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Plasmid-Mediated Novel \textit{bla}_{\text{NDM-17}} Gene Encoding a Carbapenemase with Enhanced Activity in a ST48 \textit{Escherichia coli} Strain

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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*<sub>NDM-17</sub>) 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*<sub>NDM-17</sub>. 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.
INTRODUCTION

Carbapenem-resistant Enterobacteriaceae (CRE) have been recognized as an urgent antibiotic resistance threat by the Centers for Disease Control in the US, and have become a global problem in recent years (1). The resistance exhibited by CRE is largely mediated 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 (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, 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 non-human sources of NDM (7,8). In comparison with the high prevalence of NDM-positive Enterobacteriaceae of human origin, there are few reports on CRE from food animals. Furthermore, none of the novel NDM variants described to date were originally identified in bacteria isolated from food animals. Here we describe the characterization of a novel NDM variant in *Escherichia coli* isolated from a chicken.
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
DNA sequencing were conducted to screen for known β-lactamase genes (MBL genes \( \text{bla}^{\text{DIM}}, \text{bla}^{\text{GIM}}, \text{bla}^{\text{IMP}}, \text{bla}^{\text{NDM}}, \text{bla}^{\text{SIM}}, \text{bla}^{\text{SPM}}, \) and \( \text{bla}^{\text{VIM}} \)) as described previously (12). A 150-bp paired-end library was constructed following 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 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 (13).

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

MLST was performed as described previously to identify the sequence type of isolate AD-19R (14). Southern blot analysis was used with specific \( \text{bla}^{\text{NDM}} \) digoxigenin-labeled probes to locate \( \text{bla}^{\text{NDM}} \) genes. Transconjugation assays were used to evaluate the horizontal transferability of \( \text{bla}^{\text{NDM}} \), with \( E. \ coli \) J53 as the recipient, and isolate AD-19R as the donor. The transconjugants were selected on MacConkey agar containing 100 mg/l sodium azide and 1 mg/l meropenem and the transfer frequency was calculated by transconjugants/donors. PCR with specific primers was used to confirm the presumptive transconjugants (15). Plasmid incompatibility groups were determined by two PCR-based replicon typing methods (16,17).
Cloning of bla\textsubscript{NDM-17}, bla\textsubscript{NDM-5} and bla\textsubscript{NDM-1}.

To compare the beta-lactamase activities of both NDM-1 and NDM-5 with NDM-17, the respective genes (bla\textsubscript{NDM-1}, bla\textsubscript{NDM-5} and bla\textsubscript{NDM-17}) with their native promoters were amplified by PCR using primers NP-NDM-F (5′-CGGGATCCACCTCATGTTTGAATTCGC-3′) and NP-NDM-R (5′-CCCAAGCTTCTCTGTCACATCGAAATCGC-3′), and cloned into the pHSG398 vector (Takara Bio, Dalian, China). The resulting plasmids were named pHSG398/NP-NDM-1, pHSG398/NP-NDM-5 and pHSG398/NP-NDM-17, respectively.

The complete bla\textsubscript{NDM-1}, bla\textsubscript{NDM-5} and bla\textsubscript{NDM-17} ORFs were obtained by PCR using primers NDM-F (5′-CGGGATCCATGGAA TTGCCCAATATTATG-3′) and NDM-R (5′-CCCAAGCTTTCAGCGCAGCTTGTCGGCCAT-3′), cloned into pHSG398, and named pHSG398/NDM-1, pHSG398/NDM-5 and pHSG398/NDM-17, respectively. Subsequently, pHSG398/NDM-1, pHSG398/NDM-5, pHSG398/NDM-17, pHSG398/NDM-1, pHSG398/NDM-5 and pHSG398/NDM-17 were transformed into \textit{E. coli} DH5α by electroporation, and confirmed by PCR and DNA sequencing (18,19).

