BMC Microbiology

RESEARCH

Open Access

Virulence plasmid with *IroBCDN* deletion promoted cross-regional transmission of ST11-KL64 carbapenem-resistant hypervirulent *Klebsiella pneumoniae* in central China

Han-xu Hong[1,2], Bing-Hui Huo[1,2], Tian-Xin Xiang[3], Dan-Dan Wei[1,5], Qi-Sen Huang[1], Peng Liu[1], Wei Zhang[4], Ying Xu[2,6*] and Yang Liu[1,5,7*]

Abstract

Background Carbapenem-resistant and hypervirulent *Klebsiella pneumoniae* (CR-hvKP) caused infections of high mortality and brought a serious impact on public health. This study aims to evaluate the epidemiology, resistance and virulence characteristics of CR-hvKP and to identify potential drivers of cross-regional transmission in different regions of China, in order to provide a basis for developing targeted prevention measures.

Methods Clinical *K. pneumoniae* strains were collected from Jiujiang and Nanchang in Jiangxi province between November 2021 to June 2022. Clinical data of patients (age, sex, source of infection, and diagnosis) were also gathered. We characterized these strains for their genetic relatedness using PFGE, antimicrobial and virulence plasmid structures using whole-genome sequencing, and toxicity using *Galleria mellonella* infection model.

Results Among 609 strains, 45 (7.4%) CR-hvKP were identified, while the strains.

isolated from Nanchang and Jiujiang accounted for 10.05% (36/358) and 3.59% (9/251). We observed that ST11-KL64 CR-hvKP had an overwhelming epidemic dominance in these two regions. Significant genetic diversity was identified among all ST11-KL64 CR-hvKP cross-regional transmission between Nanchang and Jiujiang and this diversity served as the primary driver of the dissemination of clonal groups. Virulence genes profile revealed that ST11-KL64 CR-hvKP might harbour incomplete pLVPK-like plasmids and primarily evolved from CRKP by acquiring the hypervirulence plasmid. We found the predominance of truncated-IncFIB/IncHI1B type virulence plasmids with a 25 kb fragment deletion that encoded *iroBCDN* clusters.

Conclusion ST11-KL64 is the most cross-regional prevalent type CR-hvKPs in Jiangxi province, which mainly evolved from CRKPs by acquiring a truncated-IncHI1B/IncFIB virulence plasmid with the deletion of *iroBCDN*. Stricter surveillance and control measures are urgently needed to prevent the epidemic transmission of ST11-KL64 CR-hvKP.

Keywords Carbapenem-resistant hypervirulent *Klebsiella pneumoniae*, Virulence plasmid, Whole genome sequencing, MLST, Capsular serotypes

*Correspondence:
Ying Xu
344803452@qq.com
Yang Liu
ly13767160474@sina.com
Full list of author information is available at the end of the article

Introduction

Klebsiella pneumoniae can be divided into classical *K. pneumoniae* (cKp) and hypervirulent *K. pneumoniae* (hvKp) that causes various diseases [1, 2]. Classical *K. pneumoniae* strains have been implicated in a higher rate of resistance to multiple antimicrobial agents and are primarily restricted in causing infections among immunocompromised patients [3]. However, compared to the cKp strains, hvKp has more intense clinical manifestation and often carries a large virulence plasmid, which include *iuc/iro*, *rmpA/rmpA2* and *peg*-344 [4]. It can cause not only nosocomial infections in patients with low immune function but also community-acquired infections in young people with normal immune function, and can present migratory spread.

In recent years, hypervirulent carbapenem-resistant *Klebsiella pneumoniae* (CR-hvKP) has been widely reported and poses a global threat, which may lead to even higher mortality and morbidity [5, 6]. These CR-hvKP strains may differentiate in two possible evolutionary pathways: (1) hvKP strains acquired carbapenem-resistance plasmids; (2) CRKP strains acquired pLVPK-like virulence plasmids [7, 8]. ST11 CR-hvKP was found to have evolved from ST11 CRKP via the acquisition of pLVPK-like virulence plasmid, which was mainly associated with *rmpA/rmpA2*, aerobactin (*iucABCD* and *iutA*) and salmochelin (*iroBCDN* gene cluster) [9, 10] with several current variant sequence types identified in different regions of China, including Beijing [11, 12], Shenzhen [13], Hangzhou [14], Zhengzhou [15], and Nanchang [16]. Furthermore, it has been reported that ST11-KL64 has gradually displaced ST11-KL47 as the most prevalent clone in China [17, 18]. Resistance, virulence and prevalence due to CR-hvKP increases the failure rate of patient treatment. Therefore, to further investigate the potential for transmission of CR-hvKP, we investigated the genetic characteristics, plasmid content and clonal relationship of CR-hvKP strains isolated from Nanchang and Jiujiang, in central China. Our study combines epidemiological data and whole-genome sequencing data from clinical isolates across different regions to investigate the transmission dynamics of CR-hvKP, providing insights into the widespread prevalence of ST11-KL64 CR-hvKP across different regions in central China.

Materials and methods

Specimen collection and identification

We collected a total of 609 *Klebsiella pneumoniae* strains between November 2021 to June 2022, comprising 358 strains from Nanchang and 251 strains from Jiujiang in central China. All clinical isolates used in the this study were resistant to carbapenems (meropenem, imipenem, or ertapenem) according to the guidelines of the Clinical Laboratory Standards Institute (CLSI, 2022) [19]. The clinical data of the patients (age, sex, acquisition of infection, and diagnosis) were collected from the electronic medical records. *K. pneumoniae* isolates were identified using matrix-assisted laser desorption/ionization-time of flight mass spectrometry (Bruker Daltonics). Carbapenem-resistant *Klebsiella pneumoniae* isolates that tested positive for the virulence genes *peg-344* and *rmpA/rmpA2* using loop-mediated isothermal amplification (LAMP) [20] and showed positive results in the string test (> 5 mm) were defined as CR-hvKP. The Ethics Committee determined that patient consent was not required because the present study was retrospective, and the identities of the patients were anonymized.

