

Characterization of blaNDM-1- and blaSHV-12-Positive IncX3 Plasmid in an *Enterobacter Hormaechei* New Sequence Type 1000 from China

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Purpose: Carbapenem-resistant *Enterobacter cloacae* complex has been reported worldwide and becomes a new challenge for clinical management. The present study was to characterize the IncX3 plasmid encoding bla_{NDM-1} and bla_{SHV-12} gene in *E. hormaechei* sequence.

Materials and Methods: EchK001 was recovered from the sputum sample of a patient. Species identification and antimicrobial susceptibility testing were performed using the VITEK 2 system, while further classification was carried out by hsp60 typing. The presence of NDM-1 was detected by PCR and sequencing. Conjugation experiments and southern blotting were carried out to determine the transferability of the NDM-1-carrying plasmid. Whole-genome sequencing and analysis were conducted to better understand the molecular characteristics of the multi-drug resistant isolate.

Results: Strain EchK001 was classified as *E. hormaechei* of new sequence type 1000. Multiple drug-resistant genes were detected. The bla_{NDM-1} and bla_{SHV-12} genes were located on a self-transferable IncX3 plasmid. Synonymous mutations were identified in the genes encoding TEM-1 and ACT-17. Phylogenetic analysis indicated that EchK001 clustered into a different clade from domestic strains.

Conclusion: The rapid spread of the recurrent IncX3 plasmid highlights the need for continuous surveillance of the NDM-1 dissemination. The presence of mutations in existing carbapenem-resistant genes may generate potential new variants and raise serious challenges for clinical treatment.

Keywords: multidrug-resistant, NDM-1, SHV-12, enterobacter hormaechei

Introduction

New Delhi metallo-β-lactamase-1 (NDM-1) is an enzyme with the ability to hydrolyze most β-lactams. Since first detected in 2009 from a *Klebsiella pneumoniae* strain,¹ 21 NDM variants have been reported from a variety of *Enterobacterales* species.² The widespread of NDM is mainly due to diverse self-transferable plasmids. The co-existence of NDM and other resistance genes in a single plasmid is increasingly detected and confers higher resistance to carbapenems, raising great challenges for clinical management.^{3,4}

As important opportunistic pathogens, *Enterobacter cloacae* complex is responsible for a variety of human infections in the respiratory tract, wound and urine.⁵ Among the members of the *Enterobacter cloacae* complex, *E. hormaechei* was first reported in 1989⁶ and has been increasingly detected worldwide with the acquisition of different beta-lactamase genes.^{7,8} In this study, we report a carbapenem-resistant *E. hormaechei*

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strain with an IncX3 plasmid coproducing NDM-1 and SHV-12. Susceptibility test, conjugation experiment and whole-genome sequencing were performed to investigate the molecular characteristics of the multiple drug-resistant strain.

Materials and Methods

Bacterial Strain Identification

The NDM-1-producing strain EcHK001 was isolated from the sputum sample of an 83-year-old male patient through routine surveillance in Haikou, China, in 2015. The *E. cloacae* strain was identified by the VITEK2 compact system (bioMérieux, France) and 16S rRNA gene sequencing.⁹ The *Enterobacter cloacae* complex can be further classified by the heat shock protein 60-encoding (*hsp60*) gene,^{10,11} the sequence of which was obtained through amplification and sequencing using the primers of Hsp60-F (5'-GGT AGA AGA AGG CGT GGT TGC-3') and Hsp60-R (5'-ATG CAT TCG GTG GTG ATC ATC AG-3') as previously described.¹² Phylogenetic analysis of the *hsp60* sequences from GenBank was performed to confirm the identification of *E. hormaechei*.

The presence of NDM, SHV, TEM, and ACT genes was detected by PCR and sequencing as previously described.^{13–16}

Antimicrobial Susceptibility Testing

The minimum inhibitory concentrations (MICs) of *E. hormaechei* strain EcHK001 and the transconjugants were determined using the VITEK2 system with AST-GN09 card (bioMérieux) and interpreted according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI).¹⁷ The following agents were tested: Ampicillin, Ampicillin/sulbactam, Piperacillin, Piperacillin/tazobactam, Cefazolin, Cefuroxime, Cefotetan, Ceftazidime, Ceftriaxone, Cefepime, Aztreonam, Imipenem, Meropenem, Amikacin, Gentamicin, Tobramycin, Ciprofloxacin, Levofloxacin, Nitrofurantoin, and Sulfamethoxazole/trimethoprim.

Southern Blotting and Conjugation Experiments

DNA fragments of strain EcHK001 were prepared in agarose plugs by electrophoresis and digested with S1 endonuclease (Takara, Dalian, China). Subsequent fragments were further separated by PFGE through a CHEF-DR III system (Bio-Rad, Hercules, USA). A sheet of nylon membrane (Roche) was used to transfer the plasmid DNA to the membrane due to the negative charge of the DNA and positive charge of the membrane. Southern blotting specific to *bla*_{NDM-1} was performed

using the Probe-NDM-F (5'-GGC GGA ATG GCT CAT CAC GA-3') and Probe-NDM-R (5'-CGC AAC ACA GCC TGA CTT TC-3') as previously described.¹⁸

Conjugation experiment was carried out by broth and filter mating using strain EcHK001 and *E. coli* strain J53 Az^r as the donor and recipient, respectively. The mixture was then incubated at 30°C for 18 hrs. Transconjugants were selected on MacConkey agar plates containing meropenem (4 µg/mL) plus sodium azide (100 µg/mL) at 37°C for 48 hrs. PCR amplification and sequencing were further performed to determine the presence of the *bla*_{NDM-1} and *bla*_{SHV-12} genes in the transconjugants.

