Complex Class 1 Integron in a Clinical Escherichia coli Strain From Vietnam Carrying Both mcr-1 and blaNDM-1

Hong-Ngoc Le-Vo, Phuong Thi-Bich Tran, Lien Le, Yuki Matsumoto, Daisuke Motooka, Shota Nakamura, James W. Jones, Tetsuya Iida and Van Cao

1 Department of Immunology and Microbiology, Pasteur Institute in Ho Chi Minh City, Ho Chi Minh City, Vietnam, 2 Genome Information Research Center, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan, 3 Department of Bacterial and Parasitic Diseases, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, 4 Department of Bacterial Infections, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan

The co-production of MCR and carbapenemase in Enterobacteriaceae has been previously reported. Here, we describe a clinical strain of Escherichia coli from Vietnam carrying both mcr-1 and blaNDM-1. Whole-genome sequencing showed that the genome of this strain consists of a 4,975,832-bp chromosome and four plasmids. The mcr-1 and blaNDM-1 genes are located on IncI2 and IncA/C2-type plasmids, respectively. Genetic analysis revealed the presence of a multidrug-resistant region with the structure of a novel complex class 1 integron including a class 1 integron region bearing two 5′ conserved segments and one 3′ conserved segment and two complete structures of ISCR1. The complex integron contains aminoglycoside resistance genes aadA2, aadB, strA, strB, and aphA6, quinolone resistance gene qnrA1, extended-spectrum β-lactamase gene blaOXA-4, and a Tn125-like transposon bearing blaNDM-1. In addition, the dfrA12-gcuF-aadA2-cmlA1-aadA1-qacH gene cassette array belonging to the sul3-type integron was also identified, but the region found downstream of the gene cassette array is the IS440-tet(M)-IS26 element instead of the sul3 gene. The results further support that Enterobacteriaceae isolates co-harboring mcr and blaNDM are widely being distributed. The structural characteristics of the complex integron reveal that ISCR1 elements play an important role in the mobilization of blaNDM-1 and the development of multidrug-resistant regions.

Keywords: mcr-1, blaNDM-1, colistin, carbapenemase, complex class 1 integron, sul3-type integron, Escherichia coli

INTRODUCTION

Infections caused by carbapenem-resistant Enterobacteriaceae (CRE) are a serious problem for global health. The main mechanism for carbapenem resistance in bacteria is the production of carbapenemases, out of which New Delhi metallo-lactamase (NDM) is one of the most common groups (Iovleva and Doi, 2017). The blaNDM-1 gene was mainly found on multiple plasmid types, including IncA/C, IncFIA, IncFIB, IncFII, and IncX3 (Wu et al., 2019). This indicates that the horizontal gene transfer mediated by plasmids plays a vital role in the widespread dissemination of blaNDM-1. Infections caused by NDM-1-producing Enterobacteriaceae are of major concern
because those isolates tend to spread among patients, which poses a challenge to control the outbreak of CRE carrying blαNDM-1. In addition, NDM-1-producing plasmids also carry additional genes conferring resistance to other antibiotics, such as aminoglycosides (aacA29 and adaA16), chloramphenicol (catA1 and cmlA1), and tetracyclines (tetB) (Rojas et al., 2016), resulting in the limited choices of antibiotics in treatment. In several plasmids, the blαNDM-1 gene was flanked by two copies of insertion sequences (e.g., ISAba125, IS26, and IS3000), which generate the composite transposons carrying blαNDM-1 (Wu et al., 2019). Class 1 integrons were found linked to the complete structures of insertion sequence common region 1 (ISCR1) including a copy of ISCR1, a variable region (VR) bearing resistance genes, and a copy of the 3′ conserved segment (CS) of the class 1 integron to construct large genetic elements that are usually called “complex class 1 integrons” (Toleman et al., 2006). Some reports showed that blαNDM-1 was also inserted into the ISCR1-linked VR of complex class 1 integrons (Chen et al., 2014; Li et al., 2014), suggesting that the mobilization of blαNDM-1 is most likely associated with ISCR1 as well as the formation of composite transposons.

