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SHORT REPORT

# Genome Characteristic of NDM-5-Producing *Klebsiella Quasipneumoniae* and *Klebsiella Pneumoniae* in China

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**Abstract:** The global dissemination of carbapenem-resistant Enterobacteriaceae poses a significant threat to public health. In this study, we identified two clinical *Klebsiella* isolates, *K. quasipneumoniae* ACESH02628 and *K. pneumoniae* ACESH02857, harboring the $bla_{NDM-5}$ gene in China. Both strains exhibited multidrug resistance, including reduced susceptibility to carbapenems, and carried transferable NDM-5-bearing plasmids. Specifically, S1-pulsed-field gel electrophoresis (PFGE), southern blotting, and whole-genome sequencing revealed that ACESH02628 contained an IncHI2/IncHI2A-type plasmid, while ACESH02857 carried an IncX3-type plasmid, both associated with the regional spread of NDM-5. Comparative analyses showed high genetic similarity with previously reported NDM-5 plasmids from *Salmonella enterica* and *Escherichia coli* in China, underscoring the ease of horizontal transfer and potential for broader dissemination. Our findings highlight the emergence of NDM-5-producing *K. quasipneumoniae* of clinical origin and reinforce the need for vigilant surveillance and infection control measures to curb the proliferation of these highly resistant pathogens.

**Keywords:** $bla_{NDM-5}$, *Klebsiella spp*., carbapenem-resistant, whole-genome sequencing

## Introduction

Antimicrobial resistance (AMR) is considered a major threat to public health and modern healthcare worldwide.[1] It is estimated that more than ten million deaths will be caused by antibiotic-resistant bacterial infections by 2050.[2] The prevalence of carbapenem-resistant *Klebsiella* spp. (CRK) has the potential to cause a global health crisis because of its resistance to carbapenems, which are a last-resort antibiotic used against gram-negative bacteria. Among the various carbapenemases, NDM is one of the most clinically significant owing to its increased resistance and rapid dissemination. Compared to NDM-1, NDM-5 conferred higher carbapenem resistance. Moreover, NDM-5 is primarily endemic to *K. pneumoniae* in *Klebsiella* spp. However, two studies have reported *Klebsiella quasipneumoniae*-related NDM-5 events. Lauren et al identified an NDM-5-carrying *K, quasipneumoniae* strain in neonates in a Nigerian hospital.[3] Yang et al investigated the characteristics of NDM-5-bearing *K. quasipneumoniae* in the animal breeding area based on the perspective of "One-Health".[4]

These reports highlight that the genetic background supporting NDM-5 dissemination can be diverse and complex, involving various plasmid types and resistance determinants. Such complexity not only challenges infection control and

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treatment strategies but also emphasizes the importance of understanding the molecular epidemiology and host range of NDM-5-bearing plasmids.[5]

In this study, we describe two NDM-5-producing *Klebsiella* spp. strains isolated from the same hospital in China. Through genomic characterization, antimicrobial susceptibility testing, and analyses of plasmid transmission mechanisms, we aim to provide further insights into the evolving epidemiology of NDM-5-producing *Klebsiella* and inform strategies to mitigate their spread.

# Methods

## Strains Isolation and Antimicrobial Susceptibility Testing

Isolates were collected from the Antimicrobial Resistance Comprehensive Etiology Study (ACES) Development Project and were isolated from the stool samples of patients from the Neurosurgery and Surgery departments at a tertiary hospital in Pingguo, Guangxi Province, China, during routine screening for carbapenem-resistant bacteria in July 2021. All isolates used in this study were handled in compliance with the Declaration of Helsinki. Patient-related data were anonymized to ensure privacy, and the study was approved by the ethics committee of the First Affiliated Hospital of Zhejiang University (approval number: No. 2021-IIT-631). Species identification was performed using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF/MS) (Bruker Daltonik GmbH, Bremen, Germany).

The susceptibility of *K. quasipneumoniae* ACESH02628, *K. pneumoniae* ACESH02857, and its transconjugants to antibiotics was determined using the agar dilution method, except for colistin and tigecycline, which was performed using the broth microdilution method.[6] The results were interpreted according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) and the European Committee for Antimicrobial Susceptibility Testing (EUCAST) guidelines. *Pseudomonas aeruginosa* ATCC27853 and *Escherichia coli* ATCC25922 were used as the quality controls.

