Molecular Characteristics of First IMP-4-Producing Enterobacter cloacae Sequence Type 74 and 194 in Korea

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The worldwide dissemination of carbapenemase-producing Enterobacteriaceae (CPE) has become a major therapeutic concern in clinical settings. Enterobacter cloacae is a major pathogen that causes serious hospital-acquired infections. We investigated the clinical characteristics and molecular mechanisms of the first IMP-4-producing E. cloacae clinical isolates in Korea. Five carbapenemase-producing E. cloacae strains out of 792 E. cloacae clinical isolates, which have been identified at a university hospital in Korea between March 2014 and February 2016, were included in this study. Antimicrobial susceptibilities to imipenem, meropenem, and ertapenem were tested using E-test. Carbapenemase determinant screening, genetic environment, and multilocus sequence typing were conducted using PCR and sequencing analysis. All isolates were not susceptible to at least one of the tested carbapenems and presented highly similar pulsed-field gel electrophoresis (PFGE) patterns, evidencing hospital-wide clonal dissemination. Among all isolates harboring the blaIMP−4 carbapenemase gene, four isolates identified as predominant ST74, also contained blaCMY−2. One strain, designated as rare ST194, carried blaCMY−1. The E. cloacae strain, harboring both blaIMP−4 and blaCMY−1, was resistant to all three tested carbapenems. The blaIMP−4 gene was located on a highly mobile class 1 integron, showing a new form of the blaIMP−4-qacG-aacA4 array. This is the first description of IMP-4-producing E. cloacae strains in Korea. This observation implicates the widespread of blaIMP−4 in Enterobacteriaceae clinical isolates and provides insights into the epidemic potential and clinical therapeutic importance of IMP-4-producing E. cloacae for healthcare-associated infections.

Keywords: IMP-4, CMY, carbapenem, class 1 integron, Enterobacter cloacae

INTRODUCTION

The spread of carbapenemase-producing Enterobacteriaceae (CPE) has become a prominent health-care challenge worldwide in the treatment of infectious diseases. Carbapenemases, including Klebsiella pneumoniae carbapenemase (KPC), imipenemase (IMP), New Delhi metallo-β-lactamase (NDM), Verona integron-encoded metallo-β-lactamase (VIM), and oxacillinase (OXA)-48 medicated antibiotic resistance (Nordmann et al., 2011; Shi et al., 2017). IMP-type CPEs have been reported globally (Queenan and Bush, 2007; Tzouvelekis et al., 2012) and have become the...
predominant form in Australia (Espedido et al., 2008; Leung et al., 2013; Sidjabat et al., 2015) since the first report of IMP-1 from Pseudomonas aeruginosa in Japan (Watanabe et al., 1991). One of the most commonly observed IMP variants was IMP-4 in clinical Enterobacteriaceae isolates (Leung et al., 2013; Hu et al., 2014), which was firstly detected in Hong Kong (Chu et al., 2001). Among more than 11 different species of IMP-4-producing CPE, Enterobacter cloacae has emerged as the predominant species (Sidjabat et al., 2015; Cao et al., 2017). IMP-type E. cloacae isolates have been found in Taiwan (IMP-8), China (IMP-1 and IMP-34), Thailand (IMP-14), Japan (IMP-1 and IMP-11), Spain (IMP-13), United Kingdom (IMP-1), and South Africa (Figure 1; Chen et al., 2009; Shet et al., 2011; Hayakawa et al., 2014; Wang et al., 2015; Osei Sekyere, 2016; Matsumura et al., 2017). IMP-4-producing E. cloacae was particularly reported in Australia and caused clinical outbreaks, which brought greater challenges to infection control (Leung et al., 2013; Chapuis et al., 2016; Pang et al., 2016). The highly mobile class 1 integron facilitates global spread of the blaIMP-4 gene (Espedido et al., 2008; Partridge et al., 2012).

Until now, carbapenem-resistant E. cloacae has rarely been reported in Korea since the initial VIM-2-producing isolate in 2003 (Jeong et al., 2003). Here, we described the clinical characteristics and molecular mechanisms of the first IMP-4-producing E. cloacae clinical isolates in Korea.

