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Genotypic Resistance in *Helicobacter pylori* Strains Correlates with Susceptibility Test and Treatment Outcomes after Levofloxacin- and Clarithromycin-Based Therapies[▽]

Jyh-Ming Liou,[1,2] Chi-Yang Chang,[3] Wang-Huei Sheng,[1] Yu-Chi Wang,[4] Mei-Jyh Chen,[1] Yi-Chia Lee,[1] Hsu-Wei Hung,[5] Hung Chian,[5] San-Chun Chang,[1] Ming-Shiang Wu,[1,6]* and Jaw-Town Lin[1,3]

Departments of Internal Medicine[1] and Primary Care Medicine,[6] National Taiwan University Hospital, College of Medicine, and Graduate Institute of Epidemiology, College of Public Health,[2] National Taiwan University, and Department of Molecular Medicine, Taipei Institute of Pathology,[5] Taipei, and Department of Internal Medicine, E-DA Hospital,[3] and Biological Science and Technology, I-Shou University,[4] Kaohsiung County, Taiwan

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The accuracy of genotypic resistance to levofloxacin (*gyrA* mutations) and its agreement with treatment outcomes after levofloxacin-based therapy have not been reported. We aimed to assess the correlation. *Helicobacter pylori* strains isolated from patients who received levofloxacin-based and clarithromycin-based triple therapies in a previous randomized trial were analyzed for point mutations in *gyrA* and 23S rRNA. PCR followed by direct sequencing was used to assess the *gyrA* and 23S rRNA mutations. An agar dilution test was used to determine the MICs of clarithromycin and levofloxacin. We found that the agreement between genotypic and phenotypic resistance to levofloxacin was best when the MIC breakpoint was >1 μg/ml (kappa coefficient, 0.754). The eradication rates in patients with and without *gyrA* mutations were 41.7% and 82.7%, respectively ($P = 0.003$). The agreement between genotypic and phenotypic resistance to clarithromycin was best when the MIC breakpoint was >2 μg/ml (kappa, 0.694). The eradication rates in patients with and without 23S rRNA mutations were 7.7% and 93.5%, respectively ($P < 0.001$). The agreements (kappa coefficient) between therapeutic outcomes after clarithromycin-based triple therapy and genotypic and phenotypic resistance were 0.671 and 0.356, respectively. The agreements (kappa coefficient) between therapeutic outcomes after levofloxacin-based triple therapy and genotypic and phenotypic resistance were 0.244 and 0.190, respectively. In conclusion, *gyrA* and 23S rRNA mutations in *H. pylori* strains appeared to be better markers than phenotypic resistance in the prediction of treatment outcomes. The optimal breakpoints for levofloxacin and clarithromycin resistance appeared to be >1 μg/ml and >2 μg/ml, respectively.

Helicobacter pylori plays a crucial role in the pathogenesis of gastroduodenal diseases (34, 35). Eradication of *H. pylori* has been shown to reduce the risk of recurrent peptic ulcer diseases and even the development of gastric cancer (9, 39, 40). Clarithromycin-based triple therapy for 7 to 14 days is the first-line therapy currently recommended by U.S. and European guidelines (4, 23). Levofloxacin-based triple therapy has been shown to be more effective and to be associated with fewer adverse effects than quadruple therapy in second- and third-line treatments for *H. pylori* infection (11, 12, 30, 31). Reports from some countries have also shown that levofloxacin-based triple therapy is effective as first-line therapy, although the result remained controversial (13, 21, 28). Resistance to clarithromycin or levofloxacin is the major reason for eradication failure after clarithromycin- or levofloxacin-based triple therapy, respectively (21, 24, 37). Therefore, the detection of antibiotic resistance is crucial in the management of *H. pylori* infection.

The determination of phenotypic resistance by MICs with the agar dilution test or the Epsilometer test (Etest) is the most commonly used method for the detection of clarithromycin and levofloxacin resistance (24, 37). In recent years, point mutations in the 23S rRNA and gyrase A (*gyrA*) genes have been shown to be associated with clarithromycin and levofloxacin resistance, respectively (24, 26, 36–38). The A2143G, A2142G, and A2142C mutations were identified in 69.8%, 11.7%, and 2.6% of *H. pylori* strains with phenotypic resistance to clarithromycin, respectively (24). Mutations at amino acid positions 87 and 91 were the most commonly identified mutations in strains with phenotypic resistance to quinolones (1, 3, 5, 15–19, 25, 26, 36). Several more-rapid and more-convenient methods have also been developed to detect the 23S rRNA and *gyrA* mutations using either *H. pylori* strains, gastric biopsy specimens, or gastric juice (2, 22, 27). However, most of the previous studies analyzed the genotypes only in strains with phenotypic resistance to levofloxacin (1, 15, 16, 17, 19, 26, 36). The *gyrA* genotypes in levofloxacin-susceptible strains were only partly determined in some studies (3, 5, 25). Therefore, the accuracy of the new molecular method and its agreement with standard MIC tests in the detection of levofloxacin resistance remain unknown. More importantly, whether the determination of the genotypic resistance of strains to levofloxacin is correlated with therapeutic outcomes after levofloxacin-based triple therapy has not been reported. The recommended MIC

