Dissemination of Blandm-5 in Escherichia Coli via the Incx3 Plasmid From Different Regions in China

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Research

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Abstract

Background: Recently, the spread of NDM-5-producing *Escherichia coli* has become a severe challenge in clinical therapy, which necessitates reliable detection and surveillance methods. However, limited information is available regarding the prevalence and dissemination of the bla_{NDM-5} gene in *Escherichia coli* in China. Therefore, we investigated the dissemination of the bla_{NDM-5} gene in carbapenem-resistant *Escherichia coli* isolates from different regions in China.

Methods: A total of 1,180 carbapenem-resistant *enterobacteriaceae* strains were obtained from patients admitted to the 20 sentinel hospitals in eight cities. Strains positive for bla_{NDM-5} were detected using the Vitek 2 compact system, 16S rRNA gene sequencing, PCR, the S1-pulsed-field gel electrophoresis assay, and Southern blot hybridization. The horizontal-transfer capability of the bla_{NDM} gene was assessed by filter mating with a standard *E. coli* J53 azide-resistant strain as the recipient. Genotyping, susceptibility testing, and whole genome sequencing were performed.

Results: Seven strains of bla_{NDM-5}-positive *E.coli* was detected in 1180 clinical strains from different regions in China. The bla_{NDM-5}-carrying strains showed resistance to multiple tested antibiotics and belonged to two widespread sequence types, ST167 and ST405. Antimicrobial resistance genes including bla_{CTX-M}, bla_{OXA}, bla_{CMY}, and two novel bla_{TEM} variants ($bla_{TEM-230}$ and $bla_{TEM-231}$) were also identified. Southern blotting located the bla_{NDM-5} gene on 46-kb IncX3 plasmids in all isolates, which showed only two single nucleotide differences between EJN003 and the other strains.

Conclusions: This study further confirms the increasing occurrence of bla_{NDM-5}-carrying IncX3 plasmids and the dissemination of carbapenem resistance in *E. coli* isolates via the plasmid from different parts in China, which warrants stringent surveillance and control measures.

1. Introduction

Escherichia coli is one of the most common causative agents of infection in humans, and the emergence of resistance to third-generation cephalosporins by[1] extended-spectrum beta-lactamases (ESBLs) has led to an increased use of carbapenem compounds. The growing incidence of resistance to carbapenems among *Enterobacteriaceae* is of major concern worldwide. Among the newly emerged lactamases, New Delhi metallo-lactamase (NDM) represents the latest threat for public health. Since NDM-1 was first reported in 2009 in a Swedish patient of Indian origin[2], NDM-producing bacteria have spread globally and have caused various types of clinical infections[3]. To date, 24 bla_{NDM} gene variants have been described worldwide, and some variants confer elevated carbapenem resistance[4,5]. The rapid evolution and dissemination of NDMs represent a crucial challenge for clinical infection treatments, which necessitates reliable detection and surveillance method[6].

NDM-5, a variant with higher carbapenemase activity than NDM-1, was first identified in an *E. coli* strain isolated in 2011 from a patient in the UK with a recent history of hospitalization in India[4]. It has two

amino acid substitutions (Val88Leu and Met154Leu) relative to NDM-1, and confers increased resistance to extended-spectrum cephalosporins and carbapenems. Since then, NDM-5-producing strains have been identified in many areas including China. The widespread occurrence of NDM-5 in recent years highlights the need for international attention. Previous studies have reported the prevalence and dissemination of *bla*$_{NDM-5}$ among E.coli from different regions in China[7,8]. Herein, we further evaluated the potential transmission of *bla*$_{NDM-5}$-harboring *E. coli* from different regions in China in the current study. Whole genome analysis and molecular analysis were performed to provide a clear and solid molecular epidemiological description of NDM-5-producing *E. coli*.

2. Materials And Methods

2.1 Bacterial Strains, Detection of the NDM Gene, and Antimicrobial Susceptibility Testing

A total of 1,180 carbapenem-resistant *enterobacteriaceae* strains obtained from patients admitted to the 20 sentinel hospitals in eight cities from January 2014 to December 2015 in China were identified using the VITEK 2 system (BioMérieux, France). In a retrospective study, the presence of *bla*$_{NDM}$ was screened by PCR amplification and sequencing using primers described in our previous study[9]. Seven *bla*$_{NDM-5}$-positive *E.coli* strains were identified. The antimicrobial susceptibility testing was performed using the VITEK 2 system (BioMérieux). The minimal inhibitory concentration (MIC) results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines[10]. *E. coli* ATCC25922 was used for quality control. Collection of the clinical isolates was part of the routine surveillance of carbapenem-resistant *Enterobacteriaceae*. This study was approved by the institutional ethics committees of Academy of Military Medical Sciences.

