

Characterization Of *bla*_{NDM-5}-Positive *Escherichia coli* Prevalent In A University Hospital In Eastern China

This article was published in the following Dove Press journal:
Infection and Drug Resistance

Pengfei Sun^{1,2,*}
Wenying Xia^{1,2,*}
Genyan Liu^{1,2}
Xu Huang^{1,2}
Chenjie Tang^{1,2}
Chengcheng Liu^{1,2}
Yuqiao Xu^{1,2}
Fang Ni^{1,2}
Yaning Mei^{1,2}
Shiyang Pan^{1,2}

¹Department of Laboratory Medicine, Jiangsu Province Hospital and Nanjing Medical University First Affiliated Hospital, Nanjing, People's Republic of China; ²National Key Clinical Department of Laboratory Medicine, Nanjing, People's Republic of China

*These authors contributed equally to this work

Purpose: The emergence and spread of carbapenem-resistant *Enterobacteriaceae* deserves special concern worldwide. Unlike the epidemiological characteristics reported in other studies, we found that the production of New Delhi metallo- β -lactamase 5 was the main mechanism for the resistance of *Escherichia coli* to carbapenems.

Methods: All carbapenem-resistant strains were collected from July 2017 to July 2018 of the First Affiliated Hospital of Nanjing Medical University. The presence of carbapenemase-encoding genes was detected using PCR and gene sequencing. Genetic relatedness of the *bla*_{NDM-5}-positive *E. coli* strains was determined with PFGE and MLST. Susceptibility profiles were measured with broth microdilution method and E-test strips. Transferability features of *bla*_{NDM-5} gene were assessed by conjugation experiments, S1-PFGE, southern blotting and PCR-based replicon typing methods. The genetic structures surrounding *bla*_{NDM-5} were acquired by whole genome sequencing and PCR mapping.

Results: Among the 28 carbapenem-resistant *E. coli* strains, 18 (64%) were verified as NDM-5 producers. The 18 *bla*_{NDM-5}-positive *E. coli* strains showed high resistance to most antibiotics, but 100% were sensitive to colistin and tigecycline. In addition, the 18 *bla*_{NDM-5}-positive *E. coli* strains belonged to eight STs, among which ST167, ST410 and ST101 were found to cause clonal spread in the hospital. Further studies found that the *bla*_{NDM-5} gene was located on an IncX3-type plasmid, and all plasmids harbored an IS3000-*AISAb125*-IS5-*bla*_{NDM-5}-*ble*MBL-*trpF*-*dsbC*-IS26 structure.

Conclusion: The clonal spread of *bla*_{NDM-5}-positive *E. coli* strains and horizontal dissemination via the pNDM-MGR 194-like plasmids should draw more attention. Appropriate infection control operations should be performed to prevent the further spread of *bla*_{NDM-5}.

Keywords: *Escherichia coli*, carbapenem-resistant *Enterobacteriaceae*, New Delhi metallo- β -lactamase, *bla*_{NDM-5}, IncX3 type plasmid

Introduction

Carbapenems were normally thought to be the last resort for treating multidrug resistant gram-negative pathogens.¹ However, the situation has changed due to the emergence and spread of carbapenem-resistant *Enterobacteriaceae* (CRE) strains worldwide over the past twenty years, posing a great challenge to human health.² Previous studies have demonstrated that carbapenemases such as KPC, IMP, VIM and OXA-48 were the main mechanism for bacterial resistance to carbapenems.³⁻⁵ In 2009, a newly emerged Amber class B carbapenemase, New Delhi metallo- β -lactamase 1 (NDM-1), was identified in a *Klebsiella pneumoniae* strain isolated from a tourist who had a hospitalization history in India.⁶ Because NDM-1 has high affinity for almost all β -lactamases, has coexistence with other drug-resistant genes

Correspondence: Genyan Liu
Department of Laboratory Medicine,
Jiangsu Province Hospital, Guangzhou
Street No. 300, Nanjing 210029, People's
Republic of China
Tel +8625 6830 7332
Fax +8625 8372 4440
Email liugenyan@njmu.edu.cn

and is located on transferable elements, NDM-1 has rapidly drawn extensive attention around the world and is even being called a “superbug” by the media. Shortly after, NDM-1 has already been discovered among multiple species in more than 40 countries.⁷ The Indian subcontinent, the Balkan states and the Middle East are thought to be three main reservoirs for the dissemination of NDM-1; according to many reports, NDM-1 strains have been recovered from patients that had travelled or received medical treatment in these places.⁸

Escherichia coli is a member of the human gut microbiota and is one of the most common gram-negative bacteria isolated from hospital specimens such as urine, fecal, sputum and bloodstream.⁹ In addition, *Escherichia coli* also plays an important role in community-acquired urinary tract infectious disease. To date, the production of ESBLs is still the main mechanism for the resistance of *E. coli* to third generation cephalosporins, and the detectable rate of ESBLs in *E. coli* was from 62% to 100% in India and almost up to 62% in China.¹⁰ Clinicians generally empirically prefer the use of carbapenems to treat infections caused by these strains.¹¹ However, during the past 10 years, the isolation of carbapenemases, especially NDM-1-producing *E. coli*, has been reported at an increasing rate worldwide. Therefore, we should pay more attention to the emergence of NDM-1-producing genes (*bla*_{NDM-1}) or their variants in *E. coli* strains to avoid encountering a situation where antibiotic options are limited. In this study, we describe a high prevalence of *bla*_{NDM-5} in carbapenem-resistant *E. coli* strains in a university hospital in Eastern China and further conduct molecular research to investigate the transmission features of this gene.

