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Integron Mobilization Unit as a Source of Mobility of Antibiotic Resistance Genes[▽]

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Antibiotic resistance genes are spread mostly through plasmids, integrons (as a form of gene cassettes), and transposons in gram-negative bacteria. We describe here a novel genetic structure, named the integron mobilization unit (IMU), that has characteristics similar to those of miniature inverted transposable elements (MITEs). Two IMUs (288 bp each) were identified from a carbapenem-resistant *Enterobacter cloacae* isolate that formed a composite structure encompassing a defective class 1 integron containing the carbapenem resistance gene bla_{GES-5}. This ß-lactamase gene was located on a 7-kb IncQ-type plasmid named pCHE-A, which was sequenced completely. The plasmid pCHE-A was not self conjugative but was mobilizable, and it was successfully transferred from *E. cloacae* to *Pseudomonas aeruginosa*. The in silico analysis of the extremities of the IMU elements identified similarities with those of insertion sequence IS*Sod9* from *Shewanella oneidensis* MR-1. The mobilization of the IMU composite structure was accomplished by using the transposase activity of IS*Sod9* that was provided in *trans*. This is the first identification of MITE-type structures as a source of gene mobilization, implicating here a clinically relevant antibiotic resistance gene.

The mobilization of antibiotic resistance genes in gram-negative bacteria occurs through several mechanisms. The transfer of plasmids (via transformation or conjugation) or of phages (via transduction) is known to be the key factor for the horizontal transfer of resistance genes. Additionally, their acquisition is related mainly to different mechanisms, including recombination, the integron-mediated mobilization of gene cassettes, classical transposition, and rolling-circle transposition mediated by the recently described IS*CR* elements (3, 19, 49, 54, 55). A large variety of antibiotic resistance determinants (resistance to ß-lactams, aminoglycosides, chloramphenicol, rifampin, trimethoprim, and sulfonamides) have been identified as gene cassettes located in class 1 integrons. An integron is a non-self-transferable genetic unit that is capable of capturing and expressing gene cassettes. The dissemination of class 1 integrons usually is related to their location in transposon structures, being mostly of Tn*21* and Tn*402* types in gram-negative bacteria (26, 30).

Among the ß-lactamases identified in *Enterobacteriaceae*, the carbapenemases have the broadest spectrum of activity and belong to Ambler classes A, B, and D (22, 43, 46, 56). The class A carbapenemases are inhibited by clavulanic acid, and their genes are located on chromosomes (e.g., bla_{NMC-A} or bla_{SME}) or plasmids (e.g., bla_{IMI}, bla_{GES}, or bla_{KPC}). The bla_{GES} genes have been identified in *Enterobacteriaceae* and *Pseudomonas aeruginosa*, and the ß-lactamases GES-2, GES-4, GES-5, and GES-8 possess carbapenemase activity (35, 43). All of the bla_{GES}-type genes have been identified as part of the class 1 integrons, with the exception of one report describing the bla_{GES-1} gene as part of a class 3 integron in *Klebsiella pneumoniae* (13). The only class A carbapenemase reported to date in *Enterobacter cloacae* is NMC-A (34).

This work was initiated by the isolation of a carbapenem-resistant *E. cloacae* strain harboring the bla_{GES-5} gene that was located in a defective class 1 integron structure, in association with a novel genetic element, termed the integron-mobilization unit (IMU), which lacks self-transposition ability. However, transposition events were obtained using a transposase from *Shewanella oneidensis* provided in *trans*. That finding represents a novel mechanism of the transfer of antibiotic resistance genes that may be of more general interest.

MATERIALS AND METHODS

Bacterial strains. *E. cloacae* CHE-2 was identified according to biochemical testing (API20E system; bioMérieux, Marcy-l'Etoile, France). A GES-1-positive control was *Klebsiella pneumoniae* isolate ORI-1 (42). *Escherichia coli* TOP10 was used for cloning experiments, whereas azide-resistant *E. coli* J53 was used as the recipient for conjugation and transformation experiments (32). A wild-type *E. cloacae* isolate (strain 5390) and *P. aeruginosa* PU21 (reference strain) were used as recipients for transformation experiments (32). *E. coli* JF703 and JF701 (kindly provided by G. Jacoby) expressing either OmpC or OmpF, respectively, were used as reference strains for outer membrane protein (OMP) analysis (23). *S. oneidensis* strain MR-1 was used to obtain the DNA template for cloning the transposase gene of insertion sequence element IS*Sod9* as a source of transposase activity in transposition experiments (20, 39).

