasizing the importance of continuous surveillance and infection control measures to limit the spread of multidrug-resistant and virulent strains within healthcare settings. This comprehensive phylogenetic and epidemiological analysis underscores the need for integrated genomic surveillance to monitor the transmission dynamics and evolutionary patterns of *K. pneumoniae*, particularly in hospital environments where such strains pose a significant threat to patient

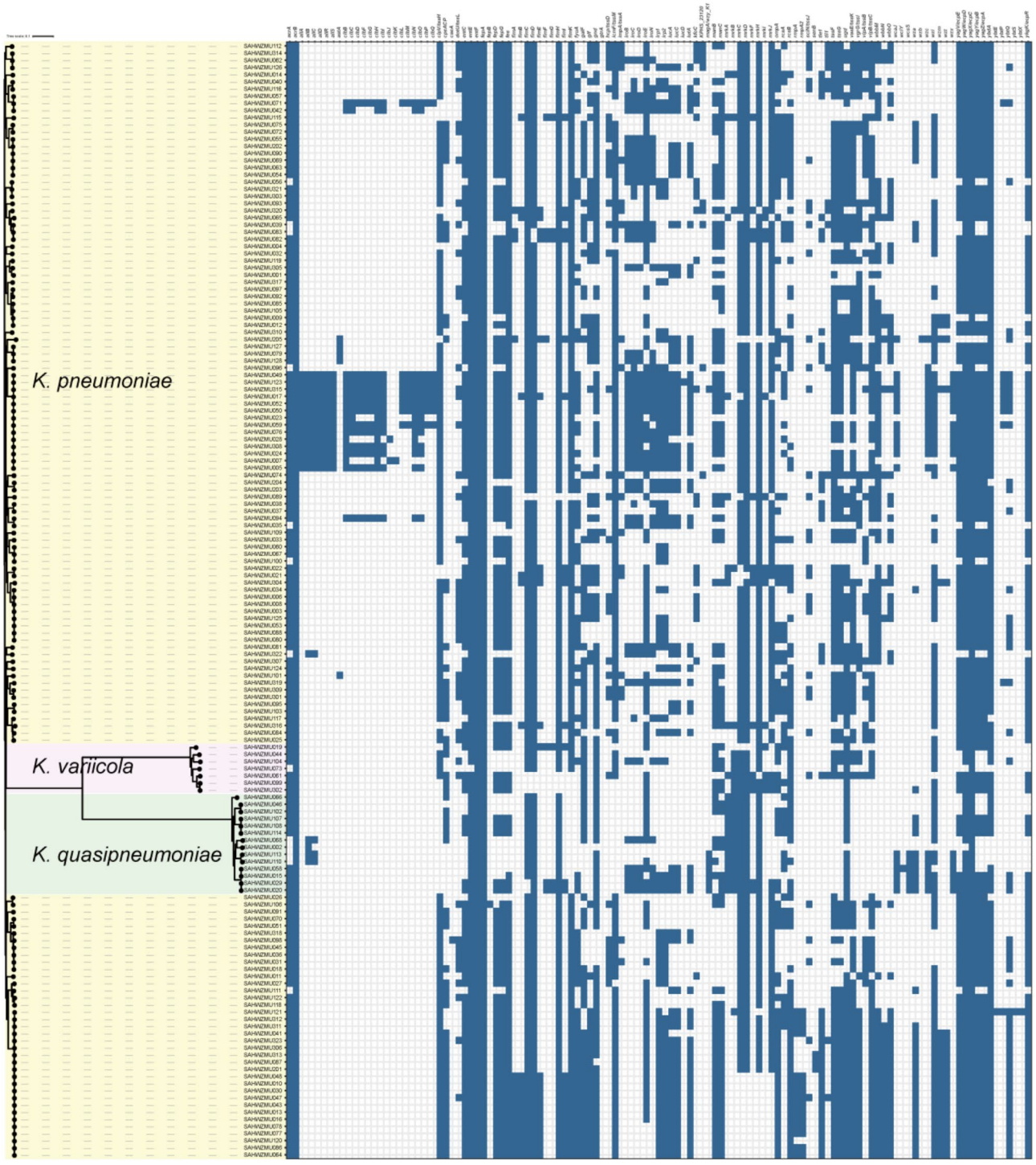


**Fig. 6** Prevalence of virulence genes. Prevalence of key virulence genes among the 156 KpSC isolates. The *rmpA* and *rmpA2* genes, which regulate the hypermucoviscosity phenotype, were detected in 14% of the isolates. Siderophore systems, including aerobactin, salmochelin, and yersiniabactin, were present in 27%, 19%, and 22% of the isolates, respectively

health. While our cgSNP analysis focused on local transmission dynamics, the dominant ST11 lineage identified here aligns with pandemic CRKP clones reported across China. Future studies integrating global genomes could further elucidate the geographic spread of these high-risk clones.

