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# Emergence of a multidrug-resistant clinical isolate of *Escherichia coli* ST8499 strain producing NDM-13 carbapenemase in the Republic of Korea☆,☆☆

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ABSTRACT

We described a carbapenem-resistant *Escherichia coli* ST8499 strain producing New Delhi metallo-β-lactamase-13 (NDM-13) from patient in Korea. The isolate exhibited multidrug resistance, but remained susceptible to colistin and tigecycline. The $bla_{NDM-13}$ gene was located on a 130-kb self-transmissible plasmid. This is the first report of NDM-13 carbapenemase in Korea.



Carbapenems are considered the most effective treatment option for severe infections caused by extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae*. Recently, carbapenem-resistant strains, particularly carbapenemase-producing *Enterobacteriaceae*, have been found worldwide and are a rising threat to public health (Queenan and Bush, 2007). New Delhi metallo-β-lactamase (NDM), an ambler class B, zinc-dependent carbapenemase, confers resistance to most β-lactams except a monobactam. Since the first description of an NDM-1 producer recovered from a Swedish patient in 2008 (Yong et al., 2009), 21 NDM variants have been identified in multiple countries (ftp://ftp.ncbi.nlm.nih.gov/pathogen/betalactamases/Allele.tab). However, NDM-13, which harbors two amino acid substitutions (Asp95Asn and Met154Leu) resulting in higher catalytic efficiency for cefotaxime compared with NDM-1 (Khan et al., 2017), has rarely been described, except for recent reports from Nepal (Shrestha et al., 2015) and China (Lv et al., 2016). To our knowledge, this is the first report of NDM-13-producing *Enterobacteriaceae* in Korea.

In July 2018, a carbapenem-resistant strain (SECR18–0956) was isolated from rectal swab sample of a 57-year-old male patient with diabetes and dementia upon intensive care unit (ICU) admission. The patient had no travel history and had received no recent antibiotic therapy in the last 6 months. He was discharged the following day; follow-up specimens were not collected. Bacterial identification was performed using Bruker Biotyper MALDI-TOF MS (Bruker Daltonics, Bremen, Germany) and VITEK 2 (bioMérieux, Marcy l'Etoile, France). Antimicrobial susceptibility testing was carried out by a broth microdilution using customized Sensititre plates (TREK Diagnostic Systems, Cleveland, OH, USA) and by tigecycline E-test strip (bioMérieux). Minimum inhibitory concentrations (MICs) were interpreted according to CLSI guidelines, with the exception of colistin and tigecycline, where EUCAST criteria were used (Lee et al., 2018). The synergy test with an imipenem disc (Oxoid, Basingstoke, UK) and EDTA was performed to confirm the presence of metallo-carbapenemase. PCR and subsequent sequencing were used to detect genes encoding carbapenemase ($bla_{KPC}$, $bla_{GES}$, $bla_{NDM}$, $bla_{IMP}$, $bla_{VIM}$, $bla_{OXA-48}$, $bla_{SIM}$, $bla_{SPM}$, $bla_{SME}$ and $bla_{GIM}$), beta-lactamases ($bla_{TEM}$, $bla_{DHA}$, $bla_{SHV}$, $bla_{OXA}$, $bla_{CMY-II}$ and $bla_{CTX-M}$) and

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**Table 1**
MICs of antimicrobial agents for NDM-13-producing *E. coli* and transconjugant strains.

