

## RESEARCH ARTICLE

# Emergence and transmission of New Delhi metallo-beta-lactamase-5-producing *Escherichia coli* Sequence Type 361 in a Tertiary Hospital in South Korea

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**Abstract**

**Background:** The emergence of carbapenem-resistant *Escherichia coli* (*E coli*) is a serious global health threat, but little is known about carbapenemase-producing *E coli* in Daejeon, South Korea. The aim of this study was to investigate characteristics of thirteen carbapenem-resistant *E coli* isolates in a tertiary hospital.

**Methods:** Thirteen non-duplicate carbapenem-resistant *E coli* strains were collected from October 2017 to January 2018. Antimicrobial susceptibility was determined with the E test or disk diffusion method. The carbapenem minimum inhibitory concentrations (MICs) were determined by the agar dilution method. The colistin and tigecycline MICs were determined by broth microdilution. The resistance genes, including carbapenemase genes, were evaluated by polymerase chain reaction, and DNA sequencing was performed to characterize the genes. Pulsed-field gel electrophoresis and multilocus sequence typing (MLST) were performed to evaluate the clonal relatedness of isolates. The clinical data of patients were retrospectively reviewed.

**Results:** All the *E coli* isolates harbored *bla*<sub>NDM-5</sub> gene and were resistant to most of the antimicrobial agents, such as carbapenem, cephalosporins, ciprofloxacin, and chloramphenicol, excluding amikacin and colistin. Other resistant genes, such as *bla*<sub>TEM-1</sub>, *bla*<sub>CTX-M-15</sub>, *bla*<sub>CMY-2</sub>, *aac*(6')-Ib-cr, and *qepA*, were detected. The *E coli* isolates harboring *bla*<sub>NDM-5</sub> belonged to ST361 (n = 11), ST12 (n = 1), ST410 (n = 1), and PFGE types A (n = 11), B (n = 1), and C (n = 1).

**Conclusions:** This study reports on an outbreak of a predominant epidemic clone, the NDM-5 producing, multidrug-resistant *E coli* ST361 isolate. These findings suggest that we should pay attention to infection control measures to limit the spread of NDM-5-producing pathogens.

**KEYWORDS**

Carbapenemase-producing *Enterobacteriaceae*, *Escherichia coli* ST 361, New Delhi metallo-beta-lactamase

**Abbreviations:** AM, ampicillin; AMK, amikacin; ATM, aztreonam; CAZ, ceftazidime; CFZ, cefazolin; CHL, chloramphenicol; CIP, ciprofloxacin; CST, colistin; CTX, cefotaxime; ETP, ertapenem; FEP, cefepime; GEN, gentamicin; IPM, imipenem; ST, sequence typing; TGC, tigecycline; TMP/SMX, trimethoprim/sulfamethoxazole; TZP, piperacillin/tazobactam.

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## 1 | INTRODUCTION

The *Enterobacteriaceae* family are leading causes of infections, such as urinary tract infections, hospital- and healthcare-associated pneumonia, and bloodstream infections.<sup>1</sup> Carbapenems that exhibit broad antibacterial activity among beta-lactams are used as effective antibiotics against *Enterobacteriaceae*-producing extended-spectrum beta-lactamases (ESBL) and plasmidic AmpC (pAmpC).<sup>2</sup>

However, as the emergence of carbapenemase-producing *Enterobacteriaceae* (CPE) is increasingly reported worldwide, bacterial resistance to antibiotics has become a major source of concern for public health in recent years.<sup>3–6</sup> According to the report of the European Survey on carbapenemase-producing *Enterobacteriaceae* (EuSCAPE), 19% (77/402) of *E coli* clinical isolates not susceptible to carbapenem, are carbapenemase producers<sup>7</sup> (eg, KPC, NDM, VIM, or OXA-48-like). Carbapenemases mainly belong to Ambler classes A, B, and D of beta-lactamase. Among them, New Delhi metallo-beta-lactamase (NDM) belongs to Ambler class B and can hydrolyze almost all beta-lactam antibiotics, including penicillins, cephalosporins, and carbapenems, except monobactams, such as aztreonam.<sup>8,9</sup>

