

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

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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-*bla*_{TEM-1B}, p673-*bla*_{CTX-M-55}, p673-*bla*_{NDM-5}, p673-13272, and p673-6468. p673-*bla*_{NDM-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 IS26 elements in p673-*bla*_{NDM-5}, indicating that IS26 could mediate the integration of *bla*_{NDM-5} into p673-*bla*_{NDM-5}. p673-*bla*_{CTX-M-55} is an IncFII-type plasmid harboring *fosA*, *aadA1*, and *bla*_{CTX-M-55}. p673-*bla*_{TEM-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 IS26 mediates the integration of *bla*_{NDM-5} into p673-*bla*_{NDM-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

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 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 IS26, IS150, IS3, and IS609, 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-*bla*TEM-1B, p673-*bla*CTX-M-55, p673-*bla*NDM-5, p673-13272, and p673-6468. p673-*bla*NDM-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-*bla*NDM-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-*bla*NDM-5 and pEC6622-1 possess *bla*_{NDM-5} (Figure 2A). Thus, p673-*bla*NDM-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-*bla*NDM-5 are flanked by IS5 and IS26, indicating that IS26 and IS5 may mediate the integration of these 11 drug resistance genes into p673-*bla*NDM-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 IS26 elements in p673-*bla*NDM-5 and pE-T84-1-NDM5. In p673-*bla*NDM-

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

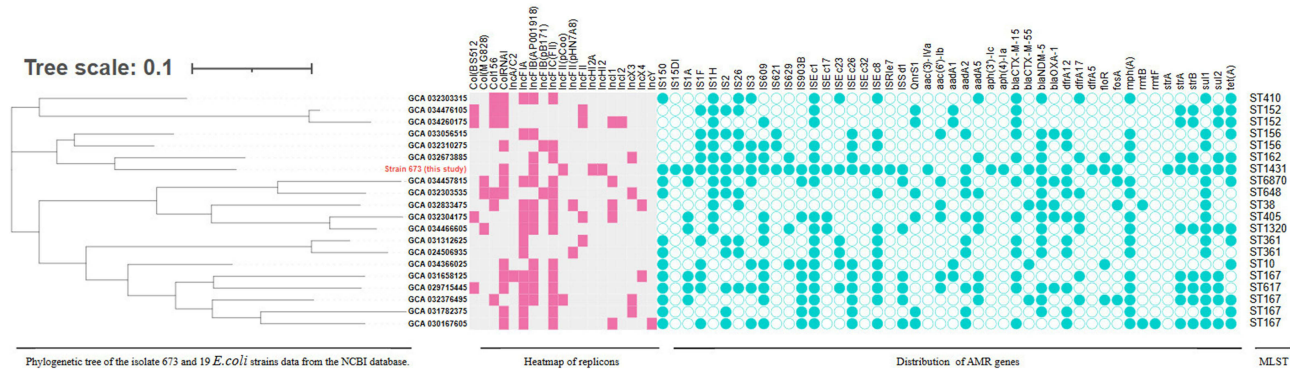


Figure 1 Phylogenetic relationships between strain 673 and 19 *E. coli* strains. Plasmid replicon types are labeled with pink squares, IS elements and drug resistance genes are labeled with green circles. *Adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, and *recA* were used as MLST sequences. MEGA version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

