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Novel Mutation in 23S rRNA That Confers Low-Level Resistance to Clarithromycin in *Helicobacter pylori*▽

In many countries, the occurrence of clarithromycin-resistant *Helicobacter pylori* has become increasingly common. The MICs of clarithromycin in most clinical *H. pylori* strains are either ≥2 mg/liter or ≤0.125 mg/liter (3). Recently, we isolated two stable *H. pylori* isolates with low-level clarithromycin resistance from different patients (TS1900 and TS1776), and these isolates showed MICs of 1 mg/liter and 0.5 mg/liter, respectively. It has been previously shown that clarithromycin-resistant strains with MICs exceeding 2 mg/liter were characterized by mutations in the 23S rRNA gene at positions 2058 and 2059 (by *Escherichia coli* numbering, corresponding to positions 2142 and 2143 in *H. pylori*), which resulted in a decreased affinity of clarithromycin for ribosomes (9, 11). However, we failed to detect mutations at those positions of the 23S rRNA gene in the weakly clarithromycin-resistant isolates TS1900 and TS1776. Fontana et al. recently reported that a mutation at position 2628 in *E. coli* numbering (corresponding to position 2717 in *H. pylori*) also conferred low-level clarithromycin resistance (1); however, both TS1900 and TS1776 had wild-type nucleic acids at that position.

Upon comparing the sequence of the 23S rRNA gene of ATCC 700392 (a clarithromycin-susceptible strain) to the sequence of the same gene in TS1900, we identified 13 substitutions corresponding to positions 720, 759, 1513, 1563, 1564, 1644, 1687, 1821, 1826, 1830, 2182 (2098), 2190 (2106), and 2694 (2611) of the *H. pylori* 23S rRNA gene (*E. coli* numbering in parentheses). In order to determine the contribution of these 23S rRNA gene mutations to low-level clarithromycin resistance, ATCC 700392 was transformed with regions of the 23S rRNA gene of TS1900 by using a transformation method described previously (5). We obtained transformants with low-level clarithromycin resistance when ATCC 700392 was transformed with the PCR products of regions of the TS1900 23S rRNA gene consisting of positions 626 to 2774, 1931 to 2774, 2276 to 2774, and 2569 to 2774 (Table 1). Intriguingly, all transformants with low-level clarithromycin resistance that were obtained in this study possessed the mutation C2611A in *E. coli* numbering (corresponding to position C2694 of *H. pylori*) in the 23S rRNA gene. Notably, because the size of the colony was not influenced by the transformation and MICs for the transformants were also stable in serial subcultures, the presence of the mutation C2611A had no effect on the overall growth of the transformants. Moreover, while the mutation T2098C (corresponding to T2182C of *H. pylori*) has been reported to confer clarithromycin resistance (2), the results of this study suggested that this mutation was not required for clarithromycin resistance to be conferred, at least in a background also containing the C2611A mutation.

In addition to TS1900, the weakly clarithromycin-resistant isolate TS1776 also possessed the C2611A mutation in its 23S rRNA gene. DNA sequencing analysis of the 23S rRNA gene from 58 clinical isolates in Japan from 1996 to 2006 indicated that the C2611A mutation was not detected in any clarithromycin-susceptible ($n = 21$) or clarithromycin-resistant ($n = 37$) isolate. Furthermore, the C2611A mutation was not detected in any 23S rRNA gene of *H. pylori* reported in the DNA Data Bank of Japan. These data suggest that the C2611A mutation can be associated with low-level clarithromycin resistance in *H. pylori*.

It was previously reported that mutations of base pairs 2057 to 2611 in 23S rRNA affect the efficacy of erythromycin by reducing the affinity of erythromycin for the ribosome in many bacterial species (4, 6, 8, 10). Since clarithromycin targets the same region of 23S rRNA as erythromycin (7), these data strongly suggest that the C2611A mutation in 23S rRNA of our isolated *H. pylori* strains may introduce a steric hindrance that prevents efficient clarithromycin binding, thus conferring the low-level resistance to clarithromycin observed in our study.

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TABLE 1. Gene mutations in 23S rRNA and clarithromycin susceptibility in transformants with low-level clarithromycin resistance

Strain	Donor DNA (region of the 23S rRNA gene)[a]	MIC (mg/liter)	Nucleic acid in *E. coli* (*H. pylori*[a]) 23S rRNA at position:				
			2058 (2142)	2059 (2143)	2098 (2182)	2106 (2190)	2611 (2694)
ATCC 700392 (recipient)		≤0.008	A	A	T	T	C
S1900 (donor)		1	A	A	C	C	A
TF1	626–2774	0.5	A	A	C	C	A
TF2	1931–2774	0.25	A	A	C	C	A
TF3	2276–2774	1	A	A	T	T	A
TF4	2569–2774	1	A	A	T	T	A

[a] Positions correspond to the 23S rRNA gene of UA802 (GenBank accession no. U27270).

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