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Gatifloxacin Resistance and Mutations in *gyrA* after Unsuccessful *Helicobacter pylori* Eradication in Japan

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A high resistance rate (47.9%) to gatifloxacin (GAT; 8-methoxy fluoroquinolone) in *Helicobacter pylori* (*H. pylori*) strains from 48 Japanese patients is observed after unsuccessful *H. pylori* eradication. A significant association between MICs for GAT equal to or above 1 μg/ml and mutations of the *gyrA* gene of *H. pylori* was demonstrated.

Resistance to antibiotics is the major cause of the failure to eradicate *Helicobacter pylori*. For such cases, alternative regimens need to be developed.

Perri et al. have reported an unacceptable low eradication rate of only 68% with a 7-day regimen of levofloxacin (LVX), amoxicillin (AMX), and pantoprazole (12). However, Sharara et al. have recently reported an excellent eradication rate of 92% with a 7-day regimen of gatifloxacin (GAT), AMX, and rabeprazole (14). It is important to note the superior in vitro activity of GAT over that of LVX against *H. pylori* (1). Therefore, GAT-based triple therapy might be a promising option for an alternative treatment regimen.

Several studies have shown that the "quinolone resistance-determining region" (QRDR) of the *gyrA* gene plays a critical role in the resistance of *H. pylori* to ciprofloxacin (CIP) (8, 15). *H. pylori* does not contain genes for topoisomerase IV, an important fluoroquinolone target in other bacteria (15). The present study demonstrated a correlation between MICs to GAT and mutations of the *gyrA* and *gyrB* genes in *H. pylori* isolated from Japanese patients after unsuccessful eradication of infection.

A total of 48 patients (32 males and 16 females; mean age, 57.8) with *H. pylori* infection after treatment failure were enrolled at Keio University Hospital between September 2004 and June 2005. Of the total, 42 patients had one treatment failure, 4 patients had two treatment failures, and 2 patients had three treatment failures (first-line treatment, triple therapy with clarithromycin [CLR], AMX, and proton pump inhibitor [PPI]; second-line treatment, triple therapy with metronidazole [MNZ], AMX, and PPI; and third-line treatment, triple therapy with LVX, AMX, and PPI). All patients underwent upper gastrointestinal endoscopy and gastric biopsy at Keio University Hospital.

The susceptibility of *H. pylori* isolates to CRL, GAT, and MNZ was determined by the agar dilution method according to the guidelines established by the CLSI (formerly NCCLS) (9). Isolates were considered resistant to MNZ if the MIC of the drug was ≥8 μg/ml and resistant to CLR and GAT if the MIC of these drugs was ≥1 μg/ml (3, 7, 10). The resistance rates to GAT, CLR, and MNZ were 47.9%, 79.2%, and 6.3%, respectively. The GAT resistance rates were 22.2% (2/9) in the strains susceptible to both CLR and MNZ, 50% (18/36) in the strains resistant to CLR but susceptible to MNZ, 100% (1/1) in the strains resistant to MNZ but susceptible to CLR, and 100% (2/2) in the strains resistant to both CLR and MNZ.

Studies in Europe have suggested that the primary resistance rate might be as low as 8% for CIP (2, 16) and that only 9% of the isolates show resistance to CIP after failure of primary eradication (4, 13). In contrast, our drug susceptibility tests showed a high resistance rate to GAT. Therefore, quinolone-resistant strains may be more prevalent in Japan than in Europe, due to wider use of fluoroquinolones.

We amplified by PCR and sequenced the QRDRs of *gyrA* (from codon 38 to 154) and *gyrB* (from codon 392 to 500) genes: *gyrA* (forward), 5′-TTTRGCTTATTCMATGAGCGT-3′; *gyrA* (reverse), 5′-GCAGACGGCTTGGTARAATA-3′; *gyrB* (forward), 5′-YGCAAAAGCCAGAGAAGCCA-3′; and *gyrB* (reverse), 5′-ACATGCCCTTGTTCAATCAGC-3′). The sequences obtained were compared with the published sequences of the *H. pylori gyrA* and *gyrB* genes (GenBank accession no. L29481) (3). Twenty-two of the 23 (95.7%) GAT-resistant strains had point mutations in the *gyrA* gene at codon 87 Asn or 91 Asp. On the other hand, only 1 of the 25 (4.0%) susceptible strains had the mutation, with a substitution at amino acid 87 (Tables 1 and 2). The sensitivity and specificity of detection of mutations in the *gyrA* gene in the GAT-resistant strains were 95.7% and 95.7%, respectively. Significant association was observed between mutations in the *gyrA* gene and resistance of the strains to GAT ($P < 0.001$). Mutations of the *gyrB* gene, on the other hand, were not encountered in either the susceptible or the resistant strains. The examination of mutations in the *gyrA* gene may be useful as a marker of GAT resistance.