Genetic support and plasmid analysis. Mating-out assays were performed between *E. cloacae* CHE-2 as a donor and azide-resistant *E. coli* J53 as a recipient strain, as described previously (32). Plasmid DNAs of *E. cloacae* CHE-2 and of the *E. coli* transformants were extracted using the Kieser method (27). They were separated on a 0.8% agarose gel, transferred onto a nylon membrane (Hybond N+; GE Healthcare, Orsay, France), and hybridized with bla_{GES-5} and bla_{SHV-5} internal probes. The labeling of the probe and signal detection were carried out using a nonradioactive ECL labeling and detection kit according to the manufacturer's instructions (GE Healthcare).

Plasmids were classified according to their incompatibility group using the

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TABLE 1. MICs of β-lactams for *E. cloacae* CHE-2 clinical isolate, *E. coli* J53 harboring plasmids pCHE-A and pCHE-B expressing β-lactamases GES-5 and SHV-5, respectively, *P. aeruginosa* PU21 harboring pCHE-A, and reference strains *E. coli* J53 and *P. aeruginosa* PU21

β-Lactam(s)	MIC for:					
	E. cloacae CHE-2 (GES-5 + SHV-5)	*E. coli* J53(pCHE-A) (GES-5)	*E. coli* J53(pCHE-B) (SHV-5)	*E. coli* J53	*P. aeruginosa* PU21(pCHE-A) (GES-5)	*P. aeruginosa* PU21
Amoxicillin	>512	>512	>512	2	>512	>512
Amoxicillin + CLA[a]	>256	16	4	2	>512	>512
Ceftazidime	>512	128	256	0.06	256	1
Ceftazidime + CLA	256	16	4	0.06	64	1
Cefoxitin	>512	128	4	4	>512	>512
Imipenem	>32	1	0.06	0.06	16	2
Meropenem	>32	0.25	0.06	0.03	>32	0.5
Ertapenem	>32	0.12	0.06	0.03	>32	8

[a] CLA, clavulanic acid at 4 μg/ml.

PCR-based replicon typing method described by Carattoli et al. (6). This technique discriminates 18 types of enterobacterial plasmids according to incompatibility groups. Positive control strains were based on *E. coli* strain DH5α and contained replicons of the incompatibility groups cloned into a TA cloning vector. PCR products were sequenced to confirm the specificity of the amplicon.

Since GES-type enzymes have been identified in unrelated gram-negative species, including *Pseudomonas* spp. (8, 16, 38, 44), electrotransformation assays of natural plasmid pCHE-A were performed in electrocompetent *P. aeruginosa* PU21 and in the recipient strain *E. cloacae* 5390, as described previously (38, 44). The selection of *E. cloacae* and *P. aeruginosa* transformants harboring plasmid pCHE-A was performed on ticarcillin (100 μg/ml)-containing agar plates.

Susceptibility testing. Antibiotic-containing disks were used for routine antibiograms that were performed by disk diffusion testing (Sanofi-Diagnostic Pasteur, Marnes-la-Coquette, France). MICs were determined by an agar dilution technique, except for colistin susceptibility, which was determined with an Etest strip (AB Biodisk, Solna, Sweden). MICs of ß-lactams were determined alone or in combination with a fixed concentration of clavulanic acid (4 μg/ml), and results were interpreted according to the guidelines of the Clinical Laboratory Standards Institute (CLSI) (10).

PCR and hybridization experiments. Total DNA of enterobacterial isolates were extracted as described previously (42) and were used as templates in standard PCR conditions with a series of primers designed for the detection of Ambler class A ß-lactamase genes: bla_{TEM}, bla_{SHV}, bla_{CARB}, bla_{VEB}, bla_{PER}, bla_{GES}, and $bla_{CTX\text{-}M}$ (44). Southern hybridizations were performed using the ECL nonradioactive labeling and detection kit (GE Healthcare, Orsay, France). An internal probe for $bla_{GES\text{-}5}$ obtained by PCR was used for Southern hybridization experiments.

