Mobilization of \textit{qnrB2} and ISCR1 in Plasmids

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ABSTRACT

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’-CS, and a qnrB2. Comparative analyses provide support for the proposed ISCR1-mediated gene mobilization.
Encoding a putative product of 513 amino acids, \textit{orf513} was initially identified adjacent to integrons In6 and In7 (11). Together with non-cassette resistance genes, it was commonly found between truncated and full-length 3’ conserved sequence (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 sequences to which it is attached (4, 12). It was proposed that \textit{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 facilitates the formation of complex class 1 integrons associated to ISCR1 (13). In addition to this putative recombinase function, 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 plasmid-mediated quinolone resistance determinant, \textit{qnr} (5), as well as genes encoding resistance to chloramphenicol, trimethoprim, aminoglycosides, and \(\beta\)-lactams (8, 13, 14). However, lacking the 59 base elements required for site-specific recombination, these \textit{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 co-mobilization 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 \textit{Enterobacter cloacae}, the association of Qnr with the IMP-8 metallo-\(\beta\)-
lactamase (MBL) was investigated (15). From 56 IMP-8 MBL producers, eight \textit{qnrB}-positive, \textit{bla}_{IMP-8}-positive transconjugants and four \textit{qnrB}-negative \textit{bla}_{IMP-8}-positive transconjugants were obtained. Restriction pattern analysis on these plasmids gave very similar patterns, suggesting the occurrence of horizontal mobility of the \textit{qnrB2} (15). To investigate the possible horizontal transfer mechanisms responsible for the \textit{qnrB2}, we have conducted complete DNA sequencing and comparative analysis on two of the plasmids, the \textit{qnrB2}-positive plasmid, pEC-IMPQ, and the \textit{qnrB2}-negative plasmid, pEC-IMP.

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 the \textit{qnrB2}, a truncated 3’-CS, and an IS\textit{CRI}. Outside this region, single nucleotide substitutions were found in 5 positions, and a 20-kb inversion, probably facilitated by the two flanking IS\textit{26}, were detected (Fig. 1a). Three β-lactamase genes, \textit{bla}_{TEM-1}, \textit{bla}_{IMP-8} and \textit{bla}_{SHV-12}, were found in both pEC-IMP and pEC-IMPQ. Among them, only \textit{bla}_{IMP-8} was located within an integron. The \textit{bla}_{TEM-1} and \textit{bla}_{SHV-12} were associated with Tn3 and IS26, respectively. There were also other resistance genes including those encoding tetracycline efflux pump and its regulator, \textit{tetAR}, dihydropteroate synthetases \textit{sul1} and \textit{sul2}, chloramphenicol acetyltransferases \textit{catA2} and \textit{catB3}, putative multidrug tolerance proteins \textit{hipBA}, a dihydrofolate reductase \textit{dfrA19}, a putative rifampin ADP-ribosyl transferase, a putative aminoglycoside 3’-phosphotransferase, and several aminoglycoside acetyltransferase genes \textit{aac3}, \textit{aacA4}, \textit{aac6}, and \textit{aac(6’)-Ile}. 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 \textit{qnrB2}-containing region of \textit{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 \textit{bla}\textsubscript{IMP-8} MBL gene, an aminoglycoside acetyltransferase \textit{aacA4}, a chloramphenicol acetyltransferase \textit{catB3}, quaternary ammonium transporter gene \textit{qacEdelta1}, and dihydropteroate synthetase gene \textit{sul1}. In \textit{pEC-IMP}, an \textit{ISCR1} and a dihydrofolate reductase \textit{dfrA19} was identified downstream of the \textit{sul1}. In \textit{pEC-IMPQ}, duplication of the \textit{ISCR1} and the 3’-end of the nearby \textit{sul1} were identified. A \textit{qnrB2}, a truncated \textit{qacEdelta1}, and another \textit{sul1} were identified between the duplicated \textit{ISCR1} (Fig. 1b).

In \textit{pEC-IMPQ}, the 5.8-kb sequence at 149,926~155,739-bp flanked by the duplications is identical to a recently described \textit{qnrB2}-containing sequence found between two \textit{ISCR1} on a plasmid from a \textit{Salmonella enterica} serovar Keurmassar strain (5). This suggests the region in common that includes \textit{qnrB2} was derived from the same immediate ancestor (Fig. 1b). The two \textit{sul1} genes differ at one position as indicated in the figure.

On the basis of sequence analyses and the proposed mobilizing function of \textit{ISCR1}, a model was made (Fig. 1b). In this model, a circular intermediate that carries the \textit{qnrB2}-truncated-\textit{qacEdelta1-sul1} was produced by RC replication initiated from the replication origin, \textit{oriIS}, of the \textit{ISCR1} element. The circular intermediate was then inserted into \textit{pEC-IMP} by homologous recombination somewhere between the 3’ moiety of the \textit{sul1} gene and the \textit{oriIS}. This would explain the formation of the so-called complex class 1 integron of \textit{pEC-IMPQ}, in which the \textit{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 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 IS*CR1s*. Our comparative analyses provide support for the proposed IS*CR1*-mediated gene mobilization.

**Nucleotide sequence accession number.** 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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FIG. 1. (a) Schematic diagram of the plasmids pEC-IMPQ, and pEC-IMP. The qnrB2-containing region in pEC-IMPQ is depicted in a white box. The inversion is indicated by dotted lines. The five nucleotide substitutions are indicated by black arrowheads. The four class 1 integrons are depicted in gray boxes. The other features indicated are: repH1A and repH12, replication origins; ter, tellurite resistance gene cluster; pbr, lead resistance gene cluster; mer, mercury resistance gene cluster; ars, arsenic resistance gene cluster; aph, putative aminoglycoside phosphotransferase; ereA2*, erythromycin esterase pseudogene; arr, putative rifampin ADP-riboseyl transferase. The ISCR1-containing regions are detailed in the lower panel. (b) The genetic contexts near the extra region found in pEC-IMPQ. The repeats of the redundant ISCR1 in pEC-IMPQ are marked. The dotted line indicates the 5.8-kb region identical to a previously reported plasmid from a Salmonella enterica serovar Keurmassar. The proposed circular intermediate of an aberrant RC replication carrying the qnrB2-truncated-qacEdelta1-sul1 is shown above. The proposed integration event between this intermediate and pEC-IMP are marked (the big ‘X’). The integrase gene intI1 of class 1 integrons are shaded. The resistance genes that co-mobilized with the ISCR1 are shown in gray. Other resistance genes are shown in white. The single nucleotide differences of the sul1 genes are indicated (T and A) at the positions of variation. Truncated orfs are marked with an asterisk after the gene name. str encode streptomycin resistance proteins.