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RAPID COMMUNICATION

# Characterization of a Novel Sequence Type (ST) 6758 *Klebsiella Pneumoniae* and the Role of IncX3 Plasmid in the Transmission of $bla_{NDM}$

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**Purpose:** Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) has emerged as a significant public health threat, particularly as a superbug responsible for nosocomial infections. In this study, we report a novel sequence type 6758 of *K. pneumoniae* harboring the $bla_{NDM-1}$ gene.

**Material and Methods:** Antimicrobial susceptibility testing was conducted according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI). The complete genome sequence of the strain was determined using the Illumina NovaSeq 6000 platform and long-read MinION sequencer. Genomic features and resistance mechanisms of the strain were further comprehensively analysed using various bioinformatics approaches.

**Results:** Antimicrobial susceptibility testing revealed that this strain exhibited resistance to multiple antimicrobials, including ceftazidime, ceftriaxone, cefazolin, cefepime, imipenem, meropenem, ampicillin/sulbactam, and sulfamethoxazole/trimethoprim. The genome analysis identified sixteen resistance genes. The $bla_{NDM-1}$ carbapenemase gene is located on a 47,823 bp IncX3-type plasmid (pNDM-CRKP331). A total of 41 *K. pneumoniae* strains carrying similar IncX3-type plasmids were retrieved from the NCBI database, representing 20 sequence types (STs) across 11 countries. The most common resistance gene carried by these IncX3-type plasmids is $bla_{NDM}$, and all these plasmids contain only the $bla_{NDM}$ gene. The $bla_{NDM}$-carrying IncX3-type plasmids are widely prevalent in *K. pneumoniae* in China, spanning 15 STs.

**Conclusion:** In summary, our study reports the first genome sequence of an ST 6758 *K. pneumoniae* strain containing the class B β-lactamase $bla_{NDM-1}$ isolated from a clinical sample. Given the global emergence of $bla_{NDM}$, measures should be taken to prevent the spread of these $bla_{NDM}$-carrying IncX3-type plasmids. Our findings contribute to the understanding of the transmission mechanisms of $bla_{NDM}$ in *K. pneumoniae*.

**Keywords:** carbapenem-resistant *Klebsiella pneumoniae*, $bla_{NDM-1}$, whole-genome sequencing, IncX3 type plasmid, ST6758

## Introduction

*Klebsiella pneumoniae*, as one of the most prevalent Gram-negative pathogens, is a significant source of both community-acquired and nosocomial infections.[1,2] The widespread use of antibiotics has led to the emergence of multidrug resistance (MDR) in *K. pneumoniae*, particularly the rise of carbapenem-resistant *K. pneumoniae* strains (CRKP). This trend poses a substantial threat to human health, as the options for treating CRKP infections dwindle, given the diminishing efficacy of available antibiotics.[3]

The first reported instances of CRKP date back to the 1990s.[4] Infection caused by carbapenem-resistant strains exhibit significantly higher mortality rates compared to those caused by carbapenem-susceptible strains.[5] In the United States and European countries, *K. pneumoniae* ST258 (sequence type) contributes significantly to the spread of carbapenem resistance, while ST11 is predominant in China.[6,7] The New Delhi metallo-β-lactamase-1 (NDM-1) was initially identified in *K. pneumoniae* and *Escherichia coli* in 2008 and has since become a global

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concern.[8] Due to the presence of mobile genetic elements such as transposons and plasmids, *K. pneumoniae* often plays a pivotal role in facilitating the transfer of antimicrobial resistance genes between environmental strains and clinical strains.[9] The IncX type plasmid is often associated with carbapenemase production, and the IncX3 plasmid is essential for the delivery of carbapenemase genes, especially $bla_{NDM}$.[10–13]

This study focuses on the genomic characterization of a new sequence type, ST6758 CRKP strain, isolated from an inpatient at a teaching hospital in China, which carries $bla_{NDM-1}$ on an IncX3 type plasmid. The complete genome sequence of this strain was determined, and a comprehensive analysis was conducted to examine its genomic features, plasmid characteristics, and resistance mechanisms. Furthermore, the transmission mechanisms of the carbapenemase gene $bla_{NDM-1}$ in *K. pneumoniae* were investigated.