* Corresponding author. Mailing address: Departments of Internal Medicine and Primary Care Medicine, National Taiwan University Hospital, National Taiwan University, College of Medicine, No. 7, Chung-Shan S. Road, Taipei, Taiwan. Phone: 886-2-23123456, ext. 65043. Fax: 886-2-23947899. E-mail: mingshiang@ntu.edu.tw.

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breakpoints for clarithromycin and levofloxacin resistance were both 1 μg/ml on the basis of previous *in vitro* studies (29, 33). However, whether these breakpoints were in good concordance with genotypic resistance and therapeutic outcomes remained controversial.

Therefore, we aimed to evaluate whether point mutations at *gyrA* correlate with phenotypic resistance and therapeutic outcomes after levofloxacin-based triple therapy. We also aimed to assess whether the previously recommended breakpoints (1 μg/ml) of MIC tests for clarithromycin and levofloxacin are appropriate in the prediction of therapeutic outcomes after clarithromycin- and levofloxacin-based triple therapies.

MATERIALS AND METHODS

Patients. This was a post-hoc subgroup study from a previous randomized comparative multicenter trial conducted in Taiwan from May 2007 to April 2009. Naïve *H. pylori*-infected patients as defined by at least two positive results among the rapid urease test, histology, and culture were enrolled in that study. The enrollment and eradication treatment are described in our previous report (21). In brief, eligible patients (n = 432) were randomized to receive either (i) levofloxacin (750 mg once a day [q.d.]), amoxicillin (1,000 mg twice a day [b.i.d.]), and lansoprazole (30 mg b.i.d.) (LAL) for 7 days (217 patients) or (ii) clarithromycin (500 mg b.i.d.), amoxicillin (1,000 mg b.i.d.), and lansoprazole (30 mg b.i.d.) (CAL) for 7 days (215 patients). Posttreatment *H. pylori* status was determined by the ^{13}C-Urea breath test (^{13}C-UBT) at least 6 weeks after the completion of treatment. Patients who failed first-line therapy were re-treated with another regimen for 10 days in a crossover manner.

In the present study, all patients from the previous trial for whom *H. pylori* strains were available (322 patients, of whom 162 were in the LAL group and 160 were in the CAL group) were enrolled for genotypic and phenotypic resistance analysis. The study protocol was approved by the Institutional Review Boards of the National Taiwan University Hospital. Written informed consent was obtained from all patients. The eradication rates were not significantly different between individuals for whom *H. pylori* strains were and were not available. In the LAL group, the eradication rates were 75.3% (122/162) and 70.9% (39/55) among patients enrolled and not enrolled in the present study (P, 0.519), respectively. In the CAL group, the eradication rates were 83.1% (133/160) and 85.5% (47/55) among patients enrolled and not enrolled in the present study (P, 0.686), respectively. A total of 303 and 308 strains were available for assessment of the concordance between genotypic and phenotypic resistance to levofloxacin and clarithromycin, respectively. Of the 64 patients who received second-line therapy, paired *H. pylori* strains (before and after triple therapy) were available from 11 and 9 patients who failed levofloxacin-based and clarithromycin-based therapy, respectively.

