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ORIGINAL RESEARCH

Eradication Treatment of *Helicobacter pylori* Infection Based on Molecular Pathologic Antibiotic Resistance

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Background: Unfortunately, the eradication rate of *Helicobacter pylori* (*H. pylori*) treatment is markedly decreasing in recent years and the major reason is antibiotic resistance. Our study was designed to determine the effect and safety of *H. pylori* eradication treatment based on the molecular pathologic antibiotic resistance.

Methods: 261 patients were analyzed retrospectively, including 111 patients who were treated for the first time (one group as First-treated) and 150 patients who failed at least once in bismuth quadruple therapy (another group as Re-treatment). Antibiotic resistance was examined by Real-time PCR detection and conventional PCR and sequencing method. The eradication rate (ER) was compared per intention to treat (ITT) and per protocol (PP) between the two groups.

Results: The resistance rates to amoxicillin, clarithromycin, fluoroquinolone and tetracycline were 5.5%, 42.1%, 41.7% and 12.9% in the 111 first-treated patients, and 11.7%, 79.7%, 70.7% and 30.0% in the 150 re-treatment patients. The ERs in the ITT and PP analyses were 92.79% (95% CI, 87.98–97.60%, n=111) and 98.10% (95% CI, 95.48–100%, n=105), respectively, in the first-treated patients and 90.67% (95% CI, 86.01–95.32%, n=150) and 95.10% (95% CI, 91.57–98.64%, n=143), respectively, in the re-treatment patients. No significant differences were shown in the ERs between two group patients, and no serious adverse events were found.

Conclusion: *H. pylori* eradication treatment based on molecular pathologic antibiotic resistance showed good effect and safety in both first and re-treated patients.

Keywords: *Helicobacter pylori*, antibiotic resistance, eradication, first treatment, re-treatment, molecular pathology

Introduction

Helicobacter pylori (*H. pylori*), which infects more than 50% of the entire world's population, has become a global health problem associated with numerous benign and malignant gastric diseases, including chronic gastritis, gastric and duodenal ulcers, gastric cancer and gastric MALT (mucosa-associated lymphoid tissue) lymphomas.[1–3] In developing countries, *H. pylori* has been found in the stomachs of 70% to 90% of the people, and in Western nations, 25% to 50% of the total population carries *H. pylori*.[3] Plenty of studies over the past decades have demonstrated that eradication treatment of *H. pylori* infection is of great importance to *H. pylori*-associated diseases, such as ulcer healing, regression of MALT lymphomas and decreased cancer risk.[4]

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Four important consensus reports for the management of *H. pylori* infection have been published in the past 4 years, including Management of *H. pylori* infection- the Maastricht V/Florence Consensus Report, Kyoto global consensus report on *H. pylori* gastritis, the Toronto consensus for the treatment of *H. pylori* infection in adults and Fifth Chinese National Consensus Report on the management of *H. pylori* infection.[5–8] Eradication treatment regimens for *H. pylori* infection recommended by these guidelines included clarithromycin-based triple therapy (proton pump inhibitor/PPI + amoxicillin + clarithromycin), nonbismuth quadruple regimen (sequential, concomitant and hybrid therapy) and bismuth-containing quadruple therapy.[8]

For standard bismuth quadruple therapy, our Fifth National Consensus recommends seven therapy regimens and six antibiotics are commonly used, including amoxicillin, clarithromycin, levofloxacin, metronidazole, furazolidone and tetracycline.[8] However, unfortunately, the eradication rate of *H. pylori* treatment is markedly decreasing and the major reason is the antibiotic resistance.[5–8] As far as the primary resistance is concerned, recent studies showed that the resistance rates of *H. pylori* to clarithromycin, metronidazole and levofloxacin are 20–50%, 40–70% and 20–50%, respectively.[8–11] To improve the *H. pylori* eradication rate, performing the drug sensitivity test before treatment may have a comparative advantage.[8,12]

With the rapid development of molecular pathology, some molecular markers have been confirmed to be associated with antibiotic resistance, for example the 23S rRNA point mutations, including A2143G, A2142G and A2142C of domain V, which have been found in more than 90% of patients with clarithromycin resistance.[13–15] For the antibiotic resistance in *H. pylori* infection, bacterial culture and disc diffusion method are recommended by the Clinical Laboratory Standard Institute (CLSI).[16–18] However, compared with *H. pylori* culture, molecular pathological detection based on molecular markers, such as real-time polymerase chain reaction (PCR) and conventional PCR analysis, is a very simple and economic approach which also takes less time. Our study was designed to determine the effect and safety of *H. pylori* eradication therapy based on the results of molecular pathologic antibiotic resistance.