Determination of capsular serotyping, virulence, PCR-based replicon typing, and drug resistance genes of CR-hvKP

All of template DNAs were extracted by bacterial DNA Kit. The presence of capsular serotyping, virulence, and drug resistance genes were determined by PCR as previously described [21, 22]. The positive products were sequenced by Shanghai Sangon Biotechnology, and the results were analyzed by BLAST. To identify the replicons of incompatibility plasmid groups among *K. pneumoniae*, a series of primers recognizing the FIA, FIB, FIC, HI1, HI2, I1-Iγ, L/M, N, P, W, T, A/C, K, B/O, X, Y, F, and FIIA replicons were used [23].

Analysis of homologous gene families and phylogenetic construction

According to the database (https://bigsdb.pasteur.fr/klebsiella/) for multilocus sequence typing (MLST), seven housekeeping genes, *gapA*, *infB*, *mdh*, *pgi*, *phoE*, *rpoB*, and *tonB*, were used for *K. pneumoniae* typing. Pulsed-field gel electrophoresis (PFGE) was carried out following the standard operating procedure by PulseNet International. PFGE images were imported to Bionumerics software ver. 7.6 (AppliedMaths, Belgium) to identify image bands and construct UPGMA dendrogram. Genetic relatedness was interpreted using a cluster cutoff line set at.

80% similarity.

Galleria mellonella infection model

The toxicity test was conducted using the *Galleria mellonella* model. Ten larvae weighing between 250 and 350 mg (purchased from Tianjin Huiyude Biotech Company, Tianjin, China) were used for the assessment of the virulence of each isolate. Overnight cultures of *K pneumoniae* strains were washed with phosphate-buffered saline (PBS) and further adjusted with PBS to concentrations of 1×10^6 CFU/ml. The insects were

inoculated by injecting 1×10^6 CFU per 10 µl aliquot into the hemocoel via the rear left proleg, followed by a recording of survival rate every 12 h for 4 days. All experiments were performed in triplicates. *K. pneumoniae* strain NTUH-K2044 and strain ATCC700603 were used as controls of high and low virulence strains, respectively.

Whole-genome sequencing

After genomic DNA extraction, the genomes of the 45 CR-hvKP strains were sequenced using the Illumina HiSeq platform (Illumina, San Diego, CA, USA), and 15 of them were further sequenced for long reads using the Oxford Nanopore MinION platform (Nanopore, Oxford, UK). The genome sequence was automatically annotated using National Center for Biotechnology Information (NCBI) Prokaryotic Genome Annotation Pipeline (PGAP) (http://www.ncbi.nlm.nih.gov/genome/annotation_prok/). The alignment of core-genome single-nucleotide polymorphisms (SNPs) was generated using Snippy (https://github.com/tseemann/snippy), with *K. pneumoniae* HS11286 (NC_016845.1) as the reference genome, and was used to construct a high-resolution phylogeny. The phylogenetic tree was displayed and annotated using tvBOT [24]. Antimicrobial resistance genes, virulence genes, plasmid incompatibility groups and capsular serotype were predicted by ResFinder database, virulence factor database (VFDB), PlasmidFinder database and Kleborate with the command line of kleborate -a ASSEMBLIES -k, respectively. Comparative genomics analysis was performed using hypervirulent *K. pneumoniae* NTUH-K2044 (GenBank accession no. AP006725) as a reference by BacWGSTdb server with 25 alleles difference threshold. Concerning the analysis of sequenced plasmids, insertion sequences (ISs) were identified using ISfinder. The plasmid structure was visualized by DNAplotter [25]. Plasmid comparisons were conducted using Easyfig and further visualized via Adobe Illustrator [26].

Statistical analysis

All statistical analyses were performed using SPSS version 20.0. Categorical variables are shown as frequencies with percentages; they were analyzed using the chi-squared test or Fisher's exact test. *P*-values < 0.05 were considered statistically significant.

Results

Demographics and clinical characteristics of CR-hvKP from Nanchang and Jiujiang

Of the 609 CRKP strains, 45 strains (7.39%) were CR-hvKP. Among them, 36 strains were from Nanchang (10.06%) and 9 strains were from Jiujiang (3.58%). The detection rate of CR-hvKP in Nanchang was significantly higher than that in Jiujiang ($\chi^2 = 8.437$, $P = 0.004$). In this study, for the CR-hvKP from two regions, the wards most affected by these strains were basically intensive care units and respiratory departments. Most of the patients have a history of combination antibiotic treatments. There was a difference in clinical specimens between CR-hvKP in Nanchang and Jiujiang. The CR-hvKP strains in Nanchang were mainly isolated from blood samples, while the majority of CR-hvKP strains in Jiujiang were isolated from sputum samples ($P < 0.05$). The mean age of onset for CR-hvKP was 61.02 ± 14.30 years, with a higher prevalence observed in males. CR-hvKP infected patients in Nanchang were significantly healthier and younger than those in Jiujiang ($P < 0.05$). Other clinical characteristics of the patients are summarized in Table 1.

Capsule serotype, resistance and virulence of CR-hvKP strains from Nanchang and Jiujiang

There was no statistically significant difference in the capsule serotypes and MLST sequence types (STs) of CR-hvKP strains between Nanchang and Jiujiang regions (Table 2). Five capsular types with the predominance of K64 (25/36, 69.44%), K1 (6/36, 16.67%), K2 (3/36, 8.33%) were isolated from Nanchang, while the majority of K64 (6/9, 66.67%), K5 (2/9, 22.22%), K1 (1/9, 11.11%) were isolated from Jiujiang.

In the virulence genes analysis, the different markers of CR-hvKP isolates were as follows: *magA* (15.6%, n = 7/45), *rmpA* (86.7%, n = 39/45), *fimH* (100%, n = 45/45), *rmpA2* (95.6%, n = 43/45), *iucA* (97.8%, n = 44/45), *iroB* (28.9%, n = 13/45), and *peg344* (93.3%, n = 42/45). Furthermore, All CR-hvKP isolates were multidrug-resistant (MDR). The occurrence of resistance genes in 45 CR-hvKP isolates was as follows: bla_{KPC} (80.0%, n = 36/45), bla_{NDM} (6.7%, n = 3/45), $bla_{CTX\text{-}M}$ (97.8%, n = 44/45), bla_{SHV} (100%, n = 45/45), bla_{TEM} (84.4%, n = 38/45), *rmtB* (15.6%, n = 7/45), *qnrS* (62.2%, n = 28/45), *qnrB* (2.2%, n = 1/45), *acc(6')-lb-cr* (31.1%, n = 14/45), *mphA* (4.4%, n = 2/45), and *sul* (77.8%, n = 35/45). Kleborate analysis showed that all CR-hvKP strains had a resistance score ≥ 1 and a virulence score ≥ 4, with 13 strains (28.9%) having a virulence score of 5. Further analysis revealed that all CR-hvKP strains were classified as convergent *K. pneumoniae* (convergence defined as a virulence score ≥ 3 and a resistance score ≥ 1 [27]), indicating the co-occurrence of virulence and resistance determinants at the genetic level in these strains. We infected *G.mellonella* larvae with all 45 CR-hvKP isolates obtained from Nanchang and Jiujiang. As shown in Fig. 1, all CR-hvKP isolates displayed greater virulence than ATCC 700603, and most isolates had similar or greater virulence than NTUH-K2044.

