NDM-2 carbapenemase in *Acinetobacter baumannii* from Egypt

Martin Kaase¹, Patrice Nordmann²*, Thomas A. Michelhaus³, Sören G. Gatermann¹, Rémy A. Bonnin² and Laurent Poirel²

¹National Reference Laboratory for Multidrug-Resistant Gram negative Bacteria, Department of Medical Microbiology, Ruhr-University Bochum, 44801 Bochum, Germany; ²Service de Bactériologie-Virologie, INSERM U914 ‘Emerging Resistance to Antibiotics’, Hôpital de Bicêtre, Assistance Publique/Hôpitaux de Paris, Faculté de Médecine et Université Paris-Sud, K.-Bicêtre, Paris, France; ³Institute of Medical Microbiology and Infection Control, Hospital of Goethe-University, Paul-Ehrlich-Str. 40, 60596 Frankfurt/Main, Germany

*Corresponding author. Tel: +33-1-45-21-36-32; Fax: +33-1-45-21-63-40; E-mail: nordmann.patrice@bct.aphp.fr

Received 11 February 2011; returned 25 February 2011; revised 1 March 2011; accepted 2 March 2011

**Objectives:** To analyse the mechanisms responsible for carbapenem resistance in one *Acinetobacter baumannii* isolate recovered from a patient transferred to Germany from an Egyptian hospital.

**Methods:** PCR and sequencing were used to search for β-lactamase and 16S RNA methylase genes. Multilocus sequence typing was used to determine the sequence type (ST) of the isolate.

**Results:** Sequencing of the PCR product obtained using primers for *bla*NDM-1 revealed a variant of NDM-1 that had a C to G substitution at position 82 resulting in an amino acid substitution of proline to alanine at position 28. This variant was designated NDM-2. Genes encoding extended-spectrum β-lactamases or 16S RNA methylase were not detected. The strain lacked detectable plasmids and *bla*NDM-2 was not transferred by conjugation. MLST showed that the isolate belonged to a new ST, ST103.

**Conclusions:** This work further underlines the spread of NDM carbapenemases in *A. baumannii*, and the spread of the corresponding gene in the Middle East. It also describes the first variant of NDM-1.

**Keywords:** metallo-β-lactamases, Gram-negative, carbapenems

**Introduction**

Recent studies have shown the worldwide spread of carbapenemase-producing Gram-negative bacilli. Spread of the metallo-β-lactamase (MBL) NDM-1, initially reported in *Klebsiella pneumoniae* and *Escherichia coli*, currently represents one of the most significant threats.¹ A high prevalence of NDM-1 producers has been reported among enterobacterial isolates recovered from the Indian subcontinent.² Additionally, there have been scattered reports of NDM-1 producers in Africa, Asia, Australia, America and Europe.³ Several *Acinetobacter baumannii* isolates expressing NDM-1 have been detected in India,⁴ and a recent study reported an NDM-1-producing *A. baumannii* in Germany.⁵ That strain was from a patient transferred from a hospital in Serbia to the University Hospital of Frankfurt, without an obvious link with the Indian subcontinent.

**Materials and methods**

**Bacterial isolates and susceptibility testing**

*A. baumannii* was identified by using the API20NE system (bioMérieux, Marcy l’Étoile, France). The antibiotic susceptibility of the isolate was determined by the disc diffusion technique on Mueller–Hinton agar.⁶ MICs were determined by using Etest strips (AB biolMérieux, Solna, Sweden). *E. coli* 271 was used as a source for amplification and cloning of the *blaNDM-1* gene.⁶

**PCR amplification and sequencing**

Multiplex PCR approaches were used to search for Ambler class B and carbapenem-hydrolysing class D β-lactamase genes.⁷,⁸ Amplified DNA fragments were purified with the Qiaquick PCR purification kit (Qiagen, Courtaboeuf, France). Both strands of the amplification products obtained were sequenced with an ABI 3100 sequencer (Applied Biosystems, Foster City, CA, USA). The nucleotide and deduced protein sequences were analysed with software available over the Internet at the National Center for Biotechnology Information web site (www.ncbi.nlm.nih.gov).

