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CASE REPORT

Case Report of *bla*$_{NDM-7}$-Harboring IncX3 Plasmid in ST196 *Klebsiella quasipneumoniae* in China

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Abstract: *Klebsiella quasipneumoniae* isolate SBH035 was recovered from a patient in Jiangsu Province, China. The isolate showed resistance to ampicillin, cefazolin, cefotaxime, meropenem, ceftazidime–avibactam, and fosfomycin. The carbapenemase-encoding gene *bla*$_{NDM-7}$ was identified, and whole genome sequencing analysis indicated that *bla*$_{NDM-7}$ was located in an IncX3 plasmid with a conserved structure of IS*26*-Δ*cutA*-*tat*-*trpF*-*ble*$_{MBL}$-*bla*$_{NDM-7}$-IS*Aba125*-IS*3000*-ΔTn2. To date, this is the first identification of a *bla*$_{NDM-7}$-harboring IncX3 plasmid in ST196 *K. quasipneumoniae* from a patient in China. Greater attention to controlling the dissemination of IncX3 plasmids is needed owing to potential horizontal transfer via mobile genetic elements.

Keywords: *Klebsiella quasipneumoniae*, ST196, carbapenem resistance, *bla*$_{NDM-7}$, IncX3 plasmid

Plain Language Summary

- We observed carbapenem resistance in ST196 *Klebsiella quasipneumoniae*.
- The *bla*$_{NDM-7}$ gene was located in a conjugative IncX3 plasmid associated with a commonly observed core structure.
- The *bla*$_{NDM-7}$ gene can confer ceftazidime–avibactam resistance.

Carbapenemase-producing *Enterobacterales*, especially New Delhi metallo-β-lactamase (NDM) enzyme-producing strains, has become an increasing threat to public health. Since *bla*$_{NDM-1}$ was first reported in 2009, 40 NDM allelic variants have been identified so far (https://www.ncbi.nlm.nih.gov/pathogens/refgene/#; last accessed March 2, 2022). As a member of the *Klebsiella pneumoniae* complex, *K. quasipneumoniae* has also become a cause for concern. To date, *bla*$_{NDM-1}$,[1,2] *bla*$_{NDM-5}$,[3,4] *bla*$_{KPC-2}$,[5,6] and *bla*$_{KPC-3}$[6] have been identified in *K. quasipneumoniae* and IncX3, IncX5, IncX6, and IncF are the main plasmids accounting for carbapenem resistance. ST196 *K. quasipneumoniae* has rarely been reported but can be present in health care centers worldwide, possibly leading to hospital-acquired infections.[7]

In 2013, *bla*$_{NDM-7}$ was identified for the first time in *Escherichia coli* from a patient who had traveled to Burma and was hospitalized in France.[8] Owing to Asp-130-Asn and Met-154-Leu substitutions, NDM-7 has greater carbapenem-hydrolyzing activity than NDM-1.[9] Subsequently, plasmid-mediated *bla*$_{NDM-7}$ has been found in different species such as *K. pneumoniae*,[10] *Enterobacter cloacae*,[11] *E.* aerogenes,[12] and *Citrobacter freundii*.[13] Here, we report the first detection of *K. quasipneumoniae* harboring *bla*$_{NDM-7}$ isolated from a patient in Jiangsu Province, China.

K. quasipneumoniae isolate SBH035 was recovered from a urine sample collected from a 67-year-old male patient in a tertiary hospital of Jiangsu Province in July 2018. Species identification was performed using the VITEK® MS system (bioMérieux, Marcy-l'Étoile, France). Antibiotic susceptibility testing against 17 antibiotics was performed using the agar dilution method or broth microdilution method (limited to colistin and tigecycline). The results were interpreted according to guidelines of the Clinical and Laboratory Standards Institute or European Committee on Antimicrobial Susceptibility Testing. The isolate was resistant to ampicillin, cefazolin, cefotaxime, meropenem, ceftazidime–avibactam,

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and fosfomycin but was susceptible to streptomycin, gentamicin, amikacin, tetracycline, tigecycline, chloramphenicol, florfenicol, nalidixic acid, ciprofloxacin, trimethoprim/sulfamethoxazole, and colistin (Table S1). Carbapenemase-encoding genes were further detected, as previously described,[14] and the $bla_{\text{NDM-7}}$ gene was identified.

Whole genome sequencing of SBH035 was performed using the Illumina NovaSeq platform (Illumina Inc., San Diego, CA, USA). The reads were assembled using SPAdes v. 3.10.0 and 85 contigs (>200 base pairs; bp) were obtained (GenBank accession no. PRJNA823891). The Center for Genomic Epidemiology pipeline (https://cge.cbs.dtu.dk/) was used to identify sequence type (ST), antimicrobial resistance genes, mutations, and plasmid replicon type. *K. quasipneumoniae* strain SHB035 was identified as ST196 and contained several resistance genes conferring resistance to fosfomycin (*fosA*), quinolone (*oqxAB*), and β-lactam ($bla_{\text{NDM-7}}$, $bla_{\text{OKP-A-5}}$).

