




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

Zhihai Liu,^a Yang Wang,^a Timothy R. Walsh,^b Dejun Liu,^a Zhangqi Shen,^a Rongmin Zhang,^a Wenjuan Yin,^a Hong Yao,^a  Jiyun Li,^a Jianzhong Shen^a

Beijing Advanced Innovation Center for Food Nutrition and Human Health, College of Veterinary Medicine, China Agricultural University, Beijing, China^a; Department of Medical Microbiology and Infectious Disease, Cardiff Institute of Infection & Immunity, Heath Park Hospital, Cardiff, United Kingdom^b

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

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-

Received 21 October 2016 Returned for modification 25 November 2016 Accepted 26 January 2017

Accepted manuscript posted online 27 February 2017

Citation Liu Z, Wang Y, Walsh TR, Liu D, Shen Z, Zhang R, Yin W, Yao H, Li J, Shen J. 2017. Plasmid-mediated novel *bla*_{NDM-17} gene encoding a carbapenemase with enhanced activity in a sequence type 48 *Escherichia coli* strain. *Antimicrob Agents Chemother* 61:e02233-16. <https://doi.org/10.1128/AAC.02233-16>.

Copyright © 2017 American Society for Microbiology. All Rights Reserved.

Address correspondence to Jianzhong Shen, sjz@cau.edu.cn.

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](https://www.ncbi.nlm.nih.gov/nuccore/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](https://www.ncbi.nlm.nih.gov/nuccore/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](https://www.ncbi.nlm.nih.gov/nuccore/CP014006)) isolated in China, pNDM_MGR194 (GenBank accession no. [KF220657](https://www.ncbi.nlm.nih.gov/nuccore/KF220657)) from *K. pneumoniae* isolated in India (12), pEc1929 (GenBank accession no. [KT824791](https://www.ncbi.nlm.nih.gov/nuccore/KT824791)) from *E. coli* isolated in China (16), pJEG027 (GenBank accession no. [KF220657](https://www.ncbi.nlm.nih.gov/nuccore/KF220657)) from *K. pneumoniae* isolated in Australia (17), and pKpN01-NDM7 (GenBank accession no. [CP012990](https://www.ncbi.nlm.nih.gov/nuccore/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 ($\mu\text{g/ml}$)									
	AD19 (NDM-17)	AD19/J53	J53	DH5 α (pHSG398)	DH5 α (pHSG398/NDM-1)	DH5 α (pHSG398/NDM-5)	DH5 α (pHSG398/NDM-17)	DH5 α (pHSG398/NP-NDM-1)	DH5 α (pHSG398/NP-NDM-5)	DH5 α (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	0.063	0.063	0.063	0.032	0.063
Amikacin	>256	2	2	0.5	0.5	0.5	0.5	0.5	0.25	1
Cefepime	>256	>256	0.063	0.032	2	4	2	8	16	16
Cefotaxime	>256	>256	0.125	0.063	64	32	32	128	128	128
Cefoxitin	>256	>256	8	4	>256	>256	>256	>256	>256	>256
Ceftazidime	>256	>256	0.5	0.25	>256	>256	>256	>256	>256	>256
Ciprofloxacin	16	\leq 0.008	\leq 0.008	0.016	0.016	\leq 0.008	0.008	0.016	0.008	0.016
Colistin	1	0.5	0.5	0.125	0.125	0.125	0.125	0.125	\leq 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	16	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	\geq 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

^aSXT, trimethoprim-sulfamethoxazole; 1/19 is the ratio of trimethoprim/sulfamethoxazole concentrations.

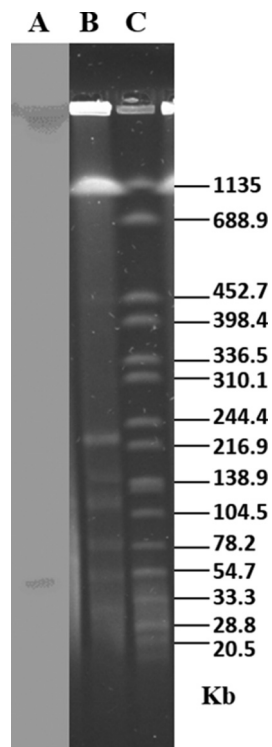


FIG 1 Location of *bla*_{NDM-17} on *E. coli* plasmid pAD-19R separated by PFGE. (A) Hybridization of the plasmid with a probe specific for *bla*_{NDM-17}; (B) plasmid from transconjugant AD19/J53; (C) Reference standard strain H9812 restricted by XbaI.