Materials And Methods

Bacterial Collection, Species Identification And Antimicrobial Susceptibility Testing

Isolates were collected from July 2017 to July 2018 in the clinical microbiology laboratory of the First Affiliated Hospital of Nanjing Medical University, a large medical center with more than three thousand beds in Jiangsu, China. All isolates were first identified using the VITEK2 compact system (bioMérieux, France) and then confirmed by the MALDI-TOF MS apparatus (Bruker Microflex LT, Germany). Antimicrobial susceptibilities were determined by measuring the minimum inhibitory concentrations (MICs) with the broth microdilution method (for piperacillin-tazobactam, ceftazidime, aztreonam, amikacin, gentamicin, levofloxacin,

imipenem, ertapenem, meropenem, and colistin), followed by E-test strips (for tigecycline). The MIC results were interpreted according to the Clinical and Laboratory Standards Institute guidelines (M100-S26),¹² except for colistin and tigecycline, for which the 2016 European Committee on Antimicrobial Susceptibility Testing breakpoints was used (http://www.eucast.org/clinical_breakpoints/). *E. coli* ATCC 25922, *K. pneumoniae* ATCC 1705 and ATCC 1706 served as the quality control strains.

Detection Of Carbapenemase Producing Isolates And Genes

Carbapenemase production was screened by the modified Hodge test and imipenem-EDTA double-disc synergy test. The presence of common carbapenemase-encoding genes (*bla*_{KPC}, *bla*_{NDM}, *bla*_{IMP}, *bla*_{VIM}, *bla*_{OXA-48}), extended-spectrum β -lactamase (ESBL) genes (*bla*_{CTX-M}, *bla*_{SHV}, *bla*_{TEM}, *bla*_{OXA-1}), 16S rRNA methylase genes (*armA*, *rmtB*) and plasmid-mediated quinolone resistance (PMQR) genes (*qnrA*, *qnrB*, *qnrS*, *aac(6)-Ib-cr*) were detected using PCR methods as described previously.^{13–15} Positive amplicons were determined by nucleotide sequencing.

Bacterial Genotyping

The *Xba*I-digested genomic DNA from *bla*_{NDM}-positive *E. coli* strains was used to investigate the genetic relationship of the strains by pulse field gel electrophoresis (PFGE). DNA fragments were separated on a CHEF Mapper apparatus (Bio-Rad, USA) according to the following parameters: voltage of 6 V/cm, pulse angle of 120°, pulse time from 6.75 to 35.38 s and run for 20 h at 14°C. *Salmonella enterica* H9812 was used as the reference marker. The PFGE patterns were analyzed with BioNumerics software (Applied Maths, Belgium) using the Dice similarity coefficient. Clusters were defined as DNA patterns sharing >85% similarity. Multilocus sequence typing (MLST) was also performed using the given primers to amplify seven house-keeping genes in the *E. coli* strains according to the protocol available on website (<http://mlst.warwick.ac.uk/mlst/dbs/Ecoli>). PCR products were sequenced and compared, and then a number was given to each allele. Sequence types (STs) were assigned according to the combination of seven allele numbers listed on the online database.

Conjugation Experiment

Conjugation experiments were performed using a parent mating conjugation assay with *bla*_{NDM-5}-positive isolates

acting as donor strains and azide-resistant *E. coli* J53 acting as a recipient strain. After overnight shaking culture in Luria-Bertani (LB) broth, the donor strain and *E. coli* J53 were mixed at a ratio of 1:1 and inoculated on Mueller-Hinton (MH) agar for 18 h at 37 °C. Subsequently, the mixture was streaked directly on MH agar supplement with sodium azide (150 µg/mL) and meropenem (2 µg/mL). Successful transconjugants were the colonies that survived on the selective MH plates with a positive *bla*_{NDM-5} gene. Other drug-resistant genes and antibiotic susceptibilities were also investigated as mentioned above.

Molecular Analysis Of Transferrable Plasmids

Molecular sizes of transferable *bla*_{NDM-5}-harboring plasmids were evaluated by S1-PFGE and the Southern blotting method.¹⁶ The whole genomic DNA of the *bla*_{NDM-5}-positive strains was digested by S1 nuclease, separated by the PFGE method and then transferred horizontally to a positively charged nylon membrane. The membranes were hybridized with a digoxigenin-labeled *bla*_{NDM} probe and detected using a nitro-blue tetrazolium/5-bromo-4-chloro-3'-indolyphosphate color detection kit (Roche, Germany). The incompatibility groups of these plasmids were determined according to PCR-based replicon typing methods.

To investigate the genetic context around the *bla*_{NDM-5} gene, we first chose the plasmid pNDM-CREC-8 which was extracted from transconjugant CREC-8-J and harbored a *bla*_{NDM-5} gene for sequencing. The whole plasmid sequence was obtained on the HiSeq 2500 platform generating 150 bp paired-end reads. Then, the derived reads were trimmed and assembled using GLC Genomics Workbench 5 (CLC Bio, Aarhus, Denmark), and gaps were closed through PCR and Sanger sequencing. Sequence analysis was conducted using the ORF Finder (<http://www.ncbi.nlm.nih.gov/gorf/gorf.html>) and BLAST functions (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Additionally, the genetic context around *bla*_{NDM-5} in the other 14 transconjugants was investigated by the PCR mapping method with primers designed according to the sequence of pNDM-CREC-8.

Results

Clinical Characteristics Of The *bla*_{NDM-5} Strains

During the study period from July 2017 to July 2018, 197 strains with decreased sensitivity to imipenem (MIC ≥ 4 µg/mL) or meropenem (MIC ≥ 4 µg/mL) were identified

as CRE, including 144 (73%) *K. pneumoniae*, 28 (14%) *E. coli* and 25 (13%) other isolates. Among the carbapenem-resistant *E. coli* strains, 18 (64%) were verified as NDM-5 producers, and the rest were KPC-2. Clinical characteristics associated with the 18 *bla*_{NDM-5}-positive *E. coli* strains are summarized in Table 1. The strains were isolated from urine (n=7), pus (n=4), sputum (n=3), blood (n=3) and cerebrospinal fluid (n=1). All patients carrying *bla*_{NDM-5}-positive strains were initially admitted to the hospital with diverse underlying diseases, and none of them had a travel history to NDM endemic countries, e.g., India and the Balkans. Medical records revealed that most of the patients had received different kinds of antimicrobial treatment during their hospital stay. However, unfortunately, only 10 of them recovered, and the rest either had poor prognosis or died. Further analysis the reasons for the poor outcomes of the patients, we found that not all the patients with poor prognosis are due to NDM-5-positive CREC infections. There are also some patients whose deaths are attributed to underlying diseases and abandonment of treatment. For example, patient 2 and 17 were finally died with blood culture negative after effectively antimicrobial treatment.

