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17 **ABSTRACT**

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

## 36 INTRODUCTION

37 Carbapenem-resistant Enterobacteriaceae (CRE) have been recognized as an urgent  
38 antibiotic resistance threat by the Centers for Disease Control in the US, and have  
39 become a global problem in recent years (1). The resistance exhibited by CRE is largely  
40 mediated by the production of carbapenemases (2), especially metallo- $\beta$ -lactamases  
41 (MBLs) such as VIM, IMP, and New Delhi metallo- $\beta$ -lactamase (NDM), which can  
42 hydrolyze almost all carbapenem  $\beta$ -lactams (3). Since its discovery in India in 2008,  
43 NDM has been identified throughout the world, and its identification in China has  
44 become common (4, 5). Currently, there are 16 NDM variants ([www.lahey.org/studies](http://www.lahey.org/studies)),  
45 with amino acid substitutions at 14 positions. The evolution and spread of NDM are  
46 rapid, and NDM-positive bacteria are found in the wider community environment, not  
47 just hospitals (6). The spread of NDM-positive bacteria depends on fecal-oral  
48 transmission, and an important route for this transmission is animal-derived food (6). The  
49 importance of minimizing the carriage of NDM-positive bacteria by food animals for  
50 public health was underlined by the discovery of non-human sources of NDM (7,8). In  
51 comparison with the high prevalence of NDM-positive Enterobacteriaceae of human  
52 origin, there are few reports on CRE from food animals. Furthermore, none of the novel  
53 NDM variants described to date were originally identified in bacteria isolated from food  
54 animals. Here we describe the characterization of a novel NDM variant in *Escherichia*  
55 *coli* isolated from a chicken.

## 56 MATERIALS AND METHODS

### 57 Identification and phenotypic characterization of the isolate.

58 A route annual surveillance of CRE of animal origin was performed to monitor its  
59 dissemination. *E. coli* AD-19R was isolated from a cloacal swab taken from a chicken at  
60 a commercial poultry farm in Shandong Province, China, in 2015. The sample was plated  
61 directly onto CHROMagar KPC selective medium (CHROMagar, Paris, France), which  
62 selects for the growth of carbapenem-resistant Enterobacteriaceae (9). The bacterial  
63 species was identified by matrix-assisted laser desorption/ionization-time of flight mass  
64 spectrometry (Bruker Daltonik, Bremen, Germany), and confirmed by 16S rRNA  
65 sequencing (10). The modified Hodge test, using imipenem and meropenem discs, was  
66 conducted to confirm the phenotype of carbapenemase production.

### 67 Antimicrobial susceptibility testing.

68 The MICs of the original isolate (AD-19R), its transconjugants and transformants,  
69 and two reference isolates (*E. coli* strain YW carrying *bla*<sub>NDM-1</sub> and DZ2-29R carrying  
70 *bla*<sub>NDM-5</sub>) to several antimicrobials (listed in Table 1) were determined using a broth  
71 microdilution method as recommended by the Clinical and Laboratory Standards  
72 Institute (11). The *E. coli* ATCC 25922 was used as a quality control strain.

### 73 Detection of $\beta$ -lactamase genes and whole-genome sequencing.

74 Whole-cell DNA was extracted from isolates AD-19R using a QiaAmp Mini kit  
75 according to the manufacturer's recommendations (Qiagen, Hilden, Germany). PCR and

76 DNA sequencing were conducted to screen for known  $\beta$ -lactamase genes (MBL genes  
77 *bla*<sub>DIM</sub>, *bla*<sub>GIM</sub>, *bla*<sub>IMP</sub>, *bla*<sub>NDM</sub>, *bla*<sub>SIM</sub>, *bla*<sub>SPM</sub>, and *bla*<sub>VIM</sub>) as described previously (12). A  
78 150-bp paired-end library was constructed following the standard Illumina (San Diego,  
79 CA, USA) paired-end protocol, and the whole genome of *E. coli* AD-19R, including  
80 plasmid pAD-19R extracted from transformants, was sequenced on the Illumina HiSeq  
81 2500 system. Results were analyzed using CLC Genomics Workbench version 9.0 (CLC  
82 bio, Aarhus, Denmark), and each predicted ORF was used as a query against the  
83 GenBank database of the National Center for Biotechnology Information using a BLAST  
84 search. Gaps in the sequence were closed by PCR and Sanger sequencing (13).

