Infection and Drug Resistance Dovepress

Open Access Full Text Article SHORT REPORT

Emergence of a *bla*$_{NDM-5}$ Carrying IncHI2/IncHI2A Plasmid in a Multidrug Resistant Clinical ST1431 *Escherichia coli* Strain

Xu Lin*, Zhiwen Lin*, Da Chen, Renhuai Huang, Hui Liang

First Department of General Surgery, Zhuhai People's Hospital (Zhuhai Hospital Affiliated to Jinan University), Zhuhai City, Guangdong Province, People's Republic of China

*These authors contributed equally to this work

Correspondence: Hui Liang, Zhuhai People's Hospital, Zhuhai, Guangdong, People's Republic of China, Email zhlianghui@126.com

Abstract: Carbapenems are the last-resort antibiotics used to treat infections caused by bacterial pathogens. Many bacterial pathogens have evolved to produce NDM carbapenemases to hydrolyze carbapenems, posing a great challenge to public health. In this study, we report a multidrug resistant clinical *E. coli* strain 673. Strain 673 belongs to sequence type (ST) 1431 and carries several plasmids, p673-blaTEM-1B, p673-blaCTX-M-55, p673-blaNDM-5, p673-13272, and p673-6468. p673-blaNDM-5 is an IncHI2/IncHI2A-type plasmid harboring several antibiotic resistance genes, including *bla*$_{NDM-5}$, *strA, strB*, and *dfrA*. The *bla*$_{NDM-5}$ gene was surrounded by two IS*26* elements in p673-blaNDM-5, indicating that IS*26* could mediate the integration of *bla*$_{NDM-5}$ into p673-blaNDM-5. p673-blaCTX-M-55 is an IncFII-type plasmid harboring *fosA, aadA1*, and *bla*$_{CTX-M-55}$. p673-blaTEM-1B is an IncFIB-type plasmid harboring *bla*$_{TEM-1B}$ and *dfrA5*. p673-13272 is a ColRNAI-type plasmid that does not carry any drug resistance genes. This is the first report that a *bla*$_{NDM-5}$-bearing IncHI2/IncHI2A-type plasmid has emerged in a clinical *E. coli* strain in China. Our findings suggest that IS*26* mediates the integration of *bla*$_{NDM-5}$ into p673-blaNDM-5. The spread of *bla*$_{NDM-5}$-bearing plasmids is a clinical challenge and endangers public health.

Keywords: NDM-5, IncHI2/IncHI2A plasmid, *E. coli*

Short Report

Carbapenems are essential antibiotics used in clinical practice. To fight these drugs, many bacterial pathogens can produce NDM carbapenemases. To date, more than 45 NDM variant genes have been identified.[1] Among these NDM variant genes, *bla*$_{NDM-1}$ and *bla*$_{NDM-5}$ are the two predominant NDM carbapenemase genes.[2] A recent study revealed that among carbapenem-resistant *Escherichia coli* strains isolated from China, the majority of these strains carry *bla*$_{NDM-5}$ instead of *bla*$_{NDM-1}$. These *E. coli* strains also encode other drug resistance genes, such as *bla*$_{CTX-M}$, *bla*$_{TEM}$, *bla*$_{SHV}$, *aadA*, and *sul2*.[3] Surveillance studies revealed that in addition to *E. coli*, other bacterial species, such as *Citrobacter freundii, E. fergusonii, Klebsiella pneumoniae, Pluralibacter gergoviae*, and *Salmonella* Typhimurium, also encode *bla*$_{NDM-5}$.[4–7] Among these diverse bacterial species, *E. coli* is recognized as the dominant host of *bla*$_{NDM-5}$.[8] Indeed, according to reports from the China Antimicrobial Resistance Surveillance System (http://www.carss.cn/), *E. coli* is the dominant gram-negative bacteria isolated in Chinese hospitals from 2020–2022. Among these *E. coli* strains, approximately 1.5% were carbapenemase producers. Therefore, carbapenem-resistant *E. coli* is a great challenge in hospitals.