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

The ORFs coding for NDM-5 and NDM-17 without signal peptide regions were amplified using primers BamHI-TEV-NDM-F (5′-ATGGATCCGAAAACCTGTATTTCACAAGGCCAGCAAATGGAAACTGCGA-3′) and BamHI-TEV-NDM-R (5′-CCCAAGCTTTGTATCATCGGAAATCGC-3′), cloned into pET-28a (+) vector (Novagen), and named pET-28a (+)/NDM-5, pET-28a (+)/NDM-17, respectively. Subsequently, pET-28a (+)/NDM-5, pET-28a (+)/NDM-17, and pET-28a (+)/NDM-17 were transformed into \textit{E. coli} DH5α by electroporation, and confirmed by PCR and DNA sequencing (18,19).
3′) and XhoI-NDM-R (5′ATCTCGAGTCAGCGCAGCTTGTCGGCCATG-3′) and then cloned into the pET28a expression vector (Merck Millipore, Danvers, MA, USA). The resulting plasmid was transformed into *E. coli* BL21 (DE3) as per the manufacturer’s instructions (TransGen Biotech, Beijing, China). Ni-nitritotriacetic 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 cleaving with Turbo 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 protein concentration was measured 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, as per 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 in 50 mM phosphate buffer (pH 7.0) containing 30 μM Zn^{2+} at 25°C (20), using a SpectraMax M5 multi-detection 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 (21,22), and by constructing a Lineweaver-Burk plot.
Accession number(s). The sequence of novel NDM variant gene has been deposited in GenBank under accession no. KX812714, and assigned to be \textit{bla}_{NDM-17} and its BioSample has also been submitted to NCBI. The complete nucleotide sequence of plasmid pAD-19R has been deposited as GenBank accession no. KX833071.

RESULTS AND DISCUSSION

Characterization of \textit{E. coli} AD19R.

The \textit{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 in the modified Hodge test demonstrated the carbapenemase production phenotype. The presence of \textit{bla}_{NDM} in AD19R was confirmed by PCR and sequencing. Analysis of the draft genome of AD19R by whole-genome sequencing revealed a novel \textit{bla}_{NDM} variant, assigned \textit{bla}_{NDM-17} (GenBank accession no. KX812714), as well as the presence of additional β-lactamase genes \textit{bla}_{CTX-M-64} and \textit{bla}_{TEM-1B}, sulfonamides resistance gene \textit{sul}2 and aminoglycoside resistance genes \textit{aph(3')-Ia, aadA5}, \textit{rmtB}. In comparison with \textit{bla}_{NDM-1}, \textit{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 the E170K being a novel substitution. Multi-locus sequence typing (MLST) analysis showed that AD19R belonged to sequence type 48 (ST48), which is most commonly associated with \textit{bla}_{CTX-M}-harboring \textit{E. coli} isolates in humans (23).
These findings suggest the possibility of transfer of *E. coli* isolates harboring \( \textit{bla}_{\text{NDM}} \) from humans to food animals (8, 24).

**Transferability and localization of \( \textit{bla}_{\text{NDM}-17} \) and plasmid analysis.**

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

The complete DNA sequence of pAD-19R (carrying \( \textit{bla}_{\text{NDM}-17} \)), 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) (Fig. 2). 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 (25,26). Although IncX3 plasmids are considered low prevalence, narrow-host-range plasmids of Enterobacteriaceae (27), they may have served as a common vehicle mediating \( \textit{bla}_{\text{NDM}} \) dissemination in China, and might be responsible for the rapid spread of NDM-carrying isolates (4,28), a theory supported by our study.
BLAST homology analysis showed that pAD-19R had 99% (46142/46161 bp) identity and 100% query coverage with pNDM5_IncX3 (GenBank accession no. KU761328), a 46,161 bp IncX3 plasmid isolated from *Klebsiella pneumoniae* (SZ204), recently reported in China (29). Notably, strain SZ204 carried a *mcr-1*-harboring plasmid, in addition to pNDM5_IncX3, which makes co-dissemination of IncX3 *bla*NDM-5*-harboring plasmids and *mcr-1*-harboring plasmids. In addition, the pAD-19R sequence was similar to other five IncX3 *bla*NDM-allele-harboring plasmids: plasmid unnamed2 from *K. pneumoniae* strain NUHL24835 (GenBank accession no. CP014006) isolated in China, pNDM_MGR194 (GenBank accession no. KF220657) from *K. pneumoniae* isolated in India (26), pEc1929 (GenBank accession no. KT824791) from *E. coli* isolated in China (30), pJEG027 (GenBank accession no. KF220657) from *K. pneumoniae* isolated in Australia (31), and pKpN01-NDM7 (GenBank accession no. CP012990) from *K. pneumoniae* isolated in Canada (32). 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-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 didn’t 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-IS5-\(\text{bla}_{\text{NDM-17}}\)-bleMBL-trpF-dsbC-IS26-\(\Delta\text{unnD}\) (Region A in Fig. S1), which plays a crucial role in horizontal transmission, and may assist in horizontal transfer of \(\text{bla}_{\text{NDM-17}}\) among Enterobacteriaceae (33). Overall, these results warn that both the genetic environment of \(\text{bla}_{\text{NDM-17}}\) and the IncX3 \(\text{bla}_{\text{NDM-17}}\)-harboring plasmids contribute to \(\text{bla}_{\text{NDM-17}}\) transmission among food-producing animals. The \(\text{bla}_{\text{NDM-17}}\) carrying isolates would pose a threat to human health once the \(E.\ coli\) AD-19R 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, 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 (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 by reference to NDM-5.