Materials and Methods

Patients, Inclusion and Exclusion Criteria

A total of 261 patients, who had been subjected to *H. pylori* treatment based on molecular pathologic antibiotic resistance, were retrospectively analyzed, including 111 patients who were treated for the first time (one group as First-treated) and 150 patients who failed at least once in bismuth quadruple therapy (another group as Re-treatment). The whole process of our research is in full compliance with the principles of the Declaration of Helsinki and our research project was approved by the Human Research Ethics Committee of our hospital. Written informed consent was obtained from all patients. Inclusion criteria included: (1) patients who were aged between 18 and 80 years old; (2) patients who were diagnosed with *H. pylori* infection by histopathological examination; and (3) patients who can be followed up successfully. Exclusion criteria included: (1) patients who had received antimicrobial therapy 2 months prior to gastroscope examination; (2) patients who had received upper gastrointestinal surgery; (3) patients who had been diagnosed with malignancies; and (4) patients who had serious diseases of heart, lung, liver or kidney.

Specimens Collection

For each patient, gastric body and/or antral biopsies were collected when they underwent upper gastrointestinal endoscopy. The gastric biopsies were prepared following the requirements of molecular pathological detection and should be sufficient for further polymerase chain reaction (PCR) and real-time PCR analysis.

PCR Detection of UreA and CYP2C19 Genotypes in Gastric Biopsies

For confirmation of the presence of *H. pylori* infection in gastric biopsies, UreA gene was detected by the conventional PCR method.[19,20] Primers for PCR amplification of UreA (F: 5′-GCCAATGGTAAATTAGTT-3′ R: 5′-CTCC TTAATTGTTTTTAC-3′)[20] was designed according to published study and synthesized at the NewLife Inc. (China). Moreover, the effect of PPI in *H. pylori* eradication therapy treatment can be affected by the CYP2C19 gene polymorphisms.[21] Three main alleles have been found, including CYP2C19*1 (wild-type), CYP2C19*2 (mutation in exon 5) and CYP2C19*3 (mutation in exon 4).[21,22] The sequences of primers were designed and synthesized for CYP2C19*2 (F: 5′-AATTACAACCAGAGCTTGGC-3′ R: 5′-TATCA CTTTCCATAAAAGCAAG-3′) and CYP2C 19*3 (F: 5′-TATTATTATCTGTTA ACTAATATGA-3′ R: 5′-ACTTCAGGGCTTGGTCAATA-3′).[21] They were detected by PCR amplification and the products were analyzed and sequenced at the NewLife Inc.

Real-Time PCR Detection of Mutations Associated with Clarithromycin Resistance

Three main 23S rRNA point mutations, including A2143G, A2142G and A2142C of domain V (Supplementary Table 1), have been found to be associated with more than 90% of patients with clarithromycin resistance.[13–15,20] A TaqMan real-time PCR allelic discrimination assay (NewLife, Beijing, China) was used to determine these point mutations, according to the description provided by previously published references.[15,20] Primers and probes were designed for both DNA wild type and mutated DNA, and the sequences are shown in Supplementary Table 2. The attachment of minor groove binder (MGB) to oligonucleotides and the shorter fluorogenic probes were used,[20,23] and the probes were labeled with two different fluorescent dyes (VIC dye and FAM dye). The real-time PCR detection was performed following the manufacturer's instructions. Positive and negative controls were used simultaneously, and all samples were detected for two times.

Conventional PCR and Sequencing for Detection of Mutations Associated with Amoxicillin, Fluoroquinolone and Tetracycline Resistance

As shown in Supplementary Table 1, gene mutations of PBP1, gyrA and 16S rRNA have been confirmed to be associated with *H. pylori* resistance to amoxicillin, fluoroquinolone and tetracycline, respectively.[24–28] Conventional PCR and sequencing method was used to detect these mutation sites, and Supplementary Table 3 shows the sequences of primers focused on target genes. All of the reactions were performed in a 25 μL volume and the PCR amplifications were performed using an MJR PTN-200 PCR machine (MJ Research Inc, Watertown, USA) according to manufacturer's directions. The PCR amplification products were separated by 2% agarose gel electrophoresis and further sequencing was done by the NewLife, China.

Eradication Treatment of *H. pylori* Infection Based on Molecular Pathologic Antibiotic Resistance

Before the initiation of eradication therapy, all the patients were diagnosed with *H. pylori* infection by histology examination (Warthin-Starry silver staining) and PCR detection of UreA gene. They were subjected to *H. pylori* eradication therapy for 14 days based on the results of antibiotic resistance by molecular pathologic tests. Four weeks after the end of eradication treatment, these patients were detected by UBT (^{13}C-urea breath test) and/or histopathological examinations to determine the status of *H. pylori* infection. Eight weeks after treatment, UBT were repeated for these patients to confirm the effect of *H. pylori* eradication therapy. At the end of treatment, liver and renal functions were examined, and adverse events were recorded and compared. The eradication rates were used as the main outcome measure.