It generally agrees that plasmid is the major vector contributing to the dissemination of *bla*$_{NDM-5}$. For example, IncF, IncFII, IncHI2, IncN, and IncX3 plasmids have been reported to harbor *bla*$_{NDM-5}$.[9–11] Among these different types of plasmids, the IncX3-type plasmid is the most prevalent type of plasmid that carries *bla*$_{NDM-5}$.[12,13] In addition, *bla*$_{NDM-5}$-bearing IncX3-type plasmids can be carried by many bacterial species that are isolates from diverse environments, animals, and humans.[11,14] A recent study revealed that the IncX3-type plasmid pX3_NDM-5 has a wide range of

Received: 27 July 2024
Accepted: 19 November 2024
Published: 3 December 2024

bacterial hosts and can be transferred between gram-negative and gram-positive bacteria.[15] Moreover, the IncHI2-type plasmid harboring bla_{NDM-5} has been increasingly reported in China. Bacterial species that carry bla_{NDM-5}-bearing IncHI2-type plasmids were isolated from eggs, chickens, ducks, pig feces, and fish.[4,11,16,17] However, neither a clinical isolate a carrying bla_{NDM-5}-bearing IncHI2-type plasmid nor an IncHI2/IncHI2A-type plasmid harboring bla_{NDM-5} has been reported.

In August 2023, strain 673 was isolated from a urine sample from Zhuhai people's hospital, Zhuhai city, Guangdong Province, China. This urine sample was obtained from a female patient who had a urinary tract infection. The symptoms include frequent urination and pain while peeing. Ceftriaxone failed to treat the infection. By using 16S rRNA sequencing and MALDI-TOF analysis, strain 673 was identified as *E. coli*. To determine the antimicrobial susceptibility of the tested strains, a broth dilution method was used as described by the Clinical & Laboratory Standards Institute (CLSI) standard, and the results were interpreted according to the CLSI 2022 guidelines (https://clsi.org).

Antimicrobial susceptibility test data revealed that strain 673 was resistant to carbapenems, including imipenem and ertapenem, indicating that it could be a carbapenemase producer. Strain 673 was resistant to many other drugs, especially cephalosporins, such as ceftriaxone, cefuroxime, ceftriaxone, cefuroxime, ceftazidime, cefepime, and cefoxitin, but was susceptible to amikacin and tigecycline (Table 1). Thus, tigecycline is likely the last choice for treating the infections caused by this multidrug-resistant *E. coli* strain or other clinical multidrug-resistant pathogenic strains isolated from our hospital.

Given that strain 673 is resistant to carbapenems, we used the Illumina and MinION platforms to sequence its genome and to determine which carbapenemase gene was harbored by this strain (GenBank accession numbers CP141196-CP141201). SeroType Finder (https://cge.food.dtu.dk/services/SerotypeFinder/) revealed that the serotype of strain 673 is H19:O8. The complete genome sequences also revealed that strain 673 has a circular chromosome and 5 plasmids. The chromosome is 5,328,997 bp in length with 50.6% GC content, encoding more than 5000 ORFs. MLST analysis of 7 core genes (PubMLST, https://pubmlst.org/), including *adk, fumC, gyrB, icd, mdh, purA*, and *recA*, revealed that strain 673 belongs to ST1431 and forms a subclade with an ST162 clinical *E. coli* strain (GenBank ID: GCA 032673885). ST1431 *E. coli* strains were isolated from animals and humans,[18] but ST1431 is not the pandemic lineage. In addition, strain 673 possesses 20 IS elements, including IS*26*, IS*150*, IS*3*, and IS*609*, much more than its phylogenetically closely related *E. coli* strains (Figure 1). Moreover, strain 673 possesses 16 antimicrobial resistance genes and 5 plasmid replicons, making this strain easy to distinguish from its phylogenetically closely related *E. coli* strains (Figure 1).

Given that strain 673 was isolated from a urine sample, we wondered which virulence genes were encoded by this strain. Using Virulence Finder (http://www.mgc.ac.cn/VFs/), many virulence genes, such as *entF, cfaC, fdeC, iutA, iroN, iucC*, and a type VI secretion system (T6SS), were identified. These results reinforced the notion that strain 673 is pathogenic. In addition, some antimicrobial resistance genes, such as *floR, sul2, aph(3')-Ia*, were identified on the chromosome of strains 673.