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, Tn3-IS3000-ΔISAba125-IS5-*bla*_{NDM-17}-*ble*_{MBL}-*trpF*-*dsbC*-IS26-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 cepheems but were susceptible to

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

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

^aThe proteins were initially modified with a His tag, which was removed after purification.

^b K_m and k_{cat} values are means \pm 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 [DH5 α (pHSG398/NP-NDM-17) and DH5 α (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 DH5 α (pHSG398/NP-NDM-17) were 2-fold higher than those for DH5 α (pHSG398/NP-NDM-1). Furthermore, DH5 α (pHSG398/NP-NDM-17) showed a 4-fold elevation in the MIC for meropenem compared to that for DH5 α (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

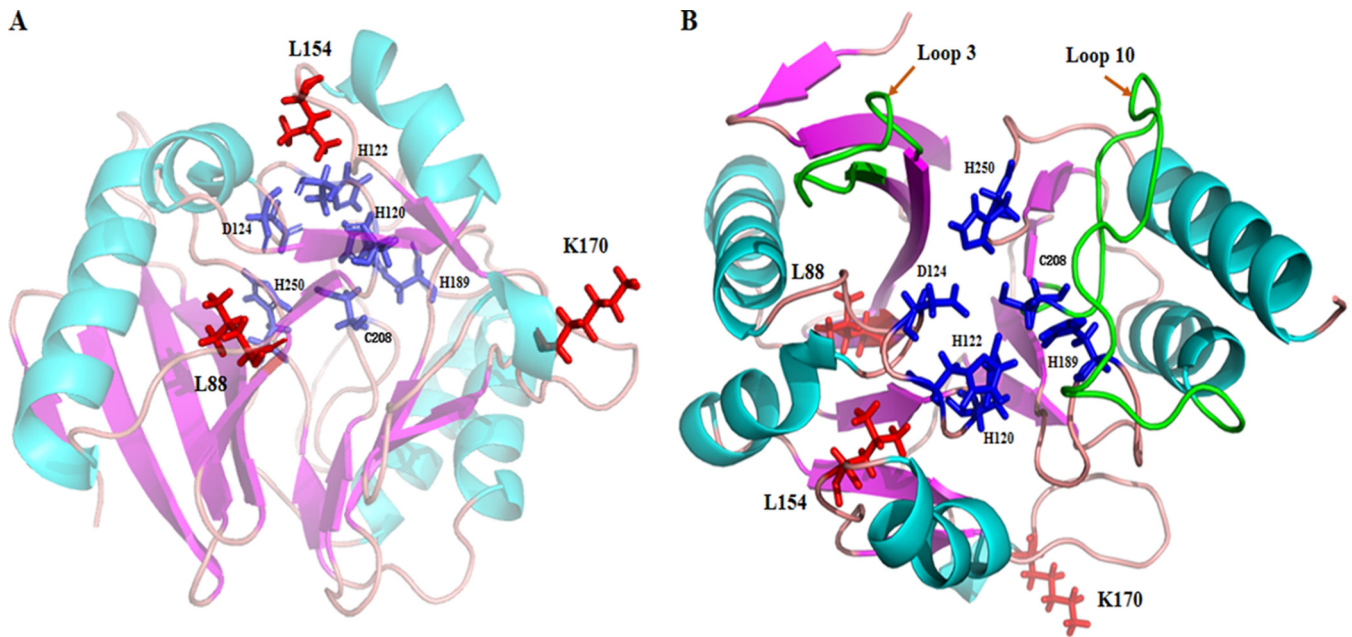


FIG 2 Homology model of NDM-17. (A) Protein backbone of NDM shown as a cartoon with the helices and strands. Amino acids binding to zinc ions (H120, H122, and H189) as well as D124, C208, and H250) and three amino acid substitutions (L88, L154, and K170) are labeled and shown in blue and red, respectively. (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*_{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*_{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'-CCCAAGCTTCTCTGTACATCGAAATCGC-3') and cloned into the pHS398 vector (TaKaRa Bio, Dalian, China). The resulting plasmids were named pHS398/NP-NDM-1, pHS398/NP-NDM-5, and pHS398/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'-CGGGATCCATGGAATTGCCAATATT ATG-3') and NDM-R (5'-CCCAAGCTTTCAGCGCAGCTTGTCCGCAT-3'), cloned into pHS398, and named pHS398/NDM-1, pHS398/NDM-5, and pHS398/NDM-17, respectively. Subsequently, pHS398/NP-NDM-1, pHS398/NP-NDM-5, pHS398/NP-NDM-17, pHS398/NDM-1, pHS398/NDM-5, and pHS398/NDM-17 were transformed into *E. coli* DH5 α 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 AACCTGTATTTCAAGGCCAGCAATGGAAGCTGGCGAC-3') and XhoI-NDM-R (5'-ATCTCGAGTCAGCGCAG CTTGTCCGCATG-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 μ M Zn²⁺ 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](https://doi.org/10.1128/AAC.02233-16) and was designated bla_{NDM-17} . The complete nucleotide sequence of plasmid pAD-19R has been deposited in GenBank under accession no. [KX833071](https://doi.org/10.1128/AAC.02233-16).

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.

ACKNOWLEDGMENTS

This study was supported by grants from the National Natural Science Foundation of China (31530076, 31422055, and 81661138002) and the National Basic Research Program of China (2013CB127200).

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.

