The research leading to these results has also received funding from the European Community’s Seventh Framework Programme FP7/2007–2013 under grant agreement no. 241742 (TEMPOtest-QC).

Transparency declarations

None to declare.

References


J Antimicrob Chemother 2012
doi:10.1093/jac/dkr431
Advance Access publication 11 October 2011

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

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Keywords: multiple co-resident carbapenemases, 16S rRNA methylase, urinary tract infections, ESBLs, Inc A/C type plasmid

Sir,
The emergence of NDM-1-producing isolates and their sources have been clearly identified in several countries worldwide.1,2 In particular, the blaNDM-1 gene was identified in various genera of Enterobacteriaceae and in non-fermenting Gram-negative bacilli from environmental samples in India.3 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.4–7

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.8

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 Antimicrobial Susceptibility Testing (EUCAST) breakpoints were applied to interpret the susceptibilities.9 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), ciprofloxacin (>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)10 and combined-disc synergy test (CDST)11 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-lactamases12 and 16S rRNA methylases13 revealed the presence of blaNDM-1, blaKPC-2, blaCTX-M-15, blaSHV-12, blaTEM-1, blaOXA-1, and rmtB genes.

Plasmid analysis using the Kieser technique14 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.15 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).15 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).15

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 replicon typing (PBRT).15 The E. coli J53-p98A transconjugant obtained
from the meropenem plate showed an MBL phenotype and elevated MICs of all the β-lactams (except aztreonam) and susceptibility to non-β-lactam antibiotics. Plasmid analysis revealed that E. coli J53-p98A harbour a 160 kb plasmid that belonged to the Inc C/3 type. Subsequently, this plasmid was found by PCR to carry the blaNDM-1 gene. In addition, the E. coli J53-p98B transconjugant grown on both ceftazidime and amikacin plates showed decreased susceptibility to aminoglycosides and cephalosporins except cefotaxin, and was positive for extended-spectrum β-lactamase (ESBL) production on DDST. Both blaCTX-M15 and rmtB genes were carried on a 120 kb IncF plasmid that was identified in E. coli J53-p98B. In contrast, the ESBL-negative transformant (E. coli TOP10-p98C) from both ceftazidime and meropenem plates was resistant to all the β-lactams except carbapenems (MICs 2 mg/L). The blaVPC-2 gene together with blaTEM-1 was carried on a 70 kb non-typeable plasmid that was identified in E. coli TOP10-p98C.

Although the co-existence of blaNDM-1 with different carbapenemase genes has been reported in India, we believe this to be the first report of the co-occurrence of blaNDM-1 with blaVPC-2 and rmtB in a clinical isolate of K. pneumoniae from India. This co-production of NDM-1 with unrelated carbapenemase and 16S RNA methylase results in very broad-spectrum antibiotic resistance profiles. The growing emergence of these powerful resistance mechanisms in India is cause for great concern as treatment options are virtually exhausted.

**Funding**

No specific funding was received.

**Transparency declarations**

None to declare.

**References**


J Antimicrob Chemother 2012
doi:10.1093/jac/dkr402
Advance Access publication 3 October 2011

**A cautionary case of microbial solidarity: concurrent isolation of VIM-1-producing Klebsiella pneumoniae, Escherichia coli and Enterobacter cloacae from an infected wound**

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