ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Dec. 2011, p. 5952–5954 Vol. 55, No. 12
0066-4804/11/$12.00 doi:10.1128/AAC.05108-11

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

Michael Hornsey,[1] Lynette Phee,[2] and David W. Wareham[1,2]*

Antimicrobial Research Group, Centre for Immunology and Infectious Disease, Blizard Institute, Barts & The London School of Medicine and Dentistry, Queen Mary University London, London, United Kingdom,[1] and Division of Infection, Barts & The London NHS Trust, London, United Kingdom[2]

Received 16 June 2011/Returned for modification 20 July 2011/Accepted 14 September 2011

A new variant of the New Delhi metallo-enzyme (NDM) carbapenemase was identified in a multidrug-resistant *Escherichia coli* ST648 isolate recovered from the perineum and throat of a patient in the United Kingdom 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 β-lactamase, New Delhi metallo-enzyme (NDM), able to confer resistance to all β-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 an MDR strain of *Escherichia coli*, designated EC405, recovered from a patient in the United Kingdom.

EC405 was isolated from a 41-year-old patient transferred directly to our institution after a 6-week stay in a medical center in Goa, India. The patient was initially admitted with herpes simplex encephalitis and treated with acyclovir but was repatriated to the United Kingdom following neurological deterioration.

EC405 was recovered from routine screening swabs (perineum and throat) plated directly onto CHROMagar KPC, a medium selective for the growth of carbapenem-resistant *Enterobacteriaceae* (9). The isolate was confirmed as *E. coli* by API 20E (bioMérieux, Marcy l'Étoile, France) and matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (Bruker UK Ltd., Coventry, United Kingdom). 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 (cefuroxime, ceftriaxone, cefotaxime [CTX], cefoxitin [FOX], ceftazidime [CAZ], cefepime; MIC, >32 μg/ml), carbapenems (ertapenem [ERT], imipenem [IMP], meropenem [MEM]; MIC, >8 μg/ml), aminoglycosides (gentamicin, tobramycin, amikacin; MIC, >32 μg/ml), and quinolones (ciprofloxacin: MIC, >2 μg/ml) tested but susceptible to colistin (MIC, 0.19 μg/ml) and tigecycline (MIC, 0.38 μg/ml). A modified Hodge test using ERT (10 μg) as the indicator disc and comparison of zone sizes surrounding IMP 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 bla_{TEM} and $bla_{CTX\text{-}M1}$-like extended-spectrum β-lactamases (ESBLs). Sequencing of these amplicons revealed that they encoded TEM-1 and CTX-M-15 β-lactamases. No bla_{SHV}, bla_{OXA}, bla_{PER}, bla_{VEB}, bla_{KPC}, bla_{GES}, bla_{IMP}, bla_{VIM}, bla_{SIM}, bla_{GIM}, bla_{SPM}, or plasmidic AmpC-like genes were detected, but the entire open reading frame of bla_{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, United Kingdom) and transformed in *E. coli* TOP10 (Invitrogen). Plasmids (pCR2.1 NDM-5 and pBAD NDM-5) were extracted, and the bla_{NDM} allele was 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, United Kingdom). All sequencing was performed at Source BioSciences Ltd. (Cambridge, United Kingdom). Chromatograms were analyzed and consensus sequences aligned using BioEdit software version 7.0.9.0. Analysis of the predicted amino acid sequence revealed two substitutions at positions 88 (Val→Leu) and 154 (Met→Leu) relative to the NDM-1 (accession no. FN396876) and NDM-2 (accession no. AEA41876)

* Corresponding author. Mailing address: Centre for Immunology and Infectious Disease, Blizard Institute, 4 Newark Street, Whitechapel, London E1 2AT, United Kingdom. Phone: 44 (0)20 7882 2317 Fax: 44 (0)20 7882 2181. E-mail: d.w.wareham@qmul.ac.uk.

▽ Published ahead of print on 19 September 2011.

TABLE 1. Antibiotic susceptibilities of *E. coli* strains and transformants used

Isolate	MIC (μg/ml) by Etest on MH agar								
	Aztreonam	FOX	CTX	CAZ	Cefpirome	Piperacillin-tazobactam	ERT	IMP	MEM
EC405	>256	>256	>256	>256	>256	>256	>32	>32	>32
TOP10	0.125	16	0.047	0.38	0.035	4	0.008	0.25	0.047
TOP10 pCR2.1 NDM-1	0.19	24	0.125[a]	2[a]	0.25	>256	0.012	0.38	0.032
TOP10 pCR2.1 NDM-5	0.125	>256	48	>256	16	>256	0.016	0.25	0.032
TOP10 pCR2.1 P+NDM-1	0.125	>256	>256	>256	>256	>256	8	8	4
TOP10 pCR2.1 P+NDM-5	0.19	>256	>256	>256	>256	>256	32	>32	32
TOP10 pBAD P+NDM-1	0.125	>256	16	>256	6	>256	0.064	0.5	0.064
TOP10 pBAD P+NDM-5	0.19	>256	>256	>256	>256	>256	4	6	1.5

[a] Colonies growing within the zone of inhibition.

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 reamplification 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_{NDM-5} 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 *E. coli* TOP10.