Susceptibility test and genotyping of *gyrA* and 23S rRNA. Biopsy specimens were cultured on plates containing *Brucella* chocolate agar with 7% sheep blood and were incubated under microaerobic conditions (5% O_2, 10% CO_2, 85% N_2) for 7 days. All isolated strains were stored at −80°C until use. The MICs of clarithromycin and levofloxacin were determined by the agar dilution test. In brief, *H. pylori* was inoculated onto antibiotic-containing Mueller-Hinton agar supplemented with 5% defibrinated sheep blood. *H. pylori* ATCC 43504 was used as the quality control strain. The MIC of each antibiotic was determined after 72 h of incubation. The technician (R.-J. Wu) who performed the agar dilution test was blinded to the genotypic resistance results and therapeutic outcomes. The Gentra DNA purification kit (Qiagen) was used to extract *H. pylori* DNA, according to the manufacturer's instructions, from the homogenous bacterial strains isolated by culture. The *H. pylori* 23S rRNA gene fragment was amplified by PCR with forward primer 5′-CCACAGCGAT GTG GTCTCAG-3 and reverse primer 5′-CTCCATAAGAGCCAAAGCCC-3′. The *gyrA* fragment was amplified by PCR with the following primers: forward, 5′-TTTRGCTTATTCM ATGAGCGT; reverse, 5′-GCAGACGGCTTGGTARAATA. The PCR products were then purified and subjected to direct sequencing using an automatic sequencer (ABI Prism genetic analyzer, model 3100; Applied Biosystems). The technician (J.-T. Hsu) who performed the genotyping was blinded to the agar dilution test results and therapeutic outcomes.

Statistical analysis. Categorical data were compared using the chi-square test employing Yates' correction for continuity or Fisher's exact test as appropriate. Continuous data were compared with Student's t test and were expressed as means and standard deviations (SD). The kappa coefficient was used to assess the agreement between genotypic resistance and phenotypic resistance as well as therapeutic outcomes. Agreement, as expressed by the kappa coefficient, was interpreted as follows: <0, poor; 0.00 to 0.20, slight; 0.21 to 0.40, fair; 0.41 to 0.6, moderate; 0.61 to 0.8, substantial; ≥0.8, excellent (18). Per-protocol analysis was used to assess the concordance between therapeutic outcomes and antibiotic resistance. Logistic regression analyses were used to compute the odds ratios (ORs) and 95% confidence intervals (95% CIs). All P values were two-tailed, and the level of statistical significance was specified as 0.05. Statistical analyses were performed using SPSS statistical software for Windows (version 12.0).

TABLE 1. Demographic data of patients analyzed for primary resistance

Characteristic[a]	Value for group[b]		
	LAL (n = 162)	CAL (n = 160)	All patients (n = 322)
No. male/no. female	68/94	72/88	140/182
Mean age (SD)	48.5 (13.1)	49.3 (12.5)	48.9 (12.8)
No. (%)			
With peptic ulcer disease	61 (37.7)	68 (42.5)	129 (40.1)
Smoking	50 (30.9)	45 (28.1)	95 (29.5)
Mean BMI (SD)	23.9 (3.7)	23.6 (3.7)	23.7 (3.7)
Eradication rate			
ITT analysis	122/162 (75.3)	133/160 (83.1)	255/322 (79.2)
PP analysis	122/152 (80.3)	133/154 (86.4)	255/306 (84.4)
23S rRNA mutation	14/159 (8.8)	13/158 (8.2)	27/317 (8.5)
gyrA mutation	13/155 (8.4)	10/158 (6.3)	23/313 (7.3)
Clarithromycin MIC, >1 μg/ml	16/157 (10.2)	13/155 (8.4)	29/312 (9.3)
Levofloxacin MIC, >1 μg/ml	11/157 (7.0)	7/155 (4.5)	18/312 (5.8)

[a] BMI, body mass index; ITT, intention to treat; PP, per protocol.
[b] Unless stated otherwise, values are given as the number of patients with the characteristic/total number of patients in the group (percentage). LAL, levofloxacin, amoxicillin, and lansoprazole; CAL, clarithromycin, amoxicillin, and lansoprazole.

RESULTS

Prevalence of primary and secondary levofloxacin and clarithromycin resistance. The prevalences of primary genotypic and phenotypic (MIC, >1 μg/ml) resistance to levofloxacin were 7.3% (23/313) and 5.8% (18/312), respectively (Table 1). The prevalences of primary genotypic and phenotypic (MIC, >1 μg/ml) resistance to clarithromycin were 8.5% (27/317) and 9.3% (29/312), respectively (Table 1). The prevalences of secondary genotypic and phenotypic resistance to levofloxacin after levofloxacin-based therapy were 43.8% (7/16) and 47.1% (8/17), respectively. The prevalences of secondary genotypic and phenotypic resistance to clarithromycin after clarithromycin-based therapy were 100% (10/10) and 83.3% (10/12), respectively.