The high prevalence of carbapenem-resistant and hypervirulent CRKP strains in our study highlights the urgent need for comprehensive antimicrobial

stewardship programs and effective infection control practices. These measures are essential to curb the spread of CRKP and mitigate its impact on patient outcomes [8]. Additionally, the integration of genomic surveillance into routine clinical practice could enhance our ability to detect and respond to emerging resistance trends and virulent strains [29].

While this study provides valuable insights into the molecular epidemiology of KpSC in Wenzhou, it has some limitations. The isolates were collected from a single hospital, which may limit the generalizability of the findings. Future studies should include a larger number of isolates from multiple hospitals to provide a more comprehensive understanding of KpSC epidemiology. Additionally, functional studies are needed to elucidate the exact mechanisms by which resistance and virulence genes contribute to the pathogenicity of CRKP [1].

In conclusion, this study underscores the complex interplay between antimicrobial resistance and virulence in KpSC isolates from a Chinese tertiary hospital. Our findings highlight the importance of continuous surveillance, targeted therapeutic strategies, and robust infection control measures to combat the threat posed by these multidrug-resistant and hypervirulent pathogens.

## Conclusion

This study provides a comprehensive analysis of 156 clinical KpSC isolates collected from a tertiary hospital in Wenzhou, China, over a three-year period (2019–2021). Our findings revealed a high prevalence of carbapenemase genes, extensive antimicrobial resistance, and considerable genetic diversity among the isolates. While this study provides valuable insights into the molecular epidemiology of KpSC, future research should include a larger number of isolates from multiple hospitals to gain a more comprehensive understanding of KpSC epidemiology. Additionally, functional studies are needed to elucidate the exact mechanisms by which resistance and virulence genes contribute to the pathogenicity of KpSC. In conclusion, this study highlights the complex interplay between antimicrobial resistance and virulence in KpSC isolates from a Chinese tertiary hospital. Our findings emphasize the importance of continuous surveillance, targeted therapeutic strategies, and robust infection control measures to combat the threat posed by these multidrug-resistant and hypervirulent pathogens.

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**Author contributions**
WJ, YC and XC wrote the main manuscript text, ML, YJ, SL, JS and XC conceived and designed the experiments, YC, ML and YJ prepared the tables, and SL and JS prepared the figures. All authors reviewed the manuscript.

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**Data availability**
The datasets generated and/or analyzed during the current study are available in the NCBI Sequence Read Archive (SRA) repository, under the BioProject number PRJNA1131666, [https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1131666].

## Declarations

**Ethics approval and consent to participate**
Not applicable. Individual patient data were not involved, and only anonymous clinical residual samples during routine hospital laboratory procedures were used in this study.

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare no competing interests.

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**References**

1. Martin RM, Bachman MA. Colonization, infection, and the accessory genome of Klebsiella pneumoniae. Front Cell Infect Microbiol. 2018;8:4. https://doi.org/10.3389/fcimb.2018.00004.
2. Morgado S, Fonseca E, Vicente AC. Genomics of Klebsiella pneumoniae species complex reveals the circulation of High-Risk Multidrug-Resistant pandemic clones in human, animal, and environmental sources. Microorganisms. 2022;10:2281. https://doi.org/10.3390/microorganisms10112281.
3. Tacconelli E, Carrara E, Savoldi A, Harbarth S, Mendelson M, Monnet DL, Pulcini C, Kahlmeter G, Kluytmans J, Carmeli Y, Ouellette M, Outterson K, Patel J, Cavaleri M, Cox EM, Houchens CR, Grayson ML, Hansen P, Singh N, Theuretzbacher U, Magrini N. WHO pathogens priority list working group, 2018. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. Lancet Infect Dis 18, 318–27. https://doi.org/10.1016/S1473-3099(17)30753-3
4. Pitout JDD, Nordmann P, Poirel L. Carbapenemase-Producing Klebsiella pneumoniae, a key pathogen set for global nosocomial dominance. Antimicrob Agents Chemother. 2015;59:5873–84. https://doi.org/10.1128/AAC.01019-15.
5. Campos-Madueno EI, Moser AI, Jost G, Maffioli C, Bodmer T, Perreten V, Endimiani A. Carbapenemase-producing Klebsiella pneumoniae strains in Switzerland: human and non-human settings May share high-risk clones. J Glob Antimicrob Resist. 2022;28:206–15. https://doi.org/10.1016/j.jgar.2022.01.016.
6. Wyres KL, Holt KE. Klebsiella pneumoniae as a key trafficker of drug resistance genes from environmental to clinically important bacteria. Curr Opin Microbiol. 2018;45:131–9. https://doi.org/10.1016/j.mib.2018.04.004.
7. Pham MH, Hoi LT, Beale MA, Khokhar FA, Hoa NT, Musicha P, Blackwell GA, Long HB, Huong DT, Binh NG, Co DX, Giang T, Bui C, Tran HN, Bryan J, Herrick A, Feltwell T, Nadjm B, Parkhill J, van Doorn HR, Trung NV, Van Kinh N, Török ME, Thomson NR. Evidence of widespread endemic populations of highly multidrug resistant Klebsiella pneumoniae in hospital settings in Hanoi, Vietnam: a prospective cohort study. Lancet Microbe. 2023;4:e255–63. https://doi.org/10.1016/S2666-5247(22)00338-X.

