Characteristics of community-onset NDM-1-producing *Klebsiella pneumoniae* isolates

So Yeon Kim,1† Ji-Young Rhee,2† Sang Yop Shin3 and Kwan Soo Ko1

1Department of Molecular Cell Biology, Samsung Biomedical Research Institute, Sungkyunkwan University School of Medicine, Suwon 440-746, South Korea
2Division of Infectious Diseases, Department of Medicine, Dankook University, Cheonan, South Korea
3Korea Medical Institute, Seoul, South Korea

Multilocus sequence typing and in vitro antimicrobial susceptibility testing were performed for three community-onset New Delhi metallo-β-lactamase-1 (NDM-1)-producing *Klebsiella pneumoniae* isolates from Korea. The genetic structure surrounding the *bla*NDM-1 gene was determined in *bla*NDM-1-harbouring plasmids. Three NDM-1-producing *K. pneumoniae* isolates were found to belong to the same clone (sequence type 340). Each of these isolates showed the same genetic structure surrounding the *bla*NDM-1 gene. The genes *bla*NDM-1, *ble*MBL, *trpF* and *dsbC* were flanked by two intact insertion sequences, IS*Aba125* and IS*26*, which may promote horizontal gene transfer. The *bla*NDM-1-harbouring plasmids conferred antimicrobial resistance to carbapenems, cephalosporins, aminoglycosides and aztreonam in transconjugants. It can be speculated that either the entire *bla*NDM-1-harbouring plasmids or just the part of the plasmid containing the *bla*NDM-1 gene may have transferred between *K. pneumoniae* and *Escherichia coli*. Following the transfer, the isolate disseminated throughout Korea. This study suggests the need for monitoring the dissemination of NDM-1-producing isolates across countries or continents due to their potential transferability via IS*Aba125*- and IS*26*-associated transposons.

**INTRODUCTION**

Since its discovery in 2009 (Yong et al., 2009), New Delhi metallo-β-lactamase-1 (NDM-1) has been of great concern worldwide (Nordmann et al., 2011). Although NDM-1 was initially reported that NDM-1-producing *Klebsiella pneumoniae* had a putative association with the Indian subcontinent (Yong et al., 2009; Kumarasamy et al., 2010), NDM-1 has been found in diverse Gram-negative bacteria throughout the world (Walsh et al., 2011; Bushnell et al., 2013). It is thought that the horizontal transferability of the *bla*NDM-1 gene via plasmids may contribute to the rapid dissemination of NDM-1-producing pathogens.

In 2010, NDM-1-producing *K. pneumoniae* isolates were identified from Korean patients with no history of travelling abroad (Kim et al., 2012). Previously, we had identified three NDM-1-producing *K. pneumoniae* isolates from a single tertiary-care hospital in Korea (Dankook University Hospital, Chunan) (Kim et al., 2013). The three patients with NDM-1-producing *K. pneumoniae* isolates had not been in contact with any person with an infection caused by an NDM-1 producer, and thus the three NDM-1-producing *K. pneumoniae* infections were considered to be of community onset. In this study, we report three cases of infection and characterize the genetic composition of the region surrounding the *bla*NDM-1 gene in these isolates.

**PATIENTS**

**Patient 1**

A 38-year-old male was admitted after a fall that produced multiple fractures. Paraplegia and multiple decubitus ulcers developed due to the thoracic vertebral fracture. The patient developed a fever of up to 39 °C and leukocytosis. Cultures were taken to determine if the fourth-grade decubitus ulcers in the sacral region might be the cause of the patient’s fever and inflammation. These cultures from the decubitus ulcers revealed carbapenem-resistant *K. pneumoniae* (isolate number A-1). Colistin was prescribed to the patient and his decubitus ulcers resolved. He was discharged from the hospital after undergoing a skin graft.
Patient 2
A 73-year-old male was admitted via the outpatient department due to jaundice. Initial laboratory tests revealed a bilirubin level of 11.38 mg dl⁻¹ and an alkaline phosphatase level of 369 IU l⁻¹. The patient complained of abdominal pain, fatigue and nausea. A 3 cm long intrapancreatic common bile duct mass and obstructive coronary heart disease/ischaemic heart disease dilatation were revealed on computed tomography. Endoscopic retrograde cholangiopancreatography (ERCP) with endoscopic sphincterotomy and balloon dilatation was performed on the second day of admission. The patient also underwent percutaneous, transhepatic gall bladder drainage because of cholangitis on the day of ERCP. Carbapenem-resistant K. pneumoniae (isolate number 2827) was cultured from the bile sample. The patient received meropenem for 14 days, after which his signs and symptoms of cholangitis were resolved. He underwent pylorus-preserving pancreaticoduodenectomy because of common bile duct cancer (T3N1). After this procedure, the patient underwent concurrent chemoradiotherapy for 2 months. He has had no recurrence of cancer.