Concordance between genotypic and phenotypic resistance for levofloxacin and clarithromycin. Genotypic resistance to levofloxacin was significantly correlated with phenotypic resistance at different MIC breakpoints (>0.5 μg/ml, >1 μg/ml, >4 μg/ml, and >8 μg/ml), as shown in Table 2. However, the kappa correlation efficient was highest (0.754) when phenotypic resistance to levofloxacin was defined as a MIC of >1 μg/ml (Table 2). Genotypic resistance to clarithromycin was also significantly correlated with phenotypic resistance at different MIC breakpoints (>0.5 μg/ml, >1 μg/ml, >2 μg/ml, and >16 μg/ml), as shown in Table 3. However, the kappa corre-

TABLE 2. Correlations between genotypic and phenotypic resistance to levofloxacin at different MIC breakpoints

MIC (μg/ml)	GyrA mutation		*P*	Kappa coefficient	Accuracy[a]	
	No	Yes			Sensitivity	Specificity
≤8	280	18	<0.001	0.277	80 (4/5)	94 (280/298)
>8	1	4			18.2 (4/22)	99.6 (280/281)
≤4	279	13	<0.001	0.522	81.8 (9/11)	95.5 (279/292)
>4	2	9			40.9 (9/22)	99.3 (279/281)
≤1	279	7	<0.001	0.754	88.2 (15/17)	97.6 (279/286)
>1	2	15			68.2 (15/22)	99.3 (279/281)
≤0.5	244	7	<0.001	0.338	28.8 (15/52)	97.2 (244/251)
>0.5	37	15			68.2 (15/22)	86.8 (244/281)

[a] Sensitivity is given as a percentage (number found positive/total number positive by the gold standard). Specificity is given as a percentage (number found negative/total number negative by the gold standard). The MIC test was used as the gold standard for the calculation of accuracy.

lation efficient was highest (0.694) when phenotypic resistance to clarithromycin was defined as a MIC of >2 μg/ml (Table 3).

Eradication rates according to genotypic and phenotypic resistance to levofloxacin. Among patients treated with levofloxacin-based triple therapy, the presence of *gyrA* mutations ($P = 0.003$) or levofloxacin resistance detected by the MIC test with a breakpoint at >1 μg/ml (*P*, 0.018 by Fisher's exact test) or >4 μg/ml (*P*, 0.021 by Fisher's exact test) was significantly associated with treatment failure (Table 4). The eradication rates were 41.7% (5/12) and 82.7% (110/133) for patients with and without *gyrA* mutations, respectively ($P = 0.003$). However, the eradication rates were not significantly different between patients with susceptible and resistant strains if the MIC breakpoints were >0.5 μg/ml or >8 μg/ml. The agreement between genotypic or phenotypic resistance to levofloxacin and the therapeutic response to levofloxacin-based triple therapy was poor. Nevertheless, the kappa coefficient appeared to be higher with genotypic resistance (0.244) and a MIC cutoff at >1 μg/ml (0.190), as shown in Table 4. The eradication rates among patients with and without Asn87 mutations were 40% and 82.7%, respectively ($P = 0.046$). Among patients with and without Asp91 mutations, the eradication rates were 50% and 82.7%, respectively ($P = 0.079$). The eradication rate for the patient with an Ala97 mutation was 0%.

TABLE 3. Correlations between genotypic and phenotypic resistance to clarithromycin at different MIC breakpoints

MIC (μg/ml)	23S rRNA mutation		*P*	Kappa coefficient	Accuracy[a]	
	No	Yes			Sensitivity	Specificity
≤16	283	12	<0.001	0.589	84.6 (11/13)	95.9 (283/295)
>16	2	11			47.8 (11/23)	99.3 (283/285)
≤8	281	9	<0.001	0.661	77.8 (14/18)	96.9 (281/290)
>8	4	14			60.9 (14/23)	98.6 (281/285)
≤2	281	8	<0.001	0.694	78.9 (15/19)	97.2 (281/289)
>2	4	15			65.2 (15/23)	98.6 (281/285)
≤1	276	8	<0.001	0.608	62.5 (15/24)	97.2 (276/284)
>1	9	15			65.2 (15/23)	96.8 (276/285)
≤0.5	271	8	<0.001	0.538	51.7 (15/29)	97.1 (271/279)
>0.5	14	15			65.2 (15/23)	95.1 (271/285)

[a] Sensitivity is given as a percentage (number found positive/total number positive by the gold standard). Specificity is given as a percentage (number found negative/total number negative by the gold standard). The MIC test was used as the gold standard for the calculation of accuracy.