8. Nordmann P, Naas T, Poirel L. Global spread of Carbapenemase-producing Enterobacteriaceae. Emerg Infect Dis. 2011;17:1791–8. https://doi.org/10.3201/eid1710.110655.
9. Liu R, Xu H, Zhao J, Hu X, Wu L, Qiao J, Ge H, Guo X, Gou J, Zheng B. Emergence of mcr-8.2-harboring hypervirulent ST412 Klebsiella pneumoniae strain from pediatric sepsis: A comparative genomic survey. Virulence. 2023;14:233–45. https://doi.org/10.1080/21505594.2022.2158980.
10. Liu R, Xu H, Guo X, Liu S, Qiao J, Ge H, Zheng B, Gou J. Genomic characterization of two Escherichia Fergusonii isolates harboring mcr-1 gene from farm environment. Front Cell Infect Microbiol. 2022;12:774494. https://doi.org/10.3389/fcimb.2022.774494.
11. Seemann T. Prokka: rapid prokaryotic genome annotation. Bioinformatics. 2014;30:2068–9. https://doi.org/10.1093/bioinformatics/btu153.
12. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol. 2012;19:455–77. https://doi.org/10.1089/cmb.2012.0021.
13. Bortolaia V, Kaas RS, Ruppe E, Roberts MC, Schwarz S, Cattoir V, Philippon A, Allesoe RL, Rebelo AR, Florensa AF, Fagelhauer L, Chakraborty T, Neumann B, Werner G, Bender JK, Stingl K, Nguyen M, Coppens J, Xavier BB, Malhotra-Kumar S, Westh H, Pinholt M, Anjum MF, Duggett NA, Kempf I, Nykäsenoja S, Olkkola S, Wieczorek K, Amaro A, Clemente L, Mossong J, Losch S, Ragimbeau C, Lund O, Aarestrup FM. ResFinder 4.0 for predictions of phenotypes from genotypes. J Antimicrob Chemother. 2020;75:3491–500. https://doi.org/10.1093/jac/dkaa345.
14. Carattoli A, Hasman H. PlasmidFinder and in Silico pMLST: identification and typing of plasmid replicons in Whole-Genome sequencing (WGS). Methods Mol Biol. 2020;2075:285–94. https://doi.org/10.1007/978-1-4939-9877-7_20.
15. Sullivan MJ, Petty NK, Beatson SA. Easyfig: a genome comparison visualizer. Bioinformatics. 2011;27:1009–10. https://doi.org/10.1093/bioinformatics/btr039.
16. Liu B, Zheng D, Zhou S, Chen L, Yang J. VFDB 2022: a general classification scheme for bacterial virulence factors. Nucleic Acids Res. 2022;50:D912–7. https://doi.org/10.1093/nar/gkab1107.
17. Croucher NJ, Page AJ, Connor TR, Delaney AJ, Keane JA, Bentley SD, Parkhill J, Harris SR. Rapid phylogenetic analysis of large samples of Recombinant bacterial whole genome sequences using Gubbins. Nucleic Acids Res. 2015;43:e15. https://doi.org/10.1093/nar/gku1196.
18. Schürch AC, Arredondo-Alonso S, Willems RJL, Goering RV. Whole genome sequencing options for bacterial strain typing and epidemiologic analysis based on single nucleotide polymorphism versus gene-by-gene-based approaches. Clin Microbiol Infect. 2018;24:350–4. https://doi.org/10.1016/j.cmi.2017.12.016.
19. Hu D, Wang S, Xu M, Zhang J, Luo X, Zhou W, Ma Q, Ma X. Double blaKPC-2 copies quadrupled minimum inhibitory concentration of ceftazidime-avibactam in hospital-derived Klebsiella pneumoniae. Microbiol Spectr. 2024;e0033124. https://doi.org/10.1128/spectrum.00331-24.
20. Ota Y, Prah I, Nukui Y, Koike R, Saito R. blaKPC-2-Encoding IncP-6 plasmids in citrobacter freundii and Klebsiella variicola strains from hospital sewage in Japan. Appl Environ Microbiol. 2022;88:e0001922. https://doi.org/10.1128/aem.00019-22.
21. Alcántar-Curiel MD, Blackburn D, Saldaña Z, Gayosso-Vázquez C, Iovine NM, De la Cruz MA, Girón JA. Multi-functional analysis of Klebsiella pneumoniae fimbrial types in adherence and biofilm formation. Virulence. 2013;4:129–38. https://doi.org/10.4161/viru.22974.
