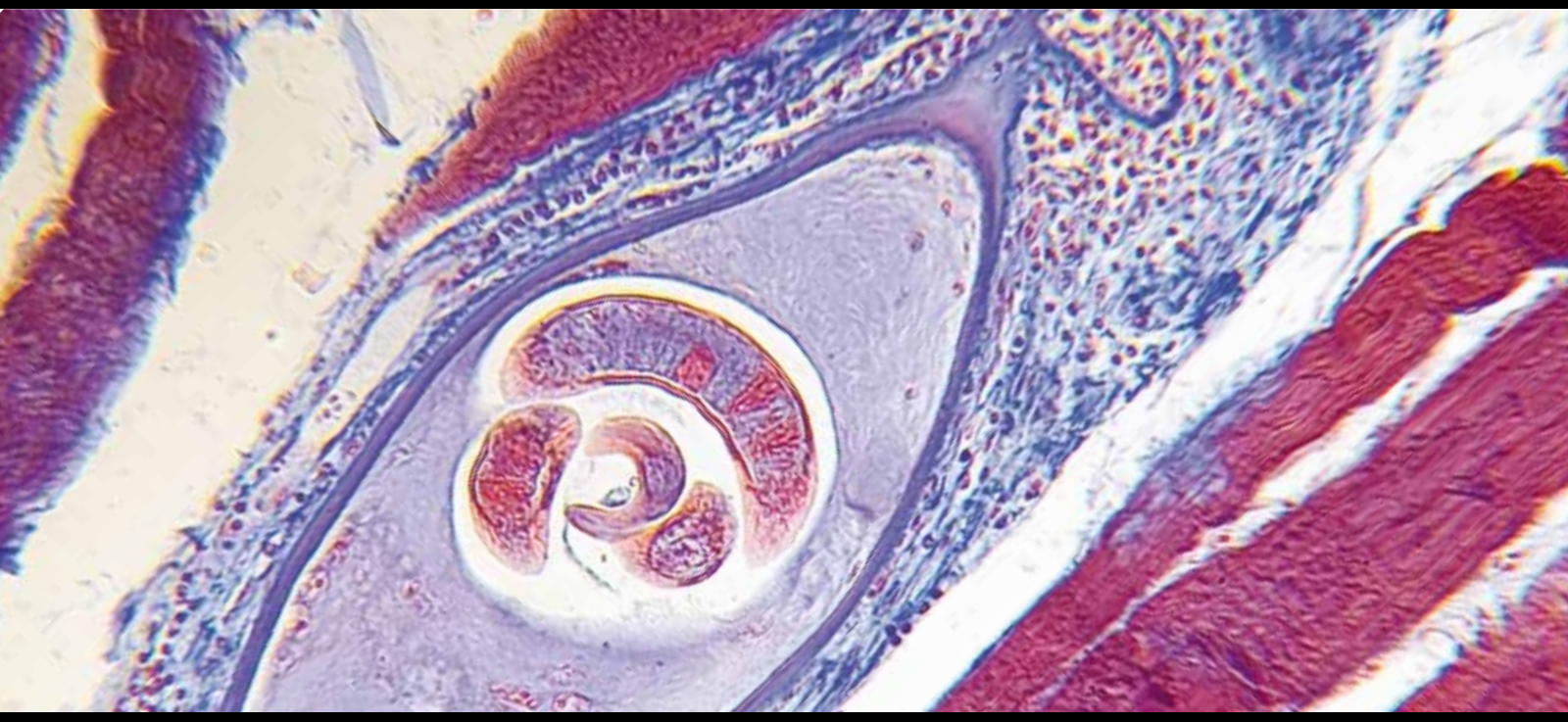
Gastroenterology Research and Practice

H. pylori Eradication Therapy

Guest Editors: Ping-I Hsu, Yoshio Yamaoka, Javier P. Gisbert, and Deng-Chyang Wu



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Editorial Board

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Editorial
H. pylori Eradication Therapy

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As a general rule for the treatment of infectious diseases, clinicians should prescribe anti-*H. pylori* regimens that have a per-protocol eradication rate ≥90%. However, the eradication rate of the standard triple therapy recommended by most guidelines has generally declined to unacceptable levels (i.e., 80% or less) recently. The reasons for this fall in efficacy with time are uncertain but may relate to the increasing incidence of clarithromycin-resistant strains of *H. pylori,* poor compliance, and rapid metabolism of proton pump inhibitor (PPI) [1, 2]. Recently, several treatment regimens have emerged to cure *H. pylori* infection. The novel first-line anti-*H. pylori* therapies include sequential therapy [3], concomitant quadruple therapy [4], hybrid (dual-concomitant) therapy [5], and bismuth-containing quadruple therapy.

After the failure of standard triple therapy, a bismuth-containing quadruple therapy regimen comprising a PPI, bismuth, metronidazole, and tetracycline as a second-line therapy is recommended. Recently, a triple therapy with the combination of a PPI, levofloxacin, and amoxicillin has been proposed as an alternative to the standard rescue therapy and can achieve a higher eradication rate than a bismuth-containing quadruple therapy in some regions. Most guidelines suggest that patients requiring a third-line therapy should be referred to medical center and treated according to the antibiotic susceptibility test. However, it has been reported that the sensitivity of culture is less than 60%. Additionally, in vitro antimicrobial sensitivity does not necessarily lead to eradication in vivo and vice versa.

The main focus of the special issue is on the recent advances in the treatment of *H. pylori* infection. This special issue reviews the novel first-line eradication regimens with a per-protocol eradication rate exceeding 90%. In addition, the emerging rescue therapies for the second-line and third-line therapies are also discussed.

In the paper entitled "*Pathogenesis of Helicobacter pylori-related gastroduodenal diseases from molecular epidemiological studies*," Y. Yamaoka presents African and Asian enigmas regarding high prevalence of *H. pylori* infection and low incidence of gastric cancer. This discrepancy could be explained in part by different types of *H. pylori* virulence factors, especially CagA, VacA, OipA, and DupA.

In the paper entitled "*Recent insights into antibiotic resistance in Helicobacter pylori eradication*," W. Wu et al. present the antibiotic resistance rates in different continental areas and the impact of antibiotic resistances on the eradication of *H. pylori*.

In the paper entitled "*Variability in prevalence of Helicobacter pylori strains resistant to clarithromycin and levofloxacin in Southern Poland*," E. Karczewska et al. compared the primary and secondary resistance of *Helicobacter pylori* strains isolated between 2006–2008 and 2009–2011 to clarithromycin and levofloxacin in Southern Poland. The data indicated the increasing amount of resistant *H. pylori* strains isolated from patients in Southern Poland to levofloxacin, with a simultaneous decreasing number of resistant strains to clarithromycin.

In the paper entitled "*The optimal first-line therapy of Helicobacter pylori infection in year 2012*," C.-H. Kuo et al. review the literature about first-line therapies for *H. pylori* infection in the recent years. The efficacies of emerging

first-line therapies including sequential therapy, concomitant therapy and hybrid therapy are well assessed.

In the paper entitled "*Helicobacter pylori eradication therapies in the era of increasing antibiotic resistance: a paradigm shift to improved efficacy,*" S. D. Georgopoulos et al. present critical issues regarding the currently available means for the management of *H. pylori* infection. The existing evidences of their clinical validation and widespread applicability are also discussed.

In the paper entitled "*7-day nonbismuth-containing concomitant therapy achieves a high eradication rate for Helicobacter pylori in Taiwan,*" S.-S. Kao et al. report that 7-day concomitant therapy achieves a very high eradication rate for *H. pylori* infection in Taiwan. The novel therapy is well tolerated. Drug compliance is an important clinical factor influencing its treatment efficacy.

In the paper entitled "*Comparison between single-dose esomeprazole- and pantoprazole-based triple therapy on the effectiveness for Helicobacter pylori eradication in Taiwanese population,*" H.-Y. Shih et al. show a higher eradication rate in esomeprazole containing triple therapy than pantoprazole containing triple therapy. The incidence of adverse effects and the compliance between two therapies are comparable.

In the paper entitled "*Rescue therapy for Helicobacter pylori infection 2012,*" J. P. Gisbert reviews current rescue therapies for *H. pylori* infection. He suggests that the choice of a "rescue" treatment depends on which treatment is used initially. If a first-line clarithromycin-based regimen was used, a second-line metronidazole-based treatment (quadruple therapy) may be used afterwards, and then a levofloxacin-based combination would be a third-line rescue option. Alternatively, a quadruple regimen may be reserved as a third rescue option if levofloxacin-based combination is used as a second-line therapy.

In the paper entitled "*Impact of Lactobacillus reuteri supplementation on anti-Helicobacter pylori levofloxacin-based second-line therapy,*" V. Ojetti et al. assessed the efficacy of *L. reuteri* supplementation in *H. pylori* eradication and in preventing gastrointestinal side effects during a second-line levofloxacin triple therapy. The data indicate that *L. reuteri* supplementation increases the eradication rate while reducing the incidence of the most common side effects associated with antibiotic therapy in a second-line treatment.

In the paper entitled "*Culture method and PCR for the detection of Helicobacter pylori in drinking water in Basrah Governorate Iraq,*" A. A. Al-Sulami et al. examined the isolated *H. pylori* from drinking water in Basrah, Iraq, on modified Columbia urea agar and HP media using the MDCS method and then confirmed that by conventional biochemical tests and 16S. rRNA PCR. The data indicate that isolating *H. pylori* from drinking water, tap and reverse osmosis water samples, by the culture method and consequent identification by biochemical tests and PCR represent a clear signal for the presence of this dangerous pathogen in the consumable water.

In the paper entitled "*A real world report on intravenous high-dose and non-high-dose proton-pump inhibitors therapy in patients with endoscopically treated high-risk peptic ulcer bleeding*," L.-S. Lu et al. conducted a retrospective case-controlled study to investigate the real world experiences in prescribing high-dose and non-high-dose proton-pump inhibitor therapy for preventing rebleeding after endoscopic treatment of high-risk peptic ulcer bleeding, a life-threatening complication of *H. pylori*-related disease. This study suggests that the effect of intravenous high-dose pantoprazole may not be superior to non-high-dose regimen in reducing rebleeding in high-risk peptic ulcer bleeding. However, selection bias may exist in a high-dose group caused by clinicians' decision on PPI dosage in patients with more severe diseases or with less manageable bleeding ulcers.

Ping-I Hsu
Nan-Jing Peng

References

[1] S. K. Chuah, F. W. Tsay, P. I. Hsu, and D. C. Wu, "A new look at anti-*Helicobacter pylori* therapy," *World Journal of Gastroenterology*, vol. 17, no. 35, pp. 3971–3975, 2011.

[2] D. Y. Graham and S. Akiko, "New concepts of resistance in the treatment of *Helicobacter pylori* infections," *Nature Clinical Practice Gastroenterology & Hepatology*, vol. 5, pp. 321–331, 2008.

[3] A. Zullo, V. De Francesco, C. Hassan, S. Morini, and D. Vaira, "The sequential therapy regimen for *Helicobacter pylori* eradication: a pooled-data analysis," *Gut*, vol. 56, no. 10, pp. 1353–1357, 2007.

[4] D. C. Wu, P. I. Hsu, J. Y. Wu et al., "Sequential and concomitant therapy with four drugs is equally effective for eradication of *H. pylori* infection," *Clinical Gastroenterology and Hepatology*, vol. 8, no. 1, pp. 36–41, 2010.

[5] P. I. Hsu, D. C. Wu, J. Y. Wu, and D. Y. Graham, "Modified sequential *Helicobacter pylori* therapy: proton pump inhibitor and amoxicillin for 14 days with clarithromycin and metronidazole added as a quadruple (Hybrid) therapy for the final 7 days," *Helicobacter*, vol. 16, no. 2, pp. 139–145, 2011.

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Letter to the Editor

High or Nonhigh Doses of Proton Pump Inhibitors for Patients with Peptic Ulcer Bleeding?

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I read with interest the article entitled "A real world report on intravenous high-dose and nonhigh-dose proton pump inhibitors therapy in patients with endoscopically treated high-risk peptic ulcer bleeding" [1]. In this study, Lu et al. retrospectively analyzed patients receiving nonhighdose (80 mg pantoprazole i.v. bolus followed by i.v. 80 mg per day for 3 days) and high-dose proton pump inhibitors (PPI, 80 mg pantoprazole i.v. bolus followed by 8 mg per hr for 3 days) after obtaining initial hemostasis. After performing case-control matching, they found no statistical difference between the high-dose and nonhigh-dose groups. Therefore, they suggest that both doses of PPI were similar in reducing rebleeding in high-risk patients after successful endoscopic therapy.

This conclusion is different from that in the consensus conference and also in our study [2, 3]. There are several key points that deserve to be mentioned with regards to this study. Lu's analysis is a retrospective study. Therefore, some important clinical variables could not be adjusted evenly between both groups. As a practice, doctors tend to use a high-dose PPI in high-risk patients after obtaining initial hemostasis. This point is demonstrated in Lu's study, Table 3. The number of patients with shock is more in the high-dose PPI group than that in the nonhigh-dose group (61.4% versus 46%).

In Lu's study, the rebleeding rate for the high-dose group (19/70, 27.1%) is much higher than our series (2/50, 4%) and another report (8/120, 6.7%) [2, 4]. This phenomenon may be explained by the high percentage of patients with renal impairment (35/70, 50%). The high proportion of enrolled patients with renal impairment is unusual as compared to the past reports. Because three days after endoscopic therapy are a critical period, high-dose PPI is needed for these three days. After three days, patients usually receive oral intake. However, in Lu's study, they still gave 80 mg i.v. per day after three days. Thus, utilizing such therapy may waste some economic resources.

In recent few years, there have been some articles supporting the use of low-dose PPI in high-risk patients after endoscopic hemostasis [5]. Many of these articles have pitfalls related to study design, such as the inclusion of patients with low-risk stigmata and the injection of epinephrine alone [6]. In vitro studies revealed that the acid environment impairs platelet function and clot stabilization [7]. Therefore, elevation of intragastric pH is mandatory to prevent rebleeding in patients with peptic ulcer bleeding, which has been confirmed in the consensus conference [2]. In our previous study, we obtained a markedly low rebleeding rate (4%) with a high-dose IV PPI [3]. Further, we found that different IV doses of PPIs have different rebleeding rates

(omeprazole 160 mg/day: 9%, 6/67; 80 mg/day: 21.2%, 14/66) [8].

Clearly, there is a bit of a grey zone in identifying stigmata of recent hemorrhage (SRH) [9]. Misinterpretation of SRH can occur for a number of reasons, such as doctors' experience and academic judgement. Therefore, one strict design (double blind study) is favored in such a clinical trial.

References

[1] L. S. Lu, S. C. Lin, C. M. Kuo et al., "A real world report on intravenous high-dose and non-high-dose proton-pump inhibitors therapy in patients with endoscopically treated high-risk peptic ulcer bleeding," *Gastroenterology Research and Practice*, vol. 2012, Article ID 858612, 7 pages, 2012.

[2] A. N. Barkun, M. Bardou, E. J. Kuipers et al., "International consensus recommendations on the management of patients with nonvariceal upper gastrointestinal bleeding," *Annals of Internal Medicine*, vol. 152, no. 2, pp. 101–113, 2010.

[3] H. J. Lin, W. C. Lo, F. Y. Lee, C. L. Perng, and G. Y. Tseng, "A prospective randomized comparative trial showing that omeprazole prevents rebleeding in patients with bleeding peptic ulcer after successful endoscopic therapy," *Archives of Internal Medicine*, vol. 158, no. 1, pp. 54–58, 1998.

[4] J. Y. W. Lau, J. J. Y. Sung, K. K. C. Lee et al., "Effect of intravenous omeprazole on recurrent bleeding after endoscopic treatment of bleeding peptic ulcers," *The New England Journal of Medicine*, vol. 343, no. 5, pp. 310–316, 2000.

[5] C. H. Wang, M. H. M. Ma, H. C. Chou et al., "High-dose vs non-high-dose proton pump inhibitors after endoscopic treatment in patients with bleeding peptic ulcer: a systematic review and meta-analysis of randomized controlled trials," *Archives of Internal Medicine*, vol. 170, no. 9, pp. 751–758, 2010.

[6] H. J. Lin, "High or low doses of PPIs for patients with peptic ulcer bleeding?" *Archives of Internal Medicine*, vol. 170, no. 18, pp. 1699–1700, 2010.

[7] F. W. Green Jr., M. M. Kaplan, L. E. Curtis, and P. H. Levine, "Effect of acid and pepsin on blood coagulation and platelet aggregation. A possible contributor to prolonged gastroduodenal mucosal hemorrhage," *Gastroenterology*, vol. 74, no. 1, pp. 38–43, 1978.

[8] H. J. Lin, W. C. Lo, Y. C. Cheng, and C. L. Perng, "Role of intravenous omeprazole in patients with high-risk peptic ulcer bleeding after successful endoscopic epinephrine injection: a prospective randomized comparative trial," *American Journal of Gastroenterology*, vol. 101, no. 3, pp. 500–505, 2006.

[9] J. Y. W. Lau, J. J. Y. Sung, A. C. W. Chan et al., "Stigmata of hemorrhage in bleeding peptic ulcers: an interobserver agreement study among international experts," *Gastrointestinal Endoscopy*, vol. 46, no. 1, pp. 33–36, 1997.

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Clinical Study

7-Day Nonbismuth-Containing Concomitant Therapy Achieves a High Eradication Rate for *Helicobacter pylori* in Taiwan

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Background. Ten-day concomitant therapy achieves a high eradication rate in Taiwan. Whether shortening the duration of concomitant therapy can still keep a high eradication rate remains unclear. *Aim.* To assess the eradication rate of 7-day pantoprazole-containing concomitant therapy in Taiwan and to investigate factors influencing the eradication outcome. *Methods.* From March 2008 to March 2012, 319 *H. pylori*-infected patients receiving a 7-day pantoprazole-containing concomitant regimen (pantoprazole 40 mg, amoxicillin 1 g, clarithromycin 500 mg, and metronidazole 500 mg twice daily for 7 days) were included. Patients were asked to return at the second week to assess drug compliance and adverse effects. Repeated endoscopy or urea breath test was performed at 8 weeks after the end of eradication therapy. *Results.* The eradication rates according to intention-to-treat and per-protocol analyses were 93.7% (299/319) and 96.4% (297/308), respectively. Adverse events occurred in 13.2% (42/319) of the patients. The compliance rate was 98.4% (314/319). Multivariate analysis disclosed that poor compliance was the only independent factor influencing the efficacy of anti-*H. pylori* therapy with an odds ratio of 0.073 (95% confidence interval, 0.011–0.483). *Conclusion.* 7-day concomitant therapy achieved a very high eradication rate for *H. pylori* infection in Taiwan. Drug compliance was the only clinical factor influencing treatment efficacy.

1. Introduction

Helicobacter pylori (H. pylori) infection is a global human pathogen and plays a cardinal role in the development of peptic ulcer disease, gastric adenocarcinoma, and mucosa-associated tissue lymphoma [1]. The Maastricht III Consensus Report has recommended that proton-pump-inhibitor-(PPI-) clarithromycin-amoxicillin or metronidazole treatment for 7 to14 days is the first choice treatment for *H. pylori* infection [2]. Initial data suggested that high eradication rates could be achieved [3, 4]. However, this gold standard has recently become declining in cure rates to unacceptable levels ($\leqq$ 80%), largely as a result of emerging resistance of the organism to clarithromycin [5–9]. In some European countries, the success rates were astonishingly low with values 25~60% [7, 10, 11]. The cure rates for first-line 7-day triple therapy in southern Taiwan declined from 84% to 80% in recent 5 years [4, 12]. Therefore, searching for more effective first-line therapies is urgently required [4, 13].

One recent therapeutic innovation is 10-day sequential regimen with a 5-day dual therapy (a PPI plus amoxicillin), followed by a 5-day triple therapy (a PPI plus clarithromycin and tinidazole (or metronidazole)) [14]. Several studies have demonstrated its satisfactory higher eradication rates than standard triple therapies [8, 9, 15]. Gatta et al. reported a rigorous systemic review that identified 13 trials evaluating 3,271 patients [16]. Most of the studies were conducted in Italy, where the patterns of clarithromycin and metronidazole resistance tend to be similar to those in United States and Europe. The data show that sequential

therapy achieves 90.7% eradication rates, with a 12% better absolute eradication rate than the standard triple therapy [16]. Our recent study also demonstrated that sequential therapy achieved a higher eradication rate than standard triple therapy in Taiwan (93% versus 80%, $P = 0.005$) [12]. However, a trial from Korea revealed that both sequential therapy and triple therapy achieved similar efficacy with unsatisfactory eradication rates (85.7% versus 76.6%, by PP analysis, $P = 0.150$) [17]. Generally speaking, sequential therapy is a good but typically not an excellent regimen (i.e., typically achieving a grade B and not grade A result) [18]. Theoretically, sequential therapy can be improved [19].

Concomitant therapy uses the same components as sequential therapy, but they are administered concomitantly [20]. It provides another novel regimen proven successful in the presence of clarithromycin resistance [21]. It is a 4-drug regimen containing a PPI (standard dose, b.i.d.), clarithromycin (500 mg, b.i.d.), amoxicillin (1 g, b.i.d.), and metronidazole (500 mg, b.i.d.) which are all given for the entire duration of therapy [22, 23]. This approach achieved more than 90% of eradication rates. A head-to-head noninferiority trial of 10-day esomeprazole-containing concomitant and 10-day esomeprazole-containing sequential therapy from our study group showed they were equivalent (93.0% versus 93.1% by per-protocol analysis) [24]. Nonetheless, a large-scaled, randomized controlled trial from Latin America revealed that the per-protocol eradication rates of 14-day lansoprazole-containing standard triple, 5-day concomitant and 10-day sequential therapies were 87%, 79%, and 81%, respectively [25]. The 5-day lansoprazole-containing concomitant therapy and 10-day sequential therapy had comparable eradicate rates. However, the eradication rate of 5-day concomitant therapy was lower than that of 14-day standard triple therapy. The insufficient treatment duration of concomitant therapy in the study was possibly an important factor accounting to the unacceptable eradication rate (< 80%) of the new therapy.

Currently, the optimal duration of concomitant therapy is unknown, and whether shortening the duration of concomitant therapy from 10 days to 7 days can still keep a high eradication rate for *H. pylori* infection remains unclear. In this study, we retrospectively assess the eradication rate of 7-day nonbismuth containing concomitant therapy in Taiwan and investigated the host factors influencing the eradication outcome.

2. Materials and Methods

2.1. Patients. From March 2008 to March 2012, 319 *H. pylori*-infected outpatients who received a 7-day pantoprazole-containing concomitant therapy in our center were included for the retrospective analysis. The exclusion criteria included (a) previous eradication therapy, (b) consumption of antibiotics, bismuth, or proton pump inhibitors within previous 4 weeks, (c) allergy to antibiotics or PPIs, (d) patients with previous gastric surgery, (e) the coexistence of serious concomitant illness (such as, decompensated liver cirrhosis, uremia), and (f) pregnant women. The *H. pylori* infection was defined by at least one positive result of following: culture, rapid urease test, histology, or urea breath test.

2.2. Study Design. In the study period, 317 *H. pylori*-infected patients received a 7-day pantoprazole-containing concomitant therapy (pantoprazole 40 mg, clarithromycin 500 mg, amoxicillin 1 g and metronidazole 500 mg twice daily). According to the standard protocol for *H. pylori* eradication therapy in our institute, all drugs were taken one hour before breakfast and dinner. Patients were asked to return at the second week to assess drug compliance and adverse effects. Repeated endoscopy with rapid urease test and histological examination or urea breath test was performed at 8 weeks after the end of anti-*H. pylori* therapy. Successful eradication was defined as (a) negative results of both rapid urease test and histology in follow-up endoscopy, or (b) a negative result of urea breath test.

2.3. Questionnaire. A complete medical history and demographic data were obtained from each patient, including age, sex, medical history, history of smoking, alcohol, coffee, and tea consumption. Adverse events were prospectively evaluated. The adverse events were retrospectively assessed according to a 4-point scale system: none; mild (discomfort annoying but not interfering with daily life); moderate (discomfort sufficient to interfere with daily life); and severe (discomfort resulting in discontinuation of eradication therapy) [26]. Compliance of patients was checked by counting unused medication at the completion of treatment. Poor compliance was defined as taking less than 80% of the total medication.

2.4. Rapid Urease Test, Histology, and Urea Breath Test. The rapid urease test, histology, and urea breath test were performed according to our previous studies [13]. A biopsy specimen was taken from the lesser curvature site of the antrum for rapid urease test. Two biopsy specimens were each taken from the lesser curvature sites of the antrum and the corpus for histological examination [26]. The cut-off value of urea breath test was set at 4.8% of $\delta^{13}CO_2$ [27].

2.5. Statistical Analysis. The primary outcome variables were the rates of eradication, adverse events, and compliance. The overall eradication rates and their 95% confidence intervals were obtained by ITT and per protocol (PP). ITT analysis included all patients who had taken at least one dose of study medication. Patients whose infection status was unknown following treatment were considered treatment failures for the purposes of ITT analysis. The PP analysis excluded the patients with unknown *H. pylori* status following therapy and those with poor compliance.

To determine the independent factors affecting the treatment response, 11 clinical and endoscopic parameters were analyzed by univariate analysis. These variables included the following: age (<60 or ≥60 years); gender; history of current smoking (<1 pack/week or ≥1 pack/week), history of current alcohol consumption (<80 g/day or ≥80 g/day),

Table 1: Demographic data and endoscopic appearance of 7-day concomitant therapy.

Characteristics	7-day concomitant therapy group ($n = 319$)
Age (yr) (mean ± SD)	53 ± 12
Gender (male/female)	189/130
Smoking	71 (22.3%)
Alcohol consumption	24 (7.5%)
Ingestion of coffee	81 (25.4%)
Ingestion of tea	124 (38.9%)
NSAID use	16 (5%)
Underlying disease	80 (25.1%)
Endoscopic findings	
Gastritis	98 (30.7%)
Gastric ulcer	100 (31.3%)
Duodenal ulcer	56 (17.6%)
Gastric ulcer + duodenal ulcer	65 (20.4%)

ingestion of coffee (<1 cup/day or ≥1 cup/day), ingestion of tea (<1 cup/day or ≥1 cup/day), coexistence of a systemic disease (yes or no); previous history of peptic ulcer disease, endoscopic appearance (ulcer or gastritis), types of PPI, and drug compliance (good or poor). Chi-square test with or without Yates correction for continuity and Fisher's exact test were used when appropriate to compare the treatment outcome and host factors using the SPSS program (version 10.1, Chicago, IL, USA). A P value less than 0.05 was considered statistically significant. Those variables found to be significant by univariate analysis were subsequently assessed by a stepwise logistic regression method to identify independent factors for eradication outcome.

3. Results

3.1. Patients. A total of 319 patients received concomitant therapy from March 2008 to March 2012. The subjects were all included in the ITT analysis for *H. pylori* eradication. Data regarding the clinical characteristics of patients at entry are summarized in Table 1. Among the subjects, five with poor compliance and six with incomplete followup were excluded from PP analysis for *H. pylori* eradication. All patients were included in intention-to-treat analysis.

3.2. Eradication of H. pylori. Table 2 lists the therapeutic outcomes of the 7-day concomitant therapy. According to the ITT analysis, *H. pylori* infection was eradicated in 93.7% (299/319) of the patients receiving concomitant therapy. By PP analysis, the treatment rate was 96.4% (297/308). Five of 319 patients (1.6%) failed to complete the treatment because of insufficient compliance. *H. pylori* was successfully eradicated in three of 5 cases (60%).

3.3. Adverse Effect and Compliances. All of the 319 patients were included in the adverse event analysis. In total, 13.2% (42/319) of the patients reported at least one adverse event during eradication therapy. The profiles and frequencies of adverse events were listed in Table 3. The most frequent symptoms were nausea (26 patients; 8.2%) and headache (11 patients; 3.4%). Less-frequent symptoms were abdominal pain (3 patients; 0.9%), abdominal constipation (2 patients; 0.6%), and diarrhea (2 patients; 0.6%). There were 5 patients who discontinued treatment as a result of adverse events during eradication therapy (nausea: 2 patient; headache: 2 patients; diarrhea: 1 patient). Overall, the compliance rate was 98.4%.

Table 2: The major outcomes of 7-day concomitant therapy.

	Outcome of 7-day concomitant therapy ($n = 319$)
Eradication rate	
Intention to treat	93.7% (299/319)
Per protocol	96.4% (297/308)
Adverse events	13.2% (42/319)
Compliance	98.4% (314/319)

Table 3: Adverse events of 7-day concomitant therapy.

Adverse events	7-day concomitant therapy group ($n = 319$)
Abdominal pain	3 (1/1/1*)
Constipation	2 (1/0/1)
Diarrhea	2 (1/0/1)
Dizziness	0 (0/0/0)
Headache	11 (7/2/2)
Nausea/vomiting	26 (14/9/3)
Taste perversion	5 (3/1/1)
Palpitation	3 (2/1/0)
Insomnia	3 (1/1/1)
Other	21 (13/4/4)

*Number of patients who suffered from mild, moderate, and severe adverse events.

3.4. Factors Influencing Efficacy of Anti-H. pylori Therapy. Table 4 lists the clinical and endoscopic factors influencing the efficacy of eradication therapy. Only patients with known follow-up *H. pylori* status ($n = 312$) were included for the analysis. The eradication rates were significantly related to drug compliance ($P = 0.015$) and smoking ($P = 0.046$) in univariate analysis. The other factors (age, sex, alcohol drinking, coffee consumption, tea consumption, previous history of ulcer, presence of ulcer, and presence of adverse event) did not markedly influence the eradication efficacy. Multivariate analysis disclosed that poor compliance was the only independent factor influencing the efficacy of anti-*H. pylori* therapy (Table 5). The odds ratios were 0.073 (95% confidence interval (CI), 0.011–0.483).

4. Discussion

The fall in *H. pylori* eradication rates with standard triple therapies resulted in a search for novel therapies for *H. pylori* infection [6]. In this study, we examined the efficacy of 7-day

Table 4: Univariate analysis of the clinical factors influencing the efficacy of *H. pylori* eradication therapy.

Principle parameter	No. of patients	Eradication rate	*P* value
Age			0.762
<60 years	220	95.5%	
≧60 years	92	96.7%	
Sex			0.010
Female	129	92.2%	
Male	183	98.4%	
Smoking			0.046
(−)	242	94.6%	
(+)	70	100%	
Alcohol consumption			0.610
(−)	290	95.5%	
(+)	22	100%	
Ingestion of coffee			0.294
(−)	231	96.5%	
(+)	81	93.8%	
Ingestion of tea			0.085
(−)	189	94.2%	
(+)	123	98.4%	
NSAID use			0.503
(−)	296	95.9%	
(+)	16	93.8%	
Previous history of peptic ulcer			
(−)			
(+)			
Presence of ulcer			0.314
(−)	65	94.9%	
(+)	234	95.1%	
Compliance			0.015
(−)	5	60%	
(+)	307	96.4%	
Side effect			0.082
(−)	270	96.7%	
(+)	42	90.5%	

Table 5: Multivariate analysis for clinical factors related to eradication efficacy of *H. pylori*.

Clinical factor	Coefficient	Standard error	Odds ratio (95% CI)	*P* value
Poor compliance	−2.617	0.964	0.073 (0.011–0.483)	0.007

pantoprazole-containing concomitant therapy in Taiwan. We found that the 7-day concomitant therapy produced 96.4% treatment success by PP analysis. The eradication rate by ITT analysis was 93.7%. In our two previous studies [4, 12], the eradication rates of 7-day pantoprazole-containing standard triple therapy by ITT analysis were 84% and 80%, respectively. The data suggested that 7-day concomitant therapy achieved a high eradication rate in Taiwanese and had a great potential to replace 7-day triple therapy as a first-line anti-*H. pylori* therapy in Taiwan.

In the initial studies from Germany and Japan for concomitant therapy, a PPI and three antibiotics (amoxicillin, clarithromycin, and metronidazole) for 5–7 days achieved high eradication rates [22, 23]. A meta-analysis published in 2009 presented the pooled eradication rate of concomitant therapy studies between 1998 to 2002 as 89.7% on ITT and 92.9% on PP analysis [20]. In recent years, concomitant therapies with duration of 5–10 days are reported 90–96% success rates on PP analysis in Asian countries, including Thailand, Taiwan, and Korea [24, 28, 29]. The high eradication rate (94.5%) was also reported in Europe, such as Greece [30]. However in Latin America, Greenberg et al. pointed that the success rate of 5-day concomitant therapy was dropped to 78.7% [25]. A recent review article of 15 studies (1723 patients) revealed that there was a tendency towards better results by longer treatments (7–10 days versus 3–5 days) [21]. Our previous study showed that 10-day esomeprazole- containing concomitant therapy achieved a 93% eradication rate [24]. In the current study, we shortened the duration of concomitant therapy from 10 days to 7 days, and still achieved high eradication rates. The results indicated that the duration of concomitant therapy could be shortened to 7 days in Taiwan.

In this investigation, 13% of the patients treated with concomitant therapy reported at least one adverse event during eradication therapy. Basically, concomitant therapy was well tolerated and had good compliance (98.4%). The most frequent symptoms were nausea (8%) and headache (3%). Only five patients discontinued treatment as a result of adverse events during eradication therapy. Multivariate analysis revealed that drug compliance was the only independent clinical factor influencing treatment efficacy. The eradication rates in patients with good and poor compliance were 96.4% and 60%, respectively. Notably, the occurrence of severe adverse events was an important cause of poor drug compliance.

Smoking has been shown to reduce the effectiveness of first line triple therapy [31]. However, in recent Taiwanese study, no significant effect of smoking was found in concomitant and sequential therapy [24]. In this study, smoking is one of clinical factors influencing treatment outcome by univariate analysis. The reason for this is possibly due to low prevalence rate of smoking. However, in multivariate analysis, it is no longer an independent factor.

This study has several limitations. Firstly, it was a retrospective study, although all patients were prospectively followed up by a standard protocol and the adverse events and compliance were assessed by trained assistants with study nurses with a standardized questionnaire. Secondly, the impacts of antibiotic resistance on the eradication rate could not be assessed by the study because a routine culture was not conducted in the first-line therapy. However, this study is the first work to investigate 7-day pantoprazole-containing concomitant therapy and the number of cases in this study was large ($n = 319$). Thirdly, in tradition, subjects need to have both positive tests of urease test and histology or

positive result of culture to be counted as infected for entry into a clinical trial. In this study, we enrolled the patients with one positive test for *H. pylori* infection. The eradication rates may be overestimated since patients with false-positive *H. pylori* infection achieve successful eradication in the trial. But generally false-positive tests for urease test are uncommon [32]. Therefore, this study represents the real world scenario for *H. pylori* detection and eradication.

In conclusion, 7-day concomitant therapy achieved a very high eradication rate for *H. pylori* infection in Taiwan. It was well tolerated. Drug compliance was the only clinical factor influencing treatment efficacy.

Authors' Contribution

S.-S. Kao and W.-C. Chen contributed equally to the work.

Conflict of Interests

The authors have no conflicts of interest to declare.

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References

[1] IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, "Infection with *Helicobacter pylori*," *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, vol. 61, pp. 177–240, 1994.

[2] P. Malfertheiner, F. Megraud, C. O'Morain et al., "Current concepts in the management of *Helicobacter pylori* infection: the Maastricht III Consensus Report," *Gut*, vol. 56, no. 6, pp. 772–781, 2007.

[3] S. K. Lam and N. J. Talley, "Report of the 1997 Asia Pacific Consensus Conference on the management of *Helicobacter pylori* infection," *Journal of Gastroenterology and Hepatology*, vol. 13, no. 1, pp. 1–12, 1998.

[4] P. I. Hsu, K. H. Lai, C. K. Lin et al., "A prospective randomized trial of esomeprazole- versus pantoprazole-based triple therapy for *Helicobacter pylori* eradication," *American Journal of Gastroenterology*, vol. 100, no. 11, pp. 2387–2392, 2005.

[5] R. J. F. Laheij, L. G. M. Van Rossum, J. B. M. J. Jansen, H. Straatman, and A. L. M. Verbeek, "Evaluation of treatment regimens to cure *Helicobacter pylori* infection—a meta-analysis," *Alimentary Pharmacology and Therapeutics*, vol. 13, no. 7, pp. 857–864, 1999.

[6] D. Y. Graham and L. Fischbach, "*Helicobacter pylori* treatment in the era of increasing antibiotic resistance," *Gut*, vol. 59, no. 8, pp. 1143–1153, 2010.

[7] E. Rimbara, L. A. Fischbach, and D. Y. Graham, "Optimal therapy for *Helicobacter pylori* infections," *Nature Reviews Gastroenterology and Hepatology*, vol. 8, no. 2, pp. 79–88, 2011.

[8] A. Zullo, V. De Francesco, C. Hassan, S. Morini, and D. Vaira, "The sequential therapy regimen for *Helicobacter pylori* eradication: a pooled-data analysis," *Gut*, vol. 56, no. 10, pp. 1353–1357, 2007.

[9] N. S. Jafri, C. A. Hornung, and C. W. Howden, "Meta-analysis: sequential therapy appears superior to standard therapy for *Helicobacter pylori* infection in patients naive to treatment," *Annals of Internal Medicine*, vol. 148, no. 12, pp. 923–931, 2008.

[10] Y. Gumurdulu, E. Serin, B. Özer et al., "Low eradication rate of *Helicobacter pylori* with triple 7-14 days and quadriple therapy in Turkey," *World Journal of Gastroenterology*, vol. 10, no. 5, pp. 668–671, 2004.

[11] M. A. Bigard, J. C. Delchier, G. Riachi, P. Thibault, and P. Barthelemy, "One-week triple therapy using omeprazole, amoxycillin and clarithromycin for the eradication of *Helicobacter pylori* in patients with non-ulcer dyspepsia: influence of dosage of omeprazole and clarithromycin," *Alimentary Pharmacology and Therapeutics*, vol. 12, no. 4, pp. 383–388, 1998.

[12] F. W. Tsay, H. H. Tseng, P. I. Hsu et al., "Sequential therapy achieves a higher eradication rate than standard triple therapy in Taiwan," *Journal of Gastroenterology and Hepatology*, vol. 27, pp. 498–503, 2012.

[13] P. I. Hsu, K. H. Lai, H. H. Tseng et al., "Correlation of serum immunoglobulin G *Helicobacter pylori* antibody titers with histologic and endoscopic findings in patients with dyspepsia," *Journal of Clinical Gastroenterology*, vol. 25, no. 4, pp. 587–591, 1997.

[14] A. Zullo, V. Rinaldi, S. Winn et al., "A new highly effective shout-term therapy schedule for *Helicobacter pylori* eradication," *Alimentary Pharmacology and Therapeutics*, vol. 14, no. 6, pp. 715–718, 2000.

[15] J. P. Gisbert, X. Calvet, A. O'Connor, F. Mégraud, and C. A. O'Morain, "Sequential therapy for *Helicobacter pylori* eradication: a critical review," *Journal of Clinical Gastroenterology*, vol. 44, no. 5, pp. 313–325, 2010.

[16] L. Gatta, N. Vakil, G. Leandro, F. Di Mario, and D. Vaira, "Sequential therapy or triple therapy for *Helicobacter pylori* infection: systematic review and meta-analysis of randomized controlled trials in adults and children," *American Journal of Gastroenterology*, vol. 104, no. 12, pp. 3069–3079, 2009.

[17] W. H. Choi, D. I. Park, S. J. Oh et al., "Effectiveness of 10 day-sequential therapy for *Helicobacter pylori* eradication in Korea," *The Korean Journal of Gastroenterology*, vol. 51, no. 5, pp. 280–284, 2008.

[18] D. Y. Graham and E. Rimbara, "Understanding and appreciating sequential therapy for helicobacterpylori eradication," *Journal of Clinical Gastroenterology*, vol. 45, no. 4, pp. 309–313, 2011.

[19] D. Y. Graham, H. Lu, and Y. Yamaoka, "Therapy for *Helicobacter pylori* infection can be improved: sequential therapy and beyond," *Drugs*, vol. 68, no. 6, pp. 725–736, 2008.

[20] A. S. Essa, J. R. Kramer, D. Y. Graham, and G. Treiber, "Meta-analysis: four-drug, three-antibiotic, non-bismuth-containing "concomitant therapy" versus triple therapy for *Helicobacter pylori* eradication," *Helicobacter*, vol. 14, no. 2, pp. 109–118, 2009.

[21] J. P. Gisbert and X. Calvet, "Review article: non-bismuth quadruple (concomitant) therapy for eradication of Helicobater pylori," *Alimentary Pharmacology and Therapeutics*, vol. 34, pp. 604–617, 2011.

[22] G. Treiber, S. Ammon, E. Schneider, and U. Klotz, "Amoxicillin/metronidazole/omeprazole/clarithromycin: a new, short quadruple therapy for *Helicobacter pylori* eradication," *Helicobacter*, vol. 3, no. 1, pp. 54–58, 1998.

[23] M. Okada, K. Oki, T. Shirotani et al., "A new quadruple therapy for the eradication of *Helicobacter pylori*. Effect of

pretreatment with omeprazole on the cure rate," *Journal of Gastroenterology*, vol. 33, no. 5, pp. 640–645, 1998.

[24] D. C. Wu, P. I. Hsu, J. Y. Wu et al., "Sequential and concomitant therapy with four drugs is equally effective for eradication of *H. pylori* infection," *Clinical Gastroenterology and Hepatology*, vol. 8, no. 1, pp. 36–41, 2010.

[25] E. R. Greenberg, G. L. Anderson, D. R. Morgan et al., "14-day triple, 5-day concomitant, and 10-day sequential therapies for *Helicobacter pylori* infection in seven Latin American sites: a randomised trial," *The Lancet*, vol. 378, pp. 507–514, 2011.

[26] P. I. Hsu, K. H. Lai, E. J. Chien et al., "Impact of bacterial eradication on the cell proliferation and p53 protein accumulation in *Helicobacter pylori*-associated gastritis," *Anticancer Research*, vol. 20, no. 2 B, pp. 1221–1228, 2000.

[27] N. J. Peng, K. H. Lai, R. S. Liu et al., "Clinical significance of oral urease in diagnosis of *Helicobacter pylori* infection by $_{13}$C urea breath test," *Digestive Diseases and Sciences*, vol. 46, no. 8, pp. 1772–1778, 2001.

[28] C. Kongchayanun, V. Mahachai, B. Pornthisarn, S. Amornsawadwattana, and R. K. Vilaichone, "Efficacy of 10-day and 5-day concomitant therapy for *Helicobacter pylori* eradication in Thai patients with non ulcer dyspepsia," *Gastroenterology*, vol. 140, Supplement 1, p. 879, 2011.

[29] S. Y. Kim, S. W. Lee, B. S. Kwon et al., "Comparative study of *Helicobacter pylori* eradication rates of 5-day quadruple 'Concomitant'therapy and 7-day standard triple therapy.," *Gastroenterology*, vol. 140, Supplement 1, p. 878, 2011.

[30] S. Georgopoulos, V. Papastergiou, E. Xirouchakis et al., "Evaluation of a four-drug, three-antibiotic, nonbismuth-containing 'concomitant'therapy as firstline *Helicobacter pylori* eradication regimen in Greece," *Helicobacter*, vol. 17, no. 1, pp. 49–53, 2012.

[31] T. Suzuki, K. Matsuo, H. Ito et al., "Smoking increases the treatment failure for *Helicobacter pylori* eradication," *American Journal of Medicine*, vol. 119, no. 3, pp. 217–224, 2006.

[32] P. Midolo and B. J. Marshall, "Accurate diagnosis of *Helicobacter pylori*: urease tests," *Gastroenterology Clinics of North America*, vol. 29, no. 4, pp. 871–878, 2000.

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Research Article

A Real World Report on Intravenous High-Dose and Non-High-Dose Proton-Pump Inhibitors Therapy in Patients with Endoscopically Treated High-Risk Peptic Ulcer Bleeding

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Background and Study Aims. The optimal dose of intravenous proton-pump inhibitor (PPI) therapy for the prevention of peptic ulcer (PU) rebleeding remains controversial. This study aimed to understand the real world experiences in prescribing high-dose PPI and non-high-dose PPI for preventing rebleeding after endoscopic treatment of high-risk PU. *Patients and Methods*. A total of 220 subjects who received high-dose and non-high-dose pantoprazole for confirmed acute PU bleeding that were successfully treated endoscopically were enrolled. They were divided into rebleeding ($n = 177$) and non-rebleeding groups ($n = 43$). Randomized matching of the treatment-control group was performed. Patients were randomly selected for non-high-dose and high-dose PPI groups ($n = 44$ in each group). *Results*. Univariate analysis showed, significant variables related to rebleeding were female, higher creatinine levels, and higher Rockall scores ($\geqq 6$). Before case-control matching, the high-dose PPI group had higher creatinine level, higher percentage of shock at presentation, and higher Rockall scores. After randomized treatment-control matching, no statistical differences were observed for rebleeding rates between the high-dose and non-high-dose groups after case-control matching. *Conclusion*. This study suggests that intravenous high-dose pantoprazole may not be superior to non-high-dose regimen in reducing rebleeding in high-risk peptic ulcer bleeding after successful endoscopic therapy.

1. Introduction

Patients with high-risk stigmata on endoscopic examination for acute upper gastrointestinal bleeding are at increased risk of recurrent bleeding [1]. Endoscopic hemostasis and continuous infusion intravenous high-dose proton-pump-inhibitor (PPI) have been proven to reduce recurrent bleeding, need for surgery, and length of hospital stay [2, 3]. Furthermore, the recently updated Vienna consensus states that intravenous high-dose PPI therapy after successful endoscopic hemostasis decreases both peptic ulcer (PU) rebleeding and mortality in patients with high-risk stigmata [4]. Despite these recent advances in the pharmacological and endoscopic treatment of acute nonvariceal upper gastrointestinal hemorrhage, the associated mortality remains high at 10% to 14% [5]. Theoretically, inhibiting gastric acid and raising the intragastric pH to 6 or more and maintaining it at that level may promote clot stability, thus decrease the likelihood of rebleeding. This is based on experimental data showing that gastric acid impairs clot formation, promotes platelet disaggregation, and favors fibrinolysis [6]. The continuous i.v. infusion of pantoprazole (80 mg bolus plus 8 mg/h continuous infusion) is able to maintain higher intragastric pH for 84% of the time during monitoring, which is higher than intermittent bolus injection (40 mg every 8 h) or lower-dose continuous infusion (40 mg bolus followed by 4 mg/h infusion) and hence should attain better control of peptic ulcer bleeding [7]. However, recent systemic review and meta-analysis of this regiment have shown inconsistent results and the optimal dosing of PPI in preventing PU rebleeding remains controversial [8–10]. This retrospective case-controlled study was conducted to

understand the real world experiences in prescribing high-dose PPI and non-high-dose PPI for preventing rebleeding after endoscopic treatment of high-risk PU.

