OPEN

Emerging Microbes and Infections (2015) 4, e72; doi:10.1038/emi.2015.72

www.nature.com/emi

LETTER TO THE EDITOR

Emergence of a New Delhi metallo-β-lactamase-1-producing *Pseudomonas aeruginosa* in Singapore

Jeanette WP Teo[1], My-Van La[2], Roland Jureen[1] and Raymond TP Lin[1,2]

Emerging Microbes and Infections (2015) **4,** e72; doi:10.1038/emi.2015.72; published online 25 November 2015

Dear Editor,

Among the clinically significant carbapenemases, the New Delhi metallo-β-lactamase (NDM) is one of the most formidable. NDM efficiently hydrolyses β-lactams and last-resort carbapenems. Hence, therapeutic options for infections by NDM-producers are restricted to a handful of antibiotics, such as colistin, tigecycline, and fosfomycin.[1] NDM is predominantly associated with Enterobacteriaceae. This carbapenemase has also been described in *Acinetobacter* spp. but has been much less frequently detected in *Pseudomonas aeruginosa*.[1]

The first report of NDM-1 production in *P. aeruginosa* came from Serbia in 2011.[2] It is now acknowledged that the NDM-1 gene is endemic to the Balkan states.[3,4] NDM-producing *P. aeruginosa* has since been isolated from other European countries,[4–7] as well as in India[8] and Egypt.[9] Local hospital laboratory surveillance suggests that approximately 12% of *P. aeruginosa* isolates are not susceptible to carbapenems.[10] Previously, only producers of the metallo-β-lactamases (MBLs) VIM and IMP had been sporadically detected.[11] Here, we describe the first observed case of NDM-1-producing *P. aeruginosa* in Southeast Asia.

P. aeruginosa was cultured from the endotracheal aspirate of a 90-year-old female patient with colon cancer in March 2015. The isolate exhibited multidrug resistance to carbapenems (meropenem, imipenem, minimum inhibitory concentrations (MICs) > 32 mg/L), cephalosporin (ceftazidime, cefepime, MICs > 256 mg/L), aminoglycosides (gentamicin, amikacin, MICs > 256 mg/L), and fluoroquinolones (ciprofloxacin, levofloxacin, MICs > 32 mg/L). The isolate was partially resistant to aztreonam (MIC 16 mg/L). Colistin susceptibility was observed at an MIC of 1 mg/L. Phenotypic testing for carbapenemases using the KPC/MBL Confirm Kit (Rosco Diagnostica A/S, Taastrup, Denmark) indicated the presence of an MBL.

Comprehensive polymerase chain reaction (PCR) screening for β-lactamase genes was performed.[12] The isolate was positive for bla_{NDM} and determined to be bla_{NDM-1} by full-length gene sequencing. The isolate was negative for other MBLs (IMP, VIM, SPM, DIM, AIM) and for genes encoding class A carbapenemases (KPC, GES). TEM and CTX-M extended spectrum β-lactamases (ESBLs) were found to be present.

Plasmid analysis using S1 nuclease pulsed-field gel electrophoresis[13] and spin column plasmid extractions (QIAprep Spin Miniprep Kit, QIAGEN, Valencia, CA, USA) did not the reveal the presence of plasmids. Southern blot analysis with a bla_{NDM-1} probe of S1 nuclease-treated DNA agarose plugs indicated that the probe hybridized to high molecular weight chromosomal DNA, suggesting that bla_{NDM-1} was situated on the chromosome (data not shown). Furthermore, solid media conjugation assays were performed to assess the transferability of bla_{NDM-1} from the clinical isolate to the azide-resistant recipient *Escherichia coli* J53. No transconjugants were obtained, suggesting the non-transmissibility of bla_{NDM-1} (at least to *E. coli*), again suggesting a chromosomal position. Because the isolate was highly resistant to most antibiotics, including rifampicin, this excluded the use of the rifampicin-resistant laboratory strain of *P. aeruginosa* for conjugation. Hence, we were unable to assess the intra-species transmissibility of bla_{NDM-1}. *P. aeruginosa* bla_{NDM-1} may be present chromosomally[2,5,6] or on a plasmid; in the latter context, bla_{NDM-1} is transmissible.[8]

The nucleotide sequences immediately flanking bla_{NDM-1} were determined by an inverse PCR and primer walking approach. Two copies of bla_{NDM-1} were detected and separated by an Insertion Sequence Common Region (IS*CR*) element (Figure 1). Sequencing of this IS*CR* element revealed a 97% nucleotide homology to IS*CR24*. IS*CR24* has been identified in the genetic environment of a novel bla_{PME-1} (*Pseudomonas aeruginosa* ESBL 1) and implicated in the acquisition of the ESBL by *P. aeruginosa*.[15] This is not surprising because IS*CR* elements mediate the mobilization of almost every class of antibiotic resistance genes, including those encoding ESBLs and carbapenemases.[16] IS*CR* elements, such as IS*CR1*, have been found to be associated with NDM-1 from *P. aeruginosa*.[3,5,7] Jovcic *et al.*[3] reported two copies of bla_{NDM-1} in *P. aeruginosa* (Figure 1), where it is presumed that the IS*CR1* element, as part of its rolling-circling mechanism of transposition, duplicates adjacent genetic segments.[16] Because IS*CR* elements are known to construct extended clusters of antibiotic resistance genes on plasmids as well as on chromosomes,[16] it would be interesting to investigate the presence of other resistance determinants surrounding bla_{NDM-1} and the IS*CR24*-like region that may contribute to its multidrug resistance.