Whole Genome Sequencing and Phylogenetic Analysis

Genomic DNA was extracted from the bacterial culture of *E. hormaechei* strain EcHK001 using the QIAamp DNA Mini Kit (Qiagen, Inc., Valencia, CA). Whole-genome sequencing was performed using Illumina HiSeq 2500 sequencer at Novogene Company (Beijing, China) with 109-fold coverage and the MinION sequencer with the nanopore library prepared using the Rapid Sequencing Kit SQK-RAD004 (Oxford Nanopore Technologies, UK). Hybrid assembly of Illumina and Nanopore sequencing reads was carried out with Unicycler (v0.4.7).¹⁹ The *bla*_{NDM}-carried plasmid was designated as pNDM-EcHK001 here and annotated by RAST.²⁰ PlasmidFinder²¹ was employed to determine the replicon type.

Strain typing was carried out by using the genomic sequence to query the seven house-keeping genes (*pyrG*, *gyrB*, *rplB*, *leuS*, *dnaA*, *rpoB* and *fusA*) of *E. cloacae* on the MLST web server.²² All 7 strains of *E. hormaechei* from China and other 11 sequences of *E. hormaechei* were obtained from NCBI for phylogenetic tree construction using kSNP (v3.0).²³ The average nucleotide identities (ANIs) between strain EcHK001 and other isolates were calculated by JSpeciesWS web service.²⁴

Nucleotide Sequence Accession Numbers

Genomic and plasmid sequences of strain EcHK001 have been deposited into GenBank under accession NZ_PEIL01000000 and NZ_CM008823, respectively.

Results

Microbiological Features of Strain EcHK001

Based on the *hsp60* gene, the *bla*_{NDM-1} carrying strain EcHK001 was further classified into the *E. hormaechei*.

Table 1 Antibiotic Susceptibilities of Strain EcHK001 and the *E. coli* J53 Transconjugants

Antimicrobial	MIC (ug/mL)/Susceptibility Result		
	EcHK001	J53	J53 (the Transconjugant)
Ampicillin	≥32/R	8/S	≥32/R
Ampicillin/sulbactam	≥32/R	4/S	≥32/R
Piperacillin	≥128/R	≤4/S	≥128/R
Piperacillin/tazobactam	≥128/R	≤4/S	64/I
Cefazolin	≥64/R	≤4/S	≥64/R
Cefuroxime	≥64/R	≤1/S	≥64/R
Cefotetan	≥64/R	≤4/S	32/I
Ceftazidime	≥64/R	≤1/S	≥64/R
Ceftriaxone	≥64/R	≤1/S	≥64/R
Cefepime	≥64/R	≤1/S	8/I
Aztreonam	≥64/R	≤1/S	32/R
Imipenem	≥16/R	≤0.05/S	≥16/R
Meropenem	≥16/R	≤1/S	8/R
Amikacin	4/S	≤2/S	≤2/S
Gentamicin	≥16/R	≤1/S	≤1/S
Tobramycin	≥16/R	≤1/S	≤1/S
Ciprofloxacin	1/S	≤0.25/S	≤0.25/S
Levofloxacin	1/S	≤0.25/S	≤0.25/S
Nitrofurantoin	128/R	≤16/S	≤16/S
Sulfamethoxazole-trimethoprim	≥320/R	≤20/S	≤20/S

Abbreviations: S, susceptible; R, resistant; I, intermediate.

EcHK001 exhibited resistance to most tested antimicrobial agents, but was still susceptible to amikacin, ciprofloxacin and levofloxacin (Table 1). S1-PFGE showed that EcHK001 contained three plasmids which sizes were ~33 kb, ~54kb and ~130 kb (Figure 1). Electrophoresis and southern blotting revealed that the *bla*_{NDM-1} gene was located on the ~54kb plasmid. This plasmid, designated as pNDM-EcHK001, was successfully transferred via conjugation at a frequency of 2.94×10^{-2} . Susceptibility testing showed the transconjugants acquired resistance to most tested β -lactams including ampicillin, ampicillin/sulbactam, piperacillin, cefazolin, cefuroxime, ceftazidime, ceftriaxone, aztreonam, imipenem and meropenem. In addition to *bla*_{NDM-1}, multiple resistance genes including *bla*_{SHV-12}, *bla*_{CTX-M-14}, *bla*_{DHA-1}, *bla*_{ACT-17}, *bla*_{TEM-1}, *qnrB4*, *brp*, *sul2*, *sul1*, *strA*, *strB*, *dfrA14* and *aac(6)-IIC* were identified through genome sequencing and analysis. Genome analysis indicated the *bla*_{DHA-1}, *bla*_{CTX-M-14} and *bla*_{ACT-17} genes were located on the chromosome, while other *bla* genes were on plasmids. The plasmid pNDM-EcHK001 had a length of 54,035 bp and carried both the *bla*_{NDM-1} and *bla*_{SHV-12} genes. The *bla*_{TEM-1} gene was located on a 124,544 bp plasmid which

