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bla$_{NDM-5}$ Carried by an IncX3 Plasmid in *Escherichia coli* Sequence Type 167

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***bla*$_{NDM-5}$ was found in *Escherichia coli* strain 0215 from a Chinese patient without travel history. Genomic sequencing and conjugation experiments were performed. Strain 0215 belonged to sequence type 167 (ST167) and had other resistance determinants, including *bla*$_{TEM-135}$, *bla*$_{CTX-M-14}$, and *aac*(6′)-*Ib*. *bla*$_{NDM-5}$ was carried by a 47-kb self-transmissible IncX3 plasmid and was in a complex genetic context similar to that of *bla*$_{NDM-1}$ on IncX3 plasmids. IncX3 plasmids might have emerged as a common vehicle mediating the spread of *bla*$_{NDM}$.**

NDM (New Delhi metallo-β-lactamase) is a type of carbapenemase with the ability to hydrolyze all β-lactams except monobactams (1). NDM enzymes confer resistance to carbapenems, which have long served as reliable and potent agents against Gram-negative bacilli. Bacterial isolates producing NDM were first identified in 2008 (2) and have emerged worldwide, representing a serious challenge for clinical management and infection control. Most NDM-producing isolates belong to various species of *Enterobacteriaceae* or *Acinetobacter*.

Until now, 12 variants of NDM enzymes (NDM-1 to -12) have been discovered and assigned in the Lahey Clinic database (see http://www.lahey.org/Studies/other.asp#table 1). The NDM-5-encoding gene, *bla*$_{NDM-5}$, was first identified in an *Escherichia coli* strain (EC045) from a patient in the United Kingdom who had a recent history of hospitalization in India (3). NDM-5 differs from NDM-1 in two amino acid substitutions (Val88Leu and Met154Leu) (3), which appear to confer increased resistance to carbapenems and broad-spectrum cephalosporins (3). The goal of this study was to characterize a Chinese *E. coli* strain carrying *bla*$_{NDM-5}$.

Strain 0215 was recovered from a routine screening rectal swab of a 75-year-old male patient with an acute exacerbation of chronic obstructive pulmonary disease in September 2013. The rectal swab was streaked directly onto a ChromID CARBA agar plate (bioMérieux, Lyon, France) selective for carbapenem-resistant *Enterobacteriaceae*. Strain 0215 was identified as *E. coli* by partially sequencing the 16S rRNA gene amplified with the universal primers 27F and 1492R (4). The MICs of imipenem, meropenem, amikacin, ceftazidime, and ciprofloxacin were determined using the microdilution broth method following the recommendations of the Clinical and Laboratory Standards Institute (5). Strain 0215 was resistant to imipenem (MIC, 512 μg/ml), meropenem (MIC, 256 μg/ml), ceftazidime (MIC, >256 μg/ml), and ciprofloxacin (MIC, 128 μg/ml) but was susceptible to amikacin (MIC, 16 μg/ml).

We screened the carbapenemase-encoding genes *bla*$_{GES}$, *bla*$_{KPC}$, *bla*$_{IMP}$, *bla*$_{NDM}$, *bla*$_{OXA-48}$, and *bla*$_{VIM}$ for strain 0215 using PCR as described previously (6–9), and only *bla*$_{NDM}$ was detected. The complete coding sequence of *bla*$_{NDM}$ was amplified with an additional pair of primers (NDM-up and NDM-dw) (6), and sequencing revealed the presence of *bla*$_{NDM-5}$. Since the discovery of *bla*$_{NDM-5}$ in *E. coli* strain EC045, *bla*$_{NDM-5}$ has also been found in a *Klebsiella pneumoniae* strain (MGR-K165, GenBank accession number KF220657) and three *E. coli* strains (GenBank accession numbers KJ150692, KF284078, and KF284079) (10) in India. Very recently, *bla*$_{NDM-5}$ was also found in three *E. coli* strains in Algeria (11) and one *E. coli* strain in Spain (12). In addition, a recent report claimed that *bla*$_{NDM-5}$ was also detected in another *E. coli* strain, KOEC3, in India (13). However, only 240 bp of the 813-bp *bla*$_{NDM}$ coding sequence are available; therefore, we were unable to confirm *bla*$_{NDM-5}$ in this case. Nonetheless, *bla*$_{NDM-5}$ has been found in different locations in India, including Lucknow (10) and Chennai (GenBank accession numbers KF220657 and KJ150692) and potentially in Goa (3) and Umiam (13), suggesting that *bla*$_{NDM-5}$ might have been circulating in India. However, the patient from whom strain 0215 was recovered had no history of travel to Africa, Europe, or South Asia during his lifetime. To our knowledge, this is the first report of *bla*$_{NDM-5}$ in China.