Since NDM-1 producing *Klebsiella pneumoniae* isolates were initially described in 2008,<sup>9</sup> to date, 21 NDM subtype variants have been reported worldwide.<sup>10</sup> The NDM subtypes contain one to five amino-acid substitutions that confer different levels of hydrolyzing activity against carbapenems and other  $\beta$ -lactam substrates.<sup>3</sup>

Among NDM variants, the first NDM-5 was identified in 2011 from a multidrug-resistant *E coli* ST648 isolate in the United Kingdom, from a patient previously hospitalized in India, and differed from NDM-1 with two amino acid substitutions at positions 88 (Val  $\rightarrow$  Leu) and 54 (Met  $\rightarrow$  Leu).<sup>11</sup>

In South Korea, the *bla*<sub>NDM-5</sub> gene was first detected in 2015, in *Klebsiella pneumoniae* clinical isolates co-producing oxacillinase 181,<sup>12</sup> and has been reported intermittently since then. However, an outbreak of NDM-5-producing isolates, especially *E coli* ST 361, has not yet been described.

In this study, we report an outbreak of NDM-5-producing *E coli* in a tertiary hospital in South Korea and characterize the molecular epidemiology and antibiotic resistance profiles of the isolates.

## 2 | MATERIALS AND METHODS

### 2.1 | Bacterial strains

Chungnam National University Hospital is a 1300-bed tertiary care hospital located in the Daejeon of South Korea, a city of about 1 490 000 residents. Prior to this outbreak, carbapenem-resistant *E coli* isolates were rarely detected and no case involving carbapenemase-producing *E coli* had been detected in this hospital.

CR-ECO1, which is resistant to carbapenem, including ertapenem and imipenem, was isolated from a urine specimen, obtained

from a patient in the neurosurgery ward on October 25, 2017. On October 31, 2017, another carbapenem-resistant CR-ECO2 was isolated from a urine sample of a patient hospitalized in the same ward, within the same period. Until January 23, 2018, a total of thirteen non-duplicate carbapenem-resistant *E coli* isolates were collected in the hospital. The identification of isolates was performed using the VITEK 2 ID-GNB cards (bioMérieux SA) according to the manufacturer instructions. The modified Hodge test, using ertapenem disks and Carba NP (bioMérieux SA), was conducted to confirm the phenotypic identification of carbapenemase production.<sup>13</sup> Both *E coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control strains for antimicrobial susceptibility testing. *Salmonella enterica serovar Braenderup* strain H9812 (ATCC BAA 664) was used as a reference marker for pulsed-field gel electrophoresis (PFGE). The clinical data for each patient were retrospectively reviewed.

### 2.2 | Antimicrobial susceptibility testing

The minimum inhibitory concentrations (MICs) of the carbapenems, such as ertapenem and imipenem, were determined by the agar dilution method, according to the Clinical and Laboratory Standards Institute (CLSI) guideline.<sup>14</sup> Antimicrobial susceptibility to nine drugs (piperacillin/tazobactam, cefepime, cefotaxime, ceftazidime, gentamicin, amikacin, ciprofloxacin, trimethoprim/sulfamethoxazole, and chloramphenicol) was evaluated using the E test (bioMérieux) on Mueller-Hinton (MH) agar (Difco Laboratories) in accordance with CLSI guidelines.<sup>15</sup> Antimicrobial susceptibility to three drugs (ampicillin, cefazolin, and aztreonam) was evaluated using the disk diffusion method on MH agar (Difco Laboratories) in accordance with CLSI guidelines.<sup>15</sup> The MICs for colistin and tigecycline were assessed by the broth microdilution method with MH broth (Difco Laboratories) in accordance with the recommendations of the joint CLSI-EUCAST (2016)<sup>16</sup> and European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria.<sup>17</sup>

