A Novel Variant (NDM-5) of the New Delhi Metallo-β-lactamase (NDM) in a Multidrug Resistant *Escherichia coli* ST648 Isolate Recovered from a Patient in the United Kingdom

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ABSTRACT

A new variant of the NDM carbapenemase was identified in a multidrug resistant *E. coli* ST648 isolate recovered from the perineum and throat of a patient in the UK with a recent history of hospitalization in India. NDM-5 differed from existing enzymes due to substitutions at positions 88 (Val→Leu) and 154 (Met→Leu) and reduced the susceptibility of *E. coli* TOP10 transformants to expanded spectrum cephalosporins and carbapenems when expressed under its native promoter.
The treatment of Gram-negative infections is increasingly complicated by the emergence of antimicrobial resistance. The recent identification of a new \( \beta \)-lactamase - New Delhi metallo-enzyme (NDM) - able to confer resistance to all \( \beta \)-lactams with the exception of aztreonam (12) has been met with considerable alarm. The rapid spread of NDM-producing strains across the globe and the dissemination of the gene into multiple Gram-negative species via multidrug resistance (MDR) plasmids has raised serious concerns that common infections with these organisms may soon be untreatable (11). In this report we describe the first detection of a new sequence variant of the NDM enzyme in a MDR strain of \textit{E. coli}, designated EC405, recovered from a patient in the UK.

EC405 was isolated from a 41 year old patient transferred directly to our institution after a six week stay in a medical centre in Goa, India. The patient was initially admitted with herpes simplex encephalitis and treated with aciclovir but was repatriated to the UK following neurological deterioration.

EC405 was recovered from routine screening swabs (perineum and throat) plated directly onto CHROMagar KPC, a media selective for the growth of carbapenem resistant Enterobacteriaceae (9). The isolate was confirmed as \textit{E. coli} by API 20E (bioMérieux, Marcy l’Étoile, France) and MALDI-TOF mass spectrometry (Bruker UK Ltd, Coventry, UK). Susceptibility testing was performed using the MicroScan WalkAway Negative Combo 36 panel (Siemens Healthcare Diagnostics, Deerfield, IL) and the Etest method (bioMérieux) for susceptibility to colistin and tigecycline. Resistance was defined according to current European Committee on Antimicrobial
Susceptibility Testing (EUCAST) criteria. Enterobacteriaceae breakpoints (EUCAST) were used in the case of tigecycline.

The isolate was resistant to all cephalosporins (CXM, CRO, CTX, FOX, CAZ, FEP: MIC >32 µg/ml), carbapenems (ERT, IMP, MEM: MIC >8 µg/ml), aminoglycosides (GEN, TOB, AMK: MIC >32 µg/ml) and quinolones (CIP: MIC >2 µg/ml) tested but susceptible to colistin (CST: MIC 0.19 µg/ml) and tigecycline (TGC: MIC 0.38 µg/ml).

A modified Hodge test using ertapenem (10 µg) as the indicator disc and comparison of zone sizes surrounding imipenem discs supplemented with and without 750 µg EDTA suggested the production of a metallo-carbapenemase (6).

A number of multiplex PCRs (3), (4) were used to identify genes encoding \( \text{bla}_{\text{TEM}} \) and a \( \text{bla}_{\text{CTX-M1-like}} \) extended-spectrum \( \beta \)-lactamases (ESBLs). Sequencing of these amplicons revealed that they encoded TEM-1 and CTX-M-15 \( \beta \)-lactamases. No SHV, OXA, VEB, KPC, GES, IMP, VIM, SIM, GIM, SPM or plasmidic AmpC–like genes were detected, but the entire open reading frame of \( \text{bla}_{\text{NDM}} \) was successfully amplified using the following primers NDM-Full F: 5’ ATG GAA TTG CCC AAT ATT ATG CAC; NDM-Full R: 5’ TCA GCG CAG CTT GTC GGC. Purified amplicons were ligated into pCR2.1 and the expression vector pBAD (Invitrogen, Paisley, UK) and transformed in \textit{E. coli} TOP10 (Invitrogen). Plasmids (pCR2.1 NDM-5 and pBAD NDM-5) were extracted and the \( \text{bla}_{\text{NDM}} \) allele sequenced multiple times on both strands using a combination of the amplification and vector-specific (M13) primers with an ABI 3730xl DNA Analyzer (Applied Biosystems, Warrington, UK). All sequencing was performed at Source BioSciences Ltd (Cambridge, UK).

Chromatograms were analyzed and consensus sequences aligned using BioEdit...
Analysis of the predicted amino acid sequence revealed two substitutions at positions 88 (Val → Leu) and 154 (Met → Leu) relative to the NDM-1 (Acc no. FN396876) and NDM-2 (Acc no. AEA41876) peptide sequences available in GenBank. The point mutations at positions 262 (G → T) and 460 (A → C) responsible for the amino acid substitutions were confirmed by re-amplification and sequencing of the gene from a fresh EC405 DNA preparation. The new enzyme variant was designated NDM-5 by the curators of the Lahey database of β-lactamases (http://www.lahey.org/Studies/webt.asp) and deposited in GenBank under the accession number JN104597.

Primers external to the bla<sub>NDM-5</sub> coding sequence were used to amplify the gene along with its native promoter using primers and conditions previously described (5). These amplicons were also cloned in pCR2.1 and pBAD TOPO, generating plasmids pCR2.1P+NDM-5 and pBADP+NDM-5, which were then transformed into <i>E. coli</i> TOP10.

