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Transparency declarations

None to declare.

References

1 Cuzon G, Naas T, Truong H *et al*. Worldwide diversity of *Klebsiella pneumoniae* that produces β-lactamase bla_{KPC-2} gene. *Emerg Infect Dis* 2010; **16**: 1349–56.

2 Samuelsen O, Naseer U, Tofteland S *et al*. Emergence of clonally related *Klebsiella pneumoniae* isolates of sequence type 258 producing plasmid-mediated KPC carbapenemase in Norway and Sweden. *J Antimicrob Chemother* 2009; **63**: 654–8.

3 Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing: Twenty-first Informational Supplement M100-S21*. CLSI, Wayne, PA, USA, 2011.

4 Carattoli A, Bertini A, Villa L *et al*. Identification of plasmids by PCR-based replicon typing. *J Microbiol Methods* 2005; **63**: 219–28.

5 Wu Q, Liu Q, Han L *et al*. Plasmid-mediated carbapenem-hydrolyzing enzyme KPC-2 and ArmA 16S rRNA methylase conferring high-level aminoglycoside resistance in carbapenem-resistant *Enterobacter cloacae* in China. *Diagn Microbiol Inf Dis* 2010; **66**: 326–8.

6 Zacharczuk K, Piekarska K, Szych J *et al*. Emergence of *Klebsiella pneumoniae* coproducing KPC-2 and 16S rRNA methylase ArmA in Poland. *Antimicrob Agents Chemother* 2011; **55**: 443–6.

7 Lascols C, Hackel M, Marshall SH *et al*. Increasing prevalence and dissemination of NDM-1 metallo-β-lactamase in India: data from the SMART study (2009). *J Antimicrob Chemother* 2011; **66**: 1992–7.

8 Mushtaq S, Irfan S, Sarma JB *et al*. Phylogenetic diversity of *Escherichia coli* strains producing NDM-type carbapenemases. *J Antimicrob Chemother* 2011; **66**: 2002–5.

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

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Sir,

The emergence of NDM-1-producing isolates and their sources have been clearly identified in several countries worldwide.[1,2] 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.[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-lactamases[12] and 16S rRNA methylases[13] 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[14] 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).[16] 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 harboured a 160 kb plasmid that belonged to the Inc A/C type. Subsequently, this plasmid was found by PCR to carry the bla_{NDM-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 cefoxitin, and was positive for extended-spectrum β-lactamase (ESBL) production on DDST.[8] Both $bla_{CTX-M15}$ 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 bla_{KPC-2} gene together with bla_{TEM-1} was carried on a 70 kb non-typeable plasmid that was identified in *E. coli* TOP10-p98C.

Although the co-existence of bla_{NDM-1} with different carbapenemase genes has been reported in India,[6] we believe this to be the first report of the co-occurrence of bla_{NDM-1} with bla_{KPC-2} and *rmtB* in a clinical isolate of *K. pneumoniae* from India. This co-production of NDM-1 with an 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.

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Transparency declarations

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References

1 Rolain JM, Parola P, Cornaglia G. New Delhi metallo-β-lactamase (NDM-1): towards a new pandemia? *Clin Microbiol Infect* 2010; **16**: 1699–701.

2 Kumarasamy KK, Toleman MA, Walsh TR *et al*. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. *Lancet Infect Dis* 2010; **10**: 597–602.

3 Walsh TR, Weeks J, Livermore DM *et al*. Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: an environmental point prevalence study. *Lancet Infect Dis* 2011; **11**: 355–62.

4 Karthikeyan K, Toleman M, Giske CG *et al*. First report of the co-existence of bla_{OXA-48} or $bla_{OXA-48-like}$ gene with bla_{NDM-1} in Enterobacteriaceae from India. *Clin Microbiol Infect* 2010; **16** Suppl 2: S187.

5 Poirel L, Ros A, Carricajo A *et al*. Extremely drug-resistant *Citrobacter freundii* isolate producing NDM-1 and other carbapenemases identified in a patient returning from India. *Antimicrob Agents Chemother* 2011; **55**: 447–8.

6 Lascols C, Hackel M, Marshal SH *et al*. Increasing prevalence and dissemination of NDM-1 metallo-β-lactamase in India: data from the SMART study (2009). *J Antimicrob Chemother* 2011; **66**: 1992–7.

7 Karthikeyan K, Thirunarayan MA, Krishnan P. Coexistence of bla_{OXA-23} with bla_{NDM-1} and *armA* in clinical isolates of *Acinetobacter baumannii* from India. *J Antimicrob Chemother* 2010; **65**: 2253–4.

8 Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing: Twentieth Informational Supplement M100-S20*. CLSI, Wayne, PA, USA, 2010.

9 European Committee on Antimicrobial Susceptibility Testing. *Breakpoint Tables for Interpretation of MICs and Zone Diameters, Version 1.3, January 2011*. www.eucast.org/clinical_breakpoints(22 Sep 2011, date last accessed).

10 Lee K, Lim YS, Yong D *et al*. Evaluation of the Hodge test and the imipenem-EDTA double-disk synergy test for differentiating metallo-β-lactamase-producing isolates of *Pseudomonas* spp. and *Acinetobacter* spp. *J Clin Microbiol* 2003; **41**: 4623–9.

11 Pournaras S, Poulou A, Tsakris A. Inhibitor-based methods for the detection of KPC carbapenemase-producing Enterobacteriaceae in clinical practice by using boronic acid compounds. *J Antimicrob Chemother* 2010; **65**: 1319–21.

12 Voets GM, Fluit AC, Scharringa J *et al*. A set of multiplex PCRs for genotypic detection of extended-spectrum β-lactamases, carbapenemases, plasmid-mediated AmpC β-lactamases and OXA β-lactamases. *Int J Antimicrob Agents* 2011; **37**: 356–9.

13 Berçot B, Poirel L, Nordmann P. Plasmid-mediated 16S rRNA methylases among extended-spectrum β-lactamase-producing Enterobacteriaceae isolates. *Antimicrob Agents Chemother* 2008; **52**: 4526–7.

14 Kieser T. Factors affecting the isolation of CCC DNA from *Streptomyces lividans* and *Escherichia coli*. *Plasmid* 1984; **12**: 19–36.

15 Mammeri H, Van De Loo M, Poirel L *et al*. Emergence of plasmid-mediated quinolone resistance in *Escherichia coli* in Europe. *Antimicrob Agents Chemother* 2005; **49**: 71–6.

16 Carattoli A, Bertini A, Villa L *et al*. Identification of plasmids by PCR-based replicon typing. *J Microbiol Methods* 2005; **63**: 219–28.

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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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