pJIE137 Carrying \( \text{bla}_{\text{CTX-M-62}} \) Is Closely Related to p271A Carrying \( \text{bla}_{\text{NDM-1}} \)

Sally R. Partridge, Ian T. Paulsen, and Jonathan R. Iredell

Centre for Infectious Diseases and Microbiology, University of Sydney, Westmead Hospital, Sydney, New South Wales, Australia, and Department of Chemistry and Biomolecular Sciences, Macquarie University, Sydney, New South Wales, Australia

Complete sequencing of pJIE137 revealed a backbone closely related to p271A, encoding a novel RepA protein but with a similar organization and up to ~70% nucleotide identity to IncN plasmids. A region in pJIE137 resembling the IncN CUP regulon is mostly missing from p271A, presumably due to recombination. The class 1 In/Tn and IS pJIE137 backbone revealed three contigs as repeated regions and the other three as segments (data not shown). Here we present the complete sequence of this plasmid, pJIE137.

Resistance to clinically important extended-spectrum \( \beta \)-lactams and carbapenems in the Enterobacteriaceae is often encoded by genes carried on plasmids (3), some of which cannot be typed using available PCR-based replication typing methods (PBRT) (5, 8). We identified \( \text{bla}_{\text{CTX-M-62}} \), encoding a Pro167Ser variant (Ambler numbering) of CTX-M-3, in Klebsiella pneumoniae JIE137. Transconjugants of JIE137 with Escherichia coli DH5αRf were obtained by filter mating with selection on cefotaxime (2 \( \mu \)g/ml) and rifampin (80 \( \mu \)g/ml) at a frequency of \( 1.33 \times 10^{-7} \) recipient and carried \( \text{bla}_{\text{CTX-M-62}} \) but gave no ampiclons by PBRT (19). Mapping and sequencing revealed a rearranged IS\( \text{Ecp1-bl}\) transposition unit separated from a class 1 integron by a region related to IncN plasmids (18). S1 nuclease digestion and pulsed-field gel electrophoresis (2, 12) of a selected transconjugant carrying \( \text{bla}_{\text{CTX-M-62}} \) (TX37) gave a single band of ~60 kb (data not shown). Here we present the complete sequence of this plasmid, pJIE137.

DNA was extracted from Tx37 and amplified, quantified, and sequenced (GS-FLX; Roche 454 life sciences, Mannheim, Germany) as described previously (11). Newbler (version 2.3; Roche) assembled seven contigs (0.5 to 37 kb, 56\% coverage), of which three cannot be typed using available PCR-based replication typing methods (PBRT), and five were identified as IncN plasmids. A region in pJIE137 resembling the IncN CUP regulon is mostly missing from p271A, presumably due to recombination. The class 1 In/Tn and IS pJIE137 backbone revealed three contigs as repeated regions and the other three as segments (data not shown). Here we present the complete sequence of this plasmid, pJIE137.

BLASTn searches (http://blast.ncbi.nlm.nih.gov/) with the pJIE137 backbone revealed >90\% identity to p271A, which carries \( \text{bla}_{\text{NDM-1}} \), from E. coli 271 (Fig. 1) (13). Although both plasmids were isolated in Sydney, Australia, isolate JIE137 was obtained in May 2006 while isolate 271 was obtained in December 2009 (P. Taylor, personal communication) from a patient at a different hospital who had been transferred from Bangladesh (14), suggesting that similar plasmids may have acquired different antibiotic resistance genes in different geographic locations.

pJIE137 and p271A backbones. The pJIE137 and p271A (13) backbones are organized similarly to the backbones of IncN plasmids, typified by plasmid R46 (Fig. 1A). Many of the proteins that they encode are also most closely related to those from IncN plasmids (see Table S1 in the supplemental material), but nucleotide identity is limited (Fig. 1) and the replicon regions and RepA proteins of pJIE137 and p271A are unrelated to those of IncN plasmids.

The p271A backbone lacks a 5.2-kb region found in pJIE137 that corresponds to the CUP (conserved upstream repeat)-controlled regulon of the IncN plasmid R46 (6). The CUP repeats contain a strong promoter and in R46 are associated with the \( \text{ardA} \) and \( \text{ardB} \) genes, encoding antirestriction proteins, several other CUP-controlled genes (\( \text{ccg} \)), and \( \text{ardD} \) and \( \text{ardR} \), which encode regulatory proteins that bind to the CUP repeats (6). Some IncN plasmids have fewer CUP repeats and only subsets of these genes (4) (Fig. 1B), which could be explained by recombination between repeats (6). In pJIE137, two copies of a 582-bp region (14 nucleotide differences) encompassing a shorter segment related to IncN CUP repeats are present, plus two partial copies of this shorter segment (Fig. 1B). These repeats are interspersed with some genes equivalent to those in R46 and other hypothetical genes (see Table S1 in the supplemental material) and contain a promoter and other features found in CUP repeats (see Fig. S1 in the supplemental material). p271A includes only one partial repeat; the rest of the region present in pJIE137 may have been lost by recombination (Fig. 1B).

