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Distribution of Spontaneous *gyrA* Mutations in 97 Fluoroquinolone-Resistant *Helicobacter pylori* Isolates Collected in France

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We determined the prevalence of *gyrA* mutations conferring fluoroquinolone resistance in 97 *Helicobacter pylori* isolates collected in France from 2007 to 2010. Ninety-four harbored one or two mutations already found in the quinolone resistance determining region (QRDR) of *gyrA* (for T87I, *n* = 23; for N87K, *n* = 32; for D91N, *n* = 30; for D91G, *n* = 7; for D91Y, *n* = 6), 2 harbored a mutation never previously described (D91H and A88P), and one strain was resistant (ciprofloxacin MIC of 8 mg/liter) without a detected mutation conferring this resistance in *gyrA* or *gyrB* genes.

Helicobacter pylori infects one-half of the world's population (9, 15), leading to different gastroduodenal and nondigestive diseases (9, 15, 17). All guidelines worldwide recommend the eradication of *H. pylori* in diagnosed patients (8, 17). This treatment consists in Europe of a triple therapy with a double-dose proton pump inhibitor and two antibiotics, such as amoxicillin, clarithromycin, or metronidazole, for 7 to 14 days, leading to an eradication rate ranging from 60 to 70% (7, 17, 18). Treatment failure is due mainly to antibiotic resistance. The primary resistance rates reached 19.1% for clarithromycin and 58.9% for metronidazole in strains isolated from 2004 to 2007 in France (20). Levofloxacin, a fluoroquinolone, then constitutes an efficient rescue therapy in third-line treatment (17) even though resistance is emerging worldwide, reaching 12% in France (20). In *H. pylori*, the only target of fluoroquinolones is a DNA gyrase consisting of two subunits, GyrA and GyrB. The main event leading to fluoroquinolone resistance is mutation in the quinolone resistance determining region (QRDR) of the *gyrA* gene (18). Eleven mutations have already been reported. They are located at codons 86, 87, 88, and 91 (3, 5, 6, 7, 11, 12, 19, 22, 23). Efflux does not play an important role in fluoroquinolone resistance of *H. pylori* (2).

The aim of this study was to determine the prevalence of *gyrA* mutations among 97 quinolone-resistant strains isolated from French patients and to attempt to describe new mechanisms involved in resistance.

The 97 fluoroquinolone-resistant strains were isolated from 2007 to 2010 from gastric biopsy specimens from symptomatic patients (67 in the University Hospital of Poitiers and 30 in the University Hospital of Cochin, Paris). During this period, the primary rate of resistance to fluoroquinolone of *H. pylori* isolated in Poitiers and Paris was 17% (personal data). Our population consisted of 43 men and 54 women. The mean age was 56.6 years (21 to 87). The endoscopy showed the presence of ulcers in 23 cases, gastritis in 45 cases, and normal mucosa in 23 cases; for 6 cases, we have no data. Among the 97 patients, 60 (62%) had never received eradication treatment for *H. pylori* infection and 37 (38%) had already been treated, only 1 of them by fluoroquinolones.

The resistance to fluoroquinolones was established using Etest (AB bioMérieux, Sweden) and confirmed by the agar dilution method; the breakpoint was 1 mg/liter as recommended by EUCAST (10, 13). Ciprofloxacin was tested because it is the most discriminant fluoroquinolone for assessing the resistance of *H. pylori* to this class of antibiotics (6). All the strains were resistant to ciprofloxacin. The MIC_{50} and MIC_{90} for ciprofloxacin were 8 mg/liter and 16 mg/liter, respectively.

Chromosomal DNA of the 97 strains was extracted using the Diagnosis MagNA Pure Compact system (Roche Diagnostics, Switzerland). We designed 4 pairs of primers to amplify the 2,487 bp of the *gyrA* gene: GYRA5 (forward), 5′-ATG-CAA-GAT-CAT-TTA-GTC-AAT-GA-3′ (bp 1 to 23), and GYRA2 (reverse), 5′-GCA-GAC-GGC-TTG-GTA-RAA-TA-3′ (bp 483 to 502) (6); GYRA6 (forward), 5′-GAA-TGA-CCA-AGG-CGA-GTG-AA-3′ (bp 389 to 408), and GYRA9 (reverse), 5′-TCA-ATA-TTG-TCC-AAG-GCG-ATC-3′ (bp 1158 to 1178); GYRA10 (forward), 5′-AGA-CGC-ACG-ATT-TTT-GAA-TTA-G-3′ (bp 1095 to 1116), and GYRA13 (reverse), 5′-GCC-AAA-TTC-GCT-CAA-ATT-GGT-3′ (bp 1876 to 1896); GYRA14 (forward), 5′-GCA-ACC-CTA-AGC-ACT-AAA-GAT-3′ (bp 1801 to 1821), and GYRA17 (reverse), 5′-TCA-CTC-AAA-CAA-ATT-TTG-CAC-C-3′ (bp 2466 to 2487) (Eurogentec, Seraing, Belgium); and a pair of primers to amplify the *gyrB* gene: GYRB1 (forward), 5′-ATG-CAA-AAT-TAC-CAG-AGC-CATA-3′ (bp 1 to 22), and GYRB3 (reverse), 5′-CAT-GCG-CTT-GGA-TAA-AGG-CT-3′ (bp 2274 to 2293). The PCR products were sequenced on both strands using the same primers.

