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

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Introduction

Qnr are proteins that mediate resistance to quinolones by protecting DNA gyrase. The plasmid was found in several Enterobacteriaceae isolated from numerous geographical regions. In small ColE1-type plasmids, an oriT is located in the plasmid sequence. This study was conducted to determine the role of site-specific recombination at oriT in plasmid evolution.

Methods

METHODS

Bacterial strains and plasmids. The plasmids pPAB19-1, pPAB19-2, pPAB19-3, and pPAB19-4 analyzed in this study were isolated from Salmonella enterica M7849, Escherichia coli M9397, E. coli M9868 and Salmonella sp. M9552, respectively (Table 1). E. coli M7849 was isolated at the Hospital Castro Rendón, Province of Neuquén (March 2006); E. coli M9397 and M9552 were isolated at the Políclinico Central de Luque, Province of San Luis (May 2007) and August 2007, respectively and Salmonella sp. M9868 was isolated at the Hospital de Niños Alesso, Province of Santa Fe (July 2007). Recombinant plasmids pPBR1 and pPBR2 were generated by ligating HindIII-digested pPAB19-1 or pPAB19-2 to HindIII-digested pUC19.

DNA sequencing and analysis. Plasmids pPAB19-1, pPAB19-2, pPAB19-3, and pPAB19-4 were screened by PCR, using the divergent primers qnrFoul (5’-GAGCTCCGAAGGGTCGACCCGCTG-3’) and qnrFoul (5’-CACATACCTTCGCTCCCTTTGAG-3’) that bind to the qnrB gene leading to amplifications of the surrounding plasmid sequences. Nucleotide sequence analyses were performed using ClustalW2 v2.0 (http://www.ncbi.nlm.nih.gov/BLAST/) and the Basic Local Alignment Search tool (BLAST) (http://www.ncbi.nlm.nih.gov/BLAST/).

Results

The genetic environment of qnrB9 in all four plasmids was identical to that in those other plasmids and in transposons such as Tn2012, Tn5387, and Tn5387-ikr. 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 the arrangement could play a role in evolution of plasmids and present a model for DNA swapping between plasmid molecules mediated by site-specific recombination at oriT and the XerRS.

Conclusions

We propose that the arrangement could play a role in evolution of plasmids and present a model for DNA swapping between plasmid molecules mediated by site-specific recombination at oriT and the XerRS.