MATERIALS AND METHODS

Bacterial Strains

A total of 792 E. cloacae clinical isolates have been identified at a university hospital in Korea between March 2014 and February 2016. Among the isolates, five carbapenemase-producing E. cloacae strains (0.6%), YUMC1, YUMC2, YUMC3, YUMC4, and YUMC5 were included in this study. The isolates were identified as E. cloacae using the Vitek GNI card (bioMérieux, Marcy l’Étoile, France) and 16S rRNA sequencing (Lane et al., 1985; Mao et al., 2012; Mezzatesta et al., 2012; Jeong et al., 2015). This study was carried out in accordance with the recommendations of Institutional Review Board of Kosin University Gospel Hospital, Busan, Korea; with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. We primarily focused on the analysis of the isolated strains and made our effort to anonymize private information of infected patients.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibilities were determined by the Vitek card AST-N246 (bioMérieux). Carbapenem producers were identified by modified Hodge test on MacConkey agar (Becton, Dickinson and Company, Sparks, MD, USA). The performance of modified Hodge test was reported to be better with MacConkey
| Class^a  | Target gene(s) or region | Primer name | Sequence (5′ to 3′) | References | Position in Figure 2 |
|---------|-------------------------|-------------|---------------------|------------|----------------------|
| Identification | 16S rRNA | 16S-F | AGAGTTTGATYMTGGCTCAG | Mao et al., 2012 |  |
| | | 16S-R | CCGTCAATTTCTAATGAGTT | Lane et al., 1985 |  |
| | blaIMP cluster | 10IMP-F | AAGGCGTTTATGCTACATTG | Hong et al., 2015 | 1 |
| | | IMP-bF | AAGGCGTTTATGCTACATTG | This study | 5 |
| | | IMP-mR | TQATGAAGCGTTTATGCTACA | This study | 4 |
| | | 10IMP-R | TTAACCGCCTGCTCAATAGTAA | Hong et al., 2015 | 2 |
| QAC | qacG | qacG-F | GGTTATTTCTGGAATGCGTCA | This study | 7 |
| | | qacG-R | AGCAAGTTGAGCGAACAGCAAC | This study | 6 |
| Integron CS | inteI1 | 5CS | CTCTTAGAAACACGGAGATGC | Jeong et al., 2003 | 3 |
| | sul1 | sul1-R | GGGTTCTCCAGAAGGAATGTT | Bae et al., 2007 | 10 |
| Fluoroquinolones | aac(6′)-ib-cr | aac(6′)-ib-F | TQACCTTGCGATGCTCATAG | This study | 9 |
| | | aac(6′)-ib-R | TTAAGGCTCAGTCGCTGCTC | This study | 8 |
| | qnrA | qnrAa-F | AAAATCCAGACATGCAATTTTTC | This study |  |
| | | qnrAa-R | AAAGGAAGGACGGGATGCTAT | This study |  |
| | qnrB1 | qnrB1-F | GCACTGTATGGCCGTTATG | This study |  |
| | | qnrB1-R | TGGGTACCGCTGCGATGAGTT | This study |  |
| | qnrB4 | qnrB4-F | GATGACTCTGGCGTTAGTGC | This study |  |
| | | qnrB4-R | CGGTCACTCGATGCTGCTGCT | This study |  |
| | qnrD | qnrD-F | CGTACGCTATGCGCAGCTAAGA | This study |  |
| | | qnrD-R | TCACTGCGATGCGTCGCGG | This study |  |
| | qnrS1 | qnrS-F | GACGGATACCGGCGGATGCT | This study |  |
| | | qnrS-R | GGACCTCATTTTCTGGAAGGATGCT | This study |  |
| ESBLs; Ambler class A | blaTEM cluster | TEM-F | TCCGCTCATGAGAACAATTAA | Bae et al., 2011 |  |
| | | TEM-R | ACGGCTCAGTGAGCAAGAAAC | Bae et al., 2011 |  |
| | blaSHV cluster | SHV-F | CCCGCGGTATTTCCGATTGTG | Bae et al., 2011 |  |
| | | SHV-R | CCCGCGGTATTTCCGATTGTG | Bae et al., 2011 |  |
| | blaVEB cluster | VEB-F | AAAATGCGAAGATAGGATGACG | Bae et al., 2011 |  |
| | | VEB-R | TCCACGTTATTTTCTGGAATTGCT | Bae et al., 2011 |  |
| | blaGES/IBC cluster | GES-F | CGTTTCATTTTCTGGAATTGCT | Bae et al., 2011 |  |
| | | GES-R | GTCCGCTGATGACGATG | Bae et al., 2011 |  |
| | blaCTX−M−1 cluster | CMT-M−1-F | CCGTCAGGCTGTGATATTAAG | Bae et al., 2011 |  |
| | | CMT-M−1-R | ACGGCTCAGTGAGAAAC | Bae et al., 2011 |  |
| | | CMT-M−9-F | CAGGGAGTCCAAGCAGGATG | Bae et al., 2011 |  |
| | | CMT-M−9-R | CAGGGAGTCCAAGCAGGATG | Bae et al., 2011 |  |
| | blakPC cluster | KPC-F | GTCAGCTGATGCGCTGCTGCA | Hong et al., 2015 |  |
| | | KPC-R | TGTGCGCGCAATAGTGGATT | Hong et al., 2015 |  |
| | blakNMC−A/M cluster | IMC-F | CATTTTTCTACAGCGCCRATACAC | This study |  |
| | | IMC-R | TGCTTTGCTACATTGCTG | This study |  |
| Ambler class B | blakVIM cluster | VIM-2F | ATCATGCTATGCGATGAGCT | Hong et al., 2015 |  |
| | | VIM-2R | ACGCTGAGGATCGGATG | Hong et al., 2015 |  |
| Ambler class C; AmpCs | blakCMY−1 cluster | CMY-1F | GTCAAGCGCGATGACGATGCT | This study |  |
| | | CMY-1R | TATTTGCGCAGTGGACG | This study |  |
| | blakCMY−2 cluster | CMY-2F | GCAAGCGATGCGGATG | This study |  |
| | | CMY-2R | GCYACCTGATGCGGAAAYG | This study |  |
| Ambler class D | blakOXA−48 cluster | OXA48-F | CAGAACGACCTATGCATATAT | This study |  |
| | | OXA48-R | GCATATCCCATCATCATCAG | This study |  |