Moreover, strain 673 carries 5 plasmids: p673-blaTEM-1B, p673-blaCTX-M-55, p673-blaNDM-5, p673-13272, and p673-6468. p673-blaNDM-5 is 216,339 bp long and is an IncHI2/IncHI2A-type plasmid. We also found that 11 antibiotic resistance genes, including bla_{NDM-5}, *strA, strB, aadA1, sul1, sul2, aadA2*, and *dfrA*, were located in this plasmid. BLAST analysis revealed that p673-blaNDM-5 shares 99.99–100% identity with pAMSH1, pEC6622-1, pJD053-230k, and pHS13-1-IncHI2, with 91–95% coverage. pAMSH1 was carried by an *E. coli* strain isolated from giant panda feces. Both pEC6622-1 and pHS13-1-IncHI2 were carried by *E. coli* strains that were isolated from patient feces and a patient, respectively. The host of pJD053-230k is an *Escherichia fergusonii* strain isolated from cecal contents. These findings indicate that these highly similar plasmids are related to human beings. Among these plasmids, only p673-blaNDM-5 and pEC6622-1 possess bla_{NDM-5} (Figure 2A). Thus, p673-blaNDM-5 is a novel bla_{NDM-5}-bearing IncHI2/IncHI2A-type plasmid. To the best of our knowledge, this is the first report that a clinical *E. coli* strain harbors a bla_{NDM-5}-bearing IncHI2/IncHI2A-type plasmid and that an ST1431 *E. coli* strain harbors bla_{NDM-5}. Moreover, 11 drug resistance genes in p673-blaNDM-5 are flanked by IS*5* and IS*26*, indicating that IS*26* and IS*5* may mediate the integration of these 11 drug resistance genes into p673-blaNDM-5. BLAST analysis revealed a similar genetic structure in pYZMc3-1_NDM-9_226k, whose host is an *E. coli* strain isolated from chicken feces. Intriguingly, similar to bla_{NDM-9} in pYZMc3-1_NDM-9_226k, the bla_{NDM-5} gene is also surrounded by two IS*26* elements in p673-blaNDM-5 and pE-T84-1-NDM5. In p673-blaNDM-

Table 1 Minimum Inhibitory Concentrations (MICs, μg/mL) of Different Antibiotics Used for E. Coli Strains and the Interpretation. S, Sensitive; R, Resistant

Strain	Amikacin	Tigecycline	Ceftriaxone	Cefuroxime	Ceftazidime	Cefepime	Cefoxitin	Ertapenem	Imipenem	Trimethoprim/sulfamethoxazole
673	2/S	0.5/S	≥64/R	≥64/R	≥64/R	≥32/R	≥64/R	≥8/R	≥16/R	≥320/R
Transconjugant	1/S	0.5/S	≥32/R	≥32/R	≥64/R	≥32/R	64	8/R	16/R	320/R

https://doi.org/10.2147/IDR.S477093

Figure 1 Phylogenetic relationships between strain 673 and 19 *E. coli* strains. Plasmid replicon types are labeled with pink squares, IS elements and drug resistance genes are labeled with green circles. *Adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, and *recA* were used as MLST sequences. MEGA version 7.0, which is based on the neighbor-joining method, was used to construct a phylogenetic tree of *E. coli* strains.

