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Genotypic Characterization of Clarithromycin-Resistant and -Susceptible *Helicobacter pylori* Strains from the Same Patient Demonstrates Existence of Two Unrelated Isolates

GE WANG, QIN JIANG, AND DIANE E. TAYLOR*

Department of Medical Microbiology and Immunology, University of Alberta, Edmonton, Alberta, Canada

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Clarithromycin-susceptible and clarithromycin-resistant *Helicobacter pylori* isolates from the same patient were investigated for the mode of development and mechanism of clarithromycin resistance. The clarithromycin-resistant strain UA1182 harbors homozygous A-to-G mutations at position 2143 in both copies of the 23S rRNA gene and has a phenotype of resistance to clarithromycin and clindamycin but no significant resistance to streptogramin B. Pulsed-field gel electrophoresis patterns of *Nru*I- and *Not*I-digested genomic DNA from the Cla^s and Cla^r isolates demonstrated that they are genetically distinct, suggesting that the development of clarithromycin resistance is not from the mutation of the existing Cla^s strain but from a completely new strain.

To eradicate *Helicobacter pylori*, a human gastric pathogen, antibacterial treatment including a proton pump inhibitor (e.g., omeprazole) in association with other antibiotics, such as clarithromycin, metronidazole, or amoxicillin, is commonly recommended (11, 18). In recent years, many cases in which clarithromycin resistance developed after treatment with this macrolide antibiotic (clarithromycin) were reported (2, 6). Genetic studies have revealed that clarithromycin resistance can most often be attributed to A-to-G transition mutations at either position 2142 or 2143 of 23S rRNA genes (5, 13, 14, 16, 19, 20).

The prevalence of clarithromycin-resistant *H. pylori* varies with geographic location (6). In Alberta, Canada, only a single clarithromycin-resistant *H. pylori* strain (UA1182) has so far been isolated in this lab (in 1993) from an adult patient (15). Several years earlier (in 1990), another *H. pylori* strain (UA799) that was shown to be susceptible to clarithromycin was cultured from a gastric biopsy specimen that was obtained from the same patient. At that time, the patient had symptoms of bloating, epigastric pain, and nausea and was diagnosed as having gastritis and a duodenal ulcer. After initial diagnosis, the patient was treated with ranitidine, bismuth, and metronidazole. As the clinical consequence of the drug treatment, the ulcer disappeared and other symptoms, including nonulcer dyspepsia, remained but were less severe. The patient then traveled in Saudi Arabia on a regular basis before the isolation of the second strain, UA1182.

To examine the genetic basis of clarithromycin resistance, the nucleotide sequence within the peptidyltransferase-encoding region of the 23S rRNA gene from both strains was determined. Briefly, a 300-bp-long PCR fragment was amplified from the chromosomal DNA by using primers DP1 and ZGE23 (16), and the fragment was then sequenced with primer DP1. The DNA sequence in this region of strain UA799 is identical to that reported for the clarithromycin-susceptible wild-type strain, UA802 (16). In strain UA1182, there is an A-to-G transition mutation at position 2143 of its 23S rRNA gene sequence (Table 1), and the mutation occurs in both copies of the gene. This type of mutation was reported in many cases to be associated with clarithromycin resistance (5, 13, 16, 19–21). In addition, a T-to-C mutation at position 2182 was revealed in the 23S rRNA gene sequence of strain UA1182. However, in vitro site-directed mutagenesis experiments suggested that this additional mutation is not associated with clarithromycin resistance (data not shown).

Macrolide resistance due to mutation in the peptidyltransferase-encoding region of the 23S rRNA is often associated with cross-resistance to lincosamide and streptogramin B antibiotics (macrolide-lincosamide-streptogramin B phenotype) (4). We tested the MICs of three representative antibiotics, clarithromycin, clindamycin, and quinupristin, for both strains by the agar dilution method (Table 1). UA1182 was shown to be resistant to clarithromycin and clindamycin but not significantly resistant to quinupristin (the MIC was identical to that of reference strains UA802 and UA799).

To investigate whether UA1182 developed as a result of a point mutation in strain UA799 after drug treatment, the overall genotypic characteristics of both strains were analyzed by pulsed-field gel electrophoresis (PFGE) as previously described (3, 9). Briefly, *H. pylori* was grown for 48 h, and the cells were then embedded in low-melting-point agarose blocks and lysed by treatment with *N*-lauroylsarcosine and proteinase K.

TABLE 1. MLS[a] phenotypes and 23S rRNA genotypes of *H. pylori* UA799 and UA1182

H. pylori strain	MIC[b] (μg/ml) of:			23S rRNA genotype
	Clarithromycin	Clindamycin	Quinupristin	
UA799	0.01	32	4	Wild type
UA1182	4–8	256	4	A2143G, T2182C

[a] MLS, macrolide-lincosamide-streptogramin B.
[b] MICs were determined by the agar dilution method.

* Corresponding author. Mailing address: Department of Medical Microbiology and Immunology, University of Alberta, Edmonton, Alberta, Canada T6G 2H7. Phone: (403) 492-4777. Fax: (403) 492-7521. E-mail: diane.taylor@ualberta.ca.

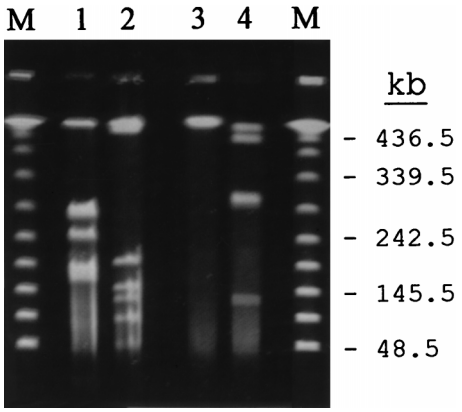

FIG. 1. PFGE patterns of *Nru*I (lanes 1 and 3)- and *Not*I (lanes 2 and 4)-digested genomic DNA from *H. pylori* UA799 (lanes 1 and 2) and UA1182 (lanes 3 and 4). The sizes of lambda DNA ladder standards (lanes M) are indicated on the right.

Subsequently, chromosomal DNA was digested with restriction endonuclease *Nru*I or *Not*I and then separated in a 1% agarose gel by using a contour-clamped homogenous electric field system (CHEF-DR-II; Bio-Rad). PFGE patterns of each strain (Fig. 1) showed that they were significantly different from one another, suggesting that the two strains were genetically unrelated. Therefore, clarithromycin-resistant UA1182 did not develop from the clarithromycin-susceptible strain UA799.

To explain the occurrence of UA1182, the following two possibilities could be considered. (i) UA1182 may have existed at the time of the first isolation in a mixed infection with UA799, but in a minor fraction or at a different site in the gastric mucosa so that it escaped identification in the first isolation. It has been documented that some patients can be concomitantly colonized by multiple *H. pylori* strains, even though this is rare (7, 17). (ii) Since the patient had traveled to Saudi Arabia, where a high proportion of the population is infected with *H. pylori* (1, 10), between the times of the two isolations, we could also speculate that the second strain may have been acquired as a new infection. Although the mode of transmission of *H. pylori* remains uncertain and the acquisition of infection in adults is rare, some studies have suggested a continuous risk of acquisition in adults, especially when the person has been exposed to an environment with a high incidence rate of *H. pylori* infection (8, 12). In conclusion, the data presented suggest that a clarithromycin-resistant *H. pylori* strain found in a patient may not necessarily be derived from a mutation of an existing strain identified in that patient; it may be a different strain, one which is involved in a multiple infection, or it may result from an entirely new infection.

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