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Complete Nucleotide Sequences of Two bla_{KPC-2}-Bearing IncN Plasmids Isolated from Sequence Type 442 *Klebsiella pneumoniae* Clinical Strains Four Years Apart

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We sequenced the oldest bla_{KPC-2}-bearing plasmid isolated in Brazil and another plasmid also carried by a *Klebsiella pneumoniae* strain of sequence type 442 (ST442), isolated 52 months later. Both plasmids present an IncN backbone and few acquired regions. Because the 2005 plasmid presented deletions and a truncated gene within Tn*4401b* compared to the 2009 plasmid, we can thus infer that IncN bla_{KPC-2}-bearing plasmids pFCF1305 and pFCF3SP had a common ancestor circulating in Brazil prior to May 2005.

Even though the first description of KPC-producing *Klebsiella pneumoniae* in Brazil was published in 2009, describing a strain isolated from a clinical sample in 2006 (1), a later publication reported the presence of a KPC-bearing strain isolated in May 2005 (2). The latter strain, FCF1305, was isolated in the state of São Paulo, Brazil, from a blood culture and belongs to sequence type 442 (ST442). We completely sequenced the bla_{KPC-2}-bearing plasmid isolated from FCF1305 and also another bla_{KPC-2}-bearing plasmid isolated from another *K. pneumoniae* strain, FCF3SP, also belonging to ST442 and also isolated from a blood culture from a patient in the state of São Paulo, but 52 months later, in September 2009. We chose to sequence the bla_{KPC-2}-bearing plasmids of these strains because FCF1305 was the oldest strain-bearing KPC in Brazil and strain FCF3SP was the strain most geographically, clinically, and epidemiologically similar to FCF1305 that we could find, but isolated 52 months later, so that differences accumulated in the wild during this period could be evaluated.

Because strain FCF1305 bore three plasmids, whereas strain FCF3SP bore five, isolation of the bla_{KPC-2}-bearing plasmids of each strain was carried out by transformation into *Escherichia coli* TOP10 (Invitrogen) as described previously (3). Plasmid DNA isolated from *E. coli* TOP10(pFCF1305) and *E. coli* TOP10 (pFCF3SP) by alkaline lysis miniprep (3) was used to construct genomic fragment libraries (one for each plasmid) which were sequenced using the Roche GS-FLX sequencer (Roche Life Sciences, Branford, CT, USA) according to the manufacturer's instructions.

Sequencing adapters were clipped from the reads and used for *de novo* assembly using Mira 4.0 (4). Plasmid *de novo* contigs were then merged by assembling *de novo* using Geneious (5) with stringent parameters (maximum gap size = 1; minimum overlap identity = 95%). Circular (with large overlapping ends) contigs of ~50 kbp were considered to be the plasmids. All other contigs generated which had no identity to the circular contigs were then subjected to a BLAST search (6) against GenBank's nonredundant (nr) database, and 100% of them had as top hits *Enterobacteriaceae* chromosomes or phages and so were discarded. The plasmid sequences were automatically annotated using the RAST server (7) and manually curated. Repeat regions were identified using UGENE (8) and insertion sequences were identified by online analysis using IS finder (9). The complete sequences of both plasmids were successfully obtained by *de novo* assembly yielding a 53,081-bp sequence for pFCF1305 (mean coverage of 261× ± 48×; minimum coverage of 39×; maximum coverage of 445×) and a 54,605-bp sequence for pFCF3SP (mean coverage of 1,013× ± 374×; minimum coverage of 67×; maximum coverage of 2,383×).

Both plasmids were found to have IncN plasmid backbones and carry a version of transposon Tn*4401b* (10). The general functional structures of both plasmids and a graphical comparison of the genetic architectures of the plasmids are presented in Fig. 1. The features present in both plasmid sequences are presented and compared to each other in Table S1 in the supplemental material.

The sequences of pFCF1305 and pFCF3SP have similar sizes and relatively minimal structures, inasmuch as both consist of a highly conserved IncN backbone with two acquired regions, one being a version of Tn*4401b*, which carries as the only resistance gene bla_{KPC-2}, and a second region containing the small insertion sequence IS*903B*. Other IncN plasmids carrying bla_{KPC} (GenBank accession numbers FJ223607, FJ223605, KC958437, and JX193301) are larger (~65 kbp to ~83 kbp) than pFCF1305