# Material and Methods

## Patient and Isolate

On May 27, 2019, a 69-year-old male was admitted to a tertiary hospital in Zhejiang Province, China, presenting with dysuria. Clinical evaluations led to the diagnosis of prostatic hyperplasia and urinary retention. *K. pneumoniae* strain CRKP331 was isolated from the patient's urine sample on May 29. The strain was initially identified using the VITEK MS system (bioMérieux, France) and further validated by whole-genome sequencing. CRKP331 was susceptible to aminoglycoside antibiotics, and the aminoglycoside antibiotic isepamicin was used to treat the urinary tract infection. The patient eventually recovered.

## Antimicrobial Susceptibility Test

Antimicrobial susceptibility testing was conducted using the VITEK 2 system (bioMérieux, France) with Gram-negative antimicrobial susceptibility testing cards (AST-GN13) and the standard broth microdilution test, adhering to the guidelines established by the Clinical and Laboratory Standards Institute (CLSI). The antimicrobial agents tested in this study included amikacin, ceftazidime, ceftriaxone, ampicillin/sulbactam, sulfamethoxazole/trimethoprim, cefepime, gentamicin, imipenem, meropenem, levofloxacin, and tobramycin. Breakpoints were interpreted in accordance with the recommendations outlined in the CLSI guidelines.[14]

## Whole-Genome Sequencing

The complete genome sequence of the strain was determined utilizing the Illumina NovaSeq 6000 platform (Illumina Inc., San Diego, CA, USA) in the 150-bp paired-end sequencing mode, with an average sequencing depth of ≥100×. Furthermore, long-read sequencing was conducted using a MinION sequencer (Nanopore, Oxford, UK). The short Illumina reads and long MinION reads were subjected to hybrid assembly using Unicycler (v0.4.7) in conservative mode. This resulted in the generation of complete circular contigs, which were then refined and corrected using Pilon with Illumina reads through multiple rounds of iteration until no further changes were detected. The resulting complete genome sequence was subsequently annotated automatically using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) server.

## Genomic Analysis

A novel sequence type of *K. pneumoniae* was assigned utilizing the conserved segments of seven essential housekeeping genes (*rpoB, gapA, mdh, pgi, phoE, infB* and *tonB*) based on the BIGSdb-Pasteur MLST (Multilocus Sequence Typing) analysis (https://bigsdb.pasteur.fr/klebsiella/). The BacWGSTdb server was employed to investigate the strain's antimicrobial resistance genes and plasmid replicons.[15,16] A comparative analysis was conducted using circular representations, depicted as concentric rings, to assess the $bla_{NDM-1}$-carrying plasmid and its resemblance to similar plasmids. This analysis was performed using the BLAST Ring Image Generator (BRIG).[17]

## Phylogenetic Analysis

The phylogenetic tree was examined using CSI Phylogeny (version 1.4),[18] which is based on a core-genome single-nucleotide polymorphism (SNP) strategy. CSI Phylogeny was employed to identify and filter *K. pneumoniae* CRKP331

SNPs, validate the SNPs, and construct a phylogeny based on a concatenated alignment of the high-quality SNPs. The maximum parsimony algorithm was then used to generate a phylogenetic tree from the resulting SNPs, which was subsequently visualised on the iTOL webpage.[19]

## Nucleotide Sequence Accession Numbers

The whole genome results for strain CRKP331 have been deposited in DDBJ/EMBL/GenBank, and the accession number is SAMN40739512.

## Ethical Approval

This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Zhejiang Provincial People's Hospital (Ethics approval number 2019KY244). In this case, the Ethics Committee of Zhejiang Provincial People's Hospital granted an exemption from written informed consent because our study focused solely on bacteria. The clinical isolate *K. pneumoniae* CRKP331 was collected as part of the routine hospital laboratory procedures.

# Results and Discussion

The minimum inhibitory concentrations (MICs) of the tested antibiotics are shown in Table S1. *K. pneumoniae* CRKP331 exhibited resistance to various antibiotics, including ceftazidime, ceftriaxone, cefazolin, cefepime, imipenem, meropenem, ampicillin/sulbactam, and sulfamethoxazole/trimethoprim. However, it remained susceptible to amikacin, gentamicin, and tobramycin, and was classified as intermediate to levofloxacin.