Table 1 CR-hvKP of infected patients from Nanchang and Jiujiang regions

Clinical features	Nanchang CR-hvKP($n=36$), n(%)	Jiujiang CR-hvKP($n=9$), n(%)	*P* value
Age, years	53.02 ± 19.34	74.22 ± 9.47	
0–30	6(16.67)	0	0.188
31–50	8(22.22)	0	0.119
51–70	17(47.22)	6(66.67)	0.297
over 70	5(13.89)	3(33.33)	0.172
Gender			
Male	29(80.56)	9(100)	0.150
Female	7(19.44)	0(0)	0.150
Samples			
Sputum	**11(30.56)**	**8(88.89)**	**0.002***
Urine	3(8.33)	0(0)	0.370
Blood	**14(38.89)**	**0(0)**	**0.024***
Bronchoalveolar lavage fluid	0(0)	1(11.11)	0.200
Ascites	2(5.56)	0(0)	0.469
Pus	4(11.11)	0(0)	0.295
Drainage fluid	1(2.78)	0(0)	0.613
Venous catheter	1(2.78)	0(0)	0.613
Wards			
ICU	21(58.33)	4(44.44)	0.667
Neurosurgical department	1(2.78)	0(0)	0.613
Neurological department	2(5.56)	1(11.11)	0.550
Respiratory department	**1(2.78)**	**3(33.33)**	**0.004***
Rehabilitation department	2(5.56)	0(0)	0.469
Other ward	9(25)	1(11.11)	0.370
Drug combination			
Yes	33(91.67)	8(88.89)	0.793
No	3(8.33)	1(11.11)	0.793
Non-medication	0(0)	0(0)	NA

Table 2 Capsule serotype and MLST STs of CR-hvKP strain

K/MLST	Nanchang CR-hvKP($n=36$), n(%)	Jiujiang CR-hvKP($n=9$), n(%)	*P* Value
Capsular serotypes			
K1	6(16.67)	1(11.11)	0.681
K2	3(8.33)	2(22.22)	0.236
K5	1(2.78)	0(0)	0.613
K47	1(2.78)	0(0)	0.613
K64	25(69.44)	6(66.67)	0.872
MLST typing			
ST11	26(72.22)	6(66.67)	0.742
ST23	6(16.67)	1(11.11)	0.681
ST65	2(5.56)	2(22.22)	0.116
ST86	1(2.78)	0(0)	0.633
ST485	1(2.78)	0(0)	0.633

Genetic analysis of CR-hvKP strains from Nanchang and Jiujiang

The MLST typing showed that all CR-hvKP isolates were grouped into 5 different STs. CR-hvKP distinct isolates from Nanchang belonged to five STs with the prevalence of ST11 (26/36, 72.22%), ST23 (6/36, 16.67%), ST65 (2/36, 5.56%), ST86 (1/36, 2.78%), and ST485 (1/36, 2.78%), while isolates from Jiujiang were ST11 (6/9, 66.67%), ST23 (1/9, 11.11%), ST65 (2/9, 22.22%).

The PFGE dendrogram was analyzed using BioNumeric software to ascertain the relatedness of these isolates. CR-hvKP strains isolated from both Nanchang and Jiujiang were grouped into six clusters, with cluster A (40%, 18/45) and cluster B (24.4%, 11/45) dominating, which were all ST11-KL64. However, the CR-hvKP strains that cross-regional transmitted between Nanchang and Jiujiang are mainly concentrated in cluster