^aQAC, quaternary ammonium compounds; CS, Conserved segment; ESBLs, extended-spectrum β-lactamases.
aggregates, containing bile compounds, than with Mueller-Hinton agar for screening carbapenemase-producing Gram-negative bacilli (K. Lee et al., 2010). Carbapenemase production was confirmed by KPC+MBL Confirm ID Kit (Rosco Diagnostica, Taastrup, Denmark) using tablets containing meropenem (10 µg) alone or supplemented with dipicolinic acid (1,000 µg), phenylboronic acid (400 µg), and clavulanic acid (575 µg). Mueller-Hinton agar (Oxoid Ltd., Basingstoke, UK). The minimum inhibitory concentrations (MICs) for imipenem, meropenem, and ertapenem were determined using E-test strips (AB Biodisk, Solna, Sweden). The breakpoints were applied according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (Clinical Laboratory Standards Institute, 2016). Double-disc synergy test (DDST) for the detection of extended-spectrum β-lactamases (ESBLs) was also performed according to the CLSI guideline.

Pulsed-Field Gel Electrophoresis
Pulsed-field gel electrophoresis (PFGE) was performed to confirm the clonality of the IMP-4-producing E. cloacae isolates. XbaI (Roche, Mannheim, Germany)-digested genomic DNA was prepared at 37°C for 12–14 h. DNA fragments were separated using a CHEF-DRII System (Bio-Rad, Hercules, CA, USA). Banding patterns were analyzed with InforQuestFP software version 4.5 (Bio-Rad) to generate a dendrogram.

Multilocus Sequence Typing
Multilocus sequence typing (MLST) for seven housekeeping genes, including dnaA, fusA, gyrB, leuS, pyrG, rplB, and rpoB, was conducted. After PCR and sequencing, nucleotide sequences were compared with those in the MLST database (http://pubmlst.org/ecloacae) to identify allelic numbers and sequence types (ST).