Statistical Analyses

SPSS 20.0 for Windows (SPSS Inc., Chicago, IL, USA) were performed for statistical analyses. Chi-square test or Fisher's exact test was used to conduct the comparison of eradication rates, most of the patients' demographic characteristics, results of antibiotic resistance by molecular pathologic examinations, treatment regimens of *H. pylori* eradication, and frequencies of adverse events. The eradication rate in each group was analyzed per intention to treat and per protocol, with 95% confidence interval. A $P<0.05$ was considered significant and all P values quoted are two-sided.

Results

Patients' Demographics

Two hundred and sixty-one patients were analyzed retrospectively, including 111 first-treated patients and 150 retreatment patients (Figure 1). The patients' demographics are shown in Table 1. For the 261 patients, the mean age was 52.3 ± 15.4 years and 116 patients were male. Upper gastrointestinal endoscopy showed that 146 patients were diagnosed with chronic superficial gastritis (CSG) and 115 patients had chronic atrophic gastritis (CAG). In addition, peptic ulcer was found in 42 patients, including 34 with duodenal ulcer and 8 with gastric ulcer (Table 1).

The diagnosis of *H. pylori* infection was confirmed by two methods, including Warthin-Starry silver staining and PCR detection of UreA gene. Before the *H. pylori* eradication treatment, antibiotic resistance was determined and the treatment regimens were chosen based on the results of molecular pathologic examinations detection. The CYP2C19 gene polymorphisms were obtained for 237 patients, including 108 patients were rapid metabolizer (RM), 105 patients were intermediate metabolizer (IM) and 24 patients were poor metabolizer

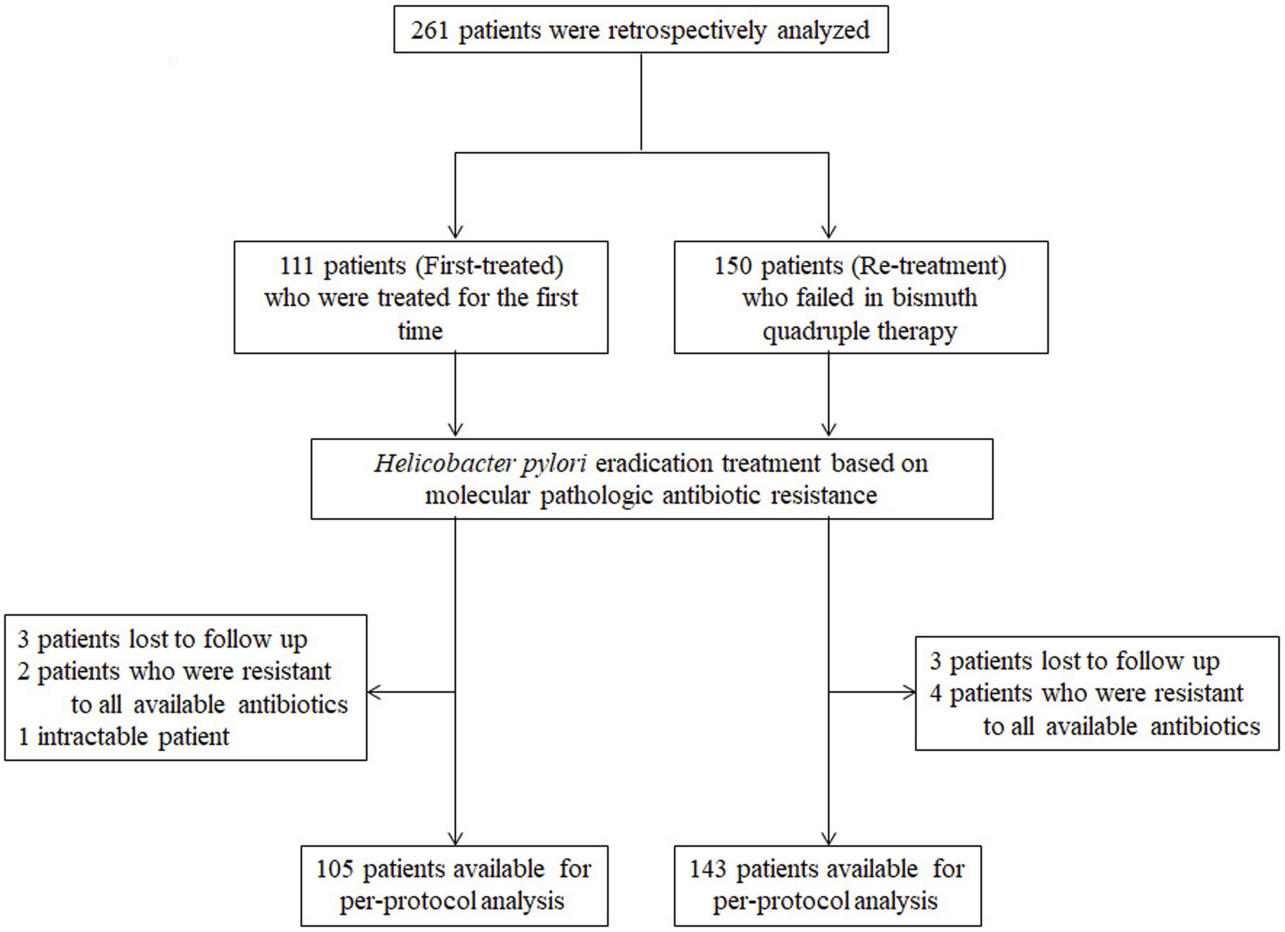

Figure 1 Flow diagram of this study.