2.2 S1- Pulsed-field gel electrophoresis (PFGE), Southern Blotting and Plasmid Conjugation

Genomic DNA from the seven strains was prepared in agarose plugs and digested with S1 nuclease (Takara, Dalian, China). Linearized plasmids and partially digested genomic DNA were separated using a CHEF-DR III system (Bio-Rad, Hercules, CA, USA). Southern blot analysis to locate the *bla*$_{NDM}$ genes was performed using specific digoxigenin-labeled probes (Roche)[11]. The NDM-1-producing *Acinetobacter* isolate 1750 reported by our laboratory[12] was used as a positive control, *S. enterica* H9812 was used as a reference size standard and a negative control.

The horizontal-transfer capability of the *bla*$_{NDM-5}$ gene was assessed by broth and filter mating using the seven strains as donors and a standard *E. coli* J53 azide-resistant strain as the recipient. The donor and recipient cultures were mixed in LB broth and incubated at 37°C for 24 hours. MacConkey agar containing 100 mg/liter sodium azide and 0.5 mg/liter meropenem was used to select *E. coli* J53

transconjugants[13]. Antimicrobial susceptibility testing and PCR amplification of the transconjugants were subsequently performed to confirm the transfer of the plasmid to the recipient.

2.3 Whole Genome Sequencing and Determination of Genetic Relatedness

Genomic DNA was extracted from seven *E. coli* isolates using the QIAamp DNA Mini Kit following the manufacturer's protocol (Qiagen, Inc., Valencia, CA, USA). Whole genome sequencing was performed on the Illumina Hiseq 2500 platform with a 350-bp insert at the Beijing Novogene Technology Co., Ltd. Paired-end reads of 150 bp were assembled using SOAPdenovo (v2.04) with coverage ranging from 113- to 157-fold. Scaffolding and gap filling were performed using SSPACE[14] and GapFiller. The plasmid pNDM-QD28[15] was selected as a reference. Gaps were closed using reference-guided assembly and manually checked by re-mapping raw reads against the reference. Plasmid sequences were annotated using RAST. Plasmid replicon type was assigned using PlasmidFinder (*Enterobacteriaceae*)[16].

The STs of seven *E. coli* isolates were identified using the MLST web server[17]. PFGE was performed after *Xba*I digestion with reference to the standard PulseNet conditions. Restriction patterns were compared visually using BioNumerics software with the *Salmonella enterica* serovar Braenderup H9812 as a size marker. *E. coli* strain K-12_DH10B (GenBank accession number CP000948) was used as the reference for alignment. Single nucleotide polymorphisms (SNPs) were identified using BWA (v0.7.12)[18] and SAMtools (v1.3). The concatenated SNPs were aligned to construct a Maximum-Likelihood phylogenetic tree using RAxML (v8.2.4) with the GTR model and the gamma distribution[19]. Genome sequences of ten pNDM5-ESY001-like plasmids were also downloaded for phylogenetic analysis as described above.

3. Results

3.1 Bacterial Strains and Antimicrobial Susceptibility Testing

Seven *bla*$_{\text{NDM-5}}$-positive isolates were recovered from seven hospitalized patients from three sources in three different cities in China. EBJ001 and EBJ003 were isolated from Beijing, ESY001, ESY002, and ESY003 from Shenyang, EJN001 and EJN003 from Jinan. (Table 1). None of the patients had ever traveled abroad. All *E. coli* strains were resistant to carbapenems, cephalosporins, quinolones, aztreonam, ampicillin, and sulfamethoxazole/trimethoprim, and were mostly susceptible to amikacin (Table 2).

3.2 Genetic Relatedness of all Strains

MLST analysis revealed that strain EBJ003 belonged to ST405, which fell into phylogenetic group D and was distinguishable from other strains by PFGE. The other six strains belonged to ST167 and phylogenetic group A **(Fig. 1)**. ESY001 and ESY002 were classified into the same pulsotype, whereas EJN001, EJN003, ESY003, and EBJ001 were classified into different pulsotypes **(Fig. 2)**, suggesting both clonal and non-clonal dissemination.

3.3 Virulence and Resistance Genes in All Strains

The seven strains harbored type II and III secretion systems. Certain virulence factors were exclusively observed in EBJ003, such as *fim* (encoding type I fimbriae regulatory proteins), *iuc* (encoding aerobactin siderophore biosynthesis proteins), and *irp* and *ybt* (encoding yersiniabactin biosynthetic proteins) genes.

