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Complete Sequence of pJIE143, a *pir*-Type Plasmid Carrying IS*Ecp1-bla*$_{CTX-M-15}$ from an *Escherichia coli* ST131 Isolate[▽]

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pJIE143 (34 kb), from an *Escherichia coli* ST131 isolate, carries *bla*$_{CTX-M-15}$ but could not be typed using the standard PCR-based replicon-typing primer set. Complete sequencing revealed a backbone with similarity to IncX plasmids, including a *pir*-like gene encoding a π-like replication protein and iterons related to those of other IncX plasmids. The 2.971-kb IS*Ecp1-bla*$_{CTX-M-15}$-orf477Δ transposition unit often found within Tn*2* is inserted just beyond the end of *pir*, flanked by 5-bp direct repeats.

The *bla*$_{CTX-M}$ genes now appear to be the dominant family of genes conferring resistance to extended spectrum β-lactam antibiotics worldwide, and *bla*$_{CTX-M-15}$ is the most common in many locations (19). *bla*$_{CTX-M-15}$ is generally found in a 2.971-kb IS*Ecp1*-mediated transposition unit that also includes a truncated orf477, usually inserted in Tn*2* (e.g., see references 17, 21, and 23) but also found directly inserted in the backbone of an IncA/C$_2$ IncY plasmid from *Salmonella* (8). Surveys (e.g., references 9 and 13) indicate that in *Escherichia coli*, *bla*$_{CTX-M-15}$ is predominantly carried by IncF or IncI1 plasmids, but some plasmids carrying this gene could not be typed by standard PCR-based replicon typing (PBRT) (4).

We identified *bla*$_{CTX-M-15}$ as the dominant extended-spectrum β-lactamase (ESBL) gene in *E. coli* in western Sydney, always linked to IS*Ecp1* (24). Fourteen of 19 *E. coli* isolates with different pulsed-field gel electrophoresis (PFGE) profiles gave transconjugants in DH5α carrying *bla*$_{CTX-M-15}$, and PBRT (4) identified replicons (IncF, $n = 9$, and IncI1, $n = 4$) in all but one (24). Isolate JIE143 gave transconjugants resistant to ampicillin (80 μg/ml) at a frequency of 6.7×10^{-7}/recipient by filter mating, but these failed to give amplicons by PBRT (24). JIE143, isolated from blood <48 h after admission of a patient to Blacktown Hospital, Sydney, in June 2006, was considered community acquired and was found to be phylogenetic group B2 by PCR (5), O25b using antisera (Denka Sieken, Coventry, United Kingdom) and by PCR (6), and sequence type 131 (ST131) by multilocus sequence typing (MLST; http://mlst.ucc.ie/mlst/dbs/Ecoli) (22). S1 nuclease-PFGE (2, 17) of DNA from a selected transconjugant (Tx143) revealed a single plasmid of ~35 kb (data not shown), designated pJIE143, which was completely sequenced.

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DNA extracted from Tx143 using the Qiagen (Hilden, Germany) HiSpeed plasmid purification system was treated with Plasmid Safe ATP-dependent DNase (Epicentre Biotechnologies, Madison WI), and 10 ng was amplified with a GenomiPhi version 2 DNA kit (GE Healthcare, Piscataway, NJ), following the manufacturers' protocols. After sodium acetate precipitation, purified DNA was resuspended in 45 μl of 10 mM Tris (pH 7.5) and quantified using the Quant-iT PicoGreen double-stranded DNA (dsDNA) assay (Invitrogen, Carlsbad, CA) following the manufacturer's protocol. A library prepared from 500 ng of plasmid DNA (*Roche Rapid Library Preparation Method Manual*, GS FLX Titanium Series, October 2009) was bar coded with Rapid Library MID adaptors (Roche 454 Life Sciences, Mannheim, Germany) and pooled with libraries from 11 other plasmids prior to emulsion PCR. Sequencing was carried out on one-quarter of a 4-region plate in a GS-FLX sequencer (Roche) (14) at the Ramaciotti Centre, Sydney.