The genome sequence of *K. pneumoniae* CRKP331 consists of five contigs, with a total length of 5,765,149 bp. Of these, one is a chromosome, measuring 5,317,817 bp, while the remaining four are plasmids. Contig 2 is 206,059 bp, contig 3 is 111,193 bp, contig 4 is 82,257 bp, and pNDM-CRKP331 is 47,823 bp in length. The novel sequence type of CRKP331 was classified as ST6758 through MLST analysis. This classification was achieved by analyzing the conserved segments of seven essential housekeeping genes (*rpoB4, gapA2, mdh1, pgi1, phoE4, infB4 and tonB174*).

The antimicrobial resistance genes identified in the genome of the isolate are presented in Table 1. We identified the β-lactam resistance genes $bla_{\text{SHV-187}}$, $bla_{\text{TEM-1B}}$, $bla_{\text{CTX-M-3}}$, and $bla_{\text{NDM-1}}$; the fosfomycin resistance gene *fosA*; the

**Table 1** Antimicrobial Resistance Genes (ARGs) in Isolate K. Pneumoniae CRKP331

| Antimicrobial Resistance Gene | Accession No. of Contig | Identity (%) | Position in Contig | Antimicrobial Resistance Category | Accession no. of Reference Gene |
|---|---|---|---|---|---|
| *oqxB* | 1 | 99.02 | 1,177,000.1180152 | Quinolone | EU370913 |
| *oqxA* | 1 | 99.15 | 1,180,176.1181351 | Quinolone | EU370913 |
| $bla_{\text{SHV-187}}$ | 1 | 99.08 | 2,632,897.2633763 | Beta-lactam | LN515533 |
| *fosA* | 1 | 94.29 | 4,574,451.4574870 | Fosfomycin | ACZD01000244 |
| *aac(6')-Ib-cr* | 2 | 100 | 112,203.112802 | Quinolone | DQ303918 |
| *ARR-3* | 2 | 99.19 | 112,859.113351 | Rifamycin | FM207631 |
| *dfrA27* | 2 | 100 | 113,484.113957 | Trimethoprim | FJ459817 |
| *aadA16* | 2 | 99.64 | 114,138.114983 | Aminoglycoside | EU675686 |
| *sul1* | 2 | 99.89 | 115,414.116280 | Sulphonamide | EU780013 |
| *mph(A)* | 2 | 99.67 | 120,475.121396 | Macrolide | U36578 |
| *tet(A)* | 2 | 100 | 123,553.124827 | Tetracycline | AF534183 |
| *floR* | 2 | 98.19 | 125,428.126641 | Phenicol | AF118107 |
| $bla_{\text{TEM-1B}}$ | 2 | 100 | 131,497.132357 | Beta-lactam | AY458016 |
| $bla_{\text{CTX-M-3}}$ | 2 | 100 | 133,139.134014 | Beta-lactam | Y10278 |
| *qnrS1* | 2 | 100 | 137,979.138635 | Quinolone | AB187515 |
| $bla_{\text{NDM-1}}$ | pNDM-CRKP331 | 100 | 33,266.34078 | Beta-lactam | FN396876 |

quinolone resistance genes *oqxB, oqxA, qnrS1* and *aac(6')-Ib-cr*; the trimethoprim resistance gene *dfrA27*; the rifamycin resistance gene *ARR-3*; the sulfonamide resistance gene *sul1*; the aminoglycoside resistance gene *addA16*; the tetracycline resistance gene *tet(A)*; the macrolide resistance gene *mph(A)* and the phenicol resistance gene *floR*.

With the exception of *oqxA, oqxB,* $bla_{\text{SHV-187}}$, and *fosA*, which are located on the chromosome, the remaining resistance genes are located on plasmids. A further analysis revealed the presence of *aac(6')-Ib-cr, ARR-3, dfrA27, aadA16, sul1, mph(A), tet(A), floR,* $bla_{\text{TEM-1B}}$, $bla_{\text{CTX-M-3}}$ and *qnrS1* on plasmid 2. In addition, the carbapenem resistance gene $bla_{\text{NDM-1}}$ was found to be located on plasmid pNDM-CRKP331. The $bla_{\text{NDM}}$-carrying plasmid pNDM-CRKP331 was designated as the IncX3-type plasmid. The similarity of pNDM-CRKP331 to other IncX3-type plasmids was analyzed using the basic local alignment search tool (Figure 1). These analogous plasmids were