### 2.3 | Resistance gene detection

Bacterial DNA was extracted using an ExiPrep Dx Bacteria Genomic DNA Kit (BIONEER) according to the manufacturer instructions. The carbapenemase genes (*bla*<sub>NDM1</sub>, *bla*<sub>IMP</sub>, *bla*<sub>VIM</sub>, *bla*<sub>KPC</sub>, *bla*<sub>OXA-48-like</sub>, *bla*<sub>GES</sub>, and *bla*<sub>OXA-181</sub>) were detected by PCR and direct sequencing was carried out for subtyping.<sup>18</sup> The presence of other resistance genes: (a) extended spectrum- $\beta$ -lactamases (ESBLs) encoding genes (*bla*<sub>CTX-M-1-like</sub>, *bla*<sub>CTX-M-9-like</sub>, *bla*<sub>TEM</sub>, and *bla*<sub>SHV</sub>); (a) AmpC genes (*bla*<sub>CIT</sub>, *bla*<sub>MOX</sub>, *bla*<sub>DHA</sub>, *bla*<sub>ACC</sub>, *bla*<sub>EBC</sub>, and *bla*<sub>FOX</sub>); (c) plasmid-mediated quinolone resistance genes, DNA gyrase protection from the action of the quinolones (*qnrA*, *qnrB*, and *qnrS*), antibiotics acetylation (*aac*( $\delta'$ )-*lb-cr*), and efflux pump production (*qepA*); and (d) 16S ribosomal methyltransferases (*armA*, *rmtA*, *rmtB*, and *rmtC*)<sup>19,20</sup> was detected by PCR with gene-specific primers. The amplicons were determined by DNA sequencing. The primers for the PCR are shown in Table 1.

**TABLE 1** The primers used to detect the resistance genes

	Gene	Primer	Sequence (5'–3')	Size of amplicon/bp	Reference
Carbapenem	<i>bla</i> <sub>NDM</sub>	F	GGTTTGGCGATCTGGTTTTTC	621	18
		R	CGGAATGGCTCATCACGATC		
	<i>bla</i> <sub>IMP</sub>	F	GGAATAGAGTGGCTTAAYTC	232	
		R	GGTTTAAAYAAAACAACCACC		
	<i>bla</i> <sub>VIM</sub>	F	GATGGTGTGGTTCGCATA	390	
		R	CGAATGCGCAGCACCAG		
	<i>bla</i> <sub>KPC</sub>	F	CGTCTAGTTCTGCTGTCTTG	798	
R		CTTGTCATCCTTGTAGGCG			
<i>bla</i> <sub>OXA-48</sub>	F	GCGTGGTTAAGGATGAACAC	438		
	R	CATCAAGTTCAACCCAACCG			
<i>bla</i> <sub>GES</sub>	F	GCTTCATTACGCACTATT	323	28	
	R	CGATGCTAGAAACCGCTC			
<i>bla</i> <sub>OXA-181</sub>	F	ATGCGTGTATTAGCCTTATCG	888	29	
	R	AACTACAAGCGCATCGAGCA			
β-lactamase	<i>bla</i> <sub>CTX-M-1</sub>	F	AGTTCACGCTGATGGCGACG	676	30
		R	AACCCAGGAAGCAGGCAGTCC		
	<i>bla</i> <sub>CTX-M-9</sub>	F	GATTGACCGTATTGGGAGTTT	947	
		R	CGGCTGGGTAAAATAGGTCA		
<i>bla</i> <sub>TEM</sub>	F	ATAAAATCTTGAAGACGAA	1080	32	
	R	GACAGTTACCAATGCTTAAT			
<i>bla</i> <sub>SHV</sub>	F	GGGTTATTCTATTGTGCGC	928		
	R	TTAGCGTTGCCAGTGCTC			
AmpC	<i>bla</i> <sub>CT</sub>	F	TGGCCAGAAGTGCAGGCAAA	462	19
		R	TTTCTCCTGAACGTG GCTGGC		
	<i>bla</i> <sub>MOX</sub>	F	GCTGCTCAAGGAGCACAGGAT	520	
		R	CACATTGACATAGGTGTGGTGC		
	<i>bla</i> <sub>DHA</sub>	F	AACTTTCACAGGTGTGCTGGGT	405	
		R	CCGTACGCATACTGGCTTTGC		
<i>bla</i> <sub>ACC</sub>	F	AACAGCCTCAGCAGCCGGTTA	346		
	R	TTGCCCGCAATCATCCCTAGC			
<i>bla</i> <sub>EBC</sub>	F	TCGGTAAAGCCGATGTTGCGG	302		
	R	CTTCCACTGCGGCTGCCAGTT			
<i>bla</i> <sub>FOX</sub>	F	AACATGGGGTATCAGGGAGATG	190		
	R	CAAAGCGCGTAACCGGATTGG			
Quinolone	<i>qnrA</i>	F	AGAGGATTTCTCACGCCAGG	580	33
		R	TGCCAGGCACAGATCTTGAC		
	<i>qnrB</i>	F	GGMATHGAAATTCGCCACTG <sup>a</sup>	264	
		R	TTTGCGYGYCGCCAGTCGAA <sup>a</sup>		
	<i>qnrS</i>	F	GCAAGTTCATTGAACAGGGT	428	
R		TCTAAACCGTCGAGTTCGGCG			
<i>aac(6)-Ib-cr</i>	F	TGACCAACAGCAACGATTCC	554	34	
	R	TTAGGCATCACTGCGTGTTCC			
<i>qepA</i>	F	GGACATCTACGGCTTCTTCG	720	35	
	R	AGCTGCAGGTAAGTGCAT			
16S rRNA methylase	<i>armA</i>	F	AGGTTGTTTCCATTTCTGAG	591	36
		R	TCTCTTCCATTCCCTTCTCC		
	<i>rmtA</i>	F	CTAGCGTCCATCCTTCTCTC	635	
		R	TTTGCTTCCATGCCCTTGCC		
<i>rmtB</i>	F	CCCAAACAGACCGTAGAGGC	585		
	R	CTCAAACCTCGGCGGGCAAGC			
<i>rmtC</i>	F	CGAAGAAGTAACAGCCAAAG	711		
	R	ATCCCAACATCTCTCCCACT			