2. Patients and Methods

2.1. Patients and Study Design. This is a 2-year retrospective chart review case-control study which began in year 2009. Two hundred and twenty patients with gastric or duodenal ulcers bleeding treated successfully via endoscopy were enrolled into this study. All subjects received intravenous PPIs. We excluded patients with malignant ulcers, upper gastrointestinal bleeding unrelated to peptic ulcer such as angiodysplasia and Mallory-Weiss tear, subjects who lost followup less than the required 30 days for reasons other than mortality, and subjects who were unsuccessfully treated during the first endoscopic hemostasis attempt or received inadequate endoscopic hemostasis therapy for high-risk ulcers such as monotherapy with Bosmin injection alone. This was based on our previous study [11] which emphasized that endoscopic epinephrine injection (EI) monotherapy in patients with high-risk ulcers should be avoided. In current studies, only those patients who received initial hemostasis with epinephrine injection combined with thermal therapy or hemoclips [4], or thermal or clip monotherapy [12] are enrolled. Patient's baseline characteristics, concomitant comorbid diseases (including cardiovascular diseases, stroke, liver cirrhosis, chronic obstructive pulmonary disease, diabetes mellitus, and hypertension), presenting hemoglobin levels, platelet counts, hemodynamic status, use of aspirin, nonsteroidal anti-inflammatory drugs (NSAIDs), warfarin/heparin and PPI prior to endoscopic therapy, were recorded using a predetermined spreadsheet. PU bleeding was defined by endoscopist's diagnosis combined with no other identifiable bleeding cause. Endoscopic findings such as ulcer locations, sizes, difficult treatment sites (lesser curvature of high body; posterior wall of bulb and superior duodenal angle), Forrest grade, Rockall scores [13, 14], and treatment methods were also recorded. The endpoints were rebleeding within 30 days after initial endoscopic hemostasis, requirement for surgical intervention, length of hospital stay and total amount of blood transfusion required, bleeding-related mortality, and all-cause mortality. According to results from medical record, these patients were classified into two groups: subjects without recurrent hemorrhage ($n = 177$) and those recurred ($n = 43$).

2.2. Definitions. Patients under non-high-dose PPI treatment were defined as those who received 80 mg pantoprazole bolus and followed by i.v. 80 mg per day, until alimentation was possible, then 40 mg per day orally. High-dose PPI therapy were defined as administering 80 mg pantoprazole i.v. bolus injection, then 8 mg per hour continuous infusion for 3 days, followed by i.v. 80 mg per day. Renal function was evaluated by estimated glomerular filtration rate calculated using the 4-variable Modification of Diet in Renal Disease Study equations and classified according to the K/DOQI Clinical Practice Guidelines for Chronic Kidney Disease [15]. High-risk ulcers were defined as Forrest grade higher or equal to 2b [3]. Rebleeding was defined as new onset of hematemesis, coffee-ground vomitus, or hematochezia, with an increasing pulse rate >110 beats/min and decreasing blood pressure below 90 mmHg after a 24-hour period of stable vital signs and hematocrit following endoscopic treatment [11, 16–18]. Total amount of blood transfusion required was defined as units given to the patients between the time PU bleeding occurred and the day of discharge. Bleeding-related mortality was defined as in-hospital death resulted solely from peptic ulcer bleeding.

2.3. Statistical Analysis. The quantitative data were compared using the Student's t-test for variables with a normal distribution. Differences between the proportions of categorical data were evaluated with Fisher's exact test when the number of expected subjects was less than five and otherwise with the χ^2 test. The results are expressed as distributions, absolute frequencies, relative frequencies, medians, and ranges, or means ± SD. A multivariate logistic regression model was used to assess the independent association between rebreeding and non-rebleeding groups. P value of <0.05 was considered statistically significant. The Statistical Package for Social Sciences (SPSS15.0, Chicago, USA) for Windows was used to analyze the data.

We employed the nearest neighbor-matching method (NCSS 2007, Kaysville, Utah 84037, USA) to reduce bias in the retrospective study. The matching algorithm was performed to find one matched control in high-dose PPIs group for each in non-high-dose group. The matching variables were stage of CKD, Forrest classification and Rockall score, and female gender. As a result, forty-four patients were randomly selected in each group.

3. Result

The difference between the two study groups (non-rebleeding versus rebleeding groups) was insignificant in terms of age, medication history such as NSAIDs, clopidogrel, warfarin, initial hemoglobin level, platelet counts, shock at presentation, percentage of high stigmata ulcers, ulcer size, and time to endoscope (Table 1). Univariate analysis revealed significant differences in the following variables: gender (female: 28.2% versus 48.8%, $P = 0.010$), initial creatinine level (2.0 ± 2.3 mg/dL versus 3.1 ± 3.2 mg/dL, $P < 0.00$), use of aspirin (17.5% versus 2.3%, $P = 0.011$), CKD stage III to V (41.2% versus 60.5%, $P = 0.013$), COPD (3.4% versus 11.6%, $P = 0.026$), Rockall score $\geqq 6$ (59.3% versus 83.7%, $P = 0.003$), amount of blood transfusion of PRBC (879.9 ± 966.4 mL versus 3220.9 ± 2824.3 mL, $P < 0.001$), surgical requirements (0 versus 4.7%, $P = 0.004$), hospital stay (10.6 ± 12.4 days versus 24.6 ± 18.6 days, $P < 0.001$); and mortality (4.5% versus 20.9%, $P = 0.001$). Multivariate analysis showed that the significant factors were sex, high Rockall score, and serum creatinine level (Table 2).

We divided our subjects into two groups: non-high dose and high dose for analysis (Table 3). There were no significant differences between the two groups (non-high dose versus high dose) in terms of patients' gender, age, initial hemoglobin and platelet, NSAIDs, aspirin,

Table 1: Univariate analysis of demographic and clinical characteristics of non-rebleeding and rebleeding patients.

Variables	Non-rebleeding group ($n = 177$)	Rebleeding group ($n = 43$)	P-value
Age (years)	63.4 ± 13.7	65.2 ± 13.5	0.941
Female gender, n (%)	50 (28.2)	21 (48.8)	0.010*
Creatinine (mg/dL)	2.0 ± 2.3	3.1 ± 3.2	<0.001*
Hb (g/L)	97.8 ± 29.4	83.1 ± 23.4	0.074
Platelet ($\times 10^9$/L)	194.8 ± 84.1	183.4 ± 147.5	0.113
Use of NSAIDs, n (%)	12 (6.8)	2 (4.7)	0.608
Use of aspirin, n (%)	31 (17.5)	1 (2.3)	0.011*
Use of clopidogrel, n (%)	18 (10.2)	5 (11.6)	0.779
Use of warfarin, n (%)	7 (4.0)	3 (7.0)	0.393
Coexisting illness, n (%)			
CKD III to V	73 (41.2)	26 (60.5)	0.013*
COPD	6 (3.4)	5 (11.6)	0.026*
CAD	29 (16.4)	8 (18.6)	0.727
DM	48 (27.1)	18 (41.9)	0.058
CVA	26 (14.7)	8 (18.6)	0.524
Liver cirrhosis	32 (18.1)	7 (16.3)	0.782
High stigmata, n (%)	173 (97.7)	41 (95.3)	0.388
Forrest classification Ia/Ib/IIa/IIb/IIc/III	9/100/18/45/5/0	5/31/1/5/0/1	
Shock on admission, n (%)	89 (50.3)	23 (53.5)	0.706
Rockall score $\geqq 6$, n (%)	105 (59.3)	36 (83.7)	0.003*
Time to endoscope (h)	14.3 ± 17.5	19.9 ± 20.2	0.129
Hemostasis methods A/B/C/D/E/F	62/48/11/50/2/4	11/14/0/15/2/1	
Ulcer size (cm)	1.0 ± 0.7	0.9 ± 0.6	0.973
Multiple ulcers, n (%)	58 (32.8)	18 (41.9)	0.261
PRBC BT (mL)	879.9 ± 966.4	3220.9 ± 2824.3	<0.001*
Surgery, n (%)	0	2 (4.7)	0.004*
Hospital stay (days)	10.6 ± 12.4	24.6 ± 18.6	<0.001*
Mortality, n (%)	8 (4.5)	9 (20.9)	0.001*
Bleeding related/other causes	1/7	3/6	

PPI: proton-pump inhibitors, Hb: hemoglobin, CKD: chronic kidney disease, NSAID: nonsteroidal anti-inflammatory drug, PPI: proton-pump inhibitor, DM: diabetes mellitus type 2, COPD: chronic obstructive pulmonary disease, CAD: coronary artery disease, CVA: cerebrovascular accident, BT: blood transfusion, PPI: proton pump inhibitor, Hemostasis methods A/B/C/D/E/F: Bosmin plus APC/heat probe=A, APC/heat probe=B, Hemoclip=C, Bosmin plus hemoclip=D, APC/heat probe plus hemoclip=E, APC plus hemoclip plus Bosmin=F, APC: argon plasma coagulation. *$P < 0.05$.

Table 2: Multivariate analysis for rebleeding and nonbleeding patients.

	Odds ratio	95% CI.	P value
Sex	0.408	0.201–0.828	0.013
High Rockall score	3.215	1.324–7.808	0.010
Creatinine	1.119	0.992–1.263	0.066

clopidogrel, warfarin use, Rockall score, ulcer pattern of Forrest, time to endoscope, duration of hospital stay, surgical interventions, rebleeding rate, and mortality. Significant variables were initial creatinine level (2.0 ± 2.4 mg/dL versus 2.6 ± 2.82 mg/dL, $P = 0.018$), diabetes (25.3% versus 40.0%, $P = 0.027$), CVA (12.0% versus 22.9%, $P = 0.038$), and shock at presentation (46.0% versus 61.4%, $P = 0.033$). Although the Rockall score was not significant between these two groups, it was higher in trend in the high-dose group (5.9 ± 1.7 versus 6.3 ± 1.5, $P = 0.106$).

To minimize the clinical characteristics difference between non-high-dose and high-dose groups, we created a treatment-control randomized match based on CKD stages, Forrest classifications, and Rockall scores. Fifty-six patients were randomly selected in each group of non-high- dose and high dose for analysis (Table 4). All of them have high-risk ulcers according to Forrest classification. As a result, there were no significant differences between the two groups (non-high dose versus high dose) in all demographic and clinical characteristics such as the rebleeding rate (18.2% versus 15.9%, $P = 0.777$), surgery needed (0 versus 0%, $P = 1.000$), and hospital stay (12.1 ± 17.2 days versus 14.3 ± 13.5 days, $P = 0.505$).

4. Discussion

After the randomized treatment-control matching process to minimize possible selection bias between the two treatment groups, current retrospective case-controlled study observed

Table 3: Comparison between the non-high-dose and high-dose PPI before case-controlled matching.

Characteristic	Non-high-dose group ($n = 150$)	High-dose group ($n = 70$)	P-value
Age (years)	64.1 ± 13.3	62.6 ± 14.4	0.558
Female gender, n (%)	105 (70.0)	44 (62.9)	0.291
Creatinine (mg/dL)	2.0 ± 2.4	2.6 ± 2.8	0.018*
Hb (g/L)	96.2 ± 28.2	92.1 ± 30.1	0.438
Platelet ($\times 10^9$/L)	195.2 ± 103.5	186.8 ± 90.4	0.592
Use of NSAIDs, n (%)	8 (5.3)	6 (8.6)	0.359
Use of aspirin, n (%)	23 (15.3)	9 (12.9)	0.628
Use of clopidogrel, n (%)	15 (10.0)	8 (11.4)	0.747
Use of warfarin, n (%)	5 (3.3)	5 (7.1)	0.206
Coexisting illness, n (%)			
CKD III, IV/V	47/17 (31.3/11.3)	23/12 (32.9/17.1)	0.422
COPD	8 (5.3)	3 (4.3)	0.740
CAD	21 (14.0)	16 (22.6)	0.102
DM	38 (25.3)	28 (40.0)	0.027*
CVA	18 (12.0)	16 (22.9)	0.038*
Liver cirrhosis	26 (17.3)	13 (18.6)	0.321
Forrest classification Ia/Ib/IIa/IIb/IIc/III	11/86/12/35/5/1	3/45/7/15/0/0	0.524
Shock on admission	69 (46.0)	43 (61.4)	0.033*
Rockall score	5.9 ± 1.7	6.3 ± 1.5	0.106
Time to endoscope (hours)	15.9 ± 19.2	14.1 ± 15.8	0.107
Hemostasis methods A/B/C/D/E/F	11/86/12/35/5/1	3/45/7/15/0/0	
PRBC BT (mL)	11101.7 ± 1495.3	1842.9 ± 2185.7	0.196
Multiple ulcers, n (%)	50 (38.7)	26 (37.1)	0.580
Rebleeding, n (%)	24 (16.0)	19 (27.1)	0.052
Surgery, n (%)	1 (0.6)	1 (1.4)	0.579
Hospital stay (days)	11.9 ± 14.9	16.5 ± 14.3	0.343
Mortality, n (%)	9 (6.0)	8 (11.4)	0.207
Bleeding related/other causes	3/6	1/7	

Hb: hemoglobin, NSAID: nonsteroidal anti-inflammatory drug, CKD: chronic kidney disease, PPI: proton-pump inhibitor, DM: diabetes mellitus type 2, COPD: chronic obstructive pulmonary disease, CAD: coronary artery disease, CVA: cerebrovascular accident, BT: blood transfusion, PPI: proton pump inhibitor, Hemostasis methods A/B/C/D/E/F: Bosmin plus APC/heat probe=A, APC/heat probe=B, Hemoclip=C, Bosmin plus hemoclip=D, APC/heat probe plus hemoclip=E, APC plus hemoclip plus Bosmin=F, APC: argon plasma coagulation. *$P < 0.05$.

that the continuous high-dose PPI regimen did not appear to be more effective in reducing rebleeding compared to non-high-dose regimen in subjects with high-risk ulcer bleeding after initial endoscopic hemostasis in real world clinical practice (18.2% versus 15.9%) as shown in Table 4. Meta-analysis performed by Wang also found that high-dose PPIs do not further reduce the rates of rebleeding, surgical intervention, or mortality after endoscopic treatment in patients with bleeding peptic ulcer [8]. This is contrary to the recently updated consensus statements on the routine use of the intensive PPI regimen for high-risk ulcer bleeding [4].

The explanation to the high rebleeding rate in the current study (43/220, 19.5%) is possibly due to the inclusion of a higher percentage ulcers with high-risk stigmata (214/220, 97.3%) and patients with more severe comorbidities (Rockall score: Mean ± SD = 6.0 ± 1.6). In real world practice, more physicians may prescribe high-dose intravenous PPIs in more severe patients. This may also explain the higher rebleeding rate in the high-dose group (27.1% versus 16.0%) before case-controlled matching. However, the rebleeding rate were identical after case-controlled matching as shown in Table 4. Although we believe that the evidence from our findings may be supportive of the aforementioned studies regarding the issue that low-dose intravenous PPI dosage may be enough in treating peptic ulcer bleeding, potential bias and the relatively small sample size may hinder the conclusion for the optimal dosing of PPIs for bleeding high risk PU.

The other explanation for the possible lower dosage needed for Taiwanese may be attributed to the metabolism of PPI via the pathway of cytochrome P450 system (CYP), where its influential role was considered substantial in this issue [19]. There are more Caucasians than Asians who belong to homozygous extensive metabolizer (EM) in the distribution of genetic polymorphisms of CYP [20, 21], and the effect to maintain intragastric pH > 6.0 in the EM patients with intravenous pantoprazole is inferior to the non-EM patients owing to the lower plasma concentration [22]. Therefore it is rational that this racial difference could suggest

Table 4: Comparison between the non-high-dose and high-dose PPI after case-controlled matching.

Characteristic	Non-high-dose group ($n = 44$)	High-dose group ($n = 44$)	P value
Age (years)	66.2 ± 12.9	61.7 ± 13.8	0.121
Female gender, n (%)	11 (25)	12 (27.3)	0.808
Creatinine (mg/dL)	2.3 ± 2.3	2.6 ± 2.8	0.615
Hb (g/L)	93.3 ± 25.3	92.5 ± 28.7	0.897
Platelet ($\times 10^9$/L)	170.4 ± 86.2	189.2 ± 82.1	0.297
Use of NSAIDs, n (%)	3 (6.8)	2 (4.5)	0.696
Use of aspirin, n (%)	4 (9.1)	5 (11.4)	0.725
Use of clopidogrel, n (%)	3 (6.8)	4 (9.1)	0.694
Use of warfarin, n (%)	1 (2.3)	2 (4.5)	0.557
Coexisting illness, n (%)			
CKD III, IV/V	19/6	13/7	0.410
COPD	1	0	0.315
CAD	6	10	0.269
DM	12	14	0.640
CVA	12	7	0.195
Liver cirrhosis	10	8	0.597
Shock oat presentation	24	28	0.386
Rockall score	6.1 ± 1.4	6.4 ± 1.5	0.387
Time to endoscope (hours)	18.3 ± 23.9	13.6 ± 17.2	0.299
PRBC BT (mL)	1369.3±1496.5	1596.6 ± 1914.0	0.537
Forrest classification Ia/Ib/IIa/IIb/IIc/III	2/28/1/13	1/28/4/11	0.513
Time to oral PPI (days)	4.5 ± 4.4	6.9 ± 4.8	0.016*
Rebleeding, n (%)	8 (18.2)	7 (15.9)	0.777
Surgery, n (%)	0	0	1.000
Hospital stay (days)	12.1 ± 17.2	14.3 ± 13.5	0.505
Mortality, n (%)	5 (11.4)	3 (6.8)	0.359
Bleeding related/other causes	3/2	3/0	

Hb: hemoglobin, NSAID: nonsteroidal anti-inflammatory drug, CKD: chronic kidney disease, PPI: proton-pump inhibitor, DM: diabetes mellitus type 2, COPD: chronic obstructive pulmonary disease, CAD: coronary artery disease, CVA: cerebrovascular accident, BT: blood transfusion, PPI: proton-pump inhibitor, Hemostasis methods A/B/C/D/E/F: Bosmin plus APC/heat probe=A, APC/heat probe=B, Hemoclip=C, Bosmin plus hemoclip=D, APC/heat probe plus hemoclip=E, APC plus hemoclip plus Bosmin=F, APC: argon plasma coagulation. *$P < 0.05$.

that PPI should have better effect in Taiwanese patients [23, 24].

In our study we observed that CKD stage III to V was the independent risk factor for recurrent bleeding. This is despite the fact that all ESRD subjects received heparin-free dialysis in our hospital. Our findings were consistent with Wu et al. [25] and Cheung et al. [26] who reported that patients with ESRD and advanced chronic kidney disease were at higher risk of peptic ulcer rebleeding. The mechanism for the excessive bleeding in patients with ESRD is still unclear but may be multifactorial [27]. Platelet dysfunction in the form of impaired platelet adhesiveness and altered platelet-vessel-wall interaction is believed to have played an important role [28]. Furthermore this platelet dysfunction is not normalized after dialysis [29, 30]. The female gender in our study had higher rebleeding rate before case-controlled matching. This is probably by chance or perhaps, the study number was not big enough, and we need larger study scale to minimize the bias. However, when we re-analyzed the case-matching between the high-dose and non-high-dose groups, this problem does not exist anymore.

We recognized several limitations in this study. First, this retrospective analysis depended heavily on the completeness of the medical charts. If incomplete chart description of ulcer morphology was encountered, we would review endoscopic images or videos to determine the location and severity of the ulcer involved. Second, the selection bias may exist in high-dose group caused by clinicians' decision on PPI dosage in patients with more severe diseases or with less manageable bleeding ulcers. One of the main purposes of the study was to attempt to minimize selection bias by the randomized treatment-control matching process after controlling the baseline conditions of subjects. Although we observed that the rebleeding rates were identical in high-dose and non-high-dose patients after case-controlled matching, the case number was too small for a solid conclusion.

In conclusion, this study suggests that the effect of intravenous high-dose pantoprazole may not be superior to non-high dose regimen in reducing the occurrence of rebleeding, mortality rate, and surgery needed in patients in high-risk peptic ulcer bleeding after successful endoscopic hemostasis. More large scale prospective studies to clarify the

issue are still mandatory. In real world practice, election bias may exist in high-dose group caused by clinicians' decision on PPI dosage in patients with more severe diseases or with less manageable bleeding ulcers.

References

[1] M. L. Freeman, O. W. Cass, C. J. Peine, and G. R. Onstad, "The non-bleeding visible vessel versus the sentinel clot: natural history and risk of rebleeding," *Gastrointestinal Endoscopy*, vol. 39, no. 3, pp. 359–366, 1993.

[2] J. Y. W. Lau, J. J. Y. Sung, K. K. C. Lee et al., "Effect of intravenous omeprazole on recurrent bleeding after endoscopic treatment of bleeding peptic ulcers," *The New England Journal of Medicine*, vol. 343, no. 5, pp. 310–316, 2000.

[3] I. M. Gralnek, A. N. Barkun, and M. Bardou, "Management of acute bleeding from a peptic ulcer," *The New England Journal of Medicine*, vol. 359, no. 9, pp. 928–937, 2008.

[4] A. N. Barkun, M. Bardou, E. J. Kuipers et al., "International consensus recommendations on the management of patients with nonvariceal upper gastrointestinal bleeding," *Annals of Internal Medicine*, vol. 152, no. 2, pp. 101–113, 2010.

[5] M. E. van Leerdam, E. M. Vreeburg, E. A. J. Rauws et al., "Acute upper GI bleeding: did anything change? Time trend analysis of incidence and outcome of acute upper GI bleeding between 1993/1994 and 2000," *American Journal of Gastroenterology*, vol. 98, no. 7, pp. 1494–1499, 2003.

[6] A. N. Barkun, A. W. Cockeram, V. Plourde, and R. N. Fedorak, "Review article: acid suppression in non-variceal acute upper gastrointestinal bleeding," *Alimentary Pharmacology and Therapeutics*, vol. 13, no. 12, pp. 1565–1584, 1999.

[7] G. Brunner, P. Luna, M. Hartmann, and W. Wurst, "Optimizing the intragastric pH as a supportive therapy in upper GI bleeding," *Yale Journal of Biology and Medicine*, vol. 69, no. 3, pp. 225–231, 1996.

[8] C. H. Wang, M. H. M. Ma, H. C. Chou et al., "High-dose vs non-high-dose proton pump inhibitors after endoscopic treatment in patients with bleeding peptic ulcer: a systematic review and meta-analysis of randomized controlled trials," *Archives of Internal Medicine*, vol. 170, no. 9, pp. 751–758, 2010.

[9] A. Andriulli, S. Loperfido, R. Focareta et al., "High- versus low-dose proton pump inhibitors after endoscopic hemostasis in patients with peptic ulcer bleeding: a multicentre, randomized study," *American Journal of Gastroenterology*, vol. 103, no. 12, pp. 3011–3018, 2008.

[10] H. C. Cheng, A. W. Kao, C. H. Chuang, and B. S. Sheu, "The efficacy of high- and low-dose intravenous omeprazole in preventing rebleeding for patients with bleeding peptic ulcers and comorbid illnesses," *Digestive Diseases and Sciences*, vol. 50, no. 7, pp. 1194–1201, 2005.

[11] M. L. Hu, K. L. Wu, K. W. Chiu et al., "Predictors of rebleeding after initial hemostasis with epinephrine injection in high-risk ulcers," *World Journal of Gastroenterology*, vol. 16, no. 43, pp. 5490–5495, 2010.

[12] R. Marmo, G. Rotondano, R. Piscopo, M. A. Bianco, R. D'Angella, and L. Cipolletta, "Dual therapy versus monotherapy in the endoscopic treatment of high-risk bleeding ulcers: a meta-analysis of controlled trials," *American Journal of Gastroenterology*, vol. 102, no. 2, pp. 279–289, 2007.

[13] T. A. Rockall, R. F. A. Logan, H. B. Devlin, and T. C. Northfield, "Risk assessment after acute upper gastrointestinal haemorrhage," *Gut*, vol. 38, no. 3, pp. 316–321, 1996.

[14] N. I. Church, H. J. Dallal, J. Masson et al., "Validity of the Rockall scoring system after endoscopic therapy for bleeding peptic ulcer: a prospective cohort study," *Gastrointestinal Endoscopy*, vol. 63, no. 4, pp. 606–612, 2006.

[15] National Kidney Foundation, "K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification," *American Journal of Kidney Diseases*, vol. 39, no. 2, supplement 1, pp. S1–S266, 2002.

[16] H. W. Xu, J. H. Wang, M. S. Tsai et al., "The effects of cefazolin on cirrhotic patients with acute variceal hemorrhage after endoscopic interventions," *Surgical Endoscopy*, vol. 25, no. 9, pp. 2911–2918, 2011.

[17] S. C. Lin, K. L. Wu, K. W. Chiu et al., "Risk factors influencing the outcome of peptic ulcer bleeding in end stage renal diseases after initial endoscopic haemostasis," *International Journal of Clinical Practice*. In press.

[18] S. C. Yang, K. L. Wu, J. H. Wang et al., "The effect of systemic antibiotic prophylaxis for cirrhotic patients with peptic ulcer bleeding after endoscopic interventions," *Hepatology International*. In press.

[19] J. M. Kang, N. Kim, D. H. Lee et al., "Effect of the CYP2C19 polymorphism on the eradication rate of Helicobacter pylori infection by 7-day triple therapy with regular proton pump inhibitor dosage," *Journal of Gastroenterology and Hepatology*, vol. 23, no. 8, pp. 1287–1291, 2008.

[20] T. Furuta, N. Shirai, M. Sugimoto, A. Nakamura, A. Hishida, and T. Ishizaki, "Influence of CYP2C19 pharmacogenetic polymorphism on proton pump inhibitor-based therapies," *Drug Metabolism and Pharmacokinetics*, vol. 20, no. 3, pp. 153–167, 2005.

[21] P. W. Y. Lim, K. L. Goh, and B. C. Y. Wong, "CYP2C19 genotype and the PPIs—focus on rabeprazole," *Journal of Gastroenterology and Hepatology*, vol. 20, no. 3, supplement, pp. S22–S28, 2005.

[22] J. H. Oh, M. G. Choi, M. S. Dong et al., "Low-dose intravenous pantoprazole for optimal inhibition of gastric acid in Korean patients," *Journal of Gastroenterology and Hepatology*, vol. 22, no. 9, pp. 1429–1434, 2007.

[23] L. Bertilsson, "Geographical/interracial differences in polymorphic drug oxidation. Current state of knowledge of cytochromes P450 (CYP) 2D6 and 2C19," *Clinical Pharmacokinetics*, vol. 29, no. 3, pp. 192–209, 1995.

[24] B. S. Sheu, A. W. Kao, H. C. Cheng et al., "Esomeprazole 40 mg twice daily in triple therapy and the efficacy of Helicobacter pylori eradication related to CYP2C19 metabolism," *Alimentary Pharmacology and Therapeutics*, vol. 21, no. 3, pp. 283–288, 2005.

[25] C. Y. Wu, M. S. Wu, K. N. Kuo, C. B. Wang, Y. J. Chen, and J. T. Lin, "Long-term peptic ulcer rebleeding risk estimation in patients undergoing haemodialysis: a 10-year nationwide cohort study," *Gut*, vol. 60, no. 8, pp. 1038–1042, 2011.

[26] J. Cheung, A. Yu, J. LaBossiere, Q. Zhu, and R. N. Fedorak, "Peptic ulcer bleeding outcomes adversely affected by end-stage renal disease," *Gastrointestinal Endoscopy*, vol. 71, no. 1, pp. 44–49, 2010.

[27] A. B. Toke, "GI bleeding risk in patients undergoing dialysis," *Gastrointestinal Endoscopy*, vol. 71, no. 1, pp. 50–52, 2010.

[28] P. Boccardo, G. Remuzzi, and M. Galbusera, "Platelet dysfunction in renal failure," *Seminars in Thrombosis and Hemostasis*, vol. 30, no. 5, pp. 579–589, 2004.

[29] R. Sreedhara, I. Itagaki, B. Lynn, and R. M. Hakim, "Defective platelet aggregation in uremia is transiently worsened by hemodialysis," *American Journal of Kidney Diseases*, vol. 25, no. 4, pp. 555–563, 1995.

[30] G. Remuzzi, M. Livio, and G. Marchiaro, "Bleeding in renal failure: altered platelet function in chronic uraemia only partially corrected by haemodialysis," *Nephron*, vol. 22, no. 4–6, pp. 347–353, 1978.

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Clinical Study

Comparison between Single-Dose Esomeprazole- and Pantoprazole-Based Triple Therapy on the Effectiveness for *Helicobacter pylori* Eradication in Taiwanese Population

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Background and Study Aims. To compare the effectiveness of two regimens, single-dose esomeprazole- and pantoprazole-based triple therapy, for *Helicobacter pylori* (*H. pylori*) eradication. *Patients and Methods.* A total of 453 patients were enrolled for *H. pylori* eradication. They were randomly assigned to either EAC group (Esomeprazole 40 mg once daily, Amoxicillin 1 g twice daily, Clarithromycin 500 mg twice daily for 7 days) or PAC group (Pantoprazole 40 mg twice daily, Amoxicillin 1 g twice daily, Clarithromycin 500 mg twice daily for 7 days). Follow-up endoscopy or urea breath test was scheduled 12–16 weeks after the eradication to evaluate the therapeutic response. *Results.* Higher eradication rate in EAC group than PAC group was shown by intention-to-treat analysis (EAC 72% versus PAC 55%, $P < 0.05$) and per-protocol analysis (EAC 91% versus PAC 72%, $P < 0.05$). The incidence of adverse effects (EAC 19% versus PAC 17%, $P = 0.712$) and the compliance (EAC 87% versus PAC 91%, $P = 0.083$) were comparable between these 2 groups. *Conclusions.* Single-dose esomeprazole-based triple therapy is effective for *H. pylori* eradication.

1. Introduction

Chronic *Helicobacter pylori* (*H. pylori*) infection is responsible for gastritis, peptic ulcer disease, gastric mucosa-associated lymphoid tissue lymphoma (MALT lymphoma) [1], and gastric adenocarcinoma [2]. Consequently, eradication of *H. pylori* is indicated for patients with peptic ulcer disease, low-grade gastric MALT lymphoma, atrophic gastritis. First-degree relatives of gastric cancer patients and some extraintestinal diseases, for example, unexplained iron deficiency anemia, and chronic idiopathic thrombocytopenic purpura may benefit from *H. pylori* eradication as well [2]. According to the Maastricht III Consensus Report, the recommended first-line treatment of *H. pylori* eradication is triple therapy with a proton pump inhibitor (PPI), clarithromycin, and amoxicillin or metronidazole given twice daily [2].

Proton pump inhibitor (PPI) is superior to H2 blocker for *H. pylori* eradication [3] because PPI is the most potent drug to inhibit gastric secretion to enhance the bioavailability of the antibiotics in the stomach [4]. PPI is metabolized via hepatic enzyme cytochrome P450 system,

especially S-mephenytoin 4′-hydroxylase (CYP 2C19) and CYP 3A4 [5]. Single-nucleotide polymorphism (SNP) of these enzymes may lead to variable plasma level of PPI and affect intragastric pH level as a result. Esomeprazole is the *S*-enantiomer of omeprazole. This single enantiomer is shown to be more efficacious than the racemic mixture of omeprazole. Although esomeprazole and its metabolites are indistinguishable from omeprazole, a single oral dose of 40 mg esomeprazole generally results in peak plasma esomeprazole concentrations of 0.5–1.0 mg/L within 1–4 hours [6]. Theoretically, esomeprazole (40 mg once daily) should be as effective and economic for *H. pylori* eradication as the regular bid dose of PPI, suggested by Maastricht III consensus. Although some studies showed the effectiveness of esomeprazole-based triple therapy for *H. pylori* eradication, they studied esomeprazole 40 mg twice daily [7, 8], instead of esomeprazole 40 mg once daily. Therefore, we conducted the study to evaluate the effectiveness of single-dose 40 mg once daily esomeprazole based triple therapy for *H. pylori* eradication.

2. Patients and Methods

2.1. Patients and Study Design. A total of 501 dyspeptic patients were included and 453 patients (192 men and 261 women, mean age 52.48 years old, 16–83 years old) were enrolled at the Outpatient Department of the Division of Gastroenterology, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan, from March 2005 to March 2009. Exclusion criteria were recent use of antibiotics, bismuth, or PPIs within the prior 4 weeks; history of gastric surgery; allergy to the study medication; serious comorbid illness (decompensated liver cirrhosis, renal failure); women who are pregnant and breastfeeding; previous *H. pylori* eradicated therapy. All of them received esophagogastroduodenoscopy (EGD). In addition, all of the patients were interviewed by a trained interviewer for the personal and medical history obtained by a standardized questionnaire. Once the status of *H. pylori* infection was confirmed, participants were randomly assigned to two groups: EAC group (esomeprazole 40 mg once daily, amoxicillin 1 g twice daily, clarithromycin 500 mg twice daily for 7 days) or PAC group (pantoprazole 40 mg twice daily, amoxicillin 1 g twice daily, clarithromycin 500 g twice daily for 7 days). Follow-up endoscopy or urea breath test was scheduled 12–16 weeks after the eradication to evaluate the therapeutic response and PPI was withheld 2 weeks beforehand. This study was approved by Institutional Review Board and Ethical committee of Kaohsiung Medical University Hospital and we obtained written informed consents from all the participants.

2.2. Questionnaire. The standardized questionnaire consisted of demographic data, underlying diseases, use of nonsteroidal anti-inflammatory drug (NSAID) and personal history about smoking and alcohol, coffee, or tea drinking. Smokers were defined as consumption of more than one pack of cigarettes per week. Drinkers were defined as consumption of more than one glass of alcoholic beverage per day. Compliance was defined as good (taking more than 70% of all administered medication) and poor [9]. The adverse events included diarrhea, constipation, abdominal pain, anorexia, nausea, vomiting, skin rash, headache, dizziness, taste perversion, and fatigue. The adverse events were further divided into positive adverse events defined as those who considered the adverse events disturbing the quality of daily life and negative ones defined as those who did not experience the events or did not consider them troublesome [9].

2.3. Diagnosis of H. pylori Infection. Culture, histology, rapid urease test, and ^{13}C-urea breath test (UBT) were used in this study. Endoscopic biopsy specimens were rubbed on the surface of a Columbia blood agar plate for culture. Positive culture was considered if one or more colonies showed Gram negative, oxidase(+), catalase(+), urease(+), or spiral or curved rods in morphology. The presence of *H. pylori* in the pathology of gastric biopsy specimens was also evaluated by experienced pathologists. The result of rapid urease test (sensitivity 93–97%, specificity 98%) [10], CLO test (Delta West Bentley, WA, Australia), was interpreted as positive if the color turned to pink or red at room temperature 6 hours after the EGD examination. The ^{13}C-urea breath test used in the study was manufactured by the Institute of Nuclear Energy Research, Taiwan. *H. pylori* infection was defined as positive either culture was positive or at least two positive results of rapid urease test, histology, or UBT [11].

2.4. Statistical Analysis. The primary outcomes were rates of eradication, adverse events, and compliance. The difference of the age of the patients was analyzed by Student's t-test. The eradication rate, adverse effects and compliance between EAC and PAC groups were analyzed by Chi-square test. P value $< .05$ was considered statistically significant.

3. Results

3.1. Demographic Characteristics. The demographic characteristics, including age, gender, smoking, alcohol consumption, ingestion of coffee or tea or both and no significant difference demonstrated, and endoscopic diagnosis of both groups (EAC group and PAC group) were analyzed (Table 1). No significant difference was found between the two groups except age and alcohol consumption (Table 1). The patient disposition according to CONSORT statement was shown (Figure 1) [12].

3.2. Eradication Rate. The eradication rate of *H. pylori* between the two groups was shown in Table 2. The eradication rate in the EAC group was significantly better than the PAC group in both the intention-to-treat (ITT) and the per-protocol (PP) analyses.

3.3. Adverse Events and Compliance. There was no difference regarding adverse effects during the treatment (EAC versus PAC, 19% versus 17%) (Table 2). In our study, adverse

Table 1: Demographic distribution and Endoscopic diagnosis of two patient groups.

	EAC group ($n = 208$)	PAC group ($n = 245$)	P value
Age (years)			
Mean ± SD	50.91 ± 12.96	51 ± 12.34	.016
Gender			.873
Male	89 (42.8%)	103 (42%)	
Female	119 (57.2%)	142 (58%)	
Smoking	27 (13%)	24 (9.8%)	.591
Alcohol consumption	18 (8.7%)	9 (3.7%)	.046
Ingestion of coffee	60 (28.8%)	49 (20%)	.195
Ingestion of tea	85 (40.9%)	79 (32.2%)	.895
Endoscopic diagnosis			.705
Gastritis	60 (28.8%)	82 (33.5%)	
Gastric ulcer	31 (14.9%)	33 (13.5%)	
Duodenal ulcer	94 (45.2%)	101 (41.2%)	
Gastric and duodenal ulcer	23 (11.1%)	29 (11.8%)	

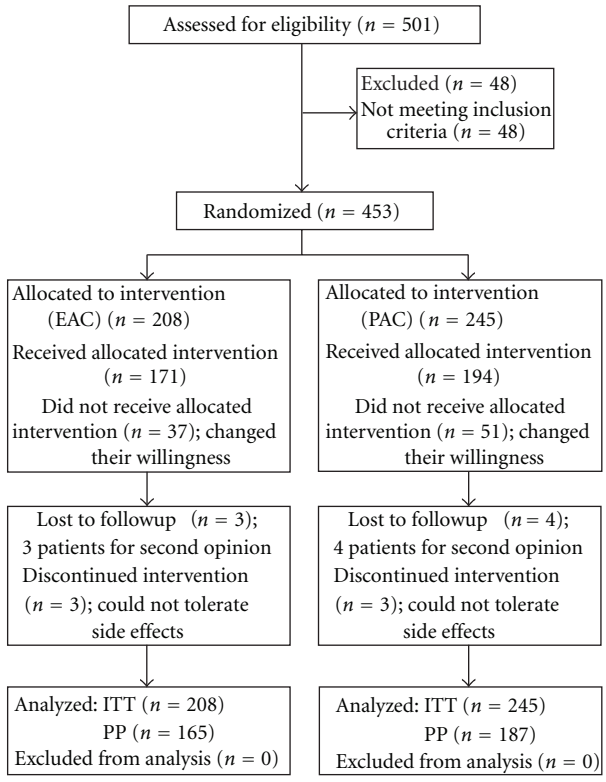

Figure 1

events included abdominal symptoms (diarrhea, constipation, abdominal pain, nausea, vomiting), taste perversion, anorexia, dizziness, headache, fatigue, and skin rash. Of all the adverse events taste perversion (EAC group 32 patients (15.4%); PAC group 29 patients (11.9%)) was the most common, followed by dizziness (EAC group 12 patients (5.8%); PAC group 11 patients (4.5%)). Fatigue (EAC 4.8%) and diarrhea (PAC 4.1%) also topped the list (Table 3). As for the compliance, 87% in the EAC group and 91% in the PAC group were noted. No significant difference was noted.

Table 2: Outcomes of esomeprazole- and pantoprazole-based triple therapy.

	EAC group ($n = 208$)	PAC group ($n = 245$)	P value
Eradication rate			
Intention-to-treat	72% (150/208)	55% (135/245)	<.05
Per-protocol	91% (150/165)	72% (135/187)	<.05
Adverse events	19% (39/208)	17% (42/245)	.712
Compliance	87% (181/208)	91% (223/245)	.083

Table 3: Adverse events during single-dosed esomeprazole- and pantoprazole-based triple therapies.

Adverse events	EAC group ($n = 208$)	PAC group ($n = 244$)	P value
Diarrhea	7 (3.4%)	10 (4.1%)	.131
Constipation	1 (0.5%)	2 (0.8%)	1.000
Abdominal pain	3 (1.4%)	9 (3.7%)	.241
Anorexia	1 (0.5%)	3 (1.2%)	.633
Nausea	7 (3.4%)	8 (3.3%)	1.000
Vomiting	1 (0.5%)	4 (1.6%)	.388
Skin rash	0 (0%)	5 (2%)	.069
Dizziness	12 (5.8%)	11 (4.5%)	.519
Headache	2 (1%)	8 (3.3%)	.196
Taste perversion	32 (15.4%)	29 (11.9%)	.209
Fatigue	10 (4.8%)	6 (2.5%)	.198

4. Discussion

Our study demonstrated higher eradication rate of *H. pylori* with single-dose esomeprazole based triple therapy (esomeprazole 40 mg once daily, amoxicillin 1 g twice daily, clarithromycin 500 mg twice daily for 7 days) than pantoprazole-based triple therapy (pantoprazole 40 mg twice daily, amoxicillin 1 g twice daily, clarithromycin 500 mg twice daily for 7 days). Similar prevalence of adverse events and compliance were observed between the two groups. Proton pump inhibitors (PPIs) are primarily metabolized via hepatic cytochrome P450(CYP)2C19 pathway. Genetic polymorphisms in CYP2C19 has been shown to have great influence on the metabolism of the PPIs. In our study, esomeprazole, s-isomer-omeprazole, is less influenced than pantoprazole. Consequently, it is more likely that esomeprazole may keep its therapeutic potency persistently [13–16].

Another issue which matters with the potency of PPI is to tackle the increasing antibiotic resistance. Increasing prevalence of resistant strain of *H. pylori* to clarithromycin was demonstrated in some studies. According to Vakil the prevalence of clarithromycin-resistant strain in the United States was 10–12% and wider range of 1–21% in the Europe. In Asia, a study from Hong Kong disclosed that

Table 4: Clinical factors of higher eradication rate in the study.

Clinical factors	*P*
Age	.016
Alcohol consumption	.046
Prescribed PPI	<.05

the prevalence was 7.8% and the prevalence in Taiwan was 6% [17–19]. PPI could enhance the bioavailability and activity of the clarithromycin by reducing gastric acid secretion. In other words, the more potent the PPI is, the more effective clarithromycin would be. Esomeprazole gets more anti-*H. pylori* activity by its potent suppression of gastric acid secretion. This may be an explanation why higher eradication rate of *H. pylori* was observed in the EAC group. The other explanation for the higher eradication rate in the EAC group is higher pKa1 and pKa2 values of esomeprazole. The PPI pharmacophore is a 2-pyridylmethyl-sulfinyl-benzimidazole. The differences of the structure of the current marketed PPIs (omeprazole, esomeprazole, lansoprazole, pantoprazole, rabeprazole) are about the substituents placed on the pyridine and benzimidazole rings. pKa1 means the pKa value of the pyridine nitrogen (the pH at which the number of inactive not protonated forms and that of active protonated forms are equal, in other words the relative acidic stability) and pKa2 is the pKa value of the benzimidazole N3. They two are crucial for the activation of the PPI and higher values are positively related to more potent and persistent effects [16, 20]. As reported by Roche et al., pKa1 and pKa2 values of esomeprazole are 4.06 and 0.79, respectively, and the values of pantoprazole are 3.83 and 0.11 [20, 21]. In addition, reports showed younger age and alcohol consumption had positive effects on *H. pylori* eradication [22, 23]. We also observed a similar correlation in our study. In the EAC group, which had a higher eradication rate, patients tended to be younger and have more frequent alcohol consumption. Therefore, we suggest that age, alcohol consumption and prescribed PPI are the clinical factors which may influence the eradication rate (Table 4). In conclusion the higher eradication rate observed in the EAC group was the accumulative results from more potency of esomeprazole, higher pKa1 and pKa2 values, less influence by genetic polymorphisms in CYP2C19, younger age, and being more frequent alcohol consumption in EAC group.

According to the results of some studies from the United States the eradication rate of *H. pylori* by first-line therapy (PPI + Amoxicillin + Clarithromycin) is decreasing in recent years from 75% (Laine, 1998) to 65% (Bochenek, 2003) [17]. As shown from our study the eradication rate in the EAC group was still as high as 91%. As mentioned above, PPIs are metabolized primarily via CYP2C19 pathway. According to the polymorphism of CYP2C19, individuals can be divided into extensive metabolizer (EM) and poor metabolizer (PM). The prevalence of PM is more frequent in Asian population (15–23%) than Caucasian population (2–5%) [24]. The therapeutic effect of PPI in terms of *H. pylori* eradication is better in PM individuals. This observation might explain the higher eradication rate in our study than studies from the United States. In addition, another major determinant for successful eradication is body mass index (BMI). According to Hsu et al. [9] the average body weight of the Asian is less than the Caucasian. Therefore, it is not surprising that higher eradication rate is found Asian populations, if the same dose of proton pump inhibitor and antibiotics are used.

The interaction between proton pump inhibitor and clopidogrel remains a controversial issue. As recent studies reported PPI and clopidogrel are both metabolized via cytochrome P450 pathway (CYP), especially 2C19 [25]. Therefore, coprescribing PPI and clopidogrel may contribute to decreased cardiovascular protection related to clopidogrel. Esomeprazole is less metabolized than pantoprazole via CYP2C19 pathway [25, 26]. In addition we could administer single-dose esomeprazole in the morning and clopidogrel in the evening or at bedtime during *H. pylori* eradication for reducing the interaction. According to Hsu et al. esomeprazole doesn't have negative effect on clopidogrel about platelet aggregation [27]. Single-dose esomeprazole-based triple therapy is a better option than pantoprazole for patients coprescribed clopidogrel.

In conclusion, our study show that single-dose esomeprazole-based first line triple therapy (esomeprazole 40 mg once daily, amoxicillin 1 g twice daily, clarithromycin 500 mg twice daily) is an effective regimen for *H. pylori* eradication in Taiwan.

Conflict of Interests

All authors have no conflict of interest to declare.

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References

[1] W. D. Chey and B. C. Y. Wong, "American College of Gastroenterology guideline on the management of *Helicobacter pylori* infection," *American Journal of Gastroenterology*, vol. 102, no. 8, pp. 1808–1825, 2007.

[2] P. Malfertheiner, F. Megraud, C. O'Morain et al., "Current concepts in the management of *Helicobacter pylori* infection: the Maastricht III Consensus Report," *Gut*, vol. 56, no. 6, pp. 772–781, 2007.

[3] J. P. Gisbert, S. Khorrami, X. Calvet, R. Gabriel, F. Carballo, and J. M. Pajares, "Meta-analysis: proton pump inhibitors vs. H2-receptor antagonists-their efficacy with antibiotics in *Helicobacter pylori* eradication," *Alimentary Pharmacology and Therapeutics*, vol. 18, no. 8, pp. 757–766, 2003.

[4] C. M. Spencer and D. Faulds, "Esomeprazole," *Drugs*, vol. 60, no. 2, pp. 321–329, 2000.

[5] T. Furuta, N. Shirai, M. Sugimoto, A. Nakamura, A. Hishida, and T. Ishizaki, "Influence of CYP2C19 pharmacogenetic polymorphism on proton pump inhibitor-based therapies.," *Drug Metabolism and Pharmacokinetics*, vol. 20, no. 3, pp. 153–167, 2005.

[6] R. C. Baselt, *Disposition of Toxic Drugs and Chemicals in Man*, Biomedical Publications, Seal Beach, Calif, USA, 8th edition, 2011.

[7] I. C. Wu, D. C. Wu, P. I. Hsu et al., "Rabeprazole- versus esomeprazole-based eradication regimens for H. pylori infection," *Helicobacter*, vol. 12, no. 6, pp. 633–637, 2007.

[8] P. I. Hsu, K. H. Lai, C. K. Lin et al., "A prospective randomized trial of esomeprazole- versus pantoprazole-based triple therapy for *Helicobacter pylori* eradication," *American Journal of Gastroenterology*, vol. 100, no. 11, pp. 2387–2392, 2005.