#### 85 **MLST, Southern blotting, transconjugation, and plasmid analysis.**

86 MLST was performed as described previously to identify the sequence type of  
87 isolate AD-19R (14). Southern blot analysis was used with specific *bla*<sub>NDM</sub>  
88 digoxigenin-labeled probes to locate *bla*<sub>NDM</sub> genes. Transconjugation assays were used to  
89 evaluate the horizontal transferability of *bla*<sub>NDM</sub>, with *E. coli* J53 as the recipient, and  
90 isolate AD-19R as the donor. The transconjugants were selected on MacConkey agar  
91 containing 100 mg/l sodium azide and 1 mg/l meropenem and the transfer frequency was  
92 calculated by transconjugants/donors. PCR with specific primers was used to confirm the  
93 presumptive transconjugants (15). Plasmid incompatibility groups were determined by  
94 two PCR-based replicon typing methods (16,17).

95 **Cloning of *bla*<sub>NDM-17</sub>, *bla*<sub>NDM-5</sub> and *bla*<sub>NDM-1</sub>.**

96 To compare the beta-lactamase activities of both NDM-1 and NDM-5 with NDM-17,  
97 the respective genes (*bla*<sub>NDM-1</sub>, *bla*<sub>NDM-5</sub> and *bla*<sub>NDM-17</sub>) with their native promoters were  
98 amplified by PCR using primers NP-NDM-F  
99 (5'-CGGGATCCCACCTCATGTTTGAATTCGC-3') and NP-NDM-R  
100 (5'-CCCAAGCTTCTCTGTTCACATCGAAATCGC-3'), and cloned into the pHSG398  
101 vector (Takara Bio, Dalian, China). The resulting plasmids were named  
102 pHSG398/NP-NDM-1, pHSG398/NP-NDM-5 and pHSG398/NP-NDM-17, respectively.  
103 The complete *bla*<sub>NDM-1</sub>, *bla*<sub>NDM-5</sub> and *bla*<sub>NDM-17</sub> ORFs were obtained by PCR using  
104 primers NDM-F (5'-CGGGATCCATGGAATTGCCCAATATTATG-3') and NDM-R  
105 (5'-CCCAAGCTTTTCAGCGCAGCTTGTCGGCCAT-3'), cloned into pHSG398, and  
106 named pHSG398/NDM-1, pHSG398/NDM-5 and pHSG398/NDM-17, respectively.  
107 Subsequently, pHSG398/NP-NDM-1, pHSG398/NP-NDM-5, pHSG398/NP-NDM-17,  
108 pHSG398/NDM-1, pHSG398/NDM-5 and pHSG398/NDM-17 were transformed into *E.*  
109 *coli* DH5 $\alpha$  by electrotransformation, and confirmed by PCR and DNA sequencing  
110 (18,19).

111 **Expression and purification of NDM-17 and NDM-5.**

112 The ORFs coding for NDM-5 and NDM-17 without signal peptide regions were  
113 amplified using primers BamHI-TEV-NDM-F  
114 (5'-ATGGATCCGAAAACCTGTATTTCGAAGGCCAGCAAATGGAACTGGCGAC-



115 3') and XhoI-NDM-R (5'ATCTCGAGTCAGCGCAGCTTGTCGGCCATG-3') and then  
116 cloned into the pET28a expression vector (Merck Millipore, Danvers, MA, USA). The  
117 resulting plasmid was transformed into *E. coli* BL21 (DE3) as per the manufacturer's  
118 instructions (TransGen Biotech, Beijing, China). Ni-nitrilotriacetic acid (NTA) agarose  
119 was used to purify the recombinant NDM proteins according to the manufacturer's  
120 instructions (Qiagen, Hilden, Germany). His tags were removed by cleaving with Turbo  
121 TEV protease (Accelagen, San Diego, CA, USA), and untagged proteins were purified by  
122 an additional passage in Ni-NTA agarose. The purity of the recombinant NDM proteins  
123 was estimated by SDS-PAGE, and protein concentration was measured using a Pierce  
124 bicinchoninic acid protein assay kit (Thermo Scientific, Waltham, MA, USA).  
125  $\beta$ -lactamase activity was monitored with nitrocefin (Oxoid Ltd., Basingstoke, United  
126 Kingdom) during the purification procedure, as per the manufacturer's instructions.