TABLE 4. Correlations between genotypic and phenotypic resistance to levofloxacin and eradication rates (per-protocol analysis) after first-line levofloxacin-based triple therapy

gyrA genotype or MIC	No. of patients for whom *H. pylori* was:		Eradication rate (%)	*P*	Kappa coefficient
	Eradicated	Not eradicated			
gyrA					
No mutation	110	23	82.7	0.003	0.244
Mutation	5	7	41.7		
MIC (μg/ml)					
≤8	121	26	82.3	0.182	0.059
>8	0	1	0		
≤4	118	23	83.7	0.021	0.173
>4	3	4	42.9		
≤1	116	22	84.1	0.018	0.190
>1	5	5	50		
≤0.5	104	19	92.7	0.084	0.160
>0.5	17	8	68		

Eradication rates according to genotypic and phenotypic resistance to clarithromycin. Among patients treated with clarithromycin-based triple therapy, the presence of 23S rRNA mutations ($P = 0.003$) or clarithromycin resistance detected by the MIC test with a breakpoint at >0.5 μg/ml ($P = 0.01$), >1 μg/ml ($P = 0.003$), >2 μg/ml ($P < 0.001$), >8 μg/ml ($P < 0.001$), or >16 μg/ml ($P = 0.014$) was significantly associated with treatment failure (Table 5). The eradication rates were 7.7% (1/13) and 93.5% (130/139) for patients with and without 23S rRNA mutations, respectively ($P < 0.001$ [Table 5]). The agreement between genotypic resistance to clarithromycin and eradication failure was good (kappa, 0.687). The agreement between phenotypic resistance to clarithromycin and the therapeutic response to clarithromycin-based triple therapy was

TABLE 5. Correlations between genotypic and phenotypic resistance to clarithromycin and eradication rates (per-protocol analysis) after first-line clarithromycin-based triple therapy

23S genotype or MIC	No. of patients for whom *H. pylori* was:		Eradication rate (%)	*P*	Kappa coefficient
	Eradicated	Not eradicated			
23S rRNA					
No mutation	130	9	93.5	<0.001	0.687
Mutation	1	12	7.7		
MIC (μg/ml)					
≤16	131	16	89.1	0.014	0.180
>16	0	2	0		
≤8	129	13	90.8	<0.001	0.356
>8	2	5	28.6		
≤2	129	13	90.8	<0.001	0.356
>2	2	5	28.6		
≤1	126	13	89.4	0.003	0.296
>1	5	5	50		
≤0.5	123	13	90.4	0.010	0.246
>0.5	8	5	61.5		

TABLE 6. Changes in phenotypic and genotypic resistance after levofloxacin-based and clarithromycin-based triple therapies

Type of therapy[a] (no. of patients) or type of resistance	No. (%) of patients with changes in resistance[b] after treatment			
	S → S	R → R	S → R	R → S
Levo-based therapy (11)				
Genotypic resistance to Levo	7 (63.6)	3 (27.3)	1 (9.1)	0
Phenotypic resistance to Levo	6 (54.5)	2 (18.2)	3 (27.3)	0
Clari-based therapy (9)				
Genotypic resistance to Clari[c]	0	4 (50)	4 (50)	0
Phenotypic resistance to Clari	2 (22.2)	3 (33.3)	4 (44.4)	0

[a] Levo, levofloxacin; Clari, clarithromycin.
[b] S, susceptible; R, resistant; S → S, susceptible before and after triple therapy; R → R, resistant before and after triple therapy; S → R, susceptible before therapy and resistant after therapy; R → S, resistant before therapy and susceptible after therapy.
[c] Genotyping for 23S rRNA was not available for one strain.

not satisfactory. Nevertheless, the kappa coefficient appeared to be higher when the MIC breakpoint was >2 μg/ml (0.356), as shown in Table 5. The eradication rates for patients with the wild type, A2143G mutants, and A2142G mutants were 93.5% (130/139), 8.3% (1/12), and 0% (0/1), respectively.

Multivariate analysis of factors associated with treatment failure for levofloxacin-based and clarithromycin-based triple therapies. After adjustment for age, gender, and peptic ulcer disease, the presence of a *gyrA* mutation (OR, 7.0; 95% CI, 2.0 to 24.6; *P*, 0.002) and phenotypic resistance to "levofloxacin" with a MIC breakpoint at >4 μg/ml (OR, 8.0; 95% CI, 1.6 to 39.7; *P*, 0.011), >1 μg/ml (OR, 6.9; 95% CI, 1.7 to 27.2; *P*, 0.006), or >0.5 μg/ml (OR, 3.0; 95% CI, 1.1 to 8.3; *P*, 0.031) were significantly associated with treatment failure after levofloxacin-based triple therapy. Similarly, the presence of a 23S rRNA mutation (OR, 202.9; 95% CI, 22.5 to 1,831; *P*, <0.001) and phenotypic resistance with a MIC breakpoint at >2 μg/ml (OR, 25.6; 95% CI, 4.4 to 151; *P*, <0.001), >1 μg/ml (OR, 10.6; 95% CI, 2.6 to 43; *P*, 0.001), or >0.5 μg/ml (OR, 6.4; 95% CI, 1.8 to 23.2; *P*, 0.005) were significantly associated with treatment failure after clarithromycin-based triple therapy.

