Funding
This work was supported by the United Kingdom Clinical Research Collaboration (UKCRC) and the National Institute for Health Research NIHR Biomedical Research Centre Oxford (OxBRC). The clinical and microbiological work in Laos was funded by the Wellcome Trust of Great Britain as part of the Wellcome Trust–Mahosot Hospital–Oxford Tropical Medicine Research Collaboration.

Transparency declarations
None to declare.

References

J Antimicrob Chemother 2012
doi:10.1093/jac/dkr426
Advance Access publication 3 October 2011

Importation of KPC-2-producing Escherichia coli from India
Anaïs Potron1, Laurent Poirel1, Delphine Verdavaine2 and Patrice Nordmann1*

1Service de Bacteriologie-Virologie, INSERM U914 ‘Emerging Resistance to Antibiotics’, Hôpital de Bicêtre, Assistance Publique/Hôpitaux de Paris, Faculté de Médecine et Université Paris-Sud, 78 rue de Général Leclerc, K.-Bicêtre, France; 2Centre Hospitalier ‘René Pleven’, Dinan, France
*Corresponding author. Tel: +33-1-45-21-36-32; Fax: +33-1-45-21-63-40; E-mail: nordmann.patrice@bct.aphp.fr

Keywords: carbapenemases, Indian subcontinent, KPC

Sir,
The production of carbapenem-hydrolysing β-lactamases is increasingly reported in Enterobacteriaceae. Among the different types of carbapenemases, the emergence of the Ambler class A KPC-type β-lactamases is of great concern, since these enzymes hydrolyse all β-lactams with the exception of cephapymcins. Enterobacterial isolates producing KPC-type β-lactamases were reported in many areas in the USA and subsequently worldwide.1 The rapid dissemination of KPC enzymes among different enterobacterial species is related to the localization of blaKPC genes on transferable broad host range plasmids and their association with a transposon.1 This dissemination has also been linked with a ‘successful’ international clone of KPC-producing Klebsiella pneumoniae of sequence type (ST) 258.2

Early in 2011, a middle-aged patient was transferred from a hospital in Mumbai, India, to the hospital in Dinan, France. The patient suffered from pleurisy due to Streptococcus pneumoniae for which he had received a combination of imipenem, vancomycin and piperacillin/tazobactam in India. Upon admission, a rectal swab revealed the presence of a multidrug-resistant Escherichia coli (designated strain GRU) with reduced susceptibility to carbapenems. No secondary local transmission occurred at the Dinan hospital following the rapid implementation of strict infection control measures.

The antibiogram determined by the disc diffusion method and MICs determined by Etest (AB bioMérieux, Solna, Sweden) and interpreted according to the CLSI guidelines3 revealed that E. coli strain GRU was resistant to all penicillins and expanded-spectrum cephalosporins, to ertapenem (MIC >32 mg/L) and to meropenem (MIC 8 mg/L) and was of intermediate susceptibility to imipenem (MIC 1.5 mg/L). The isolate was susceptible to tetracycline and fosfomycin, and MICs of tigecycline and colistin were 1 and 0.5 mg/L, respectively. However, it was resistant at a high level to all fluoroquinolones (MICs >256 mg/L). Molecular investigations performed as described previously1 identified the blaKPC-2 gene. Isolate GRU also harboured the blaTEM-1 and blaOXA-1 genes. Plasmid location of the blaKPC-2 gene was confirmed by electrophoresis of a plasmid DNA preparation obtained by the Kieser method into E. coli TOP10 with selection on Triptophane supplemented agar for ampicillin (100 mg/L).1 Molecular and phenotypic analysis of the E. coli transfectant confirmed that blaKPC-2 was located on an ~20 kb plasmid. The blaKPC-2-positive plasmid was non-typeable using PCR-based replicon typing.3 No other antibiotic resistance marker was co-transferred. PCR mapping performed as described1 showed that the blaKPC-2 gene was part of the Tn4401 transposon. It is noteworthy that E. coli GRU additionally harboured a gene encoding the 16S rRNA methylase ArmA, conferring high-level resistance to all aminoglycosides (MICs of gentamicin, netilmicin, kanamycin and tobramycin >256 mg/L). Interestingly, KPC-2 and ArmA-producing Enterobacter cloaceae and K. pneumoniae isolates have been reported in China and Poland.5,6 Multilocus sequence typing (MLST) performed according to the protocol described on the E. coli MLST web site (http://www.pasteur.fr/recherche/genopole/PGPb/mlst/EColi.html) showed that E. coli GRU belonged to ST101, recently reported to be the most frequent NDM-1-producing E. coli clone in the UK and Pakistan.7 That study reported a KPC-producing E. coli originating from India. It remains to be determined to what extent the spread of KPC-type enzymes will contribute to the problem of carbapenem resistance in India, which currently is commonly regarded as reflecting the dissemination of the NDM-1 carbapenemase.8

