Infection and Drug Resistance

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ORIGINAL RESEARCH

The First Report of *Escherichia coli* and *Klebsiella pneumoniae* Strains That Produce Both NDM-5 and OXA-181 in Jiangsu Province, China

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Objective: The aim of this study was to analyze the genetic characteristics of three Enterobacteriaceae strains (one strain of *Escherichia coli* and two strains of *Klebsiella pneumoniae*) that produce both the NDM-5 and OXA-181 carbapenemases in pediatric patients.

Methods: Carbapenem-resistant Enterobacteriaceae (CRE) strains were collected from the Children's Hospital Affiliated to Nanjing Medical University in 2022. Resistance genes were detected by PCR. CRE strains that produced both the $bla_{\text{NDM-5}}$ and $bla_{\text{OXA-181}}$ genes were further characterized by antimicrobial susceptibility testing, multilocus sequence typing (MLST), plasmid conjugation assay, S1 nuclease-PFGE, Southern blotting and whole-genome sequencing.

Results: Three Enterobacteriaceae strains carrying both the $bla_{\text{NDM-5}}$ and $bla_{\text{OXA-181}}$ resistance genes were screened. MLST results showed that the strain of *Escherichia coli* carrying both $bla_{\text{NDM-5}}$ and $bla_{\text{OXA-181}}$ was ST410; the two strains of *Klebsiella pneumoniae* with both $bla_{\text{NDM-5}}$ and $bla_{\text{OXA-181}}$ were ST2601 and ST759. Conjugation assays showed that the plasmids harboring the $bla_{\text{NDM-5}}$ and $bla_{\text{OXA-181}}$ genes were self-transmissible. S1-PFGE and Southern blotting showed that the $bla_{\text{NDM-5}}$ and $bla_{\text{OXA-181}}$ genes were located on the plasmid with the size of about 60kb~. The genotyping results showed that the plasmid types were ColKP3 and IncX3.

Conclusion: This is the first report of Enterobacteriaceae strains that produce both NDM-5 and OXA-181 isolated from pediatric patients in China. Active infection control measures are urgently needed to prevent the spread of bacteria in children.

Keywords: *Escherichia coli*, *Klebsiella pneumoniae*, NDM-5, OXA-181, plasmid

Introduction

In recent years, the continuous emergence of carbapenem-resistant Enterobacteriaceae (CRE) strains threatens public health worldwide. The main mechanism of carbapenem resistance in Enterobacteriaceae is the production of KPC (Ambler class A), NDM, VIM, IMP (class B) and OXA-48-like (class D) β-lactamases. New Delhi gold β-lactamase (NDM-1) was first discovered in 2008,[1] and since then, Enterobacteriaceae carrying the bla_{NDM} gene have been found around the world. To date, 31 gene subtypes have been found globally.[2] In 2011, Hornsey et al first identified $bla_{\text{NDM-5}}$ in a strain of *Escherichia coli* ST648 with a multidrug resistance phenotype in the UK.[3] Since then, strains carrying $bla_{\text{NDM-5}}$ have been found in many countries around the world, including Egypt,[4] South Korea,[5] Italy[6] and China.[7] Compared with NDM-1, the NDM-5 enzyme has two amino acid substitutions (Val88Leu and Met154Leu), and it has been shown that the NDM-5 enzyme causes a higher level of resistance to carbapenems and broad-spectrum cephalosporins.[3]

Since 2004, when *Klebsiella pneumoniae* carrying $bla_{\text{OXA-48}}$was isolated from a patient living in Turkey,[8] Enterobacteriaceae strains carrying $bla_{\text{OXA-48-like}}$ have been found around the world. Several OXA-48-like

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carbapenemases have been reported, including OXA-48, OXA-162, OXA-181, OXA-204, OXA-232, OXA-244, and OXA-245.[9] OXA-181, a variant of OXA-48 with four amino acid substitutions, was first reported in India in 2007.[10] Since then, it has been found mainly in *Escherichia coli* and *Klebsiella pneumoniae* strains in several countries (UK, USA, etc.). The gene encoding OXA-181 is usually located on the IncX3-type plasmid.[11] This plasmid carries several carbapenemase genes, including bla_{KPC}[12] and bla_{NDM}.[13] To date, OXA-181-producing *Escherichia coli* has been reported in Sichuan[14] and Henan,[15] and OXA-181-producing *Klebsiella pneumoniae* has been reported in Zhejiang.[16] The first strain containing $bla_{OXA-48-like}$ and bla_{NDM-} was reported in Singapore in 2013, followed by Egypt,[17] the United States,[18] Bangladesh,[19] Italy[20] and other locations. Here, we report the first *Escherichia coli* and *Klebsiella pneumoniae* strains in China that produce both the NDM-5 and OXA-181 enzymes to analyze the genetic characteristics and environment in pediatric patients.