Colistin is one of the few antibiotics that are still effective against CRE. However, the mutation of chromosomal genes involved in lipopolysaccharide production have impacts on the susceptibility of bacteria to colistin (Olaitan et al., 2014). Moreover, colistin resistance has become more serious since plasmid-mediated colistin resistance gene mcr-1 was first reported in 2015 (Liu et al., 2016). Presently, eight additional mcr genes (mcr-2 to mcr-9) and their variants have been described in Enterobacteriaceae (Xavier et al., 2016; AbuOun et al., 2017; Borowiak et al., 2017; Carattoli et al., 2017; Yin et al., 2017; Wang et al., 2018c; Yang et al., 2018; Carroll et al., 2019). Although the presence of mcr was found on chromosomes (Zhou et al., 2017), the mcr genes were mainly reported to be carried on different plasmid types, such as IncI2, IncHI2, IncX4, IncP, and IncFII (Xavier et al., 2016; Matamoros et al., 2017; Wang et al., 2018c). IncX4, IncI2, and IncHI2 are the most common types of plasmids carrying mcr-1 (Matamoros et al., 2017). On the other hand, these plasmids also harbor additional resistance genes (e.g., blαCTX-M-14, fosA3, floR, and qoxAB) and can be transferred via conjugation (Wu et al., 2018). However, the ISAPl1 composite transposon was considered as the main vehicle for the widespread distribution of mcr-1 (Wang et al., 2018a).

The mcr-1 gene was detected in colistin-resistant Enterobacteriaceae isolated from clinical samples (Du et al., 2016; Tada et al., 2017) and has been commonly found in colistin-resistant Escherichia coli recovered from farm animals and foods in China (Wang et al., 2018b; Liu et al., 2019) and Vietnam (Trung et al., 2017; Yamaguchi et al., 2018). More importantly, a recent study showed that the existence of mcr-1 is the predominant genotype of colistin-resistant E. coli isolated from healthy residents in Vietnam (Yamamoto et al., 2019). The prevalence of mcr-1 in multiple sources revealed that these sources might be potential reservoirs for the wide spread of colistin-resistant bacteria producing MCR-1 in livestock-producing countries such as Vietnam. Significantly, clinical mcr-1- and blαNDM-positive Enterobacteriaceae isolates, particularly E. coli and Klebsiella pneumoniae, were recently found in various regions (Du et al., 2016; Mediavilla et al., 2016; Zheng et al., 2016), which poses a threat to the effectiveness of carbapenems and colistin. In Vietnam, blαNDM-1 is the most common genotype reported in carbapenemase-producing E. coli (Malchione et al., 2019), and mcr-1 has been frequently found in colistin-resistant E. coli isolated from different sources (Trung et al., 2017; Yamaguchi et al., 2018). Thus, we hypothesized that multidrug-resistant Enterobacteriaceae strains that simultaneously harbor mcr-1 and blαNDM are currently circulating in Vietnam. Recently, we have obtained a clinical E. coli strain resistant to multiple antibiotics, including carbapenems and colistin. The aim of this study was to elucidate the antibiotic resistance mechanisms of the isolate, especially to detect the coexistence of mcr-1 and blαNDM.

MATERIALS AND METHODS

Bacterial Isolate

Escherichia coli EC17GD31 was recovered from the blood sample of a patient admitted to the intensive care unit of Nhan Dan Gia Dinh hospital (Ho Chi Minh City, Vietnam) in March 2017. The patient was diagnosed as having acute respiratory distress syndrome, pneumonia, and acute kidney injury and had a medical history of cerebral infarction. EC17GD31 was isolated in the routine diagnostic laboratory of the hospital and then sent to Pasteur Institute in Ho Chi Minh City for further analysis. The isolate was grown on brain heart infusion agar overnight at 37°C and species-identified by using VITEK 2 system (BioMérieux, United States).

This study received ethical approval from the Institutional Ethics Committee in Biomedical Research, Pasteur Institute in Ho Chi Minh City. Informed consent was not required for this study, since the strain was isolated as a part of the routine hospital laboratory procedures.

Antibiotic Susceptibility

The minimum inhibitory concentrations (MICs) of amikacin, gentamicin, ampicillin, amoxicillin-clavulanic acid, cefotaxime, ceftazidime, ceftriaxone, cepime, ertapenem, imipenem, meropenem, cefoxitin, ciprofloxacin, chloramphenicol, tetracycline, and trimethoprim-sulfamethoxazole were determined by agar dilution method, although MICs of colistin and tigecycline were performed with broth microdilution.

Colistin and tigecycline resistance was interpreted according to the breakpoints of the European Committee on Antimicrobial Susceptibility Testing (EUCAST®), whereas other antibiotics were identified according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2018).