Genomic DNA of strain 0215 was prepared using the QIAamp DNA minikit (Qiagen, Hilden, Germany) and was subjected to paired-end whole-genomic sequencing with a 500-bp insert size and a ca. 140× coverage using the HiSeq 2500 Sequencer (Illumina, San Diego, CA) following the manufacturer's protocol at the Beijing Genomics Institute (Beijing, China). The reads were assembled to contigs using the Velvet program (14). A total of 7,354,784 clean reads and 661,930,560 clean bases were obtained from the genomic sequencing for strain 0215. The GC content was 50.66%. There is no reference genomic sequence of sequence type 167 (ST167) *E. coli* available, and ST167 is of the ST10 clonal complex. The genomic sequence of *E. coli* strain K-12 substrain MG1655 (GenBank accession number U00096), which belongs to ST10, was therefore selected as a reference for the further assembly and annotation of the genomic sequence of strain 0215 using the

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TABLE 1 *E. coli* of ST167 carrying bla_{CTX-M} and/or bla_{NDM}

bla_{CTX-M}	bla_{NDM}	Location(s)	Host(s)	Source or reference
$bla_{CTX-M-1}$		Germany, The Netherlands	Cattle, swine, human	27, 28
$bla_{CTX-M-2}$		Germany	Cattle	27
$bla_{CTX-M-3}$		Germany	Swine	27
$bla_{CTX-M-9}$		Mongolia	Wild bird	29
$bla_{CTX-M-14}$		Spain	Human	30, 31, MLST database[a]
$bla_{CTX-M-14}$	bla_{NDM-1}	China	Human	19
$bla_{CTX-M-14}$	bla_{NDM-5}	China	Human	This study
$bla_{CTX-M-15}$	bla_{NDM-7}	France[b]	Human	20
$bla_{CTX-M-15}$		Canada, Germany, Norway, Russia, Spain	Human, swine, turkey	30, 32, 33, MLST database

[a] See http://mlst.warwick.ac.uk/mlst/dbs/Ecoli.
[b] From a French patient who had recently traveled to Myanmar.

PAGIT and Prokka programs. PAGIT is a toolkit for ordering contigs, closing gaps, correcting sequence errors, and transferring annotation (15), and Prokka is a tool for annotating prokaryotic genomes (16). Reads were assembled to 243 contigs that were ≥100 bp in length.

Phylogenetic group (A, B1, B2, and D) typing, which was performed as described previously (17), assigned strain 0215 to group A. The sequence type (ST) of strain 0215 was assigned using the assembled genomic sequence to query the seven alleles of the multilocus sequence-typing scheme for *E. coli* (see http://mlst.warwick.ac.uk/mlst/dbs/Ecoli) (18). Strain 0215 belonged to ST167. In contrast, the first *E. coli* strain carrying bla_{NDM-5} was of ST648 (3). *E. coli* strains of ST167 have been found carrying bla_{NDM-1} in China (19) and bla_{NDM-7} in a French patient after recent travel to Myanmar (20). In addition, ST167 *E. coli* strains have been found carrying a variety of extended-spectrum β-lactamase (ESBL) genes, including $bla_{CTX-M-1}$, $bla_{CTX-M-2}$, $bla_{CTX-M-3}$, $bla_{CTX-M-14}$, and $bla_{CTX-M-15}$ in various countries (Table 1).

