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Plasmid-Mediated Novel bla_{NDM-17} Gene Encoding a Carbapenemase with Enhanced Activity in a Sequence Type 48 *Escherichia coli* Strain

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ABSTRACT Carbapenem-resistant *Enterobacteriaceae* (CRE) have spread worldwide, leaving very few treatment options available. New Delhi metallo-beta-lactamase (NDM) is the main carbapenemase mediating CRE resistance and is of increasing concern. NDM-positive *Enterobacteriaceae* of human origin are frequently identified; however, the emergence of NDM, and particularly novel variants, in bacteria of food animal origin has never been reported. Here, we characterize a novel NDM variant (assigned NDM-17) identified in a β-lactam-resistant sequence type 48 (ST48) *Escherichia coli* strain that was isolated from a chicken in China. Compared to NDM-1, NDM-17 had three amino acid substitutions (V88L, M154L, and E170K) that confer significantly enhanced carbapenemase activity. Compared to NDM-5, NDM-17 had only one amino acid substitution (E170K) and slightly increased isolate resistance to carbapenem, as indicated by increased MIC values. The gene encoding NDM-17 (bla_{NDM-17}) was located on an IncX3 plasmid, which was readily transferrable to recipient *E. coli* strain J53 by conjugation, suggesting the possibility of the rapid dissemination of bla_{NDM-17}. Enzyme kinetics showed that NDM-17 could hydrolyze all β-lactams tested, except for aztreonam, and had a significantly higher affinity for all β-lactams tested than did NDM-5. The emergence of this novel NDM variant could pose a threat to public health because of its transferability and enhanced carbapenemase activity.

KEYWORDS NDM-17, carbapenemase, ST48, IncX3

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Carbapenem-resistant *Enterobacteriaceae* (CRE) have been recognized as an urgent antibiotic resistance threat by the Centers for Disease Control and Prevention in the United States and have become a global problem in recent years (1). The resistance exhibited by CRE is mediated largely by the production of carbapenemases (2), especially metallo-β-lactamases (MBLs) such as VIM, IMP, and New Delhi metallo-β-lactamase (NDM), which can hydrolyze almost all carbapenem β-lactams (3). Since its discovery in India in 2008, NDM has been identified throughout the world, and its identification in China has become common (4, 5). Currently, there are 16 NDM variants (http://www.lahey.org/studies), with amino acid substitutions at 14 positions. The evolution and spread of NDM are rapid, and NDM-positive bacteria are found in the wider community environment and not just hospitals (6). The spread of NDM-positive bacteria depends on fecal-oral transmission, and an important route for this transmission is animal-derived food (6). The importance of minimizing the carriage of NDM-positive bacteria by food animals for public health was underlined by the discovery of nonhuman sources of NDM (7, 8). In comparison to the high prevalence of NDM-

positive *Enterobacteriaceae* of human origin, there are few reports on CRE from food animals. Furthermore, none of the novel NDM variants described to date were originally identified in bacteria isolated from food animals. Here, we describe the characterization of a novel NDM variant in *Escherichia coli* isolated from a chicken.

RESULTS AND DISCUSSION

Characterization of *E. coli* AD-19R. The *E. coli* AD-19R isolate was resistant to all β-lactams tested, including imipenem, meropenem, ertapenem, and aztreonam, but was sensitive to tigecycline and colistin (Table 1). A positive result by the modified Hodge test demonstrated the carbapenemase production phenotype. The presence of bla_{NDM} in AD-19R was confirmed by PCR and sequencing. Analysis of the draft genome of AD-19R by whole-genome sequencing revealed a novel bla_{NDM} variant, assigned bla_{NDM-17} (GenBank accession no. KX812714), as well as the presence of the additional β-lactamase genes $bla_{CTX-M-64}$ and bla_{TEM-1B}, the sulfonamide resistance gene *sul2*, and the aminoglycoside resistance genes *aph(3′)-Ia*, *aadA5*, and *rmtB*. In comparison with bla_{NDM-1}, bla_{NDM-17} contained point mutations at nucleotide positions 262 (G→T), 460 (A→C), and 508 (G→A). These substitutions corresponded to amino acid variants V88L, M154L, and E170K, respectively, with E170K being a novel substitution. Multilocus sequence typing (MLST) analysis showed that AD-19R belonged to sequence type 48 (ST48), which is most commonly associated with bla_{CTX-M}-harboring *E. coli* isolates in humans (9). These findings suggest the possibility of the transfer of *E. coli* isolates harboring bla_{NDM} from humans to food animals (8, 10).