After this isolate was identified, two other NDM-1-producing *P. aeruginosa* isolates with antibiograms identical to the initial isolate were cultured from the sputum samples of two other patients in April and May 2015. One of the isolates was from a 58-year-old male with intracranial bleeding from a ruptured aneurysm, and the other was from an 84-year-old female with a femur fracture, complicated by pancreatitis and small intestine perforation. The genetic relatedness of the three NDM-1 *P. aeruginosa* isolates was investigated by DiversiLab

[1]Department of Laboratory Medicine, Division of Microbiology, National University Hospital, Singapore 119074, Republic of Singapore and [2]National Public Health Laboratory, Ministry of Health, Synapse, Singapore 138623, Republic of Singapore
Correspondence: Jeanette WP Teo
E-mail: Jeanette_Teo@nuhs.edu.sg
Received 26 August 2015; revised 21 September 2015; accepted 25 September 2015

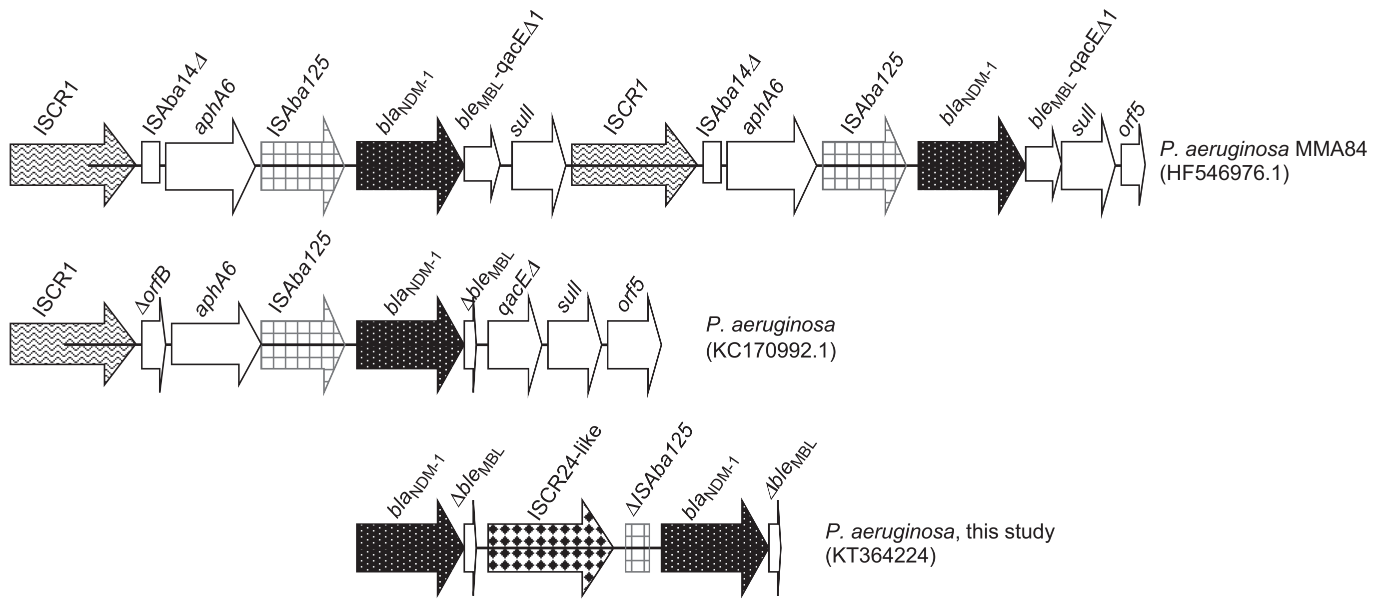

Figure 1 Comparative schematic diagrams of the diverse genetic organizations of $bla_{\mathrm{NDM-1}}$ in *P. aeruginosa*. The selected $bla_{\mathrm{NDM-1}}$ sequences are chromosomally located and associated with IS*CR* elements and class I integrons.[3,5] Typical common genetic features surrounding $bla_{\mathrm{NDM-1}}$ are the insertion sequence IS*Aba125* and the bleomycin resistance gene (ble_{MBL}).[14]

rep-PCR fingerprinting (bioMérieux, Marcy l'Etoile, France), which revealed that the rep-PCR profiles were indistinguishable, suggesting that the three isolates were clonal in nature. PCR mapping and sequencing of the two latter isolates reveal a bla_{NDM} genetic context identical to that of the first isolate. Because all three patients stayed in the same surgical intensive care unit, the detection of indistinguishable NDM-1-positive *P. aeruginosa* suggested a transmission event.

