ISEcp1-Mediated Transposition and Homologous Recombination Can Explain the Context of *bla*_{CTX-M-62} Linked to *qnrB2*[∇]

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*bla*_{CTX-M-62}, a C508T variant of *bla*_{CTX-M-3b}, was transferred from *Klebsiella pneumoniae* JIE137 on a conjugative plasmid together with a class 1 integron containing the *dfrA12-gcuF-aadA2* cassette array, ISCR1, and *qnrB2*. *bla*_{CTX-M-62} lies between intact and rearranged copies of *ISEcp1* in a configuration that can be explained by a combination of transposition and homologous recombination and which also illustrates the ability of *ISEcp1* to mobilize an adjacent gene as part of transposition units of different sizes.

The insertion sequence *ISEcp1* appears to have been responsible for the capture and mobilization of a number of unrelated antibiotic resistance genes, including several of the *bla*_{CTX-M} groups encoding a family of extended-spectrum β-lactamases that now appear dominant worldwide (18). *ISEcp1* is bounded by 14-bp inverted repeats, IR_L at the left-hand end with respect to the direction of transcription of the transposase gene and IR_R at the right-hand end. However, *ISEcp1* appears to use IR_L in conjunction with alternative sequences resembling these IR to mobilize adjacent regions, creating 5-bp duplications of flanking sequence on transposition (9, 14, 15, 19). Like *ISPMel*, which appears to operate in a similar way (2), *ISEcp1* can mobilize the same adjacent gene as part of different-size “transposition units,” as illustrated by transposition of “hybrid” units that include adjacent vector

sequence from cloned *ISEcp1*-resistance gene combinations (15, 19).

*bla*_{CTX-M} genes appear to have been captured from the chromosomes of *Kluyvera* spp. For example, a chromosomal region of *Kluyvera ascorbata* (GenBank accession no. AJ632119) is identical to the *ISEcp1*-associated, plasmid-borne *bla*_{CTX-M-3a} gene and its flanking regions (17). In this case a transposition unit that includes a 127-bp “spacer” preceding the *bla*_{CTX-M-3a} gene and 345 bp of the adjacent *orf477* appears to have been mobilized by *ISEcp1* using an alternative IR (IR₁ in Fig. 1 and 2). While the *bla*_{CTX-M-3a} gene appears widespread, CTX-M-3 may also be encoded by the *bla*_{CTX-M-3b} variant, which has 8 nucleotide differences and to date has been identified only in Japan (1) (GenBank accession no. AB059404, AB168117, AB185834 to AB185841, AB231615,

FIG. 1. Immediate genetic contexts of and differences between the *bla*_{CTX-M-3a} and *bla*_{CTX-M-3b} genes. Genes (represented by arrows) and adjacent sequences are to scale, while *ISEcp1* (represented by a pointed box) is not. The positions of the inverted repeats of *ISEcp1* (IR_R and IR_L) plus an alternative IR (IR₁) are indicated. The numbers represent the lengths of the “spacers” between IR_R and the start codon of *bla*_{CTX-M-3} and between the stop codons of *bla*_{CTX-M-3} and *orf477*. Lines marked with asterisks represent the positions at which *bla*_{CTX-M-3a} and *bla*_{CTX-M-3b} and their flanking sequences differ. The position of the C508T mutation in *bla*_{CTX-M-62} is also shown. Sequences were obtained from the following GenBank entries: *K. ascorbata bla*_{CTX-M-3a} region, accession no. AJ632119; plasmid-borne *bla*_{CTX-M-3a} region, accession no. AF550415; region containing *bla*_{CTX-M-3b}, accession no. EF382672.

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FIG. 2. (A) Configuration of *bla*_{CTX-M-62}, *ISEcpI*, and *qnrB2* in pJIE137 and comparison with related structures. Genes are indicated by labeled arrows (3b, *bla*_{CTX-M-3b}; 477Δ, *orf477Δ*), and IS are indicated by labeled pointed boxes. Gene cassettes are shown as narrow boxes, with small black boxes representing their *attC* sites. Vertical bars represent IRi and IRt of class 1 integrons and a 38-bp sequence resembling the IR of Tn3-like transposons, as indicated. Dotted lines represent different plasmid backbone sequences. Black circles represent 5-bp DR of plasmid sequence flanking different transposition units and have the following sequences: pK29 (GenBank accession no. EF382672), TACAA; pRA1 (FJ705807), ATTAT; and pJIE137, ACATA. Selected primers used to map this configuration and the extents of amplicons are shown immediately below. The regions matching pLEW517 (accession no. DQ390454) and pJIBE401 (accession no. AJ609296) are also indicated. (B) A proposed scheme for the generation of the configurations in pJIE137 and pK29. Various features are represented as in part A, and one copy of *ISEcpI* is shaded gray to make the origins of the different segments clearer. Alternative IR are indicated as IR_L, IR₂, and IR₃, and a comparison of their sequences with IR_L and IR_R is shown in the box, with bases that differ from IR_L in lowercase and bold typeface.