[9] P. I. Hsu, K. H. Lai, C. J. Wu et al., "High-dose versus low-dose esomeprazole-based triple therapy for *Helicobacter pylori* infection," *European Journal of Clinical Investigation*, vol. 37, no. 9, pp. 724–730, 2007.

[10] P. Midolo and B. J. Marshall, "Accurate diagnosis of *Helicobacter pylori*: urease tests," *Gastroenterology Clinics of North America*, vol. 29, no. 4, pp. 871–878, 2000.

[11] C. H. Kuo, H. M. Hu, F. C. Kuo et al., "Efficacy of levofloxacin-based rescue therapy for *Helicobacter pylori* infection after standard triple therapy: a randomized controlled trial," *Journal of Antimicrobial Chemotherapy*, vol. 63, no. 5, pp. 1017–1024, 2009.

[12] D. M. M. Queiroz, R. Dani, L. D. Silva et al., "Factors associated with treatment failure of *Helicobacter pylori* infection in a developing country," *Journal of Clinical Gastroenterology*, vol. 35, no. 4, pp. 315–320, 2002.

[13] S. Yasuda, Y. Horai, Y. Tomono et al., "Comparison of the kinetic disposition and metabolism of E3810, a new proton pump inhibitor, and omeprazole in relation to S-mephenytoin 4′-hydroxylation status," *Clinical Pharmacology and Therapeutics*, vol. 58, no. 2, pp. 143–154, 1995.

[14] L. Becquemont, I. Ragueneau, M. A. Le Bot, C. Riche, C. Funck-Brentano, and P. Jaillon, "Influence of the CYP1A2 inhibitor fluvoxamine on tacrine pharmacokinetics in humans," *Clinical Pharmacology and Therapeutics*, vol. 61, no. 6, pp. 619–627, 1997.

[15] D. R. Sohn, J. T. Kwon, H. K. Kim, and T. Ishizaki, "Metabolic disposition of lansoprazole in relation to the S-mephenytoin 4′-hydroxylation phenotype status," *Clinical Pharmacology and Therapeutics*, vol. 61, no. 5, pp. 574–582, 1997.

[16] T. Andersson, M. Hassan-Alin, G. Hasselgren, K. Röhss, and L. Weidolf, "Pharmacokinetic studies with esomeprazole, the (S)-isomer of omeprazole," *Clinical Pharmacokinetics*, vol. 40, no. 6, pp. 411–426, 2001.

[17] N. Vakil and F. Megraud, "Eradication Therapy for *Helicobacter pylori*," *Gastroenterology*, vol. 133, no. 3, pp. 985–1001, 2007.

[18] Q. Gu, H. H. X. Xia, J. D. Wang et al., "Update on clarithromycin resistance in *Helicobacter pylori* in Hong Kong and its effect on clarithromycin-based triple therapy," *Digestion*, vol. 73, no. 2-3, pp. 101–106, 2006.

[19] A. H. Huang, B. S. Sheu, H. B. Yang, C. C. Huang, J. J. Wu, and X. Z. Lin, "Impact of *Helicobacter pylori* antimicrobial resistance on the outcome of 1-week lansoprazole-based triple therapy," *Journal of the Formosan Medical Association*, vol. 99, no. 9, pp. 704–709, 2000.

[20] V. F. Roche, "The chemically elegant proton pump inhibitors," *American Journal of Pharmaceutical Education*, vol. 70, no. 5, article no. 101, 2006.

[21] J. Kirchheiner, S. Glatt, U. Fuhr et al., "Relative potency of proton-pump inhibitors-comparison of effects on intragastric pH," *European Journal of Clinical Pharmacology*, vol. 65, no. 1, pp. 19–31, 2009.

[22] F. Perri, M. R. Villani, V. Festa, M. Quitadamo, and A. Andriulli, "Predictors of failure of *Helicobacter pylori* eradication with the standard "Maastricht triple therapy"," *Alimentary Pharmacology and Therapeutics*, vol. 15, no. 7, pp. 1023–1029, 2001.

[23] J. M. Baena, C. López, A. Hidalgo et al., "Relation between alcohol consumption and the success of *Helicobacter pylori* eradication therapy using omeprazole, clarithromycin and amoxicillin for 1 week," *European Journal of Gastroenterology and Hepatology*, vol. 14, no. 3, pp. 291–296, 2002.

[24] J. M. Kang, N. Kim, D. H. Lee et al., "Effect of the CYP2C19 polymorphism on the eradication rate of *Helicobacter pylori* infection by 7-day triple therapy with regular proton pump inhibitor dosage," *Journal of Gastroenterology and Hepatology*, vol. 23, no. 8, pp. 1287–1291, 2008.

[25] V. P. Tan, B. P. Yan, R. H. Hunt, and B. C. Y. Wong, "Proton pump inhibitor and clopidogrel interaction: the case for watchful waiting," *Journal of Gastroenterology and Hepatology*, vol. 25, no. 8, pp. 1342–1347, 2010.

[26] P. W. Y. Lim, K. L. Goh, and B. C. Y. Wong, "CYP2C19 genotype and the PPIs - Focus on rabeprazole," *Journal of Gastroenterology and Hepatology*, vol. 20, no. 3, supplement, pp. S22–S28, 2005.

[27] P. I. Hsu, K. Lai, and C. Liu, "Esomeprazole with clopidogrel reduces peptic ulcer recurrence, compared with clopidogrel alone, in patients with atherosclerosis," *Gastroenterology*, vol. 140, no. 3, pp. 791–798, 2011.

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Review Article

Recent Insights into Antibiotic Resistance in *Helicobacter pylori* Eradication

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Antibiotics have been useful in the treatment of *H. pylori*-related benign and malignant gastroduodenal diseases. However, emergence of antibiotic resistance often decreases the eradication rates of *H. pylori* infections. Many factors have been implicated as causes of treatment failure, but the main antibiotic resistance mechanisms described to date are due to point mutations on the bacterial chromosome, a consequence of a significantly phenotypic variation in *H. pylori*. The prevalence of antibiotic (e.g., clarithromycin, metronidazole, tetracycline, amoxicillin, and furazolidone) resistance varies among different countries; it appears to be partly determined by geographical factors. Since the worldwide increase in the rate of antibiotic resistance represents a problem of relevance, some studies have been performed in order to identify highly active and well-tolerated anti-*H. pylori* therapies including sequential, concomitant quadruple, hybrid, and quadruple therapy. These represent a promising alternatives in the effort to overcome the problem of resistance. The aim of this paper is to review the current status of antibiotic resistance in *H.pylori* eradication, highlighting the evolutionary processes in detail at alternative approaches to treatment in the past decade. The underlying resistance mechanisms will be also followed.

1. Introduction

Helicobacter pylori is a spiral-shaped, microaerophilic Gram-negative flagellate bacterium that may contribute to diseases such as duodenal/gastric ulcer disease, gastritis, gastric adenocarcinoma, and mucosa-associated tissue lymphoma (MALT) and primary B-cell gastric lymphoma. Given this relationship with human diseases, eradication of *H. pylori* in individuals may be the best course of action. In fact patients who receive *H. pylori* eradication therapy (proton pump inhibitor (PPI), amoxicillin (AMO), and clarithromycin (CLA)) often encounter eradication failure over their treatment period. Moreover, the effectiveness of "legacy triple therapy" which was recommended by Maastricht III Consensus Report provides disappointingly low treatment success (i.e., below 80%) in the world. And what could account for the resulting low treatment success or eradication failure? The reasons for this fall in effectiveness are uncertain but may be mainly related to the development of antibiotic resistant strains of *H. pylori*. In this paper, we will review the latest findings on *H. pylori* and antibiotic resistance and then summarize the factors for *H. pylori* eradication failure according to the current treatment regimens.

2. Nature of *H. Pylori* and Intragastric Environment

The stomach environment where the *H. pylori* resides was thought to be a virtual desert for microbes because of its high acidity. We now know *H. pylori* dominates the microbiome in the stomach, although the effect of this dominance is unclear [1]. A major opportunity to increase our understanding of this microbiome is massive parallel pyrosequencing of bacteria 16S amplicons. This will allow us to deeply characterize the microbiota of a wide range of subjects [2]. One such study used this small subunit 16S rDNA clone library to analyze 1833 sequences generated by broad-range bacterial PCR from 23 gastric endoscopic biopsy samples. This data suggests that *H. pylori* was the only member of the genus *Helicobacter* found in these human stomach samples and was the most abundant phylotype within the libraries which tested positive for this organism by using

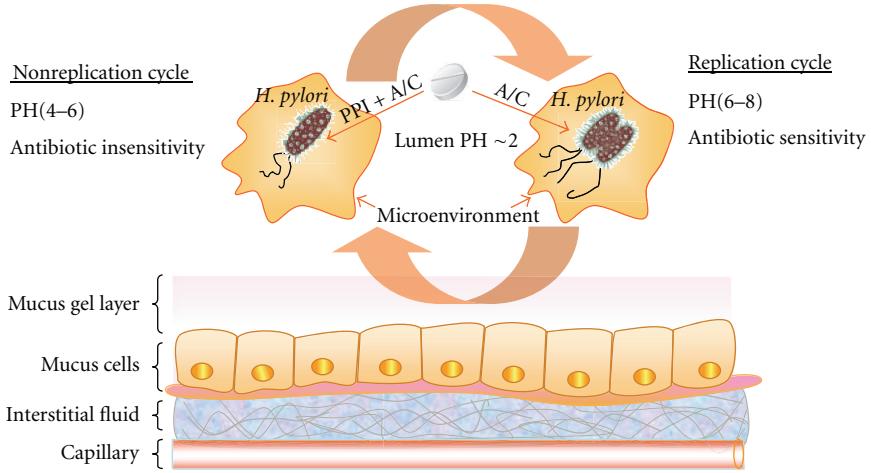

Figure 1: *H. pylori* oscillates between a replicating state (antibiotic sensitivity) and nonreplicating state (antibiotic insensitivity) according to the pH in the microenvironments, and PPI synergizes with the antibiotics by effectively increasing gastric pH and disrupts the acidic environment preferred by HP. PPI: proton pump inhibitor, A: amoxicillin, C: clarithromycin.

conventional clinical approaches [3]. The huge population of *H. pylori* is also the statistical basis of existing population of resistant organisms [4]. In addition, the bacteria oscillates between a replicating state (organism remains susceptible to the antibiotic) and nonreplicating state (the organism become phenotypically resistant) according to the pH in the microenvironments. Thus, they may enter to a nonreplicative but viable state when the pH around their microenvironments is between 4.0 and 6.0. These organisms will be difficult to eradicate, in other words, if they present the phenotypically resistant state [5] (Figure 1).

H. pylori infection also presents a unique therapeutic challenge. Determining the optimal drug therapy of such infection depends to a large extent on antimicrobial concentrations in the stomach, while it is difficult because the organism lives in an environment that is not easily accessible to some medications [6]. Upon entry into the stomach, the first hurdle for bioavailability of antibiotics is the acidity of the gastric lumen, which in humans has a median 24 h intragastric pH of 1.4 [7]. A good example of this is one of the most acid-labile antibiotics against *H. pylori*, such as clarithromycin (CLA), which is degraded in the lumen mainly through the action of acid and pepsin. Its half life is less than 1 h in this circumstance. It became clear early on that antibiotic treatment alone was relatively ineffective. Thus, increasing intragastric pH by the coadministration of potent gastric acidity inhibitors has been shown to significantly avoid eradication failure [8]. The second hurdle is the particular structure of gastric mucus. To successfully kill the bacteria present in the stomach it is necessary that the drug is delivered to the entire surface of the stomach and penetrates across the mucus layer from gastric lumen to epithelial surface (or vice versa); furthermore, the antibiotic must reach higher concentrations for a sufficient time to efficiently kill the bacteria wherever they are present [9]. Otherwise, the bacteria in such sites can recolonize the gastric epithelium, resulting in eradication failure [10]. Significant work should be undertaken in an attempt to overcome the gastric barrier, including developing several strategies to target either the transcellular or the paracellular pathway for drug delivery.

3. Epidemiology of Bacterial Resistance

It is now believed that some populations with high incidences of *H. pylori* infection, such as those in East Asian countries, have high incidences of gastric cancer, while other highly infected populations do not. This apparent anomaly has been termed the "African enigma" or "Asian enigmas". It might be explained by diverse the *H. pylori* genotypes, especially cagA and vacA, circulating in different geographic areas [11, 12]. Like the *H. pylori* infection associated with geographic areas, the prevalence of resistance rates appears to be partly determined by geographical factors; the prevalence of CLA and metronidazole (MET) resistance in China both increased from 12.8 to 23.8% and, 12.8 to 56.6%, respectively, while AMO resistance decreased from 2.1% to 0.3%, between 2000 to 2009 [13]. In Japan, adverse resistance rates to CLA increased from 7% to 15.2%, and the rate has remained fairly constant to the present day [14]. A high resistance of MET has been reported from Saudi Arabia. The rate of resistance to MET in 2008 was 69.5%, while CLA and AMO resistance rates were 21% and 0%, respectively [15]. In Europe, there are huge differences between southern and northern Europe. Higher resistance rates of clarithromycin in adults are observed in southern European countries such as Spain where the rate of CLA resistance was 35.6% in patient isolates of *H. pylori* [16]. Generally speaking, it was as high up as 20% compared to northern European countries [17, 18]. CLA resistance is seemingly common in the USA, ranging 10–15%, while MET resistance rates are 20–40% and resistance to amoxicillin appears to be infrequent [19, 20]. Mendonça et al. analyzed 90 Brazilian dyspeptic patients and revealed that resistance of *H. pylori* to clarithromycin, metronidazole, tetracycline (TET), amoxicillin (AMO), and furazolidone (FUR) was 7%, 42%, 7%, 29%, and 4%, respectively [21]. A meta-analyses reported the overall *H. pylori* antibiotic

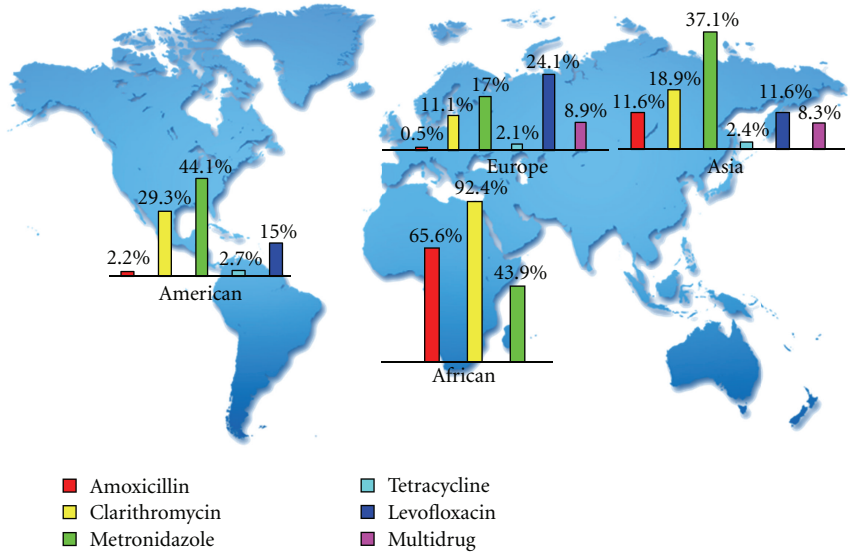

Figure 2: Antibiotic resistance rates in different continental areas.

resistance rates worldwide (31 studies from 1993 to 2009) which showed an overall *H. pylori* antibiotic resistance rate for AMO, CLA, MET, TET, levofloxacin (LEV), and multidrug-based therapies in different continental areas [22]. Detailed resistance rates towards antibiotics in different continental areas are shown in Figure 2.

Some of the reasons for these findings may include the following (1) CLA was widely administered as monotherapy for respiratory infections and as a consequence high resistance rates are reported in these countries [23]. (2) The prevalence of antibiotic resistance in various regions is correlated with the general use of antibiotics in the region, while countries with a prudent consumption of macrolides continues to be low [24]. (3) *H. pylori* strains have been divided into five major groups (east Asian type, south/central Asian type, Iberian/African type, and European type) according to geographical associations [25]. Thus, geographic differences associated with the presence of phylogeographic features of *H. pylori* may be a factor to explain the existing different antibiotic resistances [26, 27].

4. Current Anti-*H. Pylori* Regimens

H. pylori eradication therapy, including antibiotics, PPI, and/or bismuth given for one or two weeks, has emerged as the treatment of choice (Figure 3). Standard triple therapy which represents the accepted standard therapy for *H. pylori* is known to be susceptible to clarithromycin, and local antimicrobial resistance rates are below to 20% [28], while newer treatment regimens (sequential, quadruple, concomitant, and hybrid therapies) and various combinations of new and old antibiotics aimed at eradicating the organism more effectively are increasing in popularity [29, 30].

First Line Therapy. As first-line therapy in areas with a high prevalence of clarithromycin-resistant *H. pylori* strains, a novel 10 d sequential therapy should be considered. The sequential regimen containing a dual therapy (PPI and amoxicillin for 5 days) was followed by triple therapy with a PPI, clarithromycin, and tinidazole (or metronidazole) for 5 days. The eradication rate achieved with the sequential regimen has been reported significantly greater than that obtained with the standard treatment [32, 42]. However, it has shown that sequential therapy is ineffective in clearing *H. pylori* in patients with dual resistance to clarithromycin and metronidazole [23, 33]. Another new regimen term as concomitant therapy is a 4-drug non-bismuth-containing regimen (PPI, clarithromycin, amoxicillin, and metronidazole), which appears more suitable for patients in high endemic areas of dual resistance. Clinically, it is also more simple than sequential therapy as the drugs are all given together instead of changing drugs in halfway and might improve compliance. In addition, an intention-to-treat analysis demonstrated sequential or concomitant therapy with a PPI, amoxicillin, clarithromycin, and an imidazole agent has similar rates for eradication of *H. pylori* infection [42]. With regard to dual resistance, several attempts, such as the extension of sequential therapy duration and continuing the amoxicillin for the full 14 days of therapy, have been undertaken to improve the efficacy of the standard PPI triple therapy. Recently, a sequential-concomitant hybrid therapy (dual-concomitant) was designed by Hsu et al. [43]. The date showed that it provides a promising success rate of 99% by per-protocol analysis and 97% by intention-to-treat analysis. However, it must be noted that it may not work in all geographic areas, and the results will need to be confirmed in areas where different patterns of resistant are present.

Second Line Therapy. Bismuth-containing quadruple therapy as second-line and/or salvage therapy was recommended

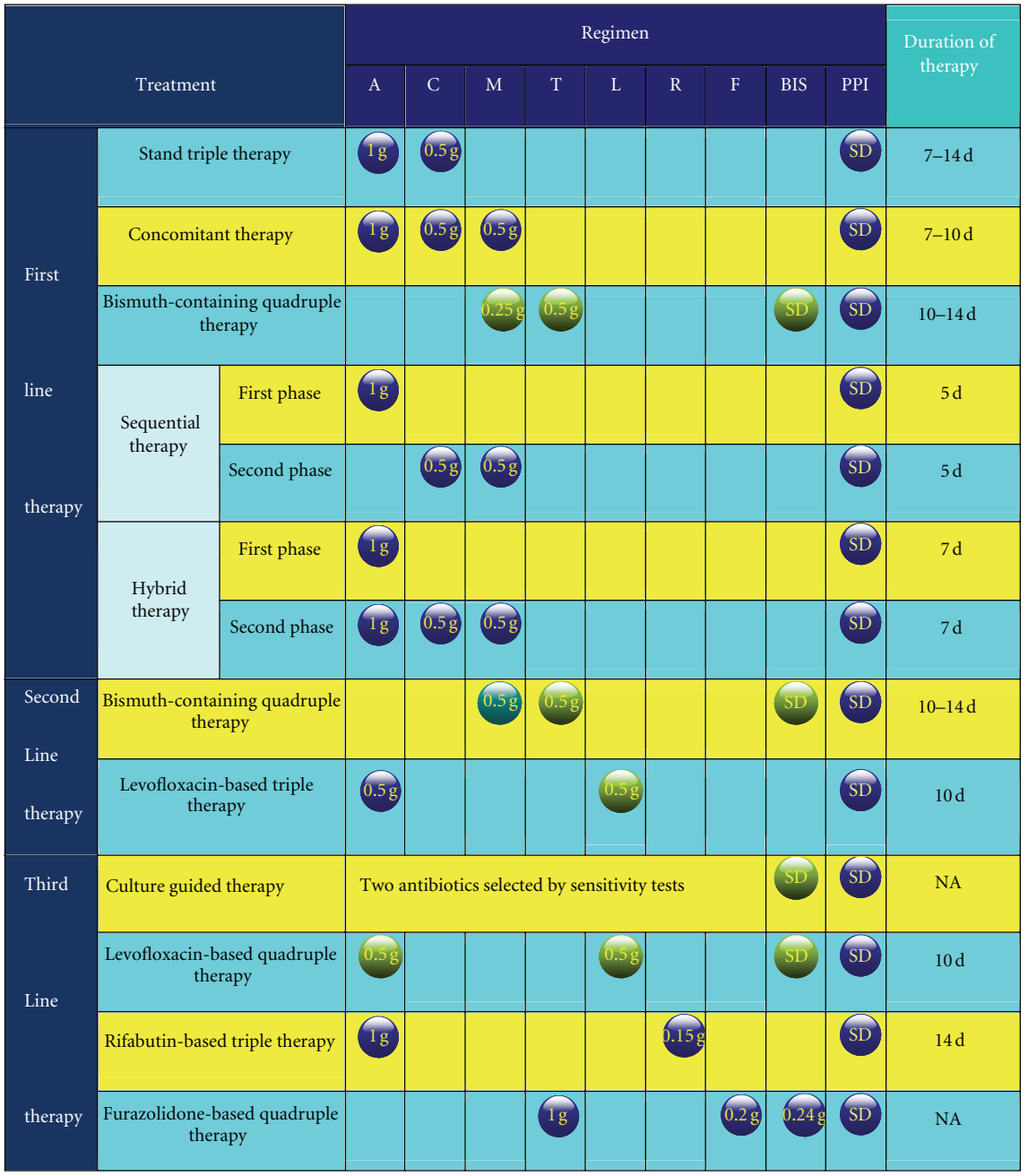

Figure 3: Current recommended regimens for *H. pylori* eradication. The figure in the ball stands for dose. Blue ball: b.i.d, purple ball: t.i.d, green ball: q.i.d. A: amoxicillin, C: clarithromycin, M: metronidazole, T: tetracycline, L: levofloxacin, R: rifabutin, F: furazolidone, SD: standard dose, BIS: bismuth, PPI: proton pump inhibitor, modified from [31–41].

by Maastricht IV/Florence Consensus Report [34]. Several multicenter studies of quadruple therapy using a single-triple (bismuth biskalcitrate, metronidazole, and tetracycline) capsule preparation with PPI have shown good efficacy for eradication of *H. pylori* [35, 36, 44]. Convenience packs that contain most of drugs in a plasticized sheet also reduce the number of pills to improve adherence. As for adverse effects, toxic effects related to bismuth are still one of the unjustified safety concerns against the quadruple therapy [37], thus, we needed to establish the reasonable bismuth dosing regimen that provides maximum eradication.

In patients who failed with clarithromycin-based triple in first line, levofloxacin-based triple therapy (levofloxacin, amoxicillin, and a PPI) has been proven in a meta-analysis which showed that this regimen was superior to quadruple therapy and fewer side effects as salvage therapy [45]. Additionally, the study revealed that antibiotics (i.e., levofloxacin) within this triple regimens cannot randomly be

changed and then switched to first line. For antibiotic resistance, rising rates of levofloxacin resistance especially in developing countries remain to be taken into account, and it appears more likely that quinolone resistance is usually relating to patients who have routinely received a fluoroquinolone for other indications [38].

Third-Line Therapy. To date, the standard third-line therapy for refractory *H. pylori* infection has not been established. Maastricht IV reports recommend that anti-*H. pylori* treatment should be guided by antimicrobial susceptibility testing after failure of second-line therapy, whenever possible [34]. Unfortunately, antimicrobial sensitivity data for patients who failed eradication therapy is still not widely available in clinical practice. For practitioners, several simple empirical management strateges are necessary.

A recent prospective study assesses the efficacy and safety of levofloxacin, amoxicillin, bismuth, and rabeprazole quadruple therapy as third-line treatment for patients who failed to eradicate *H. pylori* infection. In this investigation, the 10-day levofloxacin and amoxicillin-based quadruple rescue therapy provides superior eradication with an additional clinically important benefit of improved tolerability due to fewer side effects [30]. Other alternative candidates for third-line therapy are rifabutin; quinolones therapy is also promising [39, 40, 46], though the optimal dose and combination need further study.

5. Antibiotic Resistance Mechanisms in the Current Regimens

As a general rule for the treatment, it is defined on meeting or exceeding predefined per-protocol threshold cure rates (e.g., >90%), that is, eradication failure less than 10% [47]. *H. pylori*'s antimicrobial resistance rates vary as mentioned above. *H. pylori* eradication failures may be due to acquiring chromosomal mutations or by acquisition of foreign genes carried on mobile genetic elements (horizontal gene transfer) that cause changes in each drug's site of action [23, 48], and it cannot be reversed by increasing the dose or duration [41]. Each of these mechanisms will be elucidated in more detail below according to the current anti-*H. pylori* regimens.

Clarithromycin. In a recent study involving sequencing analysis of *H. pylori* gene 23S *rRNA* isolated from Uruguayan patients, all CLA-resistant strains point mutation were presented in position 2143 (A-to-G transition), consistent with strains studied in some developing countries worldwide. No AMO-resistant strains were identified in this study, this is most frequently reported with AMO where failure is rarely caused by acquired resistance [49, 50]. Other mutations at position 2142 (A-to-G transition) and position 2182 (C-to-T transition) have been confirmed by analysis of DNA sequencing to be the same as that described at position 2143 and are associated with CLA resistance [51]. Except for 23S *rRNA* mutations, expression of a resistance-nodulation-cell division (RND) type efflux pump, an active drug efflux mechanism responsible for rapidly transferring the drug out of the bacterial cell, preventing the binding of the antibiotic to the ribosome, plays an important role in acquiring CLA resistance [52, 53]. Nevertheless, it was shown that efflux systems are not involved in the intrinsic resistance of *H. pylor*i to antibiotics or in acquired resistance to AMO [54].

Amoxicillin. Rare tolerance to AMO has also been described and was associated to alterations in penicillin binding proteins (PBP1A) [55]. Three substitutions (Ser 414 Arg, Thr 556Ser, and Asn 562) are the most common amino acid changes in PBP1 connected to AMO resistance. Consequently, this reduces the susceptibility of these strains to the bactericidal effect of AMO [56].

Metronidazole. Metronidazole (MET) resistance in *H. pylori* is complex and is primarily associated with mutational inactivation of the redox-related gene (*frxA*, *rdxA*) [57]. *FrxA* may act indirectly by affecting cellular reductive potential in low level MET resistant isolates. *RdxA* gene inactivation confers resistance by saturation transpose on mutagenesis of the *H. pylori* genome [58, 59]. Thus, factors that lead to the loss of or inactivation of the two genes may lead to contribute to MET resistance per se. Meanwhile, there are reports that the MET resistance phenotype may arise in *H. pylori* without mutations in *rdxA* or *frxA*, suggesting the presence of additional MET resistance mechanisms [60]. Choi et al. proposed that several mutational changes in *H. pylori* Fur proteins can affect MET susceptibility via altering the balance among Fur's several competing activities and thereby eliminating bactericidal MET activation products [61].

Fluoroquinolone. The mechanism of fluoroquinolone (FLU) resistance in *H. pylori* has been found to be linked to mutations in the quinolone resistance-determining regions (QRDR) of the gyrase A (*gyrA*) gene [62]. This region, responsible for DNA cleavage and rearrangement, is also the position of action of quinolones [39]. A recent study performed in Korea has shown this resistance was considered to depend mainly on *gyrA* gene mutation at Asn87 or Asp91 [63], and mutation in the *gyrB* gene has also been identified in LEV resistant strains. This rarely occurs and often occurs together with *gyrA* mutations. This indicates that *gyrB* has little impact on primary levofloxacin resistance. In addition, *gyrA* gene has double *gyrA* mutations hot spots at N87K and D91G or D91Y which were linked to high-level fluoroquinolone resistance by laboratory mutants [64].

Rifabutin. Rifabutin (RIF) is a spiropiperidyl rifamycin-S derivative, which inhibits the B-subunit of the DNA-directed *RNA* polymerase (*rpoB*) of *H. pylori*. RIF has potential activity against *H. pylori* because the *in vitro* sensitivity is high, and it does not share resistance to either CLA or AMO [65, 66]. It is structurally related to rifampin (rifampicin) and shares many of its properties. The mechanism of *H. pylori* resistance to this group of antibiotics is not known, only some studies clearly show that it is substantial cross-resistance *in vitro* between rifabutin and rifampin, mainly

caused by point mutations occurring in the *rpo*B gene at codons 524, 525, and 585 as in other bacteria [66–68].

Tetracycline. Tetracycline (TET) is an antibiotic that is commonly used to eradicate *H. pylori* infection in several second-line regimens. The bactericidal activity of TET is a result of the drug's ability to prevent the synthesis of nascent peptide chains via binding to the 30S ribosomal subunit as well as blocking the binding of aminoacyl-*tRNA* [69]. The best-studied resistant mechanism has been mostly associated with de novo mutations in the 16S *rRNA* gene, which is based on a single, double or triple base-pair substitution in adjacent 16S *rRNA* gene [70]. In the case of mutation that cause resistance, single or double base-pair substitutions (A928C, AG926-927 → GT and A926G/A928C) as well as triple substitution (AGA926–928 → TTC) confer *H. pylori* with low and high-level TET resistance [71]. The phenotype observed in the case of this mutant is similar to those observed by Gerrits et al. [72]. Probably, decreased antibiotic binding of the drug for the ribosome reduces its antibiotic property. Resistance to TET is also related to a proton motive force (PMF)-dependent efflux of TET across the cell membrane. Consistent with efflux studies, carbonyl cyanide m-chlorophenylhydrazone (CCCP), an inhibitor that disrupts the proton gradient across the membrane, leads to antibiotic accumulation by presence or absence of it. Therefore, it plays an important role in the resistance of clinical isolates of *H. pylori* to TET [73].

6. Conclusion

H. pylori is considered pathogenic, even carcinogenic. With this simple view, eradication is considered as an obvious choice. In reality, however, the rate of eradication failure has dramatically risen in many countries due to resistance to antibiotics. On genetic support, mutation is considered as the key phenotypic variation as well as response to selection stress. Other suspected mechanisms of acquired drug resistance include: decreased permeation of the antibiotic into the bacterial cell and multidrug efflux pumps confer resistance to β-lactams [54]. An opportunity to solve this is whole-genome sequencing of multiple isolates of individual patients with dense spatial and temporal sampling. A practical application is the detection of genomic changes related to drug resistance by comparing the genomes of wild-type strains and those that survived antibiotic treatments [74, 75]. Furthermore, in the context of clinic treatment, selection pressure exerted by the long-term use of antibiotics, drug adverse effect, patient tolerability, adherence, even the patient's disease status should considered by doctors [4]. It is important to remember that antibiotic resistance can often be partially overcome by susceptibility and DNA testing and differentiation of recrudescence and reinfection. Highly active and well-tolerated regimen should be sought and appropriately tested in randomized controlled trial (RCT) instead of simply following consensus guidelines.

Conflict of Interests

There is no conflict of interest to disclose for all authors.

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References

[1] V. Necchi, M. E. Candusso, F. Tava et al., "Intracellular, intercellular, and stromal invasion of gastric mucosa, preneoplastic lesions, and cancer by*Helicobacter pylori*," *Gastroenterology*, vol. 132, no. 3, pp. 1009–1023, 2007.

[2] M. J. Friedrich, "Microbiome project seeks to understand human body's microscopic residents," *JAMA*, vol. 300, no. 7, pp. 777–778, 2008.

[3] E. M. Bik, P. B. Eckburg, S. R. Gill et al., "Molecular analysis of the bacterial microbiota in the human stomach," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 3, pp. 732–737, 2006.

[4] D. Y. Graham and L. Fischbach, "*Helicobacter pylori* treatment in the era of increasing antibiotic resistance," *Gut*, vol. 59, no. 8, pp. 1143–1153, 2010.

[5] D. Scott, D. Weeks, K. Melchers, and G. Sachs, "The life and death of *Helicobacter pylori*," *Gut*, vol. 43, supplement 1, pp. S56–S60, 1998.

[6] N. Vakil and F. Megraud, "Eradication therapy for *Helicobacter pylori*," *Gastroenterology*, vol. 133, no. 3, pp. 985–1001, 2007.

[7] S. R. Bloom and J. M. Polak, "Physiology of gastrointestinal hormones," *Biochemical Society Transactions*, vol. 8, no. 1, pp. 15–17, 1980.

[8] P. Moayyedi, P. Sahay, D. S. Tompkins, and A. T. R. Axon, "Efficacy and optimum dose of omeprazole in a new 1-week triple therapy regimen to eradicate *Helicobacter pylori*," *European Journal of Gastroenterology and Hepatology*, vol. 7, no. 9, pp. 835–840, 1995.

[9] P. D. Midolo, J. D. Turnidge, W. J. Munkhof, V. Berry, and G. Woodnutt, "Is bactericidal activity of amoxicillin against *Helicobacter pylori* concentration dependent?" *Antimicrobial Agents and Chemotherapy*, vol. 40, no. 5, pp. 1327–1328, 1996.

[10] J. C. Atherton, A. Cockayne, M. Balsitis, G. E. Kirk, C. J. Hawkey, and R. C. Spiller, "Detection of the intragastric sites at which *Helicobacter pylori* evades treatment with amoxycillin and cimetidine," *Gut*, vol. 36, no. 5, pp. 670–674, 1995.

[11] A. Kalebi, F. Rana, W. Mwanda, G. Lule, and M. Hale, "Histopathological profile of gastritis in adult patients seen at a referral hospital in Kenya," *World Journal of Gastroenterology*, vol. 13, no. 30, pp. 4117–4121, 2007.

[12] D. Y. Graham, H. Lu, and Y. Yamaoka, "African, Asian or Indian enigma, the East Asian *Helicobacter pylori*: facts or medical myths," *Journal of Digestive Diseases*, vol. 10, no. 2, pp. 77–84, 2009.

[13] W. Gao, H. Cheng, F. Hu et al., "The evolution of *Helicobacter pylori* antibiotics resistance over 10 years in Beijing, China," *Helicobacter*, vol. 15, no. 5, pp. 460–466, 2010.

[14] N. Horiki, F. Omata, M. Uemura et al., "Annual change of primary resistance to clarithromycin among *Helicobacter pylori* isolates from 1996 through 2008 in Japan," *Helicobacter*, vol. 14, no. 5, pp. 86–90, 2009.

[15] M. A. M. Marie, "Patterns of *Helicobacter pylori* resistance to metronidazole, clarithormycin and amoxicillin in Saudi Arabia," *Journal of Bacteriology and Virology*, vol. 38, no. 4, pp. 173–178, 2008.

[16] S. Agudo, G. Pérez-Pérez, T. Alarcón, and M. López-Brea, "High prevalence of clarithromycin-resistant *Helicobacter pylori* strains and risk factors associated with resistance in Madrid, Spain," *Journal of Clinical Microbiology*, vol. 48, no. 10, pp. 3703–3707, 2010.

[17] M. J. R. Janssen, L. Hendrikse, S. Y. de Boer et al., "*Helicobacter pylori* antibiotic resistance in a Dutch region: trends over time," *The Netherlands Journal of Medicine*, vol. 64, no. 6, pp. 191–195, 2006.

[18] T. Storskrubb, P. Aro, J. Ronkainen et al., "Antimicrobial susceptibility of *Helicobacter pylori* strains in a random adult Swedish population," *Helicobacter*, vol. 11, no. 4, pp. 224–230, 2006.

[19] L. A. Fischbach, K. J. Goodman, M. Feldman, and C. Aragaki, "Sources of variation of *Helicobacter pylori* treatment success in adults worldwide: a meta-analysis," *International Journal of Epidemiology*, vol. 31, no. 1, pp. 128–139, 2002.

[20] M. S. Osato, R. Reddy, S. G. Reddy, R. L. Penland, H. M. Malaty, and D. Y. Graham, "Pattern of primary resistance of *Helicobacter pylori* to metronidazole or clarithromycin in the United States," *Archives of Internal Medicine*, vol. 161, no. 9, pp. 1217–1220, 2001.

[21] S. Mendonça, C. Ecclissato, M. S. Sartori et al., "Prevalence of *Helicobacter pylori* resistance to metronidazole, clarithromycin, amoxicillin, tetracycline, and furazolidone in Brazil," *Helicobacter*, vol. 5, no. 2, pp. 79–83, 2000.

[22] V. De Francesco, F. Giorgio, C. Hassan et al., "Worldwide *H. pylori* antibiotic resistance: a systematic review," *Journal of Gastrointestinal and Liver Diseases*, vol. 19, no. 4, pp. 409–414, 2010.

[23] F. Mégraud, "*H pylori* antibiotic resistance: prevalence, importance, and advances in testing," *Gut*, vol. 53, no. 9, pp. 1374–1384, 2004.

[24] L. Perez Aldana, M. Kato, S. Nakagawa et al., "The relationship between consumption of antimicrobial agents and the prevalence of primary *Helicobacter pylori* resistance," *Helicobacter*, vol. 7, no. 5, pp. 306–309, 2002.

[25] Y. Yamaoka, E. Orito, M. Mizokami et al., "*Helicobacter pylori* in North and South America before Columbus," *FEBS Letters*, vol. 517, no. 1–3, pp. 180–184, 2002.

[26] Y. Yamaoka, "Mechanisms of disease: *Helicobacter pylori* virulence factors," *Nature Reviews Gastroenterology and Hepatology*, vol. 7, no. 11, pp. 629–641, 2010.

[27] Y. Yamaoka, M. Kato, and M. Asaka, "Geographic differences in gastric cancer incidence can be explained by differences between *Helicobacter pylori* strains," *Internal Medicine*, vol. 47, no. 12, pp. 1077–1083, 2008.

[28] P. Malfertheiner, F. Megraud, C. O'Morain et al., "Current concepts in the management of *Helicobacter pylori* infection: the Maastricht III Consensus Report," *Gut*, vol. 56, no. 6, pp. 772–781, 2007.

[29] P. P. Basu, K. Rayapudi, T. Pacana, N. J. Shah, N. Krishnaswamy, and M. Flynn, "A randomized study comparing levofloxacin, omeprazole, nitazoxanide, and doxycycline versus triple therapy for the eradication of *Helicobacter pylori*," *The American Journal of Gastroenterology*, vol. 106, no. 11, pp. 1970–1975, 2011.

[30] P. I. Hsu, D. C. Wu, A. Chen et al., "Quadruple rescue therapy for *Helicobacter pylori* infection after two treatment failures," *European Journal of Clinical Investigation*, vol. 38, no. 6, pp. 404–409, 2008.

[31] S.-K. Chuah, F.-W. Tsay, P.-I. Hsu, and D.-C. Wu, "A new look at anti-*Helicobacter pylori* therapy," *World Journal of Gastroenterology*, vol. 17, no. 35, pp. 3971–3975, 2011.

[32] J. P. Gisbert, X. Calvet, A. O'Connor, F. Mégraud, and C. A. O'Morain, "Sequential therapy for *Helicobacter pylori* eradication: a critical review," *Journal of Clinical Gastroenterology*, vol. 44, no. 5, pp. 313–325, 2010.

[33] D. C. Wu, P. I. Hsu, J. Y. Wu et al., "Sequential and concomitant therapy with four drugs is equally effective for eradication of *H pylori* infection," *Clinical Gastroenterology and Hepatology*, vol. 8, no. 1, pp. 36–41.e1, 2010.

[34] P. Malfertheiner, F. Megraud, C. A. O'Morain et al., "Management of *Helicobacter pylori* infection—the Maastricht IV/Florence consensus report," *Gut*, vol. 61, no. 5, pp. 646–664, 2012.

[35] C. O'Morain, T. Borody, A. Farley et al., "Efficacy and safety of single-triple capsules of bismuth biskalcitrate, metronidazole and tetracycline, given with omeprazole, for the eradication of *Helicobacter pylori*: an international multicentre study," *Alimentary Pharmacology and Therapeutics*, vol. 17, no. 3, pp. 415–420, 2003.

[36] L. Laine, R. Hunt, H. EI-Zimaity, B. Nguyen, M. Osato, and J. Spénard, "Bismuth-based quadruple therapy using a single capsule of bismuth biskalcitrate, metronidazole, and tetracycline given with omeprazole versus omeprazole, amoxicillin, and clarithromycin for eradication of *Helicobacter pylori* in duodenal ulcer patients: a prospective, randomized, multicenter, North American trial," *American Journal of Gastroenterology*, vol. 98, no. 3, pp. 562–567, 2003.

[37] R. H. Phillips, M. W. Whitehead, L. A. Doig et al., "Is eradication of *Helicobacter pylori* with colloidal Bismuth subcitrate quadruple therapy safe?" *Helicobacter*, vol. 6, no. 2, pp. 151–156, 2001.

[38] W. D. Chey and B. C. Y. Wong, "American College of Gastroenterology guideline on the management of *Helicobacter pylori* infection," *The American Journal of Gastroenterology*, vol. 102, no. 8, pp. 1808–1825, 2007.

[39] T. Nishizawa, H. Suzuki, and T. Hibi, "Quinolone-based third-line therapy for *Helicobacter pylori* eradication," *Journal of Clinical Biochemistry and Nutrition*, vol. 44, no. 2, pp. 119–124, 2009.

[40] D. Van Der Poorten and P. H. Katelaris, "The effectiveness of rifabutin triple therapy for patients with difficult-to-eradicate *Helicobacter pylori* in clinical practice," *Alimentary Pharmacology and Therapeutics*, vol. 26, no. 11-12, pp. 1537–1542, 2007.

[41] F. Mégraud and P. Lehours, "*Helicobacter pylori* detection and antimicrobial susceptibility testing," *Clinical Microbiology Reviews*, vol. 20, no. 2, pp. 280–322, 2007.

[42] D. Vaira, A. Zullo, N. Vakil et al., "Sequential therapy versus standard triple-drug therapy for *Helicobacter pylori* eradication: a randomized trial," *Annals of Internal Medicine*, vol. 146, no. 8, pp. 556–563, 2007.

[43] P. I. Hsu, D. C. Wu, J. Y. Wu, and D. Y. Graham, "Modified Sequential *Helicobacter pylori* therapy: proton pump inhibitor and amoxicillin for 14 days with clarithromycin and metronidazole added as a quadruple (hybrid) therapy for the final 7 days," *Helicobacter*, vol. 16, no. 2, pp. 139–145, 2011.

[44] P. Malfertheiner, F. Bazzoli, J. C. Delchier et al., "*Helicobacter pylori* eradication with a capsule containing bismuth subcitrate potassium, metronidazole, and tetracycline given with omeprazole versus clarithromycin-based triple therapy: a randomised, open-label, non-inferiority, phase 3 trial," *The Lancet*, vol. 377, no. 9769, pp. 905–913, 2011.

[45] J. M. Liou, J. T. Lin, C. Y. Chang et al., "Levofloxacin-based and clarithromycin-based triple therapies as first-line and second-line treatments for *Helicobacter pylori* infection: a randomised

comparative trial with crossover design," *Gut*, vol. 59, no. 5, pp. 572–578, 2010.

[46] S. Toracchio, S. Capodicasa, D. B. Soraja, L. Cellini, and L. Marzio, "Rifabutin based triple therapy for eradication of *H. pylori* primary and secondary resistant to tinidazole and clarithromycin," *Digestive and Liver Disease*, vol. 37, no. 1, pp. 33–38, 2005.

[47] E. Rimbara, L. A. Fischbach, and D. Y. Graham, "Optimal therapy for *Helicobacter pylori* infections," *Nature Reviews Gastroenterology and Hepatology*, vol. 8, no. 2, pp. 79–88, 2011.

[48] D. Falush, C. Kraft, N. S. Taylor et al., "Recombination and mutation during long-term gastric colonization by *Helicobacter pylori*: estimates of clock rates, recombination size, and minimal age," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 98, no. 26, pp. 15056–15061, 2001.

[49] M. E. Torres-Debat, G. Pérez-Pérez, A. Olivares et al., "Antimicrobial susceptibility of *Helicobacter pylori* and mechanisms of clarithromycin resistance in strains isolated from patients in Uruguay," *Revista Espanola de Enfermedades Digestivas*, vol. 101, no. 11, pp. 757–762, 2009.

[50] R. K. Vilaichone, V. Mahachai, and D. Y. Graham, "*Helicobacter pylori* Diagnosis and Management," *Gastroenterology Clinics of North America*, vol. 35, no. 2, pp. 229–247, 2006.

[51] S. Chen, Y. Li, and C. Yu, "Oligonucleotide microarray: a new rapid method for screening the *23S* rRNA gene of *Helicobacter pylori* for single nucleotide polymorphisms associated with clarithromycin resistance," *Journal of Gastroenterology and Hepatology*, vol. 23, no. 1, pp. 126–131, 2008.

[52] K. Hirata, H. Suzuki, T. Nishizawa et al., "Contribution of efflux pumps to clarithromycin resistance in *Helicobacter pylori*," *Journal of Gastroenterology and Hepatology*, vol. 25, supplement 1, pp. S75–S79, 2010.

[53] M. A. Webber and L. J. V. Piddock, "The importance of efflux pumps in bacterial antibiotic resistance," *Journal of Antimicrobial Chemotherapy*, vol. 51, no. 1, pp. 9–11, 2003.

[54] C. R. DeLoney and N. L. Schiller, "Characterization of an in vitro-selected amoxicillin-resistant strain of *Helicobacter pylori*," *Antimicrobial Agents and Chemotherapy*, vol. 44, no. 12, pp. 3368–3373, 2000.

[55] M. M. Gerrits, A. P. O. Godoy, E. J. Kuipers et al., "Multiple mutations in or adjacent to the conserved penicillin-binding protein motifs of the penicillin-binding protein 1A confer amoxicillin resistance to *Helicobacter pylori*," *Helicobacter*, vol. 11, no. 3, pp. 181–187, 2006.

[56] N. N. Qureshi, D. Morikis, and N. L. Schiller, "Contribution of specific amino acid changes in penicillin binding protein 1 to amoxicillin resistance in clinical *Helicobacter pylori* isolates," *Antimicrobial Agents and Chemotherapy*, vol. 55, no. 1, pp. 101–109, 2011.

[57] H. Tsugawa, H. Suzuki, K. Satoh et al., "Two amino acids mutation of ferric uptake regulator determines *Helicobacter pylori* resistance to metronidazole," *Antioxidants and Redox Signaling*, vol. 14, no. 1, pp. 15–23, 2011.

[58] J. M. Moore and N. R. Salama, "Mutational analysis of metronidazole resistance in *Helicobacter pylori*," *Antimicrobial Agents and Chemotherapy*, vol. 49, no. 3, pp. 1236–1237, 2005.