REFERENCES

- Wu W, Espedido B, Feng Y, Zong Z. 2016. *Citrobacter freundii* carrying bla_{KPC-2} and bla_{NDM-1} : characterization by whole genome sequencing. *Sci Rep* 6:30670. <https://doi.org/10.1038/srep30670>.
- Temkin E, Adler A, Lerner A, Carmeli Y. 2014. Carbapenem-resistant Enterobacteriaceae: biology, epidemiology, and management. *Ann N Y Acad Sci* 1323:22–42. <https://doi.org/10.1111/nyas.12537>.
- Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F, Balakrishnan R, Chaudhary U, Doumith M, Giske CG, Irfan S. 2010. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. *Lancet Infect Dis* 10: 597–602. [https://doi.org/10.1016/S1473-3099\(10\)70143-2](https://doi.org/10.1016/S1473-3099(10)70143-2).
- Feng Y, Yang P, Xie Y, Wang X, McNally A, Zong Z. 2015. *Escherichia coli* of sequence type 3835 carrying bla_{NDM-1} , $bla_{CTX-M-15}$, bla_{CMY-42} and bla_{SHV-12} . *Sci Rep* 5:12275. <https://doi.org/10.1038/srep12275>.
- Yong D, Toleman MA, Giske CG, Cho HS, Sundman K, Lee K, Walsh TR. 2009. Characterization of a new metallo- β -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* 53:5046–5054. <https://doi.org/10.1128/AAC.00774-09>.
- Wei WJ, Yang HF, Ye Y, Li JB. 2015. New Delhi metallo- β -lactamase mediated carbapenem resistance: origin, diagnosis, treatment and public health concern. *Chin Med J (Engl)* 128:1969–1976. <https://doi.org/10.4103/0366-6999.160566>.
- Wang Y, Wu C, Zhang Q, Qi J, Liu H, Wang Y, He T, Ma L, Lai J, Shen Z. 2012. Identification of New Delhi metallo- β -lactamase 1 in *Acinetobacter lwoffii* of food animal origin. *PLoS One* 7:e37152. <https://doi.org/10.1371/journal.pone.0037152>.
- Zhang R, Wang Y, Liu Z, Li J, Yin W, Lei L, Wu C, Shen J. 2015. Characterization of NDM-1-producing carbapenemase in *Acinetobacter* spp. and *E. coli* isolates from diseased pigs. *Front Agr Sci Eng* 2:223. <https://doi.org/10.15302/J-FASE-2015065>.
- Smet A, Martel A, Persoons D, Dewulf J, Heyndrickx M, Claeys G, Lontie M, Van Meensel B, Herman L, Haesebrouck F. 2010. Characterization of extended-spectrum β -lactamases produced by *Escherichia coli* isolated from hospitalized and nonhospitalized patients: emergence of CTX-M-15-producing strains causing urinary tract infections. *Microb Drug Resist* 16:129–134. <https://doi.org/10.1089/mdr.2009.0132>.
- Wang Y, Zhang R, Li J, Wu Z, Yin W, Schwarz S, Tyrrell JM, Zheng Y, Wang S, Shen Z, Liu Z, Liu J, Lei L, Li M, Zhang Q, Wu C, Zhang Q, Wu Y, Walsh TR, Shen J. 2017. Comprehensive resistome analysis reveals the prevalence of NDM and MCR-1 in Chinese poultry production. *Nat Microbiol* 2:16260. <https://doi.org/10.1038/nmicrobiol.2016.260>.
- Ho P, Li Z, Lo W, Cheung Y, Lin C, Sham P, Cheng VC, Ng T, Que T, Chow K. 2012. Identification and characterization of a novel incompatibility group X3 plasmid carrying bla_{NDM-1} in Enterobacteriaceae isolates with epidemiological links to multiple geographical areas in China. *Emerg Microbes Infect* 1:e39. <https://doi.org/10.1038/emi.2012.37>.
- Krishnaraju M, Kamatchi C, Jha AK, Devasena N, Vennila R, Sumathi G, Vaidyanathan R. 2015. Complete sequencing of an IncX3 plasmid carrying bla_{NDM-5} allele reveals an early stage in the dissemination of the bla_{NDM} gene. *Indian J Med Microbiol* 33:30–38. <https://doi.org/10.4103/0255-0857.148373>.
- Yang P, Xie Y, Feng P, Zong Z. 2014. bla_{NDM-5} carried by an IncX3 plasmid in *Escherichia coli* sequence type 167. *Antimicrob Agents Chemother* 58:7548–7552. <https://doi.org/10.1128/AAC.03911-14>.