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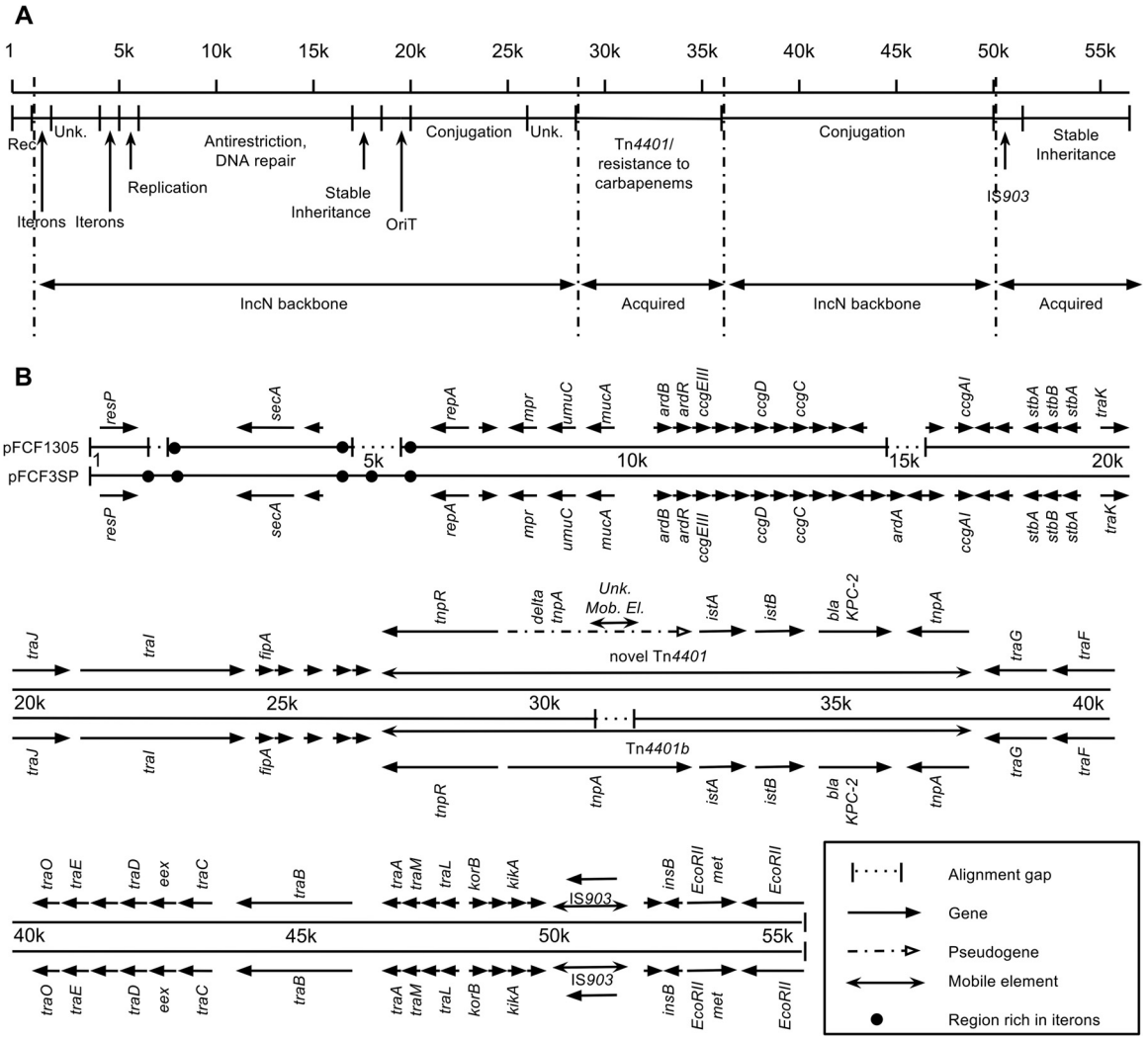

FIG 1 (A) Graphical representation of the general structures, origins, and functions of plasmids pFCF1305 (GenBank accession no. CP004366) and pFCF3SP (GenBank accession CP004367). Rec., recombination; Unk., unknown. (B) Representation of the alignment of the sequences of pFCF1305 and pFCF3SP showing features present and absent in each sequence.

and pFCF3SP, with the exception of plasmid pKP1433 (JX397875), which is about the same size but contains several non-IncN backbone replication genes (11).

The sequences of pFCF1305 and pFCF3SP are highly conserved in spite of their having been isolated 52 months apart, with their difference in size being distributed between four regions of difference (Fig. 1). The region around ~15 kbp missing in pFCF1305 contains *ardA*, an anti-restriction gene, and two small genes coding for hypothetical proteins. This deletion thus probably has a very small significance for the biological effects of inheriting either plasmid. Whereas pFCF3SP presented a Tn*4401* sequence that is >99% identical to the prototype Tn*4401b* isoform (10) (EU176012), pFCF1305 presented a Tn*4401b* isoform with the *tnpA* gene truncated due to the insertion of a 256-bp sequence which seems to be a mobile genetic element. This unknown mobile element (UME), like insertion sequences, is limited by inverted repeats of 39 bp. Unlike common insertion sequences, it does not, however, possess a gene coding for a transposase between the inverted repeats. This is the first description of the truncation of the *tnpA* gene of Tn*4401b*, which warrants its designation as a novel Tn*4401* isoform.

The novel Tn*4401b* isoform present in pFCF1305 with the truncated *tnpA* was unexpectedly present in the older molecule, pFCF1305, rather than the newer pFCF3SP. The perfect direct-repeat target site duplications generated in pFCF1305 and pFCF3SP due to transposition of Tn*4401b* are also identical in both plasmids (5′CTTCAG3′), which indicates that truncation of the *tnpA* found in pFCF1305 by the UME happened after Tn*4401b* was transposed into the common IncN ancestor of pFCF1305 and pFCF3SP and that this occurred earlier than 2005. Furthermore, because the two plasmids are different yet were both found in *K. pneumoniae* ST442 strains isolated in the same city from clinically similar cases, it can be inferred that the reason they are present in each strain is not due to vertical transmission. This can be argued because it is the plasmid from 2005 which presents small deletions of IncN backbone elements, which would have to have been "re-acquired" by the later ST442 strain bearing pFCF3SP. Also, the UME truncating *tnpA* in pFCF1305 is not present in pFCF3SP,

and although it is strictly possible that the element could be subsequently lost, it seems very unlikely that *tnpA* would have been restored to perfect functionality after such an event. We can thus infer that the IncN bla_{KPC-2}-bearing plasmids pFCF1305 and pFCF3SP had a common ancestor circulating in Brazil prior to May 2005.

Nucleotide sequence accession numbers. The nucleotide sequences for plasmids pFCF1305 and pFCF3SP have been deposited in GenBank under accession numbers CP004366 and CP004367, respectively.

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We have no conflicts of interest to declare.

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