Abbreviations: F, forward; R, reverse.

<sup>a</sup>M = A or C; H = A or C or T; Y = C or T.

## 2.4 | Pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST)

PFGE and MLST were used to investigate the homology levels among the *bla*<sub>NDM-5</sub>-positive *E coli* isolates. Bacterial DNA was prepared and cleaved with XbaI endonuclease (Roche) as described previously.<sup>21</sup> The XbaI-digested genomic DNA was subjected to PFGE using a CHEF-DR<sup>®</sup> III Variable Angle System (Bio-Rad), and then the PFGE patterns were compared using BioNumerics software (Applied Maths). Clusters were defined as DNA patterns sharing >85% similarity. PCR and sequencing for MLST were carried out for seven housekeeping genes per species: *adh*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, and *recA* for *E coli*<sup>22</sup> and the sequences were compared in the MLST database, so that allelic numbers and sequence types (STs) could be determined.<sup>3</sup> The allelic profiles and STs were assigned using an online database (<http://mlst.warwick.ac.uk/mlst/dbs/Ecoli>).

## 3 | RESULTS

### 3.1 | Clinical characteristics of the *Escherichia coli* isolates

All thirteen carbapenem-resistant *E coli* isolates showed positive phenotypic screening results for both the Modified Hodge test and the Carba-NP test. These isolates were obtained from stool (*n* = 6), urine (*n* = 5), bile fluid (*n* = 1), and pus (*n* = 1) from hospitalized patients aged from 46 to 83, with mean age of 69 years (Table 2). The stool samples were obtained using rectal swabs from patients admitted to an intensive care unit for CPE screening test. The outbreak timeline on the admission date and date of isolation of each patient is shown in Figure 1.