In addition to $bla_{\text{NDM-5}}$, other antimicrobial resistance genes were also frequently identified in the seven *E. coli* strains including $bla_{\text{CTX-M}}$, bla_{TEM}, *aac(3)-IIa*, *aada5*, *dfrA7* and *mphA*. The coexistence of a $bla_{\text{CTX-M-14}}$ gene and a $bla_{\text{CTX-M-55}}$ gene was observed in EBJ003 **(Table 1)**. A $bla_{\text{CTX-M-24}}$ gene was identified in EBJ001, and $bla_{\text{CTX-M-55}}$ was identified in EJN003, whereas other strains harbored a single $bla_{\text{CTX-M-14}}$. Also, $bla_{\text{CMY-42}}$ was detected in EJN001 and EBJ001, and $bla_{\text{OXA-1}}$ was identified in EBJ003. Strain ESY001 harbored two bla_{TEM} variants designated as $bla_{\text{TEM-230}}$ and $bla_{\text{TEM-231}}$. The deduced protein sequence of $bla_{\text{TEM-230}}$ had a single amino acid substitution at position 233 (Ser→Thr) relative to its closest homolog TEM-168. The $bla_{\text{TEM-230}}$ gene was located on an IncX6 plasmid and inserted between the *TivB5* and *TivB6* genes related to type IV secretion systems. The $bla_{\text{TEM-231}}$ gene was located on an IncFIB plasmid and inserted between a Tn*3* mobile element and *sul2*.

3.4 Characteristics of the blaNDM−5-Carrying Plasmids

In this study, all seven strains successfully transferred the $bla_{\text{NDM-5}}$ gene to the recipient with high frequency ranging from 6.56×10^{-3} to 1.33×10^{-2} per donor cell. The transfer of the $bla_{\text{NDM-5}}$ gene and carbapenem resistance to the transconjugants was confirmed by PCR amplification and antimicrobial susceptibility testing. S1-PFGE and subsequent Southern hybridization revealed that $bla_{\text{NDM-5}}$ was carried by plasmids of the same size (~45 kb) **(Fig.3)**

Sequence analysis revealed that the $bla_{\text{NDM-5}}$-carrying plasmid was identical in ESY001 (pNDM5-ESY001), ESY002 (pNDM5-ESY002), ESY003 (pNDM5-ESY003), EBJ001 (pNDM5-EBJ001), EBJ003 (pNDM5-EBJ003), and EJN001 (pNDM5-EJN001). pNDM5-ESY001 was 46,161 bp in length and had only two single nucleotide substitutions compared with the $bla_{\text{NDM-5}}$-carrying plasmid pNDM5-EJN003 in EJN003, and the two single nucleotide substitutions were located within *VirB4* and the non-coding region, respectively. A BLASTn search against the NCBI database revealed that pNDM5-ESY001 shared 100% coverage and >99% identity with plasmid pNDM5-SSH006 from Shanghai, China[20]. A single nucleotide substitution located within a truncated *cutA1* gene downstream of $bla_{\text{NDM-5}}$ was identified. A variety of

bla$_{NDM-5}$-harboring plasmids show high similarity with the plasmid pNDM5-ESY001 including pNDM_MGR194[21], pEc1929[22], pNDM-QD28, pNDM-QD29[15], pECNDM101, and pNDM5-IncX3[23]. The nucleotide differences among these plasmids were mostly located within insertion sequences and genes associated with type IV secretion systems, partition, and DNA distortion **(Fig. 4)**. The plasmids pJEG027[24] carrying *bla*$_{NDM-4}$ and pKpN01-NDM7[25] carrying *bla*$_{NDM-7}$ were also highly identical with pNDM5-ESY001 except for the variation of *bla*$_{NDM}$ alleles.

All the above plasmids shared the same genetic context of the *bla*$_{NDM-5}$ gene (IS*Swi*-IS*3000*-ΔIS*Aba125*-IS*5*-*bla*$_{NDM-5}$-*ble*-*trpF*-*tat*-Δ*ctuA1*-IS*26*-Δ*umuD*) **(Fig. 5)**. pNDM5_0215[26], which was isolated from Chengdu, China, had an insertion of IS*5* into IS*3000*. Another *bla*$_{NDM-5}$-harboring IncX3 plasmid, pP744T-NDM5[27], from China had a similar NDM genetic context except for the deletion of a segment of the truncated IS*Aba125*, whereas pTK1044[28] from Japan had a shorter IS*Aba125* remnant of 73bp, suggesting a possible dissemination of *bla*$_{NDM-5}$ via mobile elements. A recently identified fusion plasmid pBJ114T-190[29] comprising both IncFIB and IncX3 replicons from China lost the IS*SWil* and IS*3000* upstream of the *bla*$_{NDM-5}$ gene, suggesting the continuing variation of the *bla*$_{NDM-5}$ genetic context.

4. Discussion

NDM-5-producing *Enterobacteriaceae* show increased resistance to extended-spectrum cephalosporins and carbapenems, posing a significant threat to public health throughout the world. The coexistence of *bla*$_{NDM-5}$ and other resistance genes confers multidrug resistance to pathogens, which is a serious problem for clinical treatment. This study identified seven NDM-5-producing *E. coli* isolates from three different cities in China.