Cloning experiments, recombinant plasmid analysis, and DNA sequencing. Total DNA of *E. cloacae* CHE-2 was EcoRI restricted, ligated into the EcoRI site of plasmid pBK-CMV, and then transformed in *E. coli* TOP10, as described previously (42), giving rise to recombinant plasmid pBCHE-2. Strains containing recombinant plasmids were selected onto Trypticase soy (TS) agar plates containing amoxicillin (50 μg/ml) and kanamycin (30 μg/ml) and sequenced on both strands with an Applied Biosystems sequencer (ABI 3100) (Applied Biosystems, Foster City, CA).

In addition, PCR amplicons encompassing the entire sequence of the $bla_{GES\text{-}5}$ gene with or without the upstream-located IMU element were obtained with primers PRE-1 (5′-CAG AAA TGC CTC GAC TTC GC-3′) and PRE2 (5′-AAT GAG AAT CAG ATC CGC GC-3′), respectively, in combination with primer 3′-CS (29) from whole-cell DNA of *E. cloacae* CHE-2, and subsequently were cloned using the ZeroBluntTOPOPCR cloning kit (Invitrogen, Cergy-Pontoise, France), giving rise to recombinant plasmids p1 and p2, respectively. Recombinant strains *E. coli* TOP10 (p1) and *E. coli* TOP10 (p2) were used for the comparison of MICs to evaluate the possible role of the IMU in enhancing $bla_{GES\text{-}5}$ expression.

The entire sequence of the natural pCHE-A plasmid was determined by a primer-walking approach. The nucleotide and deduced amino acid sequences were analyzed and compared to sequences available over the Internet at the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov). The analyses of the IMU genetic features also were performed using the ISBiotoul website (http://www-is.biotoul.fr/).

PCR amplicons encompassing the entire sequence of the IS*Sod9* transposase gene were obtained with primers PreTnA-Fw (5′-TGT GTC CGA GAG CTC TTG TC-3′) and Pre-TnA-Rev (5′-AAA ATC GTA CGC TAA GCC GG-3′) from whole-cell DNA of *S. oneidensis* MR-1 and subsequently were cloned using ZeroBluntTOPOPCR, giving rise to recombinant plasmid pTnpA-ISSod9.

Transposition experiments. In vivo transposition experiments were performed to determine the mobility of the IMU-$bla_{GES\text{-}5}$-IMU structure. The transposition of IMU-$bla_{GES\text{-}5}$-IMU onto the pOX38-Gen conjugative plasmid was investigated with a mating-out technique in liquid medium, as described previously (41). First, natural and non-self-conjugative plasmid pCHE-A was electroporated into *E. coli* RZ211(pOX38-Gen) (selection was based on amoxicillin [100 μg/ml]). Second, nonconjugative recombinant plasmid pTnpA-ISSod9 was electroporated into *E. coli* RZ211(pOX38-Gen, pCHE-A) to provide the IS*Sod9* transposase in *trans* activity (selection was based on kanamycin [30 μg/ml]) that is necessary for transposition experiments. The transfer of the recombinant plasmids with the pOX38 backbone into *E. coli* J53AZ^R then was performed by conjugation. Transconjugants were selected on agar plates containing 7 μg per ml of gentamicin (pOX38 plasmid marker), 100 μg per ml of amoxicillin (GES-5 marker), and 100 μg per ml of azide (*E. coli* J53 chromosomal marker). The transposition frequency was calculated by dividing the number of transconjugants by the number of donors.

ß-Lactamase activity. The specific activity of the crude ß-lactamase from culture extracts of *E. cloacae* CHE-2 was obtained by UV spectrophotometry, as described previously (42), using benzylpenicillin and imipenem as substrates. One unit of enzyme activity was defined as the activity that hydrolyzed 1 μmol of substrate per min per mg of protein. The total protein content was measured with the DC protein assay kit (Bio-Rad, Ivry-sur-Seine, France).

OMP analysis. OMPs were extracted by the sarcosyl extraction of total membrane preparations, as described previously (40). Briefly, cell cultures were harvested in logarithmic phase and lysed by sonication. OMPs were obtained after the treatment of cell membranes with sodium lauryl sarcosylate (Sigma, Saint-Quentin-Fallavier, France) and subsequent ultracentrifugation. The proteins were examined by sodium dodecyl sulfate–10% polyacrylamide gel electrophoresis.