### 3.2 | Antibiotic resistance profile and distribution of resistant genes

All thirteen *E coli* isolates harbored *bla*<sub>NDM-5</sub> gene and were resistant to carbapenem (ertapenem and imipenem), ampicillin, piperacillin/tazobactam, and cephalosporins (cefazolin, cefepime, cefotaxime, and ceftazidime; Table 3). These isolates were also resistant to ciprofloxacin (92.3%, 12/13), chloramphenicol (92.3%, 12/13), tigecycline (76.9%, 10/13), gentamicin (15.4%, 2/13), and trimethoprim/sulfamethoxazole (7.7%, 1/13). Two isolates out of thirteen were resistant to aztreonam (15.4%, 2/13). All strains remained susceptible to colistin and amikacin. In addition to *bla*<sub>NDM-5</sub>, 11/13 strains harbored the *qepA* gene (Figure 2). The *bla*<sub>TEM-1</sub> was also detected in 23.1% (3/13). In particular, CR-ECO13 isolates also co-harbored *bla*<sub>CMY-2</sub>, *bla*<sub>CTX-M-15</sub>, and *aac(6)-Ib-cr* genes, as well as *bla*<sub>TEM-1</sub>. Other resistant genes that were evaluated, such as *qnrA*, *qnrB*, *qnrS*, *armA*, *rmtA*, *rmtB*, and *rmtC*, were not detected.

### 3.3 | Molecular epidemiology by MLST & PFGE

Three distinct MLST STs were observed among the thirteen isolates: ST361 with allelic profile 10-99-5-91-8-7-2 (11/13, 84.6%), ST12 (1/13, 7.7%), and ST410 (1/13, 7.7%). Moreover, three distinct PFGE patterns

(PFGE types A-C) were observed among these thirteen isolates: type A (*n* = 11), type B (*n* = 1), and type C (*n* = 1; Figure 1). Comparison of these results showed that all PFGE type A isolates corresponded to ST361, and the type B and C isolates corresponded to ST12 and ST410, respectively.

## 4 | DISCUSSION

In this study, we reported and characterized an outbreak caused by thirteen *bla*<sub>NDM-5</sub> carrying *E coli* isolates from hospitalized patients from October 2017 to January 2018. The *bla*<sub>NDM-5</sub> gene played an important role in conferring resistance to carbapenem. PFGE analysis showed that eleven out of thirteen isolates exhibited ≥90% similarities and belonged to the ST361 epidemic clone. Among them, eight isolates had the same pulsotype in PFGE, indicating they were clonally similar and showed a similar resistance phenotype in the antibiotic susceptibility testing. This finding suggests the possibility of nosocomial cross-transmission.

The predominant strain of this outbreak, *E coli* ST361, is not internationally well-known as an NDM producer. According to Yoon et al,<sup>3</sup> the distribution of NDM-5 producers in South Korea from 2010 to 2015 shows that *E coli* is the largest (14/18, 77.8%) and among them, ST 101 accounted for 77.8% (9/14), followed by ST362, ST361, ST162, ST90, and ST88 each accounted for 7.1% (1/14). The *E coli* ST361 harboring *bla*<sub>NDM-5</sub> only accounted for 7.1% (1/14) and did not belong to the most common clones, such as ST101, in South Korea. Therefore, this is the first outbreak report involving NDM-5-producing *E coli* ST361.

Here, the NDM-5-producing *E coli* ST361 strains showed high levels of multidrug resistance to carbapenem (ertapenem and imipenem), ampicillin, piperacillin/tazobactam, cephalosporins (cefazolin, cefepime, cefotaxime, and ceftazidime), ciprofloxacin, and chloramphenicol. Among the plasmid-mediated quinolone resistance genes, the co-harbored *qepA* genes encoding fluoroquinolone efflux pumps could partially contribute to the high rate of ciprofloxacin-resistance.<sup>23</sup> All ST361 strains were susceptible to amikacin, colistin, and trimethoprim/sulfamethoxazole, which mean they are treatment options with potent activity against these pathogens. A few cases of NDM-5-producing *E coli* ST361 have also been reported in China<sup>24,25</sup> and Nepal.<sup>26</sup> The *bla*<sub>NDM-5</sub>-carrying *E coli* ST361 that was isolated in Henan, China, showed resistance to sulfamethoxazole and co-harbored various resistance genes such as *bla*<sub>CTX-M-15</sub>, *bla*<sub>TEM-1</sub>, *aac(6)-Ib-cr*, and *qnrS*.<sup>25</sup> Additionally, the *bla*<sub>NDM-5</sub>-carrying *E coli* ST361 that was isolated in Zhejiang province, China carried *bla*<sub>CMY-42</sub> and *bla*<sub>TEM-1B</sub> via an IncX3 type plasmid.<sup>24</sup> However, resistance genes such as ESBLs, ampC genes, 16s ribosomal methyltransferases, and quinolone resistance genes, except for *qepA*, were not detected in NDM-5-producing *E coli* ST361 in this study.

