NDM-1-producing *Klebsiella pneumoniae* isolated in the Sultanate of Oman

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Received 22 September 2010; returned 27 September 2010; revised 16 October 2010; accepted 17 October 2010

**Objectives:** To analyse the mechanisms responsible for multidrug resistance in two carbapenem-resistant *Klebsiella pneumoniae* isolates recovered from patients hospitalized in Oman.

**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 each isolate. Clonal relationships were evaluated by PFGE.

**Results:** Both isolates carried the *bla*NDM-1 carbapenemase gene. Isolate 601 was recovered from a patient who was transferred from India, whereas isolate 419 was from an Omani patient who had not travelled abroad. The two isolates were clonally unrelated, and belonged to ST14 (isolate 601) and ST340 (isolate 419). In addition to NDM-1, the ST14 isolate expressed β-lactamases CTX-M-15, SHV-28, OXA-1, OXA-9 and TEM-1, and the aminoglycoside resistance methylase ArmA. The ST340 isolate expressed β-lactamases SHV-11, OXA-1 and ArmA. In both isolates, the *bla*NDM-1 gene was located on plasmids that were of similar size (170 kb), but of different incompatibility groups.

**Conclusion:** This is the first description of NDM-1 producers in the Arabian peninsula and in the Middle East.

Keywords: MBLs, carbapenemases, Arabian peninsula

**Introduction**

The *bla*NDM-1 gene is plasmid borne and encodes a metallo-β-lactamase (MBL) that was first identified in *Escherichia coli* and *Klebsiella pneumoniae* in Sweden in 2008 from a patient transferred from India.1 This enzyme hydrolyses all β-lactams except aztreonam and is commonly identified in multidrug-resistant isolates. The rapid emergence of this resistance determinant in a series of multiresistant enterobacterial strains in the UK, India and Pakistan, together with evidence of epidemiological links between infections occurring in these countries was reported recently.2 Subsequent reports of NDM-1-producing Gram-negative organisms from Australia, the USA and France with links to the Indian subcontinent give further cause for concern that this resistance is spreading globally.3–5 We now report the identification of two *bla*NDM-1-positive *K. pneumoniae* isolates recovered at a hospital in Muscat, Sultanate of Oman.

**Materials and methods**

**Bacterial isolates and susceptibility testing**

*K. pneumoniae* isolates 601 and 419 were identified using the API20E system (bioMérieux, Marcy l’Etoile, France). The antibiotic susceptibility of the two isolates and their corresponding *E. coli* transformants were determined by the disc diffusion technique on Mueller–Hinton agar with β-lactam and non-β-lactam antibiotic discs and interpreted according to the June 2010 updated CLSI guidelines.6 MICs were determined with Etest strips (AB bioMérieux, Solna, Sweden).

Azide-resistant *E. coli* J53 (Invitrogen, Cergy-Pontoise, France) was used as the host in conjugation experiments.

**PCR amplification and sequencing**

PCRs were performed with primers designed for the detection of Ambler class A and B β-lactamase genes.7 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 on the internet at the National Center for Biotechnology Information web site (www.ncbi.nlm.nih.gov).

**Plasmid analysis**

Conjugation assays were performed using *K. pneumoniae* 601 and 419 isolates as donors and an azide-resistant *E. coli* J53 as the recipient strain, with selection based on growth on agar in the presence of ceftazidime (30 mg/L) and azide (100 mg/L). Plasmid DNA was extracted using the Kieser method. Plasmid incompatibility groups were determined by a PCR-based replicon typing (PBRT) method as previously described.

**Strain genotyping**

Multilocus sequence typing (MLST) was performed as described previously in order to determine the sequence types (STs) of the two *K. pneumoniae* isolates, and to establish a comparison with previously reported NDM-1-producing isolates. In addition, PFGE was performed using the XbaI restriction enzyme to evaluate the clonal relationship between the different NDM-1 producers.