Nucleotide sequence accession number. The nucleotide sequences reported in this work and corresponding to the entire sequence of plasmid pCHE-A have been deposited in the GenBank nucleotide database under accession no. EU266532.

RESULTS

***E. cloacae* CHE-2 harbors a carbapenemase-encoding gene.** *E. cloacae* CHE-2 was isolated from the sputum of a patient admitted for pneumonia to an acute-care center in March 2006 in Calgary, Canada. This isolate was resistant to all β-lactam molecules, including carbapenems (Table 1). Clavulanic acid addition decreased the MIC of ceftazidime (Table 1), suggesting the production of an extended-spectrum ß-lactamase (ESBL). *E. cloacae* CHE-2 also was resistant to kanamycin, netilmicin, tobramycin, chloramphenicol, tetracycline, nalidixic acid, fluoroquinolones, rifampin, and sulfonamides, remaining susceptible only to gentamicin, amikacin, and colistin.

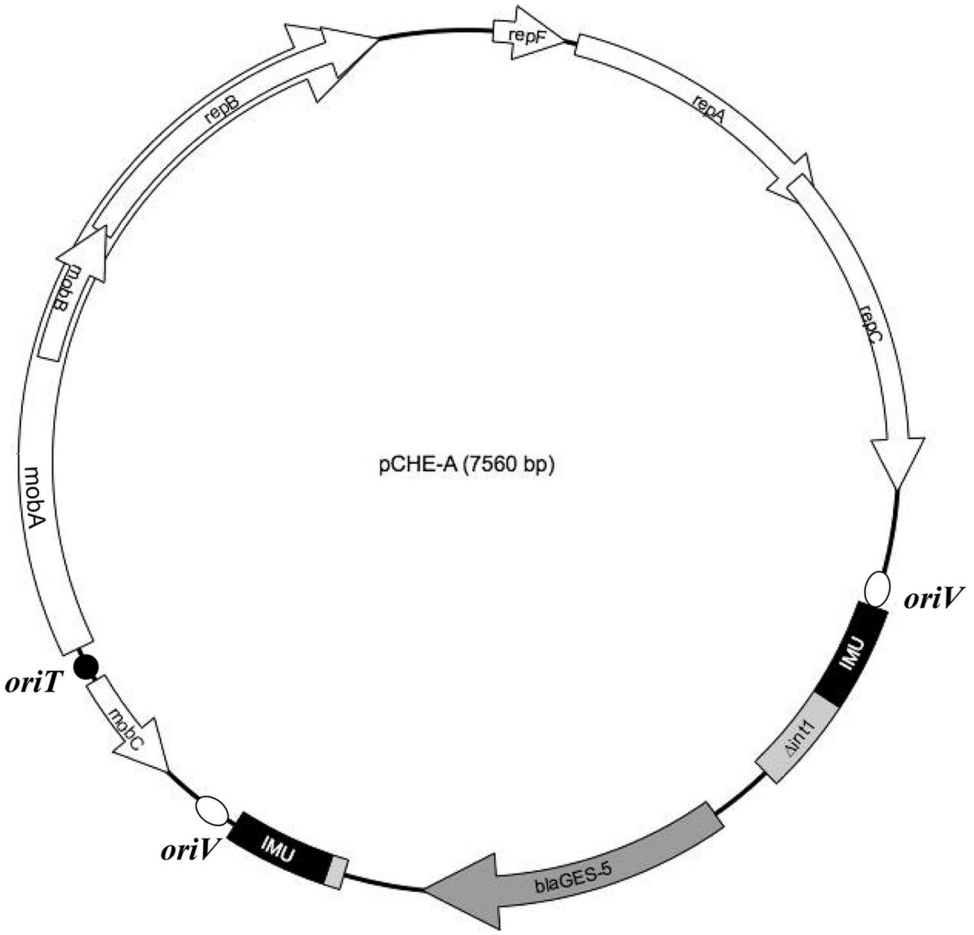


FIG. 1. Map of natural plasmid pCHE-A. A genetic map of plasmid pCHE-A is shown. Coding regions are indicated by arrows giving the direction of transcription. The plasmid is made of the replication initiation genes *repF*, *repA*, *repB*, and *repC*, the putative mobilization module genes *mobA*, *mobB*, and *mobC*, and the ß-lactamase bla_{GES-5} gene bracketed by the two IMUs. In addition, the *oriT* region that is required for mobilization and the *oriV* region that is required for replication are indicated.