In addition to bla_{NDM-5}, strain 0215 had multiple resistance genes, including $bla_{TEM-135}$ (a non-ESBL variant of bla_{TEM-1}), $bla_{CTX-M-14}$ (an ESBL gene widely distributed in China), bla_{ampC}, *aac*(6′)-*Ib*, *cmlA1* (encoding a chloramphenicol exporter), *floR* (encoding a florfenicol/chloramphenicol resistance protein), *mph* (encoding macrolide 2′-phosphotransferase I), and *tet*(A) (conferring resistance to tetracycline). Of note, the AmpC enzyme of strain 0215 is identical to the NCBI reference sequence WP_024176402 in amino acids. The same bla_{ampC} variant has also been found in $bla_{CTX-M-15}$-carrying strain EC66, which belongs to the phylogenetic group A and the O101 type from India, but its sequence type is not available (21).

Conjugation experiments were carried out in broth using azide-resistant *E. coli* strain J53 as the recipient. Transconjugants were selected on plates containing 4 μg/ml meropenem plus 150 μg/ml sodium azide. The presence of bla_{NDM} in transconjugants was confirmed using PCR, and enterobacterial repetitive intergenic consensus (ERIC)-PCR (22) was used for further distinguishing transconjugants from the donor strain. bla_{NDM-5} was transferred to *E. coli* recipient J53, although none of the other resistance genes listed above were cotransferred with bla_{NDM-5}. Therefore, bla_{NDM-5} was located on a self-transmissible plasmid, designated pNDM5_0215 here, in strain 0215. However, PCR-based replicon typing (PBRT) (23) failed to assign pNDM5_0215