[59] M. J. Matteo, C. V. Pérez, M. R. Domingo, M. Olmos, C. Sanchez, and M. Catalano, "DNA sequence analysis of *rdxA* and *frxA* from paired metronidazole-sensitive and -resistant *Helicobacter pylori* isolates obtained from patients with heteroresistance," *International Journal of Antimicrobial Agents*, vol. 27, no. 2, pp. 152–158, 2006.

[60] S. Bereswill, C. Krainick, F. Stähler, L. Herrmann, and M. Kist, "Analysis of the rdxA gene in high-level metronidazole-resistant clinical isolates confirms a limited use of rdxA mutations as a marker for prediction of metronidazole resistance in *Helicobacter pylori*," *FEMS Immunology and Medical Microbiology*, vol. 36, no. 3, pp. 193–198, 2003.

[61] S. S. Choi, P. T. Chivers, and D. E. Berg, "Point mutations in *Helicobacter pylori's* fur regulatory gene that alter resistance to metronidazole, a prodrug activated by chemical reduction," *PLoS ONE*, vol. 6, no. 3, Article ID e18236, 2011.

[62] J. Tankovic, C. Lascols, Q. Sculo, J. C. Petit, and C. J. Soussy, "Single and double mutations in gyra but not in gyrb are associated with low- and high-level fluoroquinolone resistance in *Helicobacter pylori*," *Antimicrobial Agents and Chemotherapy*, vol. 47, no. 12, pp. 3942–3944, 2003.

[63] J.-W. Chung, G. H. Lee, J.-Y. Jeong et al., "Resistance of *Helicobacter pylori* strains to antibiotics in Korea with a focus on fluoroquinolone resistance," *Journal of Gastroenterology and Hepatology*, vol. 27, no. 3, pp. 493–497, 2012.

[64] H. Miyachi, I. Miki, N. Aoyama et al., "Primary levofloxacin resistance and gyrA/B mutations among *Helicobacter pylori* in Japan," *Helicobacter*, vol. 11, no. 4, pp. 243–249, 2006.

[65] J. K. Akada, M. Shirai, K. Fujii, K. Okita, and T. Nakazawa, "In vitro anti-Helicobacter priori activities of new rifamycin derivatives, KRM-1648 and KRM-1657," *Antimicrobial Agents and Chemotherapy*, vol. 43, no. 5, pp. 1072–1076, 1999.

[66] M. Heep, D. Beck, E. Bayerdörffer, and N. Lehn, "Rifampin and rifabutin resistance mechanism in *Helicobacter pylori*," *Antimicrobial Agents and Chemotherapy*, vol. 43, no. 6, pp. 1497–1499, 1999.

[67] S. Suzuki, H. Suzuki, T. Nishizawa et al., "Past rifampicin dosing determines rifabutin resistance of *Helicobacter pylori*," *Digestion*, vol. 79, no. 1, pp. 1–4, 2009.

[68] E. Glocker, C. Bogdan, and M. Kist, "Characterization of rifampicin-resistant clinical *Helicobacter pylori* isolates from Germany," *Journal of Antimicrobial Chemotherapy*, vol. 59, no. 5, pp. 874–879, 2007.

[69] I. Chopra and M. Roberts, "Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance," *Microbiology and Molecular Biology Reviews*, vol. 65, no. 2, pp. 232–260, 2001.

[70] M. L. Ribeiro, M. M. Gerrits, Y. H. B. Benvengo et al., "Detection of high-level tetracycline resistance in clinical isolates of *Helicobacter pylori* using PCR-RFLP," *FEMS Immunology and Medical Microbiology*, vol. 40, no. 1, pp. 57–61, 2004.

[71] H. Toledo and R. López-Solís, "Tetracycline resistance in Chilean clinical isolates of *Helicobacter pylori*," *Journal of Antimicrobial Chemotherapy*, vol. 65, no. 3, pp. 470–473, 2009.

[72] M. M. Gerrits, M. R. De Zoete, N. L. A. Arents, E. J. Kuipers, and J. G. Kusters, "16S rRNA mutation-mediated tetracycline resistance in *Helicobacter pylori*," *Antimicrobial Agents and Chemotherapy*, vol. 46, no. 9, pp. 2996–3000, 2002.

[73] M. Anoushiravani, T. Falsafi, and V. Niknam, "Proton motive force-dependent efflux of tetracycline in clinical isolates of *Helicobacter pylori*," *Journal of Medical Microbiology*, vol. 58, no. 10, pp. 1309–1313, 2009.

[74] M. S. Dorer, S. Talarico, and N. R. Salama, "*Helicobacter pylori's* unconventional role in health and disease," *PLoS Pathogens*, vol. 5, no. 10, Article ID e1000544, 2009.

[75] R. Suzuki, S. Shiota, and Y. Yamaoka, "Molecular epidemiology, population genetics, and pathogenic role of *Helicobacter pylori*," *Infection, Genetics and Evolution*, vol. 12, no. 2, pp. 203–213, 2012.

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Review Article

Pathogenesis of *Helicobacter pylori*-Related Gastroduodenal Diseases from Molecular Epidemiological Studies

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Helicobacter pylori is a major human pathogen that infects the stomach and produces inflammation that is responsible for various gastroduodenal diseases. Despite the high prevalence of *H. pylori* infections in Africa and South Asia, the incidence of gastric cancer in these areas is much lower than in other countries. The incidence of gastric cancer also tends to decrease from north to south in East Asia. Data from molecular epidemiological studies show that this variation in different geographic areas could be explained in part by different types of *H. pylori* virulence factors, especially CagA, VacA, and OipA. *H. pylori* infection is thought to be involved in both gastric cancer and duodenal ulcer, which are at opposite ends of the disease spectrum. This discrepancy can also be explained in part by another *H. pylori* factor, DupA, as well as by CagA typing (East Asian type versus Western type). *H. pylori* has a genome of approximately 1,600 genes; therefore, there might be other novel virulence factors. Because genome wide analyses using whole-genome sequencing technology give a broad view of the genome of *H. pylori*, we hope that next-generation sequencers will enable us to efficiently investigate novel virulence factors.

1. Introduction

Helicobacter pylori is a gram-negative spiral bacterium whose ecological niche is the human stomach. It is a major human pathogen that infects the stomach and produces inflammation that is responsible for diseases, such as duodenal ulcer, gastric ulcer, gastric cancer, and mucosa-associated lymphoid tissue lymphoma. Despite a general decline in the incidence of gastric cancer, it remains the fourth most common cancer and second leading cause of cancer-related deaths worldwide (http://globocan.iarc.fr/). Interestingly, despite the high prevalence of *H. pylori* infections in Africa and South Asia, the incidence of gastric cancer in these areas is much lower than in other countries; these phenomena are called African enigmas and Asian enigmas [1] (Table 1). Furthermore, the incidence of gastric cancer has a tendency to decrease from north to south in East Asia. The pathogenesis of the different clinical outcomes is multifactorial with environmental factors (mainly diet) often playing a dominant role and with an influence by host factors, especially those governing the severity of the immune response as well as the virulence of the infecting organism.

H. pylori, which is highly heterogeneous, has a genome of approximately 1,600 genes, the majority of which have been functionally characterized, and 5% to 10% appear to be *H. pylori* specific [2, 3]. Genes that are specifically thought to be associated with virulence include *vacA, cagA, oipA*, and *dupA*. This paper describes the current knowledge about the pathogenesis of *H. pylori*-related diseases from the aspect of the virulence factors of *H. pylori*.

2. VacA (Vacuolating Cytotoxin)

Virtually all *H. pylori* strains have a functional VacA, which encodes a vacuolating cytotoxin. In addition to vacuolation, *vacA* can induce multiple cellular activities, including membrane channel formation, cytochrome *c* release from mitochondria leading to apoptosis, and binding to cell-membrane receptors, which is followed by the initiation of

Table 1: Incidence of gastric cancer in 2008.

Geographic region	Country	Total		Male		Female	
		Total numbers	ASR	Total numbers	ASR	Total numbers	ASR
World total		989598	14.1	640556	19.8	349042	9.1
Asia		727500	18.6	484244	25.9	243256	11.7
East Asia		601314	30.0	408208	42.4	193106	18.3
West Asia		14879	9.4	9248	12.6	5631	6.7
Southeast Asia		43281	8.6	24926	10.9	18355	6.7
South-Central Asia		68037	5.3	41871	6.7	26166	3.9
Latin America and Caribbean		65360	11.7	39401	15.7	25959	8.4
South America		47244	12.4	29312	17.3	17932	8.4
Central America		14144	10.9	7671	12.7	6473	9.3
Caribbean		3972	8.5	2418	11.2	1554	6.1
Europe		146939	10.3	87548	14.7	59391	7.0
Central-East Europe		73940	14.7	43292	22.2	30648	9.7
South Europe		32873	10.1	19953	14.0	12920	6.8
West Europe		27457	6.5	16530	9.0	10927	4.4
North Europe		12669	6.2	7773	8.6	4896	4.2
Oceania		2728	5.5	1746	7.5	982	3.7
North America		24401	4.2	15051	5.8	9350	2.8
Africa		22659	4.0	12557	4.7	10102	3.3
(1) East Asia	South Korea	27098	41.4	18200	62.2	8898	24.6
(2) East Asia	Mongolia	603	34.0	390	48.2	213	22.3
(3) East Asia	Japan	102040	31.1	69561	46.8	32479	18.2
(4) East Asia	China	464439	29.9	315843	41.3	148596	18.5
(5) Central America	Guatemala	2332	26.6	1123	27.3	1209	25.9
(6) Central America	Honduras	1245	26.6	701	31.4	544	22.3
(7) South-Central Asia	Bhutan	114	24.2	76	31.6	38	16.2
(8) South America	Ecuador	3025	23.7	1667	28.0	1358	19.8
(9) South-Central Asia	Kyrgyzstan	964	23.2	619	34.2	345	14.5
(10) Central-East Europe	Belarus	3527	22.5	2023	34.2	1504	15.0
(11) Central America	Costa Rica	946	21.8	584	28.5	362	15.6
(12) South Europe	Albania	845	21.3	459	25.4	386	17.6
(13) South America	Peru	5215	21.2	2593	22.6	2622	20.0
(14) South-Central Asia	Kazakhstan	3329	20.6	1939	31.7	1390	13.7
(15) West Africa	Mali	1177	20.3	567	21.6	610	19.3
(16) South-Central Asia	Tajikistan	716	18.9	384	22.9	332	15.6
(17) Southeast Asia	Viet Nam	15068	18.9	8429	24.4	6639	14.6
(18) Caribbean	Jamaica	522	18.3	318	24.9	204	12.3
(19) South America	Chile	3762	17.9	2497	27.3	1265	10.3
(20) Central-East Europe	Russia	40615	17.5	22876	26.9	17739	11.7
(21) South America	Colombia	6638	17.4	3959	23.4	2679	12.5
(22) West Asia	Azerbaijan	1428	17.3	805	22.9	623	12.9
(23) Central-East Europe	Ukraine	13181	16.1	7902	25.2	5279	10.3
(24) South-Central Asia	Afghanistan	1716	15.8	1036	19.5	680	12.2
(25) South-Central Asia	Iran	8641	15.6	6188	21.9	2453	9.0
(26) South-Central Asia	Turkmenistan	532	15.4	310	21.2	222	10.9
(27) West Asia	Armenia	670	15.1	414	23.0	256	9.6
(28) South Europe	FYR Macedonia	468	15.1	315	22.7	153	8.6
(29) North Europe	Lithuania	916	15.0	532	23.0	384	10.0
(30) South Europe	Montenegro	149	15.0	85	19.2	64	11.5

ASR: age-standardized incidence rates per 100,000 population.

Data are obtained from GLOBOCAN databases, which provide access to the most recent estimates (for 2008) of the incidence of and mortality from 27 major cancers worldwide and is organized by the International Agency for Research on Cancer (IARC) (http://globocan.iarc.fr/).

In addition to the ASR for geographic regions, countries with ASRs that are equal or more than 15.0 for the total (male and female) with total number of gastric cancer more than 100 are listed.

a proinflammatory response [4–6]. In addition, VacA can specifically inhibit T-cell activation and proliferation [7–9].

Differences in *vacA* structure at the signal (s) region (s1 and s2) and the middle (m) region (m1 and m2) contribute to variations in the vacuolating activity of different *H. pylori* strains [10]. s1/m1 strains are the most cytotoxic, followed by s1/m2 strains. However, s2/m2 strains have no cytotoxic activity, and s2/m1 strains are rare [10]. Many studies in Western countries, including Latin America, the Middle East, and Africa, have shown that individuals who are infected with *vacA* s1 or m1 strains have an increased risk of peptic ulcers and/or gastric cancer compared to those infected with s2 or m2 strains [10–12]. In East Asia, most of the *H. pylori* strains possess the *vacA* s1 genotype; therefore, the type of s region is independent of clinical outcomes [13, 14]. In contrast, the m1 genotype is common in areas of Northeast Asia, such as Japan and South Korea, whereas the m2 genotype is predominant in areas of Southeast Asia, such as Taiwan and Vietnam [14, 15]. Because the incidence of gastric cancer is higher in the northern regions than in the southern regions of East Asia, the *vacA* m region may play a role in the regional differences in the disease pattern in East Asia. We recently reported that the *vacA* m1 genotype was more prevalent in Hanoi than in Ho Chi Minh City in Vietnam, and the incidence of gastric cancer was higher in Hanoi than in Ho Chi Minh City [16]. These findings support the possibility that the *vacA* m region is related to clinical outcomes in East Asia.

Okinawa consists of several small islands (2,276 km^2) in southwestern Japan. Although the prevalence of *H. pylori* in Okinawa is not different from other parts of Japan [8, 13], the incidence of gastric cancer in Okinawa (6.3 deaths/100,000 population) is the lowest in Japan (mean mortality rate of Japan, 11.8 deaths/100,000 population in 2009) (Center for Cancer Control and Information Services, National Cancer Center, Japan, (http://www.ncc.go.jp/)). Interestingly, most of the *H. pylori* strains possess the *vacA* s1/m1 genotype in the mainland of Japan (e.g., Kyoto) [14]. However, we recently reported that less than 70% of the strains possessed the s1/m1 genotype in Okinawa [17]. In that study, we evaluated 337 strains and found that the *vacA* s1/m2 genotype was significantly prevalent in strains derived from gastritis than those derived from gastric ulcers (17.3% versus 7.9%, resp.; $P = 0.04$). The prevalence of the *vacA* s2/m2 genotype was significantly higher in strains derived from gastritis than those derived from gastric ulcers, duodenal ulcers, and gastric cancer (22.4% versus 11.9%, 10.5%, and 4.2%, resp.; $P = 0.04$, 0.01, and 0.04, resp.). Therefore, even in East Asia in areas where there are many cases with non-s1/m1 strains, both the *vacA* s and m genotypes can be used as markers for *H. pylori*-related diseases.

In 2007, a third disease-related region of *vacA*, which was named the intermediate (i) region, was identified between the s region and the m region [18]. All s1/m1 strains were classified as i1 type, and all s2/m2 strains were classified as i2 type. However, s1/m1 strains were classified as either i1 or i2 types and i1 strains were shown to be more pathogenic. In a recent study, a novel intermediate variant (i3) was identified. This variant was often found in Turkish strains (25.7%) [19]. In the original study [18], it was reported that the *vacA* i genotype was more effective in determining the risk of gastric cancer than in typing the s region or the m region in Iran. An additional study that was conducted by the same group showed that the *vacA* i genotype was related to the presence of peptic ulcers in Iraq and Italy [20, 21]. Interestingly, a recent study from Republic of South Korea showed that the polymorphisms at amino acid position 196 of *vacA*, which is located in the i region, were associated with severe outcomes [22]. However, in our study in East and Southeast Asia, there were no associations between the i region and diseases [23]. In a recent study from Portugal that examined patients with progression to more severe histological diagnoses after a mean of 12.8 years of follow-up, the *vacA* i genotype did not improve the prediction of progression given by the other *vacAloci*, as in s and m regions [24]. More recently, we identified a fourth disease-related region between the i region and the m region and named it the deletion (d) region [25]. The d region is divided into d1 without a deletion and d2 with a 69 to 81 bp deletion. Our study of Western strains showed that d1 was a risk factor for gastric mucosal atrophy. However, almost all East Asian strains were classified as s1/i1/d1. Although the roles of the i and d regions should be investigated in a future study, the genotypes of the s and the m regions seem to currently serve as good markers of clinical outcomes.

3. CagA (Cytotoxin-Associated Gene A Product)

cagA is located at one end of the *cag* pathogenicity island (PAI), which is an approximately 40 kbp region that is thought to have been incorporated into the *H. pylori* genome by horizontal transfer from an unknown source [26]. The *cag* PAI encodes a type IV secretion system (T4SS), through which CagA is delivered into host cells [27]. CagA has been reported to interact with various target molecules in host cells, and the best studied is the cytoplasmic Src homology-2 domain of Src homology-2 phosphatase (SHP-2), which is known to have oncogenic activity [28]. An animal study that used Mongolian gerbils showed that gastric cancer developed in animals infected with wild-type *H. pylori*, whereas it did not in gerbils infected with isogenic *cagA* mutants [29, 30]. Another study showed that gastric cancer and other malignant neoplasms occurred in some transgenic mice with an artificially introduced CagA protein [31]. These results provide strong evidence for the role of CagA as a bacterium-derived oncoprotein.

There are 2 types of clinical *H. pylori* isolates: *cagA* gene-positive strains and *cagA* gene-negative strains. Almost all *H. pylori* isolates from East Asia are *cagA* positive, whereas approximately 20% to 40% of isolates from Europe and Africa are *cagA* negative [14]. Therefore, the pathogenic differences in East Asia are difficult to explain only in terms of the presence or absence of *cagA* alone [13]. In Western countries, however, it has been reported that individuals infected with *cagA*-positive strains are at a higher risk for peptic ulcer and/or gastric cancer than those infected with *cagA*-negative strains [32, 33]. It is interesting to note that

almost all *cagA*-positive strains are classified as the *vacA* s1 strain (either m1 or m2), whereas almost all *cagA*-negative strains are classified as the *vacA* s2/m2 strain [10].

More than 10 years ago, we first reported that *cagA* could be mainly classified into 2 types (East Asian type and Western type) according to the sequence located in the 3′ region of *cagA* [34, 35]. We initially classified the repeat regions into 2 types, the first repeat and the second repeat, and found that the sequence of the second-repeat region was considerably different between East Asian strains and Western strains [14, 34–36]. Each region contains the Glu-Pro-Ile-Tyr-Ala (EPIYA) motifs, which includes a tyrosine phosphorylation site. Recently, it has been more common to name the first-repeat regions as EPIYA-A and EPIYA-B segments and the second-repeat region in Western and East Asian strains as EPIYA-C and EPIYA-D segments, respectively [28]. Each CagA sequence was assigned a sequence type that consisted of the names of the EPIYA segments in its sequence (e.g., ABC, ABCC, ABD).

In vitro experiments have shown that CagA with an EPIYA-D segment has a higher binding ability for SHP-2 than CagA with an EPIYA-C segment [28]. An animal study showed that malignant neoplasms occurred in some East Asian-type CagA-introduced transgenic mice, whereas the frequency of tumors was significantly lower in Western-type CagA-introduced transgenic mice [37]. In addition, molecular epidemiological studies from Thailand and South Korea showed that individuals infected with East Asian-type *cagA* strains have an increased risk of peptic ulcer or gastric cancer compared with those infected with Western-type *cagA* strains [22, 38]. We also recently reported that the different incidences of gastric cancer between Okinawa and mainland Japan might be explained by the high prevalence of Western-type *cagA* strains in Okinawa compared with other areas of Japan [17]. In our study from Okinawa, the East Asian-type *cagA* genotype was significantly more prevalent in strains derived from gastric ulcers (83.2%) and gastric cancer (87.5%) than those derived from gastritis (60.2%) ($P < 0.001$ and $P = 0.01$, resp.). The prevalence of the East Asian-type *cagA* genotype was also significantly higher in strains derived from gastric ulcers (83.2%) and gastric cancer (87.5%) than those derived from duodenal ulcer (64.0%) ($P = 0.001$ and 0.02, resp.). In contrast, there was no significant difference between the prevalence of East Asian-type *cagA* in duodenal ulcers and gastritis (64.0% versus 60.2%). *H. pylori* infection is thought to be involved in both gastric cancer and duodenal ulcers, which are at the opposite ends of the disease spectrum. According to our results, this discrepancy can be explained in part by the prevalence of East Asian-type *cagA,* which might be specifically related to the development of gastric cancer. Overall, both in vitro and in vivo (animal and human) data clearly show that East Asian-type CagA is more carcinogenic than Western-type CagA.

However, it should be noted that the incidence of gastric cancer is high in some regions where Western-type CagA is predominant. For example, although Western-type CagA strains have been reported to account for the majority of *H. pylori* strains in Columbia [4, 39], the incidence of gastric cancer there is substantially high (Table 1). These facts cannot be explained by the concept of East Asian-type CagA versus Western-type CagA alone. We published the first report that suggested that the number of second-repeat regions is associated with gastric cancer both in East Asia (Japan) and in Western countries, including Colombia [34, 35]. Importantly, our study of 100 *H. pylori* isolates that were derived from patients with simple gastritis (30 isolates were from Columbia and 70 were from the USA, where the incidence of gastric cancer is low (Age-Standardized Rate = 4.1)) showed that 57% of the isolates from Columbia had 2 EPIYA-C segments, whereas only 4% of the isolates from the USA had 2 EPIYA-C segments [15]. Several studies have confirmed that the incidence of gastric cancer is significantly higher in patients infected with strains with multiple EPIYA-C segments compared with those infected with a single segment in Western countries [34, 35, 40, 41]. In addition, a recent large-scale study showed that a higher number of EPIYA-C repeats was associated with gastric cancer and gastric precancerous lesions, as shown by histological gastric atrophy/metaplastic changes and decreased serum levels of pepsinogen I [42]. The prevalence of *H. pylori* infections is high in Africa, while gastric cancer is uncommon, which is known as the "African Enigma" [43]. However, the incidence of gastric cancer is extremely high in Mali, and the frequency of gastric cancer among women in Mali is higher than in Japan (Table 1). It will be interesting to investigate the *cagA* genotypes in Mali. Taken together, the number of EPIYA-C segments may explain to some extent the geographic difference in the incidence of gastric cancer in Western countries. Somewhat interestingly, although we first reported that the risk of gastric cancer development in the Japanese population increased when the number of second-repeat regions was 2 compared with 1, the structure of the second repeat was not DD, but B′D, in which the sequences of B′ were more similar to B than to D [44]. Recent in vitro data have shown that SHP2 binds EPIYA-B segments and C-terminal Src tyrosine kinase (Csk), which is another important molecule that is involved in intracellular signaling systems and prefers to bind EPIYA-A and EPIYA-B segments [45]. These results might indicate that each EPIYA segment plays a role in gastric pathogenesis, and a larger number of any type of EPIYA segments might be used as a marker for an increasing risk for gastric cancer.

As one goes southward in East Asia, the incidence of gastric cancer becomes lower, and the incidence in Vietnam is half of that in South Korea (Table 1), although most Vietnamese strains (93%) have been reported to possess the East Asian-type CagA [16]. In addition, most of the strains in both Vietnam and South Korea have only 1 EPIYA-A, EPIYA-B, and EPIYA-D segments [44]. Recently, we reported that the structure of the East Asian-type *cagA* in Vietnamese strains was slightly different from that of strains from other East Asian countries [16]. Vietnamese strains have a unique 18 bp deletion that is located slightly upstream of the EPIYA-A segment, whereas the 39 bp deletion is common in East Asian strains, such as those in Japan and South Korea, and no depletion was identified in Western strains. Further research is necessary to determine whether these subtypes are involved in the pathogenesis of gastric cancer.

4. OipA (Outer Inflammatory Protein)

OipA, which is one of the outer membrane proteins, functions in adhesion [46]. Its functional status is regulated by slipped-strand mispairing that is based on the number of CT dinucleotide repeats in the 5′ region of the genes (switch "on" = functional and switch "off" = nonfunctional) [46]. OipA was initially identified as a proinflammatory response-inducing protein based on the fact that *oipA*-isogenic mutants reduced the induction of interluekin-8 (IL-8) from gastric epithelial cell lines [46]. A recent study revealed that OipA has a function of inducing inflammation and actin dynamics through the phosphorylation of multiple signaling pathways that usually interact with *cag* PAI (CagA)-related pathways [47–52].

We previously examined the expression status or presence of multiple virulence factors (*cag* PAI, *vacA*, *iceA*, *oipA*, and *babA*) in different clinical outcomes [33]. *H. pylori* isolates were obtained from 247 patients in the USA and Colombia. An independent univariate analysis showed that the *oipA* "on," *cag* PAI-positive, *vacA* s1 genotype and the *babA*-positive type were all related to the risk of duodenal ulcer. However, a multiple logistic regression analysis showed that only the *oipA* "on" status was an independent determinant predictor of duodenal ulcer from gastritis (adjusted odds ratio (OR), 5.0; 95% confidence interval (CI) = 2.1–11.9). This finding was confirmed in a distinct study that used a nonoverlapping cohort of 200 patients that were examined by an immunoblot analysis for 4 outer-membrane proteins: OipA, BabA, BabB, and SabA [53]. A multiple logistic regression analysis showed that only the OipA-positive status was an independent determinant predictor of gastric cancer versus gastritis (OR, 4.8; 95% CI = 1.4–16.8) and duodenal ulcer versus gastritis (OR, 4.0; 95% CI = 1.6–10.2). In addition, a challenge of human volunteers with an *oipA* "on"/whole *cag* PAI-negative clinical isolate (Baylor strain 100 or ATCC BAA-945) that caused severe inflammation supports this notion [54]. In addition, an in vitro study showed that the *oipA* mutants did not induce gastric mucosal inflammation in mice that were infected for 12 weeks, whereas *cagE* mutants did induce mucosal inflammation, although the levels were milder than in the parental strains (*cagE* is an important component of *cag* PAI) [55].

The above findings suggest that the presence of OipA is a better marker of severe clinical outcomes than *cag* PAI. However, it is important to note that clinical isolates that contain the *cag* PAI typically have an *oipA* "on" status [33, 53, 56–58] despite the *oipA* gene being physically located approximately 100 kbp from the *cag* PAI on the *H. pylori* chromosome. *oipA* status is also linked to the *vacA* s region type, and it is further closely linked to the presence of the *babA* gene, which is another virulence factor that codes outer membrane proteins [59]. These linkages of the virulence factors may have a certain biological significance, and they may somehow interact with each other; therefore, it might be better to hypothesize that these factors interact synergistically with each other and induce serious diseases, rather than to discuss which of these factors is the most virulent [15]. It is interesting to note that most East Asian strains are classified as *oipA* status "on," and the CT-repeat sequences in the signal region of *oipA* were half-collapsed (e.g., CTGCCTTTCT repeat sequence), suggesting that this may result from an intentional change in the status in the course of evolution of the bacteria in order to prevent the switch from being turned "off" easily [46].

5. DupA (Duodenal Ulcer Promoting)

In 2005, we described a novel virulence factor, duodenal ulcer promoting (*dupA*) gene, which was located in the plasticity region of the *H. pylori* genome [60]. DupA pathogenesis appears to involve the induction of IL-8 production in the antrum, leading to antrum-predominant gastritis, which is a well-recognized characteristic of duodenal ulcer. Additionally, it has been reported that *H. pylori* containing intact *dupA* induces the IL-12 production of monocytes [61].

As for the molecular epidemiological studies, our initial study of a total of 500 *H. pylori* isolates, including 160 from Japan, 175 from Korea, and 165 from Colombia, showed that the positive rate for *dupA* was high in patients with duodenal ulcer and low in patients with gastric cancer, regardless of the patients' nationality (42% versus 9% on average) [60]. However, several controversial results have been reported worldwide, and an association between the presence of *dupA* and gastroduodenal diseases has appeared in some populations but not in others [15, 62]. *dupA* is generally more prevalent in Western strains than in Asian strains. In a recent review, the worldwide prevalence of *dupA* in patients with gastritis was reported to be 44.8%, and this value differed significantly between nationalities/ethnicities; *H. pylori* isolates from South America were significantly more likely to possess *dupA* (79.21% (160/202)) than those from East Asian (36.62% (130/355)), Middle Eastern (40.21% (39/97)), or European (43.75% (42/96)) countries [63]. The association between *dupA* status and disease development is primarily observed in Asian countries, such as China, Korea, Iraq, and North India. Our meta-analysis showed that infection with *dupA*-positive *H. pylori* increased the duodenal ulcer risk (OR, 1.41; 95% CI = 1.12–1.76), particularly in Asian countries (OR, 1.57; 95% CI = 1.19–2.06), but not in Western countries (OR, 1.09; 95% CI = 0.73–1.62) [64]. In contrast to the linkage among CagA, VacA, and OipA, most studies showed that there were no relationships between the presence of *dupA* and the presence of CagA, VacA, or OipA [64].

There are several possible explanations why the importance of *dupA* in gastroduodenal diseases has been controversial among studies. First, the discrepancy could be related to the limitations of the techniques used for detecting the intact *dupA* gene. All previous studies evaluated the presence of *dupA* by polymerase chain reactions and dot blot/Southern hybridization, but DupA proteins were not detected by immunoblot. However, it is well known that there are many cases with frame-shift mutations in *dupA*. Strains with these mutated sequences are not able to produce intact DupA proteins. Intriguingly, the presence of *dupA* without a stop

codon was more frequently observed in strains from patients with duodenal ulcer than in those from patients with gastritis or gastric cancer [65]. Hussein et al. recently classified a *dupA* allele with 1,884 bp as *dupA1* and a truncated version with mutations as *dupA2* [61]. Secondly, recent full-sequenced data of *H. pylori* revealed that the length of *dupA* depends on the strains, and the length of the Shi470 and G27 strains has an approximately 600-bp longer open reading frame (approximately 2,500 bp) than that of strain J99, due to the additional 5′ region of *dupA*. This suggests that *dupA* has 2 genotypes according to the location of the signal sequence of the 5′ region (long-type and short type). However, no previous studies took the additional 5′ region into account. Our preliminary data from Okinawa, Japan showed that the long-type *dupA* and not the short type *dupA* was significantly associated with severe gastroduodenal diseases (unpublished observation). A lack of concern about the 5′ region of *dupA* might be one reason for the discrepancies in the previous results. Although it is unknown whether proteins from short type *dupA* could be produced and/or functional, these data suggest that only strains that possess the long-type *dupA* without frame-shift mutations could be functional. Further analyses of the *dupA* DNA sequence will be necessary to clarify the significance of intact *dupA*. Additionally, intact *dupA* should be detected by measuring DupA protein with immunoblotting techniques.

Finally, *dupA* is predicted to form a T4SS with *vir* genes around *dupA* (*dupA* cluster). Three gene clusters that code for T4SS have been recognized in *H. pylori*: a protein translocation system encoded by the *cag* PAI, a DNA-uptake system encoded by the ComB cluster, and an unknown cluster in the plasticity region [66]. *dupA* and *virB4*, which is one of the constituents of T4SS, are highly homologous. *dupA* and the adjacent 6 *vir* gene homologs (*virB8*, *B9*, *B10*, *B11*, *virD4*, and *D2*) in the plasticity region were predicted to form the third T4SS [15]. We recently investigated the prevalence of *dupA* and *vir* gene homologs and the associations between the status of *dupA* clusters and clinical outcomes in the US population and found that the presence of a complete *dupA* cluster increases the duodenal ulcer risk compared with *H. pylori* infection with incomplete *dupA* clusters or without the *dupA* gene independent of the *cag* PAI status (adjusted OR, 2.13; 95% CI = 1.13–4.03) [66]. Therefore, although the causal relationship between the *dupA* cluster and duodenal ulcer development has not been proven, the presence of a complete *dupA* cluster and not *dupA* alone is associated with duodenal ulcer development. Overall, currently, the presence of a complete *dupA* cluster with intact *dupA* (long-type without frame-shift mutation) could be a good marker to predict the development of duodenal ulcer. Studies of the plasticity zone are only at the beginning and may be the most attractive area for future investigations.

6. Detection of Genomic Changes for Clinical Studies

The rapid advances in sequencing technology have enabled massive sequence comparisons. One of the prospective applications of the new technology to the study of *H. pylori* is the identification of novel virulence factors [67–69]. Whole-genome analyses are useful for the investigation of genetic factors that are related to differences in the virulence among strains. McNamara and El-Omar compared the genome sequences of an isolate that was obtained from a patient with gastric cancer (strain 98-10) and an isolate from a patient with gastric ulcer (strain B128) and determined strain-specific genes of strain 98-10 that were candidate genes associated with gastric cancer [70]. Kawai et al. investigated the evolution of East Asian strains using 20 whole genomes of Japanese, Korean, Amerindian, European, and West African strains [68]. A phylogenetic analysis revealed a greater divergence between the East Asian strains and the Western strains in genes related to virulence factors, especially those related to outer membrane proteins and lipopolysaccharide synthesis enzymes. Genomic changes during infection have also been studied. The whole-genome sequence of strain HPAG1 was determined with the whole-genome shotgun method, and the data obtained were used to design a custom microarray [71]. Genotyping of isolates that were obtained from patients with chronic atrophic gastritis revealed gained and lost genes during the progression of the disease, and whole-genome transcriptional profiling identified genes that were associated with the adaptation of *H. pylori* to chronic atrophic gastritis.

A chronological comparison of the whole genome was performed for 5 sets of *H. pylori* strains from Colombia with isolation intervals of 3 to 16 years using the 454 next-generation sequencing technology [72]. A comparison of the genomes revealed single-nucleotide polymorphisms and imported clusters that resulted from recombination, which is frequently found in members of the *hop* family. Data obtained with the massively parallel sequencing technology provide valuable information on candidates of new virulence factors.

7. Conclusions

It is obvious that the 4 virulence factors described in this paper are important. However, because *H. pylori* consists of approximately 1,600 genes, there remains the possibility that additional important pathogenic genes will be identified. The sequencing technology is still advancing. We believe that larger amounts of data will become available at lower costs in the near future, and other important novel virulence factors might be discovered. We must also note that the gastric cancer incidence has been changed remarkably with environmental factors, such as diet (e.g., salt intake) or immigration. Host factors (e.g., gene polymorphisms) and duration of the infection (e.g., early infection with duodenal ulcer and late infection with gastric cancer) should also be taken into account. These various factors are thought to interact in a complex manner with each other in the actual development of diseases. We hope that we will gradually understand the mechanisms underlying how *H. pylori* induces gastric inflammation and leads to severe gastroduodenal diseases, such as gastric cancer, by combining bacterial factors with other factors, such as environmental factors and host factors.

Conflict of Interests

The authors declare that they have no competing interests.

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References

[1] H. M. Malaty, "Epidemiology of *Helicobacter pylori* infection," *Best Practice and Research: Clinical Gastroenterology*, vol. 21, no. 2, pp. 205–214, 2007.

[2] R. A. Alm, L. S. L. Ling, D. T. Moir et al., "Genomic-sequence comparison of two unrelated isolates of the human gastric pathogen *Helicobacter pylori*," *Nature*, vol. 397, no. 6715, pp. 176–180, 1999.

[3] J. F. Tomb, O. White, A. R. Kerlavage et al., "The complete genome sequence of the gastric pathogen *Helicobacter pylori*," *Nature*, vol. 388, no. 6642, pp. 539–547, 1997.

[4] J. C. Atherton, "The pathogenesis of *Helicobacter pylori*-induced gastro-duodenal diseases," *Annual Review of Pathology*, vol. 1, pp. 63–96, 2006.

[5] T. L. Cover and S. R. Blanke, "*Helicobacter pylori* VacA, a paradigm for toxin multifunctionality," *Nature Reviews Microbiology*, vol. 3, no. 4, pp. 320–332, 2005.

[6] J. G. Kusters, A. H. M. Van Vliet, and E. J. Kuipers, "Pathogenesis of *Helicobacter pylori* infection," *Clinical Microbiology Reviews*, vol. 19, no. 3, pp. 449–490, 2006.

[7] M. Boncristiano, S. R. Paccani, S. Barone et al., "The *Helicobacter pylori* vacuolating toxin inhibits T cell activation by two independent mechanisms," *Journal of Experimental Medicine*, vol. 198, no. 12, pp. 1887–1897, 2003.

[8] B. Gebert, W. Fischer, E. Weiss, R. Hoffmann, and R. Haas, "*Helicobacter pylori* vacuolating cytotoxin inhibits T lymphocyte activation," *Science*, vol. 301, no. 5636, pp. 1099–1102, 2003.

[9] M. S. Sundrud, V. J. Torres, D. Unutmaz, and T. L. Cover, "Inhibition of primary human T cell proliferation by *Helicobacter pylori* vacuolating toxin (*VacA*) is independent of *vacA* effects on IL-2 secretion," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 20, pp. 7727–7732, 2004.

[10] J. C. Atherton, P. Cao, R. M. Peek, M. K. R. Tummuru, M. J. Blaser, and T. L. Cover, "Mosaicism in vacuolating cytotoxin alleles of *Helicobacter pylori*. Association of specific *vacA* types with cytotoxin production and peptic ulceration," *The Journal of Biological Chemistry*, vol. 270, no. 30, pp. 17771–17777, 1995.

[11] M. Sugimoto, M. R. Zali, and Y. Yamaoka, "The association of *vacA* genotypes and *Helicobacter pylori*-related gastroduodenal diseases in the Middle East," *European Journal of Clinical Microbiology and Infectious Diseases*, vol. 28, no. 10, pp. 1227–1236, 2009.

[12] M. Sugimoto and Y. Yamaoka, "The association of *vacA* genotype and *Helicobacter pylori*-related disease in Latin American and African populations," *Clinical Microbiology and Infection*, vol. 15, no. 9, pp. 835–842, 2009.

[13] Y. Yamaoka, T. Kodama, O. Gutierrez, J. G. Kim, K. Kashima, and D. Y. Graham, "Relationship between *Helicobacter priori iceA, cagA*, and *vacA* status and clinical outcome: studies in four different countries," *Journal of Clinical Microbiology*, vol. 37, no. 7, pp. 2274–2279, 1999.

[14] Y. Yamaoka, E. Orito, M. Mizokami et al., "*Helicobacter pylori* in North and South America before Columbus," *FEBS Letters*, vol. 517, no. 1–3, pp. 180–184, 2002.

[15] Y. Yamaoka, "Mechanisms of disease: *Helicobacter pylori* virulence factors," *Nature Reviews Gastroenterology and Hepatology*, vol. 7, no. 11, pp. 629–641, 2010.

[16] T. Uchida, L. T. Nguyen, A. Takayama et al., "Analysis of virulence factors of *Helicobacter pylori* isolated from a Vietnamese population," *BMC Microbiology*, vol. 9, article 175, 2009.

[17] O. Matsunari, S. Shiota, R. Suzuki et al., "Association between *Helicobacter pylori* virulence factors and gastroduodenal diseases in Okinawa, Japan," *Journal of Clinical Microbiology*, vol. 50, no. 3, pp. 876–883, 2012.

[18] J. L. Rhead, D. P. Letley, M. Mohammadi et al., "A new *Helicobacter pylori* vacuolating cytotoxin determinant, the intermediate region, is associated with gastric cancer," *Gastroenterology*, vol. 133, no. 3, pp. 926–936, 2007.

[19] C. Chung, A. Olivares, E. Torres, O. Yilmaz, H. Cohen, and G. Perez-Perez, "Diversity of *vacA* intermediate region among *Helicobacter pylori* strains from several regions of the world," *Journal of Clinical Microbiology*, vol. 48, no. 3, pp. 690–696, 2010.

[20] N. R. Hussein, M. Mohammadi, Y. Talebkhan et al., "Differences in virulence markers between *Helicobacter pylori* strains from Iraq and those from Iran: potential importance of regional differences in *H. pylori*-associated disease," *Journal of Clinical Microbiology*, vol. 46, no. 5, pp. 1774–1779, 2008.

[21] D. Basso, C. F. Zambon, D. P. Letley et al., "Clinical relevance of *Helicobacter pylori cagA* and *vacA* gene polymorphisms," *Gastroenterology*, vol. 135, no. 1, pp. 91–99, 2008.

[22] K. R. Jones, S. Jang, J. Y. Chang et al., "Polymorphisms in the intermediate region of *vacA* impact *Helicobacter pylori*-induced disease development," *Journal of Clinical Microbiology*, vol. 49, no. 1, pp. 101–110, 2011.

[23] H. Ogiwara, D. Y. Graham, and Y. Yamaoka, "vacA i-Region Subtyping," *Gastroenterology*, vol. 134, no. 4, p. 1267, 2008.

[24] R. M. Ferreira, C. Figueiredo, C. Bonet et al., "*Helicobacter pylorivacA* intermediate region genotyping and progression of gastric preneoplastic lesions," *American Journal of Gastroenterology* , vol. 107, no. 1, pp. 145–146, 2012.

[25] H. Ogiwara, M. Sugimoto, T. Ohno et al., "Role of deletion located between the intermediate and middle regions of the *Helicobacter pylorivacA* gene in cases of gastroduodenal diseases," *Journal of Clinical Microbiology*, vol. 47, no. 11, pp. 3493–3500, 2009.

[26] S. Censini, C. Lange, Z. Xiang et al., "*cag*, a pathogenicity island of *Helicobacter pylori*, encodes type I-specific and disease-associated virulence factors," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 93, no. 25, pp. 14648–14653, 1996.

[27] S. Backert and M. Selbach, "Role of type IV secretion in *Helicobacter pylori* pathogenesis," *Cellular Microbiology*, vol. 10, no. 8, pp. 1573–1581, 2008.

[28] M. Hatakeyama, "Oncogenic mechanisms of the *Helicobacter pylori CagA* protein," *Nature Reviews Cancer*, vol. 4, no. 9, pp. 688–694, 2004.

[29] A. T. Franco, D. A. Israel, M. K. Washington et al., "Activation of beta-catenin by carcinogenic *Helicobacter pylori*," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 30, pp. 10646–10651, 2005.

[30] A. T. Franco, E. Johnston, U. Krishna et al., "Regulation of gastric carcinogenesis by *Helicobacter pylori* virulence factors," *Cancer Research*, vol. 68, no. 2, pp. 379–387, 2008.

[31] N. Ohnishi, H. Yuasa, and S. Tanaka, "Transgenic expression of *Helicobacter pylori CagA* induces gastrointestinal and hematopoietic neoplasms in mouse," *Chemtracts*, vol. 21, no. 3, pp. 121–123, 2008.

[32] L. J. Van Doorn, C. Figueiredo, R. Sanna et al., "Clinical relevance of the *cagA, vacA*, and *iceA* status of *Helicobacter pylori*," *Gastroenterology*, vol. 115, no. 1, pp. 58–66, 1998.

[33] Y. Yamaoka, S. Kikuchi, H. M. T. ElZimaity, O. Gutierrez, M. S. Osato, and D. Y. Graham, "Importance of *Helicobacter pylori oipA* in clinical presentation, gastric inflammation, and mucosal interleukin 8 production," *Gastroenterology*, vol. 123, no. 2, pp. 414–424, 2002.

[34] Y. Yamaoka, T. Kodama, K. Kashima, D. Y. Graham, and A. R. Sepulveda, "Variants of the 3′ region of the *cagA* gene in *Helicobacter pylori* isolates from patients with different *H. pylori*-associated diseases," *Journal of Clinical Microbiology*, vol. 36, no. 8, pp. 2258–2263, 1998.

[35] Y. Yamaoka, H. M. T. El-Zimaity, O. Gutierrez et al., "Relationship between the *cagA* 3′ repeat region of *Helicobacter pylori*, gastric histology, and susceptibility to low pH," *Gastroenterology*, vol. 117, no. 2, pp. 342–349, 1999.

[36] Y. Yamaoka, M. S. Osato, A. R. Sepulveda et al., "Molecular epidemiology of *Helicobacter pylori*: separation of *H. pylori* from East Asian and non-Asian countries," *Epidemiology and Infection*, vol. 124, no. 1, pp. 91–96, 2000.

[37] M. Miura, N. Ohnishi, S. Tanaka, K. Yanagiya, and M. Hatakeyama, "Differential oncogenic potential of geographically distinct *Helicobacter pylori CagA* isoforms in mice," *International Journal of Cancer*, vol. 125, no. 11, pp. 2497–2504, 2009.

[38] R. K. Vilaichone, V. Mahachai, S. Tumwasorn, J. Y. Wu, D. Y. Graham, and Y. Yamaoka, "Molecular epidemiology and outcome of *Helicobacter pylori* infection in Thailand: a cultural cross roads," *Helicobacter*, vol. 9, no. 5, pp. 453–459, 2004.

[39] D. Kersulyte, A. K. Mukhopadhyay, B. Velapatino et al., "Differences in genotypes of *Helicobacter pylori* from different human populations," *Journal of Bacteriology*, vol. 182, no. 11, pp. 3210–3218, 2000.

[40] R. H. Argent, M. Kidd, R. J. Owen, R. J. Thomas, M. C. Limb, and J. C. Atherton, "Determinants and consequences of different levels of *CagA* phosphorylation for clinical isolates of *Helicobacter pylori*," *Gastroenterology*, vol. 127, no. 2, pp. 514–523, 2004.

[41] T. Azuma, A. Yamakawa, S. Yamazaki et al., "Correlation between variation of the 3′ region of the *cagA* gene in *Helicobacter pylori* and disease outcome in Japan," *Journal of Infectious Diseases*, vol. 186, no. 11, pp. 1621–1630, 2002.

[42] S. A. Batista, G. A. Rocha, A. M. C. Rocha et al., "Higher number of *Helicobacter pylori CagA* EPIYA C phosphorylation sites increases the risk of gastric cancer, but not duodenal ulcer," *BMC Microbiology*, vol. 11, article 61, 2011.

[43] C. Holcombe, B. A. Omotara, J. Eldridge, and D. M. Jones, "*H. pylori*, the most common bacterial infection in Africa: a random serological study," *American Journal of Gastroenterology*, vol. 87, no. 1, pp. 28–30, 1992.

[44] Y. Xia, Y. Yamaoka, Q. Zhu, I. Matha, and X. Gao, "A comprehensive sequence and disease correlation analyses for the C-terminal region of *CagA* protein of *Helicobacter pylori*," *PLoS ONE*, vol. 4, no. 11, Article ID e7736, 2009.

[45] M. Selbach, F. E. Paul, S. Brandt et al., "Host cell interactome of tyrosine-phosphorylated bacterial proteins," *Cell Host and Microbe*, vol. 5, no. 4, pp. 397–403, 2009.

[46] Y. Yamaoka, D. H. Kwon, and D. Y. Graham, "A Mr 34,000 proinflammatory outer membrane protein (*oipA*) of *Helicobacter pylori*," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 13, pp. 7533–7538, 2000.

[47] T. Kudo, H. Lu, J. Y. Wu et al., "Pattern of Transcription Factor Activation in *Helicobacter pylori*-Infected Mongolian Gerbils," *Gastroenterology*, vol. 132, no. 3, pp. 1024–1038, 2007.

[48] J. Y. Wu, H. Lu, Y. Sun, D. Y. Graham, H. S. Cheung, and Y. Yamaoka, "Balance between polyoma enhancing activator 3 and activator protein 1 regulates *Helicobacter pylori*-stimulated matrix metalloproteinase 1 expression," *Cancer Research*, vol. 66, no. 10, pp. 5111–5120, 2006.