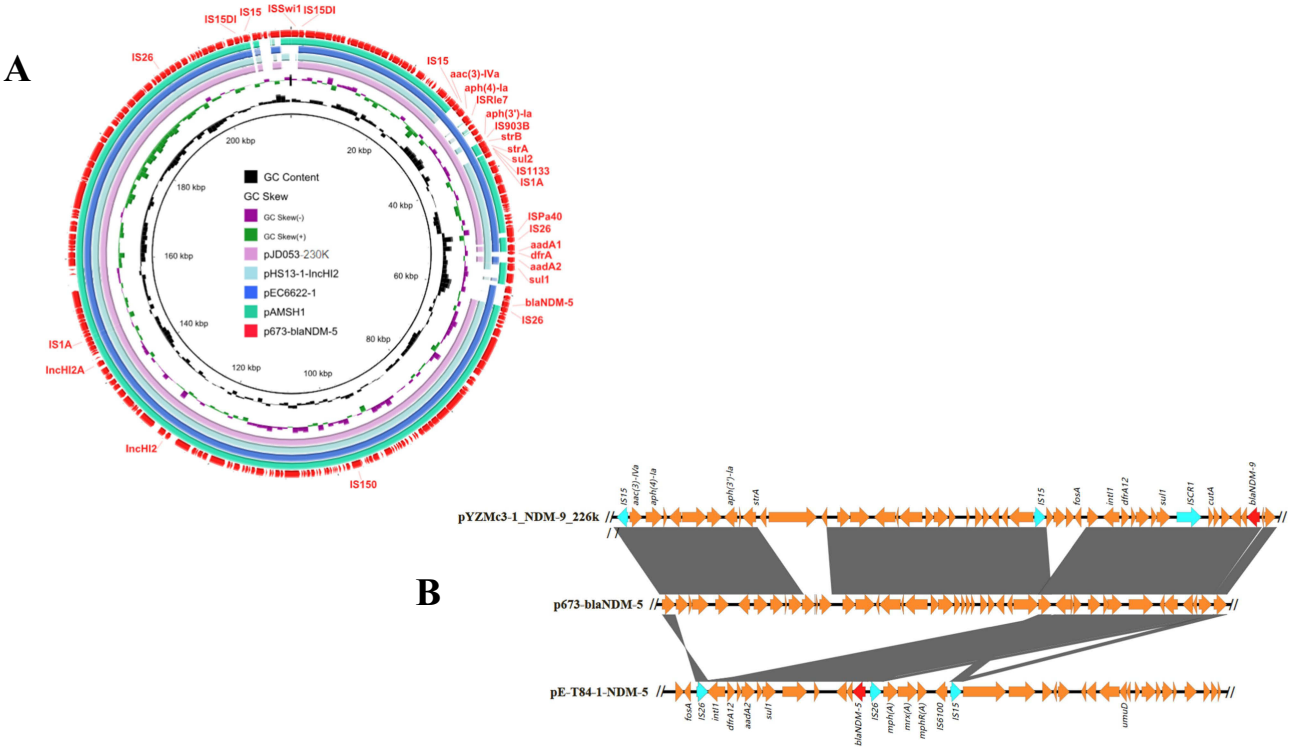

Figure 2 (A) Circular comparative analysis of p673-blaNDM-5 and other plasmids deposited in the GenBank database. Drug resistance genes and insertion sequence elements are labeled at the outermost ring. **(B)** Linear comparison of bla_{NDM-5} and bla_{NDM-9} genetic environments in p673-blaNDM-5 and pYZMc3_NDM-9. Genes are denoted by arrows and are colored on the basis of gene function classification. Red, bla_{NDM} genes; green, IS elements; yellow, other drug resistance genes and nondrug-related genes. Easyfig version 2.2.3 was used to visualize the comparison sequences.

5, two IS*26* elements form an IS26-*xerD-dfrA-orf1-aadA2-qacEΔ1-sul1-orf2-cutA-dsbD-trpF-ble*$_{MBL}$-*bla*$_{NDM-5}$-*IS26* region (Figure 2B). IS*26* may mediate the integration of *bla*$_{NDM-5}$ into p673-blaNDM-5 and pE-T84-1-NDM5, and the IS*26* element may mediate the spread of *bla*$_{NDM-5}$ into other plasmids. To test the transferability of p673-blaNDM-5, a conjugation assay was performed. The results showed that p673-blaNDM-5 could be conjugated to *E. coli* C600 with an average frequency of 3.46×10^{-6} and that the transconjugant was resistant to many drugs (Table 1).

Unlike p673-blaNDM-5, both p673-blaCTX-M-55 and p673-blaTEM-1B possess only 3 and 2 antibiotic resistance genes, respectively. p673-blaCTX-M-55 is 123,043 bp in length and is an IncFII-type plasmid harboring *fosA, aadA1*, and *bla*$_{CTX-M-55}$. This finding is consistent with a previous study showing that *bla*$_{CTX-M-55}$-bearing IncFII-type plasmids