All of the transformants were successfully cloned and confirmed by PCR. Strains containing pHSG398/NP-NDM-1, pHSG398/NP-NDM-5 and pHSG398/NP-NDM-17, with their native promoters identified no differences for all transformants by PCR and sequencing with M13 primers, exhibited resistance to all \(\beta\)-lactams tested, including meropenem and imipenem (Table 1). Interestingly, the constructs pHSG398/NDM-1,
pHSG398/NDM-5 and pHSG398/NDM-17, carrying complete ORFs without the native promoters, showed reduced susceptibility to penicillins and cephems, but were susceptible to carbapenem. This result confirmed that the wild-type promoter was crucial for carbapenem resistance (19). In addition, all transformants were susceptible to aztreonam, colistin, and tigecycline, which was consistent with previous reports (18,19). The profiles of β-lactams tested resistance for NDM-17 transformants were similar to those for corresponding NDM-5 transformants, however, the MICs of ertapenem and meropenem for pHSG398/NP-NDM-17 were slightly higher (2-fold) than those for pHSG398/NP-NDM-5 (Table 1). Importantly, the MICs of cefepime, ertapenem, and imipenem for DH5α (pHSG398/NP-NDM-17) were 2-fold higher than those of DH5α (pHSG398/NP-NDM-1). Furthermore, DH5α (pHSG398/NP-NDM-17) showed a 4-fold elevation in MIC for meropenem compared with DH5α (pHSG398/NP-NDM-1). These findings suggest that mutations outside the promoter region are responsible for the increased carbapenem resistance.

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

It is possible that the increased resistance and the higher enzyme activity of NDM-17 is conferred by the three amino acid substitutions (V88L, M154L, and E170K). The M154L substitution increases the carbapenemase activity of NDM-4 (M154L) (15), NDM-5 (V88L, M154L) (34-36), and NDM-7 (D130N, M154L) (19,37), indicating 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 NDM-4 (38). This suggests that V88L might contribute to the decreased hydrolytic activity of NDM-5 towards carbapenems. NDM-17 shares the V88L and M154L substitutions with NDM-5, in addition to E170K. Our kinetic data showed that NDM-17 had significantly higher affinity for all $\beta$-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 D130G and M154L, does not
exhibit increased hydrolytic activity for carbapenems (39). 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
the overall interaction of the three substitutions.

To determine the locations of the three amino acid substitutions and analyze their
effects on structure, a 3D model of NDM-17 was generated by homology modeling using
NDM-1 as a template (PDB accession: 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 D124, C208, and H250 (40,41). Currently, 16 amino acid substitutions
have been reported in NDMs at 14 distinct amino acids: 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. 3),
they might still indirectly affect the formation of the active site, as was previously
described (18).

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 higher affinity than NDM-5 against almost all β-lactams, as well as carbapenem confirmed by kinetic parameters, and increased carbapenemase activity compared to NDM-1 indicated by MICs. In addition, blaNDM-17 was located on an IncX3 plasmid and was surrounded by multiple insertion sequences, mediating the rapid dissemination of blaNDM. Transmission of strains carrying blaNDM-17 to humans via the food chain represents a serious threat to human health, and should be given further attention to ensure NDM-17-producing pathogens are efficiently monitored.

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COMPETING FINANCIAL INTERESTS

The authors declare that they have no competing financial interests.

AUTHORS’ CONTRIBUTIONS

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, Jianzhong Shen wrote the report. All authors revised, reviewed and approved the final report.
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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 *XbaI*.

