Nosocomial transmission of NDM-1-producing Escherichia coli ST101 in a Korean hospital

Jung Sik Yoo1, Hye Mee Kim1, Hyun Sook Koo2, Ji Woo Yang3, Jae Il Yoo1, Hwa Su Kim1, Hye Kyung Park2 and Yeong Seon Lee1*

1Division of Antimicrobial Resistance, Korea Centers for Disease Control & Prevention, 187 Osang Saengmyeong 2-ro Chungcheongbuk-do 363-951, Republic of Korea; 2Division of Infectious Disease Control, Korea Centers for Disease Control & Prevention, 187 Osang Saengmyeong 2-ro Chungcheongbuk-do, 363-951 Republic of Korea

*Corresponding author. Tel: +82-43-719-8240; Fax: +82-43-719-8269; E-mail: yslee07@nih.go.kr

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Sir,

Clonally diverse NDM-1-producing Escherichia coli (ST405, ST131, ST156 and ST101) have been identified in Australia, Canada, Germany, the UK and Pakistan. Indeed, there may be an association between isolates of NDM-1-producing E. coli ST101, phylogroup B1, that have been identified in Australia, Canada, Germany, the UK and Pakistan.

In December 2011, NDM-1-producing E. coli (CREC-36) was identified from a patient who was hospitalized in a 260 bed tertiary care hospital in Korea. Medical records were reviewed and 114 stool or rectal swab samples were taken from 67 patients on Ward 1 (19 rooms), 11 patients on the intensive care unit (ICU) and 25 healthcare workers (doctors, nurses and caregivers) working on Ward 1. We screened patients in the ICU because the first patient had been treated in the ICU for 1 month before she was transferred to room 112. In addition, 23 environmental swabs were collected from washbasins, medicine trays and linen. All samples were cultured in 5 mL of tryptic soy broth containing 10 μg meropenem discs and processed using the CDC protocol. The modified Hodge test and the double-disc synergy test were performed on representative colonies grown on MacConkey agar. The blaNDM-1 gene was identified by PCR and sequencing using the primers NDM-1 F (5'-CAGATTATGC ACCCGGTCG-3') and NDM-1 R (5'-ATCATGCTGGCCTTGGGAAC').

Three more patients who had shared a room (no. 112) with the first patient were also colonized by NDM-1-producing E. coli (CREC-45, CREC-47 and CREC-49). All four patients were elderly (>75 years) and had been in hospital for between 1 and 7 months before the NDM-1-producing E. coli were isolated. No NDM-1-producing E. coli were detected in samples from other patients, healthcare workers or the environment. NDM-1-positive individuals were isolated until decolonization had been confirmed by three consecutive negative stool cultures collected 1 week apart. None of the patients received treatment to eradicate the NDM-1 E. coli. Two patients died from their underlying disease. The remaining two were decolonized 21 and 53 days after the first isolation. No further cases of NDM-1 were detected. All the isolates were genetically related (PFGE identifying one pulsotype with >90% similarity), and the clone was identified by multilocus sequence typing (MLST; http://mlst.ucc.ie/mlst/dbs/Ecoli) as ST101, phylogenetic group B1. E. coli ST101 is an international clone, frequently associated with the Indian subcontinent. However, the four patients had no epidemiological link to the Indian subcontinent as they had been hospitalized in Korea for a long time due to other underlying diseases. All patients shared an epidemiological link in terms of transmission. Although none of the healthcare workers harboured NDM-1, personal contact between the caregivers and the patients resident in the same room is the most likely transmission route; the NDM-1-colonized patients were bedridden, the beds were very close to each other and no NDM-1 cases were detected in any other rooms on Ward 1. Transmission was probably caused by poor standards of hygiene in the hospital; there were no sinks or alcohol-based hand cleansers in the room. Neither did the hospital monitor the hygiene practices of the healthcare workers.

The MICs of antimicrobial agents for the NDM-1-producing isolates were determined using the agar dilution method and Etest. All NDM-1-producing isolates except CREC-49 showed high MICs of carbapenems (≥32 mg/L) and multidrug resistance; however, they were susceptible to tigecycline and colistin (Table 1).

Using a blaNDM-1 probe, we identified the blaNDM-1 gene by Southern blotting of S1 nuclelease-digested total DNA. The blaNDM-1 gene was identified in the 170, 180 and 120 kb plasmids. The discrepancy in blaNDM-1-bearing plasmid size may be because the plasmids are quite unstable and prone to rearrangement. Although the conjugal transfer of plasmids carried by the NDM-1-producing isolates failed, the blaNDM-1-harboursing plasmids from the four isolates were successfully transformed into electrocompetent E. coli HB101. PCR-based replicon typing of the major plasmid incompatibility groups showed that the plasmids belonged to the IncA/C incompatibility group.

All transformants co-harboured blaNDM-1, blaCTX-M-15, blacMY-4, blaDHDA-1 and arrA. The genetic elements surrounding blaNDM-1 were examined by overlapping PCR mapping. The overall structure was

ISAb125—blaNDM-1—bleMBL—trpF—blaDHDA-1—ampR—hypA—

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The sequence spanning from ISaba125 to armA was identical to that in the E. coli DVR22 plasmid (IncHI1), and the sequence from blaNDM-1 to armA was identical to that in pNDM-HK (IncL/M). The sequence sul1–ISCR1–tnpU–armA was also identified in the IncA/C incompatibility group plasmids pNDM-1_Dok01 (NC_018994) and pMR0211 (JN687470).