127 **Determination of kinetic parameters.**

128 A kinetic study was conducted to measure  $\beta$ -lactamase activity and compare the  
129 catalytic properties of NDM-17 and NDM-5. Initial hydrolysis rates were determined in  
130 50 mM phosphate buffer (pH 7.0) containing 30  $\mu$ M  $Zn^{2+}$  at 25°C (20), using a  
131 SpectraMax M5 multi-detection microplate reader (Molecular Devices, Sunnyvale, CA,  
132 USA). The  $K_m$  and  $k_{cat}$  values and the  $k_{cat}/K_m$  ratio were determined from three individual  
133 experiments using wavelengths and extinction coefficients as previously described  
134 (21,22), and by constructing a Lineweaver-Burk plot.



135       **Accession number(s).** The sequence of novel NDM variant gene has been deposited  
136       in GenBank under accession no. KX812714, and assigned to be *bla*<sub>NDM-17</sub> and its  
137       BioSample has also been submitted to NCBI. The complete nucleotide sequence of  
138       plasmid pAD-19R has been deposited as GenBank accession no. KX833071.

## 139       **RESULTS AND DISCUSSION**

### 140       **Characterization of *E. coli* AD19R.**

141       The *E. coli* AD-19R isolate was resistant to all  $\beta$ -lactams tested, including  
142       imipenem, meropenem, ertapenem, and aztreonam, but was sensitive to tigecycline and  
143       colistin (Table 1). A positive result in the modified Hodge test demonstrated the  
144       carbapenemase production phenotype. The presence of *bla*<sub>NDM</sub> in AD19R was confirmed  
145       by PCR and sequencing. Analysis of the draft genome of AD19R by whole-genome  
146       sequencing revealed a novel *bla*<sub>NDM</sub> variant, assigned *bla*<sub>NDM-17</sub> (GenBank accession no.  
147       KX812714), as well as the presence of additional  $\beta$ -lactamase genes *bla*<sub>CTX-M-64</sub> and  
148       *bla*<sub>TEM-1B</sub>, sulfonamides resistance gene *sul2* and aminoglycoside resistance genes  
149       *aph(3')-Ia*, *aadA5*, *rmtB*. In comparison with *bla*<sub>NDM-1</sub>, *bla*<sub>NDM-17</sub> contained point  
150       mutations at nucleotide positions 262 (G→T), 460 (A→C), and 508 (G→A). These  
151       substitutions corresponded to amino acid variants V88L, M154L, and E170K,  
152       respectively, with the E170K being a novel substitution. Multi-locus sequence typing  
153       (MLST) analysis showed that AD19R belonged to sequence type 48 (ST48), which is  
154       most commonly associated with *bla*<sub>CTX-M</sub>-harboring *E. coli* isolates in humans (23).

155 These findings suggest the possibility of transfer of *E. coli* isolates harboring *bla*<sub>NDM</sub>  
156 from humans to food animals (8, 24).

157 **Transferability and localization of *bla*<sub>NDM-17</sub> and plasmid analysis.**

158 Transconjugation assays showed that *bla*<sub>NDM-17</sub> was successfully transferred to *E.*  
159 *coli* J53, with a transfer frequency of  $\sim 6.32 \times 10^{-9}$  per donor. S1-pulsed-field gel  
160 electrophoresis and Southern blotting revealed that a plasmid band from the  
161 transconjugants (designated AD19/J53), with a size of  $\sim 47$  kb, hybridized with the  
162 *bla*<sub>NDM</sub> probe (Fig. 1). AD19/J53 exhibited a similar resistance profile to parental isolate  
163 AD19R, except for aztreonam.

164 The complete DNA sequence of pAD-19R (carrying *bla*<sub>NDM-17</sub>), isolated from  
165 AD19/J53 transconjugants, was obtained by whole-genome sequencing, with an average  
166 depth of coverage of 510. It was a circular, 46,161-bp plasmid with a G+C content of  
167 46.6% and 60 putative open reading frames (ORFs) (Fig. 2). pAD-19R was identified as  
168 an IncX3 plasmid, with a typical backbone structure for this plasmid type, including  
169 regions involved replication, partitioning, plasmid maintenance, transcriptional activation,  
170 and conjugation/type IV secretion (25,26). Although IncX3 plasmids are considered low  
171 prevalence, narrow-host-range plasmids of Enterobacteriaceae (27), they may have  
172 served as a common vehicle mediating *bla*<sub>NDM</sub> dissemination in China, and might be  
173 responsible for the rapid spread of NDM-carrying isolates (4,28), a theory supported by  
174 our study.