Conjugation Assay

The transferability of the mcr-1-bearing plasmid was examined by conjugation experiment. E. coli J53, which is resistant to sodium azide, was used as the recipient. E. coli J53 transconjugants that

1http://www.eucast.org/clinical_breakpoints/
harbor MCR-1-encoding plasmids were selected on brain heart infusion agar plates containing 100 μg/ml sodium azide and 2 μg/ml colistin. The presence of mcr-1 in the transconjugants was confirmed by PCR as previously described (Liu et al., 2016).

Whole-Genome Sequencing and Bioinformatics

The genomic DNA was extracted from EC17GD31 using a QIAamp DNA Mini Kit (Qiagen, Germany). Whole-genome sequencing was carried out using the MinION sequencer (Oxford Nanopore Technologies, United Kingdom). Genome assembly was performed by Canu 1.6 (Koren et al., 2017) and then corrected using Pilon 1.22 (Walker et al., 2014) with reads obtained from 250-bp paired-end Illumina MiSeq sequencing (Illumina Inc., United States). NCBI Prokaryotic Genome Annotation Pipeline was used to annotate the genomic sequences. Multilocus sequence typing of the isolate, plasmid replicon typing, and identification of resistance genes were done using tools from the Center for Genomic Epidemiology website. Insertion sequences were identified based on the ISfinder database. Sequence comparison was completed using BLASTn. The circular image and linear comparison figures of sequences were generated by CGView (Stothard and Wishart, 2004) and Easyfig (Sullivan et al., 2011), respectively.

Phylogenetic Analysis of IncI2 Plasmids

Complete sequences of IncI2 plasmids carrying mcr-1 (n = 21) were obtained from GenBank (Supplementary Table 1). A sum of 35 genes were identified as the core genome of the plasmids (including pGD31-MCR) using OrthoFinder version 2.3.3 (Emms and Kelly, 2015). The alleles of these genes were aligned as protein sequences using MAFFT version 7.427 (Katoh and Standley, 2013), and then the protein sequences of each plasmid were concatenated into a single sequence. RAXML version 8.2.12 (Stamatakis, 2014) was used to infer a phylogenetic tree with a 1000-bootstrap test. The phylogenetic tree was drawn using the iTOL web server (Letunic and Bork, 2019).

Nucleotide Sequence Accession Numbers

Complete sequences of the chromosome and plasmids of E. coli EC17GD31 have been deposited in GenBank under the accession numbers CP031293 to CP031297.

RESULTS AND DISCUSSION

Genomic Characteristics of E. coli EC17GD31

The genome of E. coli EC17GD31 consists of a 4,975,832 bp chromosome with an average 50.46% GC content and four circular plasmids (Table 1). The isolate was resistant to all tested antibiotics, except for tigecycline (Table 2). It is notable that EC17GD31 was resistant to both carbapenems (MICs ≥ 8 μg/ml) and colistin (MIC = 4 μg/ml). Identification of 22 antibiotic resistance genes showed good correlation to the antibiotic susceptibility pattern of E. coli EC17GD31 (Table 1). The detection of blaTEM-1B, blaCTX-M-55, blaOXA-4, and blaNDM-1 explained the resistance to all tested β-lactam antibiotics, including extended-spectrum cephalosporins and carbapenems (Table 2). The presence of mcr-1 and efflux pump-encoding genes (cmlA1 and floR) was related to the resistance to colistin and chloramphenicol, respectively. In addition, the genome of EC17GD31 carries genes conferring the resistance to aminoglycosides (aadA1, aadA2, aadB, aph(3′)-Ia, aphA6, strA, and strB), quinolones (qnrA1), tetracyclines [tet(A) and tet(M)], sulfonamides (sul1, sul2, and sul3), and trimethoprim (dfrA12) (Table 1). The multidrug resistance transporter-encoding gene mdf(A) was also found in EC17GD31, which probably contributed to the resistance of this isolate to tetracycline, chloramphenicol, and ciprofloxacin (Edgar and Bibi, 1997). The blaCTX-M-55 encoding extended-spectrum beta-lactamase (ESBL) and mdf(A) genes are located on the chromosome, whereas other resistance genes are present on three plasmids. Moreover, three mutations in type II topoisomerases were identified, including substitution Ser83Leu and Asp87Tyr in YгуA and Ser80Ile in ParC. These mutations are the most common quinolone resistance-associated substitutions in E. coli (Ruiz, 2003), and their coexistence confers high-level fluoroquinolone resistance (Morgan-Linnell and Zechiedrich, 2007). Chromosomal genes (mgb, phoP, phoQ, pmrB, and pmrA) involved in the susceptibility of bacteria to colistin were found to have no mutations, suggesting that colistin resistance of EC17GD31 is only related to the presence of mcr-1.