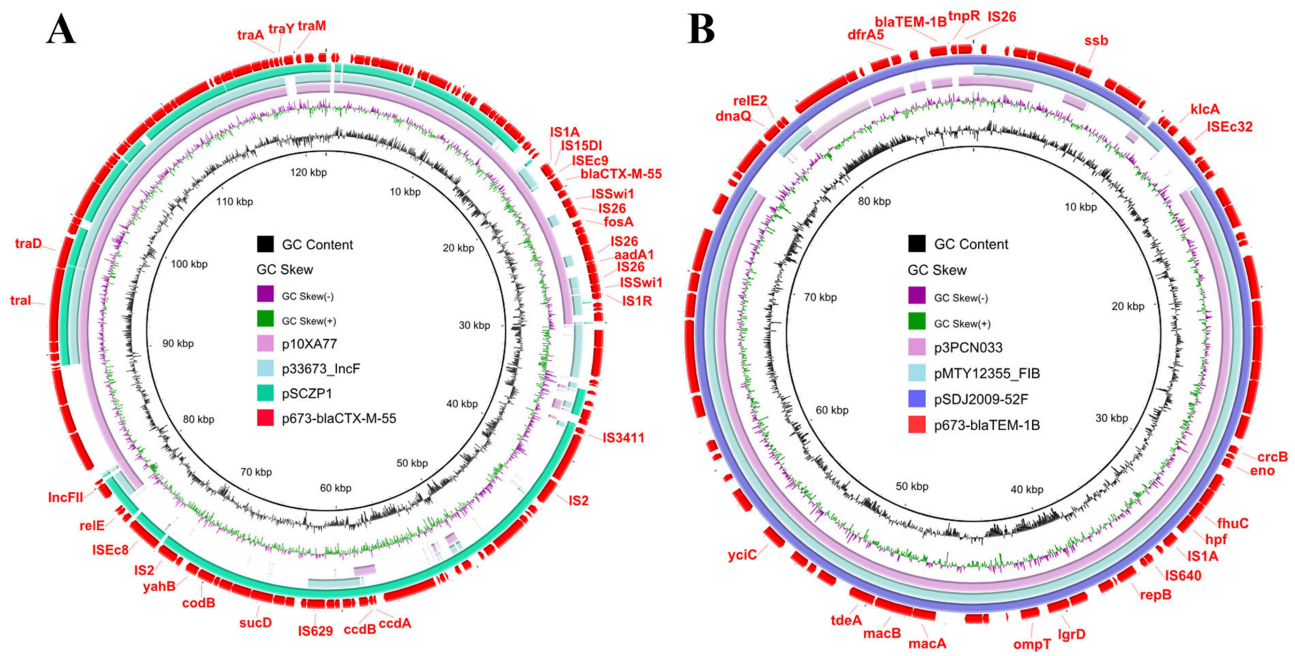

Figure 3 Circular comparative analysis of p673-blaCTX-M-55 **(A)**, p673-blaTEM-1B **(B)** and other plasmids deposited in the GenBank database. Drug resistance genes and insertion sequence elements are labeled at the outermost ring.