175 BLAST homology analysis showed that pAD-19R had 99% (46142/46161 bp)  
176 identity and 100% query coverage with pNDM5\_IncX3 (GenBank accession no.  
177 KU761328), a 46,161 bp IncX3 plasmid isolated from *Klebsiella pneumoniae* (SZ204),  
178 recently reported in China (29). Notably, strain SZ204 carried a *mcr-1*-harboring plasmid,  
179 in addition to pNDM5\_IncX3, which makes co-dissemination of IncX3  
180 *bla*<sub>NDM-5</sub>-harboring plasmids and *mcr-1*-harboring plasmids. In addition, the pAD-19R  
181 sequence was similar to other five IncX3 *bla*<sub>NDM</sub>-allele-harboring plasmids: plasmid  
182 unnamed2 from *K. pneumoniae* strain NUHL24835 (GenBank accession no. CP014006)  
183 isolated in China, pNDM\_MGR194 (GenBank accession no. KF220657) from *K.*  
184 *pneumoniae* isolated in India (26), pEc1929 (GenBank accession no. KT824791) from *E.*  
185 *coli* isolated in China (30), pJEG027 (GenBank accession no. KF220657) from *K.*  
186 *pneumoniae* isolated in Australia (31), and pKpN01-NDM7 (GenBank accession no.  
187 CP012990) from *K. pneumoniae* isolated in Canada (32). Interestingly, all six plasmids,  
188 including pNDM5\_IncX3, were carried by bacteria isolated from humans, whereas the *E.*  
189 *coli* strain carrying pAD-19R in our study was isolated from a chicken. This result  
190 further indicates the possible transfer of IncX3 *bla*<sub>NDM-17</sub>-harboring plasmids/isolates  
191 between humans and food-producing animals. Therefore, Enterobacteriaceae species  
192 carrying IncX3 *bla*<sub>NDM-17</sub>-harboring plasmids should be monitored worldwide.

193 Further analysis of the pAD-19R sequence showed that it didn't harbor other  
194 resistance genes apart from *bla*<sub>NDM-17</sub> and *ble*. The sequence surrounding *bla*<sub>NDM-17</sub> shares

195 a common genetic background with a 10,410-bp fragment,  
196 Tn3-IS3000- $\Delta$ IS*Aba125*-IS5-*bla*<sub>NDM-17</sub>-*ble*<sub>MBL</sub>-*trpF*-*dsbC*-IS26- $\Delta$ *umuD* (Region A in Fig.  
197 S1), which plays a crucial role in horizontal transmission, and may assist in horizontal  
198 transfer of *bla*<sub>NDM-17</sub> among Enterobacteriaceae (33). Overall, these results warn that both  
199 the genetic environment of *bla*<sub>NDM-17</sub> and the IncX3 *bla*<sub>NDM-17</sub>-harboring plasmids  
200 contribute to *bla*<sub>NDM-17</sub> transmission among food-producing animals. The *bla*<sub>NDM-17</sub>  
201 carrying isolates would pose a threat to human health once the *E. coli* AD-19R  
202 transferred to humans through the food chain, and vice versa.

### 203 **Functional analysis of NDM-17 and characterization of kinetic parameters.**

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

210 All of the transformants were successfully cloned and confirmed by PCR. Strains  
211 containing pHSG398/NP-NDM-1, pHSG398/NP-NDM-5 and pHSG398/NP-NDM-17,  
212 with their native promoters identified no differences for all transformants by PCR and  
213 sequencing with M13 primers, exhibited resistance to all  $\beta$ -lactams tested, including  
214 meropenem and imipenem (Table 1). Interestingly, the constructs pHSG398/NDM-1,

215 pHSG398/NDM-5 and pHSG398/NDM-17, carrying complete ORFs without the native  
216 promoters, showed reduced susceptibility to penicillins and cepheems, but were  
217 susceptible to carbapenem. This result confirmed that the wild-type promoter was crucial  
218 for carbapenem resistance (19). In addition, all transformants were susceptible to  
219 aztreonam, colistin, and tigecycline, which was consistent with previous reports (18,19).  
220 The profiles of  $\beta$ -lactams tested resistance for NDM-17 transformants were similar to  
221 those for corresponding NDM-5 transformants, however, the MICs of ertapenem and  
222 meropenem for pHSG398/NP-NDM-17 were slightly higher (2-fold) than those for  
223 pHSG398/NP-NDM-5 (Table 1). Importantly, the MICs of cefepime, ertapenem, and  
224 imipenem for DH5 $\alpha$  (pHSG398/NP-NDM-17) were 2-fold higher than those of DH5 $\alpha$   
225 (pHSG398/NP-NDM-1). Furthermore, DH5 $\alpha$  (pHSG398/NP-NDM-17) showed a 4-fold  
226 elevation in MIC for meropenem compared with DH5 $\alpha$  (pHSG398/NP-NDM-1). These  
227 findings suggest that mutations outside the promoter region are responsible for the  
228 increased carbapenem resistance.