Antimicrobial Susceptibility

Antimicrobial susceptibilities were performed on all *bla*_{NDM-5}-positive *E. coli* strains, and their susceptibility profiles are listed in Table 2. All *bla*_{NDM-5}-positive *E. coli* strains exhibited 100% resistance to ceftazidime, piperacillin/tazobactam, imipenem, meropenem, ertapenem and levofloxacin. In addition, the resistance rates of aztreonam and gentamicin were both 72.2%. In contrast, amikacin, colistin and tigecycline showed high susceptibility rates, which were 66.7%, 100% and 100%, respectively, suggesting their potential use for fighting against *bla*_{NDM-5}-positive strains infections.

Detection Of Carbapenemase Producing Isolates And Genes

The modified Hodge test and imipenem-EDTA double-disc synergy test were used to screen all *bla*_{NDM-5}-positive strains. Apart from *bla*_{NDM-5}, the other four carbapenemase-encoding genes were not discovered in any of the strains. The results of nucleotide sequencing of other drug resistance genes are listed in Table 2. ESBL genes existed in most strains (n=16, 88.9%), among which *bla*_{CTX-M} groups were the main types and accounted for 12 strains. In addition, 3 strains cocarried 2 groups of *bla*_{CTX-M} genes, including 2

Table 1 Clinical Characteristics Of bla_{NDM-5}-Positive *E. Coli* Strains

Isolate	Sex	Patient Age	Ward	Isolation Time	Specimen	Antimicrobial Treatment	Outcome	MLST	Inc	Size (~kb)
CREC-1	Female	76	General surgery	2017/7/21	Pus	ZOX	Recovered	ST167	X3	50
CREC-2	Female	51	Urology	2017/7/26	Blood	IPM	Death	ST167	X3	50
CREC-4	Female	50	Hepatology	2017/8/4	Pus	CFP/SUL+ATM	Recovered	ST10	X3	50
CREC-6	Female	33	Colorectal surgery	2017/8/10	Pus	CAZ	Recovered	ST540	X3	50
CREC-8	Male	57	Urology	2017/9/1	Urine	CFP/TZP	Recovered	ST448	X3	50
CREC-9	Female	92	Nephrology	2017/9/1	Urine	CFP/SUL	Recovered	ST167	X3	50
CREC-10	Female	53	Nephrology	2017/9/11	Sputum	TZP	Poor	ST410	X3	50
CREC-11	Female	94	Nephrology	2017/9/18	Sputum	MXF+TZP	Death	ST410	X3	50
CREC-12	Female	72	Nephrology	2017/9/18	Urine	MXF+CAZ	Recovered	ST410	X3	50
CREC-13	Male	61	Urology	2017/10/18	Urine	CFP/TZP	Poor	ST167	X3	50
CREC-14	Female	88	Urology	2017/10/23	Sputum	CAZ	Poor	ST167	X3	50
CREC-17	Male	46	Gastroenterology	2017/11/27	Blood	ATM	Poor	ST101	X3	50
CREC-19	Male	73	NICU	2018/2/1	CSF	Untreated	Death	ST167	-	-
CREC-21	Female	62	Hepatology	2018/2/14	Blood	CFP/SUL	Death	ST410	X3	50
CREC-25	Male	59	Rehabilitation	2018/3/7	Urine	Untreated	Recovered	ST359	X3	50
CREC-26	Female	40	Hematology	2018/3/17	Urine	AMK	Recovered	ST38	-	-
CREC-27	Male	83	Gastroenterology	2018/3/20	Pus	CAZ	Recovered	ST101	X3	50
CREC-28	Male	82	Urology	2018/7/8	Urine	Untreated	Recovered	ST167	-	-

Abbreviations: CREC, carbapenem-resistant *E. coli*; NICU, neurosurgical intensive care unit; CSF, cerebrospinal fluid; ZOX, ceftiozime; IPM, imipenem; CFP/SUL, ceftoperazone/sulbactam; ATM, aztreonam; CAZ, ceftazidime; TZP, piperacillin/tazobactam; MXF, moxifloxacin; CFP/TZP, ceftoperazone/tazobactam; AMK, amikacin; Inc, incompatibility.

Table 2 Antimicrobial Susceptibilities Of Additional Drug-Resistant Genes Of bla_{NDM-5}-Positive *E. Coli* Strains