Transferability and localization of bla_{NDM-17} and plasmid analysis. Transconjugation assays showed that bla_{NDM-17} was successfully transferred to *E. coli* J53, with a transfer frequency of $\sim 6.32 \times 10^{-9}$ per donor. S1-pulsed-field gel electrophoresis (PFGE) and Southern blotting revealed that a plasmid band from the transconjugants (designated AD19/J53), with a size of ~47 kb, hybridized with the bla_{NDM} probe (Fig. 1). AD19/J53 exhibited a resistance profile similar to that of parental isolate AD-19R, except for aztreonam.

The complete DNA sequence of pAD-19R (carrying bla_{NDM-17}), isolated from AD19/J53 transconjugants, was obtained by whole-genome sequencing, with an average depth of coverage of 510. It was a circular, 46,161-bp plasmid with a G+C content of 46.6% and 60 putative open reading frames (ORFs) (see Fig. S1 in the supplemental material). pAD-19R was identified as an IncX3 plasmid, with a typical backbone structure for this plasmid type, including regions involved replication, partitioning, plasmid maintenance, transcriptional activation, and conjugation/type IV secretion (11, 12). Although IncX3 plasmids are considered low-prevalence, narrow-host-range plasmids of *Enterobacteriaceae* (13), they may have served as a common vehicle mediating bla_{NDM} dissemination in China and might be responsible for the rapid spread of NDM-carrying isolates (4, 14), a theory supported by our study.

BLAST homology analysis showed that pAD-19R had 99% (46,142 bp/46,161 bp) identity and 100% query coverage with pNDM5_IncX3 (GenBank accession no. KU761328), a 46,161-bp IncX3 plasmid isolated from *Klebsiella pneumoniae* (SZ204), recently reported in China (15). Notably, strain SZ204 carried an *mcr-1*-harboring plasmid, in addition to pNDM5_IncX3, which makes coexistence of IncX3 bla_{NDM-5}-harboring plasmids and *mcr-1*-harboring plasmids. In addition, the pAD-19R sequence was similar to those of five other IncX3 bla_{NDM} allele-harboring plasmids: plasmid unnamed2 from *K. pneumoniae* strain NUHL24835 (GenBank accession no. CP014006) isolated in China, pNDM_MGR194 (GenBank accession no. KF220657) from *K. pneumoniae* isolated in India (12), pEc1929 (GenBank accession no. KT824791) from *E. coli* isolated in China (16), pJEG027 (GenBank accession no. KF220657) from *K. pneumoniae* isolated in Australia (17), and pKpN01-NDM7 (GenBank accession no. CP012990) from *K. pneumoniae* isolated in Canada (18). Interestingly, all six plasmids, including pNDM5_IncX3, were carried by bacteria isolated from humans, whereas the *E. coli* strain carrying pAD-19R in our study was isolated from a chicken. This result further indicates the possible transfer of IncX3 bla_{NDM-17}-harboring plasmids/isolates between humans and food-

TABLE 1 β-Lactam MICs for the NDM-17-carrying original *E. coli* isolate and its transconjugants and transformants