| Antimicrobial agent(s) | MICs, μg/mL | | |
|---|---|---|---|
| | *E. coli* (SECR18–0956) | *E. coli* K12 J53 | *E. coli* K12 J53 (SECR18–0956-TC) |
| Ampicillin | >64 | 4 | >64 |
| Piperacillin/Tazobactam | >128/4 | 4/4 | >128/4 |
| Cefoxitin | >64 | 8 | >64 |
| Cefepime | >16 | 4 | >16 |
| Cefpodoxime | >32 | <=0.25 | >32 |
| Ceftazidime | >128 | <=0.25 | >128 |
| Cefotaxime | >64 | <=0.12 | >64 |
| Cefotaxime/clavulanate | >128/4 | <=0.12/4 | >128/4 |
| Imipenem | 16 | <=0.5 | 4 |
| Meropenem | 32 | <=0.5 | 4 |
| Ertapenem | >32 | <=0.25 | 8 |
| Doripenem | 32 | <=0.5 | 4 |
| Nalidixic acid | >128 | <=2 | 4 |
| Ciprofloxacin | >16 | <=0.03 | <=0.03 |
| Tetracycline | >128 | 4 | 32 |
| Chloramphenicol | >32 | <=2 | 4 |
| Trimethoprim/sulfamethoxazole | >16/304 | <=1/19 | >16/304 |
| Gentamicin | >32 | <=1 | >32 |
| Amikacin | >64 | <=1 | >64 |
| Tigecycline[a] | 1.5 | 0.19 | 0.19 |
| Colistin | <=2 | <=2 | <=2 |

[a] MICs were obtained by Etest.

plasmid-mediated quinolone resistance genes (*qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, *aac(6′)-Ib-cr*, *oqxAB* and *qepA*) contributing to fluoroquinolone resistance (Ciesielczuk et al., 2013; Lee et al., 2018). Conjugation was conducted by broth mating using a sodium azide-resistant *E. coli* K12 J53 strain, and transconjugants were selected on MacConkey agar containing imipenem (1 μg/ml) and sodium azide (200 μg/ml). The $bla_{NDM-13}$ gene in the transconjugant was confirmed by PCR and sequencing. Plasmids incompatibility types on parental and transconjugant strains were determined by PCR-based replicon typing (Carattoli et al., 2005). Genomic DNA of SECR18–0956 strain was extracted with a Qiagen DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany), prepared using a Nextera XT library kit (Illumina, San Diego, CA, USA), and sequenced on a Miseq platform (Illumina). Sequence reads were assembled *de novo* using a CLC genomic workbench (CLC Bio, Arhus, Denmark) and annotated with BLAST searches (http://blast.ncbi.nlm.nih.gov/). Multilocus sequence typing (MLST) and prediction of resistance genes was performed using Enterobase (http://enterobase.warwick.ac.uk) and ResFinder 3.0 (http://cge.cbs.dtu.dk/services/ResFinder/). The DNA sequence of genetic environment containing the $bla_{NDM-13}$ has been deposited in GenBank under accession no. MK157018.

The *E. coli* strain (SECR18–0956) belonged to phylogenetic group A (Clermont et al., 2000) and a new sequence type, ST8499, assigned by the Enterobase database (allelic profile: *adk*-6, *fumC*-7, *gyrB*-5, *icd*-1, *mdh*-8, *purA*-2, *recA*-2). ST8499 is a single-locus variant of the ST206 clonal complex that has been reported as NDM-5-producing *E. coli* in China (Zheng et al., 2018). The isolate exhibited multidrug resistance to carbapenems (doripenem, imipenem, meropenem and ertapenem), piperacillin-tazobactam, cefotaxime, ciprofloxacin, tetracycline, chloramphenicol, trimethoprim-sulfamethoxazole, gentamicin and amikacin and was susceptible only to colistin and tigecycline (Table 1). Metallo-β-lactamase production was detected by the imipenem-EDTA disc synergy test. PCR and sequencing identified that strain SECR18–0956 harbored the $bla_{NDM-13}$ gene as well as the $bla_{TEM-1b}$ and *qnrS1* genes. ResFinder 3.0 detected additional genes mediating resistance to aminoglycosides (*rmtB*, *aadA5*, *aph(3′)-Ia*, *aph(3′)-Ib*, *aph(6′)-Id*), penicol (*floR*), sulfonamide (*sul1*, *sul2*), tetracycline (*tetA*), sulfamethoxazole (*dfrA17*) and a single ParC mutation (A56T) contributing to fluoroquinolone resistance (Hopkins et al., 2005). The carbapenem-resistance determinant in SECR18–0956 was successfully transferred into the recipient strain. The $bla_{TEM-1b}$ was also detected in the transconjugant, while *qnrS1* was not transferred. Transconjugants were resistant to carbapenems, beta-lactams, tetracycline, trimethoprim-sulfamethoxazole, gentamicin and amikacin. The NDM-13-positive plasmid in the transconjugant strain was assigned to the IncFIB incompatibility group and was approximately 130-kb in size (estimated by S1-nuclease restriction and subsequent pulsed-field gel electrophoresis).