Materials and Methods

Clinical Data

On January 6, 2022, a 24-day-old newborn with "poor response, abdominal distension" was admitted to the children's hospital of Nanjing Medical University. After admission, abdominal distension did not improve after symptomatic treatments, including fasting, anti-infection and defecation, so an emergency exploratory laparotomy was performed that night. Necrotizing enterocolitis was confirmed during the operation, and most of the small intestine was necrotic. "Intestinal resection, jejunostomy and intestinal adhesiolysis" were performed; the operation was successful, and the treatment was continued in the neonatal Medical Center after the operation. The patient was diagnosed with neonatal necrotizing enterocolitis, peritonitis and neonatal sepsis and was given meropenem combined with vancomycin to prevent infection. Sputum cultures and drug sensitivity testing on January 8 revealed that the identified *Escherichia coli* (EC73) strain was multidrug resistant and only sensitive to amikacin, polymyxin and tigecycline. The infant was placed in the isolation room for MDRO isolation. On January 10, vancomycin was discontinued, and immunoglobulin was given as a supportive therapy. On March 28, the infant had fever with a peak of 40.5°C and some fatigue. On March 30, a blood-bacterial culture and drug sensitivity testing revealed that there were gram-negative bacilli present. On April 1, blood-bacterial culture and drug sensitivity assays revealed that the identified *Klebsiella pneumoniae* (KP92) strain was sensitive to aztreonam. Meropenem was discontinued, and the anti-bacterial treatment aztreonam was given. After 8 days, the blood cultures were negative for CRE.

On April 24, the third strain (KP100) was extracted from the abdominal effusion of an 8-month-old child three days after enterostomy. As the drug sensitivity results showed sensitivity to aztreonam, the child was given this treatment as an anti-infective. Neither of the patients had traveled in the 30 days before their admission.

Antimicrobial Susceptibility Testing and Identification of Antibiotic Resistance Genes

This study was conducted at the Children's Hospital of Nanjing Medical University in Jiangsu Province, China. Enterobacteriaceae was identified by a VITEK-2 Compact system (bioMerieux, Marcy-L 'Etoile, France). Antimicrobial susceptibility testing was performed by broth microdilution. Susceptibility breakpoints were set according to criteria from the Institute of Clinical and Laboratory Standards (CLSI),[21] except for polymyxin E and tigecycline, whose susceptibility breakpoints were set based on criteria from the European Committee for Antimicrobial Susceptibility Testing (EUCAST) 10.0.[22] The quality control (QC) strain *Escherichia coli* ATCC 25922 was used in all tests.

PCR was used to identify bla_{NDM-5} and $bla_{OXA-181}$ using the following primers previously described in the literature: NDM-5-F, 5'-GAAGCTGAGCACCGCATTAG-3', NDM-5-R, 5'-GGGCCGTATGAGTGATTGC-3',[23] OXA-48-like-F, 5′-GCGTGGTTAAGGATGAACAC-3′ and OXA-48-like-R, 5′- CATCAAGTTCAACCCAACCG −3′.[24]

The amplification system consisted of 50 μL of ddH_2O (20 μL), Multiplex Buffer (25 μL), Primer1 (10 μM) (2 μL), Primer2 (10 μM) (2 μL), and template (1 μL). The PCR amplification conditions were 95 °C for 3 min; 35 cycles of 95 °C for 15s, 55°C for 15s, and 72°C for 15s; and 72 °C for 5 min. The PCR products were identified using a 1.0% agarose gel for electrophoresis, and the positive products were sequenced by Sanger sequencing. The complete sequences were

compared with those reported in GenBank using the BLAST algorithm (http://www.ncbi.nlm.nih.gov/blast/). Enterobacteriaceae strains carrying both $bla_{\text{NDM-5}}$ and $bla_{\text{OXA-181}}$ were collected for further study.

Multilocus Sequence Typing (MLST)

Referring to the Pasteur MLST website (http://bigsdb.Pasteur.fr/), seven housekeeping genes from *Klebsiella pneumoniae, rpoB, infB, phoE, mdh, pgi, gapA*, and *tonB*, and seven housekeeping genes from *Escherichia coli, adk, fumC, gyrB, icd, mdh, purA*, and *recA*, were amplified. DNA sequencing was performed on the positive products. The allelic profiles of the seven housekeeping genes for the respective strains were obtained from the MLST database and then submitted to the MLST website to determine the sequence typing of clinical isolates.

Conjugation Assay

Conjugation assays were performed to test the ability of plasmids carrying $bla_{\text{NDM-5}}$ and $bla_{\text{OXA-181}}$ to transfer between strains, and the rifampicin-resistant E. coli strain EC600 was used as the receptor. Overnight cultures of donor and recipient strains were mixed and dropped on sterile filter paper at a 1:3 ratio and incubated overnight at 37°C on MH agar plates. $bla_{\text{NDM-5}}$ and $bla_{\text{OXA-181}}$ conjugates were selected by screening on dual antibody plates containing imipenem (4mg/L) and rifampicin (600 mg/L).