Changes in antibiotic resistance after levofloxacin and clarithromycin treatment. Among the 11 patients who failed levofloxacin-based therapy, genotypic and phenotypic resistance to levofloxacin were selected in 1 (9.1%) and 3 (27.3%) patients, respectively (Table 6). Among the 9 patients who failed clarithromycin-based therapy, genotypic and phenotypic resistance were selected in 4 (50%) and 4 (44.4%) patients, respectively (Table 6).

DISCUSSION

The results of this study showed that the concordance between genotypic and phenotypic resistance to levofloxacin was highest (kappa coefficient, 0.754) when the MIC breakpoint was >1 μg/ml. Genotypic resistance achieved fair agreement (kappa coefficient, 0.244) with therapeutic outcomes after levofloxacin-based triple therapy. The MIC breakpoint at 1 μg/ml gave better concordance with therapeutic outcomes than those at 0.5 μg/ml and 4 μg/ml. Taking these findings together, we concluded that the optimal breakpoint of the MIC test for levofloxacin resistance was >1 μg/ml. To the best of our knowledge, this is the first study to demonstrate that the genotypic resistance of strains to levofloxacin is associated with therapeutic outcomes after levofloxacin-based triple therapy. Our results further supported previous *in vitro* studies showing that the optimal breakpoint of the MIC test for levofloxacin resistance is >1 μg/ml.

The prevalence of primary phenotypic resistance to levofloxacin was higher than 10% in France (17.2%), Belgium (16.8%), Japan (15%), Hong Kong (11.5%), and Korea (10.4%) but lower than 10% in the United Kingdom (7.5% for ciprofloxacin), Slovenia (8.3%), and Taiwan (5.8%), as shown in Table 7 (1, 3, 5, 15–19, 25, 26, 36). *gyrA* mutations occurred at positions 87 and 91 in more than 90% of the strains with

TABLE 7. Prevalence of primary quinolone resistance and molecular characteristics of resistant and susceptible *Helicobacter pylori* strains

Country, yr	Reference	Prevalence of primary resistance (% [no. with resistance/total no.])	MIC breakpoint (μg/ml)	No., type of strains tested for *gyrA* mutation	No. (%) of strains with:			
					Mutation[a]			No mutation detected
					N87	D91	Other	
Taiwan, 2010	Present study	5.8 (18/312)	>1	18, resistant	8 (44.4)	8 (44.4)	0	2 (11.1)
				287, susceptible	3 (1)	1 (0.35)	1 (0.35)	282 (98.3)
Southern Taiwan, 2009	15	5.7 (12/210)	>1	12, resistant	4 (33.3)	8 (66.7)	3 (25)	0
Hong Kong, 2008	19	11.5 (22/191)	>1	22, resistant	6 (27.3)	10 (45.5)	1 (4.5)	5 (22.7)
Korea, 2005	17	10.4 (14/135)	>1	14, resistant	0	14 (100)	0	0
Belgium, 2006	1	16.8 (82/488)	>1	70, resistant	40 (57.1)	33 47.1)	N/A	0
Slovenia, 2009	16	8.3 (33/397)	>1 (?)[b]	33, resistant	12 (36.4)	19 (57.6)		
Japan, 2006	25	15 (76/507)	≥1	70, resistant	50 (71.4)	18 (25.7)	5 (7.1)	2 (2.9)
				50, susceptible	3 (6)	4 (8)	0	43 (86)
United Kingdom, 2009	5	7.5 (19/255) (ciprofloxacin)	≥1	18, resistant	4 (22.2)	12 (67)	1 (5.6)	1
				32, susceptible	3 (9)	0	0	29 (91)
France, 2007	3	17.2 (22/128)	>1	22, resistant	10 (45.5)	12 (54.5)	1 (4.5)	0
				106, susceptible	18 (17)	0	0	88 (83)