## Plasmid Analysis and Conjugation Experiments

The number and size of plasmids in *K. quasipneumoniae* ACESH02628 and *K. pneumoniae* ACESH02857 were detected using S1-PFGE. *Salmonella enterica* serotype Braenderup H9812 was used as the size marker. The location of NDM-5 was determined by Southern blotting and hybridization with a digoxigenin-labeled NDM-5 specific probe. The transferability of $bla_{\text{NDM-5}}$-bearing plasmids was confirmed by conjugation experiments. Briefly, rifampin-resistant *P. aeruginosa* PAO1Ri was used as a recipient. Transconjugants were selected on Mueller–Hinton medium containing 200 mg/L rifampicin and 2 mg/L meropenem. Subsequently, they were identified using MALDI-TOF/MS and polymerase chain reaction (PCR).

## Whole-Genome Sequencing and in silico Analysis

Total DNA of ACESH02628 and ACESH02857 was extracted using a Bacterial DNA Kit (QIAGEN, Hilden, Germany). DNA sequencing was performed using both Illumina NovaSeq 6000 (Illumina, San Diego, CA, United States) and Oxford Nanopore (Oxford Nanopore Technologies, Oxford, United Kingdom) platforms, to produce high-quality short reads (usually 150 bp paired-end) and long-read data. Complete genome assembly was performed using Spades 3.10.1 and Unicycler 0.4.7 with default parameters.[7,8] The whole genome was annotated using Prokka.[9] Antibiotic resistance genes, plasmid replicon types, and MLST have been identified in the ResFinder, PlasmidFinder, and Pasteur MLST databases, respectively.[10,11] The plasmids were aligned by the Basic Local Alignment Search Tool (BLAST) of the National Center for Biotechnology Information (NCBI) with default settings. $bla_{\text{NDM-5}}$-carrying plasmid comparison images were visualized using the BLAST Ring Image Generator.[12]

# Results and Discussion

*K. quasipneumoniae* strains ACESH02628 and *K. pneumoniae* strain ACESH02857 were recovered from stool samples of different inpatients in July 2021, in Pingguo, China. Based on the MLST results, ACESH02628 and ACESH02857 belong to ST5309 and ST485, respectively. *K. quasipneumoniae* strain ACESH02628 was resistant to amoxicillin/

clavulanic acid (MIC = 64/32 mg/L), piperacillin/tazobactam (MIC > 128/4 mg/L), ceftazidime (MIC > 128 mg/L), ceftriaxone (MIC > 128 mg/L), cefepime (MIC = 16 mg/L), cefotaxime (MIC > 128 mg/L), imipenem (MIC = 8 mg/L), meropenem (MIC = 4 mg/L), ceftazidime/avibactam (MIC > 128/4 mg/L), and trimethoprim/sulfamethoxazole (MIC > 8/152 μg/mL). However, this strain was susceptible to ciprofloxacin (MIC = 0.25 mg/L), gentamicin (MIC = 0.5 mg/L), amikacin (MIC = 2 mg/L), aztreonam (MIC = 0.25 mg/L), fosfomycin (MIC = 4 mg/L), and tigecycline (MIC = 0.25 mg/L) (Table 1). Similarly, AST revealed that *K. pneumoniae* strain ACESH02857 was resistant to multiple antibiotics, including amoxicillin/clavulanate (MIC = 32 mg/L), piperacillin/tazobactam (MIC > 128/4 mg/L), ceftazidime (MIC > 128 mg/L), ceftriaxone (MIC > 128 mg/L), cefepime (MIC = 16 mg/L), cefotaxime (MIC > 128 mg/L), ciprofloxacin (MIC > 64 mg/L), levofloxacin (MIC > 64 mg/L), imipenem (MIC = 8 mg/L), ceftazidime/avibactam (MIC > 128/4 mg/L), and trimethoprim/sulfamethoxazole (MIC > 8/152 mg/L). The strain was only found to be susceptible to amikacin (MIC = 2 mg/L), gentamicin (MIC = 0.5 mg/L), aztreonam (MIC = 1 mg/L), fosfomycin (MIC = 4 mg/L) and tigecycline (MIC = 0.5 mg/L) and intermediate to polymyxin (MIC = 0.5 mg/L) and meropenem (MIC = 2 mg/L). According to the ResFinder results, both ACESH02628 and ACESH02857 harbored various ARGs, including $bla_{\text{NDM-5}}$, *ant(3")-Ia,* $bla_{\text{OXA-10}}$, *cmlA1,* $bla_{\text{TEM-1B}}$, *sul3, dfrA14, oqxB, oqxA, qnrS1, tet(A), arr-3*, and *fosA*, all of which confer multidrug resistance, increasing the difficulty of clinical treatment. Additionally, *K. pneumoniae* ACESH02857 carried $bla_{\text{SHV-110}}$, an extended-spectrum β-lactamase (ESBL).