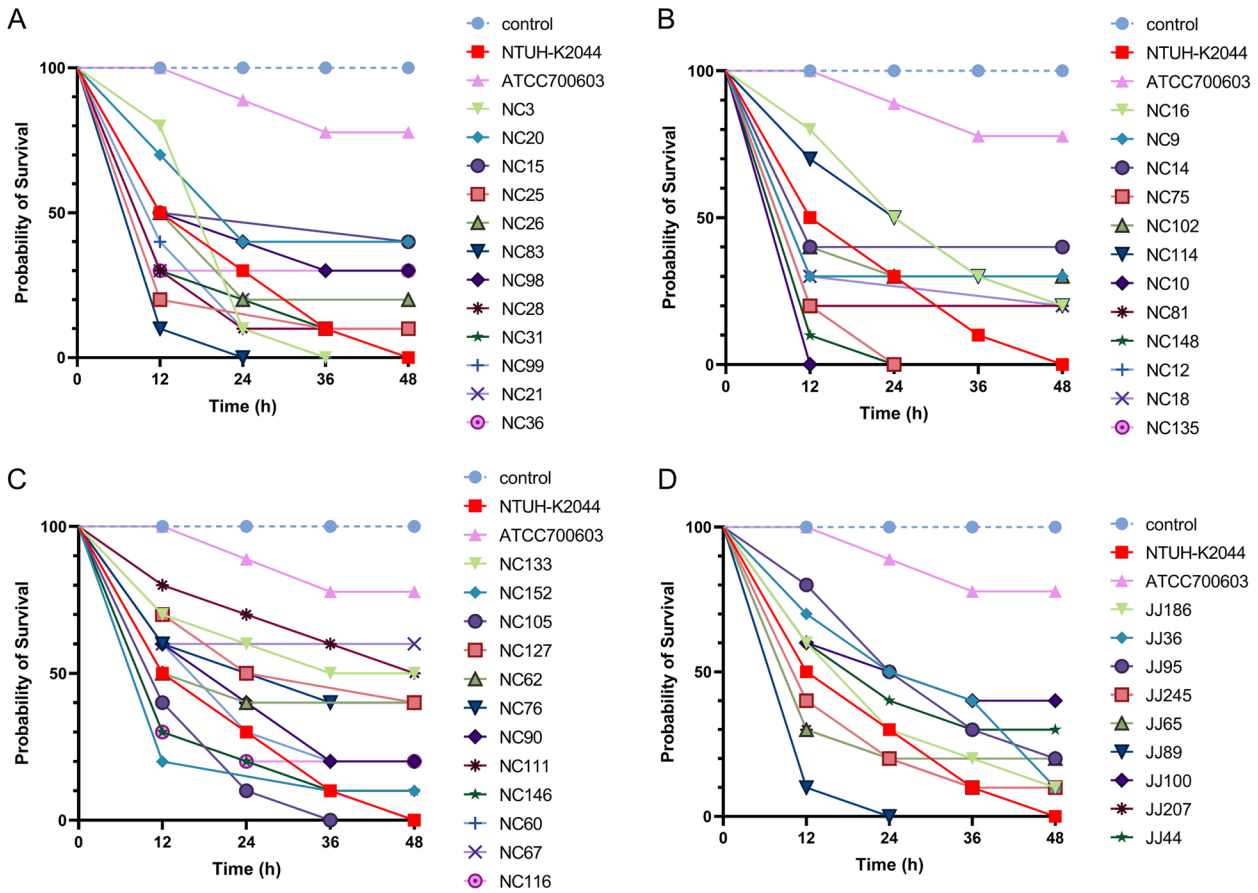

Fig. 1 The survival curves of *G. mellonella* infected by CR-hvKP isolates. **A**, **B**, **C** isolates obtained in Nanchang; **D** isolates obtained in Jiujiang

B and cluster E (Fig. 2). Additionally, we constructed a phylogenetic tree based on WGS data, which revealed a high degree of relatedness among the ST11 strains, especially the ST11-KL64 strains, while all non-ST11 strains are distantly related to ST11 strains (Fig. 3).

The epidemiology of CR-hvKP in central China is a complex and evolving phenomenon attributed to the horizontal transfer and clonal spread of major clones, predominantly ST11-KL64. Additional 8 CR-hvKP isolates were collected from patients in ICU and respiratory departments in Jiujiang (Fig. 2), who had ICU stay history in Nanchang prior to transfer to their current wards where the isolates were recovered.

Genetic environment and structural analysis of the virulence plasmid of ST11-CR-hvKP strain

The genetic context of the virulence plasmids exhibited considerable diversity from the whole-genome sequence, revealed representatives of plasmid incompatibility groups ColRNAI, IncHI1B, IncFIB, IncFII, IncR, IncQ, IncX3, respectively. Plasmid sequence analysis confirmed that all virulence plasmids belonged to the IncFIB/IncHIB type with different sizes ranging from 122.4 to 357.5 kb in length, with a GC content of 51.22% to 53.47% (Fig. 4). However, the predominant plasmid replicon types associated with the KPC and NDM genes were IncFII(k) (100%) and IncX3 (100%).

Interestingly, a 25-kbp fragment including *iroBCDN* locus with IS110 transposase were absent in the IncFIB/IncHI1B type virulence plasmid harbored in ST11-KL64 CR-hvKP isolates from Nanchang and Jiujiang (Fig. 4). According to these results, we hypothesize that IS110 might be responsible for the deletion of *iroBCDN*, and then this fragment deletion might be more conducive to promote cross-regional transmission and maintain plasmid stability in ST11-KL64 CR-hvKP isolates. Furthermore, virulence plasmids harbored in ST11-KL64 CR-hvKP isolates from Nanchang and Jiujiang exhibited a high level of homology. Virulence plasmids also harbored several virulence genes, i.e., *rmpA*, *rmpA2*, *peg344*, *iucA*, and *iutA* (Fig. 5). Comparison of genetic surroundings of virulence gene indicated that it was flanked by a similar core structure (Figure S1).