Isolate	MIC (µg/mL)													Corestant Genes
	TZP	CAZ	ATM	GEN	AMK	LVX	IPM	MEM	ETM	TGC	CST			
CREC-1	>256	>256	>256	0.5	≤1	>32	32	32	128	<1	1		bla _{CTX-M55}	
CREC-2	>256	>256	>256	>256	>256	>32	32	32	128	<1	2		bla _{CTX-M55} , bla _{CTX-M14} , rmtB	
CREC-4	>256	>256	>256	64	2	>32	32	32	32	<1	2		bla _{CTX-M14}	
CREC-6	>256	>256	>256	>256	>256	>32	32	64	128	<1	0.5		bla _{CTX-M55} , bla _{SHV-12} , bla _{TEM-1} , rmtB	
CREC-8	>256	>256	>256	1	≤1	>32	8	32	64	<1	2		bla _{CTX-M55}	
CREC-9	>256	>256	>256	>256	>256	>32	128	256	128	<1	2		bla _{CTX-M55} , bla _{TEM-1} , rmtB	
CREC-10	>256	>256	0.5	32	4	>32	64	64	128	<1	0.5		bla _{TEM-1} , bla _{OXA-11} , aac(6)-Ib-cr	
CREC-11	>256	>256	0.5	32	4	>32	64	64	128	<1	0.5		bla _{TEM-1} , bla _{OXA-11} , aac(6)-Ib-cr	
CREC-12	>256	>256	0.5	32	4	>32	64	64	128	<1	0.5		bla _{TEM-1} , bla _{OXA-11} , aac(6)-Ib-cr	
CREC-13	>256	>256	>256	>256	>256	>32	128	256	256	<1	1		bla _{CTX-M64} , bla _{CTX-M14} , aac(6)-Ib-cr	
CREC-14	>256	>256	>256	>256	>256	>32	128	256	256	<1	1		bla _{CTX-M64} , bla _{CTX-M14} , aac(6)-Ib-cr	
CREC-17	>256	>256	2	≤0.5	≤1	>32	8	32	32	<1	1		-	
CREC-19	>256	>256	1	>256	>256	>32	8	32	64	<1	2		bla _{SHV-12} , bla _{TEM-1} , rmtB	
CREC-21	>256	>256	>256	64	4	>32	8	32	64	<1	2		bla _{CTX-M55} , bla _{OXA-11} , aac(6)-Ib-cr	
CREC-25	>256	>256	>256	256	≤1	>32	64	256	128	<1	2		bla _{CTX-M55}	
CREC-26	>256	>256	32	256	2	>32	16	64	64	<1	2		bla _{CTX-M14}	
CREC-27	>256	>256	128	≤0.5	≤1	>32	8	64	64	<1	2		bla _{CTX-M14}	
CREC-28	>256	>256	>256	0.5	≤1	>32	8	32	64	<1	2		aac(6)-Ib-cr	

Abbreviations: CREC, carbapenem-resistant *E. coli*; MIC, minimal inhibitory concentration; TZP, piperacillin/tazobactam; CAZ, ceftazidime; ATM, aztreonam; GEN, gentamicin; AMK, amikacin; LVX, levofloxacin; IPM, imipenem; MEM, meropenem; ETM, ertapenem; TGC, tigecycline; CST, colistin.

with a newly emerged *bla*_{CTX-M-64} gene that evolved by homologous recombination between the *bla*_{CTX-M-15-like} and *bla*_{CTX-M-14} genes.¹⁷ The *rmtB* and *aac(6)-Ib-cr* genes were identified in 4 and 7 strains, respectively.

Genetic Relatedness

Genetic relatedness of the *bla*_{NDM-5}-positive *E. coli* strains isolated from diverse departments was investigated by PFGE and MLST. Among the *bla*_{NDM-5}-positive *E. coli* strains, ST167 (n=7) was the most prevalent, followed by ST410 (n=4) and ST101 (n=2). In addition, analysis of PFGE profiles revealed that strains in either ST167 (except CREC-8), ST410 or ST101 each shared very similar patterns (>85%), suggesting that clonal spread of *bla*_{NDM-5}-positive *E. coli* strains occurred in our hospital. Notably, when taking the hospitalization time overlap and admission units into consideration, we thought that an outbreak (CREC-10, CREC-11, CREC-12) had occurred in the department of nephrology during September 2017. The remaining strains belonged to the other 5 STs and displayed clearly different PFGE patterns, suggesting that these *E. coli* strains were not genetically related to other strains even though some of them were recovered from the same unit (Figure 1).

Transferability Of The *bla*_{NDM-5} Gene

Our study showed that the *bla*_{NDM-5} genes from 15 *E. coli* strains were successfully transferred to *E. coli* J53, whereas

CREC-19, CREC-26 and CREC-28 failed after multiple tries. Antimicrobial susceptibilities of the transconjugants showed they were resistant to all β -lactams except aztreonam. In addition to *bla*_{NDM-5}, the *bla*_{CTX-M} group, *rmtB* and *aac(6)-Ib-cr* genes could also be cotransferred in several donor strains, resulting in resistance to aztreonam and aminoglycosides in their transconjugants.

S1-PFGE found that the transconjugants all possessed nearly the same plasmids approximately 50 kb in size, and Southern blotting with a *bla*_{NDM} probe confirmed that all the plasmids contained *bla*_{NDM-5} (Figure 2). Subsequently, the plasmid incompatibility classification methods assigned them to IncX3. Considering that the *bla*_{NDM-5}-harbored transferrable plasmids that were of the same size and incompatibility group, we further selected the plasmid pNDM-CREC-8 as a reference for whole genome sequencing to better understand the genetic environment surrounding *bla*_{NDM-5}. The completed sequence length of pNDM-CREC-8 was 46,263 bp with an average GC content of 47%. When submitted to the GenBank database for sequence alignment, it showed more than 99% nucleotide identity to the previously reported IncX3 pNDM-MGR 194 plasmid (GenBank accession number KF220657) in India.¹⁸ The flanking genetic structure of *bla*_{NDM-5}, which was composed of an IS3000 and incomplete IS*Aba125* interrupted by IS5 located upstream, and the genes *ble*MBL, *trpF*, *dsbC* and IS26 downstream (IS3000-*Δ*IS*Aba125*-IS5-*bla*_{NDM-5}-*ble*MBL-*trpF*-*dsbC*-IS26) in plasmid pNDM-MGR 194