and AB432919) and Taiwan (pK29; accession no. EF382672) (5). Comparison of the only available sequence that includes regions flanking *bla*_{CTX-M-3b} (pK29) with equivalent regions flanking *bla*_{CTX-M-3a} revealed additional differences (Fig. 1), including a shorter 45-bp spacer, suggesting that *bla*_{CTX-M-3b} may have been mobilized from a different strain of *Kluyvera*. In pK29, the 345 bp of *orf477Δ* beyond *bla*_{CTX-M-3b} are followed by a unique 234-bp region that includes a partial open reading frame, designated *yhgE*. This suggests initial capture of *bla*_{CTX-M-3b} was followed by mobilization of a longer *ISEcpI* transposition unit utilizing a different alternative IR. Beyond the *yhgE* region, the first 583 bp of *ISEcpI* are found in the orientation opposite to that of the intact *ISEcpI* that lies upstream of *bla*_{CTX-M-3b} (Δ1 in Fig. 2A). The *ISEcpI*-*bla*_{CTX-M-3b}-Δ1 region is flanked by 5-bp direct repeats (DR) (TACAA) and is

apparently inserted in the plasmid backbone of pK29 as an unusual transposition unit bounded by inverted copies of IR_L.

A highly ceftazidime-resistant *Klebsiella pneumoniae* isolate from Sydney, Australia (JIE137), carries a C508T variant (stated incorrectly as G509T in reference 23) of *bla*_{CTX-M-3b}, encoding a Pro167Ser CTX-M-3 variant (Ambler numbering) designated CTX-M-62 (<http://www.lahey.org/studies/>). *bla*_{CTX-M-62} was transferred to *Escherichia coli* DH5αRf on a large conjugative plasmid (pJIE137), but PCR-based replicon typing (4) failed to identify any of the Inc groups tested for (23). Here we analyze the genetic environment of *bla*_{CTX-M-62} in detail.

ISEcpI is adjacent to *bla*_{CTX-M-62} in pJIE137 (23), and further PCR and sequencing indicated that, like *bla*_{CTX-M-3b} in pK29, *bla*_{CTX-M-62} is flanked by intact and truncated copies of *ISEcpI*, with the sequences differing only in the C508T substi-

TABLE 1. Selected primers used in this study

Primer	Sequence (5'–3')	Target	Reference
CTXM1-F	AAAAATCACTGCGCCAGTTC	bla _{CTX-M-1} group genes	22
CTXM1-R	AGCTTATTCATCGCCACGTT	bla _{CTX-M-1} group genes	22
5'ISEcp-R	CCAATGGGTGAAAGCTGAAT	ISEcp1	This study
qnrB-F ^a	GATCGTGAAGYCAAGG	qnrB genes	16
qnrB-R	ACGATGCCTGGTAGTTGTCC	qnrB genes	16
hep58	TCATGGCTTGTATGACTGT	5'-CS	20
Int1R	CCCGAGGCATAGACTGTA	5'-CS	8
3CS-F	CTATTGGTCTCGGTGTCG	3'-CS	6
IS26-F2	TGGCAAACCTGAAACGGATAA	IS26	6
dfrA12-R1	AGCTTGAATGGTTTCGGTTG	dfrA12	This study
mrxF	TTCGCGGCGACCTTCTCTT	mph(A) region	This study
bla-F1	GGACTTGCCTTGGTTCTCAA	orfRA1-14	This study
137-1	TAGGGAGCGTGAGCTGGTAG	pJIE137	This study
137-4	TCTTTACCAATTTTCCGTTA	pJIE137	This study
137-6	GAATAGGGGGAGGAGAACAA	pJIE137	This study
137-R1	TGGTAGTTTGCGCTTGAT	pJIE137	This study

^a This primer has been modified from qnrB-F in reference 16, which has C instead of the Y that is in bold type.

tution. Direct sequencing and PCR mapping of pJIE137 revealed that ISEcp1Δ1 (nucleotides [nt] 1 to 583) downstream of bla_{CTX-M-62} is followed by the end of ISEcp1 (Δ2; nt 579 to 1656) in the opposite orientation (Fig. 2A). The 898-bp abutting IR_R of ISEcp1Δ2 matches a region found adjacent to an intact ISEcp1 in the IncA/C plasmid pRA1 (FJ705807; Fig. 2A) (7). The identity ends at the boundary of a 2.554-kb ISEcp1 transposition unit in pRA1 that ends with 14 nt resembling the IR of ISEcp1 (IR₃ in Fig. 2B) and is flanked by DR (ATTAT). This region includes an open reading frame (ORF) predicted to encode a protein belonging to the β-lactamase superfamily, here designated orfRA1-14.