\( \text{Arda} \) and \( \text{Ardb} \) provide protection from the restriction enzymes of the recipient during conjugation (17). pJIE137 lacks \( \text{ardA} \) and \( \text{ardB} \) is separated from the promoter in the adjacent repeat by insertion of IS\( \text{Ecp1-bl} \), while p271A has neither \( \text{ardA} \) nor \( \text{ardB} \), which may affect conjugation efficiency. pJIE137 and p271A also lack homologs of the IncN mucAB genes, which confer increased resistance to UV radiation and increased susceptibility to mutagenesis (16). However, both pJIE137 and p271A include a gene (\( \text{ssb} \)) with no equivalent in characterized IncN plasmids. Copyright © 2012, American Society for Microbiology. All Rights Reserved.

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Address correspondence to Sally R. Partridge, sally.partridge@swahs.health.nsw.gov.au.

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Insertions in pJJE137 and p271A. pJJE137 was known to carry a class 1 integron, but the available sequence ended within IS26 (18). Here the remainder of a class 1 In/Tn structure (10) was identified, bounded by the 25-bp inverted repeats IR1 (at the intI1 end) and IR2 (at the tri end) and flanked by 5-bp direct repeats (DR) (Fig. 1C). These DR indicate insertion by transposition, and it is possible that the whole structure was inserted en bloc, but a simpler class 1 In/Tn structure may have been inserted first, with subsequent acquisition/exchange of internal components by homologous recombination (10). As in several IncN plasmids, the class 1 In/Tn in pJJE137 is inserted upstream of the resP resolvase gene, despite these resP genes being quite different (see Table S1 in the supplemental material) and in opposite orientations with respect to other backbone genes (Fig. 1A). This is explained by Tn402-like transposons and their derivatives, i.e., class 1 In/Tn structures, targeting resolvase genes, including those on plasmids (9). The complex ISEcpl-blaCTX-M-62 transposition unit (18) is inserted in the repeat upstream of ardB, and one IncN plasmid, pLEW517 (DQ390454), has Tn2 inserted between CUP repeats (Fig. 1B).

Previous analysis of p271A identified a truncated ISaba125 (interrupted by ISEc33) on one side of blaNDM-1 and ISSeq4 and...
the Tn3-like transposon Tn5403, bounded by a 38-bp IR, on the other (13). Comparison with pHIE137 confirmed that the outermost IR of Tn5403 (IR$_A$ in Fig. 1C) defined one end of the insert in p271A but revealed a third Tn3-like 38-bp IR (IR$_C$) defining the other end. A fourth 38-bp IR found 181 bp away (IR$_2$) truncates the $\text{region}$, including the 3'-mediated deletion. Insertions in the $\text{fipA}$ region are also common in IncN plasmids (Fig. 1A), and since FipA (fertility inhibition protein) inhibits conjugation of certain other plasmids (15), such insertions may have a beneficial effect (13). This analysis shows that important antibiotic resistance genes, carried by different mobile elements and inserted in different locations, have been acquired by a plasmid backbone type that current surveys may be missing.

**Nomenclature of pHIE137, p271A, and other plasmids.** Given their organizational similarity to IncN plasmids, it has been suggested that p271A and pHIE137 constitute an “IncN2” subgroup (13). While plasmid Inc groups have been subdivided, with Roman and Arabic numerals and Greek letters (or combinations of these) used to indicate various subgroups (which may or may not be compatible with one another), the meanings of the different symbols seem to lack consistency, highlighting the difficulties inherent in adapting a system that was originally based on phentypic incompatibility to the classification of plasmids based on sequence comparisons. Classification using relaxase proteins rather than replications has been suggested (7), and pHIE137 and p271A with TraI 68% identical to R46 [see Table S1 in the supplemental material] would presumably be placed in the MOB$_{p411}$ group, along with IncN, IncW, and IncP-9 plasmids. Although screening based on relaxases rather than replications may allow detection of a greater variety of plasmids, there are still problems with how to indicate “mosaic” or “hybrid” plasmids, such as p271A and pHIE137. As increasing numbers of plasmids, including those that are currently “untypeable,” are sequenced, better ways of indicating relationships between plasmids will need to be developed.

**Nucleotide sequence accession number.** GenBank accession no. EF219134 has been extended to include the complete nucleotide sequence of pHIE137.

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**REFERENCES**