Assembly with Newbler version 2.3 (Roche) gave a 34.345-kb contig (180-fold coverage), confirmed to correspond to the entire plasmid by PCR to link the ends (primers 143-1, CAATGCGGCATATCTGTCC, and 143-2, GCACGAGCGT TCCTCTCC). The typical 2.971-kb IS*Ecp1-bla*$_{CTX-M-15}$-orf477Δ transposition unit was identified, flanked by direct repeats (GGATA) indicative of insertion by IS*Ecp1*-mediated transposition using the same alternative IR$_R$ as previously observed. pJIE143 does not carry any other known antibiotic resistance genes.

BLASTn searches revealed that most of the pJIE143 backbone is closely related to pBS512_33 from *Shigella boydii* (33 kb; GenBank accession no. CP001059) and pCROD2 from *Citrobacter rodentium* (39 kb; GenBank accession no. FN543504 [18]) (Fig. 1A), neither of which carry any known antibiotic resistance genes. As noted for pCROD2 (18), pJIE143 is organized similarly to plasmids in the narrow-host-range IncX group found in the *Enterobacteriaceae*. The majority of known IncX plasmids belong to a subgroup designated IncX1, while the archetypal IncX plasmid R6K exemplifies the

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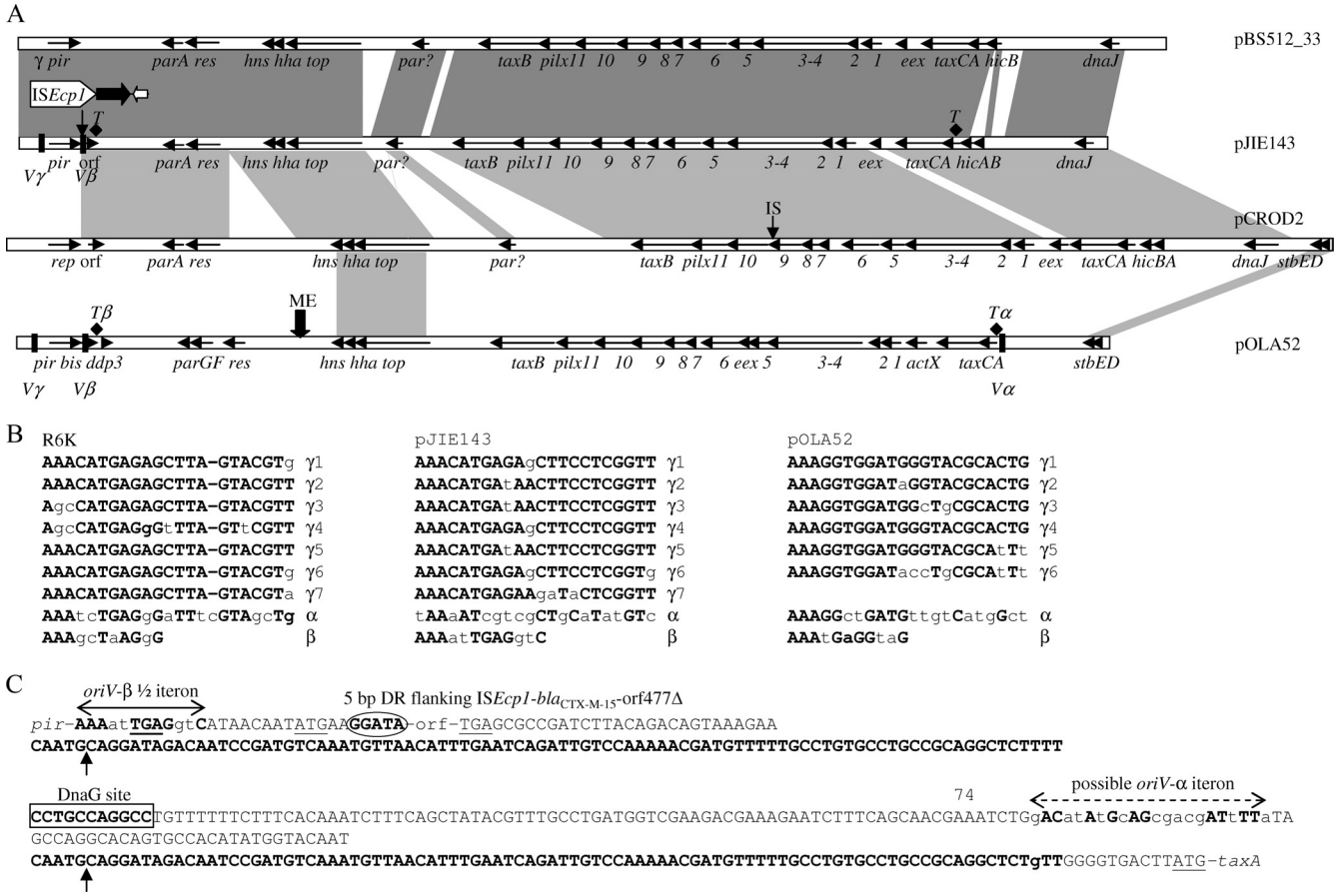

