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Small Quinolone Resistance Plasmids: a Model for Evolution Mediated by site-specific recombination at *oriT* and Xer sites

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ABSTRACT

Qnr are proteins that mediate resistance to quinolones by protecting DNA gyrase. The *qnrB19* gene was found in several *Enterobacteriaceae* isolated from numerous geographical regions within large plasmids, in IS*Ecp1C*-based transposons, and in small ColE1-type plasmids (about 3 kbp) lacking IS*Ecp1C* or any other insertion sequence. In spite of being located in such dissimilar elements, the *qnrB19* genes share a conserved genetic environment. We found 4 small plasmids harboring *qnrB19,* pPAB19-1, pPAB19-2, pPAB19-3 and pPAB19-4 in a collection of clinical enterobacteria with reduced quinolone susceptibility. Nucleotide sequencing and analysis showed that they share extensive homology among themselves and with other described small *qnrB19*-harboring plasmids. The genetic environment of *qnrB19* in all four plasmids is identical to that in those other plasmids and in transposons such as Tn*2012*, Tn*5387*, and Tn*5387-like*. These plasmids as well as those previously described showed a variable region characterized by being flanked by an *oriT* locus and a Xer recombination site (RS). Both loci were confirmed to be functional. We propose that this arrangement could play a role in evolution of plasmids and present a model for DNA swapping between plasmid molecules mediated by successive SSR events at *oriT* and the Xer RS. The first event involves SSR at *oriT*, which depends exclusively on *oriT* site and the nickase activity, and leads to integration. The cointegrate includes two directly positioned non-identical Xer RSs that may serve as substrate for a second SSR event mediated by Xer that leads to cointegrate resolution. The final products are the original plasmids but they have exchanged the fragments flanked by *oriT* and Xer. We propose that the combination *oriT*-Xer RS could be considered an element that facilitates plasmid evolution by swaping DNA regions.

INTRODUCTION

Qnr are pentapeptide repeat proteins that mediate resistance to quinolones by protecting type II DNA topoisomerases (1). Five *qnr* families (*qnrA, B, C, D* and *S*) have been found, usually hosted in large plasmids (2). The first *qnrB* gene (*qnrB1*) was identified in a plasmid from a *K. pneumoniae* strain isolated in South India (3) and 38 members of the family quickly followed (http://www.lahey.org/qnrStudies/) (4). The *qnrB19* gene has been found in several genera of *Enterobacteriaceae* isolated from humans (healthy people and clinical isolates), animals, and food of animal origin in numerous geographical regions. An interesting characteristic of the *qnrB19* allele is that it has been found within large plasmids, associated to IS*Ecp1C*-based transposons (5-7), and in small plasmids (about 3 kbp) lacking IS*Ecp1C* or any other insertion sequence (8-10) (Table 1). However, in spite of being located in such dissimilar elements, the *qnrB19* genes share a conserved genetic environment.

Here we describe their molecular features and characterize their relationships with other *qnrB19*-harboring genetic platforms. Furthermore, we propose possible pathways of evolution of the *qnrB19* environment as well as a site-specific recombination-based model for DNA modifications at a variable region found in these plasmids.

METHODS

Bacterial strains and plasmids. The plasmids pPAB19-1, pPAB19-2, pPAB19-3, and pPAB19-4 analyzed in this study were isolated from *Salmonella infantis* M7849, *Escherichia coli* M9996, *E. coli* M9888 and *Salmonella spp.* M9397, respectively (Table 1). *S. infantis* M7849 was isolated at the Hospital Castro Rendón, Province of Neuquén (March 2006); *E. coli* M9888 and M9996 were isolated at the Policlínico Central de San Luis, Province of San Luis (May 2007 and August 2008, respectively), and *Salmonella spp.* M9397 was isolated at the Hospital de Niños Alassia, Province of Santa Fe (July 2007). Recombinant plasmids pPBR1 and pPBR2 were generated by ligating *Hin*dIII-digested pPAB19-1 or pPAB19-2 to *Hin*dIII-digested pUC19. Dimers of plasmids pES and pKS492 were used as controls in dimer resolution experiments. *E. coli* DS941 (AB1157 *recF143 lacI*^{q} *lacZ*; possesses wild-type *xerC*, *xerD*, *argR*, and *pepA*), *E. coli* DS9028 (DS941 *xerD3*::*fol*), and the hyperrecombinogenic *E. coli* JC8679 (DS945 *recBC sbcA*) were used to carry out the Xer recombination experiments.