This study reports the transmission of NDM-1 E. coli ST101 among patients sharing a hospital room. It was not clear how this clone was introduced into the hospital; however, its transmission was probably related to poor hygiene standards in the hospital. No further NDM-1-producing E. coli were identified after stringent infection control procedures including contact barrier precautions, rigorous cleaning and disinfection protocols had been implemented.

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### Transparency declarations

None to declare.

### References


Sri Lanka, another country from the Indian subcontinent with NDM-1-producing Enterobacteriaceae

Laurent Dortet1*, Ludivine Brechard1, Karine Grenet2, Marie-Stéphane Nguessañ3 and Patrice Nordmann1

1INSERM U914 ‘Emerging Resistance to Antibiotics’, Faculté de Médecine Paris Sud, Le Kremlin-Bicêtre, France; 2Service de Microbiologie, Centre Hospitalier de Lagney – Marne-la-Vallée, Jossigny, France; 3Service de Médecine Interne et Maladies Infectieuses, Centre Hospitalier de Lagney – Marne-la-Vallée, Jossigny, France

*Corresponding author. Service de Bactériologie-Virologie, Hôpital de Bicêtre, 78 rue du Général Leclerc, 94275 Le Kremlin-Bicêtre, France. Tel: +33-1-45-21-20-19; Fax: +33-1-45-21-63-40; E-mail: laurent.dortet@bct.aphp.fr

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Sir,

Carbenapenems are β-lactams characterized by stability to hydrolysis by many β-lactamas, including natural and acquired extended-spectrum β-lactamas (ESBLs), which are prevalent in Enterobacteriaceae worldwide.1 Moreover, during the last decade, the emergence of acquired carbenamen-hydrolysing enzymes (carbenapenemas) has been increasingly reported.2 The metallo-β-lactamas NDM-1 is one of the most recently described carbenapenemas.3 This enzyme is mainly identified in Enterobacteriaceae, but has also been described in Acinetobacter spp. and in Pseudomonas aeruginosa. NDM-1 producers have been identified mainly in the UK, India and Pakistan, and are increasingly reported worldwide.3 The Indian subcontinent is considered to be the main reservoir of NDM-like-encoding genes.3

Our study was initiated by the isolation of Klebsiella pneumoniae from blood cultures taken from a man in his late 60s with prostatitis who was admitted to the emergency unit of Marine-la-Vallée Hospital (Paris area) in early 2013. Two weeks before admission, this patient had been hospitalized for urinary incontinence in Sri Lanka (Jaffna), where a urinary catheter had been inserted and left in place. Susceptibility testing was initially performed by disc diffusion assay and the results were interpreted according to the updated EUCAST guidelines (www.eucast.org). MICs were subsequently determined by Etest (bioMérieux, La Balme-les-Grottes, France) on Mueller–Hinton agar at 37°C. The isolate of K. pneumoniae was resistant to all β-lactams, including imipenem, meropenem, ertapenem and doripenem (Table 1). This isolate was additionally resistant to co-trimoxazole, nalidixic acid and aminoglycosides (amikacin, tobramycin and netilmicin) except gentamicin; it was susceptible to colistin, chloramphenicol, fosfomycin, tigecycline, levofoxacin, ciprofloxacin and nitrofurantoin (Table 1). On admission to hospital in France, the urinary catheter was replaced and the patient was empirically treated for 2 days with imipenem and amikacin. After 48 h, antibiotic treatment was switched to levofoxacin and gentamicin for 6 days. Since the patient still had a fever at this time, antibiotic treatment was changed to fosfomycin, colistin and gentamicin for 8 additional days. Although the fever abated, after 12 days of antibiotic therapy the patient developed a Candida albicans urinary tract infection that was successfully treated with fluconazole. After a total of 28 days treatment with antibiotics the urinary catheter was no longer colonized with carbapenem-resistant K. pneumoniae.

Carbenapenem production by the K. pneumoniae isolate was assessed using the biochemical Carbo NP test.5 The results of the Carbo NP test II (which indicates the type of carbapenemase),6 and of an MBL Etest (bioMérieux), suggested a metallo-β-lactamase. PCR amplifications of several β-lactamas genes and of the qnrS1 gene were performed as previously described,6 using whole-cell DNA extracted with the QiaAmp minikit according to the manufacturer’s instructions (Qiagen, Courtaboeuf, France). PCR amplification followed by sequencing identified a blaNDM-1 gene (http://www.lahey.org/studies/) together with blaTEM-1, blaSHV-11, blaCTX-M-15 and blaOXA-9 genes. This K. pneumoniae isolate also harboured the aac4 gene encoding the AAC(6′)-Ib aminotransferase that confers high-level resistance to aminoglycosides (except to gentamicin), and the qnrS1 gene conferring increased MICs to quinolones (Table 1). Multilocus sequence typing analysis showed that the isolate belonged to ST394.

Plasmid DNA was extracted by using the Kieser method8 and analysed by agarose gel electrophoresis. A single plasmid of ~120 kb was identified. Direct transfer of the β-lactam resistance marker into Escherichia coli J53 was attempted by liquid mating assays at 37°C. E. coli transconjugants were obtained on agar plates supplemented with sodium azide (100 mg/L) and ceftazidime (2 mg/L). MIC results mirrored those obtained with the clinical isolate, with decreased susceptibility or resistance to all β-lactams, including carbenapenems (Table 1), resistance to all aminoglycosides except to gentamicin and decreased susceptibility to quinolones. They harbour a 120 kb plasmid carrying the blaNDM-1.