FIG. 1. (A) Comparison of backbones of pBS512_33 (CP001059), pJIE143, pCROD2 (FN543504), and pOLA52 (EU370913) (GenBank accession numbers are in parentheses). Darker shading indicates regions with 99% nucleotide identity, and lighter shading 97% identity. The extents and directions of various genes are shown as arrows labeled with the gene name (as described in the text, plus *dpp3*, DNA distortion polypeptide; *actX*, antiterminator; and *dnaJ*, chaperone) or the number for *pilx* genes. The insertion point of the IS*Ecp1-bla*$_{CTX-M-15}$-orf477Δ transposition unit in pJIE143 is indicated. The vertical arrow labeled IS above pCROD2 indicates the site of insertion of IS*102.* The arrow labeled ME above pOLA52 indicates the region where several mobile elements and resistance genes are inserted. The approximate positions of proposed *oriV* (small vertical bars labeled *V*) and *oriT* (small diamonds labeled *T*) of pJIE143 and pOLA52 are indicated. (B) *oriV* iterons of R6K and proposed *oriV* iterons of pOLA52 (all taken from reference 15, except *oriV*-α of R6K, which appears to have an extra T in reference 16) and pJIE143. Bases conserved in the majority of sequences in each set are in boldface, and bases which differ are in lowercase. (C) Sequences surrounding the proposed *oriV*/*oriT* β (top) and α (bottom, reverse orientation to part A) regions of pJIE143. Proposed iterons shown in panel B are indicated by doubled-headed arrows. Stop and start codons of various genes are underlined. Vertical arrows indicate nic sites (1). The 5 bp duplicated by insertion of IS*Ecp1-bla*$_{CTX-M-15}$-orf477Δ are indicated by an oval. The DnaG site in the α region is boxed (16), and "74" indicates 74 bp corresponding to the distance between this site and one end of the *oriV*-α iteron in R6K and pOLA52 (15). Sequences are continuous from one line to the next but have been broken to enable the 94-bp *oriT* repeats (shown in boldface on the lower lines) to be aligned.

rarer IncX2 subgroup (10). The complete sequence of R6K (http://www.sanger.ac.uk/Projects/Plasmids/) has not yet been annotated or published, but this plasmid has been well studied, and pOLA52 (51.6 kb; GenBank accession no. EU370913), classified as IncX1, has also been analyzed in detail (15). The pJIE143 backbone was annotated by reference to these two plasmids.