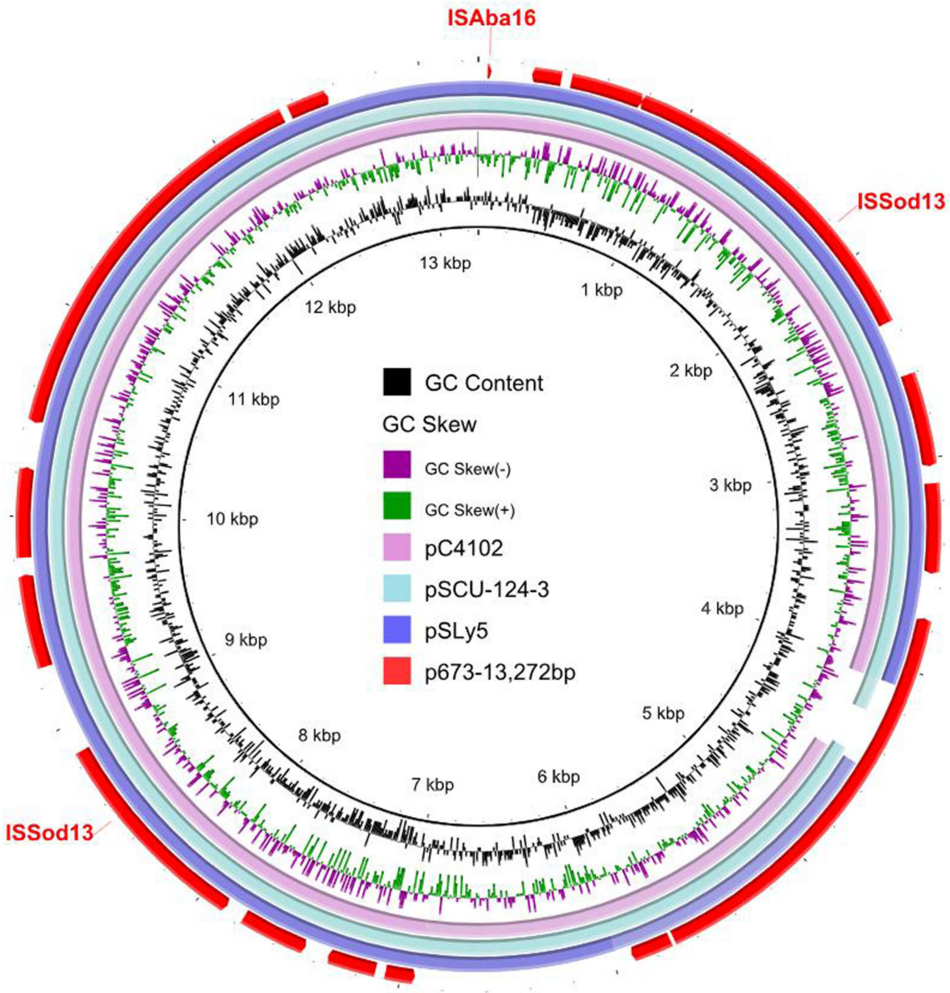

Figure 4 Circular comparative analysis of p673-13272 and other plasmids deposited in the GenBank database. Insertion sequence elements are labeled at the outermost ring.

Table 2 Drug Resistance Plasmid Replicons and Antimicrobial Resistance Genes of E. Coli 673

Plasmid	Replicon Type	Drug Resistance Genes
p673-blaNDM-5	IncHI2/IncHI2A	*bla*$_{NDM-5}$, *strA, strB, aadA1, sul1, sul2, aadA2, dfrA, aac(3)-IVa, aph(4)-Ia, aph(3')-Ia*
p673-blaCTX-M-55	IncFII	*fosA, aadA1, bla*$_{CTX-M-55}$
p673-blaTEM-1B	IncFIB	*bla*$_{TEM-1B}$, *dfrA5*

are prevalent in *E. coli* strains.[19] Moreover, the *fosA, aadA1*, and *bla*$_{CTX-M-55}$ genes are surrounded by IS*1A* and IS*1R* elements in p673-blaCTX-M-55 (Figure 3A), indicating that these IS elements may mediate the integration of *fosA, aadA1*, and *bla*$_{CTX-M-55}$ into p673-blaCTX-M-55.

p673-blaTEM-1B is 89,218 bp long and is an IncFIB-type plasmid harboring *bla*$_{TEM-1B}$ and *dfrA5*. BLAST analysis revealed that p673-blaTEM-1B shares high identity with p3PCN033, pMTY12355-FIB, and pSDJ2009-52F, with 83–97% coverage. (Figure 3B). However, p673-blaCTX-M-55 has much lower coverage, approximately 45–74% with other plasmids, p10XA77, p33673-IncF, and pSCZP1 (Figure 3A). Therefore, p673-blaTEM-1B and p673-blaCTX-M-55 are novel plasmids. Notably, similar to p673-blaNDM-5, p673-blaCTX-M-55 possesses more than 10 IS elements, including IS*2*, IS*26*, and IS*1A*, indicating that some exogenous genes could be integrated into p673-blaCTX-M-55 via these mobile elements. Further characterisation of the functions of the exogenous genes would help us to better understand the role of p673-blaCTX-M-55 in strain 673.

p673-13272 is 13,272 bp in length and is a ColRNAI-type plasmid. BLAST analysis revealed that p673-13272 has high similarity with pC4102, pSCU-124-3, and pSLy5, with 95–99% identity and 95–96% coverage (Figure 4). Unlike p673-blaNDM-5, p673-blaCTX-M-55 and p673-blaTEM-1B, p673-13272 do not possess any drug resistance genes. p673-6468 is 6468 bp in length. Neither the replicon nor the drug resistance gene was identified in this plasmid. The functions of p673-13272 and p673-6468 need further study.