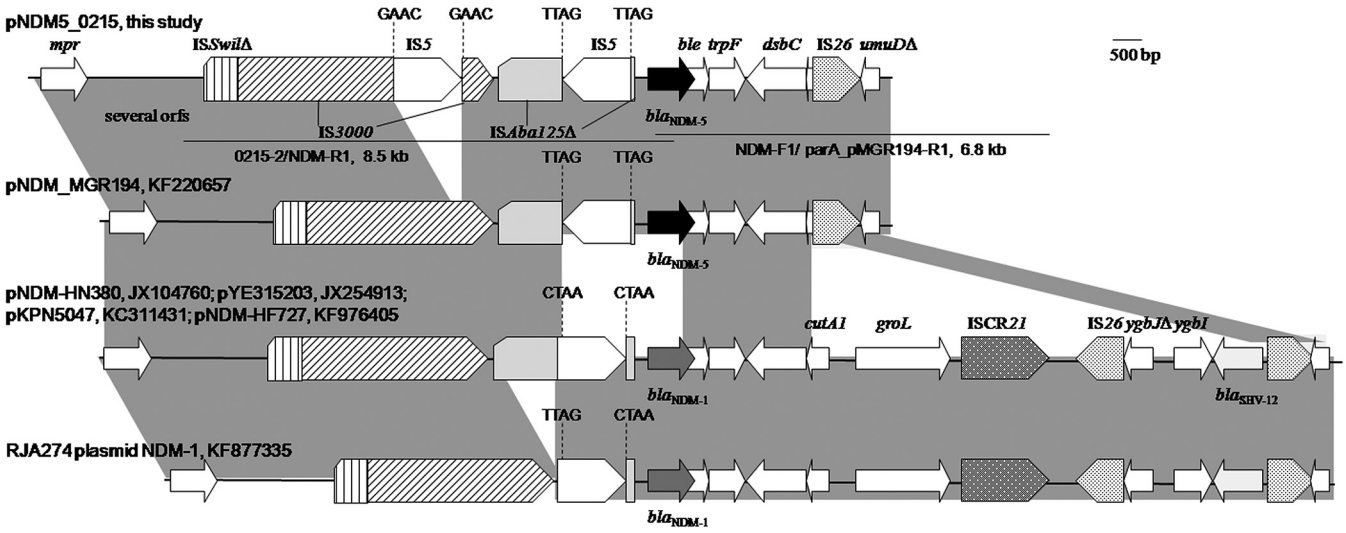

FIG 1 Genetic contexts of bla_{NDM-1} and bla_{NDM-5} on IncX3 plasmids. Plasmid names of each structure and their GenBank accession numbers are listed. The identical regions are highlighted in gray. The 4-bp flanking sequences of each IS5 are shown. Between *mpr* and IS*Swil*, there are several ORFs of unknown function, which are indicated here by "several orfs." Two long-range PCR products used to fill in the gap of pNDM5_0215 are shown by lines, and primer names and amplicon sizes are indicated. Primers NDM-F1 and NDM-R1 are from reference 6, while primers 0215-2 (TGGTGCTGGTTATCTGTGCT) and parA_pMGR194-R1 (CCGTTATCTGTCCGCTTTTC) were newly designed.

TABLE 2 IncX3 plasmids with a complete sequence available in GenBank

Plasmid	*bla* gene carried	Host species	Location	GenBank accession no.	Reference or source
pIncX-SHV	bla_{SHV-11}	*K. pneumoniae*	Italy	JN247852	34
pKP13d	bla_{KPC-2}	*K. pneumoniae*	Brazil	CP003997	35
pKPC-NY79	bla_{KPC-2}	*K. pneumoniae*	China	JX104759	36
pKpS90	bla_{KPC-2}, bla_{SHV-12}	*K. pneumoniae*	France	JX461340	37
pNDM-HN380	bla_{NDM-1}, bla_{SHV-12}	*K. pneumoniae*	China	JX104760	38
pYE315203	bla_{NDM-1}, bla_{SHV-12}	*Citrobacter freundii*	China	JX254913	
pKPN5047	bla_{NDM-1}, bla_{SHV-12}	*K. pneumoniae*	China	KC311431	
RJA274 plasmid NDM-1	bla_{NDM-1}, bla_{SHV-12}	*Raoultella planticola*	China	KF877335	
pNDM-HF727	bla_{NDM-1}, bla_{SHV-12}	*Enterobacter cloacae*	China	KF976405	36
pNDM_MGR194	bla_{NDM-5}	*K. pneumoniae*	India	KF220657	
pNDM5-0215	bla_{NDM-5}	*E. coli*	China		This study

to a replicon type, as all replicon-typing PCRs were negative. Genomic sequencing of strain 0215 revealed a 34-kb contig, which is identical to an IncX3 plasmid (pNDM_MGR194) carrying bla_{NDM-5} in *K. pneumoniae* strain MGR-K165. Therefore, the sequence of pNDM_MGR194 (GenBank accession number KF220657) was used as reference for assembling pNDM5_0215, which was completely circularized by filling the gaps using long-range PCR (Fermentas, Burlington, Canada; Fig. 1) and Sanger sequencing using an ABI 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA) at the Beijing Genomics Institute. Indeed, pNDM5_0215 was a 47-kb IncX3 plasmid and had only two minor differences from pNDM_MGR194, the absence of one copy of a 22-bp iteron that is involved in regulating plasmid replication and the presence of an additional copy of the insertion sequence IS*5* on pNDM5_0215 (Fig. 1). Otherwise, the two plasmids were identical. IncX plasmids are narrow-host-range plasmids of *Enterobacteriaceae* and include at least five subtypes, IncX1 to IncX5 (24). A few IncX3 plasmids carrying ESBL and/or carbapenemase genes from various species of *Enterobacteriaceae* found in several countries have been completely sequenced (Table 2).