The CR-ECO 13 isolate, which is *E coli* ST410, is an extensively drug-resistant strain, resistant to almost all antibiotics except for amikacin and colistin. This strain co-harbored *bla*<sub>CTX-M-15</sub>, *bla*<sub>CMY-2</sub> and *bla*<sub>TEM-1</sub>, which encodes an ESBL, conferring resistance to aztreonam and *aac(6)-Ib-cr*, which mediates high-level resistance to

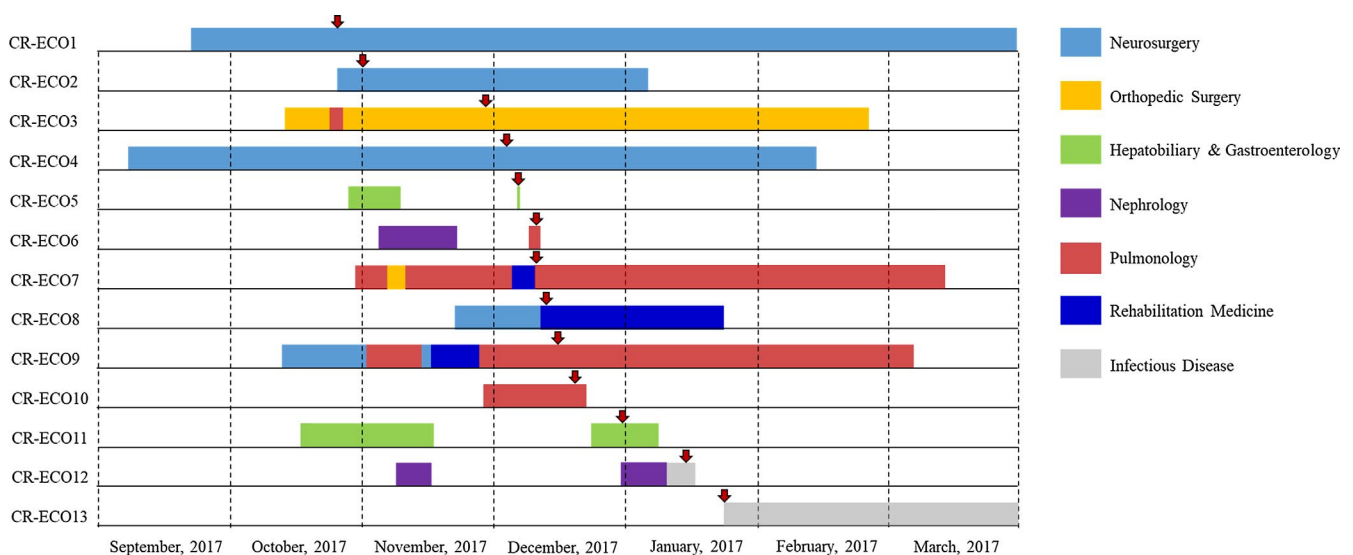
**TABLE 2** Characteristics of *bla*<sub>NDM-5</sub>-positive *Escherichia coli* isolates