229 Expression and purification experiments showed that the NDM-17 and NDM-5  
230 recombinant proteins were expressed at up to 90% purity, as evaluated by SDS-PAGE.  
231 Both NDM proteins were used to determine kinetic parameters, which revealed that  
232 NDM-17 and NDM-5 could hydrolyze all  $\beta$ -lactams tested, except for aztreonam (Table  
233 2). NDM-17 had similar  $k_{cat}/K_m$  ratios for almost  $\beta$ -lactams tested to NDM-5, except for  
234 significantly higher  $k_{cat}/K_m$  ratios for cefoxitin and penicillin G, and lower for ampicillin.

235 These results indicate that NDM-17 has similar enzymatic activity to NDM-5, which had  
236 been reported to increase the carbapenemase activity compared with NDM-1. Notably,  
237 the  $K_m$  of NDM-17 for all  $\beta$ -lactams tested was obviously lower than that of NDM-5,  
238 especially for ceftazidime, penicillin G, ertapenem, imipenem, and meropenem (Table 2).  
239 These results suggest that NDM-17 has significantly higher affinity for all  $\beta$ -lactams  
240 tested than NDM-5.

241 It is possible that the increased resistance and the higher enzyme activity of  
242 NDM-17 is conferred by the three amino acid substitutions (V88L, M154L, and E170K).  
243 The M154L substitution increases the carbapenemase activity of NDM-4 (M154L) (15),  
244 NDM-5 (V88L, M154L) (34-36), and NDM-7 (D130N, M154L) (19,37), indicating it  
245 may be responsible for the higher hydrolytic activity of NDM-17. NDM-4 and NDM-5  
246 are identical except for the V88L substitution in NDM-5, and NDM-5 has lower  $k_{cat}/K_m$   
247 values for imipenem and meropenem than NDM-4 (38). This suggests that V88L might  
248 contribute to the decreased hydrolytic activity of NDM-5 towards carbapenems.  
249 NDM-17 shares the V88L and M154L substitutions with NDM-5, in addition to E170K.  
250 Our kinetic data showed that NDM-17 had significantly higher affinity for all  $\beta$ -lactams  
251 tested, and obviously increased catalytic efficiencies for ceftazidime and penicillin G. Thus,  
252 the E170K substitution should be responsible for the higher affinity and increased  
253 catalytic efficiencies of NDM-17. Interestingly, the D130G substitution increases  
254 carbapenemase activity, but NDM-8 which contains both D130G and M154L, does not

255 exhibit increased hydrolytic activity for carbapenems (39). Thus, it is possible that  
256 certain amino acid substitutions may have different effects in different NDM variants,  
257 and the increased hydrolytic activity of NDM-17 was not the result of the cumulative  
258 effect of the individual V88L, M154L, and E170K amino acid substitutions, but rather  
259 the overall interaction of the three substitutions.

260 To determine the locations of the three amino acid substitutions and analyze their  
261 effects on structure, a 3D model of NDM-17 was generated by homology modeling using  
262 NDM-1 as a template (PDB accession: 4EXS). The previously reported crystal structure  
263 of NDM-1 shows that the active site is formed by loops 3 and 10, at the bottom of a  
264 shallow groove, and amino acid triads that bind to zinc ions are formed by H120, H122,  
265 and H189, and D124, C208, and H250 (40,41). Currently, 16 amino acid substitutions  
266 have been reported in NDMs at 14 distinct amino acids: 28, 32, 36, 69, 74, 88, 95, 130,  
267 152, 154, 200, 222, 233, and 264. E170K represents a new amino acid substitution and  
268 site, which was far from the active site and exposed to the solvent. Although positions 88,  
269 154, and 170 are not located in the active site involved in binding to zinc ions (Fig. 3),  
270 they might still indirectly affect the formation of the active site, as was previously  
271 described (18).