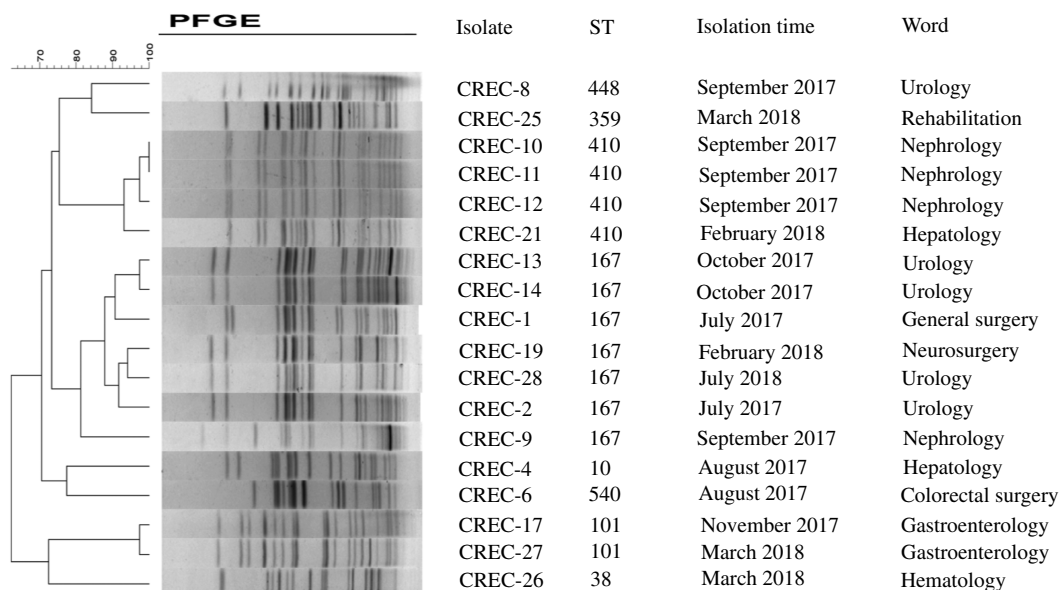


Figure 1 Dendrogram derived from the PFGE patterns of 18 *bla*_{NDM-5}-positive *E. coli* strains.

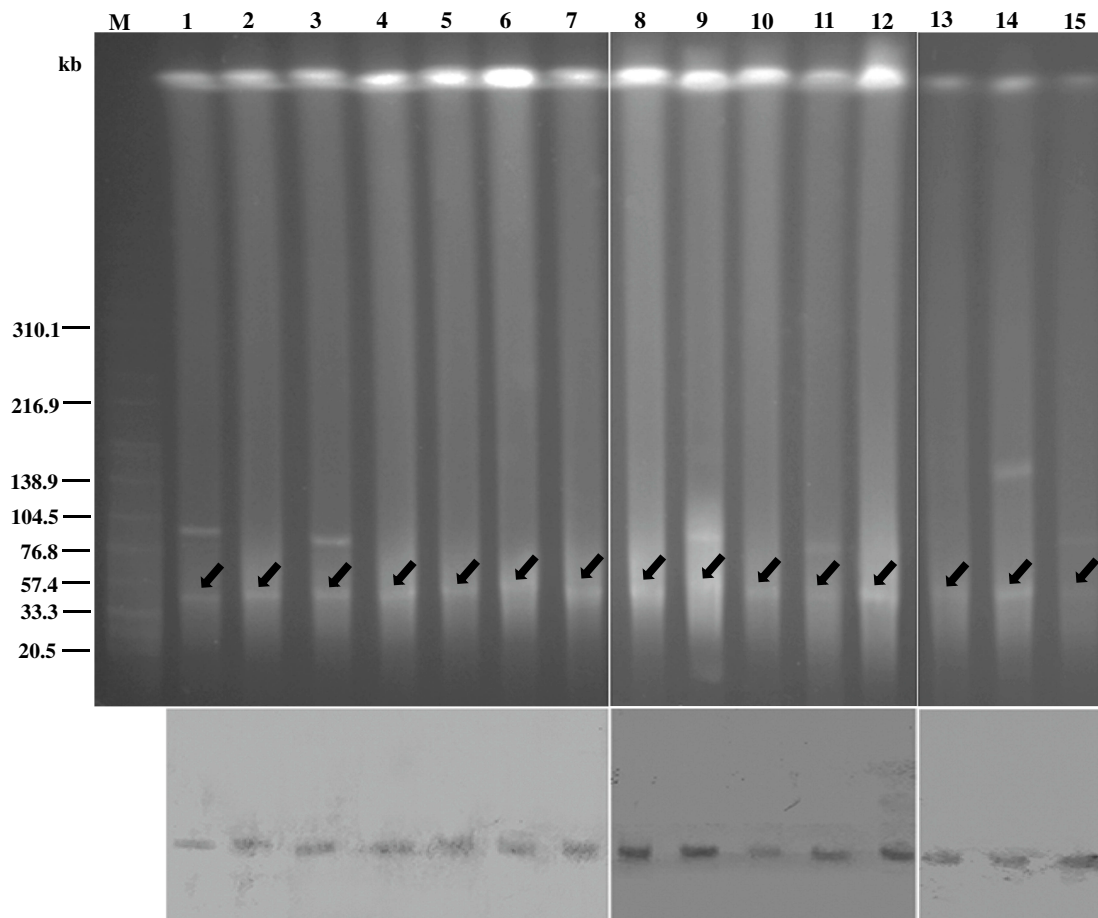


Figure 2 SI-PFGE and Southern blotting of the transferable plasmids in 15 conjugants. The arrows represent the plasmids where the bla_{NDM-5} genes located on.

were also identified in pNDM-EC-133. Furthermore, the other 14 strains were detected by PCR mapping and sequencing methods.