(PM). As shown in Table 1, compared with patients who had not received prior *H. pylori* eradication treatment, those patients who failed in bismuth quadruple therapy had a higher percentage of female patients (42.3% vs 65.3%, P=0.033). No significant differences were found by univariable analysis for mean age, endoscopic findings and CYP2C19 gene polymorphisms (Table 1).

Antibiotic Resistance of *H. pylori* Infection by Molecular Pathologic Examinations

Four antibiotics commonly used for *H. pylori* eradication treatment were examined, including amoxicillin (AMPC/A), clarithromycin (CAM/C), fluoroquinolone (FLQ/F) and tetracycline (TET/T). The choice of antibiotics in treatment regimens of *H. pylori* eradication were based on antibiotic resistance by molecular pathologic examinations. Metronidazole (MNZ) and furazolidone (FZD) was not determined in our study and furazolidone could be also used for *H. pylori* eradication treatment considering that the percentage of furazolidone resistance was very low in our country.[29,30]

Table 2 and Figure 2 show the results of molecular pathologic examinations. The total resistance rates to amoxicillin, clarithromycin, fluoroquinolone and tetracycline were 9.0%, 63.6%, 58.5% and 22.2%, respectively. For the 111 first-treated patients, the rates of antibiotic resistance were 5.5% (AMPC), 42.1% (CAM), 41.7% (FLQ) and 12.9% (TET), respectively; whereas for the 150 re-treatment patients, the rates were 11.7% (AMPC), 79.7% (CAM), 70.7% (FLQ) and 30.0% (TET), respectively. Significant differences were found by univariable analysis for the rates of antibiotic resistance to clarithromycin (P<0.001), fluoroquinolone (P<0.001) and tetracycline (P=0.002) between those first-treated patients and re-treatment patients (Table 2).

Moreover, we analyzed the percentages of patients who had drug resistance to two antibiotics, and compared the differences between first-treated and re-

Table 1 Patients' Demographics

Characteristic Data	All Patients * (n=261)	First-Treated Patients* (n=111)	Re-Treatment Patients* (n=150)	*P* value
Mean age, mean ± SD, years	52.3 ± 15.4	53.9 ± 16.9	51.1 ± 14.2	0.159
Gender, no. (%)				<0.001
Male	116 (44.4)	64 (57.7)	52 (34.7)	
Female	145 (55.6)	47 (42.3)	98 (65.3)	
Endoscopic findings, no. (%)				
Chronic superficial gastritis	146 (55.9)	56 (50.5)	90 (60.0)	0.124
Chronic atrophic gastritis	115 (44.1)	55 (49.5)	60 (40.0)	—
Duodenal ulcer	34 (13.0)	16 (14.4)	18 (12.0)	0.567
Gastric ulcer	8 (3.1)	4 (3.6)	4 (2.7)	0.943
Diagnosis of Infection before treatment, no. (%)				
Pathology	261 (100)	111 (100)	150 (100)	—
Diagnosis of Infection after treatment, no. (%)				
UBT (^{13}C-urea breath test)	239 (91.6)	104 (93.7)	135 (90.0)	0.288
Pathology	30 (11.5)	7 (6.3)	23 (15.3)	0.024
CYP2C19 genotypes[a], no. (%)				0.150
Rapid metabolizer (RM)	108 (45.6)	51 (53.1)	57 (40.4)	
Intermediate metabolizer (IM)	105 (44.3)	36 (37.5)	69 (48.9)	
Poor metabolizer (PM)	24 (10.1)	9 (9.4)	15 (10.6)	
Lost to follow up, no. (%)	6 (2.3)	3 (2.7)	3 (2.0)	1.000

Notes: *A total of 261 patients were retrospectively analyzed, including 111 patients who were treated for the first time (one group as First-treated) and 150 patients who failed at least once in bismuth quadruple therapy (another group as Re-treatment). [a]Data were available in 237 patients, including 96 in first-treated and 141 in re-treated patients.

treatment patients (Table 2). Because the rate of amoxicillin resistance was low and only 23 patients had amoxicillin resistance, the numbers of patients who had antibiotic resistance to AMPC + CAM, AMPC + FLQ and AMPC + TET were 14, 13 and 10, respectively, and no significant differences were found between those patients. As shown in Table 2, compared with first-treated patients, those re-treatment patients had higher rates of antibiotic resistance to CAM + FLQ (25.0% vs 58.0%, P<0.001), CAM + TET (9.9%