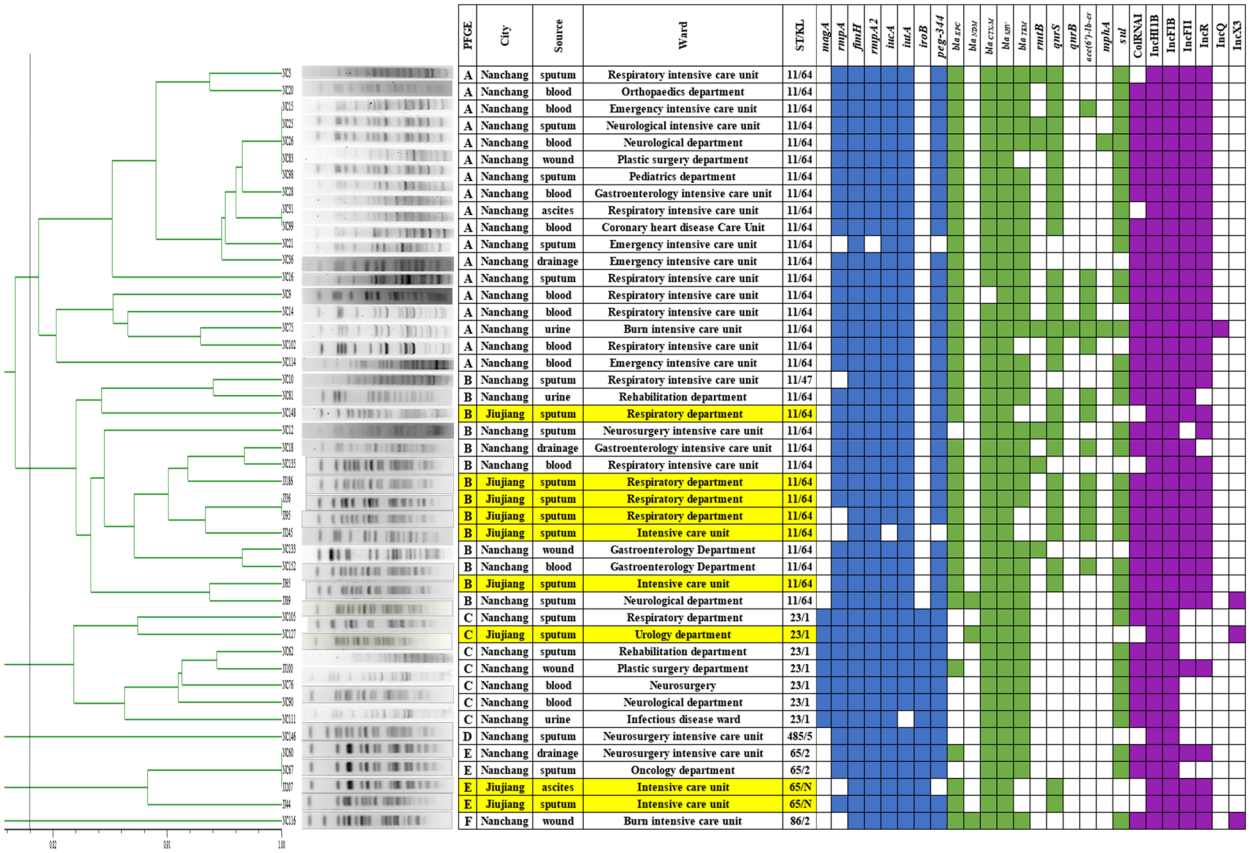

Fig. 2 Phylogenetic analysis and distribution of CR-hvKP phylogroups, Inc-type plasmid, antimicrobial resistance genes, virulence-associated genes, cluster groups

Discussion

The recent emergence of CR-hvKP, associated with considerable morbidity and mortality, poses a critical challenge to clinical and public health. ST11-KL64 was the most frequent clone for CR-hvKP globally, especially in China [28]. Virulence plasmids are demonstrated to readily convert an ST11 CRKP strain to a CR-hvKP strain via conjugation [29]. Therefore, surveillance and tracking of ST11-KL64 CR-hvKP is critical for clinicians in various clinical settings and deserves more attention. This study investigated the origin and cross-regional transmission pattern of CR-hvKP in central China.

In the retrospective study, although the specimen origins of CR-hvKP varied greatly between Nanchang and Jiujiang, the trend of virulence in CR-hvKP was similar in different regions of central China. In particular, we have identified the blood sample as an important specimen associated with invasive infection in Nanchang, while we identified a cluster of patients in the neurosurgery ward with sputum cultures positive for CR-hvKP from Jiujiang region. Our findings demonstrated that CR-hvKP from Jiujiang could colonize the respiratory tract, suggesting the strong colonization adaptability of CR-hvKP to the host. We speculate that the difference in specimen origins of CR-hvKP from Nanchang and Jiujiang may be related to differences in the administration of antimicrobial and endemic strains in these areas. Nevertheless, there is no denying that the cross-regional transmission of CR-hvKP is a serious issue.

The serotypes KL64 and KL47 were commonly present in ST11 CR-hvKP strain [30], but the epidemiological trends suggested that KL64 is prevalent among ST11 strains [31]. However, ST11-KL64 CR-hvKP is becoming hypervirulent by acquiring various virulence genes or a pLVKP-like plasmid [32]. PFGE showed that ST11-KL64 CR-hvKP from Nanchang and Jiujiang regions exhibited a high degree of clonality, suggested that clonal cross-regional transmission had occurred in central China. The related epidemiological information showed that some patients with CR-hvKP infections from Jiujiang have a history of prior hospitalization in Nanchang region. However, some patients with CR-hvKP infections from Nanchang are residents of Jiujiang region who go to seek health care at Nanchang hospitals. Current findings indicate that the two regions have the same CR-hvKP clone circulating thus

Fig. 3 Phylogenetic analysis of 45 CR-hvKP strains based on whole-genome sequencing data, serotypes, and multilocus sequence types

indicating the cross-transmission between the two regions in China. These studies emphasize the importance of genomic surveillace by WGS and phylogenetic analysis of CR-hvKP in these regions [33, 34].

ST11 *K. pneumoniae* is a widely occurring multidrug-resistant clonal lineage, including carbapenems, but not hypervirulent. In our study, carbapenemases, several extended-spectrum β-lactamase (ESBL) resistance genes, and other resistance genes, including *rmtB*, *qnrS*, *qnrB*, *acc(6')-lb-cr*, *mphA*, and *sul*, were identified in the CR-hvKP isolates. Furthermore, kleborate analysis revealed that all CR-hvKP strains harbored hypermucoviscous phenotype regulators and aerobactin synthesis, showed hypervirulent phenotype. Our data revealed a high prevalence of ST11-KL64 CR-hvKPs carrying the IncHI1B/IncFIB virulence plasmids with a 25-kbp fragment deletion including the salmonellin genes (*iroBCDN*). These results revealed virulence plasmid with *iroBCDN* deletion might promote cross-regional transmission of ST11-KL64 CR-hvKP in Jiangxi province, China. Further genomic epidemiology analysis and targeted surveillance at the national scale are urgently needed.

Conclusion

Our study identified the cross-regional transmission of ST11-KL64 CR-hvKP in China. The transmission threat of virulence plasmid with *iroBCDN* deletion in CR-hvKP represents a significant public health threat. Therefore, strengthening the clinical monitoring of CR-hvKP strains and taking adequate infection control measures to avoid transmission and prevent their further dissemination in cross-region.