Discussion

NDM-5 was first identified in a multidrug resistant *Escherichia coli* strain from a patient in United Kingdom through routine swabbing.¹⁹ Compared to the amino acid sequences, NDM-5 differs from NDM-1 at positions 88 (Val→Leu) and 154 (Met→Leu), and NDM-5 has an enhanced hydrolytic activity against carbapenems. Since then, NDM-5-producing strains have been reported in Singapore, India, Algeria, Japan, China, the Netherlands, Denmark, Australia, South Korea, Spain, Egypt and America.²⁰ Obviously, NDM-5 seems to have become a secondary popular NDM variant of the NDM-1 reported in the world. In addition, the increasing prevalence of NDM-5-producing *Enterobacteriaceae* should be of deep concern because these bacteria are usually combined with other drug-resistant mechanisms, such as cephalosporins,

quinolones, and aminoglycosides. On the other hand, NDM-5-producing *Enterobacteriaceae* have already been isolated from pets, food animals and the environment, which serve as important reservoirs in the community.^{21–23}

In this study, homology analysis indicated that ST167, ST410 and ST101 caused clonal expansion in our hospital, which revealed endemic potential and deserved extensive surveillance. Noticeably, ST167 and ST410 *E. coli* were both previously identified as successful pandemic clones for the dissemination of ESBL genes in humans and animals.^{24–27} In addition, the transmission of bla_{NDM-1} and other bla_{NDM} variants, e.g., bla_{NDM-4} and bla_{NDM-7} , in *E. coli* also had a strong association with these two sequence types worldwide.^{28–30} These findings revealed that further spread of these strains may lead to significant clinical and public health concerns. In particular, ST167, which was continuously isolated from multiple specimens in different departments during the study period, had been found carrying the bla_{NDM-5} gene in several cities (including Chengdu, Shanghai, Shandong, Shenyang, Chifeng and Haikou) with

no obvious geographical contact across China,^{20,31–33} suggesting that ST167 plays an important role in the transmission of the *bla*_{NDM-5} gene in China. In addition to a possible outbreak of *K. pneumoniae* in Europe,³⁴ our study reported the first *bla*_{NDM-5}-related outbreak of *E. coli* ST410 in mainland China. Medical records showed that two ST101 *E. coli* isolates with very similar PFGE patterns (> 95%) were isolated from two patients by endoscopic retrograde cholangiopancreatography (ERCP) with the same duodenoscope in November 2017. It remains unclear whether the ERCP was responsible for the two infectious experiences because we were unable to culture the organism from the implicated instrument. However, a few studies in the United States have identified outbreaks of NDM-1-producing *E. coli* after receiving ERCP, even though the disinfection procedure of the associated endoscope was finished following standard recommendations and guidelines,³⁵ reminding us that a potential risk of contamination exists despite high-level disinfection. The four clonally diverse STs of *E. coli* (ST10, ST38, ST448, ST540) were identified as NDM-5 or its variants producers identified previously.^{36–39} Furthermore, ST359 was first regarded as an NDM producer.

Compared with clonal expansion, mobile elements were thought to play a major role in the dissemination of *bla*_{NDM} in different species in *Enterobacteriaceae*. The results of the conjugation experiment in our study showed that 15 strains can transfer their *bla*_{NDM-5} gene to the recipient strain through IncX3 conjugative plasmids with the same molecular weight of approximately 50 kb. The IncX3-type plasmids were previously identified as a narrow host range and only transmitted in *Enterobacteriaceae*. The first emergence of the IncX3-type plasmid carrying *bla*_{NDM-1} was reported in China, and the *bla*_{NDM-1} gene was disseminated among various species worldwide. Our team recently also found a nearly same size IncX3-type plasmid responsible for the transmission of *bla*_{NDM-1} in *K. pneumoniae* in the Department of Neonatology.⁴⁰ Whether the *bla*_{NDM-5} gene carrying an ~50 kb IncX3-type plasmid was evolved from the ~50 kb IncX3-type plasmid containing *bla*_{NDM-1} gene needs further investigation. Complete sequencing of the plasmid pNDM-EC-133 shows an almost identical sequence to pNDM-MGR 194, which was first identified as an NDM-5 producer of IncX3-type in India.¹⁸ In most studies in China, the pNDM-MGR 194-like plasmids were discovered to be associated with NDM-5 and thought to be efficient vehicles for the dissemination of *bla*_{NDM-5} across the country. Except for *E. coli*, the pNDM-MGR 194-like plasmids were also found in *K. pneumoniae*, *K. michiganensis*, *C. freundii* and *P.*

mirabilis, which implies the potential to spread intra- or interspecies in *Enterobacteriaceae*.⁴¹ It is noteworthy that one study in Jiangsu Province pointed out that the pNDM-MGR 194-like plasmid played a role in the dissemination of *bla*_{NDM-5} in cows in three dairy farms distributed in scattered regions of Jiangsu Province. This finding raised the possibility of *bla*_{NDM-5} spreading from animals to humans and represented an important *bla*_{NDM-5} reservoir in the community, which may lead to enormous losses to public health and property. Therefore, there is an urgent need to implement stringent and comprehensive strategies to monitor the emergence of *bla*_{NDM-5} and avoid its further spreading.

In conclusion, our study demonstrated a high prevalence of *bla*_{NDM-5}, which was combined with clonal spread and plasmid-mediated transmission of carbapenem-resistant *E. coli* strains in a university hospital in Jiangsu Province, China. The continuous isolation of ST167 and an outbreak of ST410 *E. coli* strains in our hospital underline appropriate infection control operations, such as isolating colonized or infected patients individually, cohorting nursing, and severe hand, equipment and environmental disinfection, should be taken immediately after the first isolation of *bla*_{NDM-5}-positive strains. The pNDM-MGR 194-like plasmid acts as an important vector for the dissemination of *bla*_{NDM-5} in *E. coli*, and its emergence in diverse STs implies its potential to a further extent.