## 272 **Conclusions.**

273 In this study, a novel NDM variant, NDM-17, was identified in a ST48 *E. coli* strain  
274 isolated from a chicken. This is the first report of a new NDM variant being isolated from



275 a food animal. NDM-17 displayed higher affinity than NDM-5 against almost all  
276  $\beta$ -lactams, as well as carbapenem confirmed by kinetic parameters, and increased  
277 carbapenemase activity compared to NDM-1 indicated by MICs. In addition, *bla*<sub>NDM-17</sub>  
278 was located on an IncX3 plasmid and was surrounded by multiple insertion sequences,  
279 mediating the rapid dissemination of *bla*<sub>NDM</sub>. Transmission of strains carrying *bla*<sub>NDM-17</sub>  
280 to humans via the food chain represents a serious threat to human health, and should be  
281 given further attention to ensure NDM-17-producing pathogens are efficiently monitored.

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## 286 COMPETING FINANCIAL INTERESTS

287 The authors declare that they have no competing financial interests.

## 288 AUTHORS' CONTRIBUTIONS

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

294

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- 431

432 **FIGURE LEGENDS**

433 **FIG 1.** Location of *bla*<sub>NDM-17</sub> on *E. coli* plasmid AD-19R, separated by PFGE. Lane A,  
434 hybridization of the plasmid with a probe specific for *bla*<sub>NDM-17</sub>; lane B, plasmid from  
435 transconjugants AD19/J53; lane C, reference standard strain H9812 restricted by *Xba*I.

436

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

443 **TABLE 1**  $\beta$ -lactam MICs for the NDM17-carrying original *E. coli* isolate, and its transconjugants and transformants

Antibiotic	AD19 (NDM-17)	AD19/J53	J53	DH5 $\alpha$ (pHSG398)	DH5 $\alpha$ (pHSG398/ NDM-1)	DH5 $\alpha$ (pHSG398/ NDM-5)	DH5 $\alpha$ (pHSG398/ NDM-17)	DH5 $\alpha$ (pHSG398/ NP-NDM-1)	DH5 $\alpha$ (pHSG398/ NP-NDM-5)	DH5 $\alpha$ (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	$\leq 0.008$	$\leq 0.008$	0.016	0.016	0.008	$\leq 0.008$	0.016	0.008	0.016
Colistin	1	0.5	0.5	0.125	0.125	0.016	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	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)	$\geq 16/304$	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

444 **TABLE 2** Kinetic parameters of NDM-17 and NDM-5 enzymes<sup>a</sup>

445

$\beta$ -lactam	NDM-17 <sup>b</sup>			NDM-5 <sup>b</sup>			$k_{\text{cat}}/K_m$ ( $\mu\text{M}^{-1} \text{s}^{-1}$ ) ratio for NDM-17/NDM-5
	$K_m$ ( $\mu\text{M}$ )	$k_{\text{cat}}$ ( $\text{s}^{-1}$ ) <sup>b</sup>	$k_{\text{cat}}/K_m$ ( $\mu\text{M}^{-1} \text{s}^{-1}$ )	$K_m$ ( $\mu\text{M}$ )	$k_{\text{cat}}$ ( $\text{s}^{-1}$ ) <sup>b</sup>	$k_{\text{cat}}/K_m$ ( $\mu\text{M}^{-1} \text{s}^{-1}$ )	
Ampicillin	586 $\pm$ 53	157 $\pm$ 11	0.27	590 $\pm$ 57	267 $\pm$ 8.1	0.45	0.60
Aztreonam	NH <sup>c</sup>	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

446 <sup>a</sup>The proteins were initially modified with a His tag, which was removed after purification.

447  $^bK_m$  and  $k_{cat}$  values are means  $\pm$  standard deviations from three independent experiments.

448  $\nabla$ NH denotes no hydrolysis under conditions with substrate concentrations up to 1 mM, and enzyme concentrations up to 700 nM.

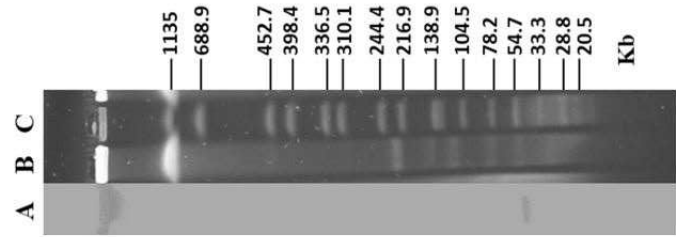


FIG 1

FIG 2