22. Mayneris-Perxachs J, Moreno-Navarrete JM, Fernández-Real JM. The role of iron in host-microbiota crosstalk and its effects on systemic glucose metabolism. Nat Rev Endocrinol. 2022;18:683–98. https://doi.org/10.1038/s41574-022-00721-3.
23. Raffatellu M, Chessa D, Wilson RP, Tükel C, Akçelik M, Bäumler AJ. Capsule-mediated immune evasion: a new hypothesis explaining aspects of typhoid fever pathogenesis. Infect Immun. 2006;74:19–27. https://doi.org/10.1128/IAI.74.1.19-27.2006.
24. Singh S, Wilksch JJ, Dunstan RA, Mularski A, Wang N, Hocking D, Jebeli L, Cao H, Clements A, Jenney AWJ, Lithgow T, Strugnell RA. LPS O antigen plays a key role in Klebsiella pneumoniae capsule retention. Microbiol Spectr. 2022;10:e0151721. https://doi.org/10.1128/spectrum.01517-21.
25. Merciecca T, Bornes S, Nakusi L, Theil S, Rendueles O, Forestier C, Miquel S. Role of Klebsiella pneumoniae type VI secretion system (T6SS) in long-term Gastrointestinal colonization. Sci Rep. 2022;12:16968. https://doi.org/10.1038/s41598-022-21396-w.
26. Rivero A, Gomez E, Alland D, Huang DB, Chiang T. K2 serotype Klebsiella pneumoniae causing a liver abscess associated with infective endocarditis. J Clin Microbiol. 2010;48:639–41. https://doi.org/10.1128/JCM.01779-09.
27. Kong ZX, Karunakaran R, Jabar A, Ponnampalavanar K, Chong S, Teh CW, C.S.J. The detection of hypermucoviscous Carbapenem-Resistant Klebsiella pneumoniae from a tertiary teaching hospital in Malaysia and assessment of hypermucoviscous as marker of hypervirulence. Microb Drug Resist. 2021;27:1319–27. https://doi.org/10.1089/mdr.2020.0096.
28. Zhao J, Zheng B, Xu H, Li J, Sun T, Jiang X, Liu W. Emergence of a NDM-1-producing ST25 Klebsiella pneumoniae strain causing neonatal sepsis in China. Front Microbiol. 2022;13:980191. https://doi.org/10.3389/fmicb.2022.980191.
29. Zheng B, Xu H, Lv T, Guo L, Xiao Y, Huang C, Zhang S, Chen Y, Han H, Shen P, Xiao Y, Li L. 2020. Stool Samples of Acute Diarrhea Inpatients as a Reservoir of ST11 Hypervirulent KPC-2-Producing Klebsiella pneumoniae. mSystems 5, e00498-20. https://doi.org/10.1128/mSystems.00498-20
30. Chen Y, Chen Y. Clinical challenges with hypervirulent Klebsiella pneumoniae (hvKP) in China. J Transl Int Med. 2021;9:71–5. https://doi.org/10.2478/jtim-2021-0004.
31. David S, Reuter S, Harris SR, Glasner C, Feltwell T, Argimon S, Abudahab K, Goater R, Giani T, Errico G, Aspbury M, Sjunnebo S, EuSCAPE Working Group, ESGEM Study Group, Feil EJ, Rossolini GM, Aanensen DM, Grundmann H. Epidemic of carbapenem-resistant Klebsiella pneumoniae in Europe is driven by nosocomial spread. Nat Microbiol. 2019;4:1919–29. https://doi.org/10.1038/s41564-019-0492-8.
32. Shi Q, Han R, Guo Y, Zheng Y, Yang Y, Yin D, Zhang R, Hu F. Emergence of ST15 Klebsiella pneumoniae clinical isolates producing Plasmids-Mediated RmtF and OXA-232 in China. Infect Drug Resist. 2020;13:3125–9. https://doi.org/10.2147/IDR.S257298.
33. Yang X, Ye L, Li Y, Chan EW-C, Zhang R, Chen S. Identification of a chromosomal integrated DNA fragment containing the rmpA2 and IucABCDiutA virulence genes in Klebsiella pneumoniae. mSphere. 2020;5:e01179–20. https://doi.org/10.1128/mSphere.01179-20.


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