Table 2 Antibiotic Resistance of *H. pylori*: Molecular Pathologic Results

Resistance to Antibiotic	Mutation Sites	All Patients* (n=261)	First-Treated Patients* (n=111)	Re-Treatment Patients* (n=150)	*P* value
Amoxicillin (A)[a], no. (%)	PBP1	23 (9.0)	6 (5.5)	17 (11.7)	0.083
Clarithromycin (C)[b], no. (%)	23S rRNA	159 (63.6)	45 (42.1)	114 (79.7)	<0.001
Fluoroquinolone (F)[c], no. (%)	gyrA	151 (58.5)	45 (41.7)	106 (70.7)	<0.001
Tetracycline (T)[d], no. (%)	16S rRNA	49 (22.2)	13 (12.9)	36 (30.0)	0.002
AMPC + CAM[e], no. (%)		14 (5.7)	5 (4.7)	9 (6.4)	0.566
AMPC + FLQ[f], no. (%)		13 (5.2)	3 (2.8)	10 (6.9)	0.147
AMPC + TET[g], no. (%)		10 (4.6)	3 (3.0)	7 (5.9)	0.470
CAM + FLQ[h], no. (%)		109 (44.1)	26 (25.0)	83 (58.0)	<0.001
CAM + TET[i], no. (%)		39 (17.8)	10 (9.9)	29 (24.6)	0.005
FLQ + TET[j], no. (%)		34 (15.5)	8 (8.1)	26 (21.7)	0.006
≥ 3 (A/C/F/T), no. (%)		43 (16.5)	12 (10.8)	31 (20.7)	0.034

Notes: *A total of 261 patients were retrospectively analyzed, including 111 patients who were treated for the first time (one group as First-treated) and 150 patients who failed at least once in bismuth quadruple therapy (another group as Re-treatment). Data were available in [a]255 (110+145), [b]250 (107+143), [c]258 (108+150), [d]221 (101+120), [e]246 (106+140), [f]252 (107+145), [g]219 (101+118), [h]247 (104+143), [i]219 (101+118), and [j]219 (99+120) patients. The numbers before the brackets indicate the total available cases in the two groups.

Abbreviations: AMPC (A), amoxicillin; CAM (C), clarithromycin; FLQ (F), fluoroquinolone; TET (T), tetracycline.

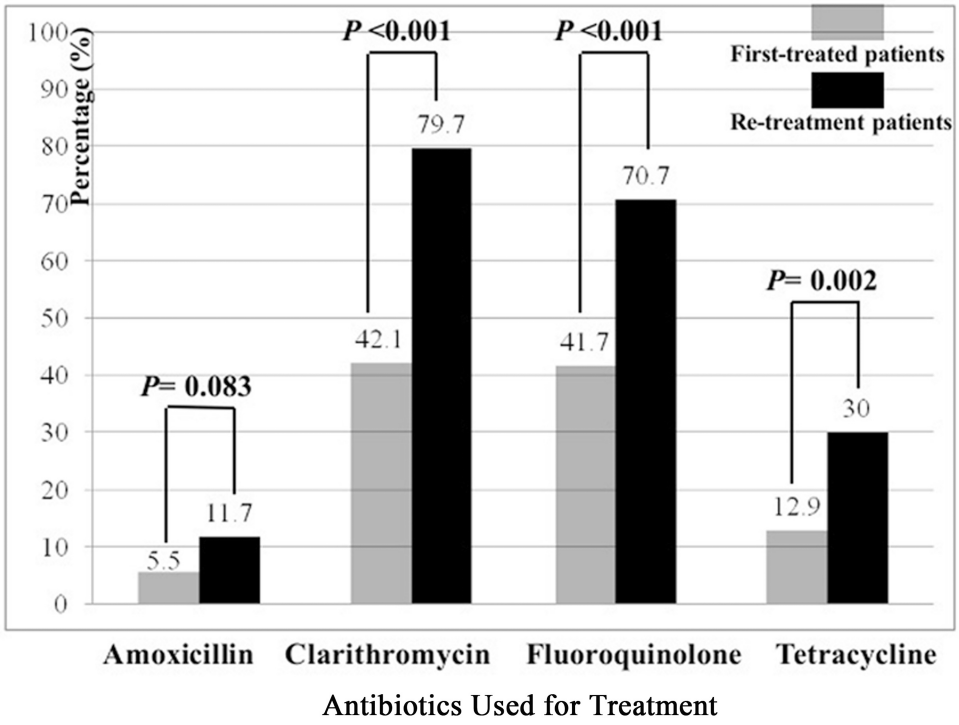

Figure 2 The prevalence of antibiotic resistance determined by molecular pathologic tests. The gray histogram indicates the percentage of antibiotic resistance in first-treated patients who were treated for the first time, and the black histogram indicates the percentage in re-treatment patients who failed in bismuth quadruple therapy. As demonstrated, the resistance rates to amoxicillin, clarithromycin, fluoroquinolone and tetracycline were 5.5%, 42.1%, 41.7% and 12.9%, in first-treated patients, whereas 11.7%, 79.7%, 70.7% and 30.0%, respectively, in re-treatment patients.

vs 24.6%, P=0.005), and FLQ + TET (8.1% vs 21.7%, P=0.006).

