Mobilization of qnrB2 and ISCR1 in Plasmids

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The DNA sequences of two IncHI2 plasmids, pEC-IMP and pEC-IMPQ, from metallo-β-lactamase-producing Enterobacter cloacae clinical isolates were determined. The two conjugative plasmids are almost identical, but pEC-IMPQ carries an additional segment containing an orf513 (ISCR1), a truncated 3′-conserved sequence, and a qnrB2. Comparative analyses provide support for the proposed ISCR1-mediated gene mobilization.

Encoding a putative product of 513 amino acids, orf513 was initially identified adjacent to integrons In6 and In7 (11). Together with noncassette resistance genes, it was commonly found between truncated and full-length 3′-conserved sequences (3′-CS) of class 1 integrons (9, 11). Their function remained mysterious until comparative analyses linked these so-called common region (CR) elements to a group of IS91-like insertion sequences (ISs) (13). The IS91-like ISs are a family of unusual IS elements that differ from most other IS elements in both structure and mode of transposition. They can perform rolling-circle (RC) transposition, in which a single IS element can mobilize the sequences to which it is attached (4, 12). It was proposed that orf513, later termed insertion sequence common region 1 (ISCR1), may have mobilized the nearby sequence and a truncated 3′-CS from one integron to the 3′-CS of another integron through RC transposition, thus facilitating the formation of complex class 1 integrons associated with ISCR1 (13). In addition to this putative recombinase function, the ISCR1 element has also been shown to play a role in the expression of nearby genes by providing a promoter (8, 10).

ISCR1 were found to be associated with many antimicrobial resistance genes, including the plasmid-mediated quinolone resistance determinant qnr (5) as well as genes encoding resistance to chloramphenicol, trimethoprim, aminoglycosides, and β-lactams (8, 13, 14). However, lacking the 59 base elements required for site-specific recombination, these orf513-linked genes could not have been acquired as gene cassettes. It was hypothesized that these antimicrobial resistance genes were added to the 3′-CS of the class 1 integron through comobilization with the nearby ISCR1 from other integrons using RC transposition and homologous recombination (1, 13).

In a recent study on the prevalence of QnrA, QnrB, and QnrS among clinical isolates of Enterobacter cloacae, the association of Qnr with the IMP-8 metallo-β-lactamase (MBL) was investigated (15). From 56 IMP-8 MBL producers, eight qnrB-positive, blaIMP,8-positive conjugants and four qnrB-negative blaIMP,8-positive conjugants were obtained. Restriction pattern analysis on these plasmids gave very similar patterns, suggesting the occurrence of horizontal mobility of qnrB2 (15). To investigate the possible horizontal transfer mechanisms responsible for qnrB2, we have conducted complete DNA sequencing and comparative analysis on two of the plasmids, the qnrB2-positive plasmid pEC-IMPQ and the qnrB2-negative plasmid pEC-IMP.

The DNA sequences of the two plasmids were determined using a whole-genome shotgun approach as described before (3). The two plasmids are 324,503 bp and 318,782 bp in length and have a common backbone similar to that of the IncHI2 plasmids (2, 6, 7). The larger one, pEC-IMPQ, carries an additional segment which contains qnrB2, a truncated 3′-CS, and an ISCR1. Outside this region, single nucleotide substitutions were found in five positions, and a 20-kb inversion, probably facilitated by the two flanking IS26s, was detected (Fig. 1a). Three β-lactamase genes, blaTEM-1, blaIMP,8, and blashv-12, were found in both pEC-IMP and pEC-IMPQ. Among them, only blaIMP,8 was located within an integron. The blaTEM-1 and blashv-12 genes were associated with Tn3 and IS26, respectively. There were also other resistance genes, including those encoding a tetracycline efflux pump and its regulator, tetAR; dihydropteroate synthetase genes sul1 and sul2; chloramphenicol acetyltransferase genes catA2 and catB3; and hipBA genes encoding putative multigand tolerance proteins; a dihydrofolate reductase gene, dfrA19; a putative rifampin-ribosyl transferase gene; a putative aminoglycoside 3′-phosphotransferase gene, and several aminoglycoside acetyltransferase genes, aac3, aacA4, aac6, and aac(3′)-Ic. Most of these antimicrobial resistance genes are located in the four integrons of both of the plasmids (Fig. 1a). Several gene clusters responsible for heavy metal resistance were also identified.

The extra qnrB2-containing region of pEC-IMPQ is located after the 3′-CS of a class 1 integron (Fig. 1b). This integron, which is present in both plasmids, contains a blaIMP,8 MBL...
gene, aminoglycoside acetyltransferase gene aacA4, chloramphenicol acetyltransferase gene catB3, quaternary ammonium transporter gene qacE/delta1, and dihydropteroate synthetase gene sul1. In pEC-IMP, an ISCR1 and dihydrofolate reductase gene dfrA19 were identified downstream of sul1. In pEC-IMPQ, the duplication of the ISCR1 and the 3’ end of the nearby sul1 were identified. A qnrB2, a truncated qacE/delta1, and another sul1 were identified between the duplicated ISCR1 (Fig. 1b).

In pEC-IMPQ, the 5.8-kb sequence at approximately bp 149926 to 155739 flanked by the duplications is identical to a recently described qnrB2-containing sequence found between two ISCR1s on a plasmid from a Salmonella enterica serovar Keurmassar strain (5). This suggests that the region in common that includes qnrB2 was derived from the same immediate ancestor (Fig. 1b). The two sul1 genes differ at one position, as indicated in the figure.

On the basis of sequence analyses and the proposed mobilizing function of ISCR1, a model was made (Fig. 1b). In this model, a circular intermediate that carries the qnrB2-truncated qacE/delta1-sul1 was produced by RC replication initiated from
the replication origin orrIS of the ISCR1 element. The circular intermediate was then inserted into pEC-IMP by homologous recombination somewhere between the 3’ moiety of the sul1 gene and the orrIS. This would explain the formation of the so-called complex class 1 integron of pEC-IMPQ, in which the qnrB2-containing extra region was found between two CRs (Fig. 1b). It is, however, also possible that the pEC-IMP was created from pEC-IMPQ by the deletion of this extra region via excision between the two repeat regions (Fig. 1b).

In summary, the major difference between two related plasmids isolated from E. cloacae was a qnrB2-containing region flanked by two ISCR1s. Our comparative analyses provide support for the proposed ISCR1-mediated gene mobilization.

Nucleotide sequence accession numbers. The annotated DNA sequences of plasmids pEC-IMPQ and pEC-IMP have been submitted to the GenBank database under accession numbers EU855788 and EU855787.

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