

Letters to the Editor

PCR-Restriction Fragment Length Polymorphism Can Also Detect Point Mutation A2142C in the 23S rRNA Gene, Associated with *Helicobacter pylori* Resistance to Clarithromycin

Clarithromycin resistance, the major cause of *Helicobacter pylori* treatment failure (2, 7), is attributed to point mutations within the peptidyltransferase-encoding region in domain V of the 23S rRNA gene (10, 15). Three distinct point mutations have been found to be associated with macrolide resistance in *H. pylori* strains (14, 15). Mutations A2142G and A2143G are the most frequently reported, whereas mutation A2142C is less common, e.g., it was found in 5 of 129 resistant strains (11). Two additional mutations, A2115G and G2141A, have been described, occurring in the same strain (4), but neither has been reported subsequently.

The three most common mutations in the 23S rRNA gene can be detected in a two-step process using different molecular methods, PCR-DNA enzyme immunoassay (5, 9), and PCR-line probe assay (12). Recently, real-time PCR-based methods have been developed to detect these mutations (1, 3, 6), but none of them can distinguish among the three common mutations. PCR-restriction fragment length polymorphism (PCR-RFLP), another two-step process, allows the identification of mutations A2142G and A2143G using the *BbsI* (8) and *BsaI* (14, 15) restriction enzymes, respectively.

In this study, detection of mutation A2142C using the enzyme *BceAI* (*BceI*), a type IIS restriction endonuclease which recognizes the two nonpalindromic sequences 5'-ACGGC-3' and 5'-GCCGT-3' (13) is reported for the first time. Novel PCR and RFLP protocols developed to detect the three most frequent *H. pylori* 23S rRNA gene mutations are also reported.

H. pylori genomic DNA was isolated using the QIAamp DNA mini kit (Qiagen SA), and a 267-bp fragment was amplified by PCR using primers 5'-AGGTTAAGAGGATGCGT CAGTC-3' (HPY-S) and 5'-CGCATGATATCCCCATTAGC AGT-3' (HPY-A), corresponding to nucleotides 1931 to 1952 and 2197 to 2175, respectively, of the 23S rRNA gene of *H. pylori* (GenBank accession number U27270). The amplification reaction was carried out in a final volume of 50 μ l containing 1 \times buffer, 1.5 mM MgCl₂, 200 μ M each of the four deoxynucleoside triphosphates, 0.2 μ M each of the primers, 1 U of *Taq* polymerase (Eurobio), and 3 μ l of extracted DNA. Amplification was performed in a Perkin Elmer GeneAmp 9700 thermocycler (Norwalk, Conn.) with the following conditions: 1 cycle at 94°C for 5 min, 40 cycles at 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min, and 1 cycle at 72°C for 7 min.

The 267-bp PCR products were precipitated and suspended in 15 μ l of H₂O, and 5 μ l was digested overnight in a final volume of 15 μ l with the restriction enzymes *BbsI* (5 U), *BsaI* (5 U) (8), and *BceAI* (0.5 U) (New England Biolabs).

PCR-RFLP allowed the identification of mutations A2142G and A2143G using the *BbsI* and *BsaI* restriction enzymes, respectively (Fig. 1A, lanes 3 and 5), as previously described (8, 14, 15). The enzyme *BceAI* recognized the two restriction sites 5'-ACGGC(N)₁₂↓N-3' and 5'-N↓(N)₁₂GCCGT-3' on the 267-bp products obtained from the wild type and the A2142G and A2143G mutants, yielding fragments of 48, 24, and 195 bp (Fig. 1A, lane 6; Fig. 1B, lane 2). On the other hand, mutation

A2142C created an additional *BceAI* recognition site, 5'-ACG GC(N)₁₂↓N-3', yielding four fragments, with the 195-bp fragment cleaved into 153- and 42-bp products (Fig. 1A, lane 7, and Fig. 1B, lane 3). For rapid detection, either the 195-bp or the 153-bp fragment could be detected on a 2% agarose gel (data not shown). These *BceAI* cleavages occurred at positions 1979, 2002 and 2155 of the 23S rRNA gene.