Polymerase Chain Reaction and Sequencing
The genomic DNA of five isolates were extracted via the boiling lysis method (L. Chen et al., 2011). The genes for 16S rRNA, carbapenemase, integron components, fluoroquinolones, ESBLs, and plasmid-mediated AmpCs were amplified by polymerase chain reaction (PCR) and sequenced using the primers (Lane et al., 1985; Jeong et al., 2003; Bae et al., 2007, 2011; Mao et al., 2012; Hong et al., 2015) described in Table 1. Briefly, the PCR program was as follows: 94°C denaturation for 5 min, followed by 30 cycles of 94°C denaturation for 30 s, then 55–60°C annealing for 30 s, and subsequently 72°C extension for 30 s, followed by 72°C final extension for 7 min. The amplified products were sequenced and the nucleotide sequences were compared by the Basic Local Alignment Search Tool (BLAST) (https://www.ncbi.nlm.nih.gov/BLAST) (Jeong et al., 2015). Genetic organization of class 1 integron carrying the bla IMP-4 gene cassette of a plasmid was investigated by PCR mapping and sequencing of the regions surrounding the gene using the primers described in Table 1 (Jeong et al., 2003; Bae et al., 2007; Hong et al., 2015). The integron variant was identified using INTEGRALL database (http://integrall.bio.ua.pt/) (Moura et al., 2009).

Nucleotide Sequence Accession Number
Nucleotide sequence data for YUMC2 are available under the GenBank accession number KY884003 and assigned In1456 for class 1 integron based on the INTEGRALL database (http://integrall.bio.ua.pt/) (Moura et al., 2009).

RESULTS
Description of the Patients
The clinical characteristics of the patients infected with five isolates are summarized in Table 2. The carbapenemase-producing E. cloacae strains were isolated from various departments and two of them were recovered from the open wounds in diabetic feet. Most of the patients suffered from underlying diseases such as hypertension, diabetes mellitus and/or cancer causing immunocompromised conditions.

Antimicrobial Susceptibility Profiles
The antimicrobial susceptibility profiles of five E. cloacae isolates with blaIMP-4 are presented in Supplementary Table 1. All five isolates were not susceptible to ampicillin, amoxicillin-clavulanic acid, cephalosporins, and carbapenems, whereas susceptible to amikacin, gentamicin, tigecycline, and ciprofloxacin, except for YUMC2. DDST for ESBL was negative for all five isolates. The antimicrobial susceptibility profiles of the other 787 E. cloacae strains are also summarized in Supplementary Table 2. The

### Table 2: Clinical characteristics of the patients infected with IMP-4-producing E. cloacae isolates.

| Strain | Sex/Age | Department | Specimen | Date of isolation (year/month) | Diagnosis | Comorbidity |
|--------|---------|------------|----------|-------------------------------|-----------|-------------|
| YUMC1  | M/44    | OS         | Wound    | 2014/9                        | Open wound on right Tox; Diabetes mellitus foot necrosis | Hypertension; Type 2 diabetes mellitus |
| YUMC2  | M/47    | PS         | Wound    | 2015/2                        | Open wound on right foot | Hypertension; Type 2 diabetes mellitus; Old cerebrovascular attack |
| YUMC3  | F/41    | GS         | Ascitic fluid | 2015/9 | Invasive carcinoma of right breast | Renal cell carcinoma; Chronic gastritis |
| YUMC4  | F/70    | NS         | Urine    | 2016/2                        | Spontaneous SAH with right PICA aneurysm | Hypertension; Cerebral infarction |
| YUMC5  | F/20    | OBGY       | Vaginal swab | 2016/2 | Vaginitis | Not specified |

Notes: OS, orthopedic surgery; PS, plastic surgery; GS, general surgery; NS, neurosurgery; OBGY, obstetrics/gynecology; SAH, subarachnoid hemorrhage; PICA, posterior inferior cerebellar artery.