[49] H. Lu, J. Y. Wu, T. Kudo, T. Ohno, D. Y. Graham, and Y. Yamaoka, "Regulation of interleukin-6 promoter activation in gastric epithelial cells infected with *Helicobacter pylori*," *Molecular Biology of the Cell*, vol. 16, no. 10, pp. 4954–4966, 2005.

[50] Y. Yamaoka, T. Kudo, H. Lu, A. Casola, A. R. Brasier, and D. Y. Graham, "Role of interferon-stimulated responsive element-like element in interleukin-8 promoter in *Helicobacter pylori* infection," *Gastroenterology*, vol. 126, no. 4, pp. 1030–1043, 2004.

[51] F. H. Tabassam, D. Y. Graham, and Y. Yamaoka, "OipA plays a role in *Helicobacter pylori*-induced focal adhesion kinase activation and cytoskeletal re-organization," *Cellular Microbiology*, vol. 10, no. 4, pp. 1008–1020, 2008.

[52] F. H. Tabassam, D. Y. Graham, and Y. Yamaoka, "*Helicobacter pylori* activate epidermal growth factor receptor- and phosphatidylinositol 3-OH kinase-dependent Akt and glycogen synthase kinase 3β phosphorylation," *Cellular Microbiology*, vol. 11, no. 1, pp. 70–82, 2009.

[53] Y. Yamaoka, O. Ojo, S. Fujimoto et al., "*Helicobacter pylori* outer membrane proteins and gastroduodenal disease," *Gut*, vol. 55, no. 6, pp. 775–781, 2006.

[54] D. Y. Graham, A. R. Opekun, M. S. Osato et al., "Challenge model for *Helicobacter pylori* infection in human volunteers," *Gut*, vol. 53, no. 9, pp. 1235–1243, 2004.

[55] Y. Yamaoka, M. Kita, T. Kodama et al., "*Helicobacter pylori* infection in mice: role of outer membrane proteins in colonization and inflammation," *Gastroenterology*, vol. 123, no. 6, pp. 1992–2004, 2002.

[56] T. Ando, R. M. Peek, D. Pride et al., "Polymorphisms of *Helicobacter pylori* HP0638 reflect geographic origin and correlate with *cagA* status," *Journal of Clinical Microbiology*, vol. 40, no. 1, pp. 239–246, 2002.

[57] A. Dossumbekova, C. Prinz, J. Mages et al., "*Helicobacter pylori* HopH (OipA) and bacterial pathogenicity: genetic and functional genomic analysis of hopH gene polymorphisms," *Journal of Infectious Diseases*, vol. 194, no. 10, pp. 1346–1355, 2006.

[58] P. Lehours, A. Ménard, S. Dupouy et al., "Evaluation of the association of nine *Helicobacter pylori* virulence factors with strains involved in low-grade gastric mucosa-associated lymphoid tissue lymphoma," *Infection and Immunity*, vol. 72, no. 2, pp. 880–888, 2004.

[59] S. Fujimoto, O. Olaniyi Ojo, A. Arnqvist et al., "*Helicobacter pylori* BabA Expression, Gastric Mucosal Injury, and Clinical

Outcome," *Clinical Gastroenterology and Hepatology*, vol. 5, no. 1, pp. 49–58, 2007.

[60] H. Lu, P. I. Hsu, D. Y. Graham, and Y. Yamaoka, "Duodenal ulcer promoting gene of *Helicobacter pylori*," *Gastroenterology*, vol. 128, no. 4, pp. 833–848, 2005.

[61] N. R. Hussein, R. H. Argent, C. K. Marx, S. R. Patel, K. Robinson, and J. C. Atherton, "*Helicobacter pylori dupA* is polymorphic, and its active form induces proinflammatory cytokine secretion by mononuclear cells," *Journal of Infectious Diseases*, vol. 202, no. 2, pp. 261–269, 2010.

[62] Y. Yamaoka, "Roles of the plasticity regions of *Helicobacter pylori* in gastroduodenal pathogenesis," *Journal of Medical Microbiology*, vol. 57, no. 5, pp. 545–553, 2008.

[63] N. R. Hussein, "The association of *dupA* and *Helicobacter pylori*-related gastroduodenal diseases," *European Journal of Clinical Microbiology and Infectious Diseases*, vol. 29, no. 7, pp. 817–821, 2010.

[64] S. Shiota, O. Matsunari, M. Watada et al., "Systematic review and meta-analysis: the relationship between the *Helicobacter pylori* dupA gene and clinical outcomes," *Gut Pathogens*, vol. 2, no. 1, article 13, 2010.

[65] D. M. M. Queiroz, G. A. Rocha, A. M. C. Rocha et al., "*DupA* polymorphisms and risk of *Helicobacter pylori*-associated diseases," *International Journal of Medical Microbiology*, vol. 301, no. 3, pp. 225–228, 2011.

[66] S. W. Jung, M. Sugimoto, S. Shiota et al., "The intact dupA cluster is a more reliable*Helicobacter pylori*virulence marker than *dupA* alone," *Infection and Immunity*, vol. 80, no. 1, pp. 381–387, 2012.

[67] W. Fischer, L. Windhager, S. Rohrer et al., "Strain-specific genes of *Helicobacter pylori*: genome evolution driven by a novel type IV secretion system and genomic island transfer," *Nucleic Acids Research*, vol. 38, no. 18, pp. 6089–6101, 2010.

[68] M. Kawai, Y. Furuta, K. Yahara et al., "Evolution in an oncogenic bacterial species with extreme genome plasticity: *Helicobacter pylori* East Asian genomes," *BMC Microbiology*, vol. 11, article 104, 2011.

[69] M. S. McClain, C. L. Shaffer, D. A. Israel, R. M. Peek, and T. L. Cover, "Genome sequence analysis of *Helicobacter pylori* strains associated with gastric ulceration and gastric cancer," *BMC Genomics*, vol. 10, article 13, 2009.

[70] D. McNamara and E. El-Omar, "*Helicobacter pylori* infection and the pathogenesis of gastric cancer: a paradigm for host-bacterial interactions," *Digestive and Liver Disease*, vol. 40, no. 7, pp. 504–509, 2008.

[71] J. D. Oh, H. Kling-Bäckhed, M. Giannakis et al., "The complete genome sequence of a chronic atrophic gastritis *Helicobacter pylori* strain: evolution during disease progression," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 26, pp. 9999–10004, 2006.

[72] L. Kennemann, X. Didelot, T. Aebischer et al., "*Helicobacter pylori* genome evolution during human infection," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 108, no. 12, pp. 5033–5038, 2011.

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Review Article

The Optimal First-Line Therapy of *Helicobacter pylori* Infection in Year 2012

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This paper reviews the literature about first-line therapies for *H. pylori* infection in recent years. First-line therapies are facing a challenge because of increasing treatment failure due to elevated antibiotics resistance. Several new treatment strategies that recently emerged to overcome antibiotic resistance have been surveyed. Alternative first-line therapies include bismuth-containing quadruple therapy, sequential therapy, concomitant therapy, and hybrid therapy. Levofloxacin-based therapy shows impressive efficacy but might be employed as rescue treatment due to rapidly raising resistance. Rifabutin-based therapy is also regarded as a rescue therapy. Several factors including antibiotics resistance, patient compliance, and CYP 2C19 genotypes could influence the outcome. Clinicians should use antibiotics according to local reports. It is recommended that triple therapy should not be used in areas with high clarithromycin resistance or dual clarithromycin and metronidazole resistance.

1. Introduction

Eradicating *Helicobacter pylori* (*H. pylori*) is the most important aspect of managing *H. pylori*-related gastrointestinal diseases. In the past decade, the Maastricht III Consensus Report has recommended that proton pump inhibitor-(PPI-) clarithromycin-amoxicillin or metronidazole treatment is the first choice for *H. pylori* infection [1]. Although some studies have revealed that the eradication rates of standard triple therapies are around 80% (by per-protocol (PP) analysis) [2, 3], most studies have demonstrated the success rate of recommended triple therapies is falling [4–7]. According to recent studies, such eradication rates have plummeted to even 25%–60% [8–10]. The many causes of fall in efficacy are varied including antibiotic resistance, poor compliance, high gastric acidity, high bacterial load, and the cytochrome P450 2C19 (CYP2C19) polymorphism [10]. Compliance is an important factor where patients with good compliance (taking more than 60% of prescribed agents) have a higher treatment success compared to patients with poor compliance (96 versus 69%) [11]. The factors that negatively affect successful eradication are an increase in body mass index and smoking [12, 13]. Besides, other factors including the patient's history of antibiotic use, the cost, and availability of the drugs would also influence the choice of regimen.

In order to overcome the challenge of decreasing eradication rates, many novel first-line therapies have been developed. According to guidelines of the Maastricht III, the minimal acceptable eradication level recommended is an 80%

eradication rate (by intention-to-treat (ITT) analysis) [1]. Graham and Fischbach recommended that clinicians should only use what works locally and ignore consensus statements and society guidelines if they are not consistent with local results [14]. According to the recommendation of the Asian Pacific *Helicobacter pylori* meeting 2012 in Singapore: (1) in areas with low clarithromycin resistance rates, standard triple therapy should be the primary choice, while bismuth-containing quadruple, sequential therapy, and concomitant therapy could be alternative first-line therapies and (2) in areas with high clarithromycin resistance, regimens including bismuth-containing quadruple, sequential therapy, and concomitance should be the better choice for first-line regimens. This paper will introduce recent novel and acceptable regimens as the first-line therapies of *H. pylori* and the factors influencing eradication.

2. Standard Triple Therapy

Triple therapies are still the most commonly used first-line therapy in the world despite decreasing efficacy [14]. Clarithromycin resistance plays the cardinal role in failure of eradication [14–16]. The standard triple therapy showed a better eradication rate in clarithromycin-sensitive strains than in clarithromycin-resistant strains (88% versus 18%) [16], so it is recommended that standard first-line therapies should be abandoned in areas with clarithromycin resistance of more than 15–20% [14], because the eradication rate often decreased to less than 85% (PP) and less than 80% (ITT) [8, 9, 15–17].

However, prolonged duration of standard triple therapy might be a good method to overcome the challenge of resistance. A systemic review showed that the distribution of clarithromycin-resistant strains ranged from 49% (Spain) to 1% (Netherlands) worldwide [18]. One American study in 2011 surveyed the efficacy of 14-day triple therapy. The eradication rates of 14-day standard therapy, concomitant therapy, and sequential therapy were 82.2% (401 of 488), 73.6% (360 of 489), and 76.5% (372 of 486), respectively. It demonstrated that fourteen-day triple therapy is preferable to 5-day concomitant or 10-day sequential four-drug regimens [19].

3. Bismuth-Containing Quadruple Therapy

The Maastricht III Consensus Report [1] and the Second Asia-Pacific Consensus Guidelines for *H. pylori* Infection [20] both recommended bismuth-containing quadruple therapy as an alternative first-line regimen for *H. pylori* infection. Three studies with this combination given for 10 days have demonstrated eradication (or successful treatment, but DC rates) rates more than 90% [21–23]. One study compared the efficacy of a 10-day bismuth-containing quadruple therapy and a 7-day triple therapy. Their data revealed that the bismuth-quadruple therapies had a higher eradication rate than the triple therapy (93% versus 70% by PP analysis) [23]. To improve compliance, one RCT presented that a capsule containing bismuth subcitrate potassium, metronidazole, and tetracycline given with omeprazole was comparable to clarithromycin-based triple therapy. The eradication rates were 80% in the quadruple therapy group versus 55% in the standard therapy group [23]. Besides, the bismuth-containing quadruple therapy provides superior eradication with similar safety and tolerability to standard therapy. So the quadruple therapy should be considered as first-line treatment in the areas of high clarithromycin resistance.

However, there is no agreement with the duration of bismuth-containing quadruple therapy now. Ten or fourteen days are often used durations in these regimens [24]. Further survey is needed.

4. Sequential Therapy

A 10-day sequential therapy consists of a 5-day dual therapy with a PPI (standard dose, b.i.d.) and amoxicillin (1000 mg, b.i.d.) followed by a 5-day triple therapy with a PPI (standard dose, b.i.d.), clarithromycin (500 mg, b.i.d.) and metronidazole (500 mg, b.i.d.). This novel therapy shows an impressive eradication rate above 90% [25–28]. The rationale of sequential therapy includes (1) Amoxicillin would decrease the bacterial load and then the risk of selection of clarithromycin-resistant mutant and (2) Amoxicillin may disrupt the efflux pump preventing clarithromycin resistance. Gatta et al. reported a meta-analysis (8 Italian studies) [26] that compared sequential therapy with standard triple therapy for 7 or 10 days, and they found the relative risk of *H. pylori* eradication was 1.21 (95% CI 1.17–1.25). This meta-analysis showed a trend preferring sequential therapy to triple therapy. Sequential therapies also demonstrated better eradication rates than standard triple therapy for clarithromycin-resistant strains (89% versus 29% by PP analysis) [25].

However, there is significant heterogeneity observed between results from Asia and Italy. One study in Asia compared the sequential therapy with standard triple therapy and found that the two methods did not have significantly different eradication rates (86% versus 77% by PP analysis) [29]. This suggests that there is likely to be a variation in eradication rates achieved by sequential therapy in different areas. Another concern is the efficacy of sequential therapy for dual resistance (clarithromycin and metronidazole resistance). Unfortunately, there is still no large study to confirm this point. Besides, sequential therapy is more complex than triple or quadruple therapies and this raises the concern about patient compliance. However, one study stated that there was no significantly different compliance between sequential therapy and concomitant therapy [30].

5. Concomitant Therapy

This regimen containing four-drug regimen: a PPI (standard dose, b.i.d.), clarithromycin (500 mg, b.i.d.), amoxicillin (1000 mg, b.i.d.), and metronidazole (500 mg, b.i.d.). All drugs are given during the course of concomitant therapy [30]. A meta-analysis was performed in 2009. It compares concomitant (293 subjects, duration 3 to 5 days) and triple therapy (283 subjects, duration 5 to 10 days) and four

other studies evaluating concomitant therapy (478 subjects, duration 3 to 7 days). Pooled data showed that concomitant therapy had obviously better eradication rates than triple therapy: with pooled adds ratio (OR) of 2.86 (95% CI: 1.73–4.73) (by ITT analysis) and pooled OR of 3.52 (95% CI: 1.95–6.38) (by PP analysis) [31]. One recent study in 2012 also supports these results [32]. Concomitant therapy is less complex than sequential therapy. One randomized control trial compared the efficacy of sequential therapy and concomitant therapy and found that these two therapies showed similar eradication rates (93.1% versus 93.0% by PP analysis) and compliance [30].

6. Hybrid Therapy

Hsu et al. [33] presented one novel therapy—The hybrid therapy. This therapy consists of two-step therapy: a dual therapy for 7 days (a PPI (standard dose, b.i.d.) and amoxicillin (1000 mg, b.i.d.)) followed by a quadruple therapy for 7 days (a PPI (standard dose, b.i.d.), amoxicillin (1000 mg, b.i.d.), clarithromycin (500 mg, b.i.d.), and metronidazole (500 mg, b.i.d.)). In this therapy, the role of fourteen-day amoxicillin is to reduce the bacterial load and try to overcome the challenge of *H. pylori* with dual resistance (metronidazole and clarithromycin). They demonstrated hybrid therapy with high eradication rates: 97% (by ITT analysis) and 99% (by PP analysis). This study also surveyed the efficacy in the treatment of *H. pylori* with dual resistance. It also showed encouraging results. Tests on the efficacy of this new regimen is needed with further studies.

7. Quinolone-Based Therapy

Levofloxacin could be used as an alternative agent for clarithromycin in either a standard triple, quadruple, or sequential regimens. The use of levofloxacin in first-line therapy has also been surveyed. The eradication rates of levofloxacin-based triple therapy ranged from 72% to 96% [34]. The variable rates may result from the difference in resistances. One study demonstrated efficacy of levofloxacin-based triple therapy had higher eradication rate than clarithromycin-based triple therapy (83% versus 66% by PP analysis) [35]. It also showed that levofloxacin-based quadruple therapy had similar eradication rates with clarithromycin-based quadruple therapy (85% versus 81% by PP analysis). Another study surveys the impact of levofloxacin on sequential therapy [36]. It demonstrated that levofloxacin-based sequential therapy had higher eradication rates than clarithromycin-based therapy (96% versus 81 % by PP analysis).

The optimal dose of levofloxacin is another interesting point. The commonly used dosage of levofloxacin was 500 mg daily in many studies in Asia [37]. Studies demonstrated that increasing the dosage of levofloxacin cannot overcome levofloxacin resistance [38, 39]. Furthermore, previous studies suggest that once-daily dosing of a levofloxacin-based triple regimen may be as efficacious as twice daily [40].

One critical point should be remembered that quinolone resistance is raised rapidly in eradication of *H. pylori*. Primary resistance to levofloxacin ranges between 8% and 31% in different countries or regions [41–43]. Inappropriate use of quinolone might result in the development of more quinolone-resistant pathogens and it might cause much trouble in controlling respiratory (especially tuberculosis) and urogenital tract infections. So the quinolone-based triple therapy is not generally recommended as first-line therapy. The regimen could be considered in those areas with clarithromycin resistance greater than 15%–20% and quinolone resistance less than 10% [34].

8. Rifabutin-Based Therapy

Rifabutin is an antituberculous agent and it is also effective for eradicating *H. pylori* [44]. The optimal duration of rifabutin-containing therapies is unclear, but most studies have recommended 10–12 days. However, there are concerns about rifabutin-based therapies. One is the side effect of myelotoxicity (22% (19–25%)) and ocular adverse events have been reported with rifabutin-based therapy [45]. Another disadvantage is popular use of rifabutin might result in the development of resistance to Mycobacterium tuberculosis and Mycobacterium avium. So it is usually used in rescue therapies only.

9. The Factors Influencing Eradication of *H. pylori* Infection

9.1. Resistance. Antibiotic resistance is the most serious problem in eradicating *H. pylori*. Resistance rates are remarkably variable in different geographic areas and therefore it is necessary to select the drugs according to local resistance patterns [46]. Clarithromycin resistance is the most important issue. The cause of high *H. pylori* clarithromycin resistance rates was mainly resulted from the long-term use of clarithromycin for respiratory tract infections [16]. A systemic review that included 11,697 cases was performed to survey the resistance rate of clarithromycin in the world in 2010. On a global scale, resistance was detected in 2014 cases (17.2%, 95% CI 16.5–17.9%). The resistance rates were obviously different among the following areas: Europe (11.1%), Asia (18.9%), and America (29.3%) [18]. A meta-analysis reported the impact of antibiotics resistance on treatment efficacy: clarithromycin resistance decreased the efficacy of PPI (standard dose, b.i.d.) + amoxicillin (1000 mg, b.i.d.) + clarithromycin (500 mg, b.i.d.) regimen by 66% (95% CI: 54–78%). The efficacy of patients receiving PPI (standard dose, b.i.d.) + metronidazole (250 mg, q.i.d.) + clarithromycin (500 mg, b.i.d.) regimen was decreased by 35% (95% CI: 24–44%) from clarithromycin resistance and decreased by 18% (95% CI: 13–23%) from metronidazole resistance [47]. Metronidazole resistance seems to have limited impact on efficacy of eradication.

The resistance to metronidazole is between 30 and 40% [48, 49], although it has less clinical impact. Metronidazole resistance can be partially overcome by increasing the dosage or treatment duration.

Resistance against amoxicillin is usually low around the world, so its resistance does not influence the use in treatment regimens.

Table 1: Recommended first-line therapies for *Helicobacter pylori* infection.

Treatment	Regimen	High clarithromycin resistance area	Low clarithromycin resistance area
Standard triple therapy	A PPI (standard dose, b.i.d.), clarithromycin (500 mg, b.i.d.), and amoxicillin (1 g, b.i.d.) for 7–14 days	x	V
Bismuth-containing quadruple therapy	A PPI (standard dose, b.i.d.), bismuth (standard dose, q.i.d.), tetracycline (500 mg, q.i.d.), and metronidazole (250 mg, q.i.d.) for 10–14 days	V	V
Sequential therapy	A 5-day dual therapy with a PPI (standard dose, b.i.d.) and amoxicillin (1 g, b.i.d.) followed by a 5-day triple therapy with a PPI (standard dose, b.i.d.), clarithromycin (500 mg, b.i.d.), and metronidazole (500 mg, b.i.d.)	V	V
Concomitant therapy	A PPI (standard dose, b.i.d.), clarithromycin (500 mg, b.i.d.), amoxicillin (1 g, b.i.d.), and metronidazole (500 mg, b.i.d.) for 7–10 days	V	V
Levofloxacin-based triple therapy	A PPI (standard dose, b.i.d.), levofloxacin (500 mg, q.d.), and amoxicillin (1 g, b.i.d.) for 10 days	V	—*
Hybrid therapy	A 7-day dual therapy with a PPI (standard dose, b.i.d.) and amoxicillin (1 g, b.i.d.) followed by a 7-day quadruple therapy with a PPI (standard dose, b.i.d.), amoxicillin (1 g, b.i.d.), clarithromycin (500 mg, b.i.d.), and metronidazole (500 mg, b.i.d.)	V	V

*Levofloxacin-based triple therapy is useful, but it might not be recommended as first-line therapy under the consideration of rapidly increasing resistance.

Resistance to levofloxacin has increased rapidly in recent years and the worldwide resistance rate is around 16.2% (95% CI 14.4–18%). In Taiwan, about five-fold increase in levofloxacin resistance was observed in primary resistance (before the year 2004, 3.2%; after the year 2004, 16.3%) [49]. Average rate of primary levofloxacin resistance to *H. pylori* in Europe (2008-2009) is around 14.1% (4.0–28.0%) [47]. Resistance to fluoroquinolones would become a serious problem. The methods for preventing the selection of resistance include using a combination of antibiotics, increasing compliance, and increasing the length of treatment.

9.2. The Polymorphism of CYP2C19. The polymorphisms of CYP2C19 lead some patients to metabolize PPI more rapidly than others. Patients are divided into three phenotypes: extensive (EM), intermediate (IM), and poor (PM) metabolizers. Ethnic differences in the frequencies of CYP2C19 gene polymorphism are well known. Asian people have a higher proportion of poor metabolizers (20 versus 5%) compared to whites [50, 51]. The different phenotypes result in different degrees of PPI metabolism. The mechanisms whereby PPIs influence the efficacy of eradicating *H. pylori* include (1) PPIs make acid-labile antibiotics more stable by increasing gastric pH value, especially clarithromycin, thereby increasing concentration and *H. pylori* sensitivity to antibiotics (2) PPIs may alter transport of antibiotics from plasma to gastric juice, increase luminal concentrations and elevating the success rate of eradication [52]. CYP2C19 genotype-dependent *H. pylori* eradication rates were noted in many kinds of PPIs [51, 53, 54]. However, rabeprazole and esomeprazole were less influenced by polymorphism of CYP2C19 [51, 52].

The effect of increasing dose is unclear. One study in China demonstrated that increasing the dosage of omeprazole (20 to 40 mg) would improve the efficacy of eradication [55], but other studies did not find a similar dose-dependent effect by use of omeprazole, rabeprazole, and lansoprazole [56, 57].

9.3. The Impact of Probiotics in Eradicating H. pylori . It is difficult to develop new effective antibiotics to eradicate *H. pylori*, so it is necessary to find alternative methods to improve eradication rate and compliance in first-line therapy. So many studies have tried new treatment approaches by using probiotics. Several studies have previously reported that certain probiotics exhibit inhibitory activity against *H. pylori* in vitro and in vivo [58, 59]. Earlier studies demonstrated that standard triple therapy plus probiotics showed better eradication rate than standard triple therapy only [60–62]. So probiotics become a promising adjunct for *H. pylori* eradication therapy because they could increase compliance by increasing tolerability and preventing side effects [63–66]. The possible mechanisms of probiotics in eradicating *H. pylori* include production of inhibitory substance, host immune modulation or competition for adhesion [64, 67, 68]. But improvement of eradication rate is not always found in every regimen. One study revealed that levofloxacin-based sequential therapy and levofloxacin based triple therapy were significantly superior to standard triple therapy plus probiotic (PP/ITT analysis: 95.5/95.5%, 89.1/86.3%, and 77.1/72.4%, resp.) [69, 70].

In previous studies, *Saccharomyces boulardii* and *Lactobacillus supp*. are the most common probiotics used in clinical trials. Several meta-analysis studies showed that

standard triple therapy accompanied with the *Saccharomyces boulardii* or *Lactobacillus supp*. could increase eradication rates and decrease therapy-related side effects, especially diarrhea and taste disturbance [71–74].

In summary, the exactly mechanism of probiotics is largely unknown and further research is greatly needed. The restoration of the normal flora in the intestine might be important in patients receiving triple therapy for *H. pylori* eradication.

9.4. Patients with Penicillin Allergy. Drug allergy to penicillin is also an important factor influencing regimen chosen. In *H. pylori* infected patients allergic to penicillin, the previously recommended first-line treatment with omeprazole-clarithromycin-metronidazole has low efficacy for curing the infection. So other regimens which include bismuth-containing, non-bismuth-conatining quadruple therapies or levofloxacin-based triple therapy should be taken into consideration. These regimens showed better and acceptable eradication outcomes [75]. So it is reasonable to choose quadruple therapy or levofloxacin-based triple therapy for patients allergic to penicillin.

9.5. Smoking. Smoking might cause failure of *H. pylori* eradication therapy. One meta-analysis of 5538 patients in 2006 revealed that the summary OR for eradication failure among smokers relative to nonsmokers was 1.95 (95% CI: 1.55–2.45; $P < 0.01$). It also showed a better eradication rate of 8.4% (95% CI: 3.3–13.5%; $P < 0.01$) in nonsmokers [13].

10. Conclusion

First-line therapies of *H. pylori* infection are facing a challenge because of increasing treatment failure. The paper reviews several new treatment strategies with the intention to overcome antibiotic resistance (Table 1). Alternative first-line therapies include bismuth-containing quadruple therapy, sequential therapy, concomitant quadruple therapy, and hybrid therapy. Levofloxacin-based therapies showed impressive efficacy, but they might be employed as rescue treatment except in areas with high clarithromycin resistance. Antimicrobial resistance is very important to clarithromycin-containing therapies because of their impact on clinical outcome and high prevalence. Antimicrobial resistance is not important for the other groups of antibiotics (amoxicillin, tetracycline) because of the low prevalence. However, it is not practical to perform culturing before first-line therapy. The impact of CYP2C19 polymorphism on eradication should be also taken into consideration. The following recommendations are important. (1) Clinicians should know the local resistance rates. (2) In areas with low clarithromycin resistance rates, standard triple therapy should be the primary choice, while bismuth-containing quadruple, sequential therapy and concomitant therapy could be alternative first-line therapies. (3) In areas with high clarithromycin resistance, regimens including bismuth-containing quadruple, sequential therapy, and concomitant should be the better choice for first-line regimens. In summary, *H. pylori* infection is a common and serious infection, and we should prescribe the first-line regimens more carefully and empirically. Clinicians should use antibiotics according to local reports.

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References

[1] P. Malfertheiner, F. Megraud, C. O'Morain et al., "Current concepts in the management of *Helicobacter pylori* infection: the maastricht III consensus report," *Gut*, vol. 56, no. 6, pp. 772–781, 2007.

[2] C. S. Qua, J. Manikam, and K. L. Goh, "Efficacy of 1-week proton pump inhibitor triple therapy as first-line *Helicobacter pylori* eradication regime in Asian patients: is it still effective 10 years on?" *Journal of Digestive Diseases*, vol. 11, no. 4, pp. 244–248, 2010.

[3] M. Sasaki, N. Ogasawara, K. Utsumi et al., "Changes in 12-year first-line eradication rate of *Helicobacter pylori* based on triple therapy with proton pump inhibitor, amoxicillin and clarithromycin," *Journal of Clinical Biochemistry and Nutrition*, vol. 47, no. 1, pp. 53–58, 2010.

[4] A. O'Connor, J. P. Gisbert, D. McNamara, and C. O'Morain, "Treatment of *Helicobacter pylori* infection 2010," *Helicobacter*, vol. 15, supplement 1, pp. 46–52, 2010.

[5] M. Selgrad and P. Malfertheiner, "Treatment of *Helicobacter pylori*," *Current Opinion in Gastroenterology*, vol. 27, no. 6, pp. 565–570, 2011.

[6] H. G. Park, M. K. Jung, J. T. Jung et al., "Randomised clinical trial: a comparative study of 10-day sequential therapy with 7-day standard triple therapy for *Helicobacter pylori* infection in naïve patients," *Alimentary Pharmacology and Therapeutics*, vol. 35, no. 1, pp. 56–65, 2012.

[7] A. O'Connor, J. P. Gisbert, D. McNamara, and C. O'Morain, "Treatment of *Helicobacter pylori* infection 2011," *Helicobacter*, vol. 16, supplement 1, pp. 53–58, 2011.

[8] Y. Gumurdulu, E. Serin, B. Özer et al., "Low eradication rate of *Helicobacter pylori* with triple 7–14 days and quadriple therapy in Turkey," *World Journal of Gastroenterology*, vol. 10, no. 5, pp. 668–671, 2004.

[9] M. A. Bigard, J. C. Delchier, G. Riachi, P. Thibault, and P. Barthelemy, "One-week triple therapy using omeprazole, amoxycillin and clarithromycin for the eradication of *Helicobacter pylori* in patients with non-ulcer dyspepsia: influence of dosage of omeprazole and clarithromycin," *Alimentary Pharmacology and Therapeutics*, vol. 12, no. 4, pp. 383–388, 1998.

[10] S. K. Chuah, F. W. Tsay, P. I. Hsu, and D. C. Wu, "A new look at anti-*Helicobacter pylori* therapy," *World Journal of Gastroenterology*, vol. 17, no. 35, pp. 3971–3975, 2011.

[11] D. Y. Graham, G. M. Lew, H. M. Malaty et al., "Factors influencing the eradication of *Helicobacter pylori* with triple therapy," *Gastroenterology*, vol. 102, no. 2, pp. 493–496, 1992.

[12] M. Abdullahi, B. Annibale, D. Capoccia et al., "The eradication of *Helicobacter pylori* is affected by body mass index (BMI)," *Obesity Surgery*, vol. 18, no. 11, pp. 1450–1454, 2008.

[13] T. Suzuki, K. Matsuo, H. Ito et al., "Smoking increases the treatment failure for *Helicobacter pylori* eradication," *American Journal of Medicine*, vol. 119, no. 3, pp. 217–224, 2006.

[14] D. Y. Graham and L. Fischbach, "*Helicobacter pylori* treatment in the era of increasing antibiotic resistance," *Gut*, vol. 59, no. 8, pp. 1143–1153, 2010.

[15] J. P. Gisbert, R. Pajares, and J. M. Pajares, "Evolution of *Helicobacter pylori* therapy from a meta-analytical perspective," *Helicobacter*, vol. 12, no. 2, pp. 50–58, 2007.

[16] F. Mégraud, "*H pylori* antibiotic resistance: prevalence, importance, and advances in testing," *Gut*, vol. 53, no. 9, pp. 1374–1384, 2004.

[17] D. Y. Graham, H. Lu, and Y. Yamaoka, "Therapy for *Helicobacter pylori* infection can be improved: sequential therapy and beyond," *Drugs*, vol. 68, no. 6, pp. 725–736, 2008.

[18] V. de Francesco, F. Giorgio, C. Hassan et al., "Worldwide *H. pylori* antibiotic resistance: a systematic review," *Journal of Gastrointestinal and Liver Diseases*, vol. 19, no. 4, pp. 409–414, 2010.

[19] E. R. Greenberg, G. L. Anderson, D. R. Morgan et al., "14-day triple, 5-day concomitant, and 10-day sequential therapies for *Helicobacter pylori* infection in seven Latin American sites: a randomised trial," *The Lancet*, vol. 378, no. 9790, pp. 507–514, 2011.

[20] K. M. Fock, P. Katelaris, K. Sugano et al., "Second Asia-Pacific consensus guidelines for *Helicobacter pylori* infection," *Journal of Gastroenterology and Hepatology*, vol. 24, no. 10, pp. 1587–1600, 2009.

[21] C. O'Morain, T. Borody, A. Farley et al., "Efficacy and safety of single-triple capsules of bismuth biskalcitrate, metronidazole and tetracycline, given with omeprazole, for the eradication of *Helicobacter pylori*: an international multicentre study," *Alimentary Pharmacology and Therapeutics*, vol. 17, no. 3, pp. 415–420, 2003.

[22] L. Laine, R. Hunt, H. EI-Zimaity, B. Nguyen, M. Osato, and J. Spénard, "Bismuth-based quadruple therapy using a single capsule of bismuth biskalcitrate, metronidazole, and tetracycline given with omeprazole versus omeprazole, amoxicillin, and clarithromycin for eradication of *Helicobacter pylori* in duodenal ulcer patients: a prospective, randomized, multicenter, North American trial," *American Journal of Gastroenterology*, vol. 98, no. 3, pp. 562–567, 2003.

[23] P. Malfertheiner, F. Bazzoli, J. C. Delchier et al., "*Helicobacter pylori* eradication with a capsule containing bismuth subcitrate potassium, metronidazole, and tetracycline given with omeprazole versus clarithromycin-based triple therapy: a randomised, open-label, non-inferiority, phase 3 trial," *The Lancet*, vol. 377, no. 9769, pp. 905–913, 2011.

[24] W. D. Chey and B. C. Y. Wong, "American College of Gastroenterology guideline on the management of *Helicobacter pylori* infection," *American Journal of Gastroenterology*, vol. 102, no. 8, pp. 1808–1825, 2007.

[25] D. Vaira, A. Zullo, N. Vakil et al., "Sequential therapy versus standard triple-drug therapy for *Helicobacter pylori* eradication: a randomized trial," *Annals of Internal Medicine*, vol. 146, no. 8, pp. 556–563, 2007.

[26] L. Gatta, N. Vakil, G. Leandro, F. di Mario, and D. Vaira, "Sequential therapy or triple therapy for *Helicobacter pylori* infection: systematic review and meta-analysis of randomized controlled trials in adults and children," *American Journal of Gastroenterology*, vol. 104, no. 12, pp. 3069–3079, 2009.

[27] P. I. Hsu, D. C. Wu, J. Y. Wu, and D. Y. Graham, "Is there a benefit to extending the duration of *Helicobacter pylori* sequential therapy to 14 days?" *Helicobacter*, vol. 16, no. 2, pp. 146–152, 2011.

[28] N. Sirimontaporn, D. Thong-Ngam, S. Tumwasorn, and V. Mahachai, "Ten-day sequential therapy of *Helicobacter pylori* infection in Thailand," *American Journal of Gastroenterology*, vol. 105, no. 5, pp. 1071–1075, 2010.

[29] W. H. Choi, D. I. Park, S. J. Oh et al., "Effectiveness of 10 day-sequential therapy for *Helicobacter pylori* eradication in Korea," *The Korean Journal of Gastroenterology* , vol. 51, no. 5, pp. 280–284, 2008.

[30] D. C. Wu, P. I. Hsu, J. Y. Wu et al., "Sequential and concomitant therapy with four drugs is equally effective for eradication of *H pylori* infection," *Clinical Gastroenterology and Hepatology*, vol. 8, no. 1, pp. 36–41, 2010.

[31] A. S. Essa, J. R. Kramer, D. Y. Graham, and G. Treiber, "Meta-analysis: four-drug, three-antibiotic, non-bismuth-containing "concomitant therapy" versus triple therapy for *Helicobacter pylori* eradication," *Helicobacter*, vol. 14, no. 2, pp. 109–118, 2009.

[32] S. Georgopoulos, V. Papastergiou, E. Xirouchakis et al., "Evaluation of a four-drug, three-antibiotic, vonbismuth-containing "concomitant" therapy as first-line *Helicobacter pylori* eradication regimen in Greece," *Helicobacter*, vol. 17, no. 1, pp. 49–53, 2012.

[33] P. I. Hsu, D. C. Wu, J. Y. Wu, and D. Y. Graham, "Modified sequential *Helicobacter pylori* therapy: proton pump inhibitor and amoxicillin for 14 days with clarithromycin and metronidazole added as a quadruple (hybrid) therapy for the final 7 days," *Helicobacter*, vol. 16, no. 2, pp. 139–145, 2011.

[34] M. Berning, S. Krasz, and S. Miehlke, "Should quinolones come first in *Helicobacter pylori* therapy?" *Therapeutic Advances in Gastroenterology*, vol. 4, no. 2, pp. 103–114, 2011.

[35] J. Molina-Infante, B. Perez-Gallardo, M. Fernandez-Bermejo et al., "Clinical trial: clarithromycin vs. levofloxacin in first-line triple and sequential regimens for *Helicobacter pylori* eradication," *Alimentary Pharmacology and Therapeutics*, vol. 31, no. 10, pp. 1077–1084, 2010.

[36] M. Romano, A. Cuomo, A. G. Gravina et al., "Empirical levofloxacin-containing versus clarithromycin-containing sequential therapy for *Helicobacter pylori* eradication: a randomised trial," *Gut*, vol. 59, no. 11, pp. 1465–1470, 2010.

[37] C. H. Kuo, H. M. Hu, F. C. Kuo et al., "Efficacy of levofloxacin-based rescue therapy for *Helicobacter pylori* infection after standard triple therapy: a randomized controlled trial," *Journal of Antimicrobial Chemotherapy*, vol. 63, no. 5, pp. 1017–1024, 2009.

[38] W. M. Wong, Q. Gu, K. M. Chu et al., "Lansoprazole, levofloxacin and amoxicillin triple therapy vs. quadruple therapy as second-line treatment of resistant *Helicobacter pylori* infection," *Alimentary Pharmacology and Therapeutics*, vol. 23, no. 3, pp. 421–427, 2006.

[39] H. C. Cheng, W. L. Chang, W. Y. Chen, H. B. Yang, J. J. Wu, and B. S. Sheu, "Levofloxacin-containing triple therapy to eradicate the persistent *H. pylori* after a failed conventional triple therapy," *Helicobacter*, vol. 12, no. 4, pp. 359–363, 2007.

[40] L. W. Chen, R. N. Chien, J. J. Chang, K. M. Fang, and L. C. Chang, "Comparison of the once-daily levofloxacin-containing triple therapy with the twice-daily standard triple therapy for first-line *Helicobacter pylori* eradication: a prospective randomised study," *International Journal of Clinical Practice*, vol. 64, no. 11, pp. 1530–1534, 2010.

[41] G. Cammarota, A. Martino, G. Pirozzi et al., "High efficacy of 1-week doxycycline- and amoxicillin-based quadruple regimen in a culture-guided, third-line treatment approach for

Helicobacter pylori infection," *Alimentary Pharmacology and Therapeutics*, vol. 19, no. 7, pp. 789–795, 2004.

[42] L. M. Best, D. J. M. Haldane, G. S. Bezanson, and S. J. Veldhuyzen van Zanten, "*Helicobacter pylori*: primary susceptibility to clarithromycin in vitro in Nova Scotia," *Canadian Journal of Gastroenterology*, vol. 11, no. 4, pp. 298–300, 1997.

[43] J. Cabrita, M. Oleastro, R. Matos et al., "Features and trends in *Helicobacter pylori* antibiotic resistance in Lisbon area, Portugal (1990–1999)," *Journal of Antimicrobial Chemotherapy*, vol. 46, no. 6, pp. 1029–1031, 2000.

[44] J. P. Gisbert and X. Calvet, "Review article: rifabutin in the treatment of refractory *Helicobacter pylori* infection," *Alimentary Pharmacology and Therapeutics*, vol. 35, no. 2, pp. 209–221, 2012.

[45] G. Apseloff, "Severe neutropenia among healthy volunteers given rifabutin in clinical trials," *Clinical Pharmacology and Therapeutics*, vol. 74, no. 6, pp. 591–592, 2003.

[46] E. Rimbara, L. A. Fischbach, and D. Y. Graham, "Optimal therapy for *Helicobacter pylori* infections," *Nature Reviews Gastroenterology and Hepatology*, vol. 8, no. 2, pp. 79–88, 2011.

[47] L. Fischbach and E. L. Evans, "Meta-analysis: the effect of antibiotic resistance status on the efficacy of triple and quadruple first-line therapies for *Helicobacter pylori*," *Alimentary Pharmacology and Therapeutics*, vol. 26, no. 3, pp. 343–357, 2007.

[48] A. O'Connor, I. Taneike, A. Nami et al., "*Helicobacter pylori* resistance to metronidazole and clarithromycin in Ireland," *European Journal of Gastroenterology and Hepatology*, vol. 22, no. 9, pp. 1123–1127, 2010.

[49] W. L. Chang, B. S. Sheu, H. C. Cheng, Y. J. Yang, H. B. Yang, and J. J. Wu, "Resistance to metronidazole, clarithromycin and levofloxacin of *Helicobacter pylori* before and after clarithromycin-based therapy in Taiwan," *Journal of Gastroenterology and Hepatology*, vol. 24, no. 7, pp. 1230–1235, 2009.

[50] F. C. Y. Cheng, S. K. Lam, and G. B. Ong, "Maximum acid output to graded doses of pentagastrin and its relation to parietal cell mass in Chinese patients with duodenal ulcer," *Gut*, vol. 18, no. 10, pp. 827–832, 1977.

[51] C. H. Kuo, S. S. W. Wang, W. H. Hsu et al., "Rabeprazole can overcome the impact of CYP2C19 polymorphism on quadruple therapy," *Helicobacter*, vol. 15, no. 4, pp. 265–272, 2010.

[52] T. Kita, Y. Tanigawara, N. Aoyama et al., "CYP2C19 genotype related effect of omeprazole on intragastric pH and antimicrobial stability," *Pharmaceutical Research*, vol. 18, no. 5, pp. 615–621, 2001.

[53] J. M. Kang, N. Kim, D. H. Lee et al., "Effect of the CYP2C19 polymorphism on the eradication rate of *Helicobacter pylori* infection by 7-day triple therapy with regular proton pump inhibitor dosage," *Journal of Gastroenterology and Hepatology*, vol. 23, part 1, no. 8, pp. 1287–1291, 2008.

[54] S. Jinda, K. Nakatani, J. Nishioka et al., "Personalized treatment in the eradication therapy for *Helicobacter pylori*," *International Journal of Molecular Medicine*, vol. 27, no. 2, pp. 255–261, 2011.

[55] J. C. Yang, H. L. Wang, H. D. Chern et al., "Role of omeprazole dosage and cytochrome P450 2C19 genotype in patients receiving omeprazole-amoxicillin dual therapy for *Helicobacter pylori* eradication," *Pharmacotherapy*, vol. 31, no. 3, pp. 227–238, 2011.

[56] X. Pan, Y. Li, Y. Qiu et al., "Efficacy and tolerability of first-line triple therapy with levofloxacin and amoxicillin plus esomeprazole or rabeprazole for the eradication of *Helicobacter pylori* infection and the effect of CYP2C19 genotype: a 1-week, randomized, open-label study in Chinese adults," *Clinical Therapeutics*, vol. 32, no. 12, pp. 2003–2011, 2010.

[57] J. H. Lee, H. Y. Jung, K. D. Choi, H. J. Song, G. H. Lee, and J. H. Kim, "The influence of CYP2C19 polymorphism on eradication of *Helicobacter pylori*: a prospective randomized study of lansoprazole and rabeprazole," *Gut and Liver*, vol. 4, no. 2, pp. 201–206, 2010.

[58] Y. Aiba, N. Suzuki, A. M. A. Kabir, A. Takagi, and Y. Koga, "Lactic acid-mediated suppression of *Helicobacter pylori* by the oral administration of Lactobacillus salivarius as a probiotic in a gnotobiotic murine model," *American Journal of Gastroenterology*, vol. 93, no. 11, pp. 2097–2101, 1998.

[59] I. V. Pinchuk, P. Bressollier, B. Verneuil et al., "*In vitro* anti-*Helicobacter pylori* activity of the probiotic strain Bacillus subtilis 3 is due to secretion of antibiotics," *Antimicrobial Agents and Chemotherapy*, vol. 45, no. 11, pp. 3156–3161, 2001.

[60] B. S. Sheu, J. J. Wu, C. Y. Lo et al., "Impact of supplement with Lactobacillus—and Bifidobacterium—containing yogurt on triple therapy for *Helicobacter pylori* eradication," *Alimentary Pharmacology and Therapeutics*, vol. 16, no. 9, pp. 1669–1675, 2002.

[61] C. G. Goldman, D. A. Barrado, N. Balcarce et al., "Effect of a probiotic food as an adjuvant to triple therapy for eradication of *Helicobacter pylori* infection in children," *Nutrition*, vol. 22, no. 10, pp. 984–988, 2006.

[62] K. Y. Wang, S. N. Li, C. S. Liu et al., "Effects of ingesting Lactobacillus- and Bifidobacterium-containing yogurt in subjects with colonized *Helicobacter pylori*," *American Journal of Clinical Nutrition*, vol. 80, no. 3, pp. 737–741, 2004.

[63] E. Lionetti, V. L. Miniello, S. P. Castellaneta et al., "Lactobacillus reuteri therapy to reduce side-effects during anti-*Helicobacter pylori* treatment in children: a randomized placebo controlled trial," *Alimentary Pharmacology and Therapeutics*, vol. 24, no. 10, pp. 1461–1468, 2006.

[64] D. Lesbros-Pantoflickova, I. Corthesy-Theulaz, L. Blum et al., "*Helicobacter pylori* and probiotics," *Journal of Nutrition*, vol. 137, supplement 3, pp. 812S–818S, 2007.

[65] K. Miki, Y. Urita, F. Ishikawa et al., "Effect of bifidobacterium bifidum fermented milk on *Helicobacter pylori* and serum pepsinogen levels in humans," *Journal of Dairy Science*, vol. 90, no. 6, pp. 2630–2640, 2007.

[66] E. Myllyluoma, L. Veijola, T. Ahlroos et al., "Probiotic supplementation improves tolerance to *Helicobacter pylori* eradication therapy—a placebo—controlled, double-blind randomized pilot study," *Alimentary Pharmacology and Therapeutics*, vol. 21, no. 10, pp. 1263–1272, 2005.

[67] E. Drouin, "*Helicobacter pylori*: novel therapies," *Canadian Journal of Gastroenterology*, vol. 13, no. 7, pp. 581–583, 1999.

[68] I. J. Broekaert and W. A. Walker, "Probiotics and chronic disease," *Journal of Clinical Gastroenterology*, vol. 40, no. 3, pp. 270–274, 2006.

[69] K. Özdil, T. Çalhan, A. Sahin et al., "Levofloxacin based sequential and triple therapy compared with standard plus probiotic combination for *Helicobacter pylori* eradication," *Hepato-Gastroenterology*, vol. 58, no. 109, pp. 1148–1152, 2011.

[70] E. Lionetti, F. Indrio, L. Pavone, G. Borrelli, L. Cavallo, and R. Francavilla, "Role of probiotics in pediatric patients with *Helicobacter pylori* infection: a comprehensive review of the literature," *Helicobacter*, vol. 15, no. 2, pp. 79–87, 2010.

[71] L. V. McFarland, "Systematic review and meta-analysis of saccharomyces boulardii in adult patients," *World Journal of Gastroenterology*, vol. 16, no. 18, pp. 2202–2222, 2010.