To determine whether resistance to carbapenems was related to any β-lactamase, imipenem hydrolysis was tested using a culture extract of *E. cloacae* CHE-2, which revealed a significant carbapenemase activity (20 mU/mg of protein^{-1}). Subsequent PCR screening using primers specific for ß-lactamase genes identified a bla_{GES}-type gene using whole-cell DNA of *E. cloacae* CHE-2 as the template. The cloning of EcoRI-restricted whole-cell DNA of *E. cloacae* CHE-2 into pBK-CMV gave recombinant plasmid pBCHE2. *E. coli* TOP10 (pBCHE2) expressed an ESBL phenotype with reduced susceptibility to imipenem (Table 1) that was consistent with the resistance profile of the clinical isolate. The DNA sequence analysis of plasmid pBCHE-2 identified a 1,620-bp insert containing the bla_{GES-5} gene, encoding a 287-amino-acid protein, GES-5. It differs from GES-1 by a serine instead of a glycine residue at Ambler position 170, and it is known to hydrolyze carbapenems (1, 24, 28). PCR screening followed by sequencing showed that *E. cloacae* CHE-2 also was positive for the bla_{SHV} gene, encoding the ESBL SHV-5, which does not hydrolyze carbapenems. Since both ß-lactamases GES-5 and SHV-5 could not explain the high MIC of imipenem for *E. cloacae* CHE-2 (Table 1), the outer membrane profile of *E. cloacae* CHE-2 was studied and compared to that of wild-type *E. cloacae* 5390 and to that of two *E. coli* reference strains. *E. cloacae* CHE-2 lacked two porins of 37 and 38 kDa, corresponding to OmpD and OmpF in *E. coli* (data not shown), respectively, therefore explaining the additional degree of resistance to carbapenems (12, 47, 53).

The bla_{GES-5} ß-lactamase gene is located on an IncQ-type plasmid. The plasmid analysis of *E. cloacae* CHE-2 identified three plasmids, pCHE-A, pCHE-B, and pCHE-C, being ca. 7, 150, and 50 kb in size, respectively (data not shown). Mating-out assays gave two types of transconjugants, both expressing an ESBL phenotype. Transconjugant *E. coli* J53 (pCHE-A) expressed the ESBL GES-5, whereas the *E. coli* J53 (pCHE-B) transconjugant expressed the ESBL SHV-5 (Table 1). Plasmid pCHE-B belonged to the IncA/C_2 incompatibility group, whereas plasmid pCHE-A was not typeable using a PCR-based technique for incompatibility grouping (6). The direct sequencing of the entire 7,560-bp-long plasmid pCHE-A by a primer-walking technique showed that it contained eight open reading frames (ORFs): RepF, RepA, RepB, RepC, MobA, MobB, MobC, and GES-5 (Fig. 1). The identification of three ORFs encoding RepF, RepA, RepB, and RepC proteins (68, 279,