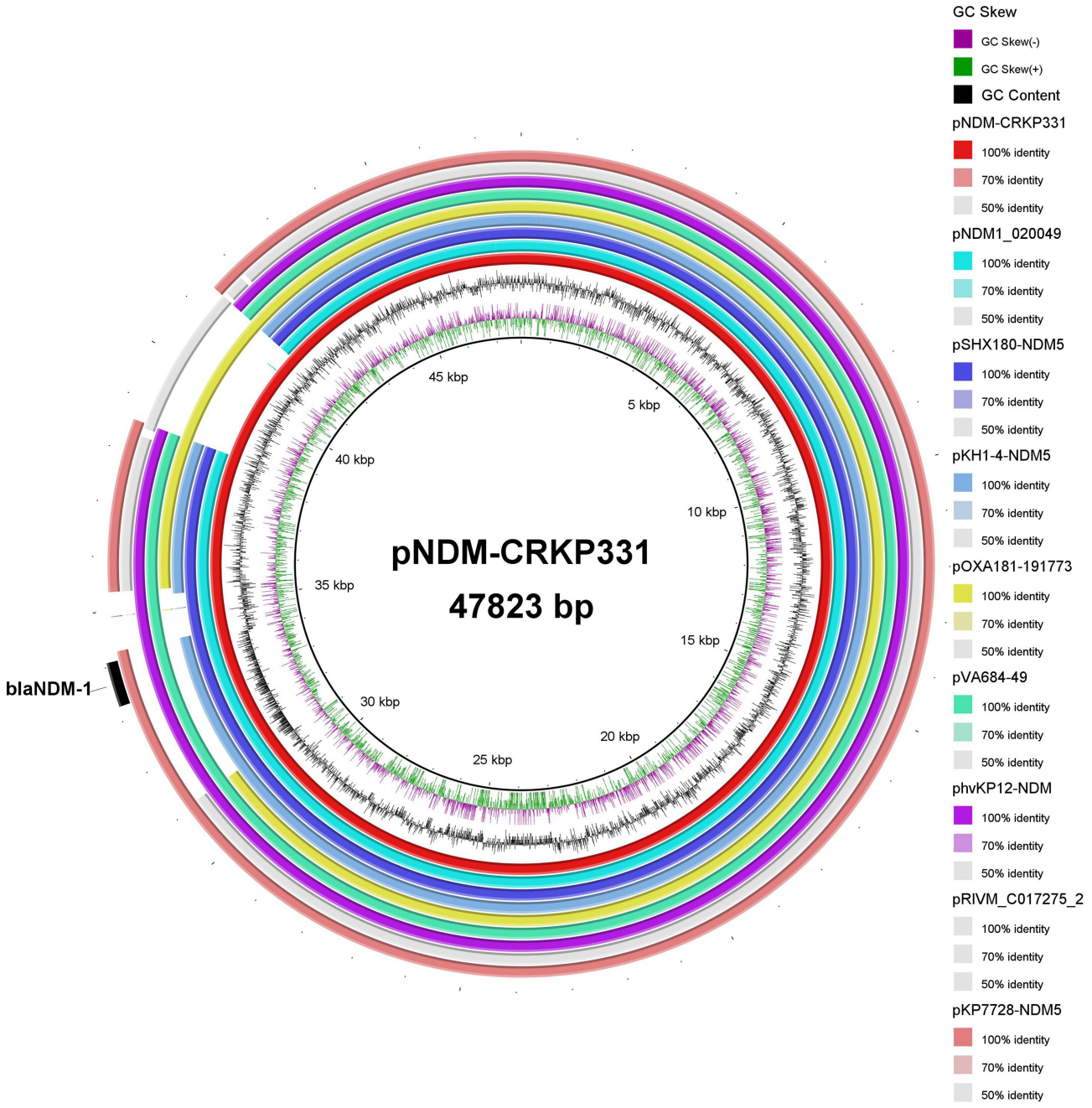


**Figure 1** Circular comparison between the $bla_{\text{NDM-1}}$-carrying plasmid pNDM-CRKP331 and similar plasmids.

all derived from *K. pneumoniae*, including pNDM1_020049 (accession no. CP028786), pSHX180-NDM5 (accession no. CP094514), pKH1-4-NDM5 (accession no. CP102881), pOXA181-191773 (accession no. CP080367), pVA684-49 (accession no. CP093461), hvKP12-NDM (accession no. CP103320), pRIVM_C017275_2 (accession no. CP068868), pKP7728-NDM5 (accession no. CP092650). All the other plasmids, with the exception of pOXA181-191773 and pRIVM_C017275_2, carry the $bla_{NDM}$ gene. This suggests that the IncX3 plasmid was an important vector for $bla_{NDM}$ gene transfer in *K. pneumoniae*. A variety of $bla_{NDM}$ sub-type were found on these IncX3 plasmid, which highlights the possibility that the IncX3 plasmid was one of the major platforms for $bla_{NDM}$ gene evolution.[20]

A simple annotation of the pNDM-CRKP331 plasmid was performed using the RAST Server. This revealed the presence of IS*6* and IS*Kox3* upstream of $bla_{NDM-1}$, while IS*Aba125* and IS*3000* were identified just downstream of $bla_{NDM-1}$. The ISFinder database was utilized to identify the IS element of pNDM-CRKP331. A total of twenty-two IS elements were identified in the plasmid, belonging to five IS families, including IS*6*, Tn*3*, IS*Kra4*, IS*L3*, and IS*30*. Among these, IS*6* (19/46) and Tn*3* (9/46) were the most prevalent. This suggests that the $bla_{NDM}$ gene may act as an external gene and recombine into the IncX3 plasmid via insertion or transposition of IS elements.

Using the Basic Local Alignment Search Tool (BLAST) with a threshold of 85% plasmid length and 80% coverage, 41 strains of *K. pneumoniae* were retrieved from the NCBI database, harboring IncX3-type plasmid similar to pNDM-CRKP331 (Table 2). Among these strains, twenty-two sequence types (STs) were identified, including ST1, ST11, ST14, ST15, ST16, ST17, ST23, ST35, ST43, ST258, ST307, ST340, ST427, ST437, ST485, ST505, ST512, ST656, ST766, ST1383, ST4523, and ST6758. The most common sequence types were ST11 (6/42), ST37 (6/42), and ST512 (5/41). These strains were