Six of the seven strains belong to the ST167 type, which is an internationally disseminated clone of the ST10 complex. ST167 *E. coli* strains carry *bla*$_{NDM-1}$, *bla*$_{NDM-5}$[26], *bla*$_{NDM-7}$[30], and a variety of *bla*$_{CTX-M}$ genes[26,31] in various countries. ST167 is the most prevalent *bla*$_{NDM}$-bearing ST in China, accounting for 42% of NDM-producing *E. coli* isolates according to the nationwide surveillance[32]. Recent study reported several cases of clinical infection related to *bla*$_{NDM-5}$-positive *E. coli* ST167 in Shanghai or Zhejiang China[33,34]. Strain EBJ003 belongs to ST405 and a different phylogenetic group D, suggesting distinct clonal dissemination from others. ST405 is a high-risk clone found in diverse hosts and it carries the *bla*$_{NDM}$ and *bla*$_{CTX-M}$ genes[35]. The *bla*$_{NDM-5}$-positive strains in this study were associated with ESBL/AmpC genes or CTX-M-types, demonstrating their ability to acquire different resistance genes. Considering that ST167 and ST405 are both widely disseminated in China, further spread of multidrug-resistant *E. coli* could become a crucial issue in the clinical setting. In addition, an increasing number of virulence factors were identified in ST405 strain EBJ003 compared with ST167 strains, underscoring the potential danger of ST405 as an epidemic pathogen.

The nucleotide sequences of the *bla*$_{NDM-5}$-carrying plasmids pNDM5-ESY001 and pNDM5-EJN003 were nearly identical to those of other IncX3 plasmids from China, suggesting that the pNDM5-ESY001-like

plasmid is an important reservoir and plays a critical role in the dissemination of *bla*$_{NDM-5}$ in China. The pNDM5-ESY001-like plasmid (pNDM_MGR194) was first reported in *Klebsiella pneumoniae* in India and later in Australia[36] and Denmark. The *bla*$_{NDM-5}$ carriers from Australia and Denmark were isolated from patients who had travelled to India, suggesting the linkage to Indian *bla*$_{NDM-5}$ dissemination. The *bla*$_{NDM-5}$ carriers isolated from China showed little association with the Indian subcontinent. However, considering that international travel is common and transmission routes between people are often unrecognized, the origin of the prevalence of pNDM5-ESY001-like plasmids in China remains unclear. IncX-type plasmids were earlier believed to have a narrow host range but were recently found to be prevalent in various members of *Enterobacteriaceae*. We previously reported the presence of an IncX3 plasmid similar to pNDM5-ESY001 in *S. enterica*[20]. The identification of pNDM5-ESY001-like plasmids in *K. pneumoniae*, *S. enterica*, and *E. coli* of different STs demonstrates their ability to mediate intra- and inter-species transfer of *bla*$_{NDM-5}$, which would facilitate the wide distribution of NDM-5 across diverse enterobacterial species. Further surveillance should be devoted to monitoring the transfer of such *bla*$_{NDM-5}$-carrying IncX3 plasmids among *Enterobacteriaceae*.

Phylogenetic analysis of plasmids revealed that the *bla*$_{NDM-4}$-carrying plasmid pJEG027[24] in Australia and the *bla*$_{NDM-7}$-carrying plasmid pKpN01-NDM7[25] in Canada are indistinguishable from the pNDM5-ESY001-like plasmids. Because *bla*$_{NDM-5}$ and *bla*$_{NDM-7}$ have a single nucleotide substitution relative to *bla*$_{NDM-4}$ (G388A and G262T, respectively), pNDM5-ESY001 and pKpN01-NDM7 are likely to have arisen from a pJEG027-like plasmid through acquisition of different variations.

In conclusion, we report seven drug-resistant *E. coli* isolates harboring *bla*$_{NDM-5}$ and multiple ESBL/AmpC genes from patients in different geographic locations in China. The coexistence of resistance to antimicrobials of different groups in clinical NDM-5-producing *E. coli* isolates is a serious concern. These isolates belonged to ST167 and ST405 and contained a transferable plasmid, pNDM5-ESY001 (pNDM5-EJN003), which serves as an important reservoir for the dissemination of *bla*$_{NDM-5}$. The present findings underscore the threat of NDM-5 carbapenemase circulation via IncX3 plasmids and the urgency of implementing stringent surveillance and control measures.

Nucleotide sequence accession number

The shotgun whole genome sequences of strains ESY001, ESY002, ESY003, EJN001, EJN003, EBJ001 and EBJ003 have been deposited in NCBI GenBank under accession numbers PUJM000000 to PUJS00000000.

Declarations

Ethics approval and consent to participate: Not applicable.

Consent for publication: Not applicable.

Availability of data and material: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests

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Authors' contributions: X.-F.H wrote the main manuscript and fully participated in all experiments. L.Y contributed to the bioinformatics data analysis. S.-F.Q designed the study. N.D and Y.-F.L participated in data collection. L.Z and X.W participated in the specimen collection. X.-D.G, Y.X, L.-Q.J and C.-F.Z participated in all experiments. H.-B.S, Y.-S.S and P.L gave final approval of the version to be submitted. All authors made substantial contributions to preparation and submission of manuscript.