In conclusion, *E. coli* 673 is a clinical strain belonging to ST1431. It carries *bla*$_{NDM-5}$ and many other drug resistance genes (Table 2). To the best of our knowledge, this is the first report in which a *bla*$_{NDM-5}$-bearing IncHI2/IncHI2A-type plasmid has emerged in a clinical *E. coli* strain in China. Our findings suggest that IS*26* mediates the integration of *bla*$_{NDM-5}$ into p673-blaNDM-5. The spread of *bla*$_{NDM-5}$-bearing plasmids is a clinical challenge and endangers public health. Given that *bla*$_{NDM-5}$-bearing *E. coli* strains have spread in China, Germany, the Czech Republic, and Zambia,[3,20–22] there is an urgent need to conduct surveillances to determine whether *bla*$_{NDM-5}$-bearing *E. coli* strains have spread in other countries and take measures to control their spread.

Data Sharing Statement

The complete sequences of strain 673 were submitted to the NCBI database under accession numbers CP141196- CP141201.

Ethics Approval Statement and Informed Consent

We confirmed that all experimental protocols were approved by the ethics committee of Zhuhai People's hospital with Approval number: (2024) Ethical Review (Research) No.15. The informed consent from patient was not a requirement due to the fact that isolation, identification, and characterization of drug-resistant clinical bacteria is a routine work in Zhuhai People's hospital. We also confirmed that the guidelines outlined in the Declaration of Helsinki were followed and the experiments were carried out in accordance with guidelines and regulations of Zhuhai People's hospital.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No. 81650003). Ethical approval was not needed.