In summary, this is the first report of the emergence of NDM-1 in *P. aeruginosa* in Southeast Asia in an unusual genetic context. The apparent intra-ward transmission of this extremely drug-resistant isolate highlights the gravity of this escalating public health issue.

Nucleotide sequence accession number

The $bla_{\mathrm{NDM-1}}$ sequence from the initial *P. aeruginosa* isolate has been deposited into Genbank under the accession number KT364224.

ACKNOWLEDGEMENTS

This work was supported by a National University Health Engineering Medicine Seed Grant (#N171-000-099-001) provided to Jeanette WP Teo.

1 Dortet L, Poirel L, Nordmann P. Worldwide dissemination of the NDM-type carbapenemases in Gram-negative bacteria. *Biomed Res Int* 2014; **2014**: 249856.
2 Jovcić B, Lepsanovic Z, Suljagic V *et al*. Emergence of NDM-1 metallo-β-lactamase in *Pseudomonas aeruginosa* clinical isolates from Serbia. *Antimicrob Agents Chemother* 2011; **55**: 3929–3931.
3 Jovcić B, Lepsanović Z, Begović J *et al*. The clinical isolate *Pseudomonas aeruginosa* MMA83 carries two copies of the blaNDM-1 gene in a novel genetic context. *Antimicrob Agents Chemother* 2013; **57**: 3405–3407.
4 Kulkova N, Babalova M, Sokolova J, Krcmery V. First report of New Delhi metallo-β-lactamase-1-producing strains in Slovakia. *Microb Drug Resist* 2015; **21**: 117–120.
5 Janvier F, Jeannot K, Tessé S *et al*. Molecular characterization of blaNDM-1 in a sequence type 235 *Pseudomonas aeruginosa* isolate from France. *Antimicrob Agents Chemother* 2013; **57**: 3408–3411.
6 Carattoli A, Fortini D, Galetti R *et al*. Isolation of NDM-1-producing *Pseudomonas aeruginosa* sequence type ST235 from a stem cell transplant patient in Italy, May 2013. *Euro Surveill* 2013; **14**: 20633.
7 Wang M, Borris L, Aarestrup FM, Hasman H. Identification of a *Pseudomonas aeruginosa* co-producing NDM-1, VIM-5 and VIM-6 metallo-β-lactamases in Denmark using whole-genome sequencing. *Int J Antimicrob Agents* 2015; **45**: 324–325.
8 Khajuria A, Praharaj AK, Kumar M, Grover N. Emergence of NDM - 1 in the clinical isolates of *Pseudomonas aeruginosa* in India. *J Clin Diagn Res* 2013; **7**: 1328–1331.
9 Zafer MM, Amin M, El Mahallawy H, Ashour MS, Al Agamy M. First report of NDM-1-producing *Pseudomonas aeruginosa* in Egypt. *Int J Infect Dis* 2014; **29**: 80–81.
10 Tan TY, Hsu LY, Koh TH *et al*. Antibiotic resistance in gram-negative bacilli: a Singapore perspective. *Ann Acad Med Singapore* 2008; **37**: 819–825.
11 Koh TH, Khoo CT, Tan TT *et al*. Multilocus sequence types of carbapenem-resistant *Pseudomonas aeruginosa* in Singapore carrying metallo-beta-lactamase genes, including the novel bla(IMP-26) gene. *J Clin Microbiol* 2010; **48**: 2563–2564.
12 Teo J, Ngan G, Balm M, Jureen R, Krishnan P, Lin R. Molecular characterization of NDM-1 producing Enterobacteriaceae isolates in Singapore hospitals. *Western Pac Surveill Response J* 2012; **3**: 19–24.
13 Barton BM, Harding GP, Zuccarelli AJ. A general method for detecting and sizing large plasmids. *Anal Biochem* 1995; **10**: 235–240.
14 Poirel L, Dortet L, Bernabeu S, Nordmann P. Genetics features of blaNDM-1-positive Enterobacteriaceae. *Antimicrob Agents Chemother* 2011; **55**: 5403–5407.
15 Tian GB, Adams-Haduch JM, Bogdanovich T, Wang HN, Doi Y. PME-1, an extended-spectrum β-lactamase identified in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 2011; **55**: 2710–2713.
16 Toleman MA, Bennett PM, Walsh TR. ISCR elements: novel gene-capturing systems of the 21st century? *Microbiol Mol Biol Rev* 2006; **70**: 296–316.

This work is licensed under a Creative Commons Attribution 4.0 Unported License. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder to reproduce the material. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/