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Tables

Table 1. Patient demographics, molecular features and *bla* resistance genes of 7 *E. coli* strains

Strain	Year	Region	Source	MLST	*bla* genes carried
ESY001	2014	Shenyang	blood	ST167	bla_{NDM-5}, $bla_{CTX-M-14}$, $bla_{TEM-230}$, $bla_{TEM-231}$
ESY002	2014	Shenyang	blood	ST167	bla_{NDM-5}, $bla_{CTX-M-14}$, $bla_{TEM-230}$
ESY003	2014	Shenyang	sputum	ST167	bla_{NDM-5}, $bla_{CTX-M-14}$, $bla_{TEM-135}$
EJN001	2014	Jinan	urine	ST167	bla_{NDM-5}, $bla_{CTX-M-14}$, bla_{CMY-42}
EJN003	2014	Jinan	blood	ST167	bla_{NDM-5}, $bla_{CTX-M-55}$
EBJ001	2014	Beijing	urine	ST167	bla_{NDM-5}, $bla_{CTX-M-24}$, bla_{CMY-42}
EBJ003	2014	Beijing	urine	ST405	bla_{NDM-5}, $bla_{CTX-M-14}$, $bla_{CTX-M-55}$, bla_{OXA-1}

TABLE 2. Antibiotic susceptibilities of seven *E. coli* strains and their J53 transconjugants

Antimicrobial	MIC (μg/ml)						
	ESY001 [J53]	ESY002 [J53]	ESY003 [J53]	EBJ001 [J53]	EBJ003 [J53]	EJN001 [J53]	EJN003 [J53]
Ampicillin	≥32 [≥32]	≥32 [≥32]	≥32 [≥32]	≥32 [≥32]	≥32 [≥32]	≥32 [≥32]	≥32 [≥32]
Amoxicillin-clavulanic acid	≥32 [≥32]	≥32 [≥32]	≥32 [≥32]	≥32 [≥32]	≥32 [≥32]	≥32 [≥32]	≥32 [≥32]
Piperacillin	≥128 [≥128]	≥128 [32]	≥128 [64]	≥128 [32]	≥128 [≥128]	≥128 [≥128]	≥128 [≥128]
Cefazolin	≥64 [≥64]	≥64 [≥64]	≥64 [≥64]	≥64 [≥64]	≥64 [≥64]	≥64 [≥64]	≥64 [≥64]
Ceftazidime	≥64 [≥64]	≥64 [≥64]	≥64 [≥64]	≥64 [≥64]	≥64 [≥64]	≥64 [≥64]	≥64 [≥64]
Ceftriaxone	≥64 [≥64]	≥64 [≥64]	≥64 [≥64]	≥64 [≥64]	≥64 [≥64]	≥64 [≥64]	≥64 [≥64]
Cefepime	≥64 [8]	≥64 [8]	≥64 [8]	≥64 [8]	≥64 [8]	≥64 [8]	≥64 [8]
Aztreonam	≥64 [≤1]	≥64 [≤1]	≥64 [≤1]	≥64 [≤1]	≥64 [≤1]	≥64 [≤1]	≥64 [≤1]
Imipenem	≥16 [8]	≥16 [8]	≥16 [8]	≥16 [8]	≥16 [4]	≥16 [≥16]	≥16 [≥16]
Meropenem	8 [2]	4 [2]	8 [2]	4 [2]	4 [2]	≥16 [2]	≥16 [1]
Amikacin	≤2 [≤2]	4 [≤2]	≤2 [≤2]	≤2 [≤2]	32 [≤2]	≥64 [≤2]	≥64 [≤2]
Gentamicin	≥16 [≤1]	≤1 [≤1]	≥16 [≤1]	≥16 [2]	≥16 [≤1]	≥16 [≤1]	≥16 [≤1]
Ciprofloxacin	≥4 [≤0.25]	≥4 [≤0.25]	≥4 [≤0.25]	≥4 [≤0.25]	≥4 [≤0.25]	≥4 [≤0.25]	≥4 [≤0.25]
Levofloxacin	≥8 [≤0.25]	≥8 [≤0.25]	≥8 [≤0.25]	≥8 [≤0.25]	≥8 [≤0.25]	≥8 [≤0.25]	≥8 [≤0.25]
Tetracycline	≥16 [≤1]	≥16 [2]	≥16 [2]	≥16 [≤1]	≥16 [2]	4 [2]	≥16 [2]
Nitrofurantoin	64 [≤16]	64 [≤16]	64 [≤16]	64 [≤16]	32 [≤16]	128 [≤16]	≤16 [≤16]
Sulfamethoxazole-trimethoprim	≥320 [≤20]	≥320 [≤20]	≥320 [≤20]	≥320 [≤20]	≥320 [≤20]	≥320 [≤20]	≥320 [≤20]

*The antibiotic susceptibilities of transconjugants are indicated in square brackets.