- Zhang F, Xie L, Wang X, Han L, Guo X, Ni Y, Qu H, Sun J. 2016. Further spread of bla_{NDM-5} in Enterobacteriaceae via IncX3 plasmids in Shanghai, China. *Front Microbiol* 7:424. <https://doi.org/10.3389/fmicb.2016.00424>.
- Du H, Chen L, Tang Y, Kreiswirth BN. 2016. Emergence of the mcr-1 colistin resistance gene in carbapenem-resistant Enterobacteriaceae. *Lancet Infect Dis* 16:287–288. [https://doi.org/10.1016/S1473-3099\(16\)00056-6](https://doi.org/10.1016/S1473-3099(16)00056-6).
- Chen D, Gong L, Walsh TR, Lan R, Wang T, Zhang J, Mai W, Ni N, Lu J, Xu J. 2016. Infection by and dissemination of NDM-5-producing *Escherichia coli* in China. *J Antimicrob Chemother* 71:563–565. <https://doi.org/10.1093/jac/dkv352>.
- Espedido BA, Dimitrijević B, van Hal SJ, Jensen SO. 2015. The use of whole-genome sequencing for molecular epidemiology and antimicrobial surveillance: identifying the role of IncX3 plasmids and the spread of bla_{NDM-4} -like genes in the Enterobacteriaceae. *J Clin Pathol* 68:835–838. <https://doi.org/10.1136/jclinpath-2015-203044>.
- Lynch T, Chen L, Peirano G, Gregson DB, Church DL, Conly J, Kreiswirth BN, Pitout JD. 2016. Molecular evolution of *Klebsiella pneumoniae* ST278 harboring bla_{NDM-7} involved in nosocomial transmission. *J Infect Dis* 214:798–806. <https://doi.org/10.1093/infdis/jiw240>.
- Wang X, Xu X, Li Z, Chen H, Wang Q, Yang P, Zhao C, Ni M, Wang H. 2014. An outbreak of a nosocomial NDM-1-producing *Klebsiella pneumoniae* ST147 at a teaching hospital in mainland China. *Microb Drug Resist* 20:144–149. <https://doi.org/10.1089/mdr.2013.0100>.
- Gottig S, Hamprecht AG, Christ S, Kempf VAJ, Wichelhaus TA. 2013. Detection of NDM-7 in Germany, a new variant of the New Delhi metallo- β -lactamase with increased carbapenemase activity. *J Antimicrob Chemother* 68:1737–1740. <https://doi.org/10.1093/jac/dkt088>.
- Zou D, Huang Y, Zhao X, Liu W, Dong D, Li H, Wang X, Huang S, Wei X, Yan X, Yang Z, Tong Y, Huang L, Yuan J. 2015. A novel New Delhi metallo- β -lactamase variant, NDM-14, isolated in a Chinese hospital possesses increased enzymatic activity against carbapenems. *Antimicrob Agents Chemother* 59:2450–2453. <https://doi.org/10.1128/AAC.05168-14>.
- Nordmann P, Boulanger AE, Poirel L. 2012. NDM-4 metallo-beta-lactamase with increased carbapenemase activity from *Escherichia coli*. *Antimicrob Agents Chemother* 56:2184–2186. <https://doi.org/10.1128/AAC.05961-11>.
- Hornsey M, Phee L, Wareham DW. 2011. A novel variant, NDM-5, of the New Delhi metallo- β -lactamase in a multidrug-resistant *Escherichia coli* ST648 isolate recovered from a patient in the United Kingdom. *Antimicrob Agents Chemother* 55:5952–5954. <https://doi.org/10.1128/AAC.05108-11>.
- Zhu Y, Zhao J, Xu C, Zhao H, Jia N, Li Y. 2016. Identification of an NDM-5-producing *Escherichia coli* sequence type 167 in a neonatal patient in China. *Sci Rep* 6:29934. <https://doi.org/10.1038/srep29934>.
- Galal D, Fernández-Martínez M, El-Defrawy I, Ocampo-Sosa AA, Martínez-Martínez L. 2016. First identification of NDM-5 associated with OXA-181 in *Escherichia coli* from Egypt. *Emerg Microbes Infect* 5:e30. <https://doi.org/10.1038/emi.2016.24>.
- Cuzon G, Bonnin RA, Normann P. 2013. First identification of novel NDM carbapenemase, NDM-7, in *Escherichia coli* in France. *PLoS One* 8:e61322. <https://doi.org/10.1371/journal.pone.0061322>.
- Makena A, Brem J, Pfeffer I, Geffen REJ, Wilkins SE, Tarhonskaya H, Flashman E, Phee LM, Wareham DW, Schofield CJ. 2015. Biochemical characterization of New Delhi metallo-beta-lactamase variants reveals differences in protein stability. *J Antimicrob Chemother* 70:463–469. <https://doi.org/10.1093/jac/dku403>.