S1-PFGE and Southern blotting indicated that NDM-5 was located on ~239 kb and ~46 kb plasmids (designated as p2628_NDM and p2857_NDM, respectively) (Figure 1A). In silico analysis confirmed that plasmid p2628_NDM belongs to the IncHI2/IncHI2A type, a prevalent plasmid type among NDM-5-bearing isolates.[13–15] The transconjugant ACESH02628-P received p2628_NDM with an MIC of 8 mg/L imipenem, suggesting that the IncHI2/IncHI2A p2628_NDM plasmid is both conjugative and responsible for carbapenem resistance. The transferability of plasmids increases the risk of the transmission of antimicrobial resistance. By searching the NCBI database, p2628_NDM showed high genetic similarity to the plasmids p23045-NDM5 (OR497833.1) and pHNBYF33-1 (CP101733.1) from *Salmonella enterica* and *E. coli*, respectively (Figure 1). Notably, all the strains isolated from China were found to be prevalent in this region. The genetic environment demonstrated a conserved structural sequence, *hin-xerC-rutD-ant1-trpF-ble-*$bla_{\text{NDM-5}}$*-hcaB-sul3-emrE-ant(3")-Ia-cmlA1-umuD*-IS*Kox3*-IS*3000*-IS*Vsa5* surrounding NDM-5 in these plasmids. Our

**Table 1** Antimicrobial Drug Susceptibility Profiles

| Antibiotic | MIC (mg/L)/antimicrobial susceptibility | | | |
|---|---|---|---|---|
| | ACESH02628 | ACESH02628-P | ACESH02857 | ACESH02857-P |
| Amoxicillin/Clavulanic acid | 64/R | >128/R | 32/R | >128/R |
| Piperacillin/Tazobactam | >128/R | 2/S | >128/R | 2/S |
| Ceftazidime | >128/R | 4/S | >128/R | 4/S |
| Ceftriaxone | >128/R | 4/R | >128/R | 4/R |
| Cefepime | 16/R | 8/R | 16/R | 16/R |
| Cefotaxime | >128/R | 8/R | >128/R | 8/R |
| Ciprofloxacin | 0.25/S | 1/R | >64/R | 1/R |
| Levofloxacin | 1/I | 1/I | >64/R | 1/I |
| Imipenem | 8/R | 8/R | 8/R | 8/R |
| Meropenem | 4/R | 1/S | 2/I | 1/S |
| Ceftazidime/Avibactam | >128/R | 1/S | >128/R | 1/S |
| Trimethoprim/Sulfamethoxazole | >8/R | 8/R | >8/R | 8/R |
| Amikacin | 2/S | 2/S | 2/S | 2/S |
| Gentamicin | 0.5/S | 1/S | 0.5/S | 1/S |
| Aztreonam | 0.25/S | 2/S | 1/S | 2/S |
| Fosfomycin | 4/S | 64/S | 4/S | 64/S |
| Tigecycline | 0.25/S | 2/I | 0.5/S | 1/I |
| Polymixin | 0.5/I | 1/I | 0.5/I | 1/I |

**Note**: S, susceptible; R, resistant; I, intermediate.


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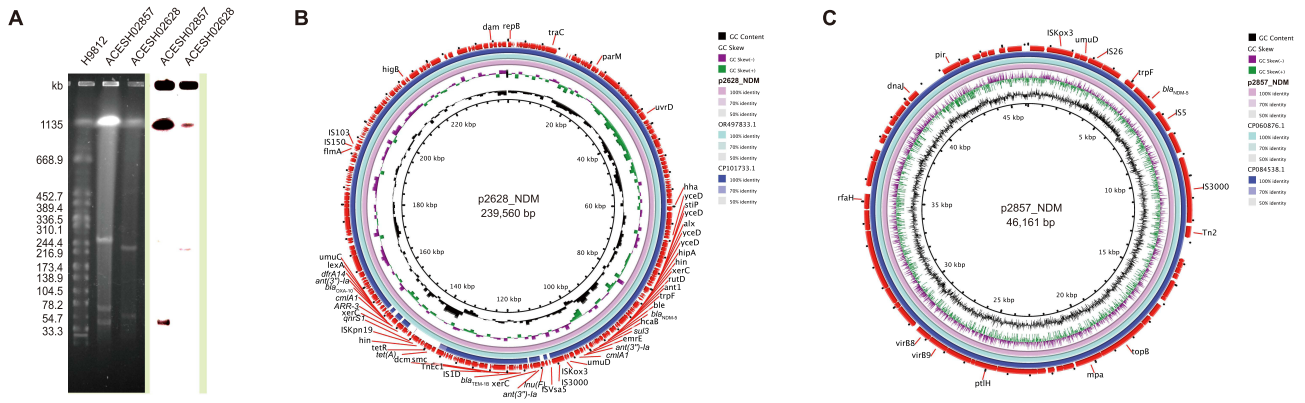