Isolate No.	Age (y)/sex	Date of isolation	Specimen	Diagnosis
CR-ECO 1	56/M	10/25/2017	Urine	Traumatic subdural hemorrhage
CR-ECO 2	83/F	10/31/2017	Urine	Spinal stenosis
CR-ECO 3	73/F	11/29/2017	Urine	Fracture of shaft of femur
CR-ECO 4	61/M	12/03/2017	Stool	Hydrocephalus
CR-ECO 5	75/M	12/05/2017	Bile fluid	Malignant neoplasm of gallbladder
CR-ECO 6	76/F	12/10/2017	Stool	Pneumonia
CR-ECO 7	82/F	12/10/2017	Urine	Pneumonia
CR-ECO 8	62/F	12/12/2017	Stool	Subarachnoid hemorrhage
CR-ECO 9	78/F	12/15/2017	Urine	Cervical myelopathy & pneumonia
CR-ECO 10	78/F	12/19/2017	Stool	Pneumonia
CR-ECO 11	53/F	12/30/2017	Pus	Ulcerative colitis
CR-ECO 12	75/M	01/14/2018	Stool	Chronic kidney disease
CR-ECO 13	46/M	01/23/2018	Stool	Meningitis

aminoglycosides and fluoroquinolones. *Escherichia coli* ST410 has been reported worldwide as a potential high-risk pathogen associated with resistance to fluoroquinolones, third generation cephalosporins, and carbapenems.<sup>27</sup> Although ST410 is not a main strain of this outbreak, it poses a significant public health risk. These findings suggest that strict infection control is essential to prevent a dissemination of these high-risk clones. The *E coli* ST12 (CR-ECO 7) was susceptible to various antimicrobial agents (eg, aztreonam, gentamicin, amikacin, ciprofloxacin, trimethoprim/sulfamethoxazole, chloramphenicol,

and colistin) and negative for other resistant genes tested, except for *bla*<sub>NDM-5</sub>.

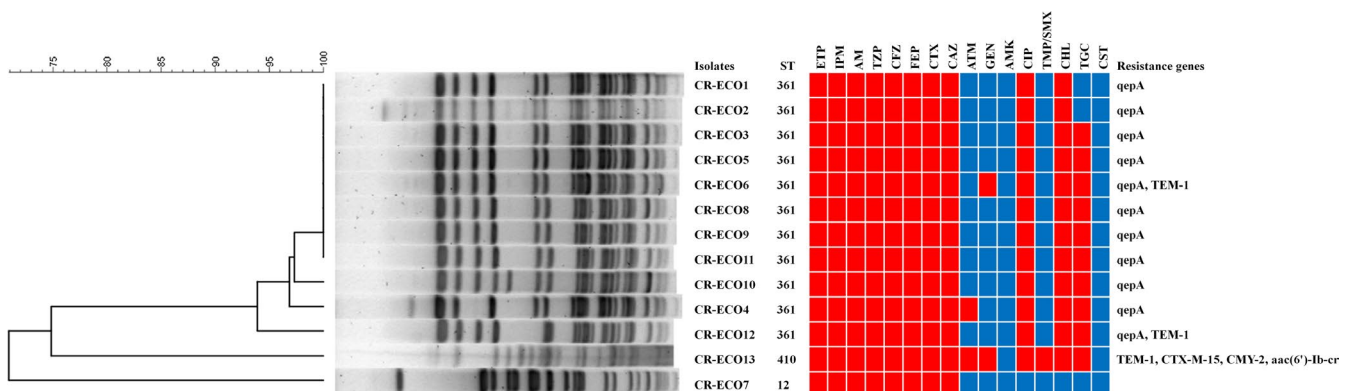
We carried out retrospective investigation with limited patient information. So, the entry and transmission route of *bla*<sub>NDM-5</sub>-producing *E coli* in this hospital is unclear. Environmental culture tests were carried out to investigate the possibility of dissemination through medical equipment and the surrounding environment, but carbapenemase-producing *E coli* were not detected. Other patients in contact with known NDM-5 carriers were also screened, but no NDM-5 producers

**FIGURE 1** Outbreak timeline of thirteen patients with *bla*<sub>NDM-5</sub>-producing *Escherichia coli* isolates. Shadows on the timeline represent the period of hospitalization and the wards. Shadows in different colors indicate different wards. The red arrow indicates the isolation date of the strains