28. Tada T, Miyoshi-Akiyama T, Dahal RK, Sah MK, Ohara H, Kirikae T, Pokhrel BM. 2013. NDM-8 metallo- β -lactamase in a multidrug-resistant *Escherichia coli* strain isolated in Nepal. *Antimicrob Agents Chemother* 57:2394–2396. <https://doi.org/10.1128/AAC.02553-12>.
29. Green VL, Verma A, Owens RJ, Phillips SEV, Carr SB. 2011. Structure of New Delhi metallo- β -lactamase 1 (NDM-1). *Acta Crystallogr Sect F Struct Biol Cryst Commun* 67:1160–1164. <https://doi.org/10.1107/S1744309111029654>.
30. Zhang H, Hao Q. 2011. Crystal structure of NDM-1 reveals a common beta-lactam hydrolysis mechanism. *FASEB J* 25:2574–2582. <https://doi.org/10.1096/fj.11-184036>.
31. Samra Z, Bahar J, Madar-Shapiro L, Aziz N, Israel S, Bishara J. 2008. Evaluation of CHROMagar KPC for rapid detection of carbapenem-resistant *Enterobacteriaceae*. *J Clin Microbiol* 46:3110–3111. <https://doi.org/10.1128/JCM.00249-08>.
32. Suzuki MT, Taylor LT, DeLong EF. 2000. Quantitative analysis of small-subunit rRNA genes in mixed microbial populations via 5'-nuclease assays. *Appl Environ Microbiol* 66:4605–4614. <https://doi.org/10.1128/AEM.66.11.4605-4614.2000>.
33. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; 25th informational supplement, M100-S25. Clinical and Laboratory Standards Institute, Wayne, PA.
34. Nordmann P, Naas T, Poirel L. 2011. Global spread of carbapenemase-producing *Enterobacteriaceae*. *Emerg Infect Dis* 17:1791–1798. <https://doi.org/10.3201/eid1710.110655>.
35. Li D, Wang Y, Schwarz S, Cai J, Fan R, Li J, Feßler AT, Zhang R, Wu C, Shen J. 2016. Co-location of the oxazolidinone resistance genes *oprA* and *cfr* on a multiresistance plasmid from *Staphylococcus sciuri*. *J Antimicrob Chemother* 71:1474–1478. <https://doi.org/10.1093/jac/dkw040>.
36. Wirth T, Falush D, Lan R, Colles F, Mensa P, Wieler LH, Karch H, Reeves PR, Maiden MCJ, Ochman H, Achtman M. 2006. Sex and virulence in *Escherichia coli*: an evolutionary perspective. *Mol Microbiol* 60:1136–1151. <https://doi.org/10.1111/j.1365-2958.2006.05172.x>.