S1-PFGE and Southern Blotting

Drug-resistant plasmids carrying the $bla_{\text{NDM-5}}$ and $bla_{\text{OXA-181}}$ genes were isolated and mapped by S1-PFGE and Southern blotting. Briefly, isolates with $bla_{\text{NDM-5}}$ and $bla_{\text{OXA-181}}$ were embedded in gold agarose and digested with S1 nuclease (TaKaRa Biotechnology Co., Dalian, China), and plasmid DNA (Bio-Rad, USA) was analyzed by PFGE electrophoresis. Then, DNA fragments were transferred to positively charged nylon membranes and hybridized specifically with $bla_{\text{NDM-5}}$ and $bla_{\text{OXA-181}}$ probes. Probes were synthesized using the DIG High Prime DNA Labeling and Detection Starter Kit I (Roche Applied Sciences, Penzberg, Germany) according to the manufacturer's instructions.

Whole-Genome Sequencing and Analysis

To get a comprehensive picture of the strains that produced both $bla_{\text{NDM-5}}$ and $bla_{\text{OXA-181}}$, the three strains that were identified in this study (EC73, KP92 and KP100) were selected for whole-genome sequencing. Genome sequencing was performed by the Personal Biotechnology Company (Shanghai, China) using the Pacific Biosciences platform and the Illumina MiSeq/Hiseq/Novaseq platform.

The whole-genome shotgun (WGS) strategy and next-generation sequencing (NGS) technology were used to construct libraries of different insertions. The libraries were sequenced on the PacBio Sequel sequencing platform which is based on the Illumina NovaSeq sequencing platform and third-generation single-molecule sequencing technology. FastQC was used for quality control. Using CARD (The Comprehensive Antibiotic Resistance Database), antibiotic resistance genes in the genome sequence can be identified through analysis of antibiotic resistance profiles. PlasmidFinder 2.1 was used to analyze plasmid replicon types. Reference sequences were searched through the NCBI blast database, comparative gene cluster analysis was performed using Easyfig, and comparative genomic circle maps were constructed using CGView.

Results

Antimicrobial Susceptibility and Resistance Gene Testing

The three CRE strains were all resistant to imipenem, meropenem, cephalosporin and enzyme inhibitor compounds, and the *Escherichia coli* strain was only sensitive to amikacin, polymyxin and tigecycline. Both *Klebsiella pneumoniae* strains were sensitive to aztreonam, amikacin, ciprofloxacin, levofloxacin, gentamicin, tobramycin, polymyxin and tigecycline. The minimum inhibitory concentration (MIC) assay results for the three CRE strains are shown in Table 1.

All three CRE strains were found to carry the carbapenemase resistance genes $bla_{\text{NDM-5}}$ and $bla_{\text{OXA-181}}$.

Table 1 The Minimum Inhibitory Concentration of Donor Bacteria, Recipient Bacteria and Conjugant (MIC, μg /Ml)

Antibiotics	EC73	J73	KP92	J92	KP100	J100	EC600
Ampicillin	**≥32**	**≥32**	**≥32**	**≥32**	**≥32**	**≥32**	4
Ampicillin/Sulbactam	**≥32**	**≥32**	**≥32**	**≥32**	**≥32**	**≥32**	≤2
Piperacillin	**≥128**	**≥128**	**≥128**	**≥128**	**≥128**	**≥128**	≤4
Piperacillin/tazobactam	**≥128**	**≥128**	**≥128**	**64**	**≥128**	**64**	≤4
Cefuroxime	**≥64**	**≥64**	**≥64**	**≥64**	**≥64**	**≥64**	4
Cefuroxime Axetil	**≥64**	**≥64**	**≥64**	**≥64**	**≥64**	**≥64**	4
Cefotetan	**≥64**	**≥64**	**≥64**	**≥64**	**≥64**	**≥64**	≤4
Ceftazidime	**≥64**	**≥64**	**≥64**	**≥64**	**≥64**	**≥64**	≤1
Ceftriaxone	**≥64**	**≥64**	**≥64**	**≥64**	**≥64**	**≥64**	≤1
Cefepime	**≥64**	**16**	**≥32**	**32**	**≥32**	**32**	≤1
Aztreonam	**16**	≤1	≤1	≤1	≤1	≤1	≤1
Imipenem	**8**	**4**	**≥16**	**8**	**≥16**	**≥16**	≤1
Meropenem	**8**	**8**	**8**	**4**	**8**	**8**	≤0.25
Amikacin	8	≤2	≤2	≤2	≤2	≤2	≤2
Gentamicin	**≥16**	≤1	≤1	≤1	≤1	≤1	≤1
Tobramycin	**≥16**	≤1	≤1	≤1	≤1	≤1	≤1
Ciprofloxacin	**≥4**	0.5	**1**	**1**	**1**	**1**	≤0.25
Levofloxacin	**≥8**	1	1	1	1	1	≤0.25
Trimethoprim/Sulfamethoxazole	**≥320**	≤20	**≥320**	≤20	**≥320**	≤20	≤20
Cefazolin	**≥64**	**≥64**	**≥64**	**≥64**	**≥64**	**≥64**	≤4
Nitrofurantoin	64	≤16	**128**	≤16	64	≤16	≤16
Colistin	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5
Tigecycline	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5