[a] N87, point mutation at codon 87 of the *gyrA* gene; D91, point mutation at codon 91 of the *gyrA* gene; N/A, not available.
[b] ?, the MIC breakpoint is not mentioned in that study.

phenotypic resistance (Table 7). This indicated that the sensitivities of molecular analysis of *gyrA* mutations at positions 87 and 91 in the detection of phenotypic resistance to levofloxacin were greater than 90% in all of these studies. Most of these studies used a breakpoint for levofloxacin resistance at either ≥1 μg/ml or >1 μg/ml based on the *in vitro* analysis of genotypic resistance. However, based on the results obtained in this study, we considered that >1 μg/ml might be the optimal breakpoint of the MIC test for levofloxacin resistance.

We also showed that the concordance between genotypic and phenotypic resistance to clarithromycin was highest (kappa coefficient, 0.694) when the MIC breakpoint was >2 μg/ml. Genotypic resistance achieved good agreement (kappa coefficient, 0.671) with therapeutic outcomes after clarithromycin-based triple therapy. The concordance between phenotypic resistance to clarithromycin and therapeutic outcomes was higher if the MIC breakpoint was >2 μg/ml (kappa coefficient, 0.356). Taking these findings together, we considered that the optimal breakpoint of the MIC test for clarithromycin resistance was >2 μg/ml. Many studies used a breakpoint of ≥1 μg/ml in the interpretation of MIC results for clarithromycin resistance based on previous *in vitro* studies. Previous clinical studies also showed a correlation between therapeutic outcomes and phenotypic resistance using the MIC breakpoint of 1 μg/ml. However, based on the results in our study, we considered that the MIC breakpoint of >2 μg/ml might be a better choice than ≥1 μg/ml.

The impact of genotypic resistance to clarithromycin on therapeutic outcomes after clarithromycin-based therapy has been reported in some studies (6–8, 10, 20). Whereas the A2143G mutation had been shown to be associated with a lower eradication rate, the impact of A2142G and A2142C mutations on the eradication rate has been controversial (6–8, 10, 20). Furuta et al. reported that the eradication rates were 48.3% and 87.3% in strains with and without the A2142G or A2143G mutation, respectively (10). However, De Francesco et al. reported that the eradication rates were 20%, 83%, and 86% for A2143G mutants, A2142C or A2142G mutants, and wild-type strains, respectively (7). Francavilla et al. reported similar results for children, with eradication rates of 50%, 80%, and 89% for A2143G mutants, A2142C or A2142G mutants, and wild-type strains, respectively (8). Recently, De Francesco et al. further showed that the concordance between genotypic and phenotypic resistance was 71.2% and that the eradication rate was lowest in the presence of concomitant phenotypic resistance and A2143G mutation (6). In this study, the eradication rates for Chinese patients with wild-type strains, A2143G mutants, and A2142G mutants were 93.5%, 8.3%, and 0% (no eradication for 1 patient), respectively. Our results were in agreement with previous studies showing that A2143G mutation confers a higher risk of treatment failure after clarithromycin-based triple therapy. However, since the A2142G mutation occurred in only one patient, it is difficult to assess the impact of A2142G on the eradication rate in this study.

Maeda et al. reported that the proportion of infection with mutant strains increased from 5/39 (13%) to 5/8 (63%) after clarithromycin-based triple therapy (22). The results in our study (Table 6) showed that selection of genotypic and phenotypic resistance to clarithromycin occurred in similar proportions of patients (50% and 44.4%, respectively) when they failed clarithromycin-based therapy. In contrast, selection of genotypic resistance to levofloxacin occurred in only 9.1% of patients, but selection of phenotypic resistance occurred in 27.3% of patients, when they failed levofloxacin-based therapy. Although the number of cases is too small to enable us to draw a conclusion, our results indicated that other mechanisms (such as efflux pump activation) for the selection of levofloxacin resistance might be responsible for this observation. Further studies on this issue are warranted.

