Novel complex class 1 integron carrying *qnrB10* and *bla*$_{CTX-M-2}$ in a twin variable region 2 found in a clinical *Citrobacter freundii* from Argentina.

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Introduction

The gene *qnrB10* is one of the plasmid-mediated mechanisms that confer low level resistance to quinolones/fluoroquinolones and *bla*$_{CTX-M-2}$ encodes the extended-spectrum β-lactamase CTX-M-2. Complex class 1 integrons (CC1Int) harboring *qnrB10* in variable region 2 (vr-2) were described as the genetic support of *qnrB10* in clinical enterobacteria from Argentina (e.g., In37::IS*CR1*::*qnrB10*). Other CC1Int bearing *bla*$_{CTX-M-2}$ in vr-2 are also broadly distributed in Argentina (e.g., In35::IS*CR1*:: *bla*$_{CTX-M-2}$). Here, we describe a new CC1Int that harbors both *qnrB10* and *bla*$_{CTX-M-2}$ in two consecutive (twin) vr-2 and is located in a 61,205-bp IncN conjugative plasmid.

Methods

Citrobacter freundii Q4143 was isolated from urine of a hospitalized patient in the Province of Chaco (Argentina, 2007). Biparental conjugation was performed using *Escherichia coli* J53 resistant to sodium azide (Az-R), as recipient. Plasmid profile was assessed by S1 nuclease. Whole genome sequencing (WGS) was performed by Illumina (MiSeq) and Oxford Nanopore Technologies (MinION). MiSeq and MinION DNA reads were used for hybrid assembly with Unicycler. Sequences were annotated with PROKKA and manually curated. PlasmidFinder, ResFinder, ISFinder and OriTfinder were used to identify incompatibility groups (Inc), resistance genes, insertion sequences and conjugative regions, respectively. Sequence comparisons were performed with Nucleotide BLAST, using the NCBI Nucleotide Collection Database (NCBI-DB), and Artemis Comparative Tool.

Results

C. freundii Q4143 harbored 6 plasmids of about 52, 60, 115, 200, 220, 245 kb. Both 60 and 200-kb plasmids were cotransferred to *E. coli* J53 Az-R, rendering transconjugant TC-M11222. WGS and hybrid assembly of TC-M11222 rendered three circular contigs: the chromosome (4.68 Mb) and two plasmids named pCfQ4143_61 (61,205 bp , IncN group) and pCfrQ4143_200 (200,887 bp , IncC group). The resistance genes *bla*$_{OXA-1,}$ *bla*$_{CTX-M-2}$, *arr-3*, *catB3*, ***aac(6')-Ib-cr,*** *qnrB10* and *sul1,* and a complete conjugative machinery were identified in pCfrQ4143_61. ISFinder showed that insertion sequences accounted for 8% of pCfrQ4143_61 sequence. pCfrQ4143_61 contained a CC1Int not previously described in NCBI-DB, which included two copies of IS*CR1* and three of 3'-conserved sequence (3'CS1, 3'CS2, 3'CS3). The 5'-conserved sequence, the cassette region or variable region 1 (vr-1) [***aac(6')-Ib-cr***/*bla*$_{OXA-1}$/*catB3*/*arr-3*] and 3'CS1 were 100% identical to In37::ISCR1::*qnrB10*. The vr-2 of the new CC1Int differed from In37::IS*CR1*::*qnrB10* because the *sapCBA* operon, located downstream to *qnrB10* was interrupted by IS*Sen4*. The remaining structures of In37::IS*CR1*::*qnrB10* were also found in the new CC1Int: *qnrB10*, ***ΔpspF*** and 3'CS2, with its characteristic 68-bp deletion. However, the 3'CS2 of the new CC1Int was followed by a second copy of IS*CR1*; the vr-2 of In35::IS*CR1*:: *bla*$_{CTX-M-2}$ harboring *bla*$_{CTX-M-2}$, and a third copy of 3'CS3, with the typical 71-bp deletion found in In35::IS*CR1*:: *bla*$_{CTX-M-2}$. The new CC1Int was named In37::IS*CR1*::*qnrB10*::IS*CR1*::*bla*$_{CTX-M-2}$.

Conclusions

These findings suggest that In37::IS*CR1*::*qnrB10*::ISCR1::*bla*$_{CTX-M-2}$ emerged from two homologous recombinations between In37::IS*CR1*::*qnrB10* and In35::IS*CR1*::*bla*$_{CTX-M-2}$ (a model is proposed). This assumption implies that the CC1Int are able to acquire new resistance genes not only as cassettes in vr-1, but also as twin vr-2 through homologous recombination between different CC1Int. Their locations in conjugative plasmids could enhance even more its role in the dissemination of antimicrobial resistance genes, contributing to multiresistance.