Antibiotic	MIC (μg/ml)									
	AD19 (NDM-17)	AD19/J53	J53	DH5α (pHSG398)	DH5α (pHSG398/NDM-1)	DH5α (pHSG398/NDM-5)	DH5α (pHSG398/NDM-17)	DH5α (pHSG398/NP-NDM-1)	DH5α (pHSG398/NP-NDM-5)	DH5α (pHSG398/NP-NDM-17)
Ampicillin	>256	>256	4	2	>256	>256	>256	>256	>256	>256
Aztreonam	256	0.063	0.063	0.032	0.063	0.032	0.063	0.063	0.032	0.063
Amikacin	>256	2	2	0.5	0.5	0.25	0.5	0.5	0.25	1
Cefepime	>256	>256	0.063	0.032	2	2	4	8	16	16
Cefotaxime	>256	>256	0.125	0.063	64	32	32	128	128	128
Cefoxitin	>256	>256	8	4	>256	128	>256	>256	>256	>256
Ceftazidime	>256	>256	0.5	0.25	>256	>256	>256	>256	>256	>256
Ciprofloxacin	16	≤0.008	≤0.008	0.016	0.016	0.008	≤0.008	0.016	0.008	0.016
Colistin	1	0.5	0.5	0.125	0.125	0.016	0.125	0.125	≤0.008	0.125
Ertapenem	256	128	0.032	0.016	0.25	2	2	64	64	128
Gentamicin	256	0.5	0.5	0.125	0.125	0.125	0.125	0.063	0.063	0.125
Imipenem	128	32	0.5	0.5	2	2	2	8	16	16
Meropenem	128	32	0.063	0.031	1	2	2	8	16	32
Penicillin G	>256	>256	64	32	>256	256	>256	>256	>256	>256
Tigecycline	0.063	0.063	0.063	0.032	0.032	0.032	0.032	0.063	0.016	0.063
SXT (1/19)[a]	≥16/304	0.063/1.2	0.032/0.61	0.5/9.5	0.5/9.5	0.25/4.75	0.25/4.75	0.032/0.61	0.032/0.61	0.063/1.2

[a]SXT, trimethoprim-sulfamethoxazole; 1/19 is the ratio of trimethoprim/sulfamethoxazole concentrations.

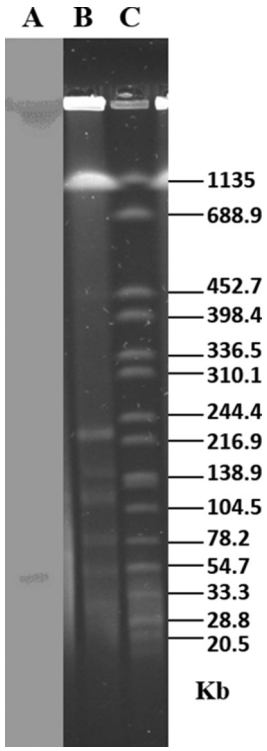

FIG 1 Location of bla_{NDM-17} on *E. coli* plasmid pAD-19R separated by PFGE. (A) Hybridization of the plasmid with a probe specific for bla_{NDM-17}; (B) plasmid from transconjugant AD19/J53; (C) Reference standard strain H9812 restricted by XbaI.

producing animals. Therefore, *Enterobacteriaceae* species carrying IncX3 bla_{NDM-17}-harboring plasmids should be monitored worldwide.

Further analysis of the pAD-19R sequence showed that it did not harbor other resistance genes apart from bla_{NDM-17} and *ble*. The sequence surrounding bla_{NDM-17} shares a common genetic background with a 10,410-bp fragment, Tn*3*-IS*3000*-ΔIS*Aba125*-IS*5*-bla_{NDM-17}-ble_{MBL}-*trpF*-*dsbC*-IS*26*-Δ*umnD* (see region A in Fig. S1 in the supplemental material), which plays a crucial role in horizontal transmission and may assist in the horizontal transfer of bla_{NDM-17} among *Enterobacteriaceae* (19). Overall, these results warn that the genetic environments of both bla_{NDM-17} and the IncX3 bla_{NDM-17}-harboring plasmids contribute to bla_{NDM-17} transmission among food-producing animals. The bla_{NDM-17}-carrying isolates would pose a threat to human health once *E. coli* AD-19R is transferred to humans through the food chain and vice versa.