Disclosure

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

References

1. Available from: https://www.ncbi.nlm.nih.gov/pathogens/refgene/#NDM.
2. Shen Y, Hu F, Wang Y, et al. Transmission of carbapenem resistance between human and animal NDM-positive *Escherichia coli* Strains. *Engineering*. 2022;15:24–33. doi:10.1016/j.eng.2021.07.030
3. Y. L, Zhang Y, Sun X, et al. National genomic epidemiology investigation revealed the spread of carbapenem-resistant *Escherichia coli* in healthy populations and the impact on public health. *Genome Med*. 2024;16(1):57. doi:10.1186/s13073-024-01310-x
4. Liu YY, Tong L, Yue HY, et al. Occurrence and characterization of NDM-5-producing *Escherichia coli* from retail eggs. *Front Microbiol*. 2023;14:1281838. doi:10.3389/fmicb.2023.1281838
5. Tang B, Guan CJ, Lin H, et al. Emergence of co-existence of *mcr-1* and $bla_{\text{NDM-5}}$ in *Escherichia fergusonii*. *Int J Antimicrob Agents*. 2023;61(3):106742. doi:10.1016/j.ijantimicag.2023.106742
6. Zeng SH, Huang YL, Zhang XW, Fu L, Sun ZH, Li XY. Molecular characterization of IncFII plasmid carrying $bla_{\text{NDM-5}}$ in a *Salmonella* enterica serovar *Typhimurium* ST34 clinical isolate in China. *mSphere*. 2023;8(6):e0048023. doi:10.1128/msphere.00480-23
7. Stehling FJ, Stehling EG. Genomic Insights into *Pluralibacter gergoviae* Sheds Light on Emergence of a Multidrug-Resistant Species Circulating between Clinical and Environmental Settings. *Pathogens*. 2023;12(11):1335. doi:10.3390/pathogens12111335
8. X. L, Y. F, Shen M, et al. Dissemination of $bla_{\text{NDM-5}}$ gene via an IncX3-type plasmid among non-clonal *Escherichia coli* in China. *Antimicrob Resist Infect Control*. 2018;7(1):59. doi:10.1186/s13756-018-0349-6
9. Nordmann P, Poirel L. Epidemiology and diagnostics of carbapenem resistance in gram-negative bacteria. *Clin Infect Dis*. 2019;69(Supplement_7):S521–S528. doi:10.1093/cid/ciz824
10. Jean SS, Harnod D, Hsueh PR. Global threat of carbapenem-resistant gram-negative bacteria. *Front Cell Infect Microbiol*. 2022;12:823684. doi:10.3389/fcimb.2022.823684
11. Lv LC, Lu YY, Gao X, et al. Characterization of *ndm-5*-producing *Enterobacteriaceae* isolates from retail grass carp (*Ctenopharyngodon idella*) and evidence of $bla_{\text{NDM-5}}$-bearing IncHI2 plasmid transfer between ducks and fish. *Zool Res*. 2022;43(2):255–264. doi:10.24272/j.issn.2095-8137.2021.426
12. Tian D, Wang B, Zhang H, et al. Dissemination of the bla NDM-5 Gene via IncX3-Type Plasmid among Enterobacteriaceae in Children. *mSphere*. 2020;5(1):e00699–00619. doi:10.1128/mSphere.00699-19
13. Zhao Q, Berglund B, Zou H, et al. Dissemination of $bla_{\text{NDM-5}}$ via IncX3 plasmids in carbapenem-resistant *Enterobacteriaceae* among humans and in the environment in an intensive vegetable cultivation area in Eastern China. *Environ Pollut*. 2021;273:116370. doi:10.1016/j.envpol.2020.116370
14. J. M, Song X, M. L, et al. Global spread of carbapenem-resistant *Enterobacteriaceae*: epidemiological features, resistance mechanisms, detection and therapy. *Microbiol Res*. 2023;266:127249. doi:10.1016/j.micres.2022.127249
15. Yang Q, Ma XD, Zeng LS, et al. Interphylum dissemination of NDM-5-positive plasmids in hospital wastewater from Fuzhou, China: a single-centre, culture-independent, plasmid transmission study. *Lancet Microbe*. 2023;S2666-5247(23):227–236.
16. Z. M, Zeng Z, Liu J, et al. Emergence of IncHI2 plasmid-harboring $bla_{\text{NDM-5}}$ from porcine *Escherichia coli* isolates in Guangdong, China. *Pathogens*. 2021;10:1–7.
17. Zhao QY, Zhu JH, Cai RM, et al. IS 26 Is Responsible for the Evolution and Transmission of blaNDM -Harboring Plasmids in Escherichia coli of Poultry Origin in China. *mSystems*. 2021;6(4):e64621. doi:10.1128/msystems.00646-21
18. Seiffert SN, Carattoli A, Schwendener S, Collaud A, Endimiani A, Perreten V. Plasmids Carrying $bla_{\text{CMY}\ -2/4}$ in *Escherichia coli* from Poultry, Poultry Meat, and Humans Belong to a Novel IncK Subgroup Designated IncK2. *Front Microbiol*. 2017;15(8):407.
19. Zeng S, Luo J, Li X, et al. Molecular Epidemiology and Characteristics of CTX-M-55 Extended-Spectrum β-Lactamase-Producing *Escherichia coli* From Guangzhou, China. *Front Microbiol*. 2021;12:730012. doi:10.3389/fmicb.2021.730012
20. Kasange M, Gajdacs M, Muleya W, et al. Genotypic Characterisation and Antimicrobial Resistance of Extended-Spectrum β-Lactamase-Producing Escherichia coli in Humans, Animals, and the Environment from Lusaka, Zambia: public Health Implications and One Health Surveillance. *Antibiotics*. 2021;13(10):951. doi:10.3390/antibiotics13100951
21. Chudejova K, Sourenian T, Palkovicova J, Working Group for Monitoring of Antibiotic Resistance, et al. Genomic characterization of ST38 NDM-5-producing *Escherichia coli* isolates from an outbreak in the Czech Republic. *Antimicrob Agents Chemother*. 16;2024:e0013324. doi:10.1128/aac.00133-24
22. Hans JB, Pfennigwerth N, Neumann B, et al. Molecular surveillance reveals the emergence and dissemination of NDM-5-producing *Escherichia coli* high-risk clones in Germany, 2013 to 2019. *Euro Surveill*. 2023;28(10):2200509. doi:10.2807/1560-7917.ES.2023.28.10.2200509