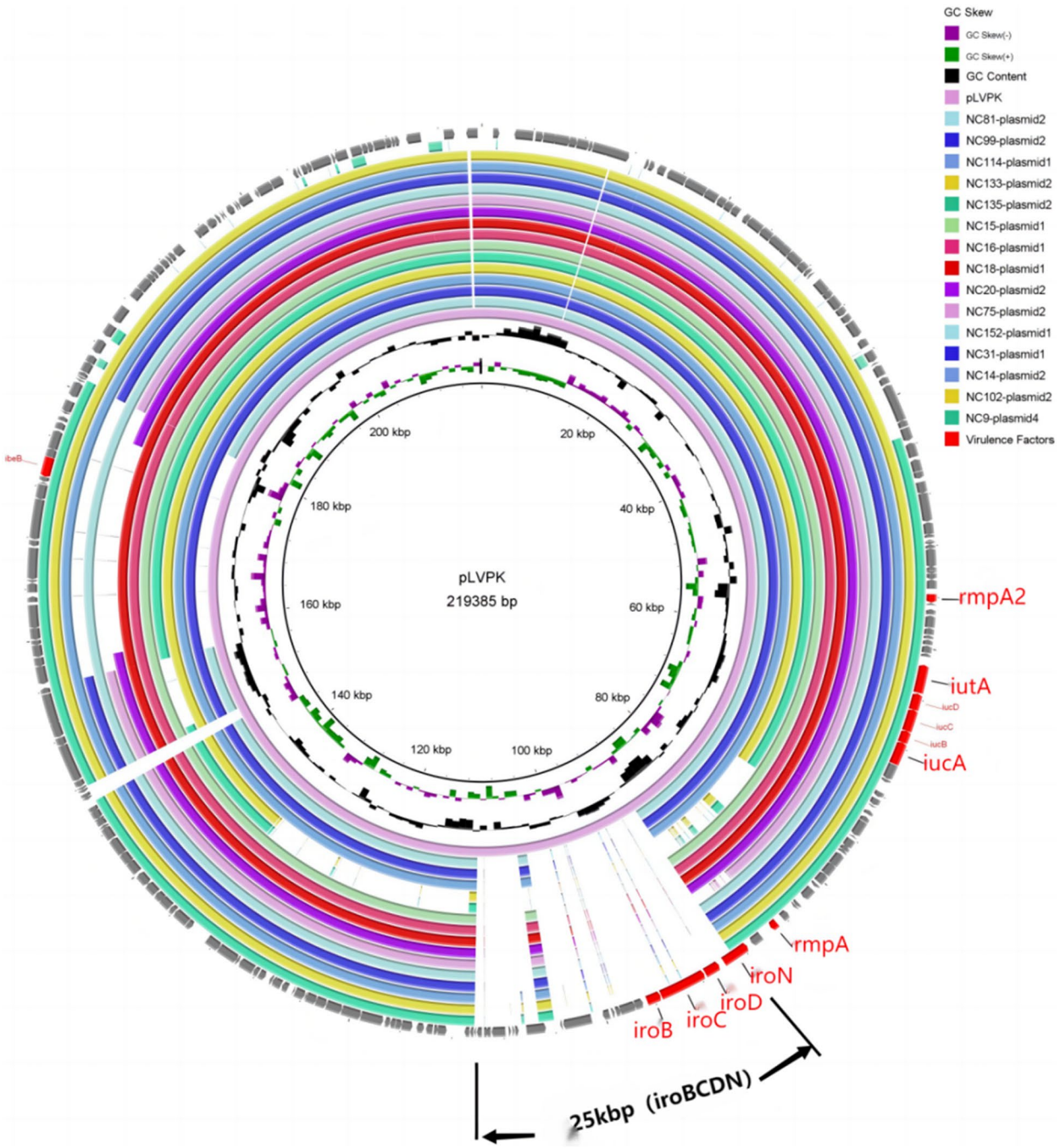

Fig. 4 Genetic comparison of virulence plasmids recovered from ST11-KL64 CR-hvKP with pLVPK plasmid in the NCBI database

Fig. 5 Structural comparison of the genetic context of virulence genes in representative pLVPK-like plasmids. Arrows indicate the direction of transcription of each gene, and different genes are shown in different colors. Regions of ≥ 90.0% nucleotide sequence identity are shaded gray

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12866-024-03564-2.

Supplementary Material 1.

Acknowledgements
We acknowledge the National Natural Youth Science Foundation of China (grant no. 82102411, 82200194); The Natural Science Key Project of Jiangxi Province (grant no. 20202ACBL206025 and 20202ACBL206023); the Project of Science and Technology Innovation Talents in Jiangxi (grant no. JSXQ2019201102); Jiangxi Provincial Department of Science and Technology (grant no. 20202ZDB01016); the Science and technology plan of Jiangxi Health Committee (grant no. SKJP-220212554); the Clinical Research Nurture Project of the First Affiliated Hospital of Nanchang University (grant no. YFYLCYJPY202001); and Youth Scientific Research Foundation of the First Affiliated Hospital of Nanchang University (grant no. YFYPY202115), and the Science and Technology Plan of Jiangxi Provincial Health Commission (grant no. 202130137).

Authors' contributions
HH, along with BH and YX, conducted experiments and wrote the manuscript. TX conceived and designed the research. DW, and QH analyzed the data. PL contributed to analytical tools. YL acquired the funding and revised the manuscript. All the authors contributed to the article and approved the final manuscript.

Funding
Not applicable.

Availability of data and materials
All sequencing data have been uploaded to NCBI, with the following accession numbers: PRJNA1139312, PRJNA1139306, PRJNA1139303, PRJNA1139259, PRJNA1139256, PRJNA1139243, PRJNA1139242, PRJNA1139239, PRJNA1139237, PRJNA1139232, PRJNA1139245, PRJNA1139252, PRJNA1139253, PRJNA1139309, PRJNA1139310.

Declarations

Ethics approval and consent to participate
Based on the rules of the Ethical Committee of our institute, this study did not require informed consent statement, because all isolates were recovered from clinical specimens during routine diagnostic procedures and these isolates were not specific to this study. In addition, the patients were not available to us. Based on the points mentioned above, the Ethical Committee of the First Affiliated Hospital of Nanchang University approved our project with reference number (2023)CDYFYYLK(01–011) and allowed this study to be conducted without informed consent statement.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Department of Clinical Laboratory, Medical Center of Burn Plastic and Wound Repair, The First Affiliated Hospital of Nanchang University, Nanchang University, Yong Wai Zheng Jie No. 17, Nanchang 330006, PR China. [2]School of Public Health, Jiangxi Medical College, Nanchang University, Bayi Avenue No. 461, Nanchang 330006, PR China. [3]Department of Infectious Diseases, The First Affiliated Hospital of Nanchang University, Nanchang University, Nanchang, Jiangxi 330006, PR China. [4]Department of Respiratory and Critical Care Medicine, The First Affiliated Hospital of Nanchang University, Nanchang University, Yong Wai Zheng Jie No. 17, Nanchang 330006, PR China. [5]China-Japan Friendship Jiang Xi Hospital, National Regional Center for Respiratory Medicine, Nanchang City, Jiangxi 330006, PR China. [6]Department of Laboratory, First People's Hospital of Jiujiang City, Taling South Road No.48, Jiujiang, Jiangxi Province 332000, PR China. [7]Jiangxi Medicine Academy of Nutrition and Health Management, Nanchang, Jiangxi 330006, PR China.