Functional analysis of NDM-17 and characterization of kinetic parameters. NDM-17 had three amino acid substitutions (V88L, M154L, and E170K) compared with NDM-1 but only one difference (E170K) in comparison with NDM-5, with which NDM-17 shares the closest relationship among the 16 reported NDM variants (http://www.lahey.org/studies). In order to determine the effects of these amino acid substitutions in NDM-17, especially E170K, cloning experiments and kinetic studies were performed with reference to NDM-5.

All of the transformants were successfully cloned and confirmed by PCR. The promoter region of the bla_{NDM} gene was identified to have no differences in pHSG398/NP-NDM-1, pHSG398/NP-NDM-5, and pHSG398/NP-NDM-17 for all corresponding DH5α transformants by PCR and sequencing with M13 primers, and all corresponding DH5α transformants exhibited resistance to all β-lactams tested, including meropenem and imipenem (Table 1). Interestingly, the constructs pHSG398/NDM-1, pHSG398/NDM-5, and pHSG398/NDM-17, carrying complete ORFs without the native promoters, showed reduced susceptibility to penicillins and cephems but were susceptible to

TABLE 2 Kinetic parameters of NDM-17 and NDM-5 enzymes[a]

	NDM-17[b]			NDM-5[b]			
β-Lactam	Mean K_m (μM) ± SD	Mean k_{cat} (s^{-1}) ± SD	k_{cat}/K_m ratio (μM^{-1} s^{-1})	Mean K_m (μM) ± SD	Mean k_{cat} (s^{-1}) ± SD	k_{cat}/K_m ratio (μM^{-1} s^{-1})	k_{cat}/K_m ratio (μM^{-1} s^{-1}) for NDM-17/NDM-5
Ampicillin	586 ± 53	157 ± 11	0.27	590 ± 57	267 ± 8.1	0.45	0.60
Aztreonam	NH	NH	NH	NH	NH	NH	NH
Cefepime	81 ± 5.5	7.5 ± 1.76	0.092	102 ± 7.9	11 ± 2.8	0.11	0.83
Cefotaxime	11 ± 2.5	11 ± 3.9	1.00	22 ± 5.4	21 ± 5.9	0.95	1.05
Cefoxitin	23 ± 3.1	5.2 ± 0.04	0.23	45 ± 0.81	6.6 ± 0.47	0.15	1.53
Ceftazidime	82 ± 8.6	10 ± 1.1	0.12	155 ± 16	21 ± 0.76	0.14	0.86
Ertapenem	237 ± 25	49 ± 2.6	0.21	571 ± 20	120 ± 7.5	0.21	1.00
Imipenem	188 ± 0.28	79 ± 2.5	0.42	396 ± 4.3	148 ± 0.64	0.37	1.14
Meropenem	453 ± 33	127 ± 15	0.28	659 ± 36	222 ± 48	0.34	0.82
Penicillin G	365 ± 33	115 ± 13	0.32	660 ± 21	93 ± 16	0.14	2.29

[a]The proteins were initially modified with a His tag, which was removed after purification.
[b]K_m and k_{cat} values are means ± standard deviations from three independent experiments. NH denotes no hydrolysis under conditions with substrate concentrations of up to 1 mM and enzyme concentrations of up to 700 nM.

carbapenem. This result confirmed that the wild-type promoter was crucial for carbapenem resistance (20). In addition, all transformants were susceptible to aztreonam, colistin, and tigecycline, which was consistent with data from previous reports (20, 21). The resistance profiles of β-lactams tested for NDM-17 transformants [DH5α(pHSG398/NP-NDM-17) and DH5α(pHSG398/NDM-17)] were similar to those for the corresponding NDM-5 transformants; however, the MICs of ertapenem and meropenem for pHSG398/NP-NDM-17 were slightly higher (2-fold) than those for pHSG398/NP-NDM-5 (Table 1). Importantly, the MICs of cefepime, ertapenem, and imipenem for DH5α(pHSG398/NP-NDM-17) were 2-fold higher than those for DH5α(pHSG398/NP-NDM-1). Furthermore, DH5α(pHSG398/NP-NDM-17) showed a 4-fold elevation in the MIC for meropenem compared to that for DH5α(pHSG398/NP-NDM-1). These findings suggest that mutations outside the promoter region are responsible for the increased carbapenem resistance.