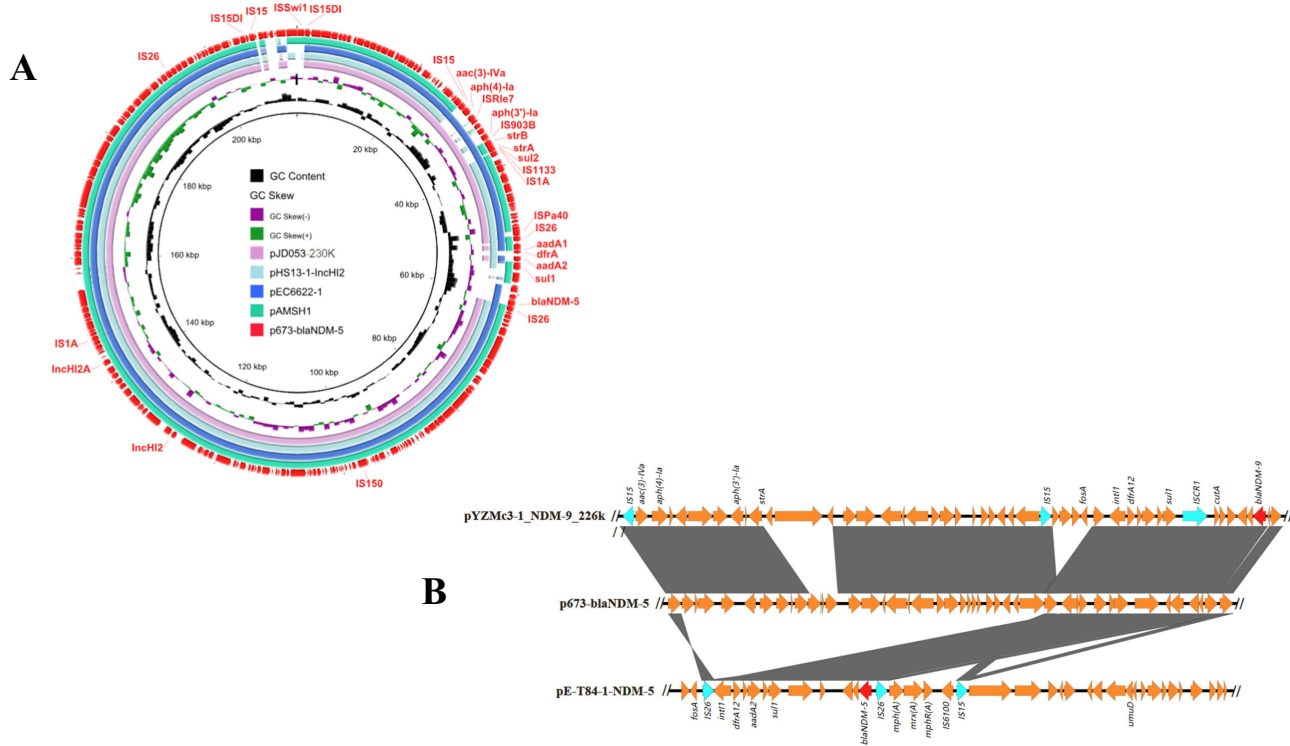


Figure 2 (A) Circular comparative analysis of p673-blaNDM-5 and other plasmids deposited in the GenBank database. Drug resistance genes and insertion sequence elements are labeled at the outermost ring. **(B)** Linear comparison of *bla*_{NDM-5} and *bla*_{NDM-9} genetic environments in p673-blaNDM-5 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 IS26 elements form an IS26-*xerD*-*dfrA*-*orf1*-*aadA2*-*qacEΔ1*-*sul1*-*orf2*-*cutA*-*dsbD*-*trpF*-*ble*_{MBL}-*bla*_{NDM-5}-IS26 region (Figure 2B). IS26 may mediate the integration of *bla*_{NDM-5} into p673-blaNDM-5 and pE-T84-1-NDM5, and the IS26 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

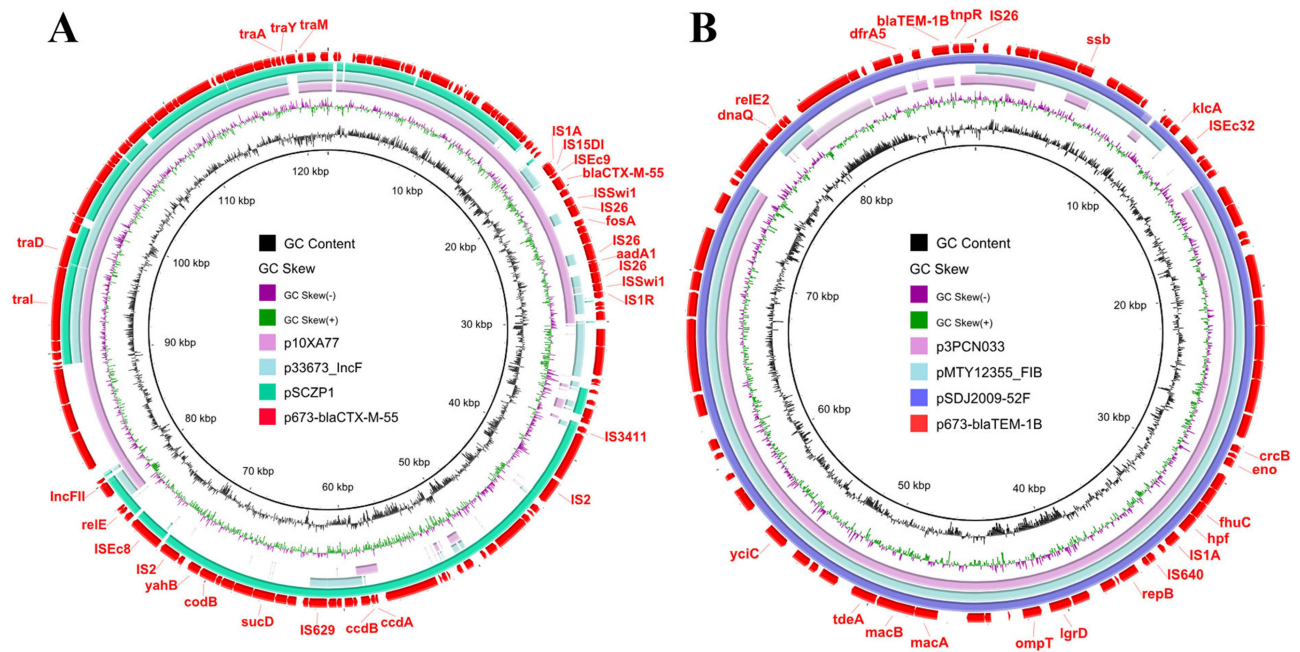


Figure 3 Circular comparative analysis of p673-blaCTX-M-55 (A), p673-blaTEM-1B (B) and other plasmids deposited in the GenBank database. Drug resistance genes and insertion sequence elements are labeled at the outermost ring.