Treatment Regimen of *H. pylori* Eradication

For all the 261 patients, whether they were treated for the first time or re-treated, bismuth quadruple therapy was used and the two antibiotics were chosen based on the results of antibiotic resistance by molecular pathologic examinations. In our study, seven patients were not treated by any regimen because 6 patients were resistant to all available antibiotics and one patient was intractable (Figure 1). In the 254 patients who received *H. pylori* eradication therapy, four treatment regimens were used for more than 90% of them (Table 3), including AMPC + CAM (59 patients, 23.2%), AMPC + FLQ (65, 25.6%), AMPC + TET (62, 24.4%), and AMPC + FZD (43, 16.9%). Between first-treated and re-treated patients, significant differences were found for all these four regimens (P<0.001, P=0.015, P=0.001 and P=0.014).

Eradication Rates of *H. pylori* Infection

As shown in Figure 1, 261 patients were included in the intention to treat (ITT) analysis, and after our study was completed, 248 patients were included in the per-protocol (PP) analysis. Six patients were lost to follow up. The eradication rates in the ITT and PP analyses were 92.79% (95% confidence interval/CI, 87.98–97.60%, n=111) and 98.10% (95% CI, 95.48–100%, n=105), respectively, in the first-treated patients and 90.67% (95% CI, 86.01–95.32%, n=150) and 95.10% (95% CI, 91.57–98.64%, n=143), respectively, in the re-treatment patients (Figure 3). No significant differences were found in the ITT (P=0.445) and PP (P=0.309) analyses.

Safety and Comparison of Adverse Events

Adverse events were recorded, including diarrhea, black stool, abdominal pain, heart burn, flatulence, nausea/vomiting, belching, skin rash, headache, dysgeusia, fever, constipation, anorexia, fatigue and dizziness. Besides the dysgeusia induced by the drug of clarithromycin, no significant differences were observed (Table 4).

Discussion

Considering the markedly decreased eradication rate of *H. pylori* infection and rapid development of molecular pathology, our study was designed to determine the effect and safety of *H. pylori* eradication therapy regimens based on the results of antibiotic resistance by molecular pathologic examinations. Our study included 111 patients who had not

Table 3 Treatment Regimen of *H. pylori* Eradication

Eradication Regimen [#]	All Patients* (n=254)	First-Treated Patients (n=108)	Re-Treatment Patients (n=146)	*P* value
AMPC + CAM, no. (%)	59 (23.2)	40 (37.0)	19 (13.0)	<0.001
AMPC + FLQ, no. (%)	65 (25.6)	36 (33.3)	29 (19.9)	0.015
AMPC + TET, no. (%)	62 (24.4)	15 (13.9)	47 (32.2)	0.001
AMPC + FZD, no. (%)	43 (16.9)	11 (10.2)	32 (21.9)	0.014
Other regimens[§], no. (%)	25 (9.8)	6 (5.6)	19 (13.0)	0.049

Notes: *Two hundred and sixty-one patients were retrospectively analyzed and 7 patients had not been treated by any regimen because 6 patients were resistant to all available antibiotics and one patient was intractable. [#] For all patients, whether they were treated for the first time or re-treated, bismuth quadruple therapy was used and the two antibiotics were shown in this Table. [§]Twenty-five patients were treated by other regimens, including CAM + FLQ for 5 patients, CAM + FZD for 2 patients, CAM + TET for 2 patients, FLQ + FZD for 7 patients, FLQ + TET for 5 patients, and FZD + TET for 4 patients.
Abbreviations: AMPC, amoxicillin; CAM, clarithromycin; FLQ, fluoroquinolone; FZD, Furazolidone; TET, tetracycline.

received prior eradication treatment and 150 patients who failed at least once in bismuth quadruple therapy, and found that good effect and safety could be obtained in both first-treated and re-treatment patients when treatment regimens were determined based on the molecular pathologic antibiotic resistance.

Primary resistance and secondary resistance are the two kinds of *H. pylori* antibiotic resistance, and secondary resistance means drug resistance after treatment failure.[8] The Fifth Chinese National Consensus Report on the management of *H. pylori* infection mentioned that the primary resistance rates to clarithromycin, metronidazole and levofloxacin are 20–50%, 40–70% and 20–50%, respectively.[8] In our study, the primary resistance rates to clarithromycin and fluoroquinolone are 42.1% and 41.7%, which are similar to the results of previous reports.