Expression and purification experiments showed that the NDM-17 and NDM-5 recombinant proteins were expressed at up to 90% purity, as evaluated by SDS-PAGE. Both NDM proteins were used to determine kinetic parameters, which revealed that NDM-17 and NDM-5 could hydrolyze all β-lactams tested, except for aztreonam (Table 2). NDM-17 had similar k_{cat}/K_m ratios for almost β-lactams tested against NDM-5, except for significantly higher k_{cat}/K_m ratios for cefoxitin and penicillin G and lower k_{cat}/K_m ratios for ampicillin. These results indicate that NDM-17 has enzymatic activity similar to that of NDM-5, which was previously reported to increase carbapenemase activity compared to NDM-1. Notably, the K_m of NDM-17 for all β-lactams tested was obviously lower than that of NDM-5, especially for ceftazidime, penicillin G, ertapenem, imipenem, and meropenem (Table 2). These results suggest that NDM-17 has a significantly higher affinity than NDM-5 for all β-lactams tested.

It is possible that the increased resistance and the higher enzyme activity of NDM-17 are conferred by the three amino acid substitutions (V88L, M154L, and E170K). The M154L substitution increases the carbapenemase activity of NDM-4 (M154L) (22), NDM-5 (V88L and M154L) (23–25), and NDM-7 (D130N and M154L) (20, 26), indicating that it may be responsible for the higher hydrolytic activity of NDM-17. NDM-4 and NDM-5 are identical except for the V88L substitution in NDM-5, and NDM-5 has lower k_{cat}/K_m values for imipenem and meropenem than does NDM-4 (27). This suggests that the V88L substitution might contribute to the decreased hydrolytic activity of NDM-5 toward carbapenems. NDM-17 shares the V88L and M154L substitutions with NDM-5, in addition to E170K. Our kinetic data showed that NDM-17 had a significantly higher affinity for all β-lactams tested and obviously increased catalytic efficiencies for cefoxitin and penicillin G. Thus, the E170K substitution should be responsible for the higher affinity and increased catalytic efficiencies of NDM-17. Interestingly, the D130G substitution increases carbapenemase activity, but NDM-8, which contains both the D130G