had an 11,452-bp resistance region containing *Δ**tnpA*, *tnpR*, *bla*_{TEM-1}, *IS*/*sa3*, *strB*, *strA*, *sul2*, *IS4321*, *Δ**tnpA* and *IS26*, same as the region from plasmid p34998-210.894kb (GenBank accession no. CP012169.1).¹⁰ Of note, synonymous mutations in the *bla*_{TEM-1} (T396G) and *bla*_{ACT-17} (C96A, T276G, T675C, T690C, A708G, G777A and G807A) genes were detected and verified by PCR, indicating the potential to generate new variants of these β -lactamase genes. The variants of *bla*_{TEM-1} and *bla*_{ACT-17} were deposited in GenBank under accession number MG818166 and MG865974.

Strain Typing and Phylogenetic Analysis

In silico MLST analysis identified a new allele of the house-keeping genes *pyrG* designated as *pyrG*-281, and strain EcHK001 was assigned a new sequence type 1000. Whole-genome phylogenetic analysis revealed EcHK001 formed a clade with strains isolated in USA and China (Figure 2). Sequence alignments revealed the ANI values between EcHK001 and three isolates from Chengdu, China ranged from 99.22% to 99.23%, while the ANI between EcHK001 and the strain UCI_5 from Irvine, California, USA was 99.30%. However, the ANI between EcHK001 and the other Chinese isolates ranged from 86.5 to 87.2. These results indicated that EcHK001 had a close relationship with overseas strains and may have originated from an earlier ancestor than domestic strains.

Characterization of Plasmid pNDM-EcHK001

Plasmid pNDM-EcHK001 had a length of 54,035 bp and 52 ORFs (Figure 3). The plasmid, assigned to replicon type IncX3, consists of a 39.6 kb backbone and a 19.2 kb multiple drug resistance (MDR) region. The backbone carries genes responsible for plasmid replication (*repB*), conjugal transfer (*taxA*, *taxB*, *taxC* and *pilx1-pilx11*, etc.) and plasmid stability (*parA*, *parB*, etc.). The MDR region is composed of Tn3, *IS3000*, the transposon Tn125 encoding *bla*_{NDM-1}, a mobile element containing *bla*_{SHV-12} and *ISL3*.

The genome sequence of pNDM-EcHK001 shared >99% similarity with plasmid pNDM-HN380 (GenBank accession no. JX104760) in *K. pneumoniae*, pNDM-HF727 (GenBank accession no. KF976405) in *E. cloacae*,²⁵ pYE315203 (GenBank accession no. JX254913) in *Citrobacter freundii* and pKPN5047 (GenBank accession no. KC311431) (Table 2), which are differed by only 14 SNPs in the *IS5* element upstream of the *bla*_{NDM-1} gene. Plasmids