Among the 97 fluoroquinolone-resistant isolates, 94 harbored one (90 isolates) or two (4 isolates) mutations already found in the QRDR of *gyrA* (N87K, *n* = 32; D91N, *n* = 30; T87I, *n* = 23; D91G, *n* = 7; D91Y, *n* = 6), 2 harbored a mutation never previously described (A88P and D91H), and 1 strain was resistant (ciprofloxacin MIC, 8 mg/liter) without any mutation conferring this resistance in the *gyrA* and *gyrB* genes (Table 1). The recipient *H. pylori* strain J99 (1) (ciprofloxacin susceptible: MIC, 0.016 mg/liter) was transformed with the QRDR DNA of the strains harbor-

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TABLE 1 Distribution of *gyrA* mutations among 97 fluoroquinolone-resistant *H. pylori* isolates

gyrA-encoded amino acid (nucleotide) mutation	No. (%) of isolates	MIC_{50}/MIC_{90} (range) [mg/liter]
T87I (C260T)	23 (23.7)	16/16 (4–32)
N87K (C261A)	26 (26.8)	8/16 (4–32)
N87K (C261G)	6 (6.2)	4/16 (2–32)
A88P (G262C)	1 (1.0)	2
D91N (G271A)	30 (30.9)	8/8 (2–32)
D91G (A272G)	7 (7.2)	8/8 (1,5–16)
D91Y (G271T)	6 (6.2)	4/8 (2–16)
D91H (G271C)	1 (1.0)	16
None	1 (1.0)	8

ing the two new mutations amplified with primers GYRA2 and GYRA5, as previously described (4). Transformation tests confirmed that these two mutations were responsible for the resistance (transfer rate: number of transformants/number of recipients = 10^{-4}). These mutations have already been described as implicated in the fluoroquinolone resistance of *Escherichia coli* and *Salmonella sp.* (A84P and D87H, corresponding to positions 88 and 91 in *H. pylori*) (16, 21).

Among the 97 resistant strains, 4 carried a double mutation in the QRDR (2 strains 1with D91N and N87K, 1 with D91Y and T87I, and 1 with D91Y and N87K). We did not find any correlation between the number of mutations and the MIC, although this has been described previously (6, 22). One strain with a MIC of 8 mg/liter was free of mutations in the QRDR. With transformation of the *H. pylori* strain J99 wild type with the whole *gyrA* gene or the whole *gyrB* gene of this isolate, transformants grew on ciprofloxacin-enriched medium (1 mg/liter) in the same numbers as spontaneous mutants (10^{-8}), whereas the concomitant transformation of the same strain by the whole *gyrA* gene of a strain harboring a D91N mutation yielded 10,000 more transformants (transfer rate, 10^{-4}). Active efflux, mutations in other genes, or plasmid-mediated resistance since cryptic plasmids were identified in *H. pylori* (14) must be explored to explain the resistance of this strain to ciprofloxacin. The distribution of the mutations differed according the geographical origins of the strains. The mutation T87I was found in 43.3% of the strains isolated in Paris versus 14.9% among those isolated in Poitiers ($P = 0.002$). The mutation D91N was found in 16.7% of the strains isolated in Paris versus 37.3% of those isolated in Poitiers ($P = 0.04$). Compiled published data show that the major mutation described around the world is D91N (29.7%), followed by D91G (29.1%), N87L (14.9%), N87K (14.1%), and D91Y (12.1%) (3, 5, 6, 7, 11, 12, 19, 22, 23). Our results highlight that resistance mutations have a local propagation, as has been observed in other areas worldwide, with prevalence varying from one country to another. This clonal diffusion of resistant clones has already been described concerning clarithromycin resistance of *H. pylori* (20).

In conclusion, fluoroquinolone resistance in *H. pylori* is due mainly (99%) to mutations in the QRDR of *gyrA*. In addition to the 11 known mutations conferring fluoroquinolone resistance, we have described 2 new mutations in the *gyrA* gene of *H. pylori*.

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