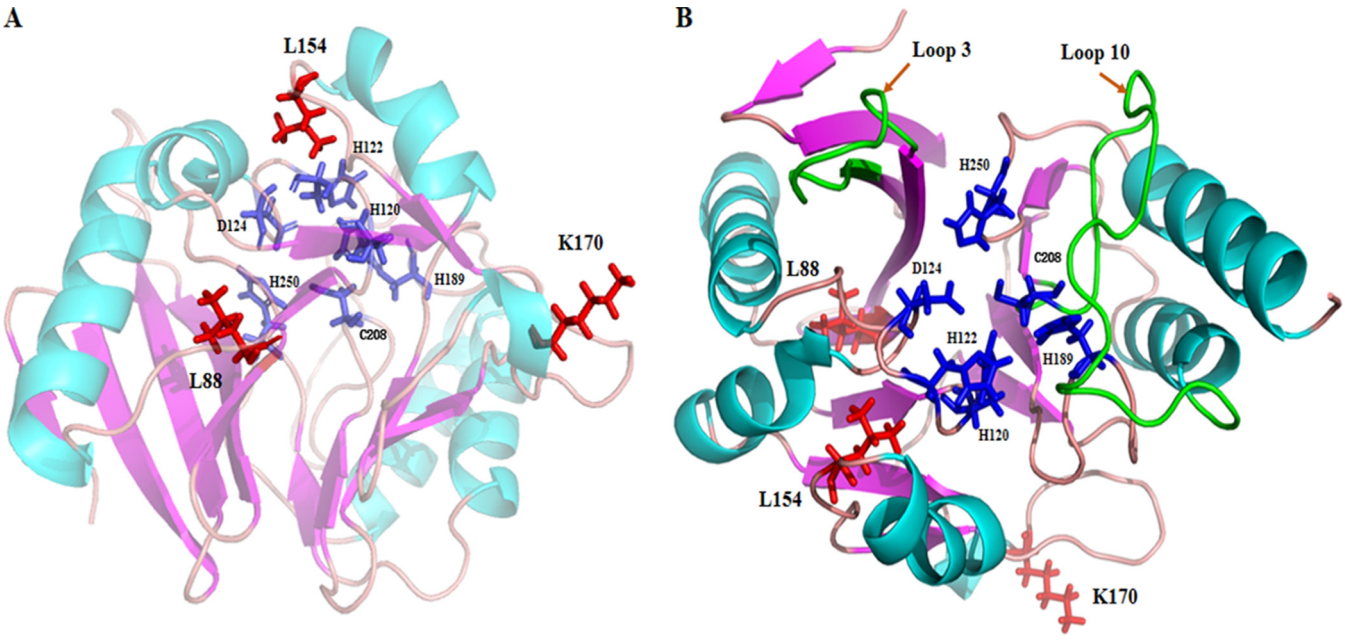

FIG 2 Homology model of NDM-17. (A) Protein backbone of NDM shown as a cartoon with the helices and strands. Amino acids binding to zinc ions (H120, H122, and H189 as well as D124, C208, and H250) and three amino acid substitutions (L88, L154, and K170) are labeled and shown in blue and red, respectively. (B) The three amino acid substitutions (red) were not located at the active sites (loop 3 [green] or loop 10 [green]) or near the amino acids binding to zinc ions (blue).

and M154L substitutions, does not exhibit increased hydrolytic activity for carbapenems (28). Thus, it is possible that certain amino acid substitutions may have different effects in different NDM variants, and the increased hydrolytic activity of NDM-17 was not the result of the cumulative effect of the individual V88L, M154L, and E170K amino acid substitutions but rather was a result of the overall interaction of the three substitutions.

To determine the locations of the three amino acid substitutions and analyze their effects on structure, a three-dimensional (3D) model of NDM-17 was generated by homology modeling using NDM-1 as a template (PDB accession no. 4EXS). The previously reported crystal structure of NDM-1 shows that the active site is formed by loops 3 and 10, at the bottom of a shallow groove, and amino acid triads that bind to zinc ions are formed by H120, H122, and H189 and by D124, C208, and H250 (29, 30). Currently, 16 amino acid substitutions have been reported in NDMs at 14 distinct amino acids at positions 28, 32, 36, 69, 74, 88, 95, 130, 152, 154, 200, 222, 233, and 264. E170K represents a new amino acid substitution and site, which was far from the active site and exposed to the solvent. Although positions 88, 154, and 170 are not located in the active site involved in binding to zinc ions (Fig. 2), they might still indirectly affect the formation of the active site, as was previously described (21).

Conclusions. In this study, a novel NDM variant, NDM-17, was identified in a ST48 *E. coli* strain isolated from a chicken. This is the first report of a new NDM variant being isolated from a food animal. NDM-17 displayed a higher affinity than NDM-5 for almost all β-lactams as well as carbapenem, as confirmed by kinetic parameters, and increased carbapenemase activity compared to that of NDM-1, as indicated by MICs. In addition, $bla_{\text{NDM-17}}$ was located on an IncX3 plasmid and was surrounded by multiple insertion sequences, mediating the rapid dissemination of bla_{NDM}. The transmission of strains carrying $bla_{\text{NDM-17}}$ to humans via the food chain represents a serious threat to human health and should be given further attention to ensure that NDM-17-producing pathogens are efficiently monitored.

