Emergence of NDM-1-producing *Klebsiella pneumoniae* in Morocco

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Objectives: To analyse the mechanisms responsible for multidrug resistance in three carbapenem-resistant *Klebsiella pneumoniae* isolates recovered from two hospitalized patients and an outpatient from the same hospital in Rabat, Morocco.

Methods: PCR and sequencing were used to search for β-lactamase genes. Clonal relationships between the isolates were analysed by multilocus sequence typing and PFGE.

Results: A history of prior hospitalization in the same ward was determined for two of the three patients. The three isolates of *K. pneumoniae* belonged to the same sequence type (ST), ST15, and were clonally related. All three isolates carried the *blaNDM-1* gene and co-expressed the extended-spectrum β-lactamases CTX-M-15 and SHV-5, as well as the narrow-spectrum β-lactamases SHV-1, OXA-1, OXA-9 and TEM-1. The *blaNDM-1* gene was located on an ~250 kb non-typeable plasmid co-harbouring the *blaOXA-1* and *blaCTX-M-15* genes. No link with the Indian subcontinent could be established for the three patients.

Conclusions: This work further emphasizes the spread of NDM-1 producers worldwide.

Keywords: plasmids, carbapenemases, Enterobacteriaceae

Introduction

Carbapenem-hydrolysing β-lactamases belonging to Ambler classes A, B and D are increasingly reported worldwide among Enterobacteriaceae.1 Whereas KPC-type (class A) producers are mostly identified in the USA, Greece and Israel, OXA-48 (class D) producers are mostly from the Mediterranean area, including Morocco.1,2 Recently, the class B producers have subsequently been identified in the UK, India and Pakistan, and identified in *K. pneumoniae* isolates recovered in Sweden from a patient previously hospitalized in India.6 NDM-1 producers have subsequently been identified in the UK, India and Pakistan, and identified in *K. pneumoniae*, *E. coli*, *Citrobacter freundii*, *Morganella morganii*, *Providencia* spp. and *Enterobacter cloacae* isolates.3 Additionally, many studies have reported identification of NDM-1-producing enterobacterial isolates in different parts of the world. NDM-1 producers have been identified in the Middle East, with two *K. pneumoniae* from the Sultanate of Oman and one from Iraq, and one *Acinetobacter baumannii* isolate producing NDM-2 from Egypt.3,5 Very recently, NDM-1 producers were found in seepage samples and public tap water in New Delhi, India, showing that the dissemination of these multidrug-resistant bacteria might be higher than expected.6

This study was initiated following isolation of multiresistant *K. pneumoniae* from three patients (two inpatients and one outpatient) attending the same hospital in Rabat, Morocco.

Materials and methods

**Bacterial isolates and susceptibility testing**

*K. pneumoniae* isolates were identified using the API20E system (bioMérieux, Marcy l’Etoile, France). Strain isolation and identification were performed locally in Morocco and strains were then further analysed in France. The antibiotic susceptibility of each isolate and their corresponding *E. coli* transformants was determined using the disc diffusion technique on Mueller–Hinton agar plates with β-lactam and non-β-lactam antibiotic-containing discs and interpreted according to the CLSI guidelines.7 Azide-resistant *E. coli* JS3 (Invitrogen, Cergy-Pontoise, France) was used as a host in conjugation experiments.2

**PCR amplification and sequencing**

PCRs were performed with a series of primers designed for the detection of Ambler class A, B and C β-lactamase genes.2 Screening of 16S rRNA methylase-encoding genes was performed using a multiplex PCR approach as described previously.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 from the National Center for Biotechnology Information web site (www.ncbi.nlm.nih.gov).

**Plasmid analysis**

Conjugation assays were performed using each of the three *K. pneumoniae* clinical isolates as donors and an azide-resistant *E. coli* J53 as the recipient strain. Selection of transconjugants was undertaken by plating on Mueller–Hinton agar containing cefoxitin (10 mg/l) and azide (100 mg/l). Plasmid incompatibility groups were determined by a PCR-based replicon typing method (PBRT) as described previously.