The strengths of this study included the relatively large sample size and the analysis of the impacts of genotypic and phenotypic resistance on therapeutic outcomes within a randomized controlled trial. Although the numbers of genotypically resistant strains were only 12 for levofloxacin and 13 for clarithromycin in the assessment of therapeutic responses, the powers of this analysis were greater than 80%. This was attributed to the relatively large overall sample size. In this study, the ratios of genotypically susceptible to resistant strains were greater than 10 for both levofloxacin (133/12) and clarithromycin (139/13). For example, in the estimation of sample size for the levofloxacin group based on a p1 of 0.42, a p2 of 0.83, an allocation ratio of 10, a power of 0.8, and an α of 0.05 in two-sided analysis, we found that the sample sizes required were 10 for N1 and 100 for N2 (our actual sample sizes were 12 for N1 and 133 for N2). Moreover, our results showed that the 95% confidence intervals of eradication rates did not overlap. The eradication rates for *gyrA* mutant and wild-type strains were 41.7% (95% CI, 13.8% to 69.6%) and 82.7% (95% CI, 76.3% to 89.1%), respectively. Besides, Fisher's exact test, which is especially applicable to small sample sizes, was used in the analysis. Therefore, the overall sample size and power were sufficient to demonstrate the difference. Nevertheless, we believe that more studies on this important issue are still warranted.

There were also several novel findings in the present study. First, this is the first study to show that *gyrA* mutations correlated well with phenotypic resistance and that the presence of *gyrA* mutations is predictive of treatment failure with levofloxacin-based triple therapy. Second, our results supported an optimal MIC breakpoint for levofloxacin resistance of >1 μg/ml. Third, our results suggested that the optimal breakpoint for clarithromycin-based triple therapy was >2 μg/ml, rather than ≥1 μg/ml, as recommended in previous *in vitro* studies. We further showed that mutations at 23S rRNA appeared to correlate better with treatment outcomes than did the phenotype resistance after clarithromycin-based triple therapy. The results of this study provided the basis for the use of genotypic resistance as guidance in the selection of appropriate antibiotics for the treatment of *H. pylori* infection, especially for patients who have already failed two or more treatments. Finally, this was the first study to provide genotypic resistance findings before and after clarithromycin- and levofloxacin-based triple therapies.

Nevertheless, this study had some limitations. First, there were a number of discrepancies between susceptibility/resistance data and treatment outcomes, e.g., failure without mutation, especially for levofloxacin resistance. Explanations for the discrepancies include the possibilities that other sufficient causes of treatment failure, such as inadequate proton pump inhibitor (PPI) dosage, inadequate compliance, and polymor-

phisms in CYP2C19 and proinflammatory cytokine genes might account for treatment failure for patients infected with levofloxacin-susceptible strains (14, 37). Further studies to assess the impacts of the CYP2C19 polymorphism and the dosage of the PPI on the eradication rate of levofloxacin-based triple therapy are warranted. We also observed that eradication was successful for 41.7% (5/12) of the patients infected with levofloxacin-resistant strains. In these five patients, the strains were all susceptible to amoxicillin. Therefore, it is possible that these strains were eradicated with dual therapy (amoxicillin plus lansoprazole), because previous studies also showed that the eradication rate with dual therapy was around 40 to 50% (14). In addition, the prevalence of mixed infections with clarithromycin-susceptible and -resistant strains in the stomach has been reported to be about 20% (32). Therefore, the question of whether detection of a *gyrA* mutation in gastric biopsy specimens is also predictive of treatment outcomes after levofloxacin-based triple therapy still awaits further studies. In the present study, we determined genotypic resistance using isolated strains rather than gastric biopsy specimens. Therefore, the prevalence of mixed infection was not reported. Besides, genotyping using isolated strains is more time-consuming, because the procedure of culture, PCR amplification, and sequencing is lengthy and tedious compared to real-time PCR performed directly on gastric biopsy specimens. Recently, some new DNA strips have been shown to be accurate in the detection of *gyrA* mutations using gastric biopsy specimens (2). Further studies to assess the concordance of *gyrA* mutations detected by these new DNA strips using gastric biopsy specimens and therapeutic outcomes after levofloxacin-based therapy are warranted. Finally, the prevalence rates of clarithromycin and levofloxacin resistance reported here cannot represent the actual prevalences of antibiotic resistance in the general population, because this was a hospital-based study.

In conclusion, *gyrA* mutations in *H. pylori* strains correlate with phenotypic resistance and treatment outcomes after levofloxacin-based triple therapy. The results of this study support previous *in vitro* studies showing that the optimal MIC breakpoint for levofloxacin resistance by the agar dilution test is >1 μg/ml. Genotypic resistance (23S rRNA mutations) appeared to correlate better with therapeutic outcomes after clarithromycin-based triple therapy than the MICs from the agar dilution test. The optimal breakpoint for clarithromycin resistance by the agar dilution test might be >2 μg/ml rather than the previously recommended breakpoint (≥ 1 μg/ml). However, more studies are warranted before we can reach a consensus about the optimal breakpoint for clarithromycin resistance in *H. pylori* infection.

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