[72] H. Szajewska, A. Horvath, and A. Piwowarczyk, "Meta-analysis: the effects of Saccharomyces boulardii supplementation on *Helicobacter pylori* eradication rates and side effects during treatment," *Alimentary Pharmacology and Therapeutics*, vol. 32, no. 9, pp. 1069–1079, 2010.

[73] J. Zou, J. Dong, and X. Yu, "Meta-analysis: lactobacillus containing quadruple therapy versus standard triple first-line therapy for *Helicobacter pylori* eradication," *Helicobacter*, vol. 14, no. 5, pp. 97–107, 2009.

[74] J. L. Tong, Z. H. Ran, J. Shen, C. X. Zhang, and S. D. Xiao, "Meta-analysis: the effect of supplementation with probiotics on eradication rates and adverse events during *Helicobacter pylori* eradication therapy," *Alimentary Pharmacology and Therapeutics*, vol. 25, no. 2, pp. 155–168, 2007.

[75] J. P. Gisbert, A. Pérez-Aisa, M. Castro-Fernández et al., "*Helicobacter pylori* first-line treatment and rescue option containing levofloxacin in patients allergic to penicillin," *Digestive and Liver Disease*, vol. 42, no. 4, pp. 287–290, 2010.

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Research Article

Culture Method and PCR for the Detection of *Helicobacter pylori* in Drinking Water in Basrah Governorate Iraq

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Helicobacter pylori is recognized by the World Health Organization to be the primary cause of peptic ulcers, chronic gastritis, and stomach cancer, though the source of human infection is not well understood. One of the problems in understanding the source of human contamination is the difficulty in isolating the organism from the environment. However, the combination of PCR results with those of culturing of 471 drinking water samples can provide a more accurate picture of *H. pylori* detection. In this method 78 presumptive *H. pylori* colonies out of 266 tap water samples were obtained in the preliminary detection on modified Columbia agar (MCUA) slant relying on urease positivity with a rate of 29.3%. However, only 11 out of them were confirmed by Gram staining and biochemical tests reducing the rate to 4.13% whereas only 3 (1.46%) from 205 reverse osmosis (RO) water samples. Furthermore, only 6 (54.5%) out of the 11 isolates from tap water and 1 (33.3%) of the 3 RO isolates were confirmed by 16SrRNA PCR. Thus PCR confirmation reduced the rate to 2.2%. In addition, only 4 (4%) of 100 tap water samples negative for *H. pylori* by culture method were *H. pylori* positive by 16SrRNA. Water samples were collected from 24 districts of Basrah Governorate from February–December 2009. The direct recovery of *H. pylori* from drinking water is both alarming and scientifically exciting in terms of the investigation of its epidemiology.

1. Introduction

Helicobacter pylori is recognized as the major cause of gastritis and peptic ulcer and gastric mucosa-associated lymphoid tissue (MALT) gastric lymphoma [1]. The mechanism of *H. pylori* pathogenic effect is unclear but is believed to be related to host bacterial interactions initiated by virulence genes, and it is possible that these effects are enhanced by invasiveness of the bacterium [2–5]. *H. pylori* changes from the normal spiral-shaped bacillary form into the coccoid form when it is exposed to water or to other adverse conditions [6]. Hence attempts have been made to develop artificial media to achieve better culture recovery results than those obtained from traditional Columbia blood agar [7, 8]. Polymerase chain reaction (PCR) methods have also been used to detect *H. pylori* as its 16SrRNA gene sequence analysis unambiguously differentiated the *Helicobacter* genus from the closely related *Campylobacter* genus and other *Helicobacter* species [9]. The presence of *H. pylori* in drinking water which was detected by PCR has been reported from several countries [10–12]. Hegarty et al. [13] also demonstrated the presence of respiring *H. pylori* from US surface water. The prevalence of disease attributed to *H. pylori* in Iraq is not available despite of its commonality.

Basrah Governorate, where Basrah city is located, has a population of about three millions; its water supply is mainly derived from three sources, Shatt-Al-Arab River, Tigris River, and Bada lake. Water from these sources is treated at 22 treatment works and distributed through approximately 13,000 Km pipe network.

Since the 1980s there has been a general marked deterioration in water quality in Iraq, reflecting the environmental degradation of the country caused by successive armed conflicts.

The aim of this study was isolating *H. pylori* from drinking water in Basrah, Iraq, on modified Columbia urea

agar (MCUA) and HP media using MDCS method [7] and then confirming that by conventional biochemical tests and 16SrRNA PCR.

2. Methods

2.1. Sample Collection and Culturing. 266 samples of tap water and 205 samples from tankers supplying Reverse osmosis (RO) were collected from 24 districts covering more than 90% of Basrah Governorate during the period from February 2008 to December 2009. Samples of 500 mL water each were collected in sterile glass flasks and examined for chlorine concentration using *o*-toluidine. Samples were transferred within 1-2 hr. to the laboratory and filtered through 0.22 μm Millipore filter membrane. Each membrane was then immersed into 2 mL of tryptic soy broth (TSB) for 1 h. After that each 2 mL TSB was taken and placed at the lower portion of the slanted MCUA tube. Each tube was tilted a few times to allow the added broth to spread bacteria on the upper part of the slant. Slanted MCUA tube, was incubated microaerophically at 37°C for 1-2 days, after which color changes from orange to pink in the solid phase, indicating urease activity. The resulting system is a simple monophasic-diphasic culture setup (MDCS), a diphasic solid liquid environment at the lower part of the test tube and a monophasic solid one above it [7]. From the bottom and the upper portions of the slanted MCUA tube subcultures were done on plates of MCUA and HP media for purification.

No controls were used in the isolation of the strains and also in PCR as they are out of reach for us in Iraq.

2.2. Primary Diagnosis of H. pylori. The suspected purified colonies were chosen according to the Gram staining and cultural characteristics.

2.3. Biochemical Tests. Biochemical tests include production of catalase, oxidase, urease, and H_2S, nitrate reduction, growing in 3.5% NaCl, growing with 1% glycine, and growing at different temperatures.

2.4. Antibiotic Sensitivity Test. The method of Piddock [14] was used to test the sensitivity of 14 isolates of *H. pylori* from drinking water to seven types of antibiotics, kanamycin 30 μg, erythromycin 15 μg, tetracycline 30 mg, ampicillin 10 μg, rifampicin 5 μg, amoxicillin 30 μg and gentamycin 30 μg (Bioanalyse, Turkey).

2.5. 16SrRNA Identification of Isolates. All isolates from tap and RO water samples which gave positive results by biochemical tests as *H. pylori* and (100) samples which were *H. pylori* negative by culture method were further confirmed by using primers specifically designed for the identification of *H. pylori* based on 16SrRNA sequence [15]. The primers for 500 bp product of the 16SrRNA sequence are represented by the forward primer sequence: 5 GCT AAG AGA TCA GCC TAT GTC C3 and the reverse one: 5 TGG CAA TCA GCG TCA GGT AAT G3.

2.6. Preparation of Bacterial Genomic DNA. Genomic DNA from each isolate was prepared by vortex after suspending a loopful of colonies in 1 mL of phosphated-buffer saline (PBS) 7.6, centrifuging at 14000 ×g for 2 min, and boiling the pellet in 1 mL of distilled water for 1 min [16]. The samples were then centrifuged at 12000 ×g for 4 min at 4°C and the supernatants were stored in sterile vials at −70°C until they were used as PCR templates. Genomic DNA from water samples, which have been cultured but did not give isolates for *H. pylori*, were prepared by centrifuging 1 mL of the liquid portion of slant MCUA tube at 14000 ×g for 2 min and washed with 1 mL of PBS to be completed by the same steps for *H. pylori* isolates. Concentration and purity were measured spectrophotometrically at OD_{260} and OD_{280} respectively, to exclude any possible contamination, and a gel of 0.8% agarose was used for electrophoresis.

2.7. PCR Amplification of 16SrRNA for H. pylori. Amplification was carried out in a 25 μL of reaction mixture containing 12.5 μL master mix, 0.5 μL forward primer, 0.5 μL reverse primer, 5 μL DNA samples, 6.5 μL distilled water and 25 μL mineral oil. PCR conditions for 16SrRNA include: denaturation step at 95°C for 5 min, followed by 39 cycles at 94°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 2 min, and an additional extension step at 72°C for 7 min. PCR products were electrophoresed in 2% agarose.

2.8. ureA Gene for H. pylori and PCR Amplification. All isolates which were confirmed by 16SrRNA have been tested for the presence of the ureA gene of *H. pylori*. The primer for 411 bp product of the ureA sequence represented by the forward primer sequence: 5 GCC AAT GGT AAA GCC TTA GTT3 and the reverse one: 5 CTC CTT AAT TGT TTT TAC 3 [17]. Amplification was carried out in a 25 μL of reaction mixture containing 12.5 μL master mix, 0.5 μL forward primer, 0.5 μL reverse primer, 5 μL DNA samples, 6.5 μL distilled water and 25 μL mineral oil. PCR conditions for ureA gene include: denaturation step at 95°C for 5 min, followed by 35 cycles at 94°C for 1 min, annealing at 45°C for. 1 min and, extension at 72°C for 1 min and an additional extension step at 72°C for 7 min. PCR products were electrophoresed in 2% agarose.

3. Results

3.1. Culture Results. Out of 471 water samples, 14 (2.76%) isolates of *H. pylori* were isolated from samples taken from 14 districts by culture method and identified by biochemical tests. They consist of 11 (4.13%) *H. pylori* that have been isolated and diagnosed from 266 samples of tap water and 3 ones (1.46%) from 205 RO samples.

The modified Columbia urea agar using MDCS method preliminarily revealed the presence of *H. pylori* in water samples, correlated with the change in the color of the slant MCUA tube from orange to pink that occurred at the same time thus giving an additional evidence for the presence of *H. pylori* in the samples (Figure 1).

Table 1: Results of biochemical tests characterizing *H. pylori* isolates from 14 districts.

District no	Catalase	Oxidase	Urease	Nitrate reduction	H_2S	Growth with 3.5% NaCl	Growth on 1% glycin	Growth at 42°C	Growth at 25°C
1	+	+	+	−	−	−	−	−	−
2	+	+	+	−	−	−	−	+	−
3	+	+	+	+	−	−	−	−	−
4	+	+	+	−	−	−	−	−	−
5	+	+	+	−	−	−	−	+	−
6	+	+	+	+	−	−	−	−	−
7	+	+	+	−	−	−	−	−	−
8	+	+	+	−	−	−	−	−	−
9	+	+	+	−	−	−	−	−	−
10	+	+	+	−	−	−	−	−	−
11	+	+	+	+	−	−	−	−	−
12	+	+	+	−	−	−	−	−	−
13	+	+	+	−	−	−	−	−	−
14	+	+	+	−	−	−	−	−	−

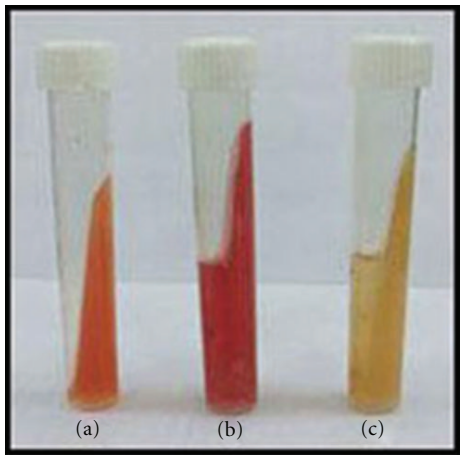

Figure 1: Change in color of slant MCUA tube, (a) slant MCUA tube only, (b) positive slant MCUA tube, culture, (c) negative slant MCUA tube, culture.

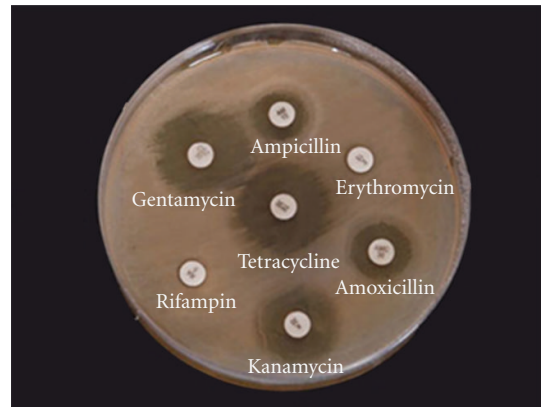

Figure 2: Antibiotic effects on *H. pylori* isolated from drinking water.

The isolation rate upon subculturing on HP medium was 14/471 (2.76%) isolates of *H. pylori*, while on MCUA medium was 6/471 (1.2%) isolates included in the 14 isolates of *H. pylori*.

On MCUA medium, the colonies of the isolated *H. pylori* were small to middle in size, rounded, and creamy in color, while, on HP medium, the isolated *H. pylori* were small in size, rounded, and transparent. Both the MCUA and HP media showed change in color from yellow/orange to red.

All *H. pylori* isolates were Gram-negative spiral to coccobacilli and shared the characteristic catalase, urease, and oxidase production, but differ slightly with respect to other tests (Table 1). Collectively, 3 isolates are being positive in nitrate reduction, 2 in being able to grow at 42°C, and 9 negatives in both traits.

3.2. Antibiotics Susceptibility. For *H. pylori* isolates from drinking water, tetracycline was found to be the most effective antibiotic, 71% of the tested isolates were sensitive to tetracycline followed by kanamycin 57% and gentamycin 36%, ampicillin 14%. Rifampicin and amoxicillin were shown to be the least effective ones (7%) against *H. pylori* isolated from drinking water,while erythromycin was a non effective antibiotic, as shown in (Figure 2) and in reference to interpretive chart of zone sizes.

3.3. PCR Results. Only 6 out of 11 (54.5%) *H. pylori* morphologically and biochemically identified isolates from tap water were found to harbor 16SrRNA gene and of the 3 R.O isolates only one (33.3%) isolate gave positive results for 16SrRNA gene by PCR. Thus leaving out 50% of the conventionally identified isolates as false positive. From the100 samples negative for *H. pylori* by culturing, only 4 (4%), gave positive results for 16SrRNA.

PCR products for 16SrRNA based primers gave bands on agarose gel corresponding to a 500 base pair product

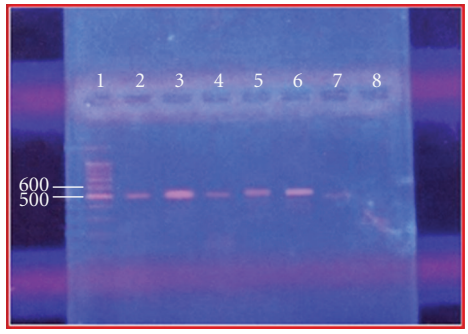

Figure 3: PCR products for 16SrRNA-based primers gave band on agarose gel corresponding to a 500 base pair product when compared to the molecular ladder. Lane 1, molecular ladder (1500–100) bp, lane (2–6) bands of PCR products for *H. pylori* with 16SrRNA.

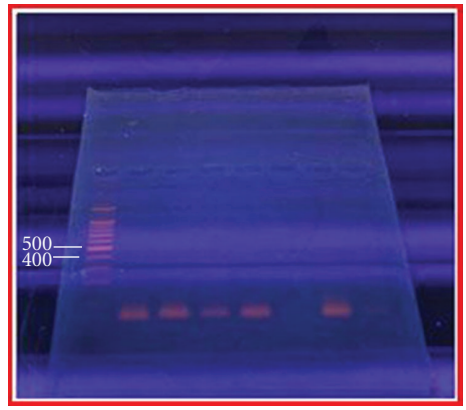

Figure 4: PCR product for *H. pylori* with ureA gene based primers. Lane 1, molecular ladder (1500–100) bp, no band of PCR product for *H. pylori* with ureA gene have been obtained.

when compared to the molecular ladder, thus identifying the isolates as *H. pylori* as shown in (Figure 3).

3.4. ureA Gene for H. pylori and PCR Amplification. All isolates of *H. pylori* which have been confirmed by 16SrRNA, did not give specific results to ureA (Figure 4), only products of 100 bp have been obtained and also a much larger bands.

4. Discussion

Natural habitat of *H. pylori* is in the human stomach, other sources of *H. pylori* and its mode of transmission are unknown [18]. In this study, *H. pylori* has been isolated and diagnosed from drinking water by culture method and a combination of biochemical and PCR test. The first indication for the presence of *H. pylori* in water came from AL-Sulami et al. [8] in which 10 isolates were identified as *H. pylori* by biochemical tests. That finding has been confirmed by current study using the same method and a combination of conventional and PCR tests in identifying recovered *H. pylori*.

A low recovery of a pathogen is not surprising considering various factors affecting its survival in water. Upon primary, isolation there were 78 urease-positive isolates obtained from 266 tap water samples and 43 urease-positive ones from RO water samples. The numbers were reduced to 11 and 3 isolates, respectively, after subjecting them to conventional tests leaving 67 and 40 false-positive ones. Urease-negative isolates were not considered. Other bacteria were mainly pseudomonads.

So far there is no published paper proving the viability of coccoid form or the possibility that coccoid form transforms to spiral bacillary form. Our results indicate that some *H. pylori* are still viable and appear as spiral bacillary after Gram staining smears from colonies on MCUA; others are not and only can be detected by PCR.

Based on the assumption that all *H. pylori* in drinking water are coccoid [6], the results implicitly indicate the possibility of the transformation of some coccoid form to spiral bacillary form.

It is difficult to compare our data with those published, because each author has used a distinct method to detect the bacterium, and all attempts to culture the organism directly from water samples [18, 19] have been unsuccessful. This may be due to the fact that overgrowth by other microorganisms on the rich media led to the difficulty of isolation of *H. pylori* from water, and another reason for the lack of recovery of *H. pylori* from the environment is the fastidious nature of *H. pylori* which has a polymorphisms phenomenon. Under these circumstances, the organism would not be recovered by traditional culture techniques; hence in our study we developed a different protocol for culturing *H. pylori* from water. The importance of this method is to provide a possibility of successful culture method for *H. pylori*.

In general, high-resistance profile to the tested antibiotics is apparent on these isolates as indicated by *H. pylori* resistance for tetracycline in 29% of the isolates, also in case of kanamycin *H. pylori* resistance of 43% which is less than Al-Sulami et al. [8] result of 60%. Amoxicillin which represents active antibiotics in treatment of this bacterium was ineffective with a resistance in 93% of the isolates.

4.1. 16SrRNA for H. pylori Detection by PCR. In this study, this is the first report on using 16SrRNA amplification and confirmation of *H. pylori* isolates from environmental samples in Iraq. The 16SrRNA was chosen for detection of *H. pylori* because it exhibits a high degree of functional and evolutionary homology within all bacteria [9]. Only 7 isolates, out of 14 morphologically and biochemically identified *H. pylori*, were confirmed by 16SrRNA as they gave positive results for 16SrRNA. The prevalence of false positive isolates by conventional tests indicates a nonspecific approach. Meanwhile, in 100 drinking water samples in which no *H. pylori* was detected by culture method, 4 samples produced positive results by 16SrRNA. This means that cells of *H. pylori* that are not detected by culture method

can be done by PCR, and hence, the MDCS provides the opportunity for simultaneous detection of both culturable and nonculturable forms.

Results of PCR products of 16SrRNA gene amplification revealed the presence of 500 base pair sequence of the gene coded the 16SrRNA molecule, and this result agrees with that of [15]. The size of PCR product was determined by comparing it with a DNA ladder, which contains DNA fragments of known size (1500–100) base pairs. Our results may shed additional light on the evidence supporting waterborne transmission which emanates from the fact that there is a direct recovery of *H. pylori* from tap water and R.O water concomitantly confirmed by PCR.

4.2. ureA Gene for H. pylori Detection by PCR. The ureA genotype was expected to be present in all *Helicobacter* positive strains. However, our study was unable to detect the ureA gene in the isolates of *H. pylori* already confirmed by 16SrRNA. This result agrees with Tiveljung et al. [20] who used ureA gene and were unable to detect it in *H. pylori* strain regarded as normal control.

Conclusion

The isolation of *H. pylori* from drinking water, tap and R.O, by culture method and consequent identification by biochemical tests and PCR represents a clear signal for the presence of this dangerous but illusive pathogen in our consumable water. It, certainly, will impact our search for a better epidemiological understanding and measures of control.

References

[1] J. C. Atherton, "The pathogenesis of *Helicobacter pylori*-induced gastro-duodenal diseases," *Annual Review of Pathology*, vol. 1, pp. 63–96, 2006.

[2] Y. Elitsur and J. Yahav, "*Helicobacter pylori* infection in pediatrics," *Helicobacter, Supplement*, vol. 10, no. 1, pp. 47–53, 2005.

[3] A. Dubois and T. Boren, "*Helicobacter pylori* is invasive and it may be a facultative intracellular organism," *Cellular Microbiology*, vol. 9, no. 5, pp. 1108–1116, 2007.

[4] M. R. Amieva and E. M. El-Omar, "Host-Bacterial Interactions in *Helicobacter pylori* Infection," *Gastroenterology*, vol. 134, no. 1, pp. 306–323, 2008.

[5] V. Necchi, M. E. Candusso, F. Tava et al., "Intracellular, intercellular, and stromal invasion of gastric mucosa, preneoplastic lesions, and cancer by *Helicobacter pylori*," *Gastroenterology*, vol. 132, no. 3, pp. 1009–1023, 2007.

[6] N. F. Azevedo, C. Almeida, L. Cerqueira, S. Dias, C. W. Keevil, and M. J. Vieira, "Coccoid form of *Helicobacter pylori* as a morphological manifestation of cell adaptation to the environment," *Applied and Environmental Microbiology*, vol. 73, no. 10, pp. 3423–3427, 2007.

[7] A. A. Al-Sulami, H. S. Al-Kiat, L. K. Bakker, and H. Hunoon, "Primary isolation and detection of *Helicobacter pylori* from dyspeptic patients: a simple, rapid method," *Eastern Mediterranean Health Journal*, vol. 14, no. 2, pp. 268–275, 2008.

[8] A. A. Al-Sulami, A. M. R. Al-Taee, and M. G. Juma'a, "Isolation and identification of *Helicobacter pylori* from drinking water in Basra governorate, Iraq," *Eastern Mediterranean Health Journal*, vol. 16, no. 9, pp. 810–816, 2010.

[9] H. Liu, A. Rahman, C. Semino-Mora, S. Q. Doi, and A. Dubois, "Specific and sensitive detection of *H. pylori* in biological specimens by real-time RT-PCR and in situ hybridization," *PLoS ONE*, vol. 3, no. 7, Article ID e2689, 2008.

[10] K. Hulten, S. W. Han, H. Enroth et al., "*Helicobacter pylori* in the drinking water in Peru," *Gastroenterology*, vol. 110, no. 4, pp. 1031–1035, 1996.

[11] J. A. Benson, K. A. Fode-Vaughan, and M. L. P. Collins, "Detection of *Helicobacter pylori* in water by direct PCR," *Letters in Applied Microbiology*, vol. 39, no. 3, pp. 221–225, 2004.

[12] M. Mazari-Hiriart, Y. López-Vidal, and J. J. Calva, "*Helicobacter pylori* in water systems for human use in Mexico City," *Water Science and Technology*, vol. 43, no. 12, pp. 93–98, 2001.

[13] J. P. Hegarty, M. T. Dowd, and K. H. Baker, "Occurrence of *Helicobacter pylori* in surface water in the United States," *Journal of Applied Microbiology*, vol. 87, no. 5, pp. 697–701, 1999.

[14] L. J. V. Piddock, "A review techniques used for the determination of antimicrobial resistance and sensitivity in bacteria," *Journal of Applied Bacteriology*, vol. 68, no. 4, pp. 307–318, 1990.

[15] T. Falsafi, R. Favaedi, F. Mahjoub, and M. Najafi, "Application of stool-PCR test for diagnosis of *Helicobacter pylori* infection in children," *World Journal of Gastroenterology*, vol. 15, no. 4, pp. 484–488, 2009.

[16] R. M. Peek Jr., G. G. Miller, K. T. Tham et al., "Detection of *Helicobacter pylori* gene expression in human gastric mucosa," *Journal of Clinical Microbiology*, vol. 33, no. 1, pp. 28–32, 1995.

[17] M. Notarnicola, F. Russo, A. Cavallini et al., "PCR identification of *Helicobacter pylori* DNA in faeces from patients with gastroduodenal pathology," *Medical Science Research*, vol. 24, no. 11, pp. 785–787, 1996.

[18] N. F. Azevedo, C. Almeida, I. Fernandes et al., "Survival of gastric and enterohepatic *Helicobacter* spp. in water: implications for transmission," *Applied and Environmental Microbiology*, vol. 74, no. 6, pp. 1805–1811, 2008.

[19] M. S. Giao, N. F. Azevedo, S. A. Wilks, M. J. Vieira, and C. W. Keevil, "Persistence of *Helicobacter pylori* in heterotrophic drinking-water biofilms," *Applied and Environmental Microbiology*, vol. 74, no. 19, pp. 5898–5904, 2008.

[20] A. Tiveljung, K. Borch, J. Jonasson, S. Mårdh, F. Petersson, and H. J. Monstein, "Identification of Helicobacter in gastric biopsies by PCR based on 16S rDNA sequences: a matter of little significance for the prediction of *H. pylori*-associated gastritis?" *Journal of Medical Microbiology*, vol. 47, no. 8, pp. 695–704, 1998.

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Review Article

Helicobacter pylori Eradication Therapies in the Era of Increasing Antibiotic Resistance: A Paradigm Shift to Improved Efficacy

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With the rising prevalence of antimicrobial resistance, the eradication rates of *Helicobacter pylori (H. pylori)* with standard treatments are decreasing to unacceptable levels (i.e., ≤80%) in most countries. After these disappointing results, several authorities have proposed that infection with *H. pylori* should be approached and treated as any other bacterial infectious disease. This implicates that clinicians should prescribe empirical treatments yielding a per protocol eradication of at least 90%. In recent years several treatments producing ≥90% cure rates have been proposed including sequential therapy, concomitant quadruple therapy, hybrid (dual-concomitant) therapy, and bismuth-containing quadruple therapy. These treatments are likely to represent the recommended first-line treatments in the near future. In the present paper, we are considering a series of critical issues regarding currently available means and approaches for the management of *H. pylori* infection. Clinical needs and realistic endpoints are taken into account. Furthermore, emerging strategies for the eradication of *H. pylori* and the existing evidence of their clinical validation and widespread applicability are discussed.

1. Introduction

Infection with *Helicobacter pylori (H. pylori)* is a global health problem affecting 20–50% of the western world's population and up to 80% of the population in developing countries [1, 2]. Presence of *H. pylori* is known to be associated with a wide range of gastrointestinal disorders including peptic ulcer, gastric carcinoma, and mucosa-associated tissue lymphoma, and, thus, ability to reliably eradicate the pathogen is important for managing these diseases [3–6]. Several factors are making infection with *H. pylori* so challenging to treat. These factors include (a) the development of *H. pylori* resistance to antibiotics, (b) the large number of bacteria in the stomach, producing an "inoculum" effect, (c) the protection of the thick gastric mucus gel layer, and (d) the intracellular (and thus inaccessible to antibiotics) location of many bacteria [7–9]. Other factors including presence of multiple strain infection and individual factors such as patient's compliance to treatment, age less than 60, the type of gastritis, and presence of nonulcer dyspepsia, where the eradication rates are lower in comparison with peptic ulcer disease, have been also linked to therapy efficacy [10, 11]. Educating the patient on the importance to take the medication as prescribed, warning in advance on the possibility of adverse events, and therefore obtain the maximum in terms of compliance to treatment poses a major clinical challenge to practicing physicians.

Historically, a wide spectrum of antimicrobial agents have been shown to be effective against *H. pylori* and successfully used in clinical practice. Most commonly are clarithromycin, amoxicillin, metronidazole, tinidazole, tetracycline, and the fluoroquinolones. As experience in treating the infection was gained, these drugs (and with the addition of an antisecretory agent or bismuth) have been used in different combinations, and developed regimens have been tailored in various parameters (dosage, dosing intervals,

duration of treatment) in order to provide the best outcome in terms of efficacy and tolerability. However, despite the continuous efforts made by the digestive disease community (and not by experts in infectious diseases), the optimal empirical treatment remains to be discovered.

In the present paper we are considering a series of critical issues regarding currently available means and approaches for the management of *H. pylori* infection. Realistic needs are taken into account with particular attention to crucial aspects for clinical practice and the importance for posttreatment testing for cure. Furthermore, emerging strategies for the eradication of *H. pylori* and the existing evidence of their clinical validation and applicability are discussed.

2. Empirical Triple Therapies: A Declining Clinical Standard

More than a decade ago, recommended therapies comprising of a proton pump inhibitor (PPI), amoxicillin, and clarithromycin (standard triple therapies) yielded high efficacy, providing eradication rates comparable to those expected for other prevalent bacterial infections such as respiratory and urinary tract infections, gonorrhea, and tuberculosis [12, 13]. Unfortunately, in successive years the eradication rates have fallen considerably with these regimens, in some countries to unacceptably low levels (<80% or even <70%), mainly because of the increasing prevalence of resistance to clarithromycin [8, 14, 15]. The widespread use of clarithromycin for infectious diseases other than *H. pylori* infection represents the main reason for the increasing development of resistance to this antibiotic; this explains the lower prevalence of clarithromycin resistance in Northern (versus Southern) European countries where policy for antibiotic use is more stringent [16]. The progressive decline in the efficacy of first-line treatments was already evident in the first meta-analyses published by the early 2000s and indirectly outlined by the European consensus recommendations (Maastricht 2000 and 2005), initially with the adoption of a "cumulative" approach to treat *H. pylori*, which introduced first- and second-line therapies, and later by the definition of a local/regional threshold of resistance to clarithromycin (15–20%), at which the antibiotic should not be used if culture was not previously performed to assess susceptibility [17–20]. In such cases, a bismuth-containing quadruple therapy (comprising of PPI, bismuth, metronidazole, and tetracycline) is recommended as an alternative empirical treatment, although its efficacy does not seem to exceed that of standard regimen according to some studies and a recent meta-analysis [21–23]. Furthermore, the threshold of resistance to clarithromycin at which triple regimens lose their efficacy seems to be substantially lower than 15–20% and may be 10% or even less [24].

Currently, standard triple therapy still remains the most widely recommended first-line treatment option worldwide and even in countries where improved alternative therapeutic options have been developed and sufficiently validated in a clinical setting [25–27]. The situation is similar in Greece: triple therapies represent the backbone of routine clinical practice but their performance is steadily declining during the past 10 years [28–31], in parallel with an increase in the incidence of clarithromycin resistance, reportedly from 6% to 26% [32–34].

To be fair, the cumulative efficacy of first and second-line treatments proposed by Maastricht 3, together with sensitivity-directed (re)treatment or administration of 3rd and 4th line rescue therapies (based on levofloxacin and rifampicin, resp.), is nearly approaching 100% [31, 35, 36]. However, for this goal to be achievable, patients must be highly compliant with repeated treatment courses. Necessity to use second-line therapies accounts for 20–30% of patients infected with *H. pylori* (intention to treat (ITT) analysis); even second-line therapy is not enough to eradicate the pathogen in 5–10% of cases [31, 37]. These rates are likely to increase further, as antimicrobial resistance becomes more prevalent worldwide. In that setting, patients may be required to complete more than one (and sometimes 3 or 4) complex treatment courses and therefore be exposed to a significant range of potential side effects which can virtually affect adherence and compromise their quality of life.

Use of an effective first-line treatment is known to provide a key advantage in the eradication of *H. pylori*, namely, prevention of secondary antibiotic resistance [38]. Much effort has been spent on improving currently recommended treatments. However, attempts to increase the duration of triple therapy, thus prolonging the exposure to antibiotics, have not resulted in a substantial benefit. There is therefore a clear need for novel therapeutic strategies.

3. A Paradigm Shift to Improved Efficacy

H. pylori is a major human pathogen which causes a serious, transmissible, infectious disease leading to significant morbidity. However, in contrast to what is common practice in other bacterial infections (where selection of the optimal therapy is usually based on susceptibility testing), first-line therapies against *H. pylori* are only prescribed empirically. This implicates that new regimens should be properly optimized (in terms of dosage, duration of treatment, dosing intervals, and local antimicrobial resistance pattern) before their introduction in clinical practice. Moreover, resolution of the infection should be always confirmed, preferably by using a noninvasive test, providing clinicians with a reliable measure of the local drug resistance. More intuitively, optimal eradication of *H. pylori* has to follow two golden rules: (1) always choose the best available first-line treatment (i.e., the one that works best locally) and (2) always confirm the success of therapy by posttreatment testing and retreat patients who fail to eradicate *H. pylori*. Adoption of these two rules will guarantee for patients the best chance to be treated, with the minimum cost in terms of treatment-related adverse events and will create a useful feedback for practicing clinicians, which will prevent them from prescribing locally unacceptable regimens.

Current approach to treatment of *H. pylori* infection is challenged by the declining efficiency of standard first-line therapies, leading to increasing need for second-line

(or more) treatment courses. Paradoxically, since the initial developments in the field, infection from *H. pylori* has been approached by the digestive disease community (and not by experts in infectious diseases), as any other gastrointestinal disease (e.g., inflammatory bowel disease or irritable bowel syndrome): in the absence of an optimal treatment, the best available therapies are offered in sequence. On the contrary, for most common infections, treatment success is expected to be near 100% (i.e., ≥95%). It becomes clear that a "paradigm shift" (i.e., a change from one way of thinking to another) is necessary in order for the field to move forward [39]. Indeed, several authorities have proposed that infection with *H. pylori* should be approached and treated as any other bacterial infectious disease [40–42]. This implicates, as a general rule, that clinicians should prescribe therapeutic regimens that have a per protocol (PP) eradication rate of at least 90% (grade B level) and probably at least 95% (grade A level), in keeping with the existing practice in the field of other common bacterial infectious diseases [40, 41].

Development of secondary resistance (i.e., as the result of failed therapy) is largely responsible for the decline in eradication rates. Owing to this conception treatment of *H. pylori* infection is becoming a hit or miss process aiming to decrease the number of eradication failures as much as possible. As stated in the present paper, infection with *H. pylori* should be treated as any other infectious disease, and, thus, ideally, a regimen should be based on pretreatment drug susceptibility testing. In spite of this, routine use of endoscopy is not feasible and not well tolerated by all patients. Moreover, the high economic burden related to this procedure together with the disappointing results often observed in vivo by following in vitro susceptibility is largely limiting cost efficacy of culture-guided therapy. On the contrary, enhancement of the eradication rate to values approaching 90% by adopting novel and possibly less expensive eradication strategies seems to represent a fascinating alternative.

In recent years, promising new treatment strategies have been proposed and largely validated in some countries and are likely to represent the recommended first-line therapies in the near future [42]. Emerging first-line treatments achieving high eradication rates of 90% or more (PP analysis) are discussed below. However, it should be noted that eradication rates reported further in this paper may be prone to wide geographic variability secondary to critically important differences in the local background rates of antibiotic resistance. As empiric treatments are given without antimicrobial susceptibility testing, the choice of an empiric therapy should rely on knowledge that the combination is successful in the local population.

4. Emerging First-Line Treatments with a Per-Protocol Eradication Rate Exceeding 90%

4.1. Sequential Therapy. One recent innovation postulated as an alternative to standard triple therapy is sequential treatment, which involves a simple dual regimen including a PPI plus amoxicillin for the first 5 days followed by a triple regimen including a PPI, clarithromycin and tinidazole for the following 5 days [43]. It represents the most extensively evaluated novel therapeutic strategy including 5 comparative meta-analyses and one pooled data analysis reporting on its efficacy and safety profile [43–48].

In the most recent meta-analysis of 15 randomized studies (published until May 2009, including 3346 patients), sequential therapy has been demonstrated to be superior to legacy triple therapy for the eradication of *H. pylori* (91.7%, 95% Confidence Interval (CI): 90–93% versus 76, 7%, 95% CI: 75–79%, ITT analysis) [43]. Interestingly, this regimen demonstrated ITT cure rates higher than 90% (grade B), even in countries with a high prevalence of resistance to clarithromycin, demonstrating higher performance (versus standard triple regimen) to eradicate clarithromycin-resistant strains [42]. In the meta-analysis by Gisbert et al., 41 out of 55 (75%) clarithromycin-resistant strains (4 studies) were eradicated after exposure to sequential therapy [43], although the total number with clarithromycin resistance in the included studies is still low for definite conclusions to be drawn. Similarly, the sequential regimen has been suggested as superior to legacy triple therapy in patients with metronidazole resistance [43, 48]. On the other hand, and despite this increased efficiency (in comparison with standard therapies) against sensitive and monoresistant strains, the performance of the sequential regimen seems to be dramatically compromised in the presence of dual antibiotic resistances (clarithromycin and imidazole) [49, 50]. Although the working mechanisms of the improved efficacy of the sequential regimen remain to be fully elucidated, some hypotheses may be put forward. It has been speculated that the disruption of the bacterial wall caused by amoxicillin could prevent the development of efflux channels for clarithromycin, which are known to rapidly transfer the drug out of the bacterial cell preventing the binding to the ribosome. However, according to another hypothesis, the improved effect with sequential therapy may be not attributed to the sequential administration itself; the bacteria may be simply "fulminated" by the larger number of antibiotics (3 together) to which the organism is exposed [51–53]. In accordance with this last scenario, concurrent administration of the same 3 antibiotics for a longer period of 7–10 days (i.e., the concomitant therapy, discussed further in this paper) has been shown to confer an acceptable eradication rate (89% by PP analysis and 87% by ITT analysis) when prescribed in a setting of high clarithromycin resistance (20%) where sequential regimen has been previously proved to be ineffective (cure rate 76%) [54, 55]. This data may represent preliminary, although indirect, evidence that sequential administration is probably more complicated than really necessary.

Indeed, a major shortcoming for the use of the sequential regimen is its complexity. Although adherence to treatment was excellent in the context of clinical trials, requiring the patient to switch from a dual to a triple therapy at midpoint could inherently interfere with compliance, if this regimen is prescribed in a real clinical practice setting [56–60]. Nonetheless, almost all studies proposing sequential therapy have been conducted in Italy. Importantly, in contrast to the initial studies showing a mean overall performance

approaching 90%, more recent studies conducted outside this country have shown a tendency towards lower eradication rates; in particular when dual antibiotic resistance is present [55, 61–68]. Further validation is therefore necessary before this regimen can be considered for widespread recommendation in clinical practice.

4.2. Nonbismuth Quadruple (Concomitant) Therapy. The concomitant regimen involves the concurrent administration of all three antibiotics used in first-line triple therapies (amoxicillin, clarithromycin, and metronidazole) given together with a PPI, all twice daily, for at least 10 days [50, 69]. This regimen is not completely novel; it has been previously evaluated with shorter durations of administration (3–7 days), in studies published between 1998 and 2002, allowing for high eradication rates (89–94% on ITT analysis) [70, 71]. It reappears nowadays as a 10-day regimen leading to eradication rates exceeding 90% on ITT analysis [50, 72]. In contrast to the sequential regimen, which has been developed and mostly evaluated in Italy, concomitant therapy has been tested in a wider range of geographical areas (including Japan, Germany, Colombia, Taiwan, and Greece) [42]. The ideal duration of administration remains an issue as direct comparisons between variable durations of treatment (e.g., 5 days versus 7 days versus 10 days) are lacking. However, one can speculate that, due to the increased antibiotic resistance rates, 3- and 5-day concomitant regimens may not be suitable today [67]. Interestingly, in a pilot study, the combination of sequential and concomitant therapies given for 14 days (hybrid therapy, PPI and amoxicillin for 7 days followed by PPI and all three antibiotics for another 7 days) achieved impressively high eradication rates (99% and 97% on PP and ITT analysis, resp.) (grade A level) [73].

In Greece, a country with high resistance rates to both clarithromycin and metronidazole (>20% for clarithromycin and >40% for metronidazole), concomitant therapy has been introduced since the beginning of 2009 achieving excellent therapeutic results with cure rates of 91.6% on ITT and 94.5% on per PP analysis (grade B) [74]. It seems that concomitant therapy eradicates more than 60% of double-resistant *H. pylori* strains and the vast majority of sensitive and monoresistant strains, thus preventing the emergence of secondary resistance [75]. At the same time, means of tolerability and safety profile are reported to be excellent and comparable to those obtained with standard triple therapy [74, 75].

A main advantage of the concomitant (versus sequential) therapy may be represented by its suitability for patients with dual resistance to antibiotics. Indeed, in a comparative study by Wu et al., patients with resistance to both clarithromycin and metronidazole had significantly lower eradication rates after sequential therapy (present versus absent: 33.3% versus 95.1%; P-value < 0.0001), but not after concomitant therapy (present versus absent: 75.0% versus 92.4%; P-value = 0.22) [50]. However, it should be noted that this study was conducted in Taiwan where the rate of antibiotic resistance is very low and even standard triple therapy is currently yielding excellent eradication rates [76]. A comparison study conducted across a broad range of patients and with a high prevalence of antibiotic-resistant *H. pylori* strains would be therefore much appreciated in order to definitely solve the issue of concurrent versus sequential administration; these two emerging treatment options seem to represent the main competitors likely to replace triple therapy in the foreseeable future.

4.3. Bismuth-Containing Quadruple Therapy. This regimen is mainly used as second-line treatment when legacy triple therapy fails, but also as an alternative first-line treatment option in regions with a high incidence of resistance to clarithromycin [77]. Other than working independently from resistance to clarithromycin, the main advantage of this regimen is represented by the limited clinical impact of metronidazole resistance which can be largely overcome by increasing the dose of metronidazole and duration of treatment. Considering that resistance to metronidazole in most countries is currently exceeding 10%, the daily dose of metronidazole prescribed should be approximately 1500 mg (3×500 or 4×400 mg in England) in order for maximal cure rates to be obtained.

Historically, in an early meta-analysis, first-line use of a bismuth-containing quadruple therapy (BQT) yielded high eradication rates (grade A or B level) [78]. These encouraging results have been mainly attributed to the efficacy against metronidazole-resistant strains, which overcome the eradication achieved with standard triple therapy over clarithromycin-resistant strains [23, 79]. However, according to a more recent meta-analysis, performance of both BQT and standard regimen was suboptimal (78.3% versus 77% on ITT analysis) [23]. In our country, BQT has been mainly used as a second-line therapy leading to rather contradictory results [28, 80]. In the only study where BQT has been used as first-line treatment and compared to standard triple therapy, both given for 10 days, results were disappointing (eradication rates 65% versus 78% on ITT analysis), whereas a higher incidence of adverse events was observed among patients receiving BQT [29].

A practical issue limiting the use of BQT is the absence of HCL tetracycline in some countries and the unavailability of bismuth salts in some other. Substitution of tetracycline with doxycycline or amoxicillin, in order to overcome this problem, was associated with rather disappointing results [81, 82]. On the contrary, high success rates were reported when BQT was used in the form of one capsule containing bismuth with both the antibiotics (metronidazole plus tetracycline). Three of these monocapsules are given four times daily in combination with a PPI twice daily for 10 days; this bismuth-based triple therapy monocapsule represents a patient-friendly formulation which is aimed to increase compliance to treatment [83, 84]. Currently, two of these monocapsules are available in the market, Helidac (USA) containing a lower dose of metronidazole (1 gr instead of 1.5 gr) and Pylera (USA and Europe) containing a lower dose of Tetracycline (1.5 gr instead of 2 gr), as compared to the classic BQT. These therapies seem to overcome *H. pylori* resistance to metronidazole since they achieve high eradication rates, reportedly exceeding 90% [85–88].

Table 1: Recommended regimens for *Helicobacter pylori* therapy.

Treatment	Regimen
First-line treatments	
Sequential therapy	A 5 d dual therapy with a PPI (standard dose, b.i.d.) and amoxicillin (1 g, b.i.d.) followed by a 5 d triple therapy with a PPI (standard dose, b.i.d.), clarithromycin (500 mg, b.i.d.), and metronidazole (500 mg, b.i.d.)
Concomitant therapy	A PPI (standard dose, b.i.d.), clarithromycin (500 mg, b.i.d.), amoxicillin (1 g, b.i.d.), and metronidazole (500 mg, b.i.d.) for 7–10 d
Hybrid therapy	A 7 d dual therapy with a PPI (standard dose, b.i.d.) and amoxicillin (1 g, b.i.d.) followed by a 7 d quadruple therapy with a PPI (standard dose, b.i.d.), amoxicillin (1 g, b.i.d.), clarithromycin (500 mg, b.i.d.), and metronidazole (500 mg, b.i.d.)
Bismuth-containing quadruple therapy	A PPI (standard dose, b.i.d.), bismuth (standard dose, q.i.d.), tetracycline (500 mg, q.i.d.), and metronidazole (500 mg, t.i.d.) for 10–14 d
Second-line/Salvage treatments	
Levofloxacin-based triple therapy	A PPI (standard dose, b.i.d.), levofloxacin (500 mg, b.i.d.), and amoxicillin (1 g, b.i.d.) for 10 d
Bismuth-containing quadruple therapy	A PPI (standard dose, b.i.d.), bismuth (standard dose, q.i.d.), tetracycline (500 mg, q.i.d.), and metronidazole (500 mg, t.i.d.) for 14 d
Standard triple therapy*	A PPI (standard dose, b.i.d.), amoxicillin (1 g, b.i.d.), and clarithromycin (500 mg, b.i.d.) for 14 days
Levofloxacin-based sequential therapy**	A 5 d dual therapy with a PPI (standard dose, b.i.d.) and amoxicillin (1 g, b.i.d.) followed by a 5 d triple therapy with a PPI (standard dose, b.i.d.), levofloxacin (250 mg, b.i.d.), and amoxicillin (1 g, b.i.d.)
Amoxicillin-based dual therapy (high dose)^	A PPI (high dose, t.i.d) and Amoxicillin (1 g, t.i.d.) for 14 days
Rifabutin-based triple therapy^	A PPI (standard dose, b.i.d.), rifabutin (150 mg b.i.d.), and amoxicillin (1 g b.i.d.) for 14 d
Furazolidone-based quadruple therapy^	A PPI (standard dose, b.i.d.), tripotassium dicitratobismuthate (240 mg, b.i.d.), furazolidone (200 mg, b.i.d.), and tetracycline (1 g, b.i.d.)

* Employed after antibiotic susceptibility testing; ** regimen under evaluation; ^ regimen usually employed as third-line therapy; PPI: proton pump inhibitor.

4.4. Alternative First-Line Therapies. In recent years, some authorities have proposed the use of levofloxacin, instead of clarithromycin, as the main compound of first-line treatments, achieving contradictory results [55, 76, 89]. Indeed, eradication rates with the use of levofloxacin-based triple therapy have been varying from 72% to 90% (ITT analysis), and this regimen has been suggested as an efficient alternative in settings of clarithromycin resistance exceeding 15%–20% and quinolone resistance less than 10% [90]. Interestingly, a novel levofloxacin-based sequential regimen was more effective than the standard clarithromycin-based sequential regimen in a setting with a high clarithromycin resistance rate (20%) where the latter has yielded suboptimal eradication rates (<80% in ITT) [89]. However, it should be noted that primary levofloxacin resistance in the study was very low (3.7%), and therefore these results may be difficult to reproduce in geographical areas with higher rates of quinolone resistance. Rapid development of resistance, as well as the high incidence of adverse events, represents further drawbacks concerning the use of levofloxacin in first-line treatment [91–96]. For these reasons, levofloxacin-based regimens are generally considered more suitable for use as second-line treatments or as salvage therapies [90, 97–101].