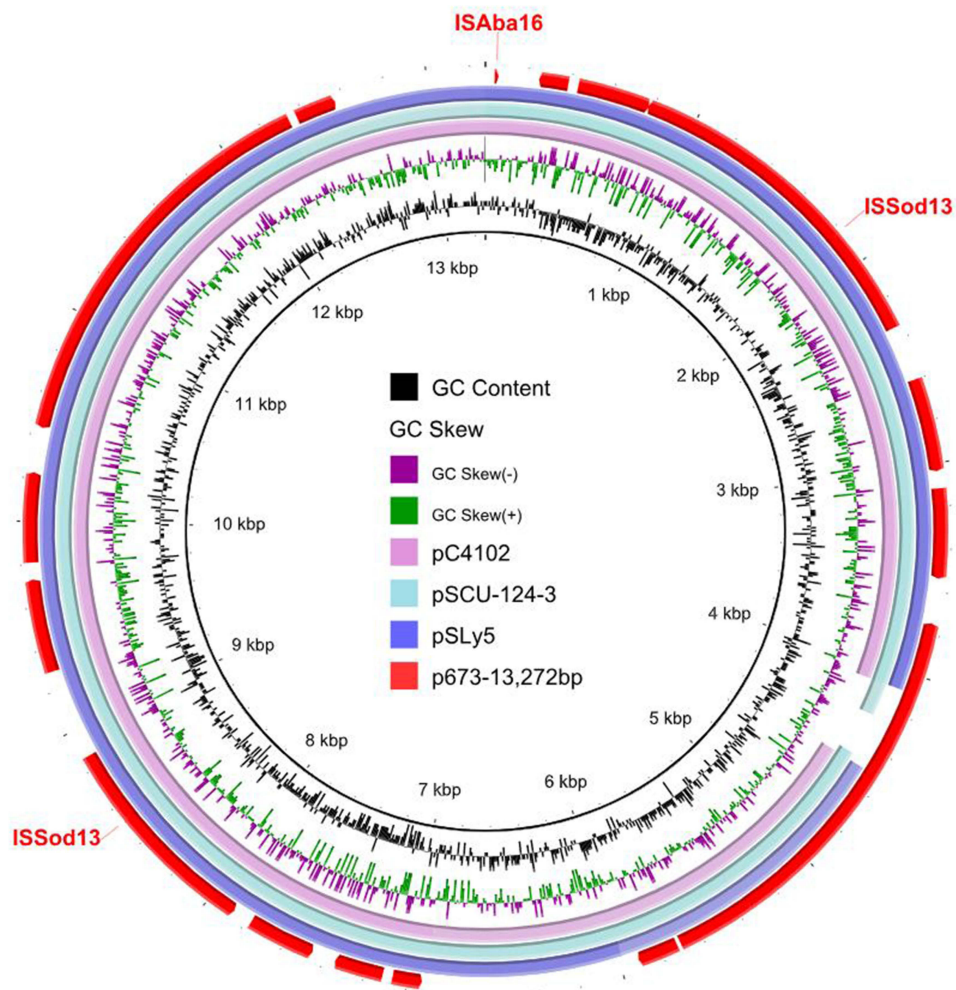


Figure 4 Circular comparative analysis of p673-13272 and other plasmids deposited in the GenBank database. Insertion sequence elements are labeled at the outermost ring.

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	<i>bla</i> _{NDM-5} , <i>strA</i> , <i>strB</i> , <i>aadA1</i> , <i>sul1</i> , <i>sul2</i> , <i>aadA2</i> , <i>dfrA</i> , <i>aac(3)-IVa</i> , <i>aph(4)-Ia</i> , <i>aph(3')-Ia</i>
p673-blaCTX-M-55	IncFII	<i>fosA</i> , <i>aadA1</i> , <i>bla</i> _{CTX-M-55}
p673-blaTEM-1B	IncFIB	<i>bla</i> _{TEM-1B} , <i>dfrA5</i>

are prevalent in *E. coli* strains.¹⁹ Moreover, the *fosA*, *aadA1*, and *bla*_{CTX-M-55} genes are surrounded by *IS1A* and *IS1R* 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 IS2, IS26, and *IS1A*, 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 *IS26* 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.

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