37. Johnson TJ, Bielak EM, Fortini D, Hansen LH, Hasman H, Debroy C, Nolan LK, Carattoli A. 2012. Expansion of the IncX plasmid family for improved identification and typing of novel plasmids in drug-resistant *Enterobacteriaceae*. *Plasmid* 68:43–50. <https://doi.org/10.1016/j.plasmid.2012.03.001>.
38. Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, Threlfall EJ. 2005. Identification of plasmids by PCR-based replicon typing. *J Microbiol Methods* 63:219–228. <https://doi.org/10.1016/j.mimet.2005.03.018>.
39. Li T, Wang Q, Chen F, Li X, Luo S, Fang H, Wang D, Li Z, Hou X, Wang H. 2013. Biochemical characteristics of New Delhi metallo- β -lactamase-1 show unexpected difference to other MBLs. *PLoS One* 8:e61914. <https://doi.org/10.1371/journal.pone.0061914>.
40. Crowder MW, Walsh TR, Banovic L, Pettit M, Spencer J. 1998. Overexpression, purification, and characterization of the cloned metallo- β -lactamase L1 from *Stenotrophomonas maltophilia*. *Antimicrob Agents Chemother* 42:921–926.
41. Queenan AM, Shang W, Flamm R, Bush K. 2010. Hydrolysis and inhibition profiles of β -lactamases from molecular classes A to D with doripenem, imipenem, and meropenem. *Antimicrob Agents Chemother* 54:565–569. <https://doi.org/10.1128/AAC.01004-09>.



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”

Zhihai Liu,^a Yang Wang,^a Timothy R. Walsh,^b Dejun Liu,^a Zhangqi Shen,^a Rongmin Zhang,^a Wenjuan Yin,^a Hong Yao,^a Jiyun Li,^a Jianzhong Shen^a

Beijing Advanced Innovation Center for Food Nutrition and Human Health, College of Veterinary Medicine, China Agricultural University, Beijing, China^a; Department of Medical Microbiology and Infectious Disease, Cardiff Institute of Infection & Immunity, Heath Park Hospital, Cardiff, United Kingdom^b

Volume 61, no. 5, e02233-16, 2017, <https://doi.org/10.1128/AAC.02233-16>. Page 8, Acknowledgments, line 2: The first grant number from the National Natural Science Foundation of China should be 31530076 instead of 31370046.

Citation Liu Z, Wang Y, Walsh TR, Liu D, Shen Z, Zhang R, Yin W, Yao H, Li J, Shen J. 2018. 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.” *Antimicrob Agents Chemother* 62:e02467-17. <https://doi.org/10.1128/AAC.02467-17>.

Copyright © 2018 American Society for Microbiology. All Rights Reserved.