5. Therapeutic Algorithm of *H. pylori* Infection in Clinical Practice

The recommended regimens for *H. pylori* therapies are summarized in Table 1. Choice of the optimal, among these regimens, has to follow the rule of what works best locally; this should be based on the knowledge of the local *H. pylori* resistance pattern and the continuous evaluation of treatment outcomes (posttreatment testing) in clinical practice [42, 102]. For 5–10% of patients, even the emerging first-line therapies, described in this paper, are expected to be unsuccessful. In these cases, empiric use of a levofloxacin-based triple therapy seems to represent a reasonable option if local resistance to this antibiotic does not exceed 10% [102–104]. Alternatively, a bismuth-based quadruple therapy can be used for 14 days, since this regimen seems to overcome, at least partially, resistance to metronidazole [105–107]. The old dual regimen of a PPI plus amoxicillin given twice daily (and abandoned because of low eradication rates (<50%)), returns nowadays with the administration of higher doses of both drugs (PPI $\times$ 3 and amoxicillin 1000 mg $\times$ 3). With the new dosing scheme this dual regimen can be used as salvage therapy in areas with high resistance rates to levofloxacin

[108]. The small minority of patients (<1%) with refractory *H. pylori* infection to both first- and second-line treatments have to be referred for antibiotic susceptibility testing in order for third-line therapies to be instituted [104, 109]. Alternatively, rifabutin-based or furazolidone-based therapies can be employed for the treatment of refractory *H. pylori* infection [110, 111].

Importantly, most of the aforementioned emerging first-line therapies have not been incorporated into international guidelines so far [25, 77], although this does not seem to be too far away according to more recent recommendations [112]. However, there is still work to be done in order for these novel regimens to be sufficiently validated and therefore possibly recommended as first choice therapies ushering in a new era of anti-*H. pylori* treatment.

References

[1] P. Moayyedi and R. H. Hunt, "Helicobacter pylori public health implications," *Helicobacter*, vol. 9, supplement 1, pp. 67–72, 2004.

[2] S. Suerbaum and P. Michetti, "Helicobacter pylori infection," *The New England Journal of Medicine*, vol. 347, no. 15, pp. 1175–1186, 2002.

[3] L. Fuccio, R. M. Zagari, L. H. Eusebi et al., "Meta-analysis: can Helicobacter pylori eradication treatment reduce the risk for gastric cancer?" *Annals of Internal Medicine*, vol. 151, no. 2, pp. 121–128, 2009.

[4] K. Fukase, M. Kato, S. Kikuchi et al., "Effect of eradication of Helicobacter pylori on incidence of metachronous gastric carcinoma after endoscopic resection of early gastric cancer: an open-label, randomised controlled trial," *The Lancet*, vol. 372, no. 9636, pp. 392–397, 2008.

[5] P. Malfertheiner, F. K. Chan, and K. E. McColl, "Peptic ulcer disease," *The Lancet*, vol. 374, no. 9699, pp. 1449–1461, 2009.

[6] A. Stathis, F. Bertoni, and E. Zucca, "Treatment of gastric marginal zone lymphoma of MALT type," *Expert Opinion on Pharmacotherapy*, vol. 11, no. 13, pp. 2141–2152, 2010.

[7] D. Y. Graham, "Antibiotic resistance in Helicobacter pylori: implications for therapy," *Gastroenterology*, vol. 115, no. 5, pp. 1272–1277, 1998.

[8] F. Mégraud, "*H pylori* antibiotic resistance: prevalence, importance, and advances in testing," *Gut*, vol. 53, no. 9, pp. 1374–1384, 2004.

[9] F. Megraud, "*Helicobacter pylori* and antibiotic resistance," *Gut*, vol. 56, no. 11, p. 1502, 2007.

[10] N. Broutet, S. Tchamgoué, E. Pereira, H. Lamouliatte, R. Salamon, and F. Mégraud, "Risk factors for failure of Helicobacter pylori therapy—results of an individual data analysis of 2751 patients," *Alimentary Pharmacology & Therapeutics*, vol. 17, no. 1, pp. 99–109, 2003.

[11] S. D. Georgopoulos, S. D. Ladas, S. Karatapanis et al., "Factors that may affect treatment outcome of triple Helicobacter pylori eradication therapy with omeprazole, amoxicillin, and clarithromycin," *Digestive Diseases and Sciences*, vol. 45, no. 1, pp. 63–67, 2000.

[12] "Current European concepts in the management of Helicobacter pylori infection. The Maastricht Consensus Report. European Helicobacter Pylori Study Group," *Gut*, vol. 41, no. 1, pp. 8–13, 1997.

[13] T. Lind, S. V. van Zanten, P. Unge et al., "Eradication of Helicobacter pylori using one-week triple therapies combining omeprazole with two antimicrobials: the MACH I study," *Helicobacter*, vol. 1, no. 3, pp. 138–144, 1996.

[14] V. De Francesco, M. Margiotta, A. Zullo et al., "Prevalence of primary clarithromycin resistance in Helicobacter pylori strains over a 15 year period in Italy," *Journal of Antimicrobial Chemotherapy*, vol. 59, no. 4, pp. 783–785, 2007.

[15] J. P. Gisbert, R. Pajares, and J. M. Pajares, "Evolution of Helicobacter pylori therapy from a meta-analytical perspective," *Helicobacter*, vol. 12, supplement 2, pp. 50–58, 2007.

[16] V. De Francesco, F. Giorgio, C. Hassan et al., "Worldwide H. pylori antibiotic resistance: a systematic review," *Journal of Gastrointestinal and Liver Diseases*, vol. 19, no. 4, pp. 409–414, 2010.

[17] L. A. Fischbach, K. J. Goodman, M. Feldman, and C. Aragaki, "Sources of variation of Helicobacter pylori treatment success in adults worldwide: a meta-analysis," *International Journal of Epidemiology*, vol. 31, no. 1, pp. 128–139, 2002.

[18] M. J. Janssen, A. H. Van Oijen, A. L. Verbeek, J. B. Jansen, and W. A. De Boer, "A systematic comparison of triple therapies for treatment of Helicobacter pylori infection with proton pump inhibitor/ranitidine bismuth citrate plus clarithromycin and either amoxicillin or a nitroimidazole," *Alimentary Pharmacology & Therapeutics*, vol. 15, no. 5, pp. 613–624, 2001.

[19] R. J. Laheij, L. G. Rossum, J. B. Jansen, H. Straatman, and A. L. Verbeek, "Evaluation of treatment regimens to cure Helicobacter pylori infection—a meta-analysis," *Alimentary Pharmacology & Therapeutics*, vol. 13, no. 7, pp. 857–864, 1999.

[20] P. Malfertheiner, F. Mégraud, C. O'Morain et al., "Current concepts in the management of Helicobacter pylori infection—the Maastricht 2-2000 Consensus Report," *Alimentary Pharmacology & Therapeutics*, vol. 16, no. 2, pp. 167–180, 2002.

[21] P. H. Katelaris, G. M. Forbes, N. J. Talley, and B. Crotty, "A randomized comparison of quadruple and triple therapies for Helicobacter pylori eradication: the QUADRATE study," *Gastroenterology*, vol. 123, no. 6, pp. 1763–1769, 2002.

[22] X. Calvet, J. Ducons, J. Guardiola et al., "One-week triple vs. quadruple therapy for Helicobacter pylori infection—a randomized trial," *Alimentary Pharmacology & Therapeutics*, vol. 16, no. 7, pp. 1261–1267, 2002.

[23] J. Luther, P. D. Higgins, P. S. Schoenfeld, P. Moayyedi, N. Vakil, and W. D. Chey, "Empiric quadruple vs. triple therapy for primary treatment of Helicobacter pylori infection: systematic review and meta-analysis of efficacy and tolerability," *American Journal of Gastroenterology*, vol. 105, no. 1, pp. 65–73, 2010.

[24] D. Y. Graham and M. P. Dore, "Helicobacter pylori therapy demystified," *Helicobacter*, vol. 16, no. 5, pp. 343–345, 2011.

[25] W. D. Chey and B. C. Wong, "American College of Gastroenterology guideline on the management of Helicobacter pylori infection," *American Journal of Gastroenterology*, vol. 102, no. 8, pp. 1808–1825, 2007.

[26] M. Caselli, A. Zullo, G. Maconi et al., "'Cervia II Working Group Report 2006': guidelines on diagnosis and treatment of Helicobacter pylori infection in Italy," *Digestive and Liver Disease*, vol. 39, no. 8, pp. 782–789, 2007.

[27] G. Maconi, C. Tosetti, G. Miroglio et al., "Management of Helicobacter pylori-related gastrointestinal diseases by general practitioners in Italy," *Alimentary Pharmacology & Therapeutics*, vol. 13, no. 11, pp. 1499–1504, 1999.

[28] S. D. Georgopoulos, S. D. Ladas, S. Karatapanis et al., "Effectiveness of two quadruple, tetracycline- or clarithromycin-containing, second-line, Helicobacter pylori eradication therapies," *Alimentary Pharmacology & Therapeutics*, vol. 16, no. 3, pp. 569–575, 2002.

[29] G. J. Mantzaris, K. Petraki, E. Archavlis et al., "Omeprazole triple therapy versus omeprazole quadruple therapy for healing duodenal ulcer and eradication of Helicobacter pylori infection: 24-month follow-up study," *European Journal of Gastroenterology and Hepatology*, vol. 14, no. 11, pp. 1237–1243, 2002.

[30] S. Karatapanis, S. D. Georgopoulos, V. Papastergiou et al., "7, 10 and 14-days rabeprazole-based standard triple therapies for H. pylori eradication: are they still effective? A randomized trial," *Acta Gastroenterol Belg*, vol. 74, no. 3, pp. 407–412, 2011.

[31] T. Rokkas, P. Sechopoulos, I. Robotis, G. Margantinis, and D. Pistiolas, "Cumulative H. pylori eradication rates in clinical practice by adopting first and second-line regimens proposed by the Maastricht III consensus and a third-line empirical regimen," *American Journal of Gastroenterology*, vol. 104, no. 1, pp. 21–25, 2009.

[32] L. Boyanova, A. Mentis, M. Gubina et al., "The status of antimicrobial resistance of Helicobacter pylori in Eastern Europe," *Clinical Microbiology and Infection*, vol. 8, no. 7, pp. 388–396, 2002.

[33] Y. Glupczynski, F. Mégraud, M. Lopez-Brea, and L. P. Andersen, "European multicentre survey of in vitro antimicrobial resistance in Helicobacter pylori," *European Journal of Clinical Microbiology and Infectious Diseases*, vol. 20, no. 11, pp. 820–823, 2001.

[34] A. F. Mentis, E. Roma, A. Pangalis, and E. Katsiyiannakis, "Susceptibilities of Helicobacter pylori strains isolated from children with gastritis to selected antibiotics," *Journal of Antimicrobial Chemotherapy*, vol. 44, no. 5, pp. 720–722, 1999.

[35] J. P. Gisbert, J. L. Gisbert, S. Marcos, I. Jimenez-Alonso, R. Moreno-Otero, and J. M. Pajares, "Empirical rescue therapy after Helicobacter pylori treatment failure: a 10-year single-centre study of 500 patients," *Alimentary Pharmacology & Therapeutics*, vol. 27, no. 4, pp. 346–354, 2008.

[36] K. Seppälä, T. U. Kosunen, L. Veijola et al., "Cure of Helicobacter pylori infection in all compliant patients: report on 644 subjects," *Scandinavian Journal of Gastroenterology*, vol. 43, no. 9, pp. 1149–1150, 2008.

[37] L. Fuccio, R. M. Zagari, and F. Bazzoli, "What is the best salvage therapy for patients with Helicobacter pylori infection?" *Nature Clinical Practice Gastroenterology and Hepatology*, vol. 5, no. 11, pp. 608–609, 2008.

[38] J. Q. Huang and R. H. Hunt, "Treatment after failure: the problem of 'non-responders'," *Gut*, vol. 45, supplement 1, pp. I40–I44, 1999.

[39] S. K. Chuah, F. W. Tsay, P. I. Hsu, and D. C. Wu, "A new look at anti-Helicobacter pylori therapy," *World Journal of Gastroenterology*, vol. 17, no. 35, pp. 3971–3975, 2011.

[40] D. Y. Graham, H. Lu, and Y. Yamaoka, "A report card to grade Helicobacter pylori therapy," *Helicobacter*, vol. 12, no. 4, pp. 275–278, 2007.

[41] D. Y. Graham, "Efficient identification and evaluation of effective Helicobacter pylori therapies," *Clinical Gastroenterology and Hepatology*, vol. 7, no. 2, pp. 145–148, 2009.

[42] D. Y. Graham and L. Fischbach, "Helicobacter pylori treatment in the era of increasing antibiotic resistance," *Gut*, vol. 59, no. 8, pp. 1143–1153, 2010.

[43] J. P. Gisbert, X. Calvet, A. O'Connor, F. Mégraud, and C. A. O'Morain, "Sequential therapy for Helicobacter pylori eradication: a critical review," *Journal of Clinical Gastroenterology*, vol. 44, no. 5, pp. 313–325, 2010.

[44] P. Moayyedi, "Sequential regimens for Helicobacter pylori eradication," *The Lancet*, vol. 370, no. 9592, pp. 1010–1012, 2007.

[45] N. S. Jafri, C. A. Hornung, and C. W. Howden, "Meta-analysis: sequential therapy appears superior to standard therapy for Helicobacter pylori infection in patients naive to treatment," *Annals of Internal Medicine*, vol. 148, no. 12, pp. 923–931, 2008.

[46] J. L. Tong, Z. H. Ran, J. Shen, and S. D. Xiao, "Sequential therapy vs. standard triple therapies for Helicobacter pylori infection: a meta-analysis," *Journal of Clinical Pharmacy and Therapeutics*, vol. 34, no. 1, pp. 41–53, 2009.

[47] L. Gatta, N. Vakil, G. Leandro, F. Di Mario, and D. Vaira, "Sequential therapy or triple therapy for Helicobacter pylori infection: systematic review and meta-analysis of randomized controlled trials in adults and children," *American Journal of Gastroenterology*, vol. 104, no. 12, pp. 3069–3079, 2009.

[48] A. Zullo, V. De Francesco, C. Hassan, S. Morini, and D. Vaira, "The sequential therapy regimen for Helicobacter pylori eradication: a pooled-data analysis," *Gut*, vol. 56, no. 10, pp. 1353–1357, 2007.

[49] D. Vaira, A. Zullo, N. Vakil et al., "Sequential therapy versus standard triple-drug therapy for Helicobacter pylori eradication: a randomized trial," *Annals of Internal Medicine*, vol. 146, no. 8, pp. 556–563, 2007.

[50] D. C. Wu, P. I. Hsu, J. Y. Wu et al., "Sequential and concomitant therapy with four drugs is equally effective for eradication of H pylori infection," *Clinical Gastroenterology and Hepatology*, vol. 8, no. 1, pp. 36.e1–41.e1, 2010.

[51] I. T. Paulsen, M. H. Brown, and R. A. Skurray, "Proton-dependent multidrug efflux systems," *Microbiological Reviews*, vol. 60, no. 4, pp. 575–608, 1996.

[52] M. A. Webber and L. J. Piddock, "The importance of efflux pumps in bacterial antibiotic resistance," *Journal of Antimicrobial Chemotherapy*, vol. 51, no. 1, pp. 9–11, 2003.

[53] M. C. Roberts, "Resistance to macrolide, lincosamide, streptogramin, ketolide, and oxazolidinone antibiotics," *Molecular Biotechnology*, vol. 28, no. 1, pp. 47–62, 2004.

[54] J. Molina-Infante, C. Pazos-Pacheco, and B. Perez-Gallardo, "Efficacy of nonbismuth quadruple "concomitant" therapy for H. pylori infection in a setting with high clarithromycin resistance," *Helicobacter*, vol. 138, article 880, supplement 1, 2011.

[55] J. Molina-Infante, B. Perez-Gallardo, M. Fernandez-Bermejo et al., "Clinical trial: clarithromycin vs. levofloxacin in first-line triple and sequential regimens for Helicobacter pylori eradication," *Alimentary Pharmacology & Therapeutics*, vol. 31, no. 10, pp. 1077–1084, 2010.

[56] W. A. de Boer, E. J. Kuipers, and J. G. Kusters, "Sequential therapy; a new treatment for Helicobacter pylori infection: but is it ready for general use?" *Digestive and Liver Disease*, vol. 36, no. 5, pp. 311–314, 2004.

[57] J. Sánchez-Delgado, X. Calvet, L. Bujanda, J. P. Gisbert, L. Titó, and M. Castro, "Ten-day sequential treatment for Helicobacter pylori eradication in clinical practice," *American Journal of Gastroenterology*, vol. 103, no. 9, pp. 2220–2223, 2008.

[58] C. A. O'Morain and J. P. O'Connor, "Is sequential therapy superior to standard triple therapy for the treatment

of Helicobacter pylori infection?" *Nature Clinical Practice Gastroenterology and Hepatology*, vol. 6, no. 1, pp. 8–9, 2009.

[59] P. Moayyedi and P. Malfertheiner, "Editorial: sequential therapy for eradication of Helicobacter pylori: a new guiding light or a false dawn?" *American Journal of Gastroenterology*, vol. 104, no. 12, pp. 3081–3083, 2009.

[60] J. Luther and W. D. Chey, "Sequential therapy for Helicobacter pylori: a two for one deal that's worth the extra effort?" *Gastroenterology*, vol. 136, no. 2, pp. 720–722, 2009.

[61] W. H. Choi, D. I. Park, S. J. Oh et al., "Effectiveness of 10 day-sequential therapy for Helicobacter pylori eradication in Korea," *The Korean Journal of Gastroenterology*, vol. 51, no. 5, pp. 280–284, 2008.

[62] X. Z. Gao, X. L. Qiao, W. C. Song, X. F. Wang, and F. Liu, "Standard triple, bismuth pectin quadruple and sequential therapies for Helicobacter pylori eradication," *World Journal of Gastroenterology*, vol. 16, no. 34, pp. 4357–4362, 2010.

[63] E. R. Greenberg, G. L. Anderson, D. R. Morgan et al., "14-day triple, 5-day concomitant, and 10-day sequential therapies for Helicobacter pylori infection in seven Latin American sites: a randomised trial," *The Lancet*, vol. 378, no. 9790, pp. 507–514, 2011.

[64] D. Y. Graham and E. Rimbara, "Understanding and appreciating sequential therapy for Helicobacter pylori eradication," *Journal of Clinical Gastroenterology*, vol. 45, no. 4, pp. 309–313, 2011.

[65] H. G. Park, M. K. Jung, J. T. Jung et al., "Randomised clinical trial: a comparative study of 10-day sequential therapy with 7-day standard triple therapy for Helicobacter pylori infection in naive patients," *Alimentary Pharmacology & Therapeutics*, vol. 35, no. 1, pp. 56–65, 2012.

[66] A. Merali and G. I. Leontiadis, "Sequential therapy—more studies are still required," *Alimentary Pharmacology & Therapeutics*, vol. 35, no. 1, pp. 194–195, 2012.

[67] J. P. Gisbert and X. Calvet, "Review article: non-bismuth quadruple (concomitant) therapy for eradication of Helicobater pylori," *Alimentary Pharmacology & Therapeutics*, vol. 34, no. 6, pp. 604–617, 2011.

[68] H. Fakheri, T. Taghvaei, V. Hosseini, and Z. Bari, "A comparison between sequential therapy and a modified bismuth-based quadruple therapy for Helicobacter pylori eradication in Iran: a randomized clinical trial," *Helicobacter*, vol. 17, no. 1, pp. 43–48, 2012.

[69] D. Y. Graham and A. Shiotani, "New concepts of resistance in the treatment of Helicobacter pylori infections," *Nature Clinical Practice Gastroenterology and Hepatology*, vol. 5, no. 6, pp. 321–331, 2008.

[70] G. Treiber, J. Wittig, S. Ammon, S. Walker, L. J. van Doorn, and U. Klotz, "Clinical outcome and influencing factors of a new short-term quadruple therapy for Helicobacter pylori eradication: a randomized controlled trial (MACLOR study)," *Archives of Internal Medicine*, vol. 162, no. 2, pp. 153–160, 2002.

[71] A. S. Essa, J. R. Kramer, D. Y. Graham, and G. Treiber, "Meta-analysis: four-drug, three-antibiotic, non-bismuth-containing "concomitant therapy" versus triple therapy for Helicobacter pylori eradication," *Helicobacter*, vol. 14, no. 2, pp. 109–118, 2009.

[72] L. A. Fischbach, L. E. Bravo, G. R. Zarama et al., "A randomized clinical trial to determine the efficacy of regimens containing clarithromycin, metronidazole, and amoxicillin among histologic subgroups for Helicobacter pylori eradication in a developing country," *Helicobacter*, vol. 14, no. 2, pp. 100–108, 2009.

[73] P. I. Hsu, D. C. Wu, J. Y. Wu, and D. Y. Graham, "Modified sequential Helicobacter pylori therapy: proton pump inhibitor and amoxicilin for 14 days with clarithromycin and metronidazole added as a quadruple (hybrid) therapy for the final 7 days," *Helicobacter*, vol. 16, no. 2, pp. 146–152, 2011.

[74] S. Georgopoulos, V. Papastergiou, E. Xirouchakis et al., "Evaluation of a four-drug, three-antibiotic, nonbismuth-containing "concomitant" therapy as first-line Helicobacter pylori eradication regimen in Greece," *Helicobacter*, vol. 16, no. 2, pp. 139–145, 2011.

[75] S. D. Georgopoulos, E. Xirouchakis, and F. Laoudi, "Clinical evaluation of a 10-day regimenwith esomeprazole, metronidazole, amoxycilline and clarithromycin for the eradication of Helicobacter pylori (E-MACH study)," *Helicobacter*, vol. 16, article A136, supplement 1, 2011.

[76] J. M. Liou, J. T. Lin, C. Y. Chang et al., "Levofloxacin-based and clarithromycin-based triple therapies as first-line and second-line treatments for Helicobacter pylori infection: a randomised comparative trial with crossover design," *Gut*, vol. 59, no. 5, pp. 572–578, 2010.

[77] P. Malfertheiner, F. Megraud, C. O'Morain et al., "Current concepts in the management of Helicobacter pylori infection: the Maastricht III Consensus Report," *Gut*, vol. 56, no. 6, pp. 772–781, 2007.

[78] L. A. Fischbach, S. van Zanten, and J. Dickason, "Meta-analysis: the efficacy, adverse events, and adherence related to first-line anti-Helicobacter pylori quadruple therapies," *Alimentary Pharmacology & Therapeutics*, vol. 20, no. 10, pp. 1071–1082, 2004.

[79] L. Fischbach and E. L. Evans, "Meta-analysis: the effect of antibiotic resistance status on the efficacy of triple and quadruple first-line therapies for Helicobacter pylori," *Alimentary Pharmacology & Therapeutics*, vol. 26, no. 3, pp. 343–357, 2007.

[80] S. Michopoulos, P. Tsibouris, H. Bouzakis et al., "Randomized study comparing omeprazole with ranitidine as anti-secretory agents combined in quadruple second-Iine Helicobacter pylori eradication regimens," *Alimentary Pharmacology & Therapeutics*, vol. 14, no. 6, pp. 737–744, 2000.

[81] F. Perri, V. Festa, A. Merla, M. Quitadamo, R. Clemente, and A. Andriulli, "Amoxicillin/tetracycline combinations are inadequate as alternative therapies for Helicobacter pylori infection," *Helicobacter*, vol. 7, no. 2, pp. 99–104, 2002.

[82] N. Garcia, X. Calvet, E. Gene, R. Campo, and E. Brullet, "Limited usefulness of a seven-day twice-a-day quadruple therapy," *European Journal of Gastroenterology and Hepatology*, vol. 12, no. 12, pp. 1315–1318, 2000.

[83] W. A. de Boer, R. J. van Etten, P. M. Schneeberger, and G. N. Tytgat, "A single drug for Helicobacter pylori infection: first results with a new bismuth triple monocapsule," *American Journal of Gastroenterology*, vol. 95, no. 3, pp. 641–645, 2000.

[84] W. A. de Boer, "A novel therapeutic approach for Helicobacter pylori infection: the bismuth-based triple therapy monocapsule," *Expert Opinion on Investigational Drugs*, vol. 10, no. 8, pp. 1559–1566, 2001.

[85] L. Laine, R. Hunt, H. EI-Zimaity, B. Nguyen, M. Osato, and J. Spénard, "Bismuth-based quadruple therapy using a single capsule of bismuth biskalcitrate, metronidazole, and tetracycline given with omeprazole versus omeprazole, amoxicillin, and clarithromycin for eradication of Helicobacter pylori in duodenal ulcer patients: a prospective, randomized, multicenter, North American trial," *American Journal of Gastroenterology*, vol. 98, no. 3, pp. 562–567, 2003.

[86] C. O'Morain, T. Borody, A. Farley et al., "Efficacy and safety of single-triple capsules of bismuth biskalcitrate, metronidazole and tetracycline, given with omeprazole, for the eradication of Helicobacter pylori: an international multicentre study," *Alimentary Pharmacology & Therapeutics*, vol. 17, no. 3, pp. 415–420, 2003.

[87] A. Saleem, A. Qasim, H. J. O'Connor, and C. A. O'Morain, "Pylera for the eradication of Helicobacter pylori infection," *Expert Review of Anti-Infective Therapy*, vol. 7, no. 7, pp. 793–799, 2009.

[88] P. Malfertheiner, F. Bazzoli, J. C. Delchier et al., "Helicobacter pylori eradication with a capsule containing bismuth subcitrate potassium, metronidazole, and tetracycline given with omeprazole versus clarithromycin-based triple therapy: a randomised, open-label, non-inferiority, phase 3 trial," *The Lancet*, vol. 377, no. 9769, pp. 905–913, 2011.

[89] M. Romano, A. Cuomo, A. G. Gravina et al., "Empirical levofloxacin-containing versus clarithromycin-containing sequential therapy for Helicobacter pylori eradication: a randomised trial," *Gut*, vol. 59, no. 11, pp. 1465–1470, 2010.

[90] M. Berning, S. Krasz, and S. Miehlke, "Should quinolones come first in Helicobacter pylori therapy?" *Therapeutic Advances in Gastroenterology*, vol. 4, no. 2, pp. 103–114, 2011.

[91] F. Perna, A. Zullo, C. Ricci, C. Hassan, S. Morini, and D. Vaira, "Levofloxacin-based triple therapy for Helicobacter pylori re-treatment: role of bacterial resistance," *Digestive and Liver Disease*, vol. 39, no. 11, pp. 1001–1005, 2007.

[92] P. Bogaerts, C. Berhin, H. Nizet, and Y. Glupczynski, "Prevalence and mechanisms of resistance to fluoroquinolones in Helicobacter pylori strains from patients living in Belgium," *Helicobacter*, vol. 11, no. 5, pp. 441–445, 2006.

[93] J. J. Carothers, M. G. Bruce, T. W. Hennessy et al., "The relationship between previous fluoroquinolone use and levofloxacin resistance in Helicobacter pylori infection," *Clinical Infectious Diseases*, vol. 44, no. 2, pp. e5–e8, 2007.

[94] P. D. van der Linden, M. C. Sturkenboom, R. M. Herings, H. G. Leufkens, and B. H. Stricker, "Fluoroquinolones and risk of achilles tendon disorders: case-control study," *British Medical Journal*, vol. 324, no. 7349, pp. 1306–1307, 2002.

[95] K. F. Croom and K. L. Goa, "Levofloxacin: a review of its use in the treatment of bacterial infections in the United States," *Drugs*, vol. 63, no. 24, pp. 2769–2802, 2003.

[96] C. Bilardi, P. Dulbecco, P. Zentilin et al., "A 10-day levofloxacin-based therapy in patients with resistant Helicobacter pylori infection: a controlled trial," *Clinical Gastroenterology and Hepatology*, vol. 2, no. 11, pp. 997–1002, 2004.

[97] R. J. Saad, P. Schoenfeld, H. M. Kim, and W. D. Chey, "Levofloxacin-based triple therapy versus bismuth-based quadruple therapy for persistent Helicobacter pylori infection: a meta-analysis," *American Journal of Gastroenterology*, vol. 101, no. 3, pp. 488–496, 2006.

[98] J. P. Gisbert, F. Bermejo, M. Castro-Fernández et al., "Second-line rescue therapy with levofloxacin after H. pylori treatment failure: a Spanish multicenter study of 300 patients," *American Journal of Gastroenterology*, vol. 103, no. 1, pp. 71–76, 2008.

[99] J. P. Gisbert, "Second-line rescue therapy of Helicobacter pylori infection," *Therapeutic Advances in Gastroenterology*, vol. 2, no. 6, pp. 331–356, 2009.

[100] J. P. Gisbert, J. L. Gisbert, S. Marcos, R. Moreno-Otero, and J. M. Pajares, "Third-line rescue therapy with levofloxacin is more effective than rifabutin rescue regimen after two Helicobacter pylori treatment failures," *Alimentary Pharmacology & Therapeutics*, vol. 24, no. 10, pp. 1469–1474, 2006.

[101] T. Nishizawa, H. Suzuki, and T. Hibi, "Quinolone-based third-line therapy for Helicobacter pylori eradication," *Journal of Clinical Biochemistry and Nutrition*, vol. 44, no. 2, pp. 119–124, 2009.

[102] S. Karatapanis, L. Skorda, S. D. Georgopoulos et al., "Levofloxacin-based triple therapy versus bismuth-based quadruple therapy as a second line treatment for the eradication of H. pylori infection," *Annals of Gastroenterology*, vol. 22, no. 4, pp. 263–267, 2009.

[103] C. Krystallis, D. Kamberoglou, D. Pistiolas et al., "A new second-line sequential regimen for Helicobacter pylori eradication based on levofloxacin: a pilot study," *Digestive Diseases and Sciences*, vol. 55, no. 12, pp. 3630–3631, 2010.

[104] S. Pontone, M. Standoli, R. Angelini, and P. Pontone, "Efficacy of H. pylori eradication with a sequential regimen followed by rescue therapy in clinical practice," *Digestive and Liver Disease*, vol. 42, no. 8, pp. 541–543, 2010.

[105] D. Y. Graham, M. S. Osato, J. Hoffman et al., "Metronidazole containing quadruple therapy for infection with metronidazole resistant Helicobacter pylori: a prospective study," *Alimentary Pharmacology & Therapeutics*, vol. 14, no. 6, pp. 745–750, 2000.

[106] B. H. Lee, N. Kim, T. J. Hwang et al., "Bismuth-containing quadruple therapy as second-line treatment for Helicobacter pylori infection: effect of treatment duration and antibiotic resistance on the eradication rate in Korea," *Helicobacter*, vol. 15, no. 1, pp. 38–45, 2010.

[107] Q. Sun, X. Liang, Q. Zheng et al., "High efficacy of 14-day triple therapy-based, bismuth-containing quadruple therapy for initial Helicobacter pylori eradication," *Helicobacter*, vol. 15, no. 3, pp. 233–238, 2010.

[108] D. Y. Graham, S. U. Javed, S. Keihanian, S. Abudayyeh, and A. R. Opekun, "Dual proton pump inhibitor plus amoxicillin as an empiric anti-H. pylori therapy: studies from the United States," *Journal of Gastroenterology*, vol. 45, no. 8, pp. 816–820, 2010.

[109] E. Rimbara, L. A. Fischbach, and D. Y. Graham, "Optimal therapy for Helicobacter pylori infections," *Nature Reviews Gastroenterology and Hepatology*, vol. 8, no. 2, pp. 79–88, 2011.

[110] J. P. Gisbert and X. Calvet, "Review article: rifabutin in the treatment of refractory Helicobacter pylori infection," *Alimentary Pharmacology & Therapeutics*, vol. 35, no. 2, pp. 209–221, 2012.

[111] G. Treiber, S. Ammon, P. Malfertheiner, and U. Klotz, "Impact of furazolidone-based quadruple therapy for eradication of Helicobacter pylori after previous treatment failures," *Helicobacter*, vol. 7, no. 4, pp. 225–231, 2002.

[112] P. Malfertheiner, F. Megraud, C. A. O'Morain et al., "Management of Helicobacter pylori infection—the Maastricht IV/Florence Consensus Report," *Gut*, vol. 61, no. 5, pp. 646–664, 2012.

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Clinical Study

Impact of *Lactobacillus reuteri* Supplementation on Anti-*Helicobacter pylori* Levofloxacin-Based Second-Line Therapy

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Introduction. *Helicobacter pylori* eradication therapy has the potential burden of antibiotic-associated gastrointestinal (GI) side effects. The occurrence of side effects is among the major drawbacks of such regimens. GI manifestations may be related to alterations in the intestinal microflora. Probiotics can prevent or reduce antibiotic-associated side effects and have an inhibitory effect on *H. pylori*. *Methods*. To define the efficacy of *Lactobacillus reuteri* supplementation in *H. pylori* eradication and in preventing GI-associated side effects during a second-line levofloxacin triple therapy. 90 *H. pylori*-positive patients receive for 7 days a second-line triple therapy with esomeprazole, levofloxacin, and amoxicillin with *L. reuteri* for 14 days (group 1) and without probiotic supplementation (group 2). Each subject received a validated questionnaire to record symptoms everyday for 4 weeks from the start of therapy. *H. pylori* status and side effects were assessed 6 weeks after treatment. *Results*. The eradication rate was significantly influenced by probiotic supplementation with *L. reuteri* (group 1: 36/45, 80%; group 2: 28/45 62%; $P < 0.05$). The incidence of nausea and diarrhoea in group 1 was significantly lower than that in group 2. *Conclusion*. In *H. pylori*-positive subjects *L. reuteri* supplementation increases the eradication rate while reducing the incidence of the most common side effects associated with antibiotic therapy in second-line treatment.

1. Introduction

Helicobacter pylori (*H. pylori*), a microaerophilic, gram negative bacterium that colonises the mucous layer of the gastric epithelium, is the causative agent of type B gastritis, peptic ulcer, gastric cancer [1–3], and extradigestive diseases [4]. At least one-third of the world's population is infected with *H. pylori*. The standard treatment recommended for *H. pylori* eradication is a combination of proton-pump inhibitor (PPI) or ranitidine bismuth citrate, clarithromycin, and either amoxicillin or nitroimidazole. These regimens have been able to achieve eradication rates ranging from 65% to 90%; however they have the disadvantage of being expensive and cause side effects which require the withdrawal of therapy and antibiotic resistance can be developed [5].

According to Maastricht III consensus the second-line treatment should be bismuth-based quadruple therapy (if available), PPI plus amoxicillin, tetracycline or metronidazole [6]. Our group report in 2009 a levofloxacin-based triple therapy as a valid alternative [7].

2 papers have shown also the superiority of levofloxacin triple over bismuth quadruple therapy [8, 9].

As regards antibiotic resistance rate a high resistance versus metronidazole and clarithromycin was reported in our country.

An interesting paper by Romano et al. report a high eradication rate with levofloxacin versus clarithromycin and the success depends at least in part on the very low prevalence in levofloxacin-resistant *H. pylori* strains in our population (3%) [10].

Antibiotic-associated gastrointestinal side effects such as diarrhoea, nausea, vomiting, bloating, and abdominal pain can represent a serious drawback to anti-*H. pylori* therapies. These manifestations have been related to quantitative and

qualitative changes in the intestinal microflora because of unabsorbed or secreted antibiotics in the intestinal content, with a resulting reduction in normal saprophytic flora, overgrowth and persistence of potentially pathogenic antibiotic-resistant indigenous strains [11].

At present, treatment failure is a significant problem in clinical practice, and the possibility to use simpler eradication schemes or new drugs should be regarded as the most promising way to improve the efficacy of eradication therapy. Some papers showed that the use of probiotics during the first-line *H. pylori* therapy improved the patients compliance and reduced gastrointestinal symptoms [12–14].

A probiotic is defined as a living microbial species that, on administration, can have a positive effect on bowel microecology with improved health conditions. At present, the most studied probiotics are lactic acid-producing bacteria, particularly *Lactobacillus* [15, 16]. Probiotics have been proven to be useful in the treatment of several gastrointestinal diseases such as acute infectious diarrhoea or pouchitis [17, 18]. Moreover, as shown in several studies, probiotics also show a direct antimicrobial effect [19]. In particular, probiotics may compete directly with *H. pylori*, possibly through the inhibition of adherence, as well as by producing metabolites and antimicrobial molecules [20].

On this basis, the Maastricht 2–2000 Consensus Report speculated on the role of probiotic supplementation in the treatment of *H. pylori* infection [21].

The implementation of standard anti-*H. pylori* regimens with probiotics could be advisable, as they are able to improve the patient's compliance by reducing antibiotic-associated adverse events, thus increasing the number of patients completing eradication therapy, resulting in improved eradication rate [22–24]. However, the number of patients enrolled in these trials was too small to achieve statistically conclusive results.

Lactobacillus reuteri (L. reuteri) in one of the most interesting lactobacillus, with some stimulating properties; in particular, it is antibiotic resistant, improves the immune response in the gastrointestinal tract, has a therapeutic effect in acute diarrhoea, reduces the incidence of antibiotic-associated side effects, and inhibits *H. pylori* in vitro and in vivo [25–28]. A recent study reports that a first-line therapy with 4-week *L. reuteri* supplementation is effective in reducing *H. pylori* bacterial load in humans and theoretically may help to control gastric inflammation [29].

The aim of our study was to define the efficacy of *L. reuteri* supplementation in *H. pylori* eradication and in preventing associated gastrointestinal side effects during anti-*H. pylori* infection second-line levofloxacin triple therapy.

2. Methods

2.1. Patients. The study was a single-centre, prospective, randomised, controlled study performed at the Gastroenterology and Internal Medicine Departments of Gemelli Hospital of Rome, Italy.

All patients are Caucasian and came from the same geographic area.

Ninety consecutive *H. pylori*-positive patients were enrolled from November 2007 to June 2008. Patients were considered eligible to enter the study if they were between 18 and 65 years old, affected by gastric *H. pylori* infection as confirmed by a 13C-urea breath test, submitted to a previous unsuccessful anti-*H. pylori* antibiotic treatment. Exclusion criteria were recent (within the previous 3 months) use of antimicrobial agents, bismuth compounds, PPI and H2 receptor antagonists, laxatives, antidiarrheal, other probiotic preparations, alcohol, or drug abuse. Patients with major concomitant diseases including psychiatric disorders and pregnant or lactating women were also excluded from the study. All patients signed a written informed consent. The study was approved by our Ethical Committee.

2.2. Treatment. Using a permuted block randomization (1 : 1), 90 patients were assigned to one of the following parallel groups.

(i) 45 patients (32 males/13 females, mean age 41.5 ± 11.7) were randomly assigned to receive a triple therapy based on esomeprazole 20 mg bid, levofloxacin 500 mg bid, and amoxicillin 1 gr bid for 7 days plus *L. reuteri* (1×10^8, CFU) (Reuflor Italchimici Pomezia, Italy) t.i.d for 14 days, during eradication therapy and 1 week thereafter.

(ii) 45 patients (28 males/17 females, mean age 43.1 ± 13.3) were randomly assigned to receive the same triple therapy without probiotics.

2.3. Side Effects. Each patient was required to complete a validated daily diary for 2 weeks, starting from the first day of eradication treatment. The diary contains a questionnaire (slightly modified from De Boer et al.)[30] evaluating the onset, intensity, and frequency of gastrointestinal side effects: taste disturbance, epigastric pain, constipation, skin rash, nausea, vomiting, abdominal pain, bloating, loss of appetite, and diarrhoea. Symptom intensity was rated using a scale, where 0, 1, 2, and 3 corresponded to absent, mild, moderate and severe symptoms, respectively. An overall judgment of tolerability was assessed by the patient at the end of both the first and second weeks of treatment. Treatment compliance was evaluated by counting the vials returned by the subject (patients who returned <80% of empty vials were not included in the per protocol population (PP) analysis). Patients were adequately informed and motivated to therapy, and strictly.

2.4. Eradication of Helicobacter pylori. H. pylori status was controlled with the 13C urea breath test performed with citric acid and 75 mg of 13C urea, with the eradication control test being performed not before 6 weeks after the end of therapy [31, 32]. A delta value higher than 3.5 units was considered the cut-off for positivity.

2.5. Statistical Analysis. To evaluate *H. pylori* eradication three variables, previously dichotomised, were analysed: *L. reuteri* supplementation (Y versus N), sex (males versus

Table 1: HP-eradication: univariate analysis.

Independent variables	Subgroups analysed	HP eradication rate	*P* value
L. reuteri supplementation	Y	36 (80%)	*0.038*
	N	27 (60%)	
Age	<42 y	33 (73.3%)	0.782
	≥42 y	30 (66.7%)	
Sex	Male	40 (88.9%)	0.329
	Female	23 (51.1%)	

Table 2: HP-eradication: multivariate analysis.

Independent variables	*P* value	Odds ratio	95% Confidence interval
L. reuteri supplementation	*0.026*	*3.055*	*1.146–8.150*
Age	0.434	1.471	0.559–3.869
Sex	0.195	0.497	0.172–1.430

females), and age (age < median age versus age ≥ median age). A univariate analysis was performed with the chi-squared test. The significant cut-off was set at $P < 0.05$. Significant parameters with a P value less than 0.25 at univariate analysis were entered in a multivariate logistic regression model to identify independent predictors of *H. pylori* eradication. Odds ratio (OR) to achieve *H. pylori* eradication with 95% confidence intervals (CI) was calculated.

The statistical analysis of side effects was performed with the chi-square univariate analysis. All variables were dichotomised into two group: symptoms Y versus symptoms N and moderate-severe symptoms versus negligible or not referred symptoms. The significant cut-off was $P < 0.05$.

All analyses were performed using SYSTAT 12.0 for Windows.

2.6. End Point. The primary end point of the study was to compare the eradication rate achieved with the triple therapy with or without *L. reuteri* supplementation.

The secondary end point were the patients compliance and the occurrence of side effects in the two groups of different treatment.

3. Results

All patients completed the study.

Forty-five patients were treated with *L. reuteri* supplementation (group 1) and 45 patients were treated without probiotic supplementation (group 2).

The per protocol and "intention to treat" analyses were shown to be the same in our study, because of the absence of drop out events. The overall patients compliance to both eradication schemes was good, with all patients completing the prescribed therapy.

A significantly higher eradication rate was achieved in group 1 with 80% eradication rate (36/45), compared to 60% (27/45) in group 2 (P: 0.038). Age (P: 0.782) and sex (P: 0.329) had no significant impact on *H. pylori* eradication rate (Table 1). *L. reuteri*, age and sex were evaluated in a multivariate model of statistical analysis and we found that *L. reuteri* supplementation was the only predicting factor in *H. pylori* eradication (P: 0.026; odds ratio: 3.055; confidence interval: 1.146–8.150) (Table 2).

As regards the analysed side effects, taste distortion was referred by 6 patients (13.3%) of group 1 and by 8 patients (17.8%) of group 2 (P: 0.561); epigastric pain was reported by 5 patients (11.1%) of group 1 and 4 patients (8.9%) of group 2 (P: 0.725); constipation was reported by 8 patients (17.8%) of group 1 and 11 patients (24.4%) of group 2 (P: 0.438); skin rash was observed in 4 patients (8.9%) of both groups (P: 1.000). No patients referred moderate or severe taste disturbance, epigastric pain, constipation and skin rash of both groups.

Thirty patients (66.7%) of group 1 reported nausea, 19 (42.2%) of them of moderate-severe intensity, while all patients (100%) of group 2 referred moderate-severe nausea ($P < 0.001$ both in absolute terms and for moderate-severe symptoms). No significant difference was reported for vomiting that was referred by 17 patients (37.8%) of group 1 and 15 patients (33.3%) of group 2 (P: 0.660), with a moderate severe score by 3 patients (6.7%) of both groups (P: 1.000). Twenty-nine patients (64.4%) of group 1 and 31 patients (68.9%) of group 2 referred abdominal pain (P: 0.655); this symptom was reported as moderate severe by 6 patients (13.3%) of group 1 and by 12 patients (26.7%) of group 2 (P: 0.114). Bloating was reported by 35 patients (77.8%) of group 1 and 37 patients (82.2%) of group 2 (P: 0.598), which was of moderate-severe intensity by 12 patients (26.7%) of group 1 and 8 patients (17.8%) of group 2, respectively (P: 0.310). 36 patients (80%) of group 1 and 33 patients (73.3%) of group 2 referred loss of appetite (P: 0.455), which was of moderate-severe intensity in 11 patients (24.4%) and 15 patients (33.3%), respectively, (P: 0.352).

Table 3: HP-eradication: univariate analysis of symptom regression.

Symptoms	*L. reuteri* group	No *L. reuteri* group	*P* value
Nausea	32 (66.7%)	45 (100%)	***<0.001***
Moderate-severe nausea	19 (42.2%)	45 (100%)	***<0.001***
Abdominal pain	29 (64.4%)	31 (68.9%)	0.655
Moderate-severe abdominal pain	6 (13.3%)	12 (26.7%)	0.114
Diarrhoea	10 (22.2%)	26 (57.7%)	***<0.004***
Moderate-severe diarrhoea	4 (10%)	15 (57.6%)	***<0.001***
Bloating	35 (77.7%)	37 (82.2%)	0.598
Moderate-severe bloating	12 (26.7%)	8 (17.8%)	0.310
Loss of appetite	36 (80%)	33 (73.3%)	0.455
Moderate-severe loss of appetite	11 (24.4%)	15 (33.3%)	0.352
Vomiting	17 (37.8%)	15 (33.3%)	0.660
Moderate-severe vomiting	3 (6.7%)	3 (6.7%)	1.000
Epigastric pain	5 (11.1%)	4 (8.9%)	0.725
Taste disturbance	6 (13.3%)	8 (17.8%)	0.561
Skin rash	4 (8.9%)	4 (8.9%)	1.000
Constipation	8 (17.8%)	11 (24.4%)	0.438

Diarrhoea was reported by 10 (22.2%) patients of group 1 and 26 (57.7%) of group 2 (*P*: 0.004); it was moderate-severe in 4 patients (40.0%) and in 15 patients (57.6%), respectively (*P*: 0.001) (Table 3).

4. Discussion

In the present randomised controlled study we have shown that patients treated with *L. reuteri* during a levofloxacin-based second-line *H. pylori* therapy experienced a lower incidence of nausea and diarrhoea compared to subjects without probiotic supplementation and with a higher eradication rate. Probiotics may act in a different way: by direct competition with *H. pylori* or by improving the patients compliance to therapy reducing the incidence of antibiotic-associated side effects [33–35]. The direct effect against *H. pylori* is supported only by animal and in vitro studies while several others have confirmed that probiotics indirectly improve eradication rate with reduced incidence of side effects and improved patients compliance [36–38].

Currently, the best studied probiotics are the lactic acid bacteria, in particular *Lactobacillus* and *Bifidobacterium* [39–43]. In our study we have used *L. reuteri* ATCC 55730 because previous clinical trials have shown that its administration is safe in both adults and children, reducing the incidence and severity of gastrointestinal side effects; moreover it is bile and acid resistant, adheres to the mucosa and to enterocytes and inhibits *H. pylori* growth in vitro and vivo [44, 45].