Notes: 1) In order to avoid duplication, only the first isolate was taken from the same specimen of the same child. In this study, a total of 3 strains were collected after removing the duplicate specimen: Escherichia coli (from sputum culture specimens) is denoted by EC73, Klebsiella pneumoniae (from blood culture specimens) is denoted by KP92, and Klebsiella pneumoniae (from ascites culture specimens) is denoted by KP100. Their conjugation products were denoted by J73, J92 and J100 respectively, and the donor bacteria was EC600. 2) The results for drug resistance have been bolded.

MLST

MLST analysis showed that the EC73 strain is ST410, the KP92 strain ST2601 and the KP100 strain ST759.

Plasmid Conjugation Assay and Gene Location

The plasmids carrying the $bla_{\text{NDM-5}}$ and $bla_{\text{OXA-181}}$ genes could be transferred from the donor strain to the recipient strain EC600. The corresponding conjugants are named J73, J92, and J100. The results of PCR amplification showed that all the conjugants carried the $bla_{\text{NDM-5}}$ and $bla_{\text{OXA-181}}$ genes. The drug sensitivity results showed that the three conjugants were resistant to carbapenems and enzyme inhibitors, as shown in Table 1.

The results of S1-PFGE and Southern blotting showed that $bla_{\text{NDM-5}}$ and $bla_{\text{OXA-181}}$ were located on plasmids of approximately 60 kb in size in the three donor strains and in the three conjugants, as shown in Figure 1.

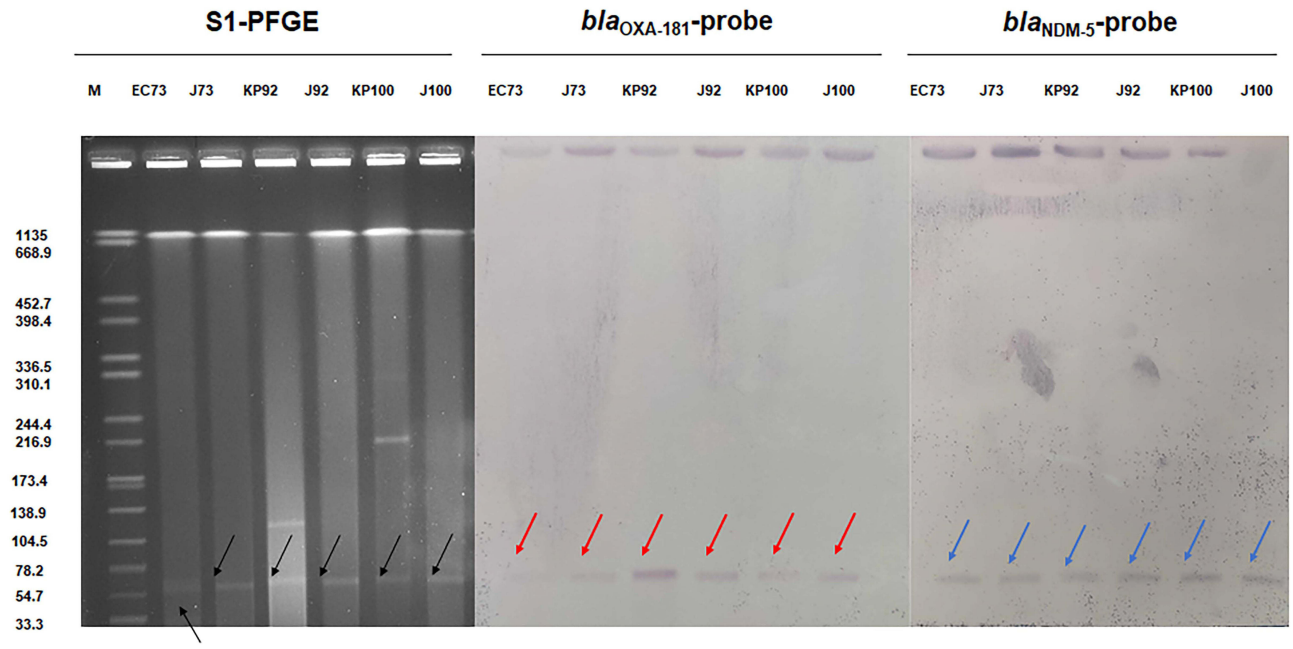

Figure 1 S1-PFGE profiles and Southern blotting results. From left to right: S1-PFGE profiles; Southern blotting results: The red arrow indicates the hybridization and localization of the plasmid carrying the *bla*$_{OXA-181}$ gene. Southern blotting results: The blue arrow indicates the hybridization and localization of the plasmid carrying the *bla*$_{NDM-5}$ gene.
Note: M: Salmonella H9812 Marker.