The integron variant was identified using INTEGRALL database (http://integrall.bio.ua.pt/) (Moura et al., 2009).
TABLE 3 | Pulsed-field gel electrophoresis (PFGE)-based dendrogram and multilocus sequence typing (MLST) of IMP-4-producing E. cloacae isolates.*

| Isolates       | PFGE-XbaI pattern | Sequence type | MIC(µg/ml) of β-lactamases | MLST       |
|----------------|-------------------|---------------|-----------------------------|------------|
|                |                   |               | IPM MEM EPM                 |            |
| YUMC 2         | 4 4               | IMP-4, CMY-1  | 7 33                        | ST74       |
| YUMC 3         | 1 1               | IMP-4, CMY-2  | 11 11                       |            |
| YUMC 4         | 2 4               | IMP-4, CMY-2  | 11 11                       |            |
| YUMC 5         | 1 1               | IMP-4, CMY-2  | 11 11                       |            |
| YUMC 1         | <0.5 0.5          | IMP-4, CMY-2  | 11 11                       |            |

*Similarity index scale is shown above the dendrogram, and % similarity indexes are indicated over the nodes.

% Similarity index

| % Similarity index | % Similarity index | % Similarity index | % Similarity index | % Similarity index | % Similarity index |
|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
| 90                 | 90                 | 95                 | 95                 | 95                 | 100                |

The MIC values of ≤1, 2, and ≥4 are susceptible, intermediate, resistant to imipenem and meropenem, respectively. The breakpoints for ertapenem are ≤0.5, 1, and ≥1 according to the interpretative criteria of Clinical and Laboratory Standards Institute (CLSI) guideline. MIC, minimum inhibitory concentration; IPM, imipenem; MEM, meropenem; EPM, ertapenem.

Resistance to Carbapenems

All five isolates were positive as carbapenem producers in the modified Hodge test and KPC+MBL Confirm ID Kit (Rosco Diagnostica). The MICs were determined using E-test strips (AB Biodisk) and the results for imipenem, meropenem, and ertapenem are presented in Table 3. All isolates were not susceptible to at least one of the carbapenems using CLSI breakpoints. Notably, YUMC2 was resistant to all tested carbapenems and had higher MICs than other isolates.

Clonality of the Isolates

YUMC4 and YUMC5 strains presented identical PFGE patterns and the other isolates also showed highly similar patterns based on the criteria of 85% similarity (Table 3). The strains, isolated same years, presented close relationship.

Sequence Type

The MLST assay assigned the isolates to two STs (Table 3). YUMC2 was assigned to predominant ST74. Four out of the five IMP-4-producing E. cloacae strains were rare ST194, showing significant clonal similarity.

Carbapenemase Genes and Genetic Environment

PCR screening demonstrated the presence of bla<sub>IMP</sub>-4 in all E. cloacae isolates (Table 3). In addition, YUMC2 was also positive for CMY-1. The other strains contained IMP-4 and CMY-2 simultaneously. The ESBL genes were not detected whereas, <i>aac(6′)-Ib-cr</i> and <i>qnrS1</i> relevant to fluoroquinolones were found. In this study, <i>bla</i><sub>CMY</sub>-1, <i>bla</i><sub>CMY</sub>-2, <i>bla</i><sub>IMP</sub>-4, <i>aac(6′)-Ib-cr</i>, and <i>qnrS1</i> were identical to previously reported sequences deposited in GenBank database under accession numbers X92508.1, X91840.1, AF244145.1, CP023487.1, and AB187515.1, respectively.

PCR mapping and sequencing generated a 3,585-bp segment that shared 99% identity with E. cloacae pEI1573 (GenBank accession no. JX101693.1) (Partridge et al., 2012). The <i>bla</i><sub>IMP</sub>-4 gene was located on class 1 integron In1456, consisted of novel <i>bla</i><sub>IMP</sub>-4-qacG2-aacA4 cassette array (Figure 2).