The MICs of a range of β-lactams for *E. coli* TOP10 transformants were determined by Etest on Mueller-Hinton (MH) agar. The medium was supplemented with arabinose (0.0002 to 0.2%) and glucose (0.2%) for the transformant harboring pBAD NDM-5. Cephalosporin, aztreonam, and carbapenem (ERT, IMP, and MEM) MICs for transformants carrying bla_{NDM-5} 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 p*lac* or p*araBAD* 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, bla_{NDM-1} with and without the same promoter region was also cloned in the *E. coli* TOP10 background. The effects of NDM-5 on the susceptibility of *E. coli* to carbapenems as well as expanded-spectrum 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.

TABLE 2. Susceptibility of *E. coli* TOP10 transformants following inducible expression of NDM-5 from the p*araBAD* promoter

Isolate	MIC (μg/ml) by Etest on MH agar			
	CAZ	IMP	ERT	MEM
TOP10 pBAD NDM-5 (0.2% glucose)	0.38	0.19	0.008	0.23
TOP10 pBAD NDM-5 (0.0002% arabinose)	0.25	0.19	0.008	0.32
TOP10 pBAD NDM-5 (0.002% arabinose)	0.38	0.19	0.008	0.32
TOP10 pBAD NDM-5 (0.02% arabinose)	0.38	0.19	0.008	0.32
TOP10 pBAD NDM-5 (0.2% arabinose)	0.75	0.19	0.008	0.23

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 bla_{NDM-5} allele revealed the presence of part of IS*Aba125*, likely derived from *Acinetobacter baumannii*, creating a hybrid (−35/−10) promoter exactly as in an NDM-1-producing *E. coli* isolate described by Poirel et al. (8). Multilocus 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 648 (ST648). This ST has been shown to cause a significant proportion of ESBL-producing *E. coli* bacteremias in a series reported from the Rotterdam area of the Netherlands (10). In another recent study, three isolates of ST648 were shown to harbor NDM, two of which were recovered from patients hospitalized in Karachi, Pakistan, while the other was isolated from a patient in the United Kingdom (7).

Plasmids carried by EC405 were extracted using a Qiagen miniprep kit (Qiagen, Crawley, United Kingdom) and separated by agarose gel electrophoresis. The NDM-5 gene was localized to a plasmid of >100 kb by PCR using gel-purified plasmid DNA as the template and Southern hybridization with a digoxigenin-labeled (Roche, Burgess Hill, United Kingdom) bla_{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 *dfrA17* genes located within a class I integron structure and the 16S rRNA methylase gene, *rmtB*, thought to account for the high-level aminoglycoside resistance.

In summary, we identified a new NDM β-lactamase gene variant, bla_{NDM-5}, encoding the fifth enzymatic variant in this rapidly emerging and troublesome family of β-lactamases. The NDM-producing isolate was recovered from a patient with a history of travel to the Indian subcontinent.

REFERENCES

1. **Carattoli, A., et al.** 2005. Identification of plasmids by PCR-based replicon typing. J. Microbiol. Methods **63:**219–228.
2. **Clermont, O., S. Bonacorsi, and E. Bingen.** 2000. Rapid and simple determination of the *Escherichia coli* phylogenetic group. Appl. Environ. Microbiol. **66:**4555–4558.
3. **Dallenne, C., A. Da Costa, D. Decre, C. Favier, and G. Arlet.** 2010. Development of a set of multiplex PCR assays for the detection of genes encoding

important beta-lactamases in *Enterobacteriaceae*. J. Antimicrob. Chemother. **65:**490–495.
4. **Ellington, M. J., J. Kistler, D. M. Livermore, and N. Woodford.** 2007. Multiplex PCR for rapid detection of genes encoding acquired metallo-beta-lactamases. J. Antimicrob. Chemother. **59:**321–322.
5. **Kaase, M., et al.** 2011. NDM-2 carbapenemase in *Acinetobacter baumannii* from Egypt. J. Antimicrob. Chemother. **66:**1260–1262.
6. **Miriagou, V., et al.** 2010. Acquired carbapenemases in Gram-negative bacterial pathogens: detection and surveillance issues. Clin. Microbiol. Infect. **16:**112–122.
7. **Mushtaq, S., et al.** 2011. Phylogenetic diversity of *Escherichia coli* strains producing NDM-type carbapenemases. J. Antimicrob. Chemother. **66:**2002–2005.
8. **Poirel, L., R. A. Bonnin, and P. Nordmann.** 2011. Analysis of the resistome of a multidrug-resistant NDM-1-producing *Escherichia coli* by high-throughput genome sequencing. Antimicrob. Agents Chemother. **55:**4224–4229.
9. **Samra, Z., et al.** 2008. Evaluation of CHROMagar KPC for rapid detection of carbapenem-resistant *Enterobacteriaceae*. J. Clin. Microbiol. **46:**3110–3111.
10. **van der Bij, A. K., et al.** 2011. Clinical and molecular characteristics of extended-spectrum-beta-lactamase-producing *Escherichia coli* causing bacteremia in the Rotterdam Area, Netherlands. Antimicrob. Agents Chemother. **55:**3576–3578.
11. **Walsh, T. R.** 2010. Emerging carbapenemases: a global perspective. Int. J. Antimicrob. Agents **36**(Suppl. 3)**:**S8–S14.
12. **Yong, D., et al.** 2009. Characterization of a new metallo-beta-lactamase gene, bla(NDM-1), and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. Antimicrob. Agents Chemother. **53:**5046–5054.