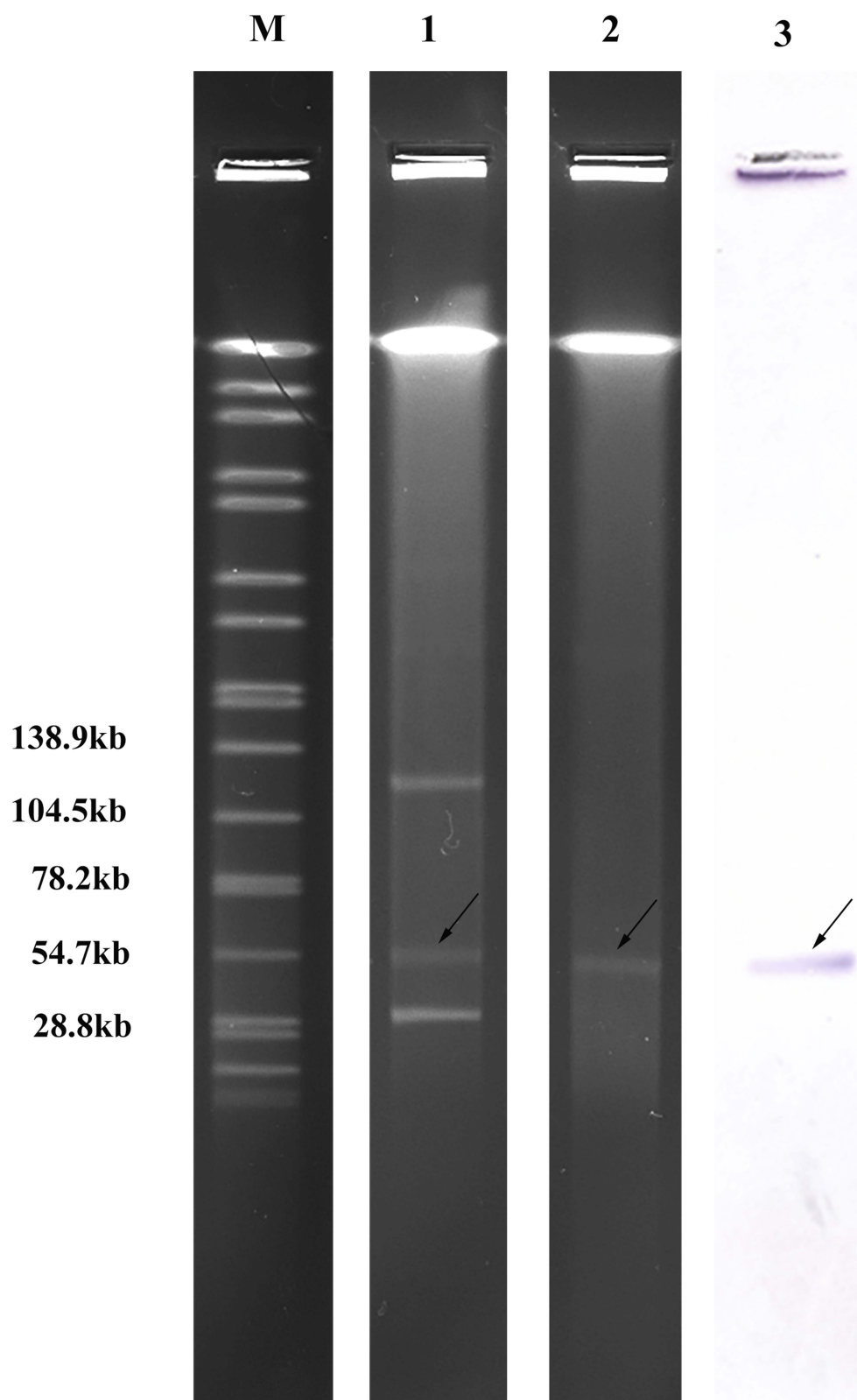


Figure 1 SI-PFGE and Southern blotting. Lane M, marker (*Salmonella* H9812); Lane 1, *E. hormaechei* EchK001; Lane 2, the transconjugants; Lane 3, Southern blotting of EchK001 with the probes specific to *bla*_{NDM-1}. The black arrows indicate the plasmid pNDM-EchK001.

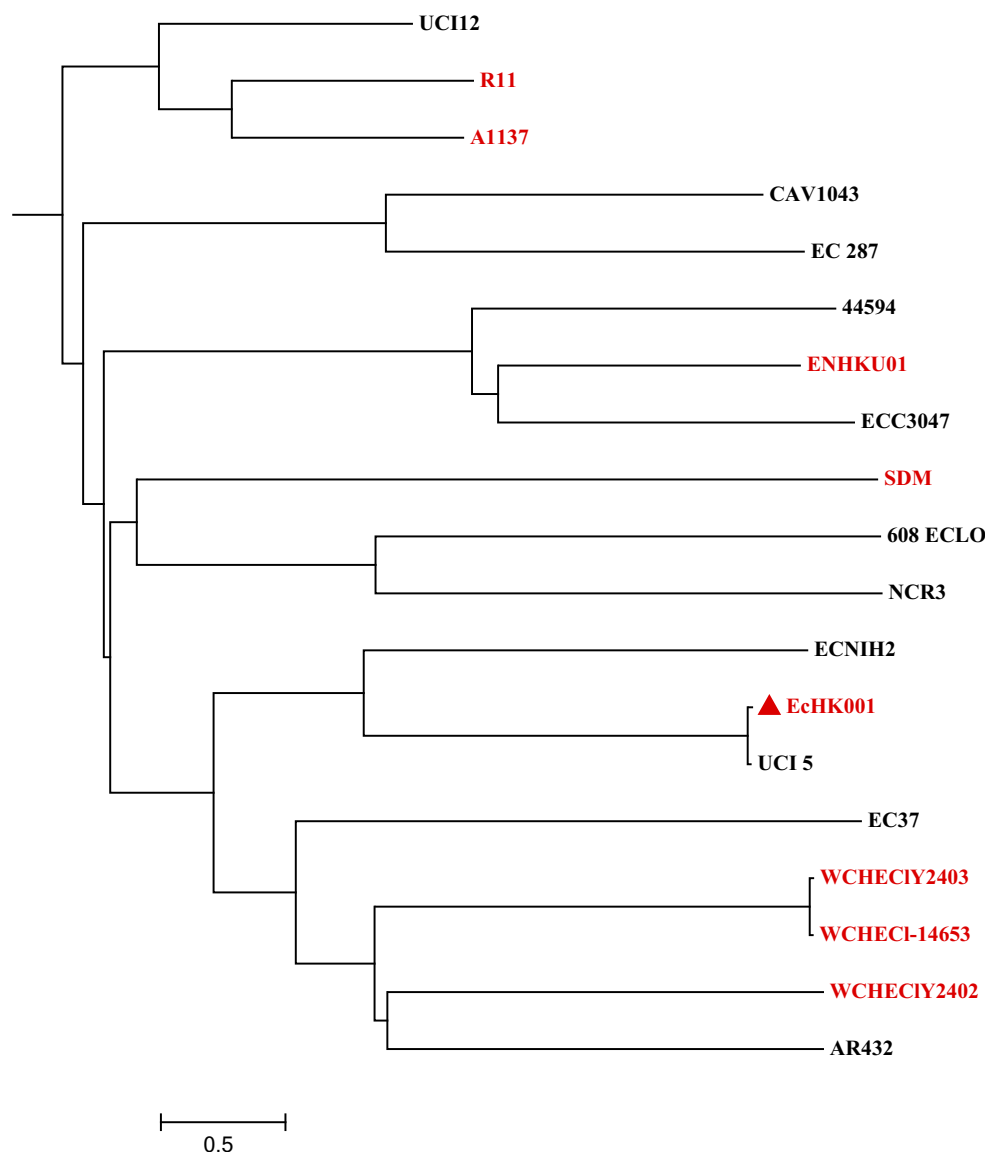
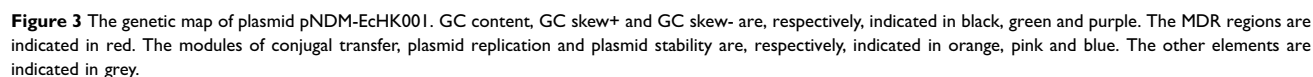


