1 **Plasmid-Mediated Novel *bla*$_{NDM-17}$ Gene Encoding a Carbapenemase with Enhanced**

2 **Activity in a ST48 *Escherichia coli* Strain**

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12 **Keywords:** NDM-17, carbapenemase, ST48, IncX3

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14 **Running title:** NDM-17 with enhanced carbapenemase activity

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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, 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, E170K) that confer significantly enhanced carbapenemase activity, When compared to NDM-5, NDM-17 had only one amino acid substitution (E170K) and slightly increased isolate resistance toward 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* J53 by conjugation, suggesting the possibility of rapid dissemination of bla_{NDM-17}. Enzyme kinetics showed that NDM-17 could hydrolyze all β-lactams tested, except for aztreonam, and had significantly higher affinity for all β-lactams tested compared to NDM-5. The emergence of this novel NDM variant could pose a threat to public health because of its 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-β-lactamases

41   (MBLs) such as VIM, IMP, and New Delhi metallo-β-lactamase (NDM), which can

42   hydrolyze almost all carbapenem β-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),

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.

3

MATERIALS AND METHODS

Identification and phenotypic characterization of the isolate.

A route annual surveillance of CRE of animal origin was performed to monitor its 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 (9). 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 (10). 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* strain YW carrying bla_{NDM-1} and DZ2-29R carrying bla_{NDM-5}) to several antimicrobials (listed in Table 1) were determined using a broth microdilution method as recommended by the Clinical and Laboratory Standards Institute (11). The *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 isolates AD-19R using a QiaAmp Mini kit according to the manufacturer's recommendations (Qiagen, Hilden, Germany). PCR and

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76   DNA sequencing were conducted to screen for known β-lactamase genes (MBL genes

77   bla_{DIM}, bla_{GIM}, bla_{IMP}, bla_{NDM}, bla_{SIM}, bla_{SPM}, and bla_{VIM}) 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_{NDM}

88   digoxigenin-labeled probes to locate bla_{NDM} genes. Transconjugation assays were used to

89   evaluate the horizontal transferability of bla_{NDM}, 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*_{NDM-17,} *bla*_{NDM-5} and *bla*_{NDM-1}.**

96   To compare the beta-lactamase activities of both NDM-1 and NDM-5 with NDM-17,

97   the respective genes (*bla*_{NDM-1}, *bla*_{NDM-5} and *bla*_{NDM-17}) with their native promoters were

98   amplified   by   PCR   using   primers   NP-NDM-F

99   (5′-CGGGATCCCACCTCATGTTTGAATTCGC-3′)   and   NP-NDM-R

100   (5′-CCCAAGCTTCTCTGTCACATCGAAATCGC-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*_{NDM-1} *bla*_{NDM-5} and *bla*_{NDM-17} ORFs were obtained by PCR using

104   primers NDM-F (5′-CGGGATCCATGGAATTGCCCAATATTATG-3′) and NDM-R

105   (5′-CCCAAGCTTTCAGCGCAGCTTGTCGGCCAT-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* DH5α 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′-ATGGATCCGAAAACCTGTATTTCCAAGGCCAGCAAATGGAAACTGGCGAC-

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   β-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 β-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 μM 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.

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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 $bla_{\text{NDM-17}}$ 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 β-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_{NDM} 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_{NDM} variant, assigned $bla_{\text{NDM-17}}$ (GenBank accession no.

147   KX812714), as well as the presence of additional β-lactamase genes $bla_{\text{CTX-M-64}}$ and

148    $bla_{\text{TEM-1B}}$, sulfonamides resistance gene *sul2* and aminoglycoside resistance genes

149   *aph(3')-Ia*, *aadA5*, *rmtB*. In comparison with $bla_{\text{NDM-1}}$, $bla_{\text{NDM-17}}$ 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 $bla_{\text{CTX-M}}$-harboring *E. coli* isolates in humans (23).

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155   These findings suggest the possibility of transfer of *E. coli* isolates harboring bla_{NDM}

156   from humans to food animals (8, 24).

157   **Transferability and localization of bla_{NDM-17} and plasmid analysis.**

158   Transconjugation assays showed that bla_{NDM-17} was successfully transferred to *E.*

159   *coli* J53, with a transfer frequency of ~6.32×10^{-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 ~47 kb, hybridized with the

162   bla_{NDM} 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_{NDM-17}), 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_{NDM} 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.