DISCUSSION

E. cloacae is frequently implicated in serious nosocomial infections with high mortality. Majority of patients were reported to be immunocompromised, similar to our patients (Qureshi et al., 2011). Clinical outbreaks of E. cloacae in the hematology ward, burns unit, and intensive care unit have persisted, despite of concerted infection control to prevent ongoing transmission (Leung et al., 2013; Chapuis et al., 2016; Pang et al., 2016). VIM-2, NDM-1, and IMP-1, frequently found in Asia, have been previously reported mechanisms of carbapenem-resistant E. cloacae in Korea (Jeong et al., 2003; Kim et al., 2015; Lee et al., 2017). Meanwhile, IMP-4-producing E. cloacae isolates have been mainly found in Australia (Peleg et al., 2005; Leung et al., 2017). Overall patterns are similar to those of five IMP-4-producing isolates, except for the carbapenems.
The first detection of IMP-4 in this study implicates that the plasmid-mediated \( \text{bla}_{\text{IMP-4}} \) eventually spread in \( \text{E. cloacae} \) clinical isolates in Korea.

IMP-4 was reported to be strongly active against imipenem and meropenem, with 0.25–16 MIC range (Chu et al., 2001). The MICs of our isolates showed that all five strains were not susceptible to at least one of the carbapenems, including imipenem, meropenem, and ertapenem. Antibiotic resistance profiles of \( \text{bla}_{\text{IMP-4}} \)-positive \( \text{Enterobacteriaceae} \) isolates showed 25% resistance, 57% intermediate resistance, and 18% susceptibility to meropenem and 6% resistance, 33% intermediate resistance, and 61% susceptibility to imipenem in a previous study (Dolejska et al., 2016). Natural antibiotic susceptibility of \( \text{E. cloacae} \) complex to carbapenems were reported to be susceptible (Stock et al., 2001), however, the presence of IMP-4 would influence on the antibiotic profiles.

The antimicrobial susceptibility profiles of \( \text{E. cloacae} \) isolates in this study were similar to the intrinsic patterns of antibiotics (Mezzatessa et al., 2012). However, 5 strains containing \( \text{bla}_{\text{IMP-4}} \) were not susceptible to carbapenems and YUMC2 was resistant to ciprofloxacin. The detected genes, \( \text{aac}(6')\text{-Ib-cr} \) and \( \text{qnrS1} \) relevant to fluoroquinolones might be associated with this results. Nevertheless, the \( cr \) variant of \( \text{aac}(6')\text{-Ib} \) confers reduced susceptibility to ciprofloxacin by \( N \)-acytlylation of its piperazinyl amine (Robicsek et al., 2006), ciprofloxacin resistance was not related to \( \text{aac}(6')\text{-Ib-cr} \) prevalence (Park et al., 2006). Interestingly, the isolates co-carrying \( \text{aac}(6')\text{-Ib-cr} \) and \( \text{qnrS1} \) were also reported to be sensitive to quinolones (Huang et al., 2012). Therefore, these genes seems to supplement other quinolone resistance mechanisms rather than confer directly to resistance. Although, the \( \text{aac}(6')\text{-Ib-cr} \) and \( \text{qnrS1} \) genes were frequently found to be co-carried with various ESBLs, becoming therapeutic threats (Huang et al., 2012; Mezzatessa et al., 2012), our isolates harbored \( \text{bla}_{\text{IMP-4}} \) without ESBLs.

The homogeneity of five strains was analyzed using PFGE. Although the strains were isolated from various clinical departments, the high similarity of PFGE patterns of isolates, especially in the same years, might be the evidence of hospital-wide clonal dissemination.

According to MLST results, YUMC2 was designated to ST74, the most predominant clonal lineage with increased epidemic potential based on previous \( \text{E. cloacae} \) clonality studies (Fernández et al., 2015; Guillard et al., 2015; Izdebski et al., 2015). \( \text{E. cloacae} \) ST74 had higher carbapenems MICs than other isolates, similar to the results of previous studies, and was assumed to confer with the spread of the resistance to carbapenems (Guillard et al., 2015; Izdebski et al., 2015). The other four IMP-4-producing \( \text{E. cloacae} \) strains were ST194, presenting significant genetic similarity. To the best of our knowledge, available studies for \( \text{E. cloacae} \) ST194 were rare, indicating that this is the first report of clinical \( \text{E. cloacae} \) ST194.