Figure 2 Phylogenetic tree of *E. hormaechei* strain EchK001 with other 19 available *E. cloacae* complex genomes from GenBank. Strain EchK001 is marked with a solid triangle. Isolates from China are indicated with red shading.

pEC55-NDM4 (GenBank accession no. KX470734) and pZHDC33 (GenBank accession no. KX094555) also shared >99% identity with pNDM-EchK001 but containing different *bla*_{NDM} alleles (Figure 4). The *bla*_{NDM-4} (T354G) and *bla*_{NDM-13} genes (C531T) differ from *bla*_{NDM-1} and *bla*_{NDM-4} by only a single nucleotide, respectively, suggesting a close relationship among these three plasmids. On these above plasmids, the *bla*_{NDM-1} gene was located in a *Tn125* variant while the *bla*_{SHV-12} gene in a composite transposon. The transposon *Tn125* served as a mobile element for the transfer of *bla*_{NDM-1} and comprised Δ IS*Aba125*, *bla*_{NDM-1} (locus tag, CS291_RS00145), the bleomycin resistance gene *ble* (locus tag, CS291_RS00140), Δ *trpF* (locus tag, CS291_RS00135),

tat (locus tag, CS291_RS00130), *dct* (locus tag, CS291_RS00125), *groES* (locus tag, CS291_RS00120), *groEL* (locus tag, CS291_RS00115) and *insE* (locus tag, CS291_RS00115) as previously described.²⁵ Compared with the prototype *Tn125*, the upstream Δ IS*Aba125* is disrupted by IS5 while the downstream copy is absent. The Δ IS*Aba125* and IS5 were each surrounded by two inverted repeats (IRs) (15 bp and 12 bp), respectively. Two direct repeats (DRs) (21 bp and 11 bp) were located at the end of *groEL* and *insE*.

The *bla*_{SHV-12}-carrying transposon is sequentially organized as Δ *ygbJ* (locus tag, CS291_RS00085), *ygbI* (locus tag, CS291_RS00080) and *bla*_{SHV-12} (locus tag, CS291_RS00075), which were located within two opposite



with an inversion of IS5 and a shorter Δ IS*Aba125* region with only 65 bp remaining, suggesting the composite transposon may serve as the major vehicle for SHV-12 (GenBank accession no. KX023261) (Figure 4). The information of all the above plasmids is shown in Table 2.

Previous studies have shown that the *bla*_{NDM-1}-positive isolates in diverse species of *Enterobacteriales* often exhibited high resistance to beta-lactam antibiotics and posed

Plasmid	Host	Location	<i>Bla</i> Gene	Coverage	Similarity	GenBank Accession	Reference
pNDM-HF727	<i>E. cloacae</i>	Haifeng, China	<i>bla</i> _{NDM-1} , <i>bla</i> _{SHV-12}	100%	99.99%	KF976405	25
pNDM-HN380	<i>K. pneumoniae</i>	Hunan, China	<i>bla</i> _{NDM-1} , <i>bla</i> _{SHV-12}	100%	99.99%	JX104760	25
pYE15203	<i>C. freundii</i>	Zhejiang, China	<i>bla</i> _{NDM-1} , <i>bla</i> _{SHV-12}	100%	99.99%	JX254913	Unpublished
pKPN5047	<i>K. pneumoniae</i>	Beijing, China	<i>bla</i> _{NDM-4} , <i>bla</i> _{SHV-12}	100%	99.99%	KC311431	Unpublished
pEC55-NDM4	<i>E. coli</i>	Henan, China	<i>bla</i> _{NDM-4} , <i>bla</i> _{SHV-12}	100%	99.99%	KX470734	Unpublished
pZHDC33	<i>E. coli</i>	Zhejiang, China	<i>bla</i> _{NDM-13} , <i>bla</i> _{TEM}	100%	99.99%	KX094555	Unpublished
pKP04NDM	<i>K. pneumoniae</i>	Hong Kong, China	<i>bla</i> _{NDM-1}	100%	99.99%	KU314941	26
p112298-NDM	<i>C. freundii</i>	Guangzhou, China	<i>bla</i> _{NDM-1} , <i>bla</i> _{SHV-2}	98.99%	99.99%	KP987216	27
pIncX-SHV	<i>K. pneumoniae</i>	Rome, Italy	<i>bla</i> _{SHV-11}	80.28%	99.99%	JN247852	28
pSCE516-2	<i>E. coli</i>	Chengdu, China	<i>bla</i> _{NDM-1}	85.6%	99.99%	KX023261	Unpublished