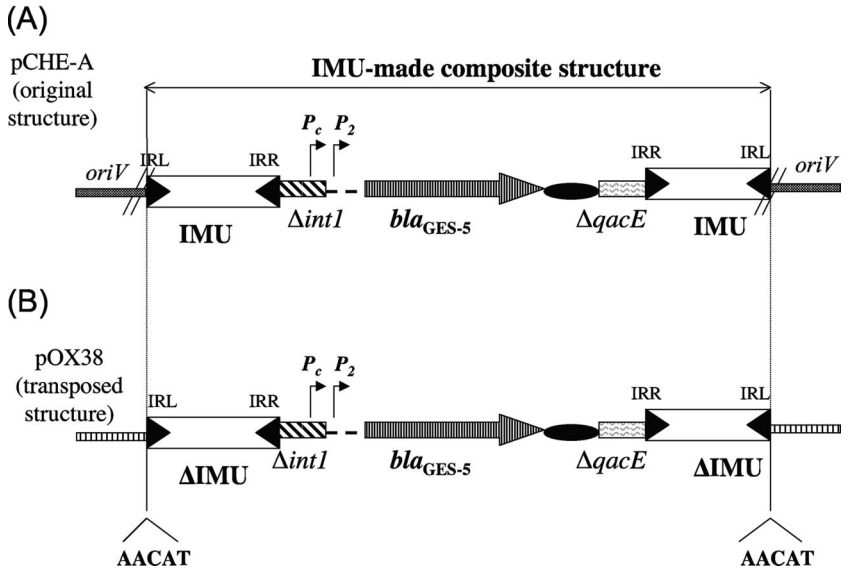


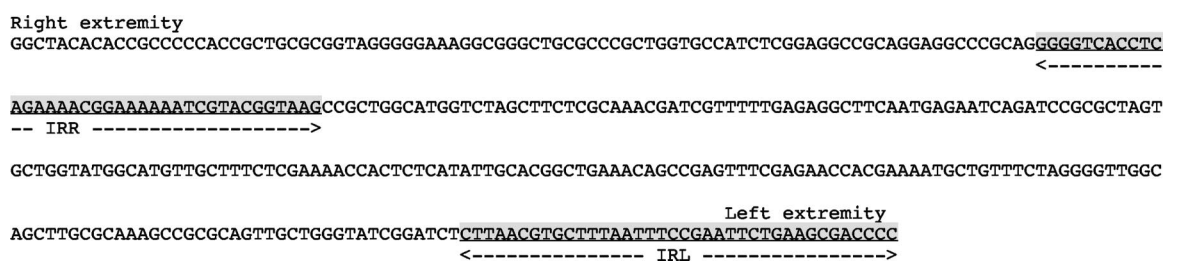
FIG. 2. Structure of the IMU-made composite structure harboring the bla_{GES-5} gene. (A) Schematic map of the putative mobilized structure on natural plasmid pCHE-A corresponding to the two IMUs bracketing the bla_{GES-5}-borne integron, with a truncated *int1* integrase gene and a truncated *qacE* gene. The IRR and IRL identified in the IMU are indicated by black triangles. The 59-bp sequence of the bla_{GES-5} gene cassette is indicated by a black oval, as is the *oriV* coding sequence region. The integron-mediated P_c and P_2 promoter sequences are indicated. (B) Schematic map of the fragment mobilized by the in *trans* transposition process onto recipient plasmid pOX38.

323, and 312 amino acids long, respectively) (Fig. 1) classified plasmid pCHE-A as a member of the MOB_Q family of plasmids, also known as mobilizable IncQ-type plasmids (they are not included in the PCR-based replicon typing scheme) (6). RepF, RepA, RepB, and RepC proteins shared 63, 93, 71, and 85% amino acid identity, respectively, with those of the reference and broad-host-range plasmid pRSF1010, which was identified in *E. coli* (50). A *mobA* gene was identified that encoded a putative 701-amino-acid MobA mobilization protein sharing 90% amino acid identity with that identified on a plasmid conferring resistance to florfenicol from *Pasteurella multocida* (25). The *repB* gene mentioned above corresponds to the same reading frame as *mobA*, but it has a different start codon that gives rise to a shorter protein (Fig. 1). Considering another reading frame overlapping the *mobA* sequence, an *mobB* gene was identified that encoded a 136-amino-acid-long MobB protein sharing 62% identity with that of plasmid pRSF1010 (50). A *mobC* gene was identified that encoded a putative 105-amino-acid MobC mobilization protein sharing 84% amino acid identity with that of plasmid pRFS1010 (50). Those Mob proteins are part of a plasmid mobilization system consisting of multifunctional proteins acting as relaxases and DNA primases, in which an *oriT* region was identified (18, 48). The sixth ORF corresponded to that of the bla_{GES-5} gene (Fig. 1). The *oriV* sequence, corresponding to the origin of replication of plasmid pCHE-A, also was identified downstream of the *repB* gene (Fig. 1).