PCR results showed the presence of CMY-1 in YUMC2 and CMY-2 in the other strains as well as IMP-4. Prior studies demonstrated that the most frequently reported AmpC \( \beta \)-lactamase was CMY, consisting of 92.7% of CMY-2 among \( \text{Enterobacteriaceae} \) isolates in the Asia-Pacific region (Sheng et al., 2013). The combination of \( \text{bla}_{\text{IMP-4}} \) and \( \text{bla}_{\text{CMY-2-like}} \) was found from one clinical \( \text{E. cloacae} \) isolate among the CPE in Australia (Sidjabat et al., 2015). In addition, the coexistence of \( \text{bla}_{\text{IMP-4}} \) and \( \text{bla}_{\text{CMY-1}} \) in \( \text{E. cloacae} \) strain was not reported previously and this is the first description of \( \text{E. cloacae} \), coproducing IMP-4 and CMY-1 with resistance to all three carbapenems.

When comparing the product of sequencing of our study to \( \text{E. cloacae} \) pEI1573 (GenBank accession no. JX101693.1) (Partridge et al., 2012), both of the \( \text{bla}_{\text{IMP-4}} \) genes of our study and pEI1573 were located on class 1 integrons. However, the gene cassettes compositions were slightly different between YUMC2 and pEI1573, containing a reference sequence of typical Australian class 1 integrons (Mezzatessa et al., 2012), the high similarity of PFGE patterns of isolates, especially in the same years, might be the evidence of hospital-wide clonal dissemination.

Figure 2 | Schematic representation of the class 1 integron gene cassettes bearing the \( \text{bla}_{\text{IMP-4}} \) genes in \( \text{E. cloacae} \) isolates. Genes and their directions of transcription are described as broad arrows. The gray box indicates recombination site. The primers, detailed in Table 1, for PCR mapping are depicted as narrow arrows with numbers. The red arrow of \( \text{bla}_{\text{IMP-4}} \) is related to carbapenemase. The yellow arrow of \( \text{qacG} \) and \( \text{aacA4} \) are associated with resistance to quaternary ammonium compounds and fluoroquinolone, respectively. The 5′ conserved segment (CS) of \text{IntI1} \) and 3′ CS of \( \text{su}1 \) are presented with green arrow. (A) \( \text{E. cloacae} \) YUMC2 in this study with Genbank accession no. KY884003. (B) \( \text{E. cloacae} \) El1573 from Sydney, Australia with Genbank accession no. JX101693.1. (C) \( \text{E. cloacae} \) from Queensland, Australia reported by Sidjabat et al. (2015).
reported previously from Queensland, Australia (Sidjabat et al., 2015). These cassette arrays, found in diverse isolates with slightly different genetic contexts, suggest movement of the array by homologous recombination and the worldwide dissemination potential of blaIMP-4 gene.

In the respect of epidemiological relationship, the class 1 integrons of Australia and Korea, containing blaIMP-4 genes of E. cloacae isolates, revealed similar gene cassettes, except for catB3 or qacG. Geographically, Australia and Korea are located at the rim of the Asian-Pacific region. Further, a large-scale transmission of blaIMP-4 of E. cloacae isolates, predominant from of CPE in Australia (Sidjabat et al., 2015), through silver gulls of Australia was previously reported (Dolejska et al., 2016).

In conclusion, we report the first IMP-4-producing E. cloacae strains identified as predominant ST74 and rare ST194 in Korea. Furthermore, it is the first description of blaIMP-4 and blaCMY-1 coexistence and a new class 1 integron cassette array form in Enterobacteriaceae. This finding implicates the emergence of plasmid-mediated blaIMP-4 on the highly mobile class 1 integron in Enterobacteriaceae clinical isolates in Korea with great concern for widespread and therapeutic threats. In addition, it provides insights into the epidemic potential and clinical importance of IMP-4-producing E. cloacae for hospital-acquired infections.

AUTHOR CONTRIBUTIONS
SJ: analyzed the data, and wrote the manuscript; IKB: designed and performed the experiments, and revised the manuscript; JHL and CHL: helped the experiments and the writing of the manuscript.

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SUPPLEMENTARY MATERIAL
The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2017.02343/full#supplementary-material

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