Received: 24 June 2024 Accepted: 3 October 2024
Published online: 10 October 2024

References

1. Russo TA, Marr CM: Hypervirulent Klebsiella pneumoniae. Clin Microbiol Rev 2019, 32(3). https://doi.org/10.1128/cmr.00001-19.
2. Catalán-Nájera JC, Garza-Ramos U, Barrios-Camacho H. Hypervirulence and hypermucoviscosity: two different but complementary Klebsiella spp. phenotypes? Virulence. 2017;8(7):1111–23. https://doi.org/10.1080/21505594.2017.1317412.
3. Wang Q, Wang X, Wang J, Ouyang P, Jin C, Wang R, Zhang Y, Jin L, Chen H, Wang Z, et al. Phenotypic and genotypic characterization of carbapenem-resistant enterobacteriaceae: data from a longitudinal large-scale CRE study in China (2012-2016). Clin Infect Dis. 2018;67(suppl_2):S196–s205. https://doi.org/10.1093/cid/ciy660.
4. Russo TA, Olson R, Fang CT, Stoesser N, Miller M, MacDonald U, Hutson A, Barker JH, La Hoz RM, Johnson JR: Identification of biomarkers for differentiation of hypervirulent Klebsiella pneumoniae from Classical K. pneumoniae. J Clin Microbiol 2018;56(9). https://doi.org/10.1128/jcm.00776-18.
5. Navon-Venezia S, Kondratyeva K, Carattoli A. Klebsiella pneumoniae: a major worldwide source and shuttle for antibiotic resistance. FEMS Microbiol Rev. 2017;41(3):252–75. https://doi.org/10.1093/femsre/fux013.
6. Lam MMC, Wyres KL, Wick RR, Judd LM, Fostervold A, Holt KE, Löhr IH. Convergence of virulence and MDR in a single plasmid vector in MDR Klebsiella pneumoniae ST15. J Antimicrob Chemother. 2019;74(5):1218–22. https://doi.org/10.1093/jac/dkz028.
7. Andrade LN, Curiao T, Ferreira JC, Longo JM, Clímaco EC, Martinez R, Bellissimo-Rodrigues F, Basile-Filho A, Evaristo MA, Del Peloso PF, et al. Dissemination of blaKPC-2 by the spread of Klebsiella pneumoniae clonal complex 258 clones (ST258, ST11, ST437) and plasmids (IncFII, IncN, IncL/M) among Enterobacteriaceae species in Brazil. Antimicrob Agents Chemother. 2011;55(7):3579–83. https://doi.org/10.1128/aac.01783-10.
8. Liu Y, Wan LG, Deng Q, Cao XW, Yu Y, Xu QF. First description of NDM-1-, KPC-2-, VIM-2- and IMP-4-producing Klebsiella pneumoniae strains in a single Chinese teaching hospital. Epidemiol Infect. 2015;143(2):376–84. https://doi.org/10.1017/s0950268814000995.
9. Chen YT, Chang HY, Lai YC, Pan CC, Tsai SF, Peng HL. Sequencing and analysis of the large virulence plasmid pLVPK of Klebsiella pneumoniae CG43. Gene. 2004;337:189–98. https://doi.org/10.1016/j.gene.2004.05.008.
10. Walker KA, Miner TA, Palacios M, Trzilova D, Frederick DR, Broberg CA, Sepúlveda VE, Quinn JD, Miller VL: A Klebsiella pneumoniae regulatory mutant has reduced capsule expression but retains hypermucoviscosity. mBio 2019;10(2).https://doi.org/10.1128/mBio.00089-19.
11. Liu Y, Liu PP, Wang LH, Wei DD, Wan LG, Zhang W. Capsular polysaccharide types and virulence-related traits of epidemic KPC-producing klebsiella pneumoniae isolates in a Chinese University Hospital. Microb Drug Resist. 2017;23(7):901–7. https://doi.org/10.1089/mdr.2016.0222.
12. Yao B, Xiao X, Wang F, Zhou L, Zhang X, Zhang J. Clinical and molecular characteristics of multi-clone carbapenem-resistant hypervirulent (hypermucoviscous) Klebsiella pneumoniae isolates in a tertiary hospital in Beijing, China. Int J Infect Dis. 2015;37:107–12. https://doi.org/10.1016/j.ijid.2015.06.023.
13. Dong N, Liu L, Zhang R, Chen K, Xie M, Chan EWC, Chen S. An IncR plasmid harbored by a hypervirulent carbapenem-resistant klebsiella pneumoniae strain possesses five tandem repeats of the bla(KPC-2)::NTE(KPC)-Id Fragment. Antimicrob Agents Chemother. 2019;63(3). https://doi.org/10.1128/aac.01775-18.
14. Du P, Zhang Y, Chen C. Emergence of carbapenem-resistant hypervirulent Klebsiella pneumoniae. Lancet Infect Dis. 2018;18(1):23–4. https://doi.org/10.1016/s1473-3099(17)30625-4.
15. Shu L, Dong N, Lu J, Zheng Z, Hu J, Zeng W, Sun Q, Chan EW, Zhou H, Hu F, et al. Emergence of OXA-232 carbapenemase-producing klebsiella pneumoniae that carries a plvpk-like virulence plasmid among elderly patients in China. Antimicrob Agents Chemother. 2019;63(3):10–128. https://doi.org/10.1128/aac.02246-18.