For the secondary resistance, our study demonstrates that the resistance rates to clarithromycin and fluoroquinolone are 79.7% and 70.7% in the patients who failed at least once in bismuth quadruple therapy. Compared with primary resistance rates, these numbers showed that the acquired drug resistances to clarithromycin and fluoroquinolone were easier than to other antibiotics, for example amoxicillin and tetracycline. The Maastricht V/Florence Consensus Report mentions that, when the resistance rate to clarithromycin is more than 15%, PPI-clarithromycin-containing triple therapy without prior susceptibility testing should be abandoned.[5] Considering the high clarithromycin and fluoroquinolone resistance in our country,[8] performing the drug sensitivity test before treatment may have a comparative advantage.

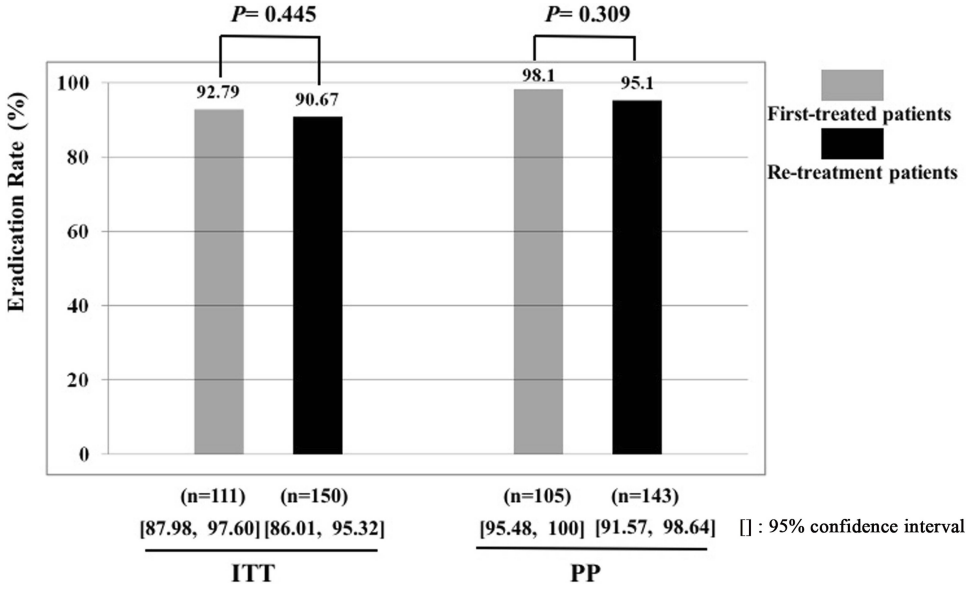

Figure 3 Eradication rates of *H. pylori* based on molecular pathologic antibiotic resistance. The gray and black histograms indicate the eradication rates in first-treated and re-treatment patients.
Abbreviations: ITT, intention-to-treat analysis; PP, per-protocol analysis.

Table 4 Safety and Comparison of Adverse Events

Adverse Event	All Patients* (n=254)	First-Treated Patients (n=108)	Re-Treatment Patients (n=146)	*P* value
Diarrhea, no. (%)	11 (4.3)	6 (5.6)	5 (3.4)	0.608
Black stool, no. (%)	246 (96.9)	105 (97.2)	141 (96.6)	1.000
Nausea/Vomiting, no. (%)	8 (3.1)	5 (4.6)	3 (2.1)	0.425
Abdominal pain, no. (%)	9 (3.5)	4 (3.7)	5 (3.4)	1.000
Flatulence, no. (%)	4 (1.6)	2 (1.9)	2 (1.4)	1.000
Belching, no. (%)	5 (2.0)	2 (1.9)	3 (2.1)	1.000
Heart burn, no. (%)	7 (2.8)	3 (2.8)	4 (2.7)	1.000
Skin rash, no. (%)	3 (1.2)	1 (0.9)	2 (1.4)	1.000
Headache, no. (%)	7 (2.8)	4 (3.7)	3 (2.1)	0.685
Dysgeusia, no. (%)	68 (26.8)	43 (39.8)	25 (17.1)	<0.001
Fever, no. (%)	4 (1.6)	2 (1.9)	2 (1.4)	1.000
Constipation, no. (%)	4 (1.6)	1 (0.9)	3 (2.1)	0.838
Anorexia, no. (%)	5 (2.0)	3 (2.8)	2 (1.4)	0.733
Fatigue, no. (%)	6 (2.4)	2 (1.9)	4 (2.7)	0.966
Dizziness, no. (%)	6 (2.4)	3 (2.8)	3 (2.1)	1.000

Notes: *Two hundred and sixty-one patients were retrospectively analyzed and 7 patients had not been treated by any regimen because 6 patients were resistant to all available antibiotics and one patient was intractable.