distributed across 11 countries, with the highest prevalence in China (22 strains, 22/42), followed by the USA (5/42) and Italy (5/42). Phylogenetic analysis of the 42 strains is shown in Figure 2. The carbapenemase genes carried by these strains were predominantly $bla_{NDM}$ (25/42). Additionally, $bla_{KPC}$ was identified in 18 strains, including $bla_{KPC-2}$ and $bla_{KPC-3}$. Notably, five strains carried both $bla_{KPC}$ and $bla_{NDM}$. The CRKP331 strain demonstrates a unique spectrum of resistance genes. Nonetheless, it possesses a set of shared resistance genes with other strains, which confer resistance to β-lactams, aminoglycosides, fluoroquinolones, macrolides, tetracyclines, and sulfonamides. These observations highlight the imperative for customized therapeutic approaches and underscore the evolving and complex nature of antimicrobial resistance within *K. pneumoniae*.

We further investigated the IncX3-type plasmids in these strains. These plasmids predominantly carried single resistance genes (39/42), with a few (3/42) carrying two resistance genes. The resistance genes were primarily $bla_{NDM}$ (25/42), encompassing three subtypes: $bla_{NDM-1}$, $bla_{NDM-5}$, and $bla_{NDM-7}$. Three strains carried $bla_{KPC}$, all of which carried two resistance genes ($bla_{KPC}$ and $bla_{SHV}$). Additionally, three strains carried only $bla_{OXA-181}$. Some strains did not carry carbapenemase genes but instead carried an Extended Spectrum β-Lactamases (ESBLs) gene, such as $bla_{SHV-12}$ (5/42) or $bla_{SHV-182}$ (5/42). Notably, the five strains from Italy (all ST512) carried an IncX3 plasmid with only $bla_{SHV-182}$, while the five strains from the USA (all ST258) carried an IncX3 plasmid, with four strains carrying only $bla_{SHV-12}$ and one strain carrying both $bla_{KPC-3}$ and $bla_{SHV-12}$. Of the 22 strains from China, all carried the IncX3 plasmid, with 20 carrying $bla_{NDM}$ and the other two carrying $bla_{OXA-181}$. The IncX3 plasmid carrying the $bla_{NDM}$ gene has been widely prevalent in *K. pneumoniae* in China, involving 15 ST types (ST1, ST11, ST14, ST15, ST17, ST23, ST35, ST340, ST485, ST505, ST656, ST766, ST1383, ST4523, ST6758).