Acknowledgments

This study was supported by the Project of the Key Laboratory for Laboratory Medicine of Jiangsu Province (No. XK201749) and the innovation project for postgraduate education of Jiangsu Province (No. SJLX15_0436). We would like to thank Professor Wenen Liu (Zhongnan University) and Huimin Qian (Jiangsu Provincial Centers for Disease Control and Prevention) for kindly providing the reference strains.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Peirano G, Schreckenberger PC, Pitout JD. Characteristics of NDM-1-producing *Escherichia coli* isolates that belong to the successful and virulent clone ST131. *Antimicrob Agents Chemother.* 2011;55(6):2986–2988. doi:10.1128/AAC.01763-10
2. Tangden T, Giske CG. Global dissemination of extensively drug-resistant carbapenemase-producing *Enterobacteriaceae*: clinical perspectives on detection, treatment and infection control. *J Intern Med.* 2015;277(5):501–512. doi:10.1111/joim.12342

3. Partridge SR, Ginn AN, Wiklendt AM, et al. Emergence of blaKPC carbapenemase genes in Australia. *Int J Antimicrob Agents*. 2015;45(2):130–136. doi:10.1016/j.ijantimicag.2014.10.006
4. Kola A, Piening B, Pape UF, et al. An outbreak of carbapenem-resistant OXA-48 - producing Klebsiella pneumonia associated to duodenoscopy. *Antimicrob Resist Infect Control*. 2015;4:8. doi:10.1186/s13756-015-0049-4
5. Yang Q, Fang L, Fu Y, Du X, Shen Y, Yu Y. Dissemination of NDM-1-producing Enterobacteriaceae mediated by the IncX3-type plasmid. *PLoS One*. 2015;10(6):e0129454. doi:10.1371/journal.pone.0129454
6. Yong D, Toleman MA, Giske CG, et al. Characterization of a new metallo-beta-lactamase gene, bla(NDM-1), and a novel erythromycin esterase gene carried on a unique genetic structure in Klebsiella pneumoniae sequence type 14 from India. *Antimicrob Agents Chemother*. 2009;53(12):5046–5054. doi:10.1128/AAC.00774-09
7. Johnson AP, Woodford N. Global spread of antibiotic resistance: the example of New Delhi metallo-beta-lactamase (NDM)-mediated carbapenem resistance. *J Med Microbiol*. 2013;62(Pt 4):499–513. doi:10.1099/jmm.0.052555-0
8. Seara N, Oteo J, Carrillo R, et al. Interhospital spread of NDM-7-producing Klebsiella pneumoniae belonging to ST437 in Spain. *Int J Antimicrob Agents*. 2015;46(2):169–173. doi:10.1016/j.ijantimicag.2015.04.001
9. Gaynes R, Edwards JR. Overview of nosocomial infections caused by gram-negative bacilli. *Clin Infect Dis*. 2005;41(6):848–854. doi:10.1086/432803
10. Liu H, Wang Y, Wang G, et al. The prevalence of Escherichia coli strains with extended spectrum beta-lactamases isolated in China. *Front Microbiol*. 2015;6:335. doi:10.3389/fmicb.2015.00335
11. Paterson DL, Ko WC, Von Gottberg A, et al. Antibiotic therapy for Klebsiella pneumoniae bacteremia: implications of production of extended-spectrum beta-lactamases. *Clin Infect Dis*. 2004;39(1):31–37. doi:10.1086/420816
12. Institute CaLS. *Performance Standards for Antimicrobial Susceptibility Testing; Twenty-third Informational Supplement. M100-S25*. Wayne, PA: Clinical and Laboratory Standards Institute; 2015.
13. Liao XP, Xia J, Yang L, et al. Characterization of CTX-M-14-producing Escherichia coli from food-producing animals. *Front Microbiol*. 2015;6:1136. doi:10.3389/fmicb.2015.01136
14. Chen L, Chen ZL, Liu JH, Zeng ZL, Ma JY, Jiang HX. Emergence of RmtB methylase-producing Escherichia coli and Enterobacter cloacae isolates from pigs in China. *J Antimicrob Chemother*. 2007;59(5):880–885. doi:10.1093/jac/dkm065
15. Cattoir V, Poirel L, Rotimi V, Soussy CJ, Nordmann P. Multiplex PCR for detection of plasmid-mediated quinolone resistance qnr genes in ESBL-producing enterobacterial isolates. *J Antimicrob Chemother*. 2007;60(2):394–397. doi:10.1093/jac/dkm204
16. Qin S, Fu Y, Zhang Q, et al. High incidence and endemic spread of NDM-1-positive Enterobacteriaceae in Henan Province, China. *Antimicrob Agents Chemother*. 2014;58(8):4275–4282. doi:10.1128/AAC.02813-13
17. Gu DX, Yu T, Wang Y, Zhang R. Detection of CTX-M-64 in Escherichia coli isolates from human patients in China. *Antimicrob Agents Chemother*. 2015;59(2):1371–1372. doi:10.1128/AAC.04158-14
18. Krishnaraju M, Kamatchi C, Jha AK, et al. Complete sequencing of an IncX3 plasmid carrying blaNDM-5 allele reveals an early stage in the dissemination of the blaNDM gene. *Indian J Med Microbiol*. 2015;33(1):30–38. doi:10.4103/0255-0857.148373
19. Hornsey M, Phee L, Wareham DW. A novel variant, NDM-5, of the New Delhi metallo-beta-lactamase in a multidrug-resistant Escherichia coli ST648 isolate recovered from a patient in the United Kingdom. *Antimicrob Agents Chemother*. 2011;55(12):5952–5954. doi:10.1128/AAC.05108-11
20. Zhang F, Xie L, Wang X, et al. Further spread of bla NDM-5 in Enterobacteriaceae via IncX3 plasmids in Shanghai, China. *Front Microbiol*. 2016;7:424.
21. Yousfi M, Mairi A, Bakour S, et al. First report of NDM-5-producing Escherichia coli ST1284 isolated from dog in Bejaia, Algeria. *New Microbes New Infect*. 2015;8:17–18. doi:10.1016/j.nmni.2015.09.002
22. Yaïci L, Haenni M, Saras E, Boudehouche W, Touati A, Madec JY. blaNDM-5-carrying IncX3 plasmid in Escherichia coli ST1284 isolated from raw milk collected in a dairy farm in Algeria. *J Antimicrob Chemother*. 2016;71(9):2671–2672. doi:10.1093/jac/dkw160
23. Almakki A, Maure A, Pantel A, et al. NDM-5-producing Escherichia coli in an urban river in Montpellier, France. *Int J Antimicrob Agents*. 2017;50(1):123–124. doi:10.1016/j.ijantimicag.2017.04.003
24. Oteo J, Diestra K, Juan C, et al. Extended-spectrum beta-lactamase-producing Escherichia coli in Spain belong to a large variety of multilocus sequence typing types, including ST10 complex/A, ST23 complex/A and ST131/B2. *Int J Antimicrob Agents*. 2009;34(2):173–176. doi:10.1016/j.ijantimicag.2009.03.006
25. Schink AK, Kadlec K, Kaspar H, Mankertz J, Schwarz S. Analysis of extended-spectrum-beta-lactamase-producing Escherichia coli isolates collected in the GERM-Vet monitoring programme. *J Antimicrob Chemother*. 2013;68(8):1741–1749. doi:10.1093/jac/dkt123
26. Schaufler K, Semmler T, Wieler LH, et al. Clonal spread and interspecies transmission of clinically relevant ESBL-producing Escherichia coli of ST410—another successful pandemic clone? *FEMS Microbiol Ecol*. 2016;92:1. doi:10.1093/femsec/fiv155
27. Timofte D, Maciucă IE, Williams NJ, Wattret A, Schmidt V. Veterinary hospital dissemination of CTX-M-15 extended-spectrum beta-lactamase-producing Escherichia coli ST410 in the United Kingdom. *Microb Drug Resist*. 2016;22(7):609–615. doi:10.1089/mdr.2016.0036
28. Qin S, Zhou M, Zhang Q, et al. First identification of NDM-4-producing Escherichia coli ST410 in China. *Emerg Microbes Infect*. 2016;5(11):e118. doi:10.1038/emi.2016.119
29. Cuzon G, Bonnin RA, Nordmann P. First identification of novel NDM carbapenemase, NDM-7, in Escherichia coli in France. *PLoS One*. 2013;8(4):e61322. doi:10.1371/journal.pone.0061322
30. Zhang X, Lou D, Xu Y, et al. First identification of coexistence of blaNDM-1 and blaCMY-42 among Escherichia coli ST167 clinical isolates. *BMC Microbiol*. 2013;13:282. doi:10.1186/1471-2180-13-282
31. Yang P, Xie Y, Feng P, Zong Z. blaNDM-5 carried by an IncX3 plasmid in Escherichia coli sequence type 167. *Antimicrob Agents Chemother*. 2014;58(12):7548–7552. doi:10.1128/AAC.03911-14
32. Zhu YQ, Zhao JY, Xu C, Zhao H, Jia N, Li YN. Identification of an NDM-5-producing Escherichia coli sequence type 167 in a neonatal patient in China. *Sci Rep*. 2016;6:29934. doi:10.1038/srep29934
33. Chen D, Gong L, Walsh TR, et al. Infection by and dissemination of NDM-5-producing Escherichia coli in China. *J Antimicrob Chemother*. 2016;71(2):563–565. doi:10.1093/jac/dkv352
34. Hammerum AM, Hansen F, Olesen B, et al. Investigation of a possible outbreak of NDM-5-producing ST16 Klebsiella pneumoniae among patients in Denmark with no history of recent travel using whole-genome sequencing. *J Glob Antimicrob Resist*. 2015;3(3):219–221. doi:10.1016/j.jgar.2015.05.003
35. Smith ZL, Oh YS, Saecian K, et al. Transmission of carbapenem-resistant Enterobacteriaceae during ERCP: time to revisit the current reprocessing guidelines. *Gastrointest Endosc*. 2015;81(4):1041–1045. doi:10.1016/j.gie.2014.11.006
36. Nakano R, Nakano A, Hikosaka K, et al. First report of metallo-beta-lactamase NDM-5-producing Escherichia coli in Japan. *Antimicrob Agents Chemother*. 2014;58(12):7611–7612. doi:10.1128/AAC.04265-14
37. Pitart C, Sole M, Roca I, et al. Molecular characterization of blaNDM-5 carried on an IncFII plasmid in an Escherichia coli isolate from a nontraveler patient in Spain. *Antimicrob Agents Chemother*. 2015;59(1):659–662. doi:10.1128/AAC.04040-14
38. Sekizuka T, Matsui M, Yamane K, et al. Complete sequencing of the bla(NDM-1)-positive IncA/C plasmid from Escherichia coli ST38 isolate suggests a possible origin from plant pathogens. *PLoS One*. 2011;6(9):e25334. doi:10.1371/journal.pone.0025334