**TABLE 3** Antibiotic susceptibilities of 13 bla<sub>NDM-5</sub>-positive *Escherichia coli* isolates

Isolate No.	MICs (µg/mL)												
	ETP	IPM	TZP	FEP	CTX	CAZ	GEN	AMK	CIP	TMP/SMX	CHL	TGC	CST
CR-ECO1	32	64	>128	>256	>256	>256	1	2	>32	0.125	>256	0.5	2
CR-ECO2	32	64	>128	>256	>256	>256	1	2	>32	0.125	>256	0.5	2
CR-ECO3	16	8	>128	>256	>256	>256	1	2	>32	0.19	>256	1	0.5
CR-ECO4	32	128	>128	>256	>256	>256	0.125	0.125	>32	0.25	>256	1	0.5
CR-ECO5	32	128	>128	>256	>256	>256	1	2	>32	0.25	>256	1	0.5
CR-ECO6	32	64	>128	>256	>256	>256	32	1	>32	0.5	>256	1	2
CR-ECO7	32	64	>128	64	>256	>256	1	2	0.016	0.064	2	0.5	1
CR-ECO8	32	256	>128	>256	>256	>256	1	1.5	>32	0.125	>256	1	2
CR-ECO9	32	64	>128	>256	>256	>256	1	2	>32	0.38	>256	4	2
CR-ECO10	32	256	>128	>256	>256	>256	1	3	>32	0.125	>256	1	2
CR-ECO11	32	64	>128	>256	>256	>256	1	1	>32	0.19	>256	1	2
CR-ECO12	32	128	>128	>256	>256	>256	1	1	>32	0.19	>256	1	2
CR-ECO13	32	128	>128	>256	>256	>256	64	8	>32	>32	>256	1	0.5

Abbreviations: AMK, amikacin (0.016–256 µg/mL); CAZ, ceftazidime (0.016–256 µg/mL); CHL, chloramphenicol (0.016–256 µg/mL); CIP, ciprofloxacin (0.002–32 µg/mL); CST, colistin; TMP/SMX, trimethoprim/sulfamethoxazole (0.002–32 µg/mL); CTX, cefotaxime (0.016–256 µg/mL); ETP, ertapenem; FEP, cefepime (0.016–256 µg/mL); GEN, gentamicin (0.064–1024 µg/mL); IPM, imipenem; MICs, minimum inhibitory concentrations; TGC, tigecycline; TZP, piperacillin/tazobactam (0.016–256 µg/mL).

**FIGURE 2** Dendrogram based on pulsed-field gel electrophoresis patterns, multilocus sequence typing, antibiogram, and distribution of resistance genes of thirteen NDM-5-producing *Escherichia coli* isolates. The red and blue squares indicate resistance and susceptibility to each antibiotic, respectively

were detected. However, PFGE patterns showed that ST361 isolates were closely related. Also, some patients admitted to the same ward during the same period, overlapped with each other. Therefore, we suspect that NDM-5-producing *E coli* was transmitted by patients or medical staff. The relatively long hospitalization period of patients may also increase the possibility of dissemination of the NDM-5-producing *E coli* isolates. To prevent further spread, enhanced infection control measures, such as strengthening of hand hygiene, contact precaution, environmental cleaning, and preemptive patient isolation, were implemented. The outbreak was interrupted in February 2018, 3 months after isolation of the first NDM-5-producing *E coli*.

Considering that *E coli* is one of the major causes of community-acquired infections, and the spread of these strains is possible not only in the hospital, but also in the surrounding environment, the

severity of dissemination of *E coli* carrying the bla<sub>NDM-5</sub> gene will be even greater.<sup>2</sup>

In conclusion, the rapid dissemination of NDM-5-producing *E coli* emphasizes that stringent infection control measures and active surveillance play important roles to prevent the spread of these pathogens.

#### CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

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**How to cite this article:** Park Y, Choi Q, Kwon GC, Koo SH. Emergence and transmission of New Delhi metallo-beta-lactamase-5-producing *Escherichia coli* Sequence Type 361 in a Tertiary Hospital in South Korea. *J Clin Lab Anal.* 2020;34:e23041. <https://doi.org/10.1002/jcla.23041>