Funding
This work was supported by a grant from the Ministère de la Recherche, Université Paris XI, Paris, and by the INSERM, France.
Sir,

The emergence of NDM-1-producing isolates and their sources have been clearly identified in several countries worldwide. In particular, the bla_{NDM-1} gene was identified in various genera of Enterobacteriaceae and in non-fermenting Gram-negative bacilli from environmental samples in India. Furthermore, the increasing co-production of NDM-1 with other carbapenemases has been detected amongst isolates of Enterobacteriaceae and Acinetobacter sp. in many parts of India.

We report here the isolation of a strain of Klebsiella pneumoniae (designated IR98) from a urine sample from a middle-aged patient admitted to the intensive care unit of a tertiary care hospital in Chennai, India in July 2010. Species identification and antibiotic susceptibility, determined using an automated system (VITEK-2, bioMérieux Inc.), showed wide-spectrum resistance to β-lactams, aminoglycoside, fluoroquinolones, co-trimoxazole, nitrofurantoin and tigecycline, but susceptibility to colistin and fosfomycin, according to CLSI guidelines.

MICs of various antibiotics were determined using the agar dilution method, while the tigecycline MIC was determined using the broth microdilution method. European Committee on Microbiological Susceptibility Testing (EUCAST) breakpoints were applied to interpret the susceptibilities. The isolate was highly resistant to imipenem (256 mg/L), meropenem (128 mg/L), ceftazidime (>256 mg/L), cefotaxime (>256 mg/L), amikacin (>512 mg/L), gentamicin (>512 mg/L), tobramycin (>512 mg/L), netilmicin (>512 mg/L), co-trimoxazole (>32 mg/L) and tigecycline (4 mg/L), but remained susceptible to colistin (0.5 mg/L). The double-disc synergy test (DDST), modified Hodge test (MHT) and combined-disc synergy test (CDST) were used for the detection of metallo-β-lactamases (MBLs), other carbapenemases and KPC, or KPC with MBLs. The simultaneous production of MBL and KPC-like carbapenemases was confirmed by positive DDST, MHT and CDST with meropenem discs containing both EDTA and phenylboronic acid (PBA) or EDTA, while meropenem discs supplemented with PBA were negative. PCR assays for genes encoding β-lactamases and 16S rRNA methylases revealed the presence of bla_{NDM-1}, bla_{KPC-2}, bla_{CTX-M-15}, bla_{SHV-12}, bla_{TEM-1}, bla_{OXA-1} and rmtB genes.

Plasmid analysis using the Kieser technique revealed that K. pneumoniae IR98 harboured four plasmids, with sizes of 160, 120, 70 and 40 kb, using Escherichia coli NCTC 50192 as a reference. To study the transferability of these plasmids (encoding the resistance determinants), transconjugation and transformation experiments were performed using E. coli J53 (Azide-R) and E. coli TOP10 as recipient strains. Transconjugants were selected on MacConkey agar plates using sodium azide (200 mg/L) with ceftazidime (2 mg/L), meropenem (0.5 mg/L) or amikacin (20 mg/L). The plasmid extract of K. pneumoniae IR98 was transformed into E. coli TOP10, and transformants were selected on MacConkey agar plates containing 2 mg/L ceftazidime. Selected colonies were replica-plated onto MacConkey agar plates with or without meropenem (0.5 mg/L) or amikacin (20 mg/L).

The genes encoding NDM-1, CTX-M15 and 16S rRNA methylase were transferred in conjugation experiments, whereas transfer of KPC-2 was successful only by transformation. The plasmids purified from the clinical isolate, transconjugants and transformants were typed by PCR-based replication typing (PBRT). The E. coli J53-p98A transconjugant obtained

**Emergence of Klebsiella pneumoniae isolate co-producing NDM-1 with KPC-2 from India**

Karthikeyan Kumarasamy and Aravindan Kalyanasundaram

**References**