The broad-host-range property of plasmid pCHE-A was assessed by obtaining transformants using *P. aeruginosa* PU21 and *E. cloacae* 5390 as recipient strains. It was not self conjugative, as indicated by the negative results of mating-out assays. However, it was mobilizable in *trans* by the $IncA/C_2$ plasmid pCHE-B (bla_{SHV-5}), as shown by the obtention of *E. coli* J53 transconjugants harboring both plasmids pCHE-A and pCHE-B.

The bla_{GES-5} gene is associated with novel genetic structures. The analysis of the DNA sequences flanking the bla_{GES-5} gene showed that it formed a gene cassette, as previously observed for bla_{GES}-like genes and some other ESBL-encoding genes (33, 38). In particular, it possessed core and inverse core sites, together with a 59-base element (52). The bla_{GES-5} gene cassette was preceded by a class 1 integrase gene and followed by a *qacE* gene, which are two classical features of class 1 integrons, but both were truncated in that present structure (Fig. 1 and 2A). Further analysis identified two repeated and perfectly identical 288-bp elements (termed IMUs) encompassing this class 1 integron remnant. These elements were located in opposite orientations on each side of the bla_{GES-5} gene (Fig. 1 and 2A). Their GC content was 50%; it was 62.3% for the overall plasmid backbone. A Blast analysis did not show any significant identity between those elements and any known sequences. However, a detailed analysis performed on the ISBiotoul website (http://www-is.biotoul.fr/) revealed that the first 38 bp of one end of the IMU element (defined arbitrarily as being its right end and consequently named the right inverted repeat [IRR]) were 95% identical (only two base pair mismatches) with the IRR of insertion sequence IS*Sod9*, which belongs to the Tn*3* family of transposons. Two copies of IS*Sod9* previously had been identified on a 160-kb plasmid of *Shewanella oneidensis* MR-1 (20). A putative left inverted repeat (IRL) sequence was identified at the opposite end of the IMU, sharing 28 out of the 38 bp of the IRR (Fig. 3). However, as opposed to insertion sequence (IS) elements, no ORF (and therefore no transposase) was identified in the IMU elements.

IMUs possess features similar to MITEs. These elements



belong to a large family of small, repeat, and nonautonomous sequences identified in several bacterial genomes, archaebacteriacae, and eukaryotes (5). Notably, no target site duplication was identified on either extremity of the IMU-Δ*int*/Δ*qac*-IMU structure that could have suggested any transposition-mediated acquisition.

Interestingly, the integration of the IMU-Δ*int*/Δ*qac*-IMU structure occurred within the *oriV* region of plasmid pCHE-A, disrupting the so-called single-strand initiation (*ssi*) signal sequences that serve for initiating the priming of single-stranded DNA synthesis during plasmid replication (18, 48). A detailed sequence analysis showed that part of these *ssi* sequences were duplicated and identified on both extremities of the IMU-Δ*int*/Δ*qac*-IMU structure, but in opposite orientations.

IMU elements may mobilize the antibiotic resistance gene by transposition. To determine whether the IMU-Δ*int*/bla_{GES-5}/Δ*qac*-IMU structure could be mobilized by transposition, experiments were conducted by providing in *trans* the IS*Sod9* transposase activity of *S. oneidensis*. Several *recA-E. coli* J53 transconjugants contained two plasmids, whereas others harbored only the plasmid pOX38 backbone. The identification of two plasmids in a single host strain corresponded to a mobilization of the natural bla_{GES-5}-positive plasmid pCHE-A by plasmid pOX38. The transposition of the IMU-Δ*int*/bla_{GES-5}/Δ*qac*-IMU overall fragment was identified in transconjugants that had a single pOX38-like plasmid. On those plasmids, the bla_{GES-5} gene was bracketed by the two IMU elements. A 5-bp target site duplication was identified on both ends of the ΔIMU-Δ*int*/Δ*qac*-ΔIMU structures, which is the signature of an in vivo transposition process (Fig. 2B). This result was in accordance with the in silico identification of 5-bp-long duplicated sequences identified on both extremities of an IS*Sod9*-made composite transposon in *S. oneidensis* MR-1 (data not shown). Since all transconjugants did not harbor recombinant pOX38 plasmids, it was not possible to deduce the transposition frequency.