Ten published genomes of ST196 *K. quasipneumoniae* were downloaded, and the phylogenetic tree was generated using Parsnp (core genome SNP tree).[15] The results showed that the SBH035 isolate in our study was clustered in one separate clade, and isolates from the United States (US) and Qatar were clustered into respective clades. All Qatar strains contained $bla_{\text{NDM-1}}$, and one US strain (CAV1947, from hospital wastewater) co-harbored $bla_{\text{KPC-2}}$ and $bla_{\text{KPC-3}}$; only SBH035 contained $bla_{\text{NDM-7}}$ (Figure 1).

The complete plasmid sequence harboring $bla_{\text{NDM-7}}$ was assembled using PCR and Sanger sequencing (Table S2) and designated pYUSBH035 (GenBank accession no. LC716358). pYUSBH035 was 46,461 bp in size, with an average GC content of 46.65% and was identified as IncX3 plasmid. BLASTn analysis showed that pYUSBH035 was identical or highly similar to other NDM-7-producing IncX3 plasmids (99.9–100% identity and 100% coverage) as well as IncX3 plasmids harboring other bla_{NDM} alleles, for example, pHN6DS3 ($bla_{\text{NDM-5}}$, MN276078), pHD6415-NDM ($bla_{\text{NDM-33}}$, MZ004933), pNDM-20 ($bla_{\text{NDM-20}}$, MF458176), and pM216_X3 ($bla_{\text{NDM-4}}$, AP018146) (Figure S1). In pYUSBH035, $bla_{\text{NDM-7}}$ was located in a region with various insertion sequence (IS) elements (IS*26*, IS*5*, IS*Aba125* and IS*3000*). The $bla_{\text{NDM-7}}$ gene was embedded in an 8906-bp structure, IS*26*-Δ*cutA-tat-trpF-ble*$_{\text{MBL}}$-$bla_{\text{NDM-7}}$-IS*Aba125*-IS*3000*-ΔTn2, in which IS*Aba125* was interrupted by IS*5*, and 4-bp direct repeats (5'-CTAA-3'; DRs) were generated. This $bla_{\text{NDM-7}}$ segment was inserted into *umuD* flanked by 3-bp DRs (5'-TGT-3') (Figure 2). This genetic context was also found in other bla_{NDM}-carrying IncX3 plasmids, except that 925 bp of the 5'-end of IS*Aba15* was absent in a $bla_{\text{NDM-7}}$-bearing plasmid pHZW25-P4 (CP025215) from *K. pneumoniae* in China (Figure 2).

The plasmid pYUSBH035 could be successfully transferred to the recipient *E. coli* C600 and the bla_{NDM}-carrying transconjugant displayed resistance to ampicillin, cefazolin, cefotaxime, meropenem, and ceftazidime–avibactam (Table S1). The $bla_{\text{NDM-7}}$-carrying plasmid pYUSBH035 was a self-transmissible plasmid, which could transfer carbapenem resistance to *E. coli*. IncX3 plasmids are efficient vectors for bla_{NDM} transmission between different species.

K. quasipneumoniae was initially considered a commensal intestinal colonizer, but recent studies have proven that it is an etiologic agent in potentially fatal infections. Plasmid-mediated carbapenem resistance has also been observed among *K. quasipneumoniae* clinical isolate strains, which may complicate treatment regimens. Until now, $bla_{\text{KPC-2}}$, $bla_{\text{KPC-3}}$, $bla_{\text{KPC-9}}$, $bla_{\text{OXA-181}}$, $bla_{\text{GES-5}}$, $bla_{\text{NMD-1}}$, and $bla_{\text{NMD-5}}$ have been reported in *K. quasipneumoniae*, and the plasmids involved mainly include IncX3, IncX5, IncX6, and IncF (Table S3).

To the best of our knowledge, this is the first identification of a $bla_{\text{NDM-7}}$-harboring IncX3 plasmid in ST196 *K. quasipneumoniae* from a patient in China. Greater attention to controlling the dissemination of IncX3 plasmids is needed owing to potential horizontal transfer via mobile genetic elements.

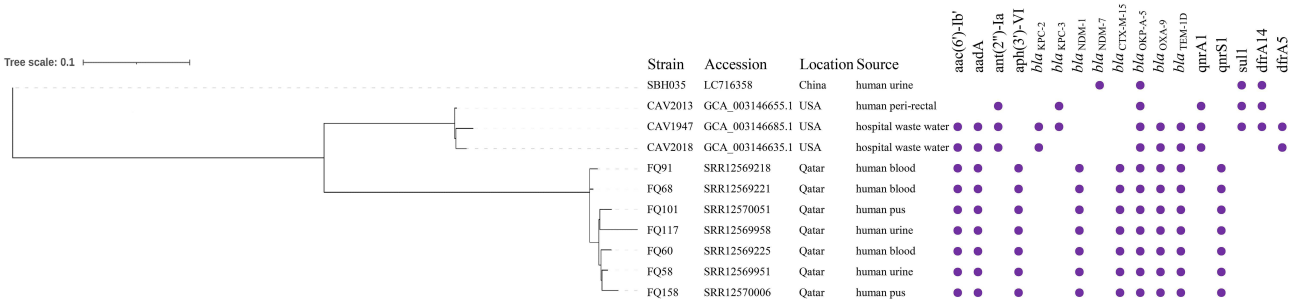

Figure 1 Phylogenetic tree based on core genome and drug resistance genes of ST196 *Klebsiella quasipneumoniae*. Antimicrobial resistance genes are shown in purple solid circles.