Plasmid Sequence Analysis Based on Whole-Genome Sequencing

The CARD database was used to analyze the whole-genome sequencing results, and the predicted resistance genes in the EC73, KP92 and KP100 strains were identical as follows: 1) the β-lactam resistance genes: *bla*$_{NDM-5}$ and *bla*$_{OXA-181}$; 2) the quinolone resistance gene: *qnrS1*; 3) the aminoglycoside resistance gene: *aadA2*; 4) the sulfa resistance gene: *sul*; 5) the methylene benacil resistance gene: *dfrA12*; and 6) the quaternary ammonium resistance gene: *qacEΔ1*. The carbapenemase resistance gene *bla*$_{OXA-181}$ was located on the 61,973 bp plasmid 2. The length of EC73 plasmid 2 was 61,973 bp, the average GC content was 48.06%, and there were 81 open reading frames. The length of KP92 plasmid 2 was 61,973 bp, the average GC content was 48.06%, and there were 81 open reading frames. The length of KP100 plasmid 2 was 61,973 bp, the average GC content was 48.06%, and there were 83 open reading frames. According to the PlasmidFinder database from the Center for Genomic Epidemiology, the bacterial drug resistance genes *bla*$_{NDM-5}$ and *bla*$_{OXA-181}$ were on the IncX3 and ColKP3 plasmids in this study. The skeleton structure of these plasmids includes the plasmid conjugation T4SS secretion system, which consists of the Vir gene family (*virB2, virB3, virB9, virB10, virB11*), the protein-related genes *parA* and *topB* that help maintain plasmid stability, and the *traG* and *trbM* elements that play a role in conjugation and transfer.

The *bla*$_{NDM-5}$ and *bla*$_{OXA-181}$ carrier genes are located on the Tn3 gene transposable unit. The complete sequences of the three strains (EC73, KP92 and KP100) have been deposited in GenBank, and the accession numbers are SRP399849, SRP398461 and SRP398463. Further BLAST plasmid sequence alignment analysis showed that the nucleotide similarity between EC73 plasmid 2 and NZ_CP024825.1 (*Escherichia coli* isolated from Korea in 2015, *bla*$_{NDM-5}$ located on the IncX3 plasmid) was more than 99%, and KP92 plasmid 2 and NZ_CP026727.1 (*Escherichia coli* isolated from the United Kingdom in 2018, *bla*$_{OXA-181}$ is located on the IncX3 plasmid) had more than 99% nucleotide similarity. The genome comparisons are shown in Figures 2 and 3.

Discussion

Carbapenems have long been considered the last line of defense for multidrug-resistant gram-negative bacterial infections. The emergence of carbapenem-resistant *Klebsiella pneumoniae* is a serious challenge as there are few clinical anti-