The IMU element does not enhance expression of the antibiotic resistance gene. Since multiple IS elements may enhance the expression of downstream-located genes, the IMU-dependent expression of the bla_{GES-5} gene was evaluated. The IMU location truncated the *int1* integrase gene (by introducing an immediate stop codon in its corresponding frame), whereas the P_c and P_2 promoter sequences specific to class 1 integrons still were clearly identified (Fig. 2A) (11). Clonings of the bla_{GES-5}^+ PCR fragments with or without the IMU structure located upstream of the bla_{GES-5} gene showed identical ß-lactam MICs for *E. coli* recombinant strains, indicating that the IMU structure did not enhance the expression of the bla_{GES-5} gene (data not shown).

DISCUSSION

This study described a carbapenem-resistant *E. cloacae* isolate producing the ß-lactamase GES-5 and is the first evidence of the dissemination of this carbapenemase in North America. The bla_{GES-5} gene was located on a small-size, broad-host-range, IncQ-type plasmid named pCHE-A. This plasmid was not self conjugative but was mobilizable by a broad-host-range IncA/C_2 plasmid (named pCHE-B). Plasmid pCHE-A was transferred to and was able to replicate into enterobacterial and *Pseudomonas* species.

By far, the most interesting result of the study is the identification of a totally novel DNA element, IMU, of which two copies were identified, in opposite orientations, encompassing a defective class 1 integron containing the carbapenem resistance gene bla_{GES-5}. The IMU elements did not code for any ORF but possessed two 39-bp imperfect inverted repeats. The IMU structure resembles MITEs, which have been identified in eukaryotes such as plants (7) and also in bacteria (5, 17). However, the IMUs differ from other MITEs in the following features: IMUs are larger (MITEs usually are <200 bp) and have a higher GC content (MITEs usually are AT rich) (14). Nevertheless, some MITEs can be large, such as SMN1 from the archaebacterium *Sulfolobus islandicus* (321 bp in size) (4). In addition, as opposed to what has been observed with the MITE-type elements from *Neisseria* spp. (Correia element) (14, 33), no integration host factor binding site was identified in the IMU sequence. The only known MITE identified in *Enterobacteriaceae* corresponded to the enterobacterial intergenic consensus, which likely is involved in the regulation of mRNA stability (21). Other bacterial MITEs are RUPs (repeat unit of *Pneumococcus*) from *Streptococcus pneumoniae* or RPE (*Rickettsia* palindromic element) in *Rickettsia* spp., and all are known to be naturally present at multiple copies in their host (31, 36, 37).

The two IMUs identified in this study formed a composite element and likely were responsible for the mobilization of this defective class 1 integron structure. We have shown that the mobility of the IMU elements is mediated by a transposition mechanism once a transposase activity (such as IS*Sod9* transposase) is provided in *trans*. The *trans*-mobilization of deleterious IS elements previously had been reported with, e.g., IS*911* (45) or IS*231* (15). Also, an in silico analysis of the whole

genome of *Geobacter uraniireducens* Rf4 allowed us to hypothesize that the *Chunjie* MITE had been mobilized in *trans* by the transposase of IS*Gur4*, which shared similar inverted repeat sequences (9). Interestingly, we identified that the IMU-Δ*int*/Δ*qac*-IMU structure inserted within the *ssi* repeats of the *oriV* region, which was duplicated in part. In silico analysis showed that the duplication of this part of the *oriV* sequence may be observed in other IncQ-type plasmids (i.e., plasmids pIE1130 and pIE1115 [51]).

Since the absence of target site duplication on plasmid pCHE-A does not correlate with the integration of the IMU-Δ*int*/Δ*qac*-IMU structure by a transposition mechanism, several hypotheses can be made. One is that the *oriV* duplication observed on each side of the two IMUs has been at the origin of a homologous recombination process, leading to the acquisition of the IMU-Δ*int*/Δ*qac*-IMU structure.

Although all known MITEs are located on chromosomes, we report in this study a plasmid location of the IMUs associated with a clinically relevant antibiotic resistance gene. Finally, we have shown that the IMUs at least can be mobilized by the transposase of an unrelated IS element. The discovery of IMUs provides a new piece of evidence in the evolutionary engineering of bacterial pathogens, playing an adaptive role for survival against selective pressure (2). Since the genetic elements have been identified in a broad-host-range plasmid, future studies will evaluate the prevalence and possible spread of IMUs among clinically relevant pathogens.

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