In conclusion, we report a new ST-type CRKP strain, ST6758, which carries 16 antimicrobial resistance genes, including $bla_{NDM}$. These resistance genes are primarily located on plasmids, with the carbapenemase gene $bla_{NDM-1}$ specifically located on an IncX3-type plasmid. A total of 41 *K. pneumoniae* strains carrying similar IncX3-type plasmids were retrieved from the NCBI database, representing 20 ST types across 11 countries. The most common resistance gene carried by these IncX3-type plasmids is $bla_{NDM}$, and all these plasmids carry only a single $bla_{NDM}$ gene. IncX3-type

**Table 2** Information of 41 Strains of K. Pneumoniae Retrieved from the NCBI Database That Harbouring Similar IncX3-Type Plasmid

| Assembly Accession | Strain | Country | Collection date | Isolation Source | Query Coverage | Percent Identity | Plasmid Length | ST Type | Antimicrobial Resistance Genes |
|---|---|---|---|---|---|---|---|---|---|
| GCA 000814305.1 | 34618 | USA | 2011 | Homo sapiens | 82% | 99.99% | 43380bp | 258 | $bla_{SHV-12}$ |
| GCA 001521895.1 | NUHL24835 | China | 2014 | Homo sapiens | 94% | 99.98% | 46161bp | 14 | $bla_{NDM-5}$ |
| GCA 001701845.2 | 20 GR 12 | Greece | 2012 | Homo sapiens | 82% | 99.99% | 43380bp | 258 | $bla_{SHV-12}$ |
| GCA 001902215.1 | MNCRE69 | USA | 2012 | Homo sapiens | 82% | 99.99% | 45288bp | 258 | $bla_{SHV-12}$ |
| GCA 001902235.1 | MNCRE53 | USA | 2012 | Homo sapiens | 82% | 99.99% | 45289bp | 258 | $bla_{SHV-12}$ |
| GCA 002852995.3 | SCKP020049 | China | 2016 | Homo sapiens | 94% | 99.99% | 54035bp | 1 | $bla_{NDM-1}$ |
| GCA 003054385.1 | SCM96 | China | 2017 | Homo sapiens | 94% | 99.98% | 46161bp | 15 | $bla_{NDM-1}$ |
| GCA 011769805.1 | 50595 | Czech Republic | 2019 | Homo sapiens | 91% | 99.98% | 51140bp | 11 | $bla_{OXA-181}$ |
| GCA 012970485.1 | B16KP0226 | South Korea | 2016 | Homo sapiens | 81% | 99.99% | 46835bp | 307 | $bla_{KPC-2}$, $bla_{SHV-106}$ |
| GCA 012971365.1 | F16KP0075 | South Korea | 2016 | Homo sapiens | 81% | 99.99% | 46836bp | 11 | $bla_{KPC-2}$, $bla_{SHV-182}$ |
| GCA 015353095.1 | 19110124 | China | 2019 | Swine | 94% | 99.98% | 46161bp | 340 | $bla_{NDM-5}$ |
| GCA 015775135.1 | ZG2017CW4-4-1-2 | China | 2017 | River | 94% | 99.98% | 49941bp | 11 | $bla_{NDM-5}$ |
| GCA 016772555.1 | 45706 | USA | 2013 | Homo sapiens | 82% | 99.99% | 43379bp | 258 | $bla_{SHV-12}$ |
| GCA 018623105.1 | KP32558 | China | 2020 | Homo sapiens | 94% | 99.97% | 46161bp | 656 | $bla_{NDM-5}$ |
| GCA 019443585.1 | K191773 | China | 2019 | Homo sapiens | 91% | 99.99% | 51479bp | 16 | $bla_{OXA-181}$ |
| GCA 019458505.1 | 1678 | China | 2021 | Homo sapiens | 94% | 99.99% | 46161bp | 485 | $bla_{NDM-5}$ |