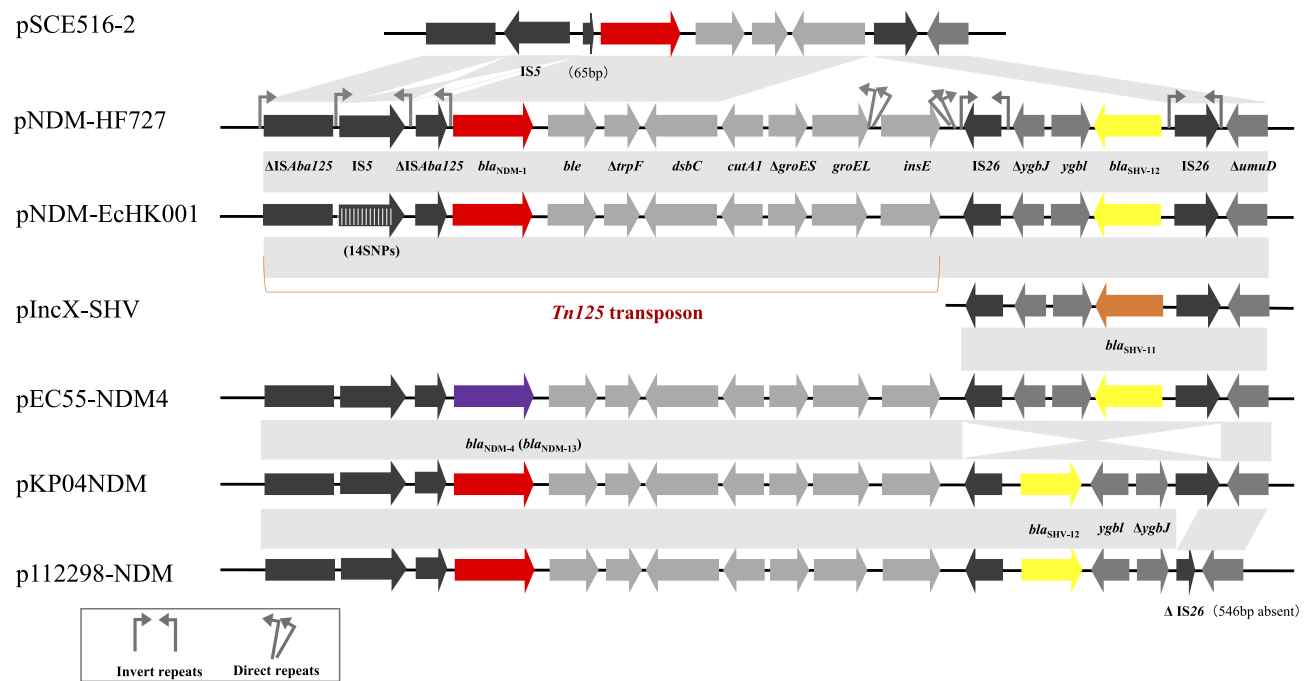


Figure 4 The genetic structure features of pNDM-EcHK001-like plasmids. The *bla*_{NDM-1} and *bla*_{SHV-12} gene are indicated in red and yellow. The *bla*_{NDM} variants and the *bla*_{SHV-11} gene are indicated in purple and dark orange. The insertion sequences *ΔISAba125*, *IS5* and *IS26* are indicated in black. Other genes in *ΔTn125* and *bla*_{SHV-12}-carrying transposon are indicated in light gray and dark gray, respectively.

a significant threat to public health.^{3,29} We provided a detailed description of a multidrug-resistant *E. hormaechei* strain EcHK001, which had an IncX plasmid pNDM-EcHK001 co-harboring *bla*_{NDM-1} and *bla*_{SHV-12}. The IncX plasmids have been repeatedly reported in *Enterobacteriales*, carrying different resistance genes.³⁰ The plasmid pNDM-EcHK001 had a relatively high conjugation frequency compared with other IncX3 plasmids,³¹ which may facilitate rapid dissemination of the *bla*_{NDM-1} gene.

The pNDM-EcHK001-like plasmids, which share the same backbone and have both *bla*_{NDM-1} and *bla*_{SHV-12}, have a broad host range and the ability of transfer among *Enterobacteriales*.³² However, it is noteworthy that there were no previous reports of such plasmids in *E. hormaechei*. The co-occurrence of *bla*_{NDM-1} and other beta-lactamase genes mediated resistance to broad-spectrum antibiotics such as carbapenems and cephalosporins, raising challenges in rapidly tailoring clinical therapy.

These plasmids were first detected and repeatedly reported from different geographical locations mainly in China. The isolates carrying these plasmids were all recovered from samples of human patients, which had no history of foreign travel or epidemiological

relationship. These evidences indicated a natural reservoir of these plasmids may exist in China. The slight variations among the pNDM-EcHK001-like plasmids suggesting they may be descended from a common ancestor. Whole-genome phylogenetic analysis showed that EcHK001 had a close relationship with foreign isolates. More data are needed to further understand the mechanisms underlying the dissemination of these pNDM-EcHK001-like plasmids.