**Plasmid analysis**

Conjugation assays were performed using *A. baumannii* ML as donor and azide-resistant *E. coli* JS3 (Invitrogen, Cergy-Pontoise, France) and *A. baumannii* CIP70.10 (rifampicin resistant) as recipient strains, using selection based on ceftazidime (30 mg/L) and azide (100 mg/L) or...
rifaximin (200 mg/L), respectively. Plasmid DNA was extracted using the Kieser method.9

Comparative analysis of NDM-1 and NDM-2

In order to compare the relative contributions of NDM-1 and NDM-2 to carbapenem resistance, the corresponding genes were cloned and expressed in an isogenic E. coli background under the control of the same promoter (E. coli TOP10; Invitrogen). Cloning experiments were performed using the pCR-BluntIII-TOPO vector (Invitrogen) following the manufacturer’s instructions with 984 bp PCR amplicons as targets that had been generated using external primers Pre-NDM-A (5′-CACCT CATGTTGAATTCCGC-3′) and Pre-NDM-B (5′-CTCTGTCACATGAATCGCC-3′) encompassing the entire blaNDM genes with the same promoter sequences.

Strain genotyping

A multilocus sequence typing (MLST) method was performed as described.10 Sequences of the seven housekeeping genes were analysed on the database (http://www.pasteur.fr/recherche/genopole/PFB/mlst/Abaumannii.html).

Results and discussion

Here we report on a young child who had a traffic accident during a holiday in Egypt. She suffered a severe cranioencephalic injury with diffuse brain oedema, pelvic fracture and splenic laceration and was hospitalized in the intensive care units of two different hospitals in Cairo. No information was available regarding antibiotic use during this time. Three years after this accident, the patient was transferred to Germany, where culture of a central venous line catheter (placed at the Egyptian hospital) performed on the day of admission grew Staphylococcus epidermidis and carbapenem-resistant A. baumannii (designated strain ML). Rectal screening for carriage of multidrug-resistant bacteria was negative. Owing to her poor prognosis, the patient did not receive any antibiotics while hospitalized in Germany and died 7 days later.

A. baumannii ML was resistant to ceftazidime and cefepime (MICs >256 mg/L), to carbapenems (MICs of imipenem, meropenem and doripenem >32 mg/L), to aminoglycosides (MICs of tobramycin, amikacin and gentamicin >32 mg/L), to chloramphenicol (MIC >256 mg/L) and to ciprofloxacin (MIC >32 mg/L). MICs for strain ML were 4 mg/L of rifampicin, 8 mg/L of tigecycline and 0.25 mg/L of colistin. A combined disc test performed using 930 μg of EDTA and a disc of imipenem was positive for MBL production. In addition, the MBL-Etest (AB Biodisk) was performed using 930 μg of tigecycline and 0.25 mg/L of colistin. A combined disc test.

Cloning gave rise to recombinant strains E. coli TOP10 (pNDM-1) and E. coli TOP10 (pNDM-2), respectively. MIC values of all β-lactams including carbapenems showed no significant differences for those two E. coli recombinant strains, indicating that NDM-2 and NDM-1 probably share an identical spectrum of hydrolysis (data not shown).

A search for ISAba1 upstream of the intrinsic blaOXA-51 gene gave a negative result, indicating that this gene was probably not expressed. In addition, genes encoding 16S rRNA methylase, which confers resistance to all aminoglycosides, were not detected by PCR, although NDM-1-producing A. baumannii possessing the armA gene has been identified previously.3 Analysis by gel electrophoresis failed to demonstrate plasmids in extracts of strain ML and attempts to transfer resistance in mating experiments were unsuccessful. This suggests that the blaNDM-2 gene was chromosomally encoded in strain ML.

This is the first report of an NDM-1 variant, although the ongoing spread of strains carrying the blaNDM-1 gene will enhance the likelihood of variants emerging. This is an important consideration when designing genetic tools to target carbapenem resistance genes. Interestingly, we have evidence that NDM-encoding genes may be widespread in A. baumannii, and further molecular surveys will be necessary to evaluate their distribution in that species. We showed here the first identification of a blaNDM gene in a clinical isolate originating from Egypt, with no obvious link with the Indian subcontinent. This clinical case suggests that NDM-producing isolates have already disseminated in the Middle East, after the recent identification of NDM producers in Iraq and the Sultanate of Oman.11,12

Funding

This work was mostly funded by the INSERM (U914), France, and by grants from the Ministère de l’Education Nationale et de la Recherche (UPRES-EA3539), Université Paris XI, France and from the European Community (TEMPTest-QC, HEALTH-2009-241742).

Transparency declarations

None to declare.

References