Multilocus sequence typing indicated that EC17GD31 belongs to ST457 according to the Warwick scheme. Several reports have described E. coli ST457 isolated from animals and hospitals also carries the mcr-1 gene (Suzuki et al., 2016; Tada et al., 2017). Furthermore, this clone and ST410 were the first identified clones of clinical E. coli from Vietnam harboring mcr-1 (Tada et al., 2017). Significantly, carbapenem resistance gene blaNDM-1 and colistin resistance gene mcr-1 coexist in EC17GD31. To the best of our knowledge, this is the first report of a clinical E. coli strain from Vietnam that coharbors mcr-1 and blaNDM-1. NDM- and MCR-coproducing Enterobacteriaceae isolates, mainly E. coli, were reported in numerous other countries, e.g., China (Zheng et al., 2016), the United States (Mediavilla et al., 2016), the Arabian Peninsula (Sonnevend et al., 2016), Venezuela (Delgado-Blas et al., 2016), and Thailand (Paveenkittiporn et al., 2017). The NDM genes blaNDM-1 (Delgado-Blas et al., 2016; Zheng et al., 2016; Paveenkittiporn et al., 2017) and blaNDM-5 (Mediavilla et al., 2016; Yang et al., 2016; Zheng B. et al., 2018), followed by blaNDM-9 (Yao et al., 2016; Lai et al., 2017), were commonly detected in E. coli isolates co-carrying blaNDM and mcr-1. The emergence and dissemination of NDM- and MCR-coproducing Enterobacteriaceae has become an urgent threat to clinical treatment and infection control.
Genetic Features of Plasmid pGD31-MCR

pGD31-MCR is a 60,038-bp circular plasmid bearing the mcr-1 gene, with an average GC content of 42.28%. The plasmid belongs to the IncI2 group, which is the most prevalent MCR-1-producing plasmid group in Asia (Matamoros et al., 2017). pGD31-MCR only carries mcr-1, whereas some other IncI2-type plasmids harbor both the mcr-1 and CTX-M ESBL genes (Sun et al., 2016; Matamoros et al., 2017). Colistin-resistant transconjugant indicated that the plasmid carrying the mcr-1 gene was transferred to the E. coli J53 recipient strain (Table 2). This result showed that pGD31-MCR is a self-conjugative plasmid. Based on the core genome of IncI2 plasmids harboring mcr-1, the phylogenetic analysis revealed that pGD31-MCR shared the highest level of sequence similarity with pSLy21 (Genbank accession no. CP016405) in E. coli strain 210221272 isolated from a pig (Meinersmann et al., 2016; Figure 1). The sequence comparison of pGD31-MCR using BLASTn showed 99% identity and 97% coverage with pHNSHP45 (Genbank accession no. KP347127), the first identified plasmid harboring mcr-1 (Liu et al., 2016). Similar to pHNSHP45, the mcr-1 gene is located in a region with a structure of nikB-ISAp11-mcr-1-pap2. The ISAp11-mediated composite transposon was considered to be the primary genetic context of mcr-1 (Snesrud et al., 2016; Wang et al., 2018a). Illegitimate recombination may result in the loss of ISAp11 flanking elements, leading to the stable existence of mcr-1 in plasmids or chromosomes (Snesrud et al., 2016).

A Novel Complex Class 1 Integron in Plasmid pGD31-NDM

Plasmid pGD31-NDM is a circular molecule of 188,230 bp with an average GC content of 51.37% that belongs to the IncA/C2 group. The plasmid harbors numerous antibiotic resistance genes, which together provide resistance to multiple classes of antibiotics, including aminoglycosides, β-lactams, quinolones, and sulfonamides (Table 1). Interestingly, all resistance genes of the plasmid are located in a 30,659-bp multidrug-resistant region with the structure of a complex class 1 integron consisting of two 5′ CSs, three 3′ CSs, two ISCR1s, and four VRs bearing resistance genes (Figure 2). The class 1 integron region contains a complete copy and a partial copy of the 5′ CS and a copy of the 3′ CS, similar to that of Acinetobacter baumannii strain D4 (Genbank accession no. KP054476.2) (Hamidian et al., 2015). The cassette regions of the class 1 integron in pGD31-NDM carry five resistance genes (blaOXA-4, aadA2, strA, strB, and aadB), three of which were found in those of A. baumannii strain D4. The blaOXA-4 gene encoding an ESBL is usually found in Pseudomonas aeruginosa (Marumo et al., 1999; Kainuma et al., 2018).