The MICs of a range of β-lactams for <i>E. coli</i> TOP10 transformants were determined by Etest on Mueller-Hinton (MH) agar. Media was supplemented with arabinose (0.0002 – 0.2 %) and glucose (0.2 %) for the transformant harbouring pBAD NDM-5. Cephalosporin, aztreonam and carbapenem (ERT, IMP and MEM) MICs for transformants carrying bla<sub>NDM-5</sub> expressed constitutively from the pCR2.1 lac promoter, inducibly when under the control of the araBAD promoter in pBAD, and under the control of the NDM-5 native promoter are shown in Tables 1 and 2. Interestingly, expression in TOP10 from either plac or paraBAD had only limited effects on the susceptibility of the TOP10 transformants to carbapenems. Only when
the native promoter was used were marked increases in the carbapenem MICs observed (Table 1). As a comparator, \textit{bla}_{\text{NDM-1}} with and without the same promoter region was also cloned in the \textit{E. coli} TOP10 background. The effects of NDM-5 on the susceptibility of \textit{E. coli} to carbapenems as well as third and fourth-generation cephalosporins appeared to be greater than those of NDM-1 (Table 1). Whether the amino acid substitutions unique to NDM-5 could enhance the hydrolytic activity of the enzyme and be responsible for the differences observed will need to be assessed in kinetic studies using the purified enzyme.

Additional molecular analyses were undertaken to further characterize strain EC405. Phylotyping using a multiplex PCR method (2) defined it as a member of phylogroup D. Analysis of the region upstream of the \textit{bla}_{\text{NDM-5}} allele revealed the presence of part of \textit{ISAba125}, likely derived from \textit{Acinetobacter baumannii}, creating a hybrid (-35 / -10) promoter exactly as in a NDM-1-producing \textit{E. coli} isolate described by Poirel \textit{et al} (8). Multi-locus sequence typing performed according to the protocol available at http://mlst.ucc.ie/mlst/dbs/Ecoli/documents/primersColi_html identified the isolate as a member of sequence type (ST) 648. This ST has been shown to cause a significant proportion of ESBL-producing \textit{E. coli} bacteraemias in a series reported from the Rotterdam area of the Netherlands (10). In another recent study, three isolates of ST648 were shown to harbour NDM; two of which were recovered from patients hospitalized in Karachi, Pakistan while the other was isolated from a patient in the UK (7).

Plasmids carried by EC405 were extracted using a Qiagen Miniprep kit (Qiagen, Crawley, UK) and separated by agarose gel electrophoresis. The NDM-5 gene was
localized to a plasmid of >100 Kb by: i) PCR using gel-purified plasmid DNA as template; Southern hybridization with a digoxygenin labeled (Roche, Burgess Hill, UK) $\text{bla}_{\text{NDM}}$ specific probe. PCR replicon typing (1) revealed that the plasmid encoding NDM-5 belonged to the incF incompatibility group. Other resistance determinants detected included aadA5 and $\text{dfrA17}$ genes located within a class I integron structure and the 16S rRNA methylase gene, $\text{rmtB}$ thought to account for the high-level aminoglycoside resistance.

In summary, we identified a new NDM $\beta$-lactamase gene variant, $\text{bla}_{\text{NDM}-5}$, encoding the fifth enzymatic variant in this rapidly emerging and troublesome family of $\beta$-lactamases. Consistent with previous reports (5) the NDM-producing isolate was recovered from a patient with a history of travel to the Indian subcontinent.
<table>
<thead>
<tr>
<th>Isolate</th>
<th>MIC (µg/mL) by Etest on MH agar</th>
<th>ATM</th>
<th>FOX</th>
<th>CTX</th>
<th>CAZ</th>
<th>Cefpirome</th>
<th>TZP</th>
<th>ERT</th>
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<tr>
<td>EC405</td>
<td></td>
<td>&gt;256</td>
<td>&gt;256</td>
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<td>&gt;32</td>
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<tr>
<td>TOP10</td>
<td></td>
<td>0.125</td>
<td>16</td>
<td>0.047</td>
<td>0.38</td>
<td>0.035</td>
<td>4</td>
<td>0.008</td>
<td>0.25</td>
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<td>TOP10 pCR2.1 NDM-1</td>
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<td>24</td>
<td>0.125*</td>
<td>2*</td>
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<td>&gt;256</td>
<td>0.012</td>
<td>0.38</td>
<td>0.032</td>
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<td>16</td>
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<td>0.016</td>
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<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>8</td>
<td>8</td>
<td>4</td>
<td></td>
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<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>32</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>&gt;32</td>
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<tr>
<td>TOP10 pBAD P+NDM-1</td>
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<td>&gt;256</td>
<td>16</td>
<td>&gt;256</td>
<td>6</td>
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<td>0.064</td>
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<tr>
<td>TOP10 pBAD P+NDM-5</td>
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<td>0.19</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>4</td>
<td>6</td>
<td>1.5</td>
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* Colonies growing within the zone of inhibition
Table 2. Susceptibility of *E. coli* TOP10 transformants following inducible expression of NDM-5 from the paraBAD promoter.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>MIC (µg/mL) by Etest on MH agar</th>
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<tr>
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<td>TOP10 pBAD NDM-5 (0.2% gluc)</td>
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<td>TOP10 pBAD NDM-5 (0.0002% arab)</td>
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<td>TOP10 pBAD NDM-5 (0.002% arab)</td>
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<td>TOP10 pBAD NDM-5 (0.2% arab)</td>
<td>0.75</td>
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References


genetic structure in Klebsiella pneumoniae sequence type 14 from India.