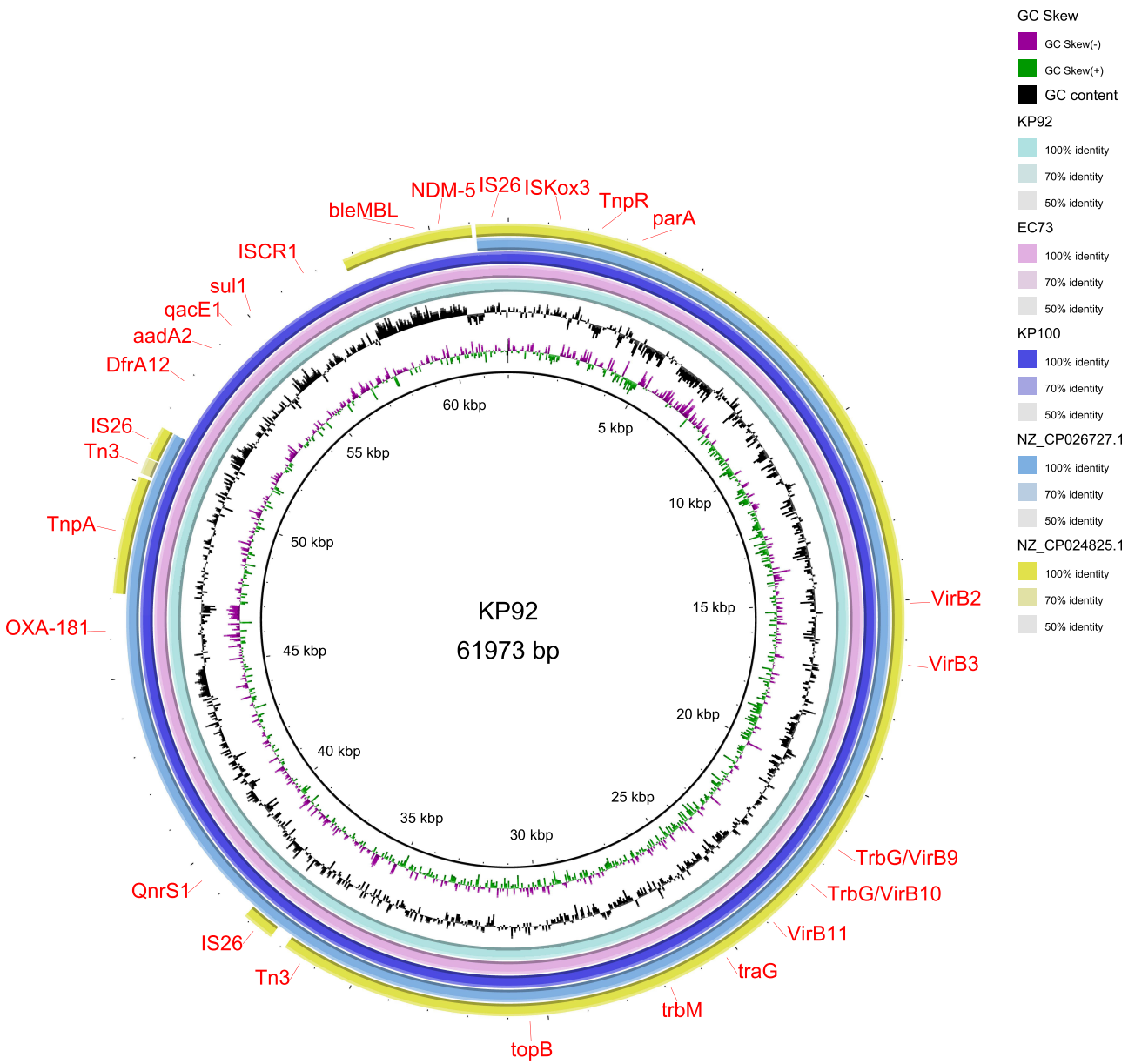

Figure 2 Comparison of NZ_CP024825.1, NZ_CP026727.1, KP100, KP92 and EC73 genomes from the outside in.

infective treatments, especially in pediatric patients. The purpose of this study was to investigate the resistance-related characteristics of CRE strains isolated in the Children's Hospital Affiliated to Nanjing Medical University that produce both the NDM-5 and OXA-181 enzymes to help prevent large-scale hospitalization of patients with CRE in the Children's Hospital. To the best of our knowledge, this is the first time in China that *Escherichia coli* and *Klebsiella pneumoniae* strains that produce both the NDM-5 and OXA-181 enzymes have been found in children.

The two children whose samples were used in this study were both preterm infants who had very low birth weights. Since preterm birth and low birth weight are both risk factors for CRE infection in children,[25] children with high risk factors should receive more attention when implementing prevention and control measures in the future. In this study, all clinical strains were sensitive to polymyxin and tigecycline in vitro. Tigecycline is not recommended for children because of the risk of tooth coloring, and polymyxin is not used for the clinical treatment of children in China.[26] In addition, aminoglycoside antibiotics and quinolones are rarely used in children because of their ototoxicity and nephrotoxicity.[27,28] Clinical data showed that both children had good clinical effects after treatment with aztreonam,