Conclusions

In summary, our study characterized a novel *E. hormaechei* ST1000 strain EcHK001, which had a different origination with domestic isolates through phylogenetic analysis. The strain carried multiple *bla* genes and conferred increased resistance to carbapenems. The coexistence of the *bla*_{NDM-1} and *bla*_{SHV-12} genes is repeatedly reported on the pNDM-EcHK001-like plasmids, which had a high transferability and may serve as a common vehicle for rapid dissemination of carbapenemase-encoding genes. Our findings further highlight the spread of NDM-1 in *Enterobacteriales*/*Enterobacteriaceae*. More attention should be devoted to the surveillance of the rapid dissemination of the NDM-1 gene.

Ethics Approval

The authors state that all experimental protocols were approved by the institutional ethics committees of Academy of Military Medical Sciences.

Data Sharing Statement

Genomic and plasmid sequences of strain EcHK001 were deposited in GenBank under accession NZ_PEIL01000000 and NZ_CM008823. The variants of blaTEM-1 and blaACT-17 were deposited in GenBank under accession number MG818166 and MG865974.

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Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

References

- Yong D, Toleman MA, Giske CG, et al. Characterization of a new metallo-beta-lactamase gene, bla(NDM-1), and a novel erythromycin esterase gene carried on a unique genetic structure in klebsiella pneumoniae sequence type 14 from India. *Antimicrob Agents Chemother.* 2009;53(12):5046–5054. doi:10.1128/AAC.00774-09
- Liu L, Feng Y, McNally A, Zong Z. blaNDM-21, a new variant of blaNDM in an escherichia coli clinical isolate carrying blaCTX-M-55 and rmtB. *J Antimicrob Chemother.* 2018;73(9):2336–2339. doi:10.1093/jac/dky226
- Kumarasamy KK, Toleman MA, Walsh TR, et al. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. *Lancet Infect Dis.* 2010;10(9):597–602. doi:10.1016/S1473-3099(10)70143-2
- Naas T, Cuzon G, Bogaerts P, Glupczynski Y, Nordmann P. Evaluation of a DNA microarray (check-MDR CT102) for rapid detection of TEM, SHV, and CTX-M extended-spectrum beta-lactamases and of KPC, OXA-48, VIM, IMP, and NDM-1 carbapenemases. *J Clin Microbiol.* 2011;49(4):1608–1613. doi:10.1128/JCM.02607-10
- Mezzatesta ML, Gona F, Stefani S. Enterobacter cloacae complex: clinical impact and emerging antibiotic resistance. *Future Microbiol.* 2012;7(7):887–902. doi:10.2217/fmb.12.61
- O'Hara CM, Steigerwalt AG, Hill BC, Farmer JJ 3rd, Fanning GR, Brenner DJ. Enterobacter hormaechei, a new species of the family enterobacteriaceae formerly known as enteric group 75. *J Clin Microbiol.* 1989;27(9):2046–2049.
- Castanheira M, Sader HS, Deshpande LM, Fritsche TR, Jones RN. Antimicrobial activities of tigecycline and other broad-spectrum antimicrobials tested against serine carbapenemase- and metallo-beta-lactamase-producing enterobacteriaceae: report from the SENTRY antimicrobial surveillance program. *Antimicrob Agents Chemother.* 2008;52(2):570–573. doi:10.1128/AAC.01114-07
- Sampaio JL, Ribeiro VB, Campos JC, et al. Detection of OXA-370, an OXA-48-related class D beta-lactamase, in enterobacter hormaechei from Brazil. *Antimicrob Agents Chemother.* 2014;58(6):3566–3567. doi:10.1128/AAC.02510-13
- Frank JA, Reich CI, Sharma S, Weisbaum JS, Wilson BA, Olsen GJ. Critical evaluation of two primers commonly used for amplification of bacterial 16S rRNA genes. *Appl Environ Microbiol.* 2008;74(8):2461–2470. doi:10.1128/AEM.02272-07
- Chavda KD, Chen L, Fouts DE, et al. Comprehensive genome analysis of carbapenemase-producing enterobacter spp.: new insights into phylogeny, population structure, and resistance mechanisms. *MBio.* 2016;7(6):e02093–16. doi:10.1128/mBio.02093-16
- Hoffmann H, Roggenkamp A. Population genetics of the nomenclotype Enterobacter cloacae. *Appl Environ Microbiol.* 2003;69(9):5306–5318. doi:10.1128/AEM.69.9.5306-5318.2003
- Morand PC, Billoet A, Rottman M, et al. Specific distribution within the enterobacter cloacae complex of strains isolated from infected orthopedic implants. *J Clin Microbiol.* 2009;47(8):2489–2495. doi:10.1128/JCM.00290-09
- Aminov RI, Chee-Sanford JC, Garrigues N, et al. Development, validation, and application of PCR primers for detection of tetracycline efflux genes of gram-negative bacteria. *Appl Environ Microbiol.* 2002;68(4):1786–1793. doi:10.1128/AEM.68.4.1786-1793.2002
- Coque TM, Novais A, Carattoli A, et al. Dissemination of clonally related escherichia coli strains expressing extended-spectrum beta-lactamase CTX-M-15. *Emerg Infect Dis.* 2008;14(2):195. doi:10.3201/eid1402.070350
- Tijet N, Alexander DC, Richardson D, et al. New delhi metallo-beta-lactamase, Ontario, Canada. *Emerg Infect Dis.* 2011;17(2):306. doi:10.3201/eid1702.101561
- Yan -J-J, Wu S-M, Tsai S-H, Wu -J-J, Su I-J. Prevalence of SHV-12 among clinical isolates of klebsiella pneumoniae producing extended-spectrum beta-lactamases and identification of a novel AmpC enzyme (CMY-8) in Southern Taiwan. *Antimicrob Agents Chemother.* 2000;44(6):1438–1442. doi:10.1128/AAC.44.6.1438-1442.2000
- CLSI C. Performance standards for antimicrobial susceptibility testing; twenty-fourth informational supplement. *M100-S24 January.* 2014.
- Fu Y, Du X, Ji J, Chen Y, Jiang Y, Yu Y. Epidemiological characteristics and genetic structure of blaNDM-1 in non-baumannii acinetobacter spp. in China. *J Antimicrob Chemother.* 2012;67(9):2114–2122. doi:10.1093/jac/dks192
- Wick RR, Judd LM, Gorrie CL, Holt KE, Phillippy AM. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. *PLoS Comput Biol.* 2017;13(6):e1005595. doi:10.1371/journal.pcbi.1005595
- Aziz RK, Bartels D, Best AA, et al. The RAST server: rapid annotations using subsystems technology. *BMC Genomics.* 2008;9(1):75. doi:10.1186/1471-2164-9-75
- Carattoli A, Zankari E, García-Fernández A, et al. In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. *Antimicrob Agents Chemother.* 2014;58(7):3895–3903. doi:10.1128/AAC.02412-14
- Larsen MV, Cosentino S, Rasmussen S, et al. Multilocus sequence typing of total-genome-sequenced bacteria. *J Clin Microbiol.* 2012;50(4):1355–1361. doi:10.1128/JCM.06094-11