**Strain genotyping**

Multilocus sequence typing (MLST) was performed as described previously. In addition, PFGE was performed using the XbaI restriction enzyme to evaluate the clonal relationship between the different NDM-1 producers.

**Results and discussion**

Isolate 1 was from the urine of an elderly male patient hospitalized in a medical department who had previously received broad-spectrum cephalosporins and fluoroquinolones for recurrent urinary tract infections. Once the *K. pneumoniae* strain was isolated, the treatment was changed to amikacin and colistin, and the patient recovered. This patient had a history of prior hospitalization 1 month before in the same hospital in the surgery and intensive care departments for treatment of vessel cancer. Isolate 2 was recovered 10 days later from a blood culture of an aplastic female in her 60s who was hospitalized in the same hospital and in the same intensive care department. She was treated with imipenem and metronidazole, but died. This patient had a history of prior hospitalization 1.5 months before in the same hospital in the surgery department for treatment of throat cancer and hospitalization in another hospital 15 days before. Isolate 3 was recovered from a pancreatic abscess of a middle-aged female patient who was an outpatient from the same hospital. This patient had been hospitalized in another hospital for treatment of this pancreatic abscess. No information is available concerning her treatment and outcome.

The three isolates had the same antibiotic susceptibility pattern, being susceptible to imipenem and doripenem with MICs of 1 mg/l, of intermediate susceptibility to meropenem with MICs of 2 mg/l and resistant to ertapenem with MICs of 4 mg/l. They were also resistant to aminoglycosides (except to amikacin, being of intermediate susceptibility), fluoroquinolones, chloramphenicol, sulphonamides and rifampicin. They remained susceptible to fosfomycin, tigecycline, colistin and nitrofurantoin. The three isolates were positive for *bla*<sub>NDM-1</sub> and *bla*<sub>TEM-1</sub> and sequencing revealed that they were producing NDM-1.

PFGE analysis showed an identical pattern for all isolates. The clonal relationship was further confirmed by MLST, which showed that the three *K. pneumoniae* isolates belonged to the same sequence type (ST), ST15. Notably, *bla<sub>CTX-M-15</sub>*-positive but *bla<sub>NDM-1</sub>*-negative *K. pneumoniae* strains of ST15 have been found to be endemic both in Hungary and Denmark. No screening for carriage of other NDM-1 producers was performed among neighbouring hospitalized patients since the *K. pneumoniae* isolates were analysed retrospectively.

A search for narrow-spectrum β-lactamase, extended-spectrum β-lactamase (ESBL) and AmpC genes revealed that all isolates harboured the narrow-spectrum β-lactamase genes *bla<sub>TEM-1</sub>*, *bla<sub>SHV-1</sub>*, *bla<sub>OXA-1</sub>* and *bla<sub>OXA-9</sub>*. They were also resistant to aztreonam (which indicates a synergy was determined with clavulinate), and having decreased susceptibility to carbapenems (MICs of imipenem, meropenem, ertapenem and doripenem being 1, 1, 2 and 0.5 mg/l, respectively (the MICs of these compounds being 0.12 mg/l for the *E. coli* J53 recipient strain)). The *E. coli* transconjugants were consequently sensitive to ticarcillin, and the patient recovered. This patient had a history of prior hospitalization 1.5 months before in the same hospital in the surgery department for treatment of vessel cancer. Isolate 2 was recovered 10 days later from a blood culture of an aplastic female in her 60s who was hospitalized in the same hospital and in the same intensive care department. She was treated with imipenem and metronidazole, but died. This patient had a history of prior hospitalization 1.5 months before in the same hospital in the surgery department for treatment of throat cancer and hospitalization in another hospital 15 days before. Isolate 3 was recovered from a pancreatic abscess of a middle-aged female patient who was an outpatient from the same hospital. This patient had been hospitalized in another hospital for treatment of this pancreatic abscess. No information is available concerning her treatment and outcome.

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**Transparency declarations**

None to declare.

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