The R6K and pOLA52 replication regions include a *pir* gene, encoding the π protein for initiation of replication, and three vegetative origins, *oriV*-α, *oriV*-β, and *oriV*-γ (see reference 20 and references therein). pJIE143 encodes a protein related to π of R6K (39% identical/63% similar) and pOLA52 (35%/60%) and more closely related (75%/86%) to the putative π protein of pJARS35 from *Yersinia pestis* (35 kb; GenBank accession no. CP002179). pCROD2 has a quite different replication protein (<29%/<50%). The *bis* gene found downstream of *pir* in R6K and pOLA52, which has an uncertain role in replication from *oriV*-β (16), is missing from pJIE143. A different open reading frame (ORF) (Fig. 1A) is found in this position, interrupted after 10 bp by IS*Ecp1-bla*$_{CTX-M-15}$-orf477Δ (Fig. 1A and C), and equivalent ORFs are present in pCROD2 (95% identical) and pJARS35 (69% identical).

In R6K, *oriV*-γ is located upstream of *pir* (Fig. 1A) and contains seven 22-bp iterons to which π binds. *oriV*-β, downstream of *pir*, has a half-iteron and *oriV*-α, found some distance away near a DnaG binding site, has a single iteron (16). Seven *oriV*-γ iterons related to those of R6K were identified in pJIE143 in the expected location (Fig. 1B). A putative *oriV*-β half iteron was identified close to the end of

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pir in pJIE143, and although a probable DnaG binding site was also found, potential α iterons in this region were not convincing (Fig. 1B and C).

The conjugation region of pJIE143 encodes equivalents (31 to 55% identical/46 to 71% similar) of Pilx1 to Pilx11 involved in pilus synthesis and assembly in R6K (GenBank accession no. AJ006342) and pOLA52. pJIE143 also encodes equivalents of TaxA, the TaxB coupling protein, and the TaxC relaxase of R6K and pOLA52 (25 to 40% identical/45 to 59% similar) (16). A putative *eex* (entry exclusion) gene encodes a protein 57% identical/72% similar to Eex of pOLA52 but is located in a different position (Fig. 1A). Like R6K (1) and pOLA52 (15), pJIE143 appears to have two origins of transfer (*oriT*) situated in inverted repeats (94 bp) (Fig. 1C).

pJIE143 and pCROD2 do not include the *parFG* plasmid partitioning locus found in pOLA52, but both have other putative *par* genes and a resolvase gene (>93% identical) (*parA*, *par?* and *res* in Fig. 1A). pJIE143 lacks the *stbDE* toxin/antitoxin genes found in pOLA52 and pCROD2 but does have the *hicAB* genes also present pCROD2 (99% identical). These are proposed to encode another toxin/antitoxin system in which HicA inhibits translation, causing mRNA cleavage, while HicB appears to be an unstable repressor that neutralizes HicA (11). pJIE143 also includes a region 97% identical to both pCROD2 and pOLA52 and containing *hns*, *hha*, and *topB* genes, putatively encoding a histone-like nucleotide structuring protein (H-NS), a modulator of H-NS activity (12), and a topoisomerase, respectively. These factors may all contribute to plasmid maintenance.

R6K carries Tn*3* (bla_{TEM-1a}) inserted in Tn*5393* (*strAB*), while pOLA52 carries *oqxAB*, encoding an efflux pump conferring resistance to olaquindox, in an IS*26*-mediated composite transposon inserted in Tn*2* (bla_{TEM-1b}). Other "IncX-like" resistance plasmids are also beginning to be identified; e.g., pE001 (GenBank accession no. JF776874) carries the bla_{TEM-52} extended-spectrum β-lactamase gene in Tn*2* (3). However, these plasmids may be being missed in surveys, as the R6K-derived probe used for hybridization-based replicon typing did not hybridize with all IncX plasmids (7) and the PBRT IncX primers were designed from this probe (4). These primers flank the *oriV*-γ iteron region of R6K and have essentially no similarity with this region in other characterized IncX-like plasmids, would not be expected to detect pOLA52, and did not detect pE001 (3) or pJIE143. Similarly, primers in the pOLA52 or pE001 *pir* gene (3) would not be expected to detect pJIE413. Thus, evaluation of different targets and/or the development of a set of primers may be required to enable the detection of subsets of "IncX-like" plasmids that appear to have similar organization but quite variable sequences.

Nucleotide sequence accession number. The complete nucleotide sequence of pJIE143 has been submitted to GenBank under accession no. JN194214.

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