Figures

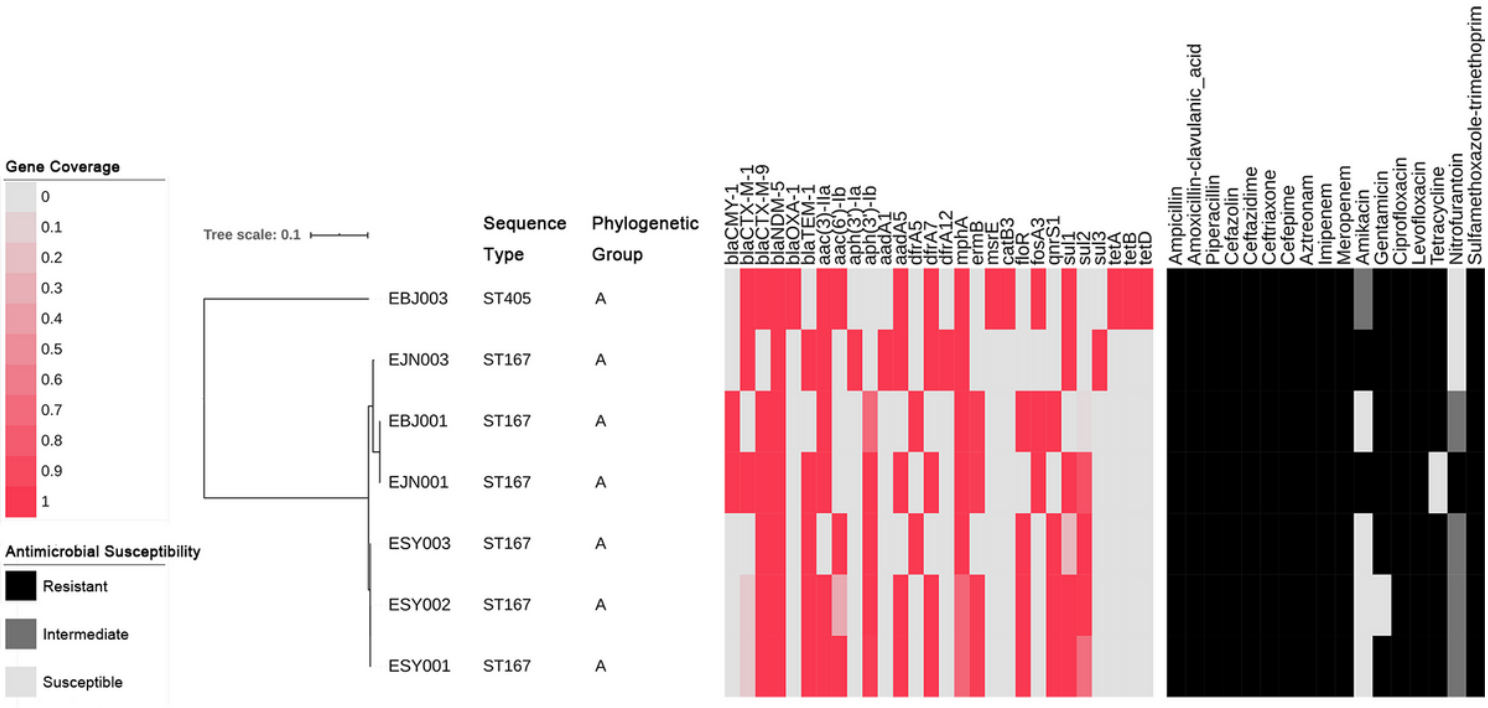
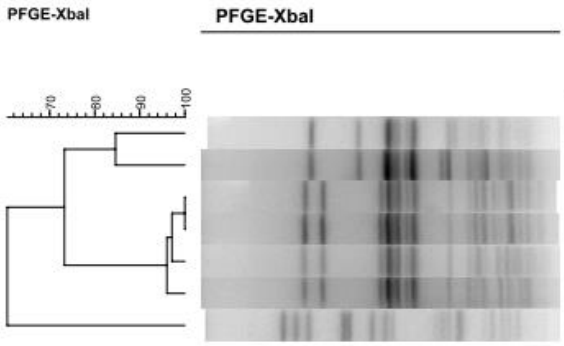


Figure 1

Phylogenetic tree of seven blaNDM-5-positive E. coli isolates with antimicrobial resistance phenotypes and genotypes. Red indicates the query coverage against resistance genes. Black, dark gray and light gray indicate “resistant”, “intermediate” and “susceptible” to antimicrobials, respectively



Strain number	ST	blaNDM	Species	Year	Region	Ward	Source
EBJ001	167	5	E.coli	2014	Beijing	pediatric surgery	urine
EJN001	167	5	E.coli	2014	Jinan	intensive care unit	urine
ESY001	167	5	E.coli	2014	Shenyang	haematology	blood
ESY002	167	5	E.coli	2014	Shenyang	haematology	blood
EJN003	167	5	E.coli	2014	Jinan	haematology	blood
ESY003	167	5	E.coli	2014	Shenyang	intensive care	sputum
EBJ003	405	5	E.coli	2014	Beijing	neonatology	urine

Figure 2

Dendrogram of patterns generated by pulsed-field gel electrophoresis (PFGE) of seven strains using the BioNumerics software program.