Sequencing analysis revealed a truncated IS*Aba125* upstream of the $bla_{NDM-13}$ gene with the −35 promoter region for $bla_{NDM}$ expression. The genes $ble_{MBL}$ (mediating bleomycin resistance), *trpF* (encoding a phosphoribosylanthranilate isomerase) and *dsbC* (encoding an oxidoreductase) were found in the downstream region (Fig. 1). The same genetic structure of ΔIS*Aba125*-$bla_{NDM-13}$-$ble_{MBL}$-*trpF*-*dsbC* was observed in two previously sequenced $bla_{NDM-13}$ *E. coli* strains IOMUT558 (ST101) from Nepal and DC33 (ST5138) from China (GenBank accession nos. LC012596 and KX094555, respectively). However, the $bla_{NDM-13}$ gene is found on different genetic backgrounds (on chromosome of IOMUT558, IncX3 plasmid of DC33 and IncFIB plasmid of SECR18–0956). In addition, the ~12 kb sequenced region in our isolate, including further downstream genes, *aadA5*, *dhfrI* and *IntI*, was nearly identical to that of a plasmid pKBN10P04869A harboring the $bla_{NDM-5}$ gene (GenBank accession no. CP026474) of *E. coli* ST410 isolate from Korea in 2017, except for an intact IS*1294* insertion into IS*Aba125* observed in a SECR18–0956 strain.

The emergence of NDM-producing *Enterobacteriaceae* has become a global public health challenge. The environmental structure of the $bla_{NDM}$ and plasmid replicon in our isolate SECR18–0956 was different from those of other NDM-13-producers, implying that the $bla_{NDM-13}$ gene in this study might have emerged independently by nucleotide mutations. Monitoring of NDM-variants on the self-transmissible plasmid is warranted for preventing the dissemination of carbapenemase-producing *Enterobacteriaceae*.

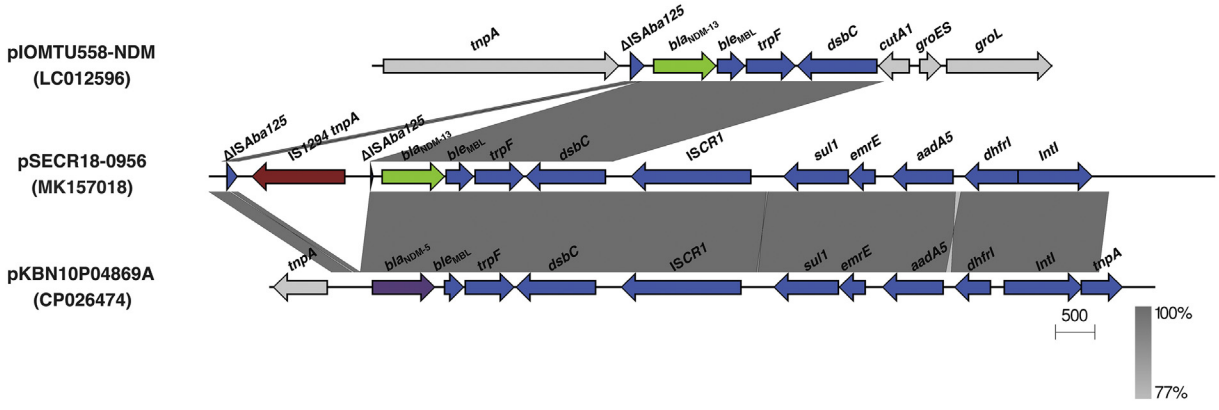


**Fig. 1.** Schematic presentation of genetic structures surrounding $bla_{NDM}$.

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