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

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>AD19 (NDM-17)</th>
<th>AD19 J53</th>
<th>J53</th>
<th>DH5a (pHSG398)</th>
<th>DH5a (pHSG398/NDM-1)</th>
<th>DH5a (pHSG398/NDM-5)</th>
<th>DH5a (pHSG398/NDM-17)</th>
<th>DH5a (pHSG398/NDM-5)</th>
<th>DH5a (pHSG398/NDM-17)</th>
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<tr>
<td>Ampicillin</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>4</td>
<td>2</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
</tr>
<tr>
<td>Aztreonam</td>
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<td>0.063</td>
<td>0.032</td>
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<tr>
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<td>2</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
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<td>&gt;256</td>
<td>&gt;256</td>
<td>0.063</td>
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<td>2</td>
<td>2</td>
<td>4</td>
<td>8</td>
<td>16</td>
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<tr>
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<td>&gt;256</td>
<td>0.125</td>
<td>0.063</td>
<td>64</td>
<td>32</td>
<td>32</td>
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<td>128</td>
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<tr>
<td>Cefoxitin</td>
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<td>&gt;256</td>
<td>8</td>
<td>4</td>
<td>&gt;256</td>
<td>128</td>
<td>&gt;256</td>
<td>&gt;256</td>
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<tr>
<td>Ceftazidime</td>
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<td>&gt;256</td>
<td>0.5</td>
<td>0.25</td>
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<td>&gt;256</td>
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<tr>
<td>Ciprofloxacin</td>
<td>16</td>
<td>≤0.008</td>
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<tr>
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<td>0.5</td>
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<td>Imipenem</td>
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<td>0.5</td>
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<td>8</td>
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<td>&gt;256</td>
<td>64</td>
<td>32</td>
<td>&gt;256</td>
<td>256</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
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<tr>
<td>Tigecycline</td>
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<td>0.063</td>
<td>0.063</td>
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<td>0.032</td>
<td>0.032</td>
<td>0.063</td>
<td>0.016</td>
</tr>
<tr>
<td>SXT (1/19)</td>
<td>≥16/304</td>
<td>0.063/1.2</td>
<td>0.032/0.61</td>
<td>0.59/5.5</td>
<td>0.59/5.5</td>
<td>0.254/7.5</td>
<td>0.254/7.5</td>
<td>0.032/0.61</td>
<td>0.032/0.61</td>
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</tbody>
</table>
Table 2: Kinetic parameters of NDM-17 and NDM-5 enzymes

<table>
<thead>
<tr>
<th>β-lactam</th>
<th>K_m (μM)</th>
<th>k_cat (s⁻¹)</th>
<th>k_cat/K_m (μM⁻¹ s⁻¹)</th>
<th>K_m (μM)</th>
<th>k_cat (s⁻¹)</th>
<th>k_cat/K_m (μM⁻¹ s⁻¹)</th>
<th>k_cat/K_m (μM⁻¹ s⁻¹) ratio for NDM-17/NDM-5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>586±53</td>
<td>157±11</td>
<td>0.27</td>
<td>590±57</td>
<td>267±8.1</td>
<td>0.45</td>
<td>0.60</td>
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<tr>
<td>Aztreonam</td>
<td>NH</td>
<td>NH</td>
<td>NH</td>
<td>NH</td>
<td>NH</td>
<td>NH</td>
<td>NH</td>
</tr>
<tr>
<td>Cefepime</td>
<td>81±5.5</td>
<td>7.5±1.76</td>
<td>0.092</td>
<td>102±7.9</td>
<td>11±2.8</td>
<td>0.11</td>
<td>0.83</td>
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<tr>
<td>Cefotaxime</td>
<td>11±2.5</td>
<td>11±3.9</td>
<td>1.00</td>
<td>22±5.4</td>
<td>21±5.9</td>
<td>0.95</td>
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<tr>
<td>Cefoxitin</td>
<td>23±3.1</td>
<td>5±0.04</td>
<td>0.23</td>
<td>45±0.81</td>
<td>6±0.47</td>
<td>0.15</td>
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</tr>
<tr>
<td>Ceftazidime</td>
<td>82±8.6</td>
<td>10±1.1</td>
<td>0.12</td>
<td>155±16</td>
<td>21±0.76</td>
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<tr>
<td>Ertapenem</td>
<td>237±25</td>
<td>49±2.6</td>
<td>0.21</td>
<td>571±20</td>
<td>120±7.5</td>
<td>0.21</td>
<td>1.00</td>
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<tr>
<td>Imipenem</td>
<td>188±0.28</td>
<td>79±2.5</td>
<td>0.42</td>
<td>396±4.3</td>
<td>148±0.64</td>
<td>0.37</td>
<td>1.14</td>
</tr>
<tr>
<td>Meropenem</td>
<td>453±33</td>
<td>127±15</td>
<td>0.28</td>
<td>659±36</td>
<td>222±48</td>
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<td>Penicillin G</td>
<td>365±33</td>
<td>115±13</td>
<td>0.32</td>
<td>660±21</td>
<td>93±16</td>
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<td>2.29</td>
</tr>
</tbody>
</table>

*The proteins were initially modified with a His tag, which was removed after purification.*
and k\textsubscript{cat} values are means ± standard deviations from three independent experiments.

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