Patient 3
A 67-year-old male visited the emergency department because of abdominal pain. His body temperature was 38.3 °C and he had severe abdominal tenderness on physical examination. Computed tomography revealed a pancreatic pseudocyst (3 x 10 cm) and severe peripancreatic inflammation. He was admitted following a diagnosis of acute pancreatitis and pseudocyst. Endoscopic, ultrasonography-guided aspiration of the pseudocyst was performed to address a suspected pancreatic pseudocyst was performed to address a suspected pancreatic pseudocyst. Gastropancreatostomy with drainage of the pancreatic pseudocyst (cystogastrostomy with a metal stent) was performed and colistin was prescribed for 29 days and tigecycline for 12 days. The patient was discharged after removal of the cystogastrostomy metal stent.

METHODS

Identification of species and blaNDM-1 gene. Three isolates were first identified with VITEK2 (bioMérieux) and were confirmed using 16S rRNA gene sequencing. To detect metallo-β-lactamase (MBL) activity, the EDTA-imipenem disc synergy test was performed for all carbapenem-resistant Enterobacteriaceae isolates (Cardoso et al., 2008). MBL genes including blalSP, blaVIM, blaIMP, blaKPC, blaOXA-24 and blalNDM and blaKPC and blalNDM-1 were detected by gene amplification and sequencing of PCR products.

Antimicrobial susceptibility testing and multilocus sequence typing. In vitro antimicrobial susceptibility testing was performed using the broth microdilution method according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2013). Twenty antimicrobial agents were tested, including imipenem and meropenem.

Multilocus sequence typing was performed as described previously (http://www.pasteur.fr/recherche/genopole/PSB/mlst) (Diancourt et al., 2005).

Conjugation and sequencing of the genetic environment. The genetic region surrounding the blaNDM-1 gene was characterized using primer walking after the blaNDM-1-bearing plasmids were extracted by transconjugation into Escherichia coli DH5α (Shin et al., 2012).

RESULTS AND DISCUSSION

The three carbapenem-resistant K. pneumoniae isolates showed a very similar PFGE pattern, and belonged to the same clone, sequence type (ST)340, which is a single-locus variant of ST11, a CTX-M-15-, NDM-1- or K. pneumoniae carbapenemase-producing clone that is prevalent worldwide. ST340 had been identified in the first reported NDM-1-producing K. pneumoniae isolates found in Korea (Kim et al., 2012). The three micro-organisms were isolated within 48 h after patients’ admission to hospital. Because there was no evidence that the patients had visited a healthcare facility or had been in contact with healthcare workers prior to the isolation of the micro-organisms, the NDM-1-producing K. pneumoniae infections in this study were regarded as being of community onset. Thus, the NDM-1-producing K. pneumoniae ST340 clone may have already disseminated through parts of Korea.

The size of plasmids bearing the blaNDM-1 gene was estimated to be 100–120 kb. It has been reported that NDM-1-producing K. pneumoniae isolates from Korea had plasmids of the IncN group (Kim et al., 2012), but we could not determine the compatibility group of our plasmids. The three NDM-1-producing isolates showed identical genetic structures in the regions surrounding the blaNDM-1 gene (Fig. 1). The blaNDM-1, blaMBL, trpF and dsbC genes were flanked by two insertion sequences, ISAba125 (tnp) and IS26 (tnpA), which may promote horizontal gene transfer. This structure is identical to that of E. coli BJ01 (JX296013.1) from China (Liu et al., 2013), and only two nucleotide substitutions resulting in alterations of amino acids in the dsbC gene were identified among two genetic structures. In addition, these genetic environments are similar to those of E. coli pNDM-HK (HQ451071) from Hong Kong, E. coli DVR22 (JF922606) from Spain and K. pneumoniae pNDM-MAR (JN420336) from Morocco (Ho et al., 2011; Solé et al., 2011; Villa et al., 2012). Unlike these similar structures, both insertion sequences (IS26 and ISAba125) were preserved as intact, but the catA1 gene was truncated in our NDM-1-producing K. pneumoniae isolates, as well as in E. coli BJ01. In addition, the blalA1 and ampR genes were not identified in these isolates, although some similar structures carried them.