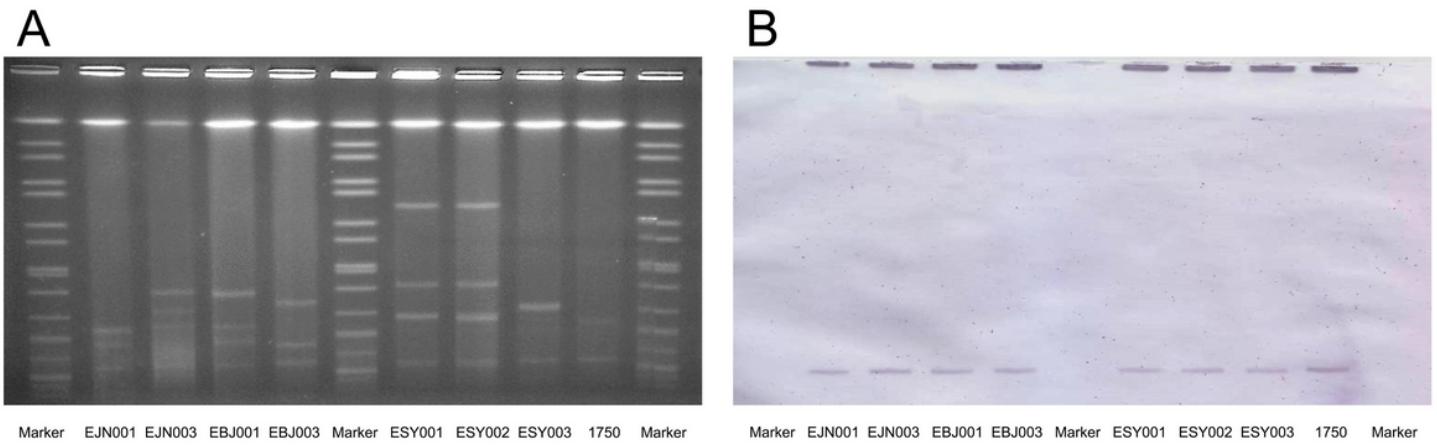


Figure 3

S1-pulsed-field gel electrophoresis (S1-PFGE) patterns of the seven blaNDM−5-producing isolates (A) and Southern hybridization with a probe specific for blaNDM−5 (B). Marker: S.enterica serotype Braenderup H9812 as a reference size standard.

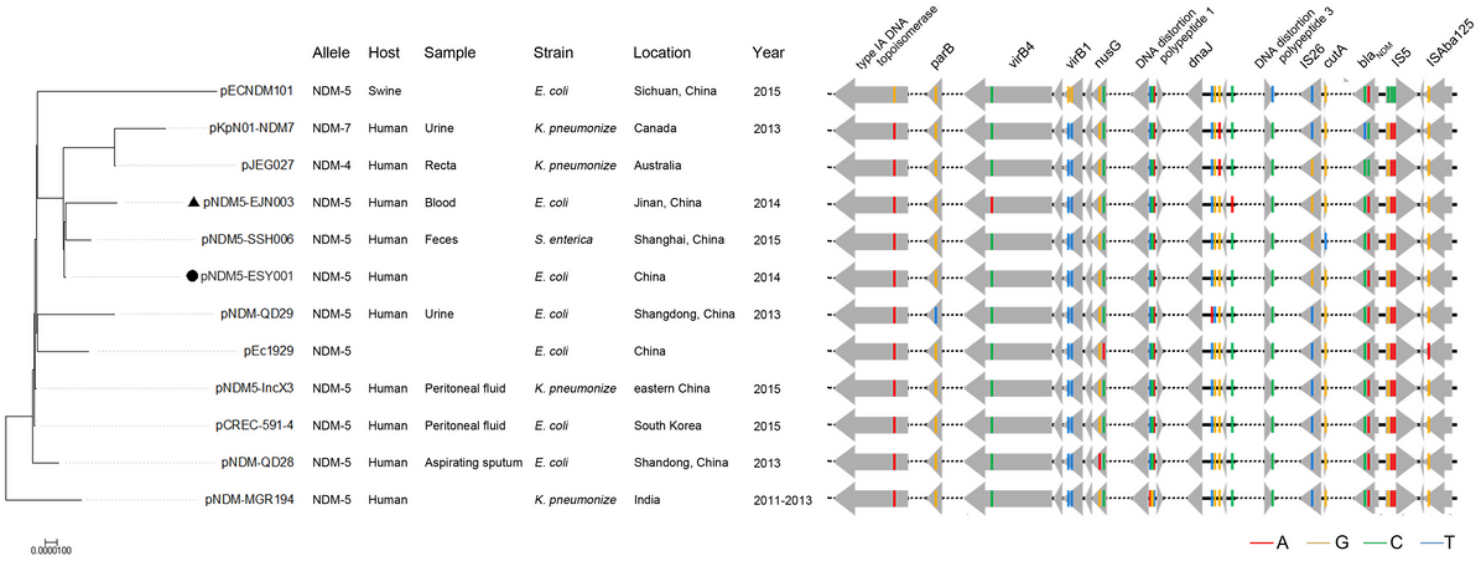


Figure 4

Phylogenetic tree of pNDM5-ESY001-like plasmids. pNDM5-ESY001 and pNDM5-EJN003 were marked with a solid circle and triangle, respectively. Open reading frames (ORFs) were indicated by gray arrows. The nucleotides at SNP loci were indicated by colored lines.