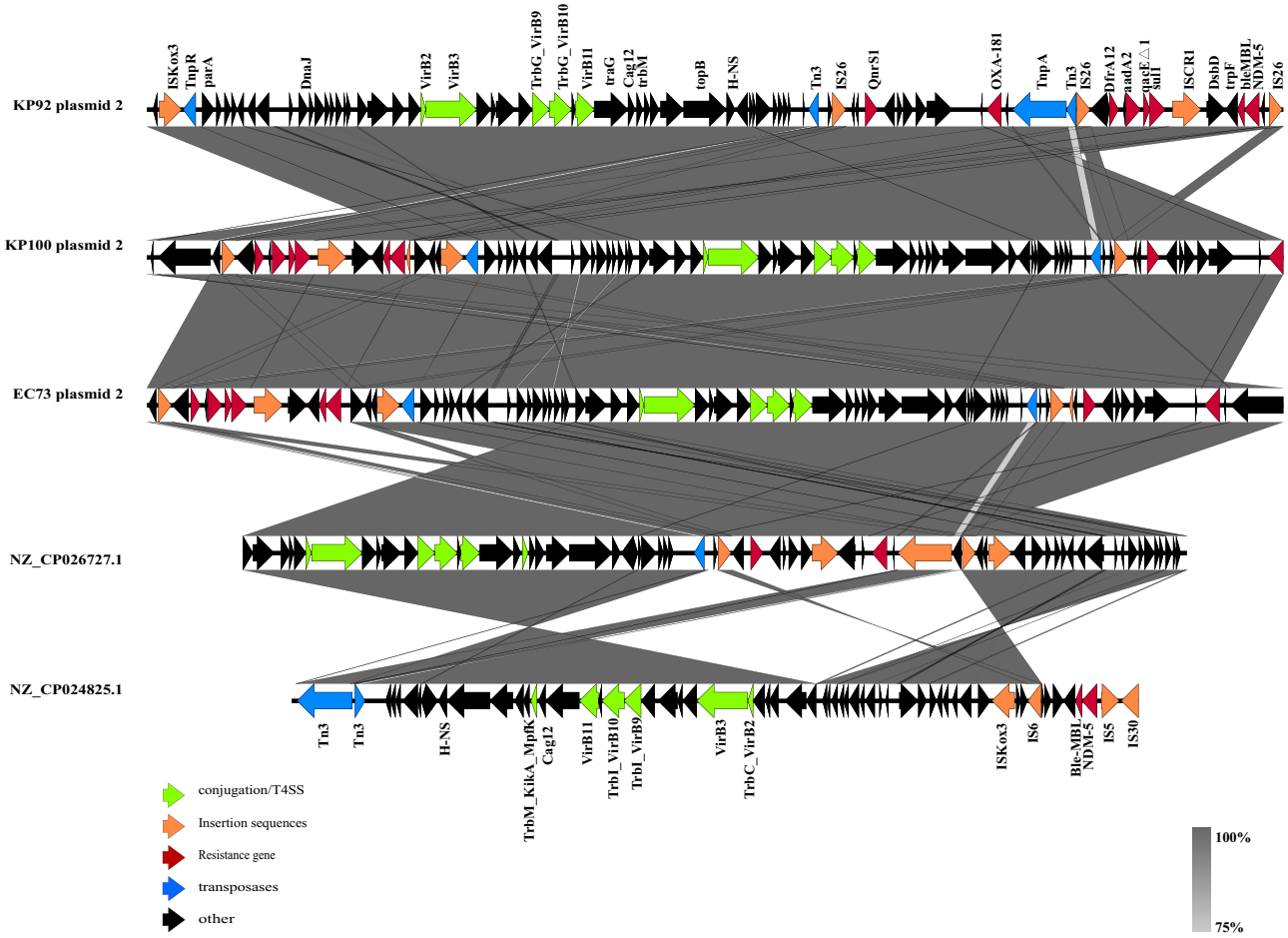

Figure 3 Comparative gene cluster analysis. Arrows indicate open reading frames arranged in the direction of transcription. The gene functions are represented by different colors. The red region represents drug resistance genes, the green region represents genes related to plasmid conjugation, the Orange regions represent insertion elements, the blue regions represent transposons, and the black regions encode other genes. Gray shaded areas indicate sequences that are highly similar sequences between plasmids.

which was consistent with the drug resistant profiles of the clinical isolates. Therefore, it is possible that the strains isolated from sputum specimens may be a part of the colonizing flora in the respiratory tract rather than true pathogens. Because this study was retrospective, we were unable to conduct an in-depth analysis of the environment surrounding the patients and the source of the strains.

According to the MLST results in this study, the EC73 strain is ST410, the KP92 strain is ST2601, and the KP100 strain is ST759. The *Escherichia coli* strain identified in this study is ST410, which belongs to the ST23 clonal complex and has been considered the origin of the ST23 complex. *Escherichia coli* strains that are ST410 are widely distributed in Europe (Poland,[29] Denmark,[30] Italy,[31] etc.), Asia (South Korea,[32] Singapore,[33] India,[34] China,[35] etc.), Africa,[36] North America (United States,[37] Mexico,[38] etc.) and South America (Brazil,[39] Chile[40] et al), etc. However, *Klebsiella pneumoniae* strains that are ST2601 and ST759 and that produce both NDM-5 and OXA-181, such as the strains used in this study, have never been reported globally.

The results of conjugation experiments showed that the resistance genes $bla_{\text{NDM-5}}$ and $bla_{\text{OXA-181}}$ in the three CRE strains could be successfully transferred into the recipient strain EC600 by plasmid, which made the conjugants resistant to carbapenems. Plasmid analysis showed that $bla_{\text{NDM-5}}$ and $bla_{\text{OXA-181}}$ are located on the IncX3 and ColKP3 plasmids, respectively, both of which are the same size (approximately 61 kb). The ColKP3 plasmid is not self-transmitted but can move with the help of other plasmids.[9] The IncX3 plasmid plays an important role in the transmission of the $bla_{\text{NDM-5}}$ gene among Enterobacteriaceae.[7] IncX3-type plasmids have also been frequently reported to mediate the spread of other NDM

subtypes, including *bla*$_{\text{NDM-1}}$, *bla*$_{\text{NDM-4}}$, *bla*$_{\text{NDM-5}}$, *bla*$_{\text{NDM-6}}$, *bla*$_{\text{NDM-7}}$, *bla*$_{\text{NDM-13}}$, *bla*$_{\text{NDM-17}}$, *bla*$_{\text{NDM-19}}$, *bla*$_{\text{NDM-20}}$, and *bla*$_{\text{NDM-21}}$.[41] Therefore, we need to take effective measures to control the spread of this resistance plasmid among strains.