The same genetic environment of blaNDM-1 gene structure between two different species from East Asia may reflect interspecies transferability, as indicated previously (Walsh et al., 2011). In addition, the fact that we observed isolates sharing a similar blaNDM-1 sequence in diverse bacterial
Δ IS Aba125

IS 26 (tnpA)

IS Aba 125 (tnp)

bla

ble

MBL

Delta blaDHA-1

Delta ampR

cutA1

bla

NDM-1

ble

MBL

trpF

Δ trpF

E. coli

pNDM-HK (HQ451071)

E. coli DVR22 (JF922606)

K. pneumoniae pNDM-MAR (JN420336)

E. coli BJ01 (JX296013.1)

K. pneumoniae A-1, B-1 & 2827

Fig. 1. Schematic representation of the genetic structure flanking the blaNDM-1 gene in the three NDM-1-producing K. pneumoniae isolates in this study and other published strains. Δ, truncated genes; IRR, right inverted repeat; IRL, left inverted repeat. IRR and IRL are indicated as short vertical bars.

Table 1. MICs of NDM-1-producing K. pneumoniae isolates and transconjugants containing the blaNDM-1-bearing plasmid

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Isolate</th>
<th>MIC (mg l⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A-1</td>
<td>B-1</td>
</tr>
<tr>
<td>Imipenem</td>
<td>8</td>
<td>&gt;64</td>
</tr>
<tr>
<td>Meropenem</td>
<td>16</td>
<td>64</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>&gt;128</td>
<td>&gt;128</td>
</tr>
<tr>
<td>Cefazidime</td>
<td>&gt;64</td>
<td>&gt;64</td>
</tr>
<tr>
<td>Cefepime</td>
<td>&gt;64</td>
<td>&gt;64</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>&gt;128</td>
<td>&gt;128</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>&gt;64</td>
<td>&gt;64</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Amikacin</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>&gt;64</td>
<td>&gt;64</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>32</td>
<td>&gt;64</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>&gt;64</td>
<td>&gt;64</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>&gt;64</td>
<td>&gt;64</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>32</td>
<td>64</td>
</tr>
<tr>
<td>Colistin</td>
<td>4</td>
<td>0.06</td>
</tr>
<tr>
<td>Polymyxin B</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Ampicillin/sulbactam</td>
<td>&gt;64/32</td>
<td>&gt;64/32</td>
</tr>
<tr>
<td>Trimethoprim/sulfamethoxazole</td>
<td>0.5/9.5</td>
<td>8/152</td>
</tr>
<tr>
<td>Piperacillin/tazobactam</td>
<td>&gt;256/4</td>
<td>&gt;256/4</td>
</tr>
</tbody>
</table>

*Values showing higher MICs in transconjugants than in recipient (DH5α) are represented in bold.
species from Europe, Africa and Asia suggests that rapid dissemination of NDM-1-producing isolates has occurred worldwide. Although an ST340 clone producing NDM-1 has been identified in the Sultanate of Oman and Canada (Peirano et al., 2011; Poirel et al., 2011), it is now more plausible that blaNDM-1-bearing plasmids have been transferred among E. coli and K. pneumoniae isolates in East Asia, rather than that the NDM-1-producing K. pneumoniae ST340 clone has disseminated across continents.

The three transconjugants with the blaNDM-1-bearing plasmid showed increased MICs for 20 antimicrobial agents (Table 1). The results suggest that blaNDM-1-bearing plasmids confer resistance to several antimicrobial agents. No resistance to fluoroquinolones, tetracyclines or polymyxins was observed. However, the MICs of imipenem and meropenem in transconjugants were less than those of the original NDM-1-producing K. pneumoniae isolates. In addition, because the three NDM-1-producing K. pneumoniae isolates had different MIC profiles as antimicrobial agents, despite having the same MICs as transconjugants, the different genetic backgrounds of the original isolates may have influenced antimicrobial activity.

In short, we identified three NDM-1-producing K. pneumoniae isolates in Korea. These isolates belonged to the same clone and shared the same genetic composition surrounding the blaNDM-1 gene. The plasmids bearing the blaNDM-1 gene, which contribute resistance to carbapenems, cephalosporins and aminoglycosides, may have been transferred from E. coli or K. pneumoniae. In addition, our study reveals that NDM-1-producing K. pneumoniae isolates may have disseminated in Korea, including in the community.

ACKNOWLEDGEMENTS

This research was supported by the Korea Medical Institute. The authors have no conflicts of interest to declare.

REFERENCES