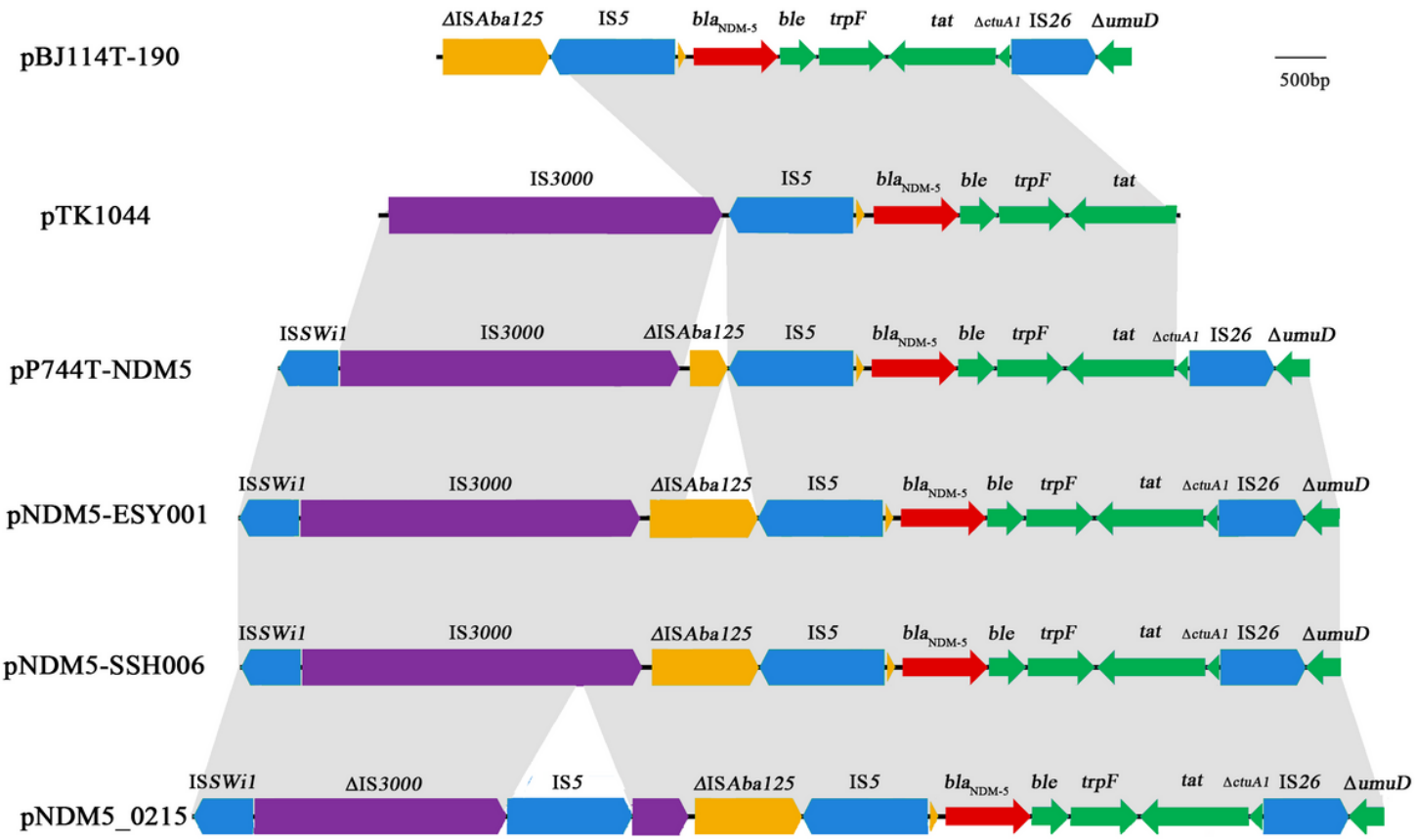


Figure 5

The genetic structure of blaNDM-5 context in pBJ114T-190, pTK1044, pP744T-NDM5, pNDM5-ESY001, pNDM5-SSH006 and pNDM5_0215. The arrows represent ORFs. The blaNDM-5 gene is indicated in red. IS3000, ISAba125 and other insertion sequences are indicated in purple, yellow and blue, respectively. The remaining genes are indicated in green. Homology regions are denoted by light gray shading.