16. Wei DD, Wan LG, Deng Q, Liu Y. Emergence of KPC-producing Klebsiella pneumoniae hypervirulent clone of capsular serotype K1 that belongs to sequence type 11 in Mainland China. Diagn Microbiol Infect Dis. 2016;85(2):192–4. https://doi.org/10.1016/j.diagmicrobio.2015.03.012.
17. Solgi H, Shahcheraghi F, Bolourchi N, Ahmadi A: Molecular characterization of carbapenem-resistant serotype K1 hypervirulent Klebsiella pneumoniae ST11 harbouring bla(NDM-1) and bla(OXA-48) carbapenemases in Iran. Microb Pathog 2020;149:104507.https://doi.org/10.1016/j.micpath.2020.104507
18. Yang Q, Jia X, Zhou M, Zhang H, Yang W, Kudinha T, Xu Y. Emergence of ST11-K47 and ST11-K64 hypervirulent carbapenem-resistant Klebsiella pneumoniae in bacterial liver abscesses from China: a molecular, biological, and epidemiological study. Emerg Microbes Infect. 2020;9(1):320–31. https://doi.org/10.1080/22221751.2020.1721334.
19. Institute CaLS. Performance standards for antimicrobial susceptibility testing. 31st ed. Wayne (PA): Clinical and Laboratory Standards Institute; 2022.
20. Liao W, Long D, Huang Q, Wei D, Liu X, Wan L, Feng Y, Zhang W, Liu Y. Rapid Detection to Differentiate Hypervirulent Klebsiella pneumoniae (hvKp) From Classical K. pneumoniae by Identifying peg-344 With Loop-Mediated Isothermal Amplication (LAMP). Front Microbiol. 2020;11:1189. https://doi.org/10.3389/fmicb.2020.01189.
21. Fan LP, Yu Y, Huang S, Liao W, Huang QS, Du FL, Xiang TX, Wei DD, Wan LG, Zhang W, et al. Genetic characterization and passage instability of a novel hybrid virulence plasmid in a ST23 hypervirulent Klebsiella pneumoniae. Front Cell Infect Microbiol. 2022;12:870779. https://doi.org/10.3389/fcimb.2022.870779.
22. Huang QS, Liao W, Xiong Z, Li D, Du FL, Xiang TX, Wei D, Wan LG, Liu Y, Zhang W. Prevalence of the NTE(KPC)-I on IncF plasmids among hypervirulent Klebsiella pneumoniae isolates in Jiangxi Province, South China. Front Microbiol. 2021;12:622280.https://doi.org/10.3389/fmicb.2021.622280.
23. Zhou H, Zhang K, Chen W, Chen J, Zheng J, Liu C, Cheng L, Zhou W, Shen H, Cao X. Epidemiological characteristics of carbapenem-resistant enterobacteriaceae collected from 17 hospitals in Nanjing district of China. Antimicrob Resist Infect Control. 2020;9(1):15. https://doi.org/10.1186/s13756-019-0674-4.
24. Xie J, Chen Y, Cai G, Cai R, Hu Z, Wang H. Tree Visualization By One Table (tvBOT): a web application for visualizing, modifying and annotating phylogenetic trees. Nucleic Acids Res. 2023;51(W1):W587–w592. https://doi.org/10.1093/nar/gkad359.
25. Carver T, Thomson N, Bleasby A, Berriman M, Parkhill J. DNAPlotter: circular and linear interactive genome visualization. Bioinformatics. 2009;25(1):119–20. https://doi.org/10.1093/bioinformatics/btn578.
26. Sullivan MJ, Petty NK, Beatson SA. Easyfig: a genome comparison visualizer. Bioinformatics. 2011;27(7):1009–10. https://doi.org/10.1093/bioinformatics/btr039.
27. Lam MMC, Wick RR, Watts SC, Cerdeira LT, Wyres KL, Holt KE. A genomic surveillance framework and genotyping tool for Klebsiella pneumoniae and its related species complex. Nat Commun. 2021;12(1):4188. https://doi.org/10.1038/s41467-021-24448-3.
28. Wang J, Feng Y, Zong Z. Worldwide transmission of ST11-KL64 carbapenem-resistant Klebsiella pneumoniae: an analysis of publicly available genomes. mSphere. 2023;8(4):e0017323. https://doi.org/10.1128/msphere.00173-23.
29. Zhou Y, Wu C, Wang B, Xu Y, Zhao H, Guo Y, Wu X, Yu J, Rao L, Wang X, et al. Characterization difference of typical KL1, KL2 and ST11-KL64 hypervirulent and carbapenem-resistant Klebsiella pneumoniae. Drug Resist Updat. 2023;67:100918. https://doi.org/10.1016/j.drup.2023.100918.
30. Liu C, Du P, Xiao N, Ji F, Russo TA, Guo J. Hypervirulent Klebsiella pneumoniae is emerging as an increasingly prevalent K. pneumoniae pathotype responsible for nosocomial and healthcare-associated infections in Beijing, China. Virulence. 2020;11(1):1215–24. https://doi.org/10.1080/21505594.2020.1809322.
31. Zhou K, Xiao T, David S, Wang Q, Zhou Y, Guo L, Aanensen D, Holt KE, Thomson NR, Grundmann H, et al. Novel subclone of carbapenem-Resistant Klebsiella pneumoniae Sequence Type 11 with Enhanced Virulence and Transmissibility, China. Emerg Infect Dis. 2020;26(2):289–97. https://doi.org/10.3201/eid2602.190594.
32. Gu D, Dong N, Zheng Z, Lin D, Huang M, Wang L, Chan EW, Shu L, Yu J, Zhang R, et al. A fatal outbreak of ST11 carbapenem-resistant hypervirulent Klebsiella pneumoniae in a Chinese hospital: a molecular epidemiological study. Lancet Infect Dis. 2018;18(1):37–46. https://doi.org/10.1016/s1473-3099(17)30489-9.
33. Zhang Y, Jin L, Ouyang P, Wang Q, Wang R, Wang J, Gao H, Wang X, Wang H. Evolution of hypervirulence in carbapenem-resistant Klebsiella pneumoniae in China: a multicentre, molecular epidemiological analysis. J Antimicrob Chemother. 2020;75(2):327–36. https://doi.org/10.1093/jac/dkz446.
34. de Campos TA, Gonçalves LF, Magalhães KG, de Paulo Martins V, Pappas Júnior GJ, Peirano G, Pitout JDD, Gonçalves GB, Furlan JPR, Stehling EG, et al. A fatal bacteremia caused by hypermucousviscous KPC-2 producing extensively drug-resistant K64-ST11 Klebsiella pneumoniae in Brazil. Front Med (Lausanne). 2018;5:265. https://doi.org/10.3389/fmed.2018.00265.

Publisher's Note