The PCR protocol developed here could also be used to amplify sequences containing the two rare mutations A2115G and G2141A, described by Hultén et al. (4). RFLP with *BbsI*, *BsaI*, and *BceAI* would lead to the same restriction patterns as obtained with the amplified wild-type sequence, but RFLP with *EcoRI* and *BpI* would allow the identification of the

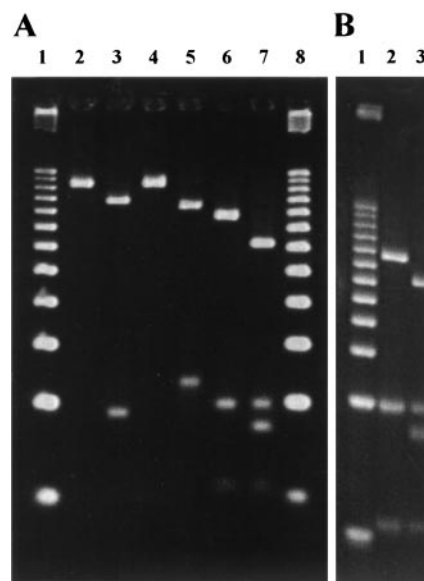


FIG. 1. Detection of mutation A2142C by *BceAI*-mediated restriction digestion. The restriction fragments of the 267-bp PCR products were analyzed by electrophoresis on a 5% agarose Resophor gel (A) or on a 12% polyacrylamide gel (B) stained with ethidium bromide. (A) PCR-RFLP analysis of mutations A2142G, A2143G, and A2142C occurring in domain V of the 23S rRNA gene of *H. pylori*. Lanes 1 and 8, 25-bp DNA Step Ladder molecular size markers (Promega). Lanes 2 and 3, PCR products of the wild-type and A2142G *H. pylori* strains digested with *BbsI*, respectively. Lanes 4 and 5, PCR products of the wild-type and A2143G *H. pylori* strains digested with *BsaI*, respectively. Lanes 6 and 7, PCR products of the wild-type and A2142C *H. pylori* strains digested with *BceAI*, respectively. (B) PCR product of the *H. pylori* strain with mutation A2142C digested with *BceAI*. Lanes 2 and 3, amplified wild-type PCR product and amplified PCR product presenting the A2142C mutation, respectively. Lane 1, 25-bp DNA Step Ladder (Promega). The wild-type *H. pylori* reference strain CIP 101260 and strains 683, 677, and 825, with mutations A2142G, A2142C, and A2143C, respectively, in the 23S rRNA gene were used as controls in this study (8).

A2115G and A2141T mutations, respectively. Lack of access to strains containing these rare mutations precluded obtaining their restriction profiles.

In conclusion, it is possible to detect by PCR-RFLP the three most frequent point mutations of the 23S rRNA gene conferring clarithromycin resistance on *H. pylori*. This method can be applied to *H. pylori* strains and also to gastric biopsy samples, allowing the detection of the three genotypes with one PCR sample: 7 of 200 *H. pylori*-positive biopsies with the A2142C mutation were detected (data not shown).

Real-time PCR-based methods have enormous potential, but limited availability of LightCycler instruments restricts their implementation in many clinical laboratories. For this reason, although more time-consuming, a simple method with the same potential for detection is highly desirable. By extending the highly specific PCR-RFLP technique to the detection of the A2142C mutation, the protocol proposed here achieves this goal.

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Armelle Ménard*

Adriana Santos

Francis Mégraud

Laboratoire de Bactériologie

Université Victor Segalen Bordeaux 2

Bordeaux, France

Mónica Oleastro

Laboratoire de Bactériologie

Université Victor Segalen Bordeaux 2

Bordeaux, France, and

Centro Bacteriologia

Instituto Nacional Saúde Dr Ricardo Jorge

Lisbon, Portugal

*Phone: 33 (0) 5 57 57 12 88

Fax: 33 (0) 5 56 51 41 82

E-mail: Armelle.Menard@labhel.u-bordeaux2.fr