**Results and discussion**

The first isolate was recovered from a urinary catheter of an Omani patient previously admitted to an Indian hospital (precise location unknown) for pneumonia in March 2009. The patient was transferred to Oman and admitted to the intensive care unit (ICU) where endotracheal secretions grew a multidrug-resistant *Acinetobacter baumannii* together with a methicillin-resistant *Staphylococcus aureus*. The patient was treated with meropenem and colistin inhalation for 10 days and recovered. Systematic surveillance for urine colonization identified a multidrug-resistant *K. pneumoniae* (designated strain 601) that was resistant to all β-lactams including carbapenems (MICs of imipenem, ertapenem and meropenem all >32 mg/L). PCR and sequencing revealed that the isolate harboured the blαNDM-1 gene, with additional β-lactamase genes including the extended-spectrum β-lactamase genes blaCTX-M-15 and blaSHV-28, and blaOXA-1, blaOXA-9 and blaTEM-1. In addition, the armA and aacA4 genes encoding resistance to aminoglycosides were identified. Mating assays identified the blαNDM-1 gene on a 170 kb transferable plasmid. This plasmid (p601) also harboured the armA gene, which co-transferred resistance to all aminoglycosides, and belonged to the IncL/M incompatibility group. The MICs of imipenem, ertapenem and meropenem for an *E. coli* transconjugant containing p601 were 1, 1 and 0.5 mg/L, respectively.

The second patient, who had been involved in a road traffic accident, was admitted to the same ICU in the autumn of accident, was admitted to the same ICU in the autumn of 2009. Approximately 3 months after the first patient had left. This patient undergone orthopaedic surgery, and received a 5 day regimen of piperacillin/tazobactam and amikacin following onset of a fever. Again, surveillance of urine showed colonization with a multidrug-resistant *K. pneumoniae* (strain 419). However, this patient remained asymptomatic and was discharged 3 months later without any other febrile episode. Isolate 419 was resistant to penicillins, cephalosporins, ertapenem (MIC 2 mg/L) and meropenem (MIC 6 mg/L) and had decreased susceptibility to imipenem (MIC 2 mg/L), but was susceptible to aztreonam. It was also resistant to all aminoglycosides, sulphonamides, tetracycline and fluoroquinolones, and remained susceptible only to tigecycline and colistin. Isolate 419 harboured the blαNDM-1 gene with β-lactamase genes blαOXA-1 and blαSHV-11. In addition, the armA and aacA4 genes encoding resistance to aminoglycosides were identified. Mating assays demonstrated that the blαNDM-1 gene was on a 170 kb transferable plasmid co-harbouring the armA gene as observed for isolate 601. However, the PBRT method did not allow us to determine the Inc type of plasmid p419. An *E. coli* transconjugant (p419) exhibited imipenem, ertapenem and meropenem MICs of 1, 1 and 0.5 mg/L, respectively. In addition to resistance to β-lactams and aminoglycosides, it was also resistant to trimethoprim.

MLST identified *K. pneumoniae* 601 as an ST14 strain, which matches the genetic background of the index NDM-1-producing *K. pneumoniae* isolate originating from India, and of the NDM-1-producing *K. pneumoniae* isolates recently identified from Kenya (L. Poirel, G. Revathi, S. Bernabeu and P. Nordmann, unpublished data). PFGE analysis confirmed that *K. pneumoniae* 601 was clonally related to *K. pneumoniae* 05-506 from India and to NDM-1-producing isolates from Kenya (Figure 1). However, isolate 419 (which was not thought to be an imported strain) was not clonally related by PFGE analysis, and MLST typing revealed that it belonged to ST340, which is significantly different from ST14. This finding suggests that the ST340 clone may have originated from Oman or from another country. *K. pneumoniae* 419 harboured a blαNDM-1-positive plasmid (p419), the replicase gene of which was untypeable, thus suggesting that plasmids p419 and p601 were different. To further assess the relationship between the two plasmids, both were analysed by restriction fragment length polymorphisms using different endonucleases. Despite repeated attempts, self-degradation of plasmid p419 did not allow comparison using this technique (data not shown).

This is the first identification of NDM-1 producers in the Arabian peninsula. Interestingly, one of the two isolates was clonally related to the strain previously identified in India, strongly suggesting its importation from India. This further emphasizes that the Indian subcontinent may be an important

![Figure 1. PFGE DNA patterns generated by XbaI restriction enzyme treatment of several NDM-1-producing K. pneumoniae isolates. Lanes 1 and 2, KP1 and KP2 from Kenya; lanes 3, reference strain from India; lanes 4 and 5, KP601 and KP419 from Oman (this study).](image-url)
reservoir of NDM-1. This is especially of concern in the Sultanate of Oman where up to 450000 Indian or Pakistani citizens live.

Acknowledgements
We thank T. R. Walsh for the gift of *K. pneumoniae* 05-506 used as a control strain.

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

Transparency declarations
None to declare.

References