DNA sequencing and analysis. Plasmids pPAB19-1, pPAB19-2, pPAB19-3, and pPAB19-4 were screened by PCR, using the divergent primers *qnrB*-Fout (5'-GACGTTCAGTGGTTCAGATCTCTC) and *qnrB*-Rout (5'-GACTAAAATTGCACCCTTTCTGACT) that bind to the *qnrB19* gene leading to amplifications of its surrounding plasmid sequences. Nucleotide sequence analyses were performed using ClustalX2, v2.0.9 (ftp://ftp.ebi.ac.uk/pub/software/clustalw2) (11) and the Basic Local Alignment Search tool (BLAST) (http://www.ncbi.nlm.nih.gov/BLAST/) (12).

Nucleotide sequence accession numbers. The nucleotide sequence of plasmids pPAB19-1, pPAB19-2, pPAB19-3, and pPAB19-4 have been deposited in GenBank under the accession numbers GQ412195, JN979787, JN985534, and JN995611, respectively.

RESULTS

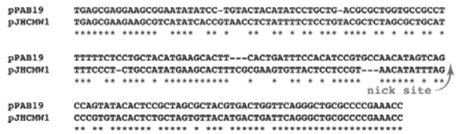

Fig. 2. Comparison of *oriT* regions. Comparison was carried out using ClustalW2. The *oriT* sites from pPAB19-1, pPAB19-2, pPAB19-3, and pPAB19-4 are identical

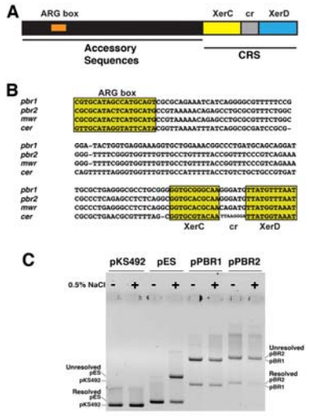

Fig. 3. A. Schematic diagram of plasmid's Xer recombination sites. B. Comparison of the nucleotide sequences of *pbr1* (present in pPAB19-1 and pPAB19-4), *pbr2* (present in pPAB19-2 and pPAB19-3), *mwr*, and *cer*. C. Dimer resolution assay. Dimers of plasmids pKS492 (*cer*), pES (*mwr*), pPBR1 (*pbr1*), and pPBR2 (*pbr2*) were introduced by transformation into *E. coli* DS941. The cells were cultured for 20 generations and the plasmid content was analyzed.

Fig. 4. Model of exchange of the DNA region flanked by *oriT* and Xer recombination sites.

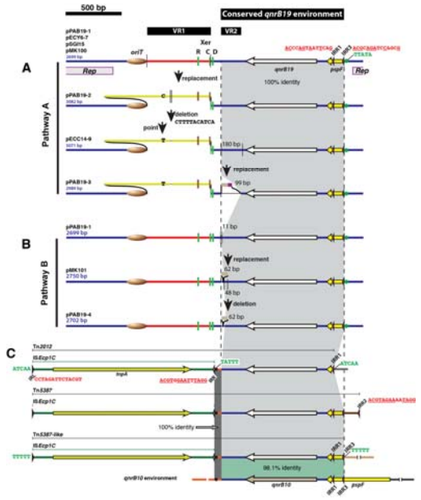

Fig. 1. Comparative diagram of *qnrB19*-harboring elements. Colors indicate identical nucleotide sequences. For the sake of clarity in two regions across the different elements a gray shading was added to indicate the portions with identical sequences. In the case of the *qnrB10* environment a green shading was added to show the 98.1 identity region. Variable regions VR1 and VR2 as well as the conserved *qnrB19* environment are indicated with black bars on top of the genetic maps. The thin vertical lines in the plasmid maps indicate the edges of the DNA fragments replaced or deleted in each rearrangement. The Xer recombination sites components are indicated. The different colors of XerC, XerD, and ArgR binding sites indicate that they have different sequences. The *oriT* is shown as a brown oval. IRL, IRR, IRR1, IRR2, and IRR3 are indicated by slender arrowheads and their sequences are shown in red (underlined nucleotides correspond to a perfect reverse complement of the IRL sequence). The target site duplications of IS*Ecp1C* or IS*Ecp1C*-based transposons are shown in green (the TTATA sequence after IRR3 in the different plasmids and the TATTT after IRR in the transposons are emphasized by green and black dots, respectively). The location of the replication region (Rep) of all plasmids is shown below the pPAB19-1 genetic map.

CONCLUSIONS

The genetic environment of *qnrB19* in all four plasmids is identical to that in those other plasmids and in transposons such as Tn*2012*, Tn*5387*, and Tn*5387-like*.

Nucleotide sequence comparisons among these and previously described plasmids showed a variable region characterized by being flanked by an *oriT* locus and a Xer recombination site.

We propose that this arrangement could play a role in evolution of plasmids and present a model for DNA swapping between plasmid molecules mediated by site specific recombination events at *oriT* and a Xer target site.

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