A wealth of research suggests that *H. pylori* resistance to clarithromycin is the most important factor for eradication failure.[31,32] Are there any definite time span and/or external factors which influence led to the occurrence of clarithromycin resistance and 23S rRNA point mutations? One study was performed to evaluate the primary clarithromycin resistance and associated mutations in children in Poland during 1998–2000.[33] The authors found that most of the primary resistance was caused by an A2143G mutation and the prevalence of clarithromycin resistance in children was increased from 17.0% to 23.5% over the 3 years.[33] Some researchers from Spain showed that clarithromycin resistance was significantly associated with patients who were previously treated, with patients born in Spain, and with pediatric patients.[34] In addition, they found the relationship between clarithromycin resistance, the vacA s2/m2 genotype and cagA negative-expression.[34] Hakemi Vala et al deduced that the primary resistance could be related to cross-reactivity between other macrolides.[32]

As shown in Table 1, the percentage of female patients who had failed reaction to bismuth quadruple therapy was higher than male patients. It is in line with some previous studies.[35,36] One study showed that female gender (OR, 1.73; 95% CI, 1.15 to 4.25) and clarithromycin resistance (OR, 19.13; 95% CI, 9.35 to 35.09) were strongly associated with eradication failure.[35] Another study also showed that female gender and smoking were associated with the treatment failure.[36] The reason may be also related to clarithromycin resistance and point mutation. Research suggested that the 2143G point mutation in the 23S rRNA was a risk factor for eradication failure and the presence of 2143G was significantly associated with female sex.[37]

Our study also shows that the primary and secondary resistance rates of *H. pylori* to amoxicillin are 5.5% and 11.7%, whereas these rates to tetracycline are 12.9% and 30.0%, respectively. A prospective serial study conducted in Beijing of China found that the primary resistance rates of *H. pylori* were 6.7% and 4.9% to amoxicillin and tetracycline.[9] Another study conducted in Shanghai of China reported the amoxicillin resistance rate was 8.3%.[38] For amoxicillin resistance, we obtained the similar result; however, for tetracycline, the rate is slightly higher than others and the reason may be associated with the patient population and prior used same class drugs.[8] Because the primary resistance rates to amoxicillin and tetracycline remain low in our country, it may be not necessary to consider the drug sensitivity test before use.[8]

For the effect and safety of *H. pylori* eradication treatment, our study showed that the ERs in the ITT and PP analyses were 92.79% and 98.10%, respectively, in the first-treated patients and 90.67% and 95.10%, respectively, in the re-treated patients. We compared our results with others from different geographical regions.[39–41] One study

which was conducted in the Japan used vonoprazan-based eradication therapy and found that the ERs were 91.24% (427/468) and 95.45% (42/44), respectively, for the first-line therapy and the second-line therapy.[39] The researchers from the Iran evaluated the efficacy of bismuth-based quadruple therapy and showed that the ERs in the ITT and PP analyses were 90.2% and 91.9%, respectively.[40] Another study from the Northwest Ethiopia adopted the standard triple therapy and reached the overall eradication rate of 90.3% (379/421).[41] Compared with these studies, good effect and safety were obtained in our study for both first-treated and re-treatment patients, although the high resistance rates to some antibiotics were found, and more than half the patients failed in bismuth quadruple therapy. Our results indicated that satisfactory ER could be also obtained based on the molecular pathologic antibiotic resistance.

The best effort was that the antibiotic resistance of *H. pylori* was determined by molecular pathologic examinations, including Real-time PCR detection and conventional PCR and sequencing method, especially the TaqMan real-time PCR allelic discrimination assay which was used to detect the clarithromycin resistance. Compared with the E-test and agar dilution method,[42,43] the molecular pathologic examinations gave the similar results. Moreover, satisfactory eradication rates were obtained which indicated that these examinations could be used routinely.

The first limitation may be that our study was conducted in one large-scale medical center of China and the selection bias may not be avoided. The patient population may be different from those patients treated in the small and medium-sized hospitals, not to speak of the hospitals located in remote regions and ethnic minority regions. In addition, a meaningful portion of the fees for molecular pathologic examinations were afforded by the patients. The second limitation may be that only 261 patients with *H. pylori* infection were included in our study. We wish that our results and conclusion could be validated in more patients, more hospitals and more studies.

In conclusion, good effect and safety are obtained in both first-treated and re-treated patients when treatment regimens were determined based on the molecular pathologic antibiotic resistance, although more prospective studies are required. Compared with *H. pylori* culture and disc diffusion method, molecular pathological detection based on molecular markers is a very simple and economic approach which also takes less time, we hope that molecular pathologic antibiotic resistance could be used widely.

Ethics Approval and Informed Consent

The whole process of our research is in full compliance with the principles of the Declaration of Helsinki and our research project was approved by the Human Research Ethics Committee of China-Japan Friendship Hospital, Ministry of Health. Written informed consent was obtained from all patients.

Author Contributions

All authors contributed toward data analysis, drafting and revising the paper, gave final approval of the version to be published and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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