MATERIALS AND METHODS

Identification and phenotypic characterization of the isolate. A routine annual surveillance of CRE of animal origin was performed to monitor their dissemination. *E. coli* AD-19R was isolated from a cloacal

swab taken from a chicken at a commercial poultry farm in Shandong Province, China, in 2015. The sample was plated directly onto CHROMagar KPC selective medium (CHROMagar, Paris, France), which selects for the growth of carbapenem-resistant *Enterobacteriaceae* (31). The bacterial species was identified by matrix-assisted laser desorption ionization–time of flight mass spectrometry (Bruker Daltonik, Bremen, Germany) and confirmed by 16S rRNA sequencing (32). The modified Hodge test, using imipenem and meropenem discs, was conducted to confirm the phenotype of carbapenemase production.

Antimicrobial susceptibility testing. The MICs of the original isolate (AD-19R), its transconjugants and transformants, and two reference isolates (*E. coli* strains YW carrying bla_{NDM-1} and DZ2-29R carrying bla_{NDM-5}) for several antimicrobials (listed in Table 1) were determined by using a broth microdilution method as recommended by the Clinical and Laboratory Standards Institute (33). *E. coli* ATCC 25922 was used as a quality control strain.

Detection of β-lactamase genes and whole-genome sequencing. Whole-cell DNA was extracted from isolate AD-19R by using a QIAamp minikit according to the manufacturer's recommendations (Qiagen, Hilden, Germany). PCR and DNA sequencing were conducted to screen for known β-lactamase genes (MBL genes bla_{DIM}, bla_{GIM}, bla_{IMP}, bla_{NDM}, bla_{SIM}, bla_{SPM}, and bla_{VIM}) as described previously (34). A 150-bp paired-end library was constructed according to the standard Illumina (San Diego, CA, USA) paired-end protocol, and the whole genome of *E. coli* AD-19R, including plasmid pAD-19R extracted from transformants, was sequenced on the Illumina HiSeq 2500 system. Results were analyzed by using CLC Genomics Workbench version 9.0 (CLC Bio, Aarhus, Denmark), and each predicted ORF was used as a query against the GenBank database of the National Center for Biotechnology Information using a BLAST search. Gaps in the sequence were closed by PCR and Sanger sequencing (35).

MLST, Southern blotting, transconjugation, and plasmid analysis. MLST was performed as described previously to identify the sequence type of isolate AD-19R (36). Southern blot analysis was used with specific bla_{NDM} digoxigenin-labeled probes to locate bla_{NDM} genes. Transconjugation assays were used to evaluate the horizontal transferability of bla_{NDM}, with *E. coli* J53 as the recipient and isolate AD-19R as the donor. The transconjugants were selected on MacConkey agar containing 100 mg/liter sodium azide and 1 mg/liter meropenem, and the transfer frequency was calculated by transconjugants/donors. PCR with specific primers was used to confirm the presumptive transconjugants (22). Plasmid incompatibility groups were determined by two PCR-based replicon typing methods (37, 38).