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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_{NDM-5}-harboring plasmids and *mcr-1*-harboring plasmids. In addition, the pAD-19R

181   sequence was similar to other five IncX3 bla_{NDM}-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_{NDM-17}-harboring plasmids/isolates

191   between humans and food-producing animals. Therefore, Enterobacteriaceae species

192   carrying IncX3 bla_{NDM-17}-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_{NDM-17} and *ble*. The sequence surrounding bla_{NDM-17} shares

195   a   common   genetic   background   with   a   10,410-bp   fragment,

196   Tn*3*-IS*3000*-ΔIS*Aba125*-IS*5*-*bla*$_{NDM-17}$-*ble*$_{MBL}$-*trpF*-*dsbC*-IS*26*-Δ*umnD* (Region A in Fig.

197   S1), which plays a crucial role in horizontal transmission, and may assist in horizontal

198   transfer of *bla*$_{NDM-17}$ among Enterobacteriaceae (33). Overall, these results warn that both

199   the genetic environment of *bla*$_{NDM-17}$ and the IncX3 *bla*$_{NDM-17}$-harboring plasmids

200   contribute to *bla*$_{NDM-17}$ transmission among food-producing animals. The *bla*$_{NDM-17}$

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). 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 β-lactams tested, including

214   meropenem and imipenem (Table 1). Interestingly, the constructs pHSG398/NDM-1,

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215   pHSG398/NDM-5 and pHSG398/NDM-17, carrying complete ORFs without the native

216   promoters, showed reduced susceptibility to penicillins and cephems, 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 β-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 DH5α (pHSG398/NP-NDM-17) were 2-fold higher than those of DH5α

225   (pHSG398/NP-NDM-1). Furthermore, DH5α (pHSG398/NP-NDM-17) showed a 4-fold

226   elevation in MIC for meropenem compared with DH5α (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 β-lactams tested, except for aztreonam (Table

233   2). NDM-17 had similar k_{cat}/K_m ratios for almost β-lactams tested to NDM-5, except for

234   significantly higher k_{cat}/K_m ratios for cefoxitin and penicillin G, and lower for ampicillin.

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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 β-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 β-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 β-lactams

251   tested, and obviously increased catalytic efficiencies for cefoxitin 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-8which 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 β-lactams, as well as carbapenem confirmed by kinetic parameters, and increased

277 carbapenemase activity compared to NDM-1 indicated by MICs. In addition, bla_{NDM-17}

278 was located on an IncX3 plasmid and was surrounded by multiple insertion sequences,

279 mediating the rapid dissemination of bla_{NDM}. Transmission of strains carrying bla_{NDM-17}

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.

282 **ACKNOWLEDGMENTS**

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.

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431

FIGURE LEGENDS

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

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, H189; D124, C208, H250) and three amino acid substitutions (L88, L154, and K170) are labeled and colored 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).

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

Antibiotic	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)	≥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

24

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

445

β-lactam	NDM-17[b]			NDM-5[b]			k_{cat}/K_m (μM^{-1} s^{-1}) ratio for NDM-17/NDM-5
	K_m (μM)	k_{cat} (s^{-1})[b]	k_{cat}/K_m (μM^{-1} s^{-1})	K_m (μM)	k_{cat} (s^{-1})[b]	k_{cat}/K_m (μM^{-1} s^{-1})	
Ampicillin	586±53	157±11	0.27	590±57	267±8.1	0.45	0.60
Aztreonam	NH[c]	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

446 [a]The proteins were initially modified with a His tag, which was removed after purification.

25

447 $^{b}K_{m}$ and k_{cat} values are means ± standard deviations from three independent experiments.

448 ^{c}NH denotes no hydrolysis under conditions with substrate concentrations up to 1 mM, and enzyme concentrations up to 700 nM.
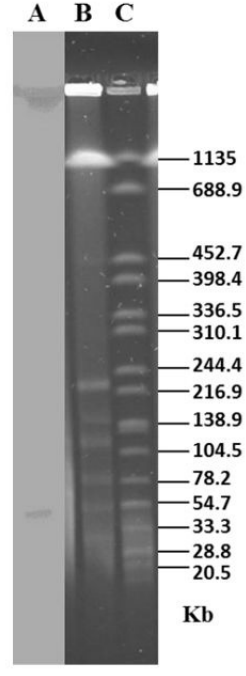
FIG 1

FIG 2