Although bla_{NDM-5} has been identified in several cases, its genetic context was largely uninvestigated. Nonetheless, the available complete sequences of pNDM5_0215 and pNDM_MGR194 allowed us to reveal the genetic context of bla_{NDM-5}. On the two IncX3 plasmids, bla_{NDM-5} is in the same genetic context, except there is an insertion of an additional copy of IS*5* into IS*3000* on pNDM5_0215, as evidenced by the presence of the characteristic 4-bp direct target repeats (Fig. 1). In such a context, bla_{NDM-5} is adjacent to an incomplete IS*Aba125*, which is interrupted by the insertion of IS*5* and is also truncated by the insertion of IS*3000* at its left end upstream. A zinc metalloproteinase-encoding gene, *mpr*, lies further upstream of bla_{NDM-5}. Several putative open reading frames (ORFs) of unknown function and a truncated IS*Swil* are present between *mpr* and IS*3000*. Downstream of bla_{NDM-5}, there are *ble* (mediating bleomycin resistance), *trpF* (encoding a phosphoribosylanthranilate isomerase), *dsbC* (encoding an oxidoreductase), a remnant of *ctuA1* (encoding an ion-tolerant protein) that is truncated by the insertion of IS*26*, and a truncated *umuD* gene (encoding a mutagenesis protein) (Fig. 1).

Such a context of bla_{NDM-5} on IncX3 plasmids is highly similar to those of bla_{NDM-1} on several other IncX3 plasmids (Fig. 1). All contexts of bla_{NDM-1} and bla_{NDM-5} on IncX3 plasmids are bounded by *mpr* at one end and by Δ*umuD* at the other. The highly similar contexts suggest that bla_{NDM-5} might have evolved from bla_{NDM-1} via point mutations in the *mpr*-Δ*umuD* region of an IncX3 plasmid. Of note, an IS*26*-bounded region containing bla_{SHV-12}, *ygbI* (a putative dehydrogenase gene), and a truncated *ygbJ* (a putative DEOR-type transcriptional regulator gene) is present downstream of bla_{NDM-1} on IncX3 plasmids (Fig. 1). It is therefore reasonable to propose that the absence of *groL*, IS*CR21*, and the region containing bla_{SHV-12} from the context of bla_{NDM-5} might be explained by the insertion of an addition of IS*26* into *cutA1* and the following homologous recombination between two copies of IS*26*, which resulted in the deletion. The IncX3 plasmids carrying bla_{NDM-1} or bla_{NDM-5} also had nearly identical backbones, further supporting the idea that bla_{NDM-5} might have emerged on IncX3 plasmids. A few bla_{NDM}-carrying IncX3 plasmids have been found in China but at various locations, suggesting that a common IncX3 plasmid carrying bla_{NDM} is circulating in various *Enterobacteriaceae* species in China. However, IncX3 plasmids carrying bla_{NDM} are not restricted to China but also have been identified in Germany (bla_{NDM-7}, in an *E. coli* isolate from a Yemeni patient) (25), India (bla_{NDM-5}, pNDM_MGR194), and the United Arab Emirates (bla_{NDM-1}) (26), suggesting IncX3 plasmids might have emerged as a common platform mediating the spread of bla_{NDM}. In addition, two nearly identical bla_{NDM-5}-carrying plasmids, pNDM5_0215 and pNDM_MGR194, were found in China and India separately. This might suggest unrecognized international transfers of the same IncX3 plasmid or, less likely, the independent emergence of bla_{NDM-5} on the same plasmid in each country. The association of IncX3 plasmids and bla_{NDM} variants and the epidemiology of IncX3 plasmids in *Enterobacteriaceae* species warrant more studies.

Nucleotide sequence accession number. Reads of the 0215 genomic sequence have been deposited into the NCBI database under BioProject identification no. PRJNA244631 and submission identification no. SUB492451.

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