The ISCR1-1-linked VR-3 in pGD31-NDM contains two genes of unknown function, apha6 and a Tn125 transposon carrying the blaNDM-1 gene (Figure 2). However, ISAba125 lying downstream of blaNDM-1 was interrupted by the insertion of ISaba24. Interestingly, the ISCR1-1-associated VR in plasmid pC1394 (Genbank accession no. MH457126) is identical to that found in pGD31-NDM, except for the truncated ISaba125 lying downstream of blaNDM-1 and the lack of apha6. ISCR1 was found linked to various VRs carrying blaNDM-1 in E. coli plasmid pEcNDM1 (Genbank accession no. JX469383) and Proteus mirabilis plasmid pNDM-PM58 (Genbank accession no. KP662515). The construction of complex class 1 integrons harboring blaNDM-1 indicated that ISCR1 is also an element responsible for the mobilization of the blaNDM-1 gene.

Similar to pEcNDM1, pGD31-NDM harbors a second copy of ISCR1 followed by quinolone-resistant gene qnrA (Figure 2). In pGD31-NDM, however, a duplicate of the 3′ CS of the class 1 integron is located immediately downstream of the qnrA1 gene, revealing the presence of a complete structure of ISCR1.

Table 1 | Genomic features of E. coli EC17GD31.

| Chromosome/plasmid | Type | Size (bp) | GC content (%) | Resistance gene(s) |
|--------------------|------|-----------|----------------|--------------------|
| EC17GD31           | ST457| 4,975,832  | 50.46          | blACTXM-55, mdfA1   |
| pGD31-MCR          | IncI2| 60,038    | 42.28          | mcr-1              |
| pGD31-NDM          | IncA/C2| 188,230   | 51.62          | aadA2, aadB, apha6, blalem-1, blamMBL, qacEΔ1, qnrA1, strA, strB, sulI |
| pGD31-F25          | IncFIB (pLFR2) | 110,686 | 45.70          | -                  |
| pGD31-F1928        | IncFIB (pAP001918), IncFil, IncR | 245,305 | 51.37          | aadA1, aadA2, apha(3′)-la, blatem-1B, cmlA1, dfrA12, floR, ΔmfE(B), Δmig-14, qacH, sul2, sul3, tetA, tetM |

Table 2 | Minimum inhibitory concentrations of tested antibiotic agents for E. coli EC17GD31, the transconjugant, and E. coli J53 recipient strain.

| Isolate          | AMK | GEN | AMP | AMC | CTX | CRO | FEP | ETP | IPM | MEM | FOX | CIP | CHL | CST | TET | TGC | SXT |
|------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| EC17GD31         | 128 | >256| >256| >256| >256| 24  | 32  | 8   | 128 | >256| 128 | 256 | 4   | 256 | 0.06| >32 |
| Transconjugant   | 2   | 1.5 | 6   | 4   | 0.06| 1   | 0.03| 0.16| 0.008| 1   | 0.015| 8   | 0.03| 8   | 4   | 2   | 0.06| 0.47|
| E. coli J53      | 2   | 1.5 | 4   | 3   | 0.125| 0.25| 0.03| 0.016| 0.008| 1   | 0.015| 8   | 0.03| 8   | 4   | 0.5 | 2   | 0.125| 0.047|

AMK, amikacin; GEN, gentamicin; AMP, ampicillin; AMC, amoxicillin-clavulanic acid; CTX, cefotaxime; CAZ, ceftazidime; CRO, ceftriaxone; FEP, cepfime; ETP, ertapenem; IPM, imipenem; MEM, meropenem; FOX, cefoxitin; CIP, ciprofloxacin; CHL, chloramphenicol; CST, colistin; TET, tetracycline; TGC, tigecycline; SXT, trimethoprim-sulfamethoxazole.
FIGURE 1 | Phylogenetic analysis on pGD31-MCR and 21 other IncI2 mcr-1-carrying plasmids obtained from GenBank. The accession numbers of the plasmids are shown in Supplementary Table 1. Bootstrap values are shown next to nodes.