Cloning of bla_{NDM-17}, bla_{NDM-5}, and bla_{NDM-1}. To compare the beta-lactamase activities of both NDM-1 and NDM-5 with that of NDM-17, the respective genes (bla_{NDM-1}, bla_{NDM-5}, and bla_{NDM-17}) with their native promoters were amplified by PCR using primers NP-NDM-F (5′-CGGGATCCCACCTCATGTTT GAATTCGC-3′) and NP-NDM-R (5′-CCCAAGCTTCTCTGTCACATCGAAATCGC-3′) and cloned into the pHSG398 vector (TaKaRa Bio, Dalian, China). The resulting plasmids were named pHSG398/NP-NDM-1, pHSG398/NP-NDM-5, and pHSG398/NP-NDM-17, respectively. The complete bla_{NDM-1}, bla_{NDM-5}, and bla_{NDM-17} ORFs were obtained by PCR using primers NDM-F (5′-CGGGATCCATGGAATTGCCCAATATT ATG-3′) and NDM-R (5′-CCCAAGCTTTCAGCGCAGCTTGTCGGCCAT-3′), cloned into pHSG398, and named pHSG398/NDM-1, pHSG398/NDM-5, and pHSG398/NDM-17, respectively. Subsequently, pHSG398/NP-NDM-1, pHSG398/NP-NDM-5, pHSG398/NP-NDM-17, pHSG398/NDM-1, pHSG398/NDM-5, and pHSG398/NDM-17 were transformed into *E. coli* DH5α by electrotransformation and confirmed by PCR and DNA sequencing (20, 21).

Expression and purification of NDM-17 and NDM-5. The ORFs coding for NDM-5 and NDM-17 without signal peptide regions were amplified by using primers BamHI-TEV-NDM-F (5′-ATGGATCCGAA AACCTGTATTTCCAAGGCCAGCAAATGGAAACTGGCGAC-3′) and XhoI-NDM-R (5′-ATCTCGAGTCAGCGCAG CTTGTCGGCCATG-3′) and then cloned into the pET28a expression vector (Merck Millipore, Danvers, MA, USA). The resulting plasmid was transformed into *E. coli* BL21(DE3) according to the manufacturer's instructions (TransGen Biotech, Beijing, China). Ni-nitrilotriacetic acid (NTA) agarose was used to purify the recombinant NDM proteins according to the manufacturer's instructions (Qiagen, Hilden, Germany). His tags were removed by cleavage with Turbo tobacco etch virus (TEV) protease (Accelagen, San Diego, CA, USA), and untagged proteins were purified by an additional passage in Ni-NTA agarose. The purity of the recombinant NDM proteins was estimated by SDS-PAGE, and the protein concentration was measured by using a Pierce bicinchoninic acid protein assay kit (Thermo Scientific, Waltham, MA, USA). β-Lactamase activity was monitored with nitrocefin (Oxoid Ltd., Basingstoke, United Kingdom) during the purification procedure, according to the manufacturer's instructions.

Determination of kinetic parameters. A kinetic study was conducted to measure β-lactamase activity and compare the catalytic properties of NDM-17 and NDM-5. Initial hydrolysis rates were determined with 50 mM phosphate buffer (pH 7.0) containing 30 μM Zn^{2+} at 25°C (39), using a SpectraMax M5 multidetection microplate reader (Molecular Devices, Sunnyvale, CA, USA). The K_m and k_{cat} values and the k_{cat}/K_m ratio were determined from three individual experiments using wavelengths and extinction coefficients as previously described (40, 41) and by constructing a Lineweaver-Burk plot.

Accession number(s). The sequence of the novel NDM variant gene has been deposited in GenBank under accession no. KX812714 and was designated bla_{NDM-17}. The complete nucleotide sequence of plasmid pAD-19R has been deposited in GenBank under accession no. KX833071.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.02233-16.

SUPPLEMENTAL FILE 1, PDF file, 0.2 MB.

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We declare that we have no competing financial interests.

Jianzhong Shen designed the study. Zhihai Liu, Dejun Liu, Rongmin Zhang, Jiyun Li, and Wenjuan Yin collected the data. Zhihai Liu, Yang Wang, Zhangqi Shen, Timothy R. Walsh, and Hong Yao analyzed and interpreted the data. Zhihai Liu, Yang Wang, Timothy R. Walsh, and Jianzhong Shen wrote the report. All authors revised, reviewed, and approved the final report.

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ERRATUM

Erratum for Liu et al., "Plasmid-Mediated Novel bla_{NDM-17} Gene Encoding a Carbapenemase with Enhanced Activity in a Sequence Type 48 *Escherichia coli* Strain"

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