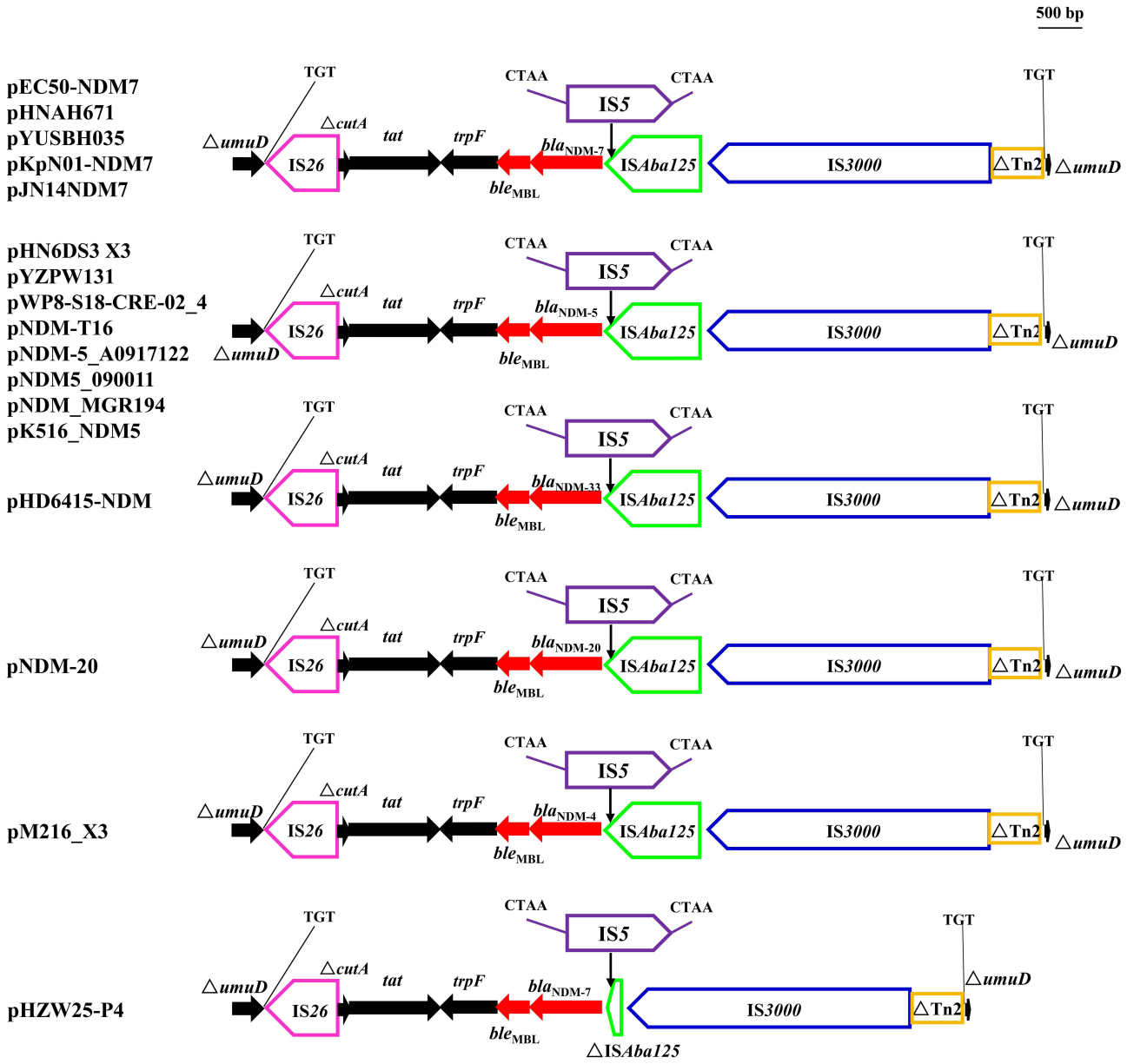

Figure 2 Genetic environment of multidrug resistance region in pYUSBH035 and homologous plasmids.

Patient Consent and Ethics Statement

The patient provided informed consent for the case details to be published. This study was approved by Jiangsu Key Laboratory of Zoonosis and Clinical Medical College, Yangzhou University.

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Disclosure

The authors report no conflicts of interest in this work.

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