FIGURE 2 | Schematic representation of the complex class 1 integron in pGD31-NDM and structural comparison with Acinetobacter baumannii strain D4, Vibrio alginolyticus plasmid pC1394, E. coli plasmid pEcNDM1, and Proteus mirabilis plasmid pNDM-PM58. Boxed arrows indicate the positions of open reading frames and their directions of transcription. Open reading frames encoding hypothetical proteins are portrayed by gray-shaded arrows. Gray-shaded areas denote more than 99% DNA identity between sequences. CS, conserved segment; VR, variable region.
containing \textit{qnrA1}. The \textit{ampR} gene, which is involved in the induction of class C β-lactamase, is usually found adjacent to \textit{qnrA1} in IS\textit{CR1}-linked regions (Rui et al., 2018), whereas it was missing in that of pGD31-NDM.

Unlike other plasmids, the complex class 1 integron in pGD31-NDM carries the class 1 integron region bearing two 5' CSs and one 3' CS and the two complete structures of IS\textit{CR1}. The presence of these two complete structures of IS\textit{CR1} may be the result of successive integration events following the model of Toleman et al. (2006). Free circular intermediates carrying the orf-orf-Tn125-like transposon-\textit{aphA6-Δ3’ CS-IS\textit{CR1} element} and \textit{qnrA1-Δ3’ CS-IS\textit{CR1} element} were generated via aberrant rolling-circle replications. These circular intermediates might then be rescued by homologous recombination via the 3' CS of the class 1 integron. Thus, it would be possible for the complex integron to expand structure. Surprisingly, the overall form of

![Circular representation of plasmid pGD31-F1928](image-url)

\textbf{FIGURE 3} | Circular representation of plasmid pGD31-F1928. The outermost circle indicates genetic elements, including resistance region, transposon, integron, and transfer region. The second and third circles show coding sequences on the forward and reverse strand of the plasmid, respectively. The fourth and fifth circles show the GC plot and GC skew graph, respectively. The innermost circle shows size in kb.
the complex class 1 integron in pGD31-NDM is identical to that of pC1394 in *Vibrio alginolyticus* strain Vb1394 isolated from shrimp (Zheng Z. et al., 2018), except for the structure of the class 1 integron, the truncation of ISAba1, and the existence of aphA6 (Figure 2). Two copies of IS5075 are adjacent to the boundaries of the complex integron, leading to putative genesis of an IS5075-mediated composite transposon, which may move the complex integron via transposition or homologous recombination. The formation of potential IS5075-mediated composite transposon raises a concern about the prevalence of this complex integron.

The linkage of the type 1 integron and ISCR1 results in the extension of multidrug-resistant regions by adding resistance genes. In addition, the mcr and *blaNDM* genes may be transferred to a same transconjugant, although they are located on various self-transmissible plasmids (Long et al., 2019). Altogether, ISCR1-mediated construction of complex class 1 integrons and co-transfer of mcr and *blaNDM* play a significant role in forming extensive drug-resistant and pandrug-resistant bacteria, thus causing great challenges in the fight against antibiotic resistance.

**sul3-Type Integron in Plasmid pGD31-F1928**

pGD31-F1928 is a 245,305-bp circular plasmid with an average GC content of 51.37% (Table 1). The plasmid harbors three replication systems (IncR, IncFIB, and IncFII), two conjugation regions, *sitABCD* operon, copper/silver resistance genes, *Tn2* transposon, a class 1 integron, and some resistance genes bounded by various insertion sequences (Figure 3). Antibiotic resistance genes are associated with resistance to aminoglycosides, β-lactams (penicillins and first-generation cephalosporins), chloramphenicol, florfenicol, trimethoprim, sulfonamides, and tetracyclines. The genetic context of sulfonamide resistance gene *sul2* and florfenicol resistance gene *floR*, *sul2-glmM-ISCR2-lysR-floR-virD2-ΔISC2*, was identified (Figure 4). This genetic environment is identical to that of plasmid pHSNHP45-2 (Genbank accession no. KU341381) and Tn6540 transposon of *Proteus mirabilis* strain SNGY17 (Genbank accession no. MF805806) (Chen et al., 2018). The *sul2* and *floR* genes are usually found associated with ISCR2, the amino acid sequence of which is 65% similar to that of ISCR1, but ISCR2 is not responsible for the formation of the complex class 1 integron (Toleman et al., 2006). In addition, pGD31-F1928 also carries a truncated mig-14 gene, which relates to antimicrobial peptide resistance in *Salmonella enterica* (Brodsky et al., 2002) and *P. aeruginosa* (Jochumsen et al., 2011).