39. Zhang Y, Liao K, Gao H, et al. Decreased fitness and virulence in ST10 *Escherichia coli* Harboring blaNDM-5 and mcr-1 against a ST4981 strain with blaNDM-5. *Front Cell Infect Microbiol.* 2017;7:242. doi:10.3389/fcimb.2017.00517
40. Huang X, Cheng X, Sun P, Tang C, Ni F, Liu G. Characteristics of NDM-1-producing *Klebsiella pneumoniae* ST234 and ST1412 isolates spread in a neonatal unit. *BMC Microbiol.* 2018;18(1):186. doi:10.1186/s12866-018-1202-z
41. Li X, Fu Y, Shen M, et al. Dissemination of blaNDM-5 gene via an IncX3-type plasmid among non-clonal *Escherichia coli* in China. *Antimicrob Resist Infect Control.* 2018;7:59. doi:10.1186/s13756-018-0349-6

Infection and Drug Resistance

Dovepress

Publish your work in this journal

Infection and Drug Resistance is an international, peer-reviewed open-access journal that focuses on the optimal treatment of infection (bacterial, fungal and viral) and the development and institution of preventive strategies to minimize the development and spread of resistance. The journal is specifically concerned with the epidemiology of

antibiotic resistance and the mechanisms of resistance development and diffusion in both hospitals and the community. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <https://www.dovepress.com/infection-and-drug-resistance-journal>