Whole-genome sequencing of the CRE strains revealed *bla*$_{\text{NDM-5}}$ and *bla*$_{\text{OXA-181}}$ to be present on the IncX3 and ColKP3 plasmids that were 61,973 bp in size. BLAST analysis of plasmid sequences showed that plasmid 2 of EC73 is highly similar to that in NZ_CP024825.1 (*Escherichia coli* isolated from Korea in 2015) and that plasmid 2 of KP92 is highly similar to that in NZ_CP026727.1 (*Escherichia coli* isolated from the UK in 2018). These findings suggest that the CRE plasmids that carry both of the carbapenemase genes may be derived from *Escherichia coli*. In plasmids, drug resistance genes are often associated with mobile genetic elements, such as transposons (Tn) and insertion sequences (IS). IS26 belongs to the IS6 family, which plays a pivotal role in the transmission of antibiotic resistance genes among gram-negative bacilli.[42] IS26 encloses multiple resistance genes and lays the groundwork for expression of resistance genes.[43] In the three CRE strains in this study, *bla*$_{\text{NDM-5}}$ is surrounded by two IS26 on both sides to form the complex transposon in the core region of *dfrA12-aadA2-qacEΔ1-sul*-ISCR1-*dsbD-trpF-bleMBL-bla*$_{\text{NDM-5}}$ (not seen in NZ_CP024825.1), and *bla*$_{\text{OXA-181}}$ is flanked by two IS26 envelopes to form the *qnrS1-bla*$_{\textit{OXA-181}}$ resistance gene cassette (also present in NZ_CP026727.1), with the *qnrS1* gene being associated with low levels of fluoroquinolone resistance. The downstream elements of the *bla*$_{\text{NDM-5}}$ gene (*bleMBL, trpF* and *dsbD*) often appeared in various plasmids, and a large number of drug resistance genes were found to coexist upstream and downstream of NDM-5.[44,45] The drug resistance genes included *dfrA12, aadA2, qacEΔ1* and *sul*. The ISCR1 element was first reported by the Australian scholar Stokes.[46] The ISCR1 element can recognize and carry downstream drug resistance genes via transposition by rolling loop replication. Because ISCR1 can easily carry drug resistance genes, it leads to the spread of drug resistance.[47] In addition, the three CRE strains in this study were found to carry the disinfectant resistance gene *qacEΔ1*, indicating that Enterobacteriaceae strains have increased resistance to disinfectants that are commonly used in hospitals. The disinfectants commonly used in clinical practice include iodophor, Pasteurian disinfectant, 533 (chlorine-containing disinfectant), Aijiajia (chlorhexidine gluconate), chlorhexidine, and benzalkonium chloride. However, due to the widespread and unreasonable use of various disinfectants, selective pressure on bacteria causes them to become insensitive to disinfectants or makes conventionally used disinfectant concentrations ineffective. The *qacEΔ1* gene encodes a transmembrane protein that is resistant to quaternary ammonium disinfectants (such as benzylammonium bromide and neogeramine). Due to the linkage of the *qacEΔ1* and *sul1* genes, these bacteria are resistant to sulfonamides.[48] Therefore, we should switch the disinfectants that are regularly used in the clinic to minimize bacterial tolerance to disinfectants.

Although international travel has been reported to accelerate the spread of NDM and OXA-48-like enzymes, neither the children nor the mothers in this study had a history of international travel. At present, more epidemiological studies are urgently needed to better understand the emergence and transmission mechanism of the *bla*$_{\text{NDM-5}}$ and *bla*$_{\text{OXA-181}}$ genes in China.

Conclusion

In conclusion, this study is the first report of *Escherichia coli* and *Klebsiella pneumoniae* strains that produce carbapenemases (NDM-5 and OXA-181) in pediatric patients in Jiangsu Province, China. These strains are a major threat because they make treatment and recovery more difficult in young children. Plasmid analysis showed the *bla*$_{\text{NDM-5}}$ and *bla*$_{\text{OXA-181}}$ genes to be located on the IncX3 and ColKP3 plasmids with a size of 61 kb. The IncX3 plasmid, which is a carrier of drug resistance genes and is associated with a risk of spread in pediatric wards, showed a high degree of autonomous transfer between strains. Therefore, we need to actively monitor the genetics of CRE strains, especially in immunocompromised children, and take more stringent control measures to prevent the further spread of these multi-drug-resistant strains in pediatric wards. In addition, clinicians need to fully understand the characteristics of disinfectant resistance genes and their current status to establish sound norms for the use of disinfectants and to reduce the spread of these resistance genes caused by irregular use or abuse of disinfectants.

Ethical Approval

The guardian of the pediatric patients signed informed consent to participate in the study before the study began and this study was conducted in accordance with the Declaration of Helsinki. The Clinical Research Ethics Committee of the Children's Hospital of Nanjing Medical University approved the study (202205054-1), as all samples collected in this work were initially used to diagnose patient care without increasing the patient's medical costs and suffering.

Consent for Publication

Written informed consent was provided by the patient guardian for the publication of the case details.

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Disclosure

The authors report no conflicts of interest in this work.

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