| GCA 020459305.1 | C11 | China | 2018 | Homo sapiens | 94% | 99.99% | 54034bp | 1383 | $bla_{NDM-1}$ |
| GCA 021390015.1 | LZKP00001 | China | 2020 | Homo sapiens | 94% | 99.98% | 46162bp | 35 | $bla_{NDM-5}$ |
| GCA 021397585.1 | KP46 | China | 2017 | Homo sapiens | 92% | 99.98% | 53096bp | 15 | $bla_{NDM-1}$ |
| GCA 022453565.1 | KP7728 | China | 2021 | Homo sapiens | 91% | 99.99% | 54048bp | 17 | $bla_{NDM-5}$ |
| GCA 022649725.1 | WCHKP115038 | China | 2018 | Homo sapiens | 94% | 99.99% | 46161bp | 11 | $bla_{NDM-5}$ |
| GCA 023612215.1 | S01 | Australia | 2016 | Homo sapiens | 91% | 99.99% | 54048bp | 15 | $bla_{NDM-7}$ |
| GCA 024226755.1 | LH13-d | Switzerland | 2022 | Gallus gallus domesticus | 80% | 99.95% | 46338bp | 427 | $bla_{NDM-1}$ |
| GCA 024396875.1 | KP82 | China | 2013 | Homo sapiens | 94% | 99.99% | 54035bp | 437 | $bla_{NDM-5}$ |
| GCA 024637895.1 | SHX180 | China | 2018 | Homo sapiens | 94% | 99.99% | 53065bp | 4523 | $bla_{NDM-5}$ |
| GCA 024734155.1 | KH1 | China | 2019 | Homo sapiens | 91% | 99.98% | 52085bp | 37 | $bla_{NDM-5}$ |
| GCA 024734195.1 | KW2 | China | 2019 | Well water | 94% | 99.98% | 46161bp | 766 | $bla_{NDM-5}$ |
| GCA 024762135.1 | hvKP12 | China | 2021 | Homo sapiens | 94% | 99.99% | 46161bp | 11 | $bla_{NDM-5}$ |
| GCA 025677745.1 | BSIKPN-11 | China | 2017 | Homo sapiens | 94% | 99.98% | 46161bp | 11 | $bla_{NDM-5}$ |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| GCA 026247925.1 | LC-1873/18 | Italy | 2018 | Homo sapiens | 82% | 99.99% | 43380bp | 512 | $bla_{SHV-182}$ |
| GCA 026247945.1 | LC-79/19 | Italy | 2019 | Homo sapiens | 82% | 99.99% | 43380bp | 512 | $bla_{SHV-182}$ |
| GCA 026247965.1 | LC-394/19 | Italy | 2019 | Homo sapiens | 82% | 99.99% | 43380bp | 512 | $bla_{SHV-182}$ |
| GCA 026247985.1 | LC-395/19 | Italy | 2019 | Homo sapiens | 82% | 99.99% | 43380bp | 512 | $bla_{SHV-182}$ |
| GCA 026248005.1 | LC-422/19 | Italy | 2019 | Homo sapiens | 82% | 99.99% | 43380bp | 512 | $bla_{SHV-182}$ |
| GCA 028472725.1 | SCKP090351 | China | 2019 | Homo sapiens | 94% | 99.99% | 54035bp | 23 | $bla_{NDM-1}$ |
| GCA 028554845.1 | S5 CRE5a | USA | 2017 | Homo sapiens | 82% | 99.99% | 53321bp | 258 | $bla_{KPC-3}$, $bla_{SHV-12}$ |
| GCA 029623595.1 | GIMC1009:Kpn-52ICU-2H | Russia | 2021 | Homo sapiens | 80% | 99.99% | 48359bp | 307 | $bla_{NDM-1}$ |
| GCA 029834605.1 | IITJ BC16 | India | 2021 | Homo sapiens | 87% | 99.99% | 46612bp | 16 | $bla_{NDM-5}$ |
| GCA 030061645.1 | VA684 | Chile | 2019 | Homo sapiens | 94% | 99.99% | 48523bp | 505 | $bla_{NDM-7}$ |
| GCA 030544725.1 | T877-PC | China | 2021 | Homo sapiens | 91% | 99.99% | 51479bp | 43 | $bla_{OXA-181}$ |
| GCA 030552975.1 | KP9 | China | 2021 | Homo sapiens | 94% | 99.98% | 46162bp | 37 | $bla_{NDM-5}$ |