**Figure 1** The plasmid profiles of *K. quasipneumoniae* ACESH02628 and *K. pneumoniae* ACESH02857. (**A**) Southern blot-hybridization of S1-nuclease digested DNA using a specific probe ($bla_{NDM}$). Marker: *Salmonella enterica* serotype Braenderup H9812. (**B**) Genomic map of the $bla_{NDM-5}$ producing IncHI2/IncHI2A p2628_NDM plasmid with three closely related plasmids (OR497833.1 and CP101733.1). (**C**) Genomic map of the $bla_{NDM-5}$ producing IncX3 p2857_NDM plasmid with three closely related plasmids (CP060876.1 and CP084538.1). ORFs are portrayed by arrows and colored according to their putative functions. The alignment of the plasmids was performed and visualized by BLAST ring image generator (BRIG) software.

study further highlights the emergence of NDM-5-carrying *K. quasipneumoniae* of clinical origin in China and emphasizes that an effective strategy should be adopted to prevent further dissemination.

Whole-genome sequencing confirmed that the plasmid p2857_NDM belonged to the IncX3 type plasmid, with a length of 46,161 bp and an average GC content of 46.6%. NCBI BLAST analysis further revealed that p2857_NDM shared the highest similarity (100% identity) with *K. pneumoniae* plasmid pIncX3 (CP080676.1) and *E. coli* plasmid p399-4 (CP084538.1). A conserved genetic context around $bla_{NDM-5}$ (IS*Kox3*-*umuD*-IS*26*-*trpF*-$bla_{NDM-5}$-IS*5*-IS*3000*-Tn*2*) was identified in p2857_NDM. Previous reports have indicated that IncX3 plasmids are prevalent in *E. coli*, followed by *K. pneumoniae*.[16,17] NDM has rapidly disseminated in China, primarily because of the national spread of the NDM-bearing IncX3 plasmid.[18] Our study stressed that the IncX3 plasmid might accelerate the occurrence of the clinical origin of *K. pneumoniae* harboring NDM-5 isolates in China.

In summary, our study documents the emergence of NDM-5-producing *K. quasipneumoniae* and *K. pneumoniae* isolates recovered from stool samples in China, providing a critical snapshot of the evolving resistance landscape in this region. To date, much of the surveillance data on resistance gene prevalence in specific regions of China are derived from larger-scale national surveys or comprehensive AMR monitoring programs,[19] rather than localized studies focused on *K. quasipneumonia*e.[20] Our current work contributes to filling this knowledge gap by highlighting the presence of NDM-5-carrying isolates within a particular clinical setting. The identification of an IncHI2/IncHI2A-type plasmid carrying the NDM-5 gene in *K. quasipneumoniae* is particularly noteworthy, as it expands our understanding of the plasmid types associated with this resistance determinant and suggests a broader host range than previously recognized. Furthermore, the persistence and dissemination of NDM-5-bearing IncX3 plasmids highlight their role in facilitating the regional spread of carbapenem resistance among Enterobacteriaceae. These findings underscore the urgent need for enhanced infection control measures, robust surveillance, and targeted interventions to limit the further proliferation of multidrug-resistant organisms in both hospital and community settings. By situating our results within the context of regional and global antimicrobial resistance trends, our work contributes valuable epidemiological insights that may guide future research, inform policy decisions, and support the development of more effective strategies to combat the ongoing threat of carbapenem-resistant pathogens.

Nonetheless, we acknowledge that drawing more explicit connections to broader regional patterns would strengthen our manuscript. In the revised version, we plan to incorporate references to recent large-scale AMR surveillance reports and studies that detail the regional dissemination of NDM-5 and related resistance determinants in China. By doing so, we aim to provide a more substantial regional context, thus emphasizing the relevance and urgency of our findings. We trust that this additional information will better illustrate how our results fit into, and potentially inform, the ongoing efforts to understand and mitigate the spread of multidrug-resistant organisms in China.

## Ethics Approval and Consent to Participate

The authors declare that they have no conflicts of interest. This study was conducted in accordance with the Declaration of Helsinki and approved by the Clinical Research Ethics Committee of The First Affiliated Hospital, Zhejiang University School of Medicine [No. 2021-IIT-631].

## Data Sharing Statement

The complete genomes of *K. quasipneumoniae* ACESH02628 and *K. pneumoniae* ACESH02857 were deposited at NCBI under the biosamples SAMN41221222 and SAMN41221227, respectively.

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## Disclosure

The authors report no conflicts of interest in this work.

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