The association of extended-spectrum β-lactamase (ESBL) genes and plasmid-mediated quinolone resistance determinants is not unusual (e.g., see reference 10), and screening for qnrB genes (qnrB-F/qnrB-R; Table 1) revealed qnrB2 in pJIE137, although both JIE137 and its transconjugant were sensitive to ciprofloxacin (MIC ≤ 0.5 μg/ml). As expected, qnrB2 was located between ISCR1 and a partial copy of the 3' conserved segment (3'-CS) of class 1 integrons, and the common (13) dfrA12-gcuF-aadA2 cassette array was identified upstream of ISCR1. The chrA-mph(A)-IS26 configuration found in several multiresistance regions (see reference 6) was identified beyond qnrB2. Long PCR linked ISEcp1-bla_{CTX-M-62}-Δ1/Δ2-orfRA1-14 to int11, and additional PCR mapping enabled the whole resistance region to be sequenced (Fig. 2A).

The region between orfRA1-14 and IRi of the integron contains genes predicted to encode a zinc metalloproteinase (80% identical/89% similar to Mpr of the IncN plasmid R46) and an ArdK-like protein (48% identical/65% similar to ArdK of R46) separated by a gene potentially encoding a single-strand DNA-binding protein (ssb). A gene predicted to encode a protein ~70% identical to plasmid RepA from several different bacterial species follows ardK. This region also appears to have organization similar to that of part of the IncN resistance plasmids (e.g., R46), suggesting that it corresponds to part of a plasmid backbone and that bla_{CTX-M-62} and qnrB2 are found in separate resistance regions in pJIE137. The sequence adjacent to IR_L of intact ISEcp1 upstream of bla_{CTX-M-62} in pJIE137 includes a gene apparently encoding an ArdB-like

protein (49% identical/66% similar to ArdB of R46 [3]), suggesting it is also part of the plasmid backbone. The 5 bp (ACATA) adjacent to IR_L of this ISEcp1 are identical to those immediately after the end of the orfRA1-14 region (as identified by comparison with pRA1), suggesting that a 5.759-kb ISEcp1-bla_{CTX-M-62}-Δ1/Δ2-orfRA1-14 transposition unit was inserted in this plasmid backbone (Fig. 2A).

The scheme in Fig. 2B outlines one way in which this complex transposition unit could have arisen. First, ISEcp1 inserts into the *Kluyvera* chromosome 45 bp from the start of bla_{CTX-M-3b} and uses IR_L and IR₁ to transfer this gene plus orf477Δ to a new location. Next, a slightly longer region flanked by IR_L and IR₂ and including the yhgE segment is mobilized by ISEcp1 as part of a 3.2-kb transposition unit. This unit is inserted into a second copy of ISEcp1 upstream of orfRA1-14 (as seen in pRA1), with the ISEcp1 elements in opposite orientations, generating 5-bp DR (TGATA). Homologous recombination between the duplicated, inversely oriented left ends of ISEcp1 would invert the region between them. Transposition of the 5.759-kb region between IR_L and IR₃, including orfRA1-14, to a new location would give the configuration in pJIE137, and a single point mutation of bla_{CTX-M-3b} to give bla_{CTX-M-62} is also required. Transposition of the 3.783-kb region bounded by the two inverted copies of IR_L to a new location, with duplication of the target sequence (TACAA), yields the related configuration found in pK29.

The configuration of the bla_{CTX-M-62} region can therefore be explained by several ISEcp1-mediated transposition events utilizing different alternative IR combined with homologous recombination. The relationship of the qnrB2 region to other characterized resistance regions could also be explained by homologous recombination. For example, in the IncN plasmid pLEW517 (GenBank accession no. DQ390454) (21), dfrA12-gcuF-aadA2 is linked to the chrA-mph(A)-IS26 structure, but the ISCR1-qnrB2 region is not present, while pJIBE401 (accession no. AJ609296), an IncL/M plasmid from Sydney (6), has both ISCR1-qnrB2 and chrA-mph(A)-IS26 but the bla_{IMP-4}-qacG-aacA4-catB3 cassette array (Fig. 2A). Homologous recombination in the duplicated 3'-CS flanking the ISCR1-qnrB2 region can explain its insertion/deletion (12), while double

crossover in both the 5'-CS and 3'-CS allows switching of entire cassette arrays between different integrons (11). Thus, the resistance regions of pJIE137 illustrate how combinations of transposition and homologous recombination may be involved in the movement of resistance genes and the evolution of multiresistance regions.

Nucleotide sequence accession number. The nucleotide sequence of the *bla*_{CTX-M-62} gene available under GenBank accession no. EF219134 has been extended to include sequence obtained here.

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