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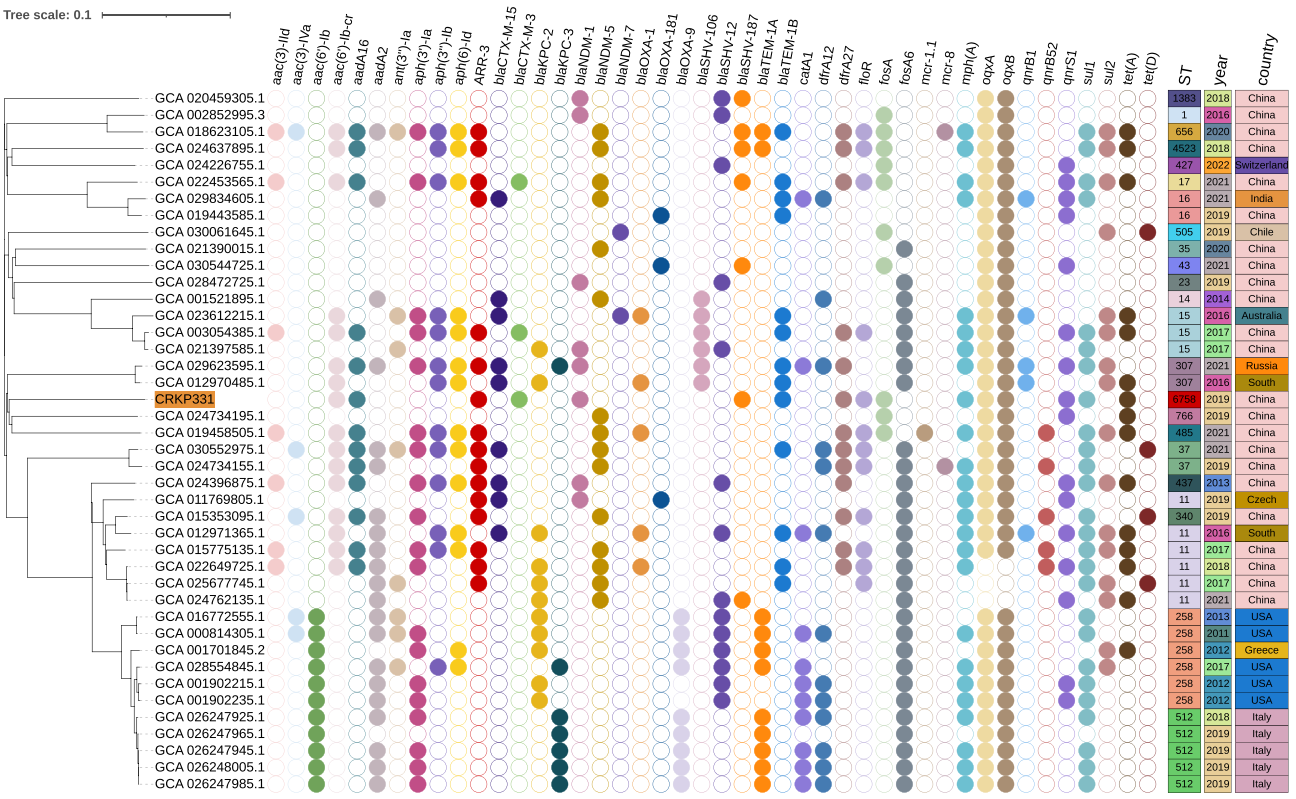


**Figure 2** Phylogenetic analyses of CRKP331 and 41 *K. pneumoniae* strains carrying an IncX3-type plasmid similar to pNDM-CRKP331. The antimicrobial resistance genes are represented by different colours in the cells, whereas the gene is absent in the empty cells. Each square colour indicates a specific sequence type.

plasmids carrying the $bla_{\mathrm{NDM}}$ gene are widely prevalent in *K. pneumoniae* in China, spanning 15 ST types. Measures should be taken to prevent the spread of these $bla_{\mathrm{NDM}}$-carrying IncX3-type plasmids. Our findings contribute to the understanding of the transmission mechanisms of carbapenemase genes in *K. pneumoniae*.

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## Disclosure

The author reports no conflicts of interest in this work.

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