23. Gardner SN, Slezak T, Hall BG. kSNP3. 0: SNP detection and phylogenetic analysis of genomes without genome alignment or reference genome. *Bioinformatics*. 2015;31(17):2877–2878. doi:10.1093/bioinformatics/btv271
24. Richter M, Rossello-Mora R. Shifting the genomic gold standard for the prokaryotic species definition. *Proc Natl Acad Sci U S A*. 2009;106(45):19126–19131. doi:10.1073/pnas.0906412106
25. Ho PL, Li Z, Lo WU, et al. Identification and characterization of a novel incompatibility group X3 plasmid carrying bla NDM-1 in enterobacteriaceae isolates with epidemiological links to multiple geographical areas in China. *Emerg Microbes Infect*. 2012;1(11):e39. doi:10.1038/emi.2012.37
26. Lai CC, Chen CC, Lu YC, et al. Simultaneous three enterobacteriaceae with different bla NDM-1-encoding plasmids in a patient transferred from mainland China to Taiwan. *Infect Drug Resist*. 2018;11:2555–2560. doi:10.2147/IDR.S179024
27. Feng J, Qiu Y, Yin Z, et al. Coexistence of a novel KPC-2-encoding MDR plasmid and an NDM-1-encoding pNDM-HN380-like plasmid in a clinical isolate of *Citrobacter freundii*. *J Antimicrob Chemother*. 2015;70(11):2987–2991. doi:10.1093/jac/dkv232
28. Garcia-Fernandez A, Villa L, Carta C, et al. *Klebsiella pneumoniae* ST258 producing KPC-3 identified in Italy carries novel plasmids and OmpK36/OmpK35 porin variants. *Antimicrob Agents Chemother*. 2012;56(4):2143–2145. doi:10.1128/AAC.05308-11
29. Deshpande P, Rodrigues C, Shetty A, Kapadia F, Hedge A, Soman R. New Delhi metallo-beta lactamase (NDM-1) in enterobacteriaceae: treatment options with carbapenems compromised. *J Assoc Physicians India*. 2010;58(3):147–149.
30. Kopotsa K, Osei Sekyere J, Mbelle NM. Plasmid evolution in carbapenemase-producing enterobacteriaceae: a review. *Ann N Y Acad Sci*. 2019. doi:10.1111/nyas.14223
31. Wang Y, Tong MK, Chow KH, et al. Occurrence of highly conjugative IncX3 epidemic plasmid carrying bla NDM in enterobacteriaceae isolates in geographically widespread areas. *Front Microbiol*. 2018;9:2272. doi:10.3389/fmicb.2018.02272
32. Tardif G, Grant RB. Transfer of plasmids from *Escherichia coli* to *Pseudomonas aeruginosa*: characterization of a *Pseudomonas aeruginosa* mutant with enhanced recipient ability for enterobacterial plasmids. *Antimicrob Agents Chemother*. 1983;24(2):201–208. doi:10.1128/AAC.24.2.201

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