The *aadA1, aadA2, cmlA1, dfrA12, and qacH* genes are located in a gene cassette array of class 1 integron (Figure 4). The integron with a structure of intI1-dfrA12-gcuF-aadA2-cmlA1-aadA1-qacH-IS440-sul3 is known as sul3-associated integron type I (Antunes et al., 2007). Surprisingly, only IS440 was found downstream of the *qacH* gene, whereas sul3 and a duplicate of IS440 were located in another region of the plasmid (Figure 4). The IS440-sul3 element lay upstream of an untyped integrase-encoding intI gene and linked to the region harboring a putative oxidoreductase gene (*orfJ*) and *mef(B)* truncated by an IS26 insertion. The linkage of sul3 with *mef(B)*, a macrolide efflux gene, was first described in *E. coli* isolated from pigs (Liu et al., 2009). The intI1-dfrA12-gcuF-aadA2-cmlA1-aadA1-qacH-IS440 region is adjacent to the tetM-IS26 element, creating a new structure of a non-classic class 1 integron. This structure was also observed in plasmid pSCE516-4 (Genbank accession no. KX023259), but the integron is located upstream of the *TnAs1* *tnpA-tnpR* element and *Tn21* *tnpM* instead of the *Tn21* *tnpA-tnpR-tnpM* element as in pGD31-F1928 and pHYC7-110 (Genbank accession no. KX518744) (Figure 4). In addition, the genetic context of sul3 in pSCE516-4 bears a truncated IS440 and complete *mef(B)*, whereas pGD31-F1928 contains a complete IS440 and truncated *mef(B)*. Altogether, we propose a hypothesis that the original
Additionally, this study revealed that the 

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health officials to the presence of 

A multidrug-resistant 

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\textit{tet(M)}-associated class 1 integron. The connection of the class 1 integron and the \textit{tet(M)}-IS26 element may construct a putative \textit{tet(M)}-associated class 1 integron.

**CONCLUSION**

A multidrug-resistant \textit{E. coli} strain EC17GD31 simultaneously harboring \textit{mcr-1} and \textit{bla}\textsubscript{NDM-1} is identified, which alerts health officials to the presence of \textit{mcr-} and \textit{bla}\textsubscript{NDM}-positive \textit{Enterobacteriaceae} in Vietnam. The close monitoring of MCR- and NDM-coproducing \textit{Enterobacteriaceae} isolates is essential in order to safeguard the effectiveness of last-resort antibiotics. Additionally, this study revealed that the \textit{bla}\textsubscript{NDM-1} gene is located in a novel complex class 1 integron containing a class 1 integron region bearing two VRs and two complete structures of ISCR1. The characterization of the complex integron further demonstrates that ISCR1 is a powerful genetic element that can mobilize a single gene as well as a composite transposon (e.g., Tn125-like transposon), therefore, the spread of resistance genes including \textit{bla}\textsubscript{NDM-1} can be anticipated. Although additional intermediates and progenitor plasmids were not found, the formation of the complex integron was most likely based on successive events of resistance gene mobilization. The findings of this study further support that complex interactions between antibiotic resistance genes and mobile elements play an important role in the creation and evolution of multidrug-resistant regions, thus facilitating bacteria to achieve either extensive drug resistance or pandrug resistance.

**DATA AVAILABILITY STATEMENT**

The raw data supporting the conclusions of this manuscript will be shared upon request submitted to the corresponding author.

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**ETHICS STATEMENT**

The studies involving human participants were reviewed and approved by the Institutional Ethics Committee in Biomedical Research, Pasteur Institute in Ho Chi Minh City, Vietnam. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

**AUTHOR CONTRIBUTIONS**

VC and JJ conceived and designed the study. PT and LL performed the microbiological and molecular experiments. TI, SN, and DM performed the whole-genome sequencing experiments. H-NL-V, PT, and YM analyzed the data and prepared figures. H-NL-V wrote the manuscript with support from VC, PT, and LL. All authors contributed to manuscript revision, read, and approved the submitted version.

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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.2019.02472/full#supplementary-material
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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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