Although many isolates bear the same *gyrA* mutation, their GAT MICs vary over an eightfold range. It might be due to the other mechanisms determining GAT MICs, such as an active

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TABLE 1. List of GAT-resistant strains

Strain	Substitution in *gyrA* QRDR	Nucleotide mutation	GAT MIC (μg/ml)	CLR MIC (μg/ml)	MNZ MIC (μg/ml)	Prior treatment(s)[a]
KS0141	Asp 91 Asn	G 271 A	1	16	1	LAC
KS0148	Asp 91 Asn	G 271 A	1	32	2	LAC
KS0149	Asp 91 Asn	G 271 A	1	32	2	LAC
KS0154	Asp 91 Asn	G 271 A	1	16	1	LAC
KS0142	Asp 91 Gly	A 272 G	1	16	1	LAC
KS0161	Asn 87 Lys	C 261 A	1	0.06	4	LAC, LAM, RAL
KS0162	Asn 87 Lys	C 261 A	1	16	1	LAC
KS0187	Asp 91 Tyr	G 271 T	1	8	1	LAC
KS0150	Asp 91 Asn	G 271 A	2	32	2	LAC
KS0151	Asp 91 Asn	G 271 A	2	64	2	LAC
KS0155	Asn 87 Lys	C 261 A	2	64	8	LAC
KS0170	Asn 87 Lys	C 261 G	2	≤0.015	4	LAC, LAM
KS0177	Asn 87 Lys	C 261 G	2	8	1	LAC
KS0186	ND[b]	ND	2	16	2	LAC
KS0188	Asp 91 Gly	A 272 G	2	8	0.5	LAC
KS0189	Asp 91 Asn	G 271 A	2	16	0.5	LAC
KS0146	Asn 87 Lys	C 261 A	4	16	2	LAC
KS0171	Asn 87 Lys	C 261 A	4	0.12	32	LAC, LAM, RAL
KS0185	Asn 87 Lys	C 261 A	4	16	1	LAC
KS0143	Asn 87 Lys	C 261 A	8	32	2	LAC
KS0157	Asn 87 Lys	C 261 A	8	16	2	LAC
KS0145	Asp 91 Asn	G 271 A	8	64	64	LAC, LAM
KS0147	Asp 91 Tyr	G 271 T	8	64	2	LAC

[a] LAC, triple therapy with lansoprazole, amoxicillin, and clarithromycin; LAM, triple therapy with lansoprazole, amoxicillin, and metronidazole; and RAL, triple therapy with rabeprazole, amoxicillin, and levofloxacin.
[b] ND, not detected.

multidrug efflux mechanism (5). Since the CmeABC efflux pump is linked to the acquired fluoroquinolone resistance in *Campylobacter* isolates (6), active efflux systems may also be involved in *H. pylori* susceptibility to GAT.

Four GAT- and CLR-susceptible strains were serially plated onto GAT (0.12 μg/ml)- or CLR (0.015 μg/ml)-containing agar with increasing agar density. Although none of the strains plated on CLR-containing agar developed resistance to GAT until the 10th generation of repeated culture, all four strains plated on GAT-containing agar developed GAT resistance and

TABLE 2. List of GAT-susceptible strains

Strain	Substitution in *gyrA* QRDR	Nucleotide mutation	GAT MIC (μg/ml)	CLR MIC (μg/ml)	MNZ MIC (μg/ml)	Prior treatment(s)[a]
KS0175	ND[b]	ND	0.06	16	2	LAC
KS0176	ND	ND	0.06	16	0.5	LAC
KS0178	ND	ND	0.06	8	2	LAC
KS0179	ND	ND	0.06	8	1	LAC
KS0180	ND	ND	0.06	0.03	1	LAC
KS0184	ND	ND	0.06	8	4	LAC
KS0153	ND	ND	0.12	32	1	LAC
KS0158	ND	ND	0.12	16	1	LAC
KS0159	ND	ND	0.12	4	0.5	LAC
KS0160	ND	ND	0.12	8	1	LAC
KS0164	ND	ND	0.12	8	1	LAC
KS0165	ND	ND	0.12	64	1	LAC, LAM
KS0168	ND	ND	0.12	≤0.015	1	LAC
KS0172	ND	ND	0.12	16	2	LAC
KS0173	ND	ND	0.12	8	1	LAC
KS0174	ND	ND	0.12	16	2	LAC
KS0181	ND	ND	0.12	≤0.015	1	LAC, LAM
KS0182	ND	ND	0.12	8	2	LAC
KS0183	ND	ND	0.12	8	1	LAC
KS0152	ND	ND	0.25	4	1	LAC
KS0163	ND	ND	0.25	0.12	1	LAC
KS0167	ND	ND	0.25	0.03	2	LAC
KS0144	ND	ND	0.5	0.25	2	LAC
KS0166	ND	ND	0.5	8	1	LAC
KS0169	Asp 91 Gly	A 272 G	0.5	≤0.015	1	LAC

[a] LAC, triple therapy with lansoprazole, amoxicillin, and clarithromycin; and LAM, triple therapy with lansoprazole, amoxicillin, and metronidazole.
[b] ND, not detected.

three strains showed mutations of *gyrA*, suggesting that a high incidence of GAT resistance could not arise through mutation of susceptible bacteria during the course of therapy with CLR, AMX, and PPI and could be due to primary infection with resistant bacteria or to history of fluoroquinolone treatment.

Oram and Fisher reported that examination of silent nucleotide changes in the *gyrA* genes revealed four different patterns of DNA polymorphism in *Escherichia coli* (11). In the present study, compared with the reported sequence (GenBank accession no. L29481), there were C→T substitutions at position 108, 132, 210, 396, and 438, G→A substitutions at positions 276, 277, 291, and 318, T→C substitutions at positions 183 and 225, and A→G substitutions at positions 135 and 366. While KS0149 and KS0150 shared the same patterns of polymorphism, the other strains showed different patterns, indicating that these strains were not genetically related and that each strain developed GAT resistance during the previous fluoroquinolone usage.

In conclusion, a high resistance rate to GAT in *H. pylori* strains from Japanese patients is observed after unsuccessful eradication. Mutation in the *gyrA* gene of *H. pylori* was significantly associated with MICs for GAT equal to or above 1 μg/ml. Although GAT may be one of the promising candidates for third-line therapy, the choice of GAT should be made after drug susceptibility tests or *gyrA* gene analysis.

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