Previous studies reported that *L. reuteri* has the cell surface protein that inhibits in vitro the binding of *H. pylori* to receptor glycolipids (asialo-GM1 and sulfatide) [19]. To confirm this data Canducci et al. [26] have recently published a randomised placebo-controlled study and have shown an inhibitory effect of *L. reuteri* on *H. pylori* growth with a significant decrease in both 13C-UBT and *H. pylori*. Thus, *L. reuteri* seems to exert a beneficial effect during *H. pylori* infection resulting in a reduction of bacterial load and gastric inflammation. In the literature, many clinical trials are reported on the use of single or multiple probiotic strains administered for *H. pylori* treatment. Unfortunately, it is hard to compare these trials because of different randomisation, probiotic administration, doses, and concomitant therapy.

The second aim of our study was to assess whether *L. reuteri* could be of help in ameliorating symptoms during *H. pylori* triple therapy. We have shown that patients receiving the probiotic experienced a significant improvement of some gastrointestinal symptoms compared to those without probiotic supplementation.

In particular symptoms with a lower incidence in group 1 treated with *L. reuteri* were diarrhoea and nausea. Previous studies have shown that oral probiotic treatments during first-line anti-*H. pylori* regimens were able to reduce the incidence of diarrhoea, nausea and taste disturbance. It is well known that antibiotic-associated side effects are common and are usually the first cause of therapy withdrawal. In fact, antibacterial drugs, can alter the equilibrium between bacterial concentration and colonic mucosal cells, causing a prevalence of pathogens over the normal microflora. Probiotic supplementation may partially restore the intestinal physiological microecology [46–48].

A Cochrane analysis showed that antibiotics alter the microbial balance within the gastrointestinal tract and probiotics [49]. In particular, *Lactobacillus spp.* and *Saccharomyces boulardii* can prevent antibiotic-associated diarrhoea by restoring the gut microflora [42–51].

A recently published paper has shown that *L. reuteri* is effective in reducing gastrointestinal symptoms during *H. pylori* eradication therapy in children [29–33].

A meta-analysis on the effects of probiotic supplementation on eradication rates and adverse events during *H. pylori*

eradication therapy suggests that probiotics are effective in increasing the eradication rate and can be considered helpful for patients with eradication failure [52]. However, there are only two trials which confirm this conclusion: this is the reason why to confirm this result; we have designed a randomised controlled trial in a large population.

A major drawback of our study is the lack of a double-blind controlled design; so the difference in side effects needs to be judged with caution.

Our study is the first that has evaluated the administration of *L. reuteri* in levofloxacin second-line therapy. It is well known that a second-line therapy results in success rates from less than 60% to 90% with the most relevant determinant of success being microbial sensitivity and patients compliance.

In summary, we confirm that *L. reuteri* supplementation during a second-line *H. pylori* therapy is recommended first of all for a better eradication rate and second for reduced gastrointestinal side effects. Although this pilot study opens new areas of investigation, further studies on a larger number of patients are required to define its real clinical application.

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References

[1] D. Palli, M. Menegatti, G. Masala et al., "*Helicobacter pylori* infection, anti-cagA antibodies and peptic ulcer: a case-control study in Italy," *Alimentary Pharmacology and Therapeutics*, vol. 16, no. 5, pp. 1015–1020, 2002.

[2] E. Bayerdörffer and A. Morgner, "Gastric marginal zone B-cell lymphoma of the mucosa-associated lymphoid tissue type: management of the disease," *Digestive and Liver Disease*, vol. 32, no. 3, pp. 192–194, 2000.

[3] N. Uemura, S. Okamoto, S. Yamamoto et al., "*Helicobacter pylori* infection and the development of gastric cancer," *The New England Journal of Medicine*, vol. 345, no. 11, pp. 784–789, 2001.

[4] A. Gasbarrini, F. Franceschi, A. Armuzzi et al., "Extradigestive manifestations of *Helicobacter pylori* gastric infection," *Gut*, vol. 45, no. 1, pp. I9–I12, 1999.

[5] I. Adamsson, C. E. Nord, P. Lundquist, S. Sjöstedt, and C. Edlund, "Comparative effects of omeprazole, amoxycillin plus metronidazole versus omeprazole, clarithromycin plus metronidazole on the oral, gastric and intestinal microflora in *Helicobacter pylori*-infected patients," *Journal of Antimicrobial Chemotherapy*, vol. 44, no. 5, pp. 629–640, 1999.

[6] P. Malfertheiner, F. Megraud, C. O'Morain et al., "Current concepts in the management of *Helicobacter pylori* infection: the Maastricht III Consensus Report," *Gut*, vol. 56, no. 6, pp. 772–781, 2007.

[7] S. Di Caro, F. Franceschi, A. Mariani et al., "Second-line levofloxacin-based triple schemes for *Helicobacter pylori* eradication," *Digestive and Liver Disease*, vol. 41, no. 7, pp. 480–485, 2009.

[8] R. J. Saad, P. Schoenfeld, M. K. Hyungjin, and W. D. Chey, "Levofloxacin-based triple therapy versus bismuth-based quadruple therapy for persistent *Helicobacter pylori* infection: a meta-analysis," *American Journal of Gastroenterology*, vol. 101, no. 3, pp. 488–496, 2006.

[9] S. Karatapanis, L. Skorda, S. Georgopoulos et al., "Levofloxacin-based triple therapy versus bismuth-based quadruple therapy as a second line treatment for the eradication of *H. pylori* infection," *Annals of Gastroenterology*, vol. 22, no. 4, pp. 263–267, 2009.

[10] M. Romano, A. Cuomo, A. G. Gravina et al., "Empirical levofloxacin-containing versus clarithromycin-containing sequential therapy for *Helicobacter pylori* eradication: a randomised trial," *Gut*, vol. 59, no. 11, pp. 1465–1470, 2010.

[11] M. De Vrese, "Health benefits of probiotics and prebiotics in women," *Menopause International*, vol. 15, no. 1, pp. 35–40, 2009.

[12] J. Zou, J. Dong, and X. Yu, "Meta-analysis: lactobacillus containing quadruple therapy versus standard triple first-line therapy for *Helicobacter pylori* eradication," *Helicobacter*, vol. 14, no. 5, pp. 97–107, 2009.

[13] M. Candelli, E. C. Nista, E. Carloni et al., "Treatment of *H. pylori* infection: a review," *Current Medicinal Chemistry*, vol. 12, no. 4, pp. 375–384, 2005.

[14] B. J. Egan, M. Katicic, H. J. O'Connor, and C. A. O'Morain, "Treatment of *Helicobacter pylori*," *Helicobacter*, vol. 12, no. 1, pp. 31–37, 2007.

[15] Y. Aiba, N. Suzuki, A. M. A. Kabir, A. Takagi, and Y. Koga, "Lactic acid-mediated suppression of *Helicobacter pylori* by the oral administration of Lactobacillus salivarius as a probiotic in a gnotobiotic murine model," *American Journal of Gastroenterology*, vol. 93, no. 11, pp. 2097–2101, 1998.

[16] C. G. Goldman, D. A. Barrado, N. Balcarce et al., "Effect of a probiotic food as an adjuvant to triple therapy for eradication of *Helicobacter pylori* infection in children," *Nutrition*, vol. 22, no. 10, pp. 984–988, 2006.

[17] S. J. Lewis and A. R. Freedman, "Review article: the use of biotherapeutic agents in the prevention and treatment of gastrointestinal disease," *Alimentary Pharmacology and Therapeutics*, vol. 12, no. 9, pp. 807–822, 1998.

[18] C. Hedin, K. Whelan, and J. O. Lindsay, "Evidence for the use of probiotics and prebiotics in inflammatory bowel disease: a review of clinical trials," *Proceedings of the Nutrition Society*, vol. 66, no. 3, pp. 307–315, 2007.

[19] T. Mukai, T. Asasaka, E. Sato, K. Mori, M. Matsumoto, and H. Ohori, "Inhibition of binding of *Helicobacter pylori* to the glycolipid receptors by probiotic *Lactobacillus reuteri*," *FEMS Immunology and Medical Microbiology*, vol. 32, no. 2, pp. 105–110, 2002.

[20] R. W. Jack, J. R. Tagg, and B. Ray, "Bacteriocins of gram-positive bacteria," *Microbiological Reviews*, vol. 59, no. 2, pp. 171–200, 1995.

[21] P. Malfertheiner, F. Mégraud, C. O'Morain et al., "Current concepts in the management of *Helicobacter pylori* infection—the Maastricht 2-2000 Consensus Report," *Alimentary Pharmacology and Therapeutics*, vol. 16, no. 2, pp. 167–180, 2002.

[22] E. C. Nista, M. Candelli, F. Cremonini et al., "Bacillus clausii therapy to reduce side-effects of anti-*Helicobacter pylori* treatment: randomized, double-blind, placebo controlled trial," *Alimentary Pharmacology and Therapeutics*, vol. 20, no. 10, pp. 1181–1188, 2004.

[23] A. Armuzzi, F. Cremonini, F. Bartolozzi et al., "The effect of oral administration of Lactobacillus GG on antibiotic-associated gastrointestinal side-effects during *Helicobacter pylori* eradication therapy," *Alimentary Pharmacology and Therapeutics*, vol. 15, no. 2, pp. 163–169, 2001.

[24] A. Armuzzi, F. Cremonini, V. Ojetti et al., "Effect of Lactobacillus GG supplementation on antibiotic-associated gastrointestinal side effects during *Helicobacter pylori* eradication therapy: a pilot study," *Digestion*, vol. 63, no. 1, pp. 1–7, 2001.

[25] F. Cremonini, S. Di Caro, M. Covino et al., "Effect of different probiotic preparations on anti-*Helicobacter pylori* therapy-related side effects: a parallel group, triple blind, placebo-controlled study," *American Journal of Gastroenterology*, vol. 97, no. 11, pp. 2744–2749, 2002.

[26] F. Canducci, A. Armuzzi, F. Cremonini et al., "A lyophilized and inactivated culture of *Lactobacillus acidophilus* increases *Helicobacter pylori* eradication rates," *Alimentary Pharmacology and Therapeutics*, vol. 14, no. 12, pp. 1625–1629, 2000.

[27] E. Myllyluoma, L. Veijola, T. Ahlroos et al., "Probiotic supplementation improves tolerance to *Helicobacter pylori* eradication therapy—a placebo-controlled, double-blind randomized pilot study," *Alimentary Pharmacology and Therapeutics*, vol. 21, no. 10, pp. 1263–1272, 2005.

[28] N. Valeur, P. Engel, N. Carbajal, E. Connolly, and K. Ladefoged, "Colonization and immunomodulation by *Lactobacillus reuteri* ATCC 55730 in the human gastrointestinal tract," *Applied and Environmental Microbiology*, vol. 70, no. 2, pp. 1176–1181, 2004.

[29] R. Francavilla, E. Lionetti, S. P. Castellaneta et al., "Inhibition of *Helicobacter pylori* infection in humans by *Lactobacillus reuteri* ATCC 55730 and effect on eradication therapy: a pilot study," *Helicobacter*, vol. 13, no. 2, pp. 127–134, 2008.

[30] W. A. De Boer, J. C. Thys, T. J. Borody, D. Y. Graham, C. O'Morain, and G. N. J. Tytgat, "Proposal for use of a standard side effect scoring system in studies exploring *Helicobacter pylori* treatment regimens," *European Journal of Gastroenterology and Hepatology*, vol. 8, no. 7, pp. 641–643, 1996.

[31] D. Y. Graham and P. D. Klein, "Accurate diagnosis of *Helicobacter pylori*: ^{13}C-urea breath test," *Gastroenterology Clinics of North America*, vol. 29, no. 4, pp. 885–893, 2000.

[32] R. M. Zagari, P. Pozzato, C. Martuzzi et al., "L3C-urea breath test to assess *Helicobacter pylori* bacterial load," *Helicobacter*, vol. 10, no. 6, pp. 615–619, 2005.

[33] A. Tursi, G. Brandimarte, G. M. Giorgetti, and M. E. Modeo, "Effect of Lactobacillus casei supplementation on the effectiveness and tolerability of a new second-line 10-day quadruple therapy after failure of a first attempt to cure *Helicobacter pylori* infection," *Medical Science Monitor*, vol. 10, no. 12, pp. CR662–CR666, 2004.

[34] E. Lionetti, V. L. Miniello, S. P. Castellaneta et al., "*Lactobacillus reuteri* therapy to reduce side-effects during anti-*Helicobacter pylori* treatment in children: a randomized placebo controlled trial," *Alimentary Pharmacology and Therapeutics*, vol. 24, no. 10, pp. 1461–1468, 2006.

[35] A. V. Shornikova, I. A. Casas, E. Isolauri, H. Mykkänen, and T. Vesikari, "*Lactobacillus reuteri* as a therapeutic agent in acute diarrhea in young children," *Journal of Pediatric Gastroenterology and Nutrition*, vol. 24, no. 4, pp. 399–404, 1997.

[36] I. Sakamoto, M. Igarashi, K. Kimura, A. Takagi, T. Miwa, and Y. Koga, "Suppressive effect of *Lactobacillus gasseri* OLL 2716 (LG21) on *Helicobacter pylori* infection in humans," *Journal of Antimicrobial Chemotherapy*, vol. 47, no. 5, pp. 709–710, 2001.

[37] P. Michetti, G. Dorta, P. H. Wiesel et al., "Effect of whey-based culture supernatant of *Lactobacillus acidophilus (johnsonii)* La1 on *Helicobacter pylori* infection in humans," *Digestion*, vol. 60, no. 3, pp. 203–209, 1999.

[38] S. Cruchet, M. C. Obregon, G. Salazar, E. Diaz, and M. Gotteland, "Effect of the ingestion of a dietary product containing *Lactobacillus johnsonii* La1 on *Helicobacter pylori* colonization in children," *Nutrition*, vol. 19, no. 9, pp. 716–721, 2003.

[39] M. Gotteland and S. Cruchet, "Suppressive effect of frequent ingestion of *Lactobacillus johnsonii* La1 on *Helicobacter pylori* colonization in asymptomatic volunteers," *Journal of Antimicrobial Chemotherapy*, vol. 51, no. 5, pp. 1317–1319, 2003.

[40] K. Y. Wang, S. N. Li, C. S. Liu et al., "Effects of ingesting *Lactobacillus*- and *Bifidobacterium*-containing yogurt in subjects with colonized *Helicobacter pylori*," *American Journal of Clinical Nutrition*, vol. 80, no. 3, pp. 737–741, 2004.

[41] T. Shimizu, H. Haruna, K. Hisada, and Y. Yamashiro, "Effects of *Lactobacillus gasseri* OLL 2716 (LG21) on *Helicobacter pylori* infection in children," *Journal of Antimicrobial Chemotherapy*, vol. 50, no. 4, pp. 617–618, 2002.

[42] D. Pantoflickova, I. Corthésy-Theulaz, G. Dorta et al., "Favourable effect of regular intake of fermented milk containing *Lactobacillus johnsonii* on *Helicobacter pylori* associated gastritis," *Alimentary Pharmacology and Therapeutics*, vol. 18, no. 8, pp. 805–813, 2003.

[43] M. Gotteland, L. Poliak, S. Cruchet, and O. Brunser, "Effect of regular ingestion of *Saccharomyces boulardii* plus inulin or *Lactobacillus acidophilus* LB in children colonized by *Helicobacter pylori*," *Acta Paediatrica*, vol. 94, no. 12, pp. 1747–1751, 2005.

[44] A. V. Shornikova, I. A. Casas, H. Mykkänen, E. Salo, and T. Vesikari, "Bacteriotherapy with *Lactobacillus reuteri* in rotavirus gastroenteritis," *Pediatric Infectious Disease Journal*, vol. 16, no. 12, pp. 1103–1107, 1997.

[45] B. W. Wolf, K. B. Wheeler, D. G. Ataya, and K. A. Garleb, "Safety and tolerance of *Lactobacillus reuteri* supplementation to a population infected with the human immunodeficiency virus," *Food and Chemical Toxicology*, vol. 36, no. 12, pp. 1085–1094, 1998.

[46] P. L. Conway, S. L. Gorbach, and B. R. Goldin, "Survival of lactic acid bacteria in the human stomach and adhesion to intestinal cells," *Journal of dairy science*, vol. 70, no. 1, pp. 1–12, 1987.

[47] C. Johnson, J. Dicksved, and H. Jonsson, "Anti *Helicobacter pylori* activity among lactic acid bacteria isolated from gastric biopsies and strains of *Lactobacillus reuteri*," *Helicobacter*, vol. 8, article 473, 2003.

[48] P. Marteau and J. C. Rambaud, "Potential of using lactic acid bacteria for therapy and immunomodulation in man," *FEMS Microbiology Reviews*, vol. 12, no. 1–3, pp. 207–220, 1993.

[49] B. C. Johnston, A. L. Supina, M. Ospina, and S. Vohra, "Probiotics for the prevention of pediatric antibiotic-associated diarrhea," *Cochrane Database of Systematic Reviews (Online)*, no. 2, Article ID CD004827, 2007.

[50] E. Myllyluoma, L. Veijola, T. Ahlroos et al., "Probiotic supplementation improves tolerance to *Helicobacter pylori* eradication therapy—a placebo-controlled, double-blind randomized pilot study," *Alimentary Pharmacology and Therapeutics*, vol. 21, no. 10, pp. 1263–1272, 2005.

[51] M. Gotteland, O. Brunser, and S. Cruchet, "Systematic review: are probiotics useful in controlling gastric colonization by *Helicobacter pylori*?" *Alimentary Pharmacology and Therapeutics*, vol. 23, no. 8, pp. 1077–1086, 2006.

[52] J. L. Tong, Z. H. Ran, J. Shen, C. X. Zhang, and S. D. Xiao, "Meta-analysis: the effect of supplementation with probiotics on eradication rates and adverse events during *Helicobacter pylori* eradication therapy," *Alimentary Pharmacology and Therapeutics*, vol. 25, no. 2, pp. 155–168, 2007.

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Research Article

Variability in Prevalence of *Helicobacter pylori* Strains Resistant to Clarithromycin and Levofloxacin in Southern Poland

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Background. An increasing resistance of *Helicobacter pylori* strains to antimicrobial agents is the serious therapeutic problem. The aim of this study was to compare the primary and secondary resistance of *H. pylori* strains isolated between 2006–2008 (data published) and 2009–2011 to clarithromycin and levofloxacin. *Material and Methods*. 220 dyspeptic patients (153 before treatment, 67 after), were enrolled in the study. 51 *H. pylori* strains were isolated. MIC values of clarithromycin and levofloxacin were determined by the *E*-test method. The statistical analysis was conducted with the χ^2 test with Yates correction at the 0.05 significance level ($P \leq 0.05$). *Results*. Between 2006 and 2008, 34% (39/115) of *H. pylori* strains were resistant to clarithromycin (primary 21% (19/90), secondary 80% (20/25)). 5% (6/115) of strains were resistant to levofloxacin (primary 2% (2/90), secondary 16% ((4/25); data published) Between 2009–2011, 22% (11/51) of *H. pylori* strains were resistant to clarithromycin (primary 19% (8/43), secondary 38% (3/8)). 16% (8/51) of strains were resistant to levofloxacin (primary 12% (5/43), secondary 38% (3/8)). *Conclusion*. The present study has shown the increasing amount of resistant *H. pylori* strains isolated from patients in Southern Poland to levofloxacin and decreasing number of resistant strains to clarithromycin.

1. Introduction

Helicobacter pylori (H. pylori) is a Gram-negative, microaerophilic, and urease-positive spiral shaped bacterium, which colonizes the gastric mucosa of 50% of the population worldwide [1, 2]. The incidence of the infection is associated mostly with childhood as well as socioeconomic and sanitary conditions. *Helicobacter pylori* infection plays a major role in peptic ulcer disease, low-grade mucosa-associated lymphoid tissue (MALT) lymphoma, and gastric cancer. Thanks to the discovery of this pathogen by Marshall and Warren in 1982, peptic ulcer diseases are no longer chronic but can be cured by the regimen of antibiotics and gastric antisecretory drugs [3].

The preferred eradication therapy is triple or quadruple therapy, which is combined therapy including three types of drugs: antisecretory drugs, cytoprotectants, and antibiotics and chemotherapeutic drugs. Current guidelines from the American College of Gastroenterology and the European Helicobacter Study Group (EHSG) recommend a clarithromycin-based triple therapy for the first 5 days (a proton pump inhibitor (PPI) plus amoxicillin and clarithromycin) or a bismuth quadruple therapy (a PPI plus bismuth, metronidazole and tetracycline) [4, 5]. Obligatory procedures for the management of *H. pylori* infection in Poland elaborated upon by the Working Group of the Polish Society of Gastroenterology (PTG) are based on new guidelines from the Third Maastricht Consensus Conference in 2005 [6].

Current regimens of treatment *H. pylori* infection in Poland are as follows.

(i) The First-Line Treatment. PPI, amoxicillin (1000 mg), and metronidazole (500 mg) twice a day, 10–14 days, and PPI, clarithromycin (500 mg), and metronidazole (500 mg) twice a day, 10–14 days, or PPI, amoxicillin (500 mg), and clarithromycin (500 mg) twice a day, 10–14 days.

(i) The Second-Line Treatment. PPI, amoxicillin (1000 mg), and metronidazole (500 mg) twice a day and tetracycline (250 mg) three times daily prolonged to 14 days, or PPI, amoxicillin (1000 mg), and metronidazole (500 mg) twice a day and bismuth salts (120 mg) four times daily; prolonged to 14 days.

(i) The Third-Line Treatment. Evaluation of the susceptibility of the strains to the currently used antimicrobial agents: amoxicillin, metronidazole, clarithromycin, and tetracycline; possible introduction of levofloxacin; adding a probiotic [6].

Recommendations of PTG were published in 2008 and were the first polish recommendations which allow introduction of levofloxacin in treatment of *H. pylori* infection.

The increasing level of antibiotic resistance in *H. pylori* strains had a drastic effect on the successful treatment [7, 8]. The most recent Maastricht guidelines recommend substituting metronidazole for clarithromycin in case where the resistance level exceeds 15–20% [9]. However, according to the Maastricht recommendation, if the resistance level to metronidazole exceeds 40% and for clarithromycin 15–20%, these antimicrobial agents should not be used or susceptibility testing should be done. In addition, it recommends local permanent monitoring of *H. pylori* susceptibility to antimicrobial agents [5]. Emerging evidence indicates that resistance rates to metronidazole could constitute the real problem. On the other hand, some scientists believe that the resistance might be overcome with increased doses of metronidazole [10]. The rate of clarithromycin resistance is increasing, and one of the reasons of this increase is likely to be a greater use of clarithromycin in the treatment of respiratory tract infections in the community. Clarithromycin resistance in *H. pylori* is associated with treatment failure, although geographical variations were also observed [7, 11]. In Poland the resistance of *H. pylori* to antimicrobial drugs used in the therapy is high and amounts to 28% to clarithromycin (primary resistance 22%, secondary resistance 54%) and 46% to metronidazole (primary resistance 41%, secondary resistance 68%) (data published by PTG) [6, 12]. Therefore, in accordance with the Maastricht recommendations, in Poland clarithromycin and metronidazole should not be used without previous susceptibility testing [5].

When the first-line therapy is unsuccessful, we need the effective second-line therapy. Evolving research has demonstrated that the introduction of new drugs, such as levofloxacin and rifabutin, provides new possibilities of treatment [7, 10, 11]. However, the current recommendation of PTG is to entertain the introduction of levofloxacin as the third-line empirical treatment [6]. Nevertheless, some studies carried out by Molina-Infante in Spain examined the introduction of levofloxacin in the first-line treatment in triple and sequential regimens and demonstrated the advantage of levofloxacin in both combinations. Levofloxacin may be a good alternative to clarithromycin in the region with high percentage of resistant *H. pylori* strains to clarithromycin. [7, 11]. As a result of frequent resistance of *H. pylori* to clarithromycin in Poland and recommendations of PTG (2008) that enable the introduction of levofloxacin to *H. pylori* eradication therapy, many physicians have started using the levofloxacin in first-line treatment (data not published).

Levofloxacin, a bactericidal fluoroquinolone of the 3rd generation antibiotics, has also the activity in the second-line therapy. Levofloxacin may be used as a substitute for clarithromycin in either a standard triple or sequential regimen. A large study comparing antibiotics in either of regimens shows a clear advantage to levofloxacin in both combinations. It has been proposed that levofloxacin-based regimens are the most beneficial in areas where clarithromycin resistance is higher [13–16]. The introduction of levofloxacin to the treatment scheme raises many hopes, but the resistance to levofloxacin is a growing problem in Spain (from 6% to more than 25% over the last 5 years) [17]. A rapidly increasing rate of fluoroquinolone resistance was reported in several countries [7]. The apparently rapid rate at which fluoroquinolone resistance seems to develop may limit the use of levofloxacin in *H. pylori* eradication to the second-line therapy.

Since the resistance to antimicrobials is a major cause of eradication failure, the monitoring of antimicrobial resistance of *H. pylori* in each domestic area should be warranted, especially for clarithromycin and the commonly applied metronidazole. Such monitoring is also recommended by the Maastricht III Consensus. For developing countries this monitoring should probably also include other antimicrobials used in the eradication therapy [18–20]. Therefore, the aim of this prospective study was to assess the primary and secondary resistance of *H. pylori* strains isolated from adult patients, from the Malopolska region in Poland between 2006–2008 [13] and 2009–2011, to antibacterial drugs (clarithromycin and levofloxacin) used clinically for *H. pylori* eradication.

2. Materials and Methods

2.1. Patients. The study enrolled a group of 220 dyspeptic patients aged 16–87, who underwent gastroscopy in the "Falck" Health Care Center in Krakow, Poland.

153 patients had never been treated for *H. pylori* infection, whereas 67 patients underwent the *H. pylori* eradication therapy.

The plan of the study was approved by the Bioethical Commission of the Jagiellonian University, and each patient signed the informed consent for the participation in the study.

2.2. Clinical Material. During gastroscopy two biopsy specimens (bioptates) were taken from each patient. Bioptates

were collected from the antrum and the body of the stomach. Bioptates were transferred in a transportation medium, Portagerm pylori (bioMérieux, Marcy-l'Etoile, France), and then sent for microbiological tests, which were performed at the Department of the Pharmaceutical Microbiology of the Jagiellonian University Medical College.

2.3. Bacterial Culture and Susceptibility Testing. Bioptate was homogenized in glass sterile mortars to ensure a homogeneous distribution of bacteria in the whole specimen. Homogenate was inoculated onto the solid medium, Schaedler agar with 5% sheep blood added (bioMérieux, Marcy-l'Etoile, France) and medium, Schaedler agar with 5% sheep blood, and Dent selective supplement added (Helicobacter pylori Selective Supplement-DENT, Oxoid, Basingstoke, UK). The culture was carried out for 3 to 7 days under 5% CO_2 at 37°C.

The presence of *H. pylori* in the tested material was confirmed by the visual examination of the typical colonies morphology on the plate with medium, positive biochemical tests for catalase, oxidase, and urease. Furthermore, Gram-staining preparation from the colony was performed to confirm the presence of Gram-negative spiral bacteria.

The susceptibility of *H. pylori* strains to antimicrobial agents was assessed by the quantitative method, *E*-test (AB Biodisk, Solna, Sweden), which determined the minimal inhibitory concentration (MIC) of the drug that inhibits the growth of bacterial strains. The susceptibility to clarithromycin and levofloxacin was tested for each *H. pylori* strain. From the pure *H. pylori* culture, one colony was taken to prepare the suspension in 0.85% NaCl on an equivalent of 3.0 McFarland units. The inoculum was spread on the plate with the Schaedler agar with 5% sheep blood (bioMérieux, Marcy-l'Etoile, France) within 15 minutes after the preparation. Then, *E*-test stripes with the clarithromycin and levofloxacin gradient were placed on plates according to manual of the manufacturer (AB Biodisk, *E*-test technical manual), separately for clarithromycin and levofloxacin. Plates were incubated in microaerophilic conditions at 37°C for 72 hrs.

The breakpoints used to qualify strains as resistant according to the MIC values were 1 mg/L for both tested antibiotics, as previously described [21, 22].

The determination of MIC values was carried out against the reference *H. pylori* strain from the American Type Culture Collection, ATCC 43504 *Helicobacter pylori*, to ensure the quality of susceptibility tests.

2.4. Statistical Analysis. The statistical parameters such as: mean values and chi-squared test of Independence (χ^2 test) were performed. The accepted significance level was 0.5 (results with $P \leq 0.05$ were considered statistically significant). In cases where the expected values were less than 5, the Yates correction was used.

The association between the primary and the secondary *H. pylori* resistance to the tested antibiotics was checked.

Moreover, the statistical analysis tested the differences between the level of primary and secondary *H. pylori* resistance to clarithromycin and levofloxacin in the years of our study (2009–2011) and the previous study which was carried out in the years 2006–2008, also in our Department and showed the level of *H. pylori* resistance in the same region of Southern Poland, Malopolska [13].

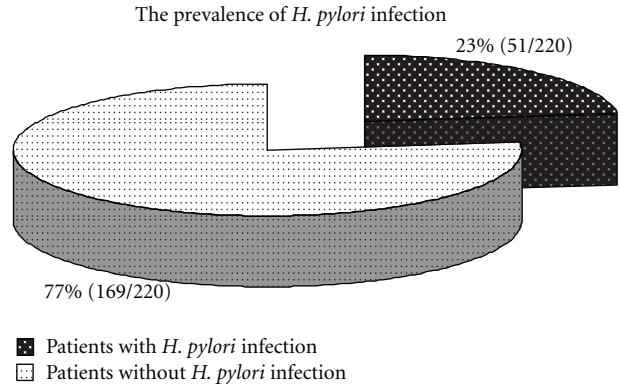

Figure 1: The prevalence of *H. pylori* infection among dyspeptic patients enrolled in the study in 2009–2011.

3. Results and Discussion

3.1. Results. Among 220 patients with dyspeptic symptoms admitted to the study between January 2009 and December 2011, the presence of *H. pylori* infections was confirmed in 51 cases. The prevalence of *H. pylori* infections among dyspeptic patients in Southern Poland amounted to 23% (51/220 Figure 1). The presence of *H. pylori* was confirmed by CLO test—rapid urease test—performed by a doctor and bacterial culture.

51 strains of *H. pylori* were successfully isolated from biopsy specimens of 51 patients who were identified as positive for *H. pylori*. The group of *H. pylori*-positive patients consisted of 28 women (55%) and 23 men (45%), which indicates that both ganders were equally represented in the study. The average age of this group of patients was 45.6 years (aged 18–75 years).

In total, 43 strains were derived from patients who had never been treated for *H. pylori* infections (primary strains 84%) and 8 strains were derived from patients after the failed therapy (secondary strains 16%) (Figure 2).

Susceptibility to clarithromycin and levofloxacin was tested for all *H. pylori* strains by the quantitative method, *E*-test. The obtained MIC values ranged from 0.016 to 12 mg/L for clarithromycin and from 0.012 to 32 mg/L for levofloxacin. Mean MIC values were as follows: 1.22 mg/L for clarithromycin and 1.42 mg/L for levofloxacin.

In total, in the years 2009–2011, the ratio of *H. pylori* strains susceptible to clarithromycin amounted to 78% (40/51), while the ratio of resistant strains amounted to 22% (11/51); primary resistance was 19% (8/43 strains) and secondary 38% (3/8 strains). The ratio of *H. pylori* strains susceptible to levofloxacin amounted to 84% (43/51 strains), while the ratio of resistant strains amounted to 16% (8/51 strains); primary resistance 12% (5/43), secondary 38% (3/8) (Table 1).

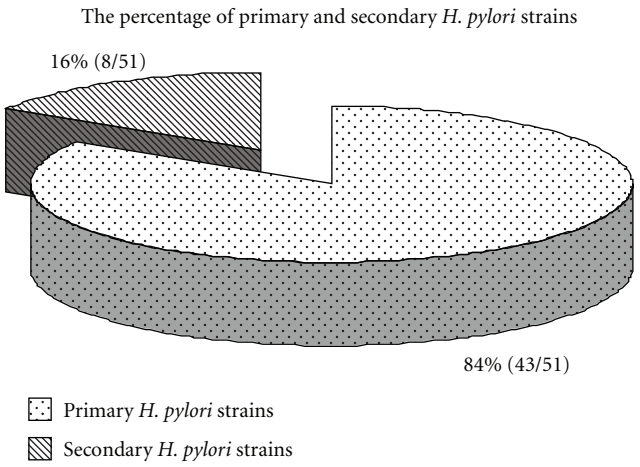

Figure 2: The percentage of primary and secondary *H. pylori* strains isolated from dyspeptic patients enrolled in the study in 2009–2011.

Table 1: Comparison of resistance of *H. pylori* primary and secondary strains to clarithromycin and levofloxacin in 2009–2011.

Antimicrobial agent	No. (%) of resistant *H. pylori* strains in the years 2009–2011		
	All strains	Primary strains	Secondary strains
	$n = 51$	$n = 43$	$n = 8$
CLA[1]	11 (22%)	8 (19%)	3 (38%)
LEV[1]	8 (16%)	5 (12%)	3 (38%)

[1] CLA: clarithromycin, LEV: levofloxacin.

In the years 2006–2008, 115 strains were isolated. 34% (39/115) of *H. pylori* strains were resistant to clarithromycin (primary 21% (19/90), secondary 80% (20/25)). 5% (6/115) of strains were resistant to levofloxacin (primary 2% (2/90), secondary 16% (4/25)) [13].

The comparison of the *H. pylori*-resistant strains to clarithromycin and levofloxacin, isolated between 2006–2008 [13] and 2009–2011, was conducted with the use of the χ^2 test. An increase of the amount of resistant strains to levofloxacin was statistically significant; 5% (6/115) between 2006 and 2008 [13] versus 16% (8/51) between 2009 and 2011, $P = 0.05$ (with the Yates correction).

Nevertheless, the amount of *H. pylori*-resistant strains to clarithromycin is decreasing. The total amount of resistant strains decrease from 34% in 2006–2008 [13] to 22% in 2009–2011; however it is statistically insignificant ($P = 0.16$ (Table 2, Figure 3)).

4. Discussion

Variations of the prevalence of resistant *H. pylori* strains depend on some factors, for instance, the use of antibiotics and chemotherapeutics in recommended patterns of antimicrobial agents, and are geographically differentiated [23].

Table 2: Comparison of resistance of *H. pylori* strains to clarithromycin and levofloxacin between 2006–2008 [13] and 2009–2011.

Antimicrobial agent	No. (%) of *H. pylori*-resistant strains		P value[1]
	2006–2008 [13] $n = 115$	2009–2011 $n = 51$	
CLA[2]	39 (34%)	11 (22%)	0,16 NS[3]
LEV[2]	6 (5%)	8 (16%)	0,05[4]

[1] P value (chi-square test) with the Yates correction. $P \leq 0.05$ was deemed statistically significant.
[2] CLA: clarithromycin, LEV: levofloxacin.
[3] NS: non significant.
[4] Statistically significant differences between the level of resistance in the years 2006–2008 and 2009–2011.

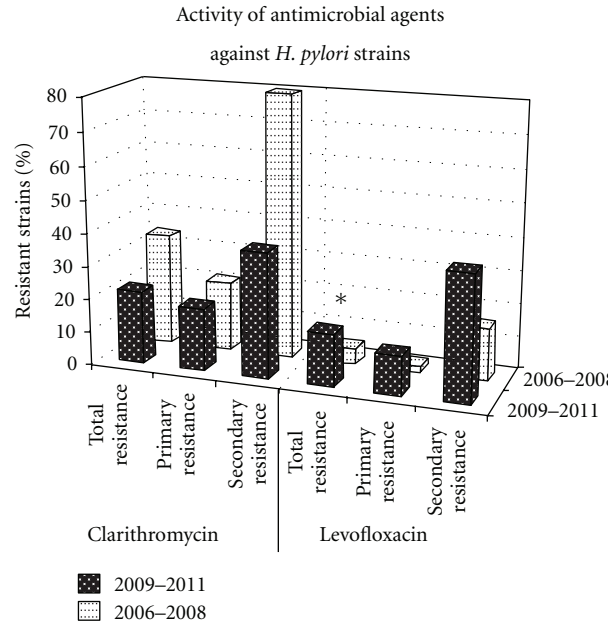

Figure 3: Activity of clarithromycin and levofloxacin against primary and secondary *H. pylori* strains. *statistically significant differences between the level of resistance to levofloxacin in the years 2006–2008 and 2009–2011.

The resistance of *H. pylori* strains to levofloxacin is quickly acquired; thus, it is the growing problem [7, 23]. For example, in France it increased from 3.3% in 1999 to 17.5% in 2003 [21]; in Spain the resistance increased from 6% to more than 25% over the last five years [17, 24]. In another country, such as Iran, the resistance of *H. pylori* to fluoroquinolones has also been increasing although it had not been reported before—primary resistance has amounted to 5.3% for levofloxacin [18].

Our study has shown that in Poland there is also a significant increase of *H. pylori* strains resistant to levofloxacin, from 5% in 2006–2008 [13] to 16% in 2009–2011 ($P = 0.05$). Many studies have shown that resistance to fluoroquinolones is easily acquired and is due to point mutations in gyrA genes [21, 23, 25, 26]. The higher rate of *H. pylori*-resistant strains may be caused by the more frequent use of levofloxacin

in the treatment of *H. pylori* infections. Studies conducted in Belgium over the last 20 years (1990–2009) show the correlation between consumption of antibiotics and the rates of resistant *H. pylori* strains [27]. Also another study, carried out by Cabrita et al. in Portugal, shows the correlation between increased use of antibiotics and the growth in prevalence of resistant *H. pylori* strains to these antibiotics [28]. Nevertheless, there is no commonly available information about usage of antibiotics and chemotherapeutics in outpatient clinic in Poland, but, as known, fluoroqinolones are used not only in *H. pylori* infection but also in treatment of infections of genitourinary tract and respiratory tract, gastrointestinal diseases, infection of skin and soft tissues, and many others [25, 29, 30]. This usage of fluoroquinolones and cited studies allows to conclude that increasing resistance of *H. pylori* strains to levofloxacin in Southern Poland may be caused by more common use of levofloxacin and other fluoroquinolones in community and also in treatment of *H. pylori* infections. Susceptibility testing has not been routinely performed and anti-*H. pylori* drugs like levofloxacin are used in the empirical therapy as suggested by many researchers. However, due to the fact that the resistance to levofloxacin is quickly acquired, susceptibility testing should be routinely carried out to enable properly selecting treatment model, or levofloxacin should not be used commonly but only in the rescue third-line therapy, when treatment with clarithromycin and metronidazole failed (as it is recommended by EHSG and PTG [5, 6]) to avoid the further increase of resistance of *H. pylori* to antimicrobial agents [31]. Moreover, Marzio et al. dealt with the role of preliminary susceptibility testing before therapy and after failed therapy. It has been suggested that triple therapy with levofloxacin, amoxicillin, and PPI should not be used without previous susceptibility test in the region where primary resistance of *H. pylori* to levofloxacin amounted to 10% [32]. In our study, 16% of *H. pylori* strains was resistant to levofloxacin and primary resistance 12%.

According to EHSG and the Polish Society of Gastroenterology recommendations, there are three schemes of treatment which suggested the use of levofloxacin as the third-line treatment [5, 6]. Moreover, several studies which showed the efficacy of the third-line rescue therapy with levofloxacin were carried out [31, 33, 34]. Furthermore, levofloxacin was also successfully tested as a good substitute of clarithromycin in the area with the high prevalence of clarithromycin-resistant *H. pylori* strains [7, 35] and as a good alternative for patients allergic to penicillin [36].

Positive results of these studies were likely to contribute to the increased use of levofloxacin instead of clarithromycin in the empirical treatment. Apart from that, fluoroquinolones as drugs with a broad spectrum of activity against bacteria are commonly used in the treatment of many diseases, not only in the treatment of *H. pylori* infections.

An interesting result shown by our research is the change in the profile of the susceptibility of *H. pylori* strains isolated from patients in Southern Poland to clarithromycin. The resistance to clarithromycin decreased in comparison to the previous years 2006–2008. The current level of resistance of *H. pylori* to clarithromycin has amounted to 22%, while in 2006–2008 it was equal to 34% [13]. This change may be caused by the lower consumption of this antimicrobial agent and higher consumption of levofloxacin instead of clarithromycin. This proposal is due to the changes in the profile of *H. pylori* susceptibility and the previously cited studies indicating the relationship between the amount of drug consumption and the amount of resistance of *H. pylori* strains to this drug [27]. It is a hypothesis which would require further detailed research and analysis. However, as the Maastricht III Consensus Report recommended, we carry out the monitoring of antibiotics resistance of *H. pylori* strains in our region of Poland—Southern Poland.

Interesting results have been obtained in Brazil, the research shows that the resistance to clarithromycin is lower than that to levofloxacin (8% versus 23%), which suggests that clarithromycin is still a good option in the treatment of *H. pylori* infections [16]. If the level of resistance to levofloxacin continues to rise and the downward trend of resistance to clarithromycin is sustained, a similar situation may occur in Poland.

5. Conclusion

All things considered, it should be noted that the resistance of *H. pylori* strains is changing and depends on commonly used antimicrobial agents, so the obligatory susceptibility testing before the treatment would be a much better solution to avoid the further increase of resistance of *H. pylori* and other bacteria to antibiotics commonly used in treatment of *H. pylori* infection [31]. Moreover, the present study shows rapidly increasing resistance of *H. pylori* strains isolated from patients in Poland, to levofloxacin. That could discourage the use of this fluoroquinolone in the empirical first-line therapy of *H. pylori* infections and suggest that it should be avoided to overuse of levofloxacin as a first-line therapy. Thus, *H. pylori* resistance to clarithromycin should be permanently monitored due to the variability of the prevalence of resistant *H. pylori* strains.

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References

[1] Review Team, *Helicobacter pylori in Developing Countries*, World Gastroenterology Organization, 2010.

[2] A. Zawadzka-Gralec, M. Wróblewska, A. Szaflarska-Popławska et al., "The drug sensitivity of *Helicobacter pylori* strains—our own observation," *Pediatria Wspolczesna*, vol. 10, no. 1, pp. 29–31, 2008.

[3] B. J. Marshall and J. R. Warren, "Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration," *The Lancet*, vol. 1, no. 8390, pp. 1311–1314, 1984.

[4] W. D. Chey and B. C. Y. Wong, "American College of Gastroenterology guideline on the management of *Helicobacter pylori*

infection," *American Journal of Gastroenterology*, vol. 102, no. 8, pp. 1808–1825, 2007.

[5] P. Malfertheiner, F. Megraud, C. O'Morain et al., "Current concepts in the management of *Helicobacter pylori* infection: the Maastricht III consensus report," *Gut*, vol. 56, no. 6, pp. 772–781, 2007.

[6] J. Dzieniszewski, M. Jarosz, W. Bartnik et al., "Consensus of the Polish Society of Gastroenterology *Helicobacter pylori* Working Group on diagnostic and therapeutic approaches for the *Helicobacter pylori* infection," *Gastroenterologia Polska*, vol. 15, no. 5, pp. 323–331, 2008.

[7] A. O'Connor, J. P. Gisbert, D. McNamara, and C. O'Morain, "Treatment of *Helicobacter pylori* Infection 2010," *Helicobacter*, vol. 16, supplement 1, pp. 53–58, 2011.

[8] W. Ziemniak, "Efficacy of *Helicobacter pylori* eradication taking into account its resistance to antibiotics," *Journal of Physiology and Pharmacology*, vol. 57, no. 3, pp. 123–141, 2006.

[9] T. Lind, S. V. van Zanten, P. Unge et al., "Eradication of *Helicobacter pylori* using one-week triple therapies combining omeprazole with two antimicrobials: the MACH I study," *Helicobacter*, vol. 1, no. 3, pp. 138–144, 1996.

[10] P. P. Basu, K. Rayapudi, T. Pacana, N. J. Shah, N. Krishnaswamy, and M. Flynn, "A Randomized study comparing levofloxacin, omeprazole, nitazoxanide and doxycycline versus triple therapy for the eradication of *Helicobacter pylori*," *The American Journal of Gastroenterology*, vol. 106, no. 11, pp. 1970–1975, 2011.

[11] J. Molina-Infante, B. Perez-Gallardo, M. Fernandez-Bermejo et al., "Clinical trial: clarithromycin vs. levofloxacin in first-line triple and sequential regimens for *Helicobacter pylori* eradication," *Alimentary Pharmacology and Therapeutics*, vol. 31, no. 10, pp. 1077–1084, 2010.

[12] E. Karczewska, I. Wojtas, and A. Budak, "Występowanie pierwotnej oporności *Helicobacter pylori* na leki przeciwbakteryjne w Polsce i na świecie," *Postępy Mikrobiologii*, vol. 48, no. 1, pp. 31–41, 2009.

[13] E. Karczewska, I. Wojtas-Bonior, E. Sito, M. Zwolińska-Wcisło, and A. Budak, "Primary and secondary clarithromycin, metronidazole, amoxicillin and levofloxacin resistance to *Helicobacter pylori* in southern Poland," *Pharmacological Reports*, vol. 63, no. 3, pp. 799–807, 2011.

[14] A. T. Abadi, T. Taghvaei, A. M. Mobarez, B. M. Carpenter, and D. S. Merrell, "Frequency of antibiotic resistance in *Helicobacter pylori* strains isolated from the Northern Population of Iran," *The Journal of Microbiology*, vol. 49, no. 6, pp. 987–993, 2011.

[15] V. De Francesco, F. Giorgio, E. Ierardi et al., "Primary clarithromycin resistance in *Helicobacter pylori*: the Multicentric Italian Clarithromycin Resistance Observational (MICRO) Study," *Journal of Gastrointestinal and Liver Diseases*, vol. 20, no. 3, pp. 235–239, 2011.

[16] E. Jaime Natan, F. M. Silva, R. C. Barbuti, T. Navarro-Rodriguez, J. P. Moraes-Filho, and J. Pedrazzoli Jr., "*Helicobacter pylori* antibiotic resistance in Brazil: clarithromycin is still a good option," *Archives of Gastroenterology*, vol. 48, no. 4, pp. 261–264, 2011.

[17] J. Molina-Infante and J. P. Gisbert, "Levofloxacin in first-line eradication regimens for *Helicobacter pylori*: better test antibiotic susceptibility before treating," *Gut*. In press.

[18] A. Talebi Bezmin Abadi, A. Ghasemzadeh, T. Taghvaei, and A. M. Mobarez, "Primary resistance of *Helicobacter pylori* to levofloxacin and moxifloxacine in Iran," *Internal and Emergency Medicine*. In press.

[19] P. Malfertheiner, F. Bazzoli, J. C. Delchier et al., "*Helicobacter pylori* eradication with a capsule containing bismuth subcitrate potassium, metronidazole, and tetracycline given with omeprazole versus clarithromycin-based triple therapy: a randomised, open-label, non-inferiority, phase 3 trial," *The Lancet*, vol. 377, no. 9769, pp. 905–913, 2011.

[20] F. Iwańczak and B. Iwańczak, "Nowe możliwości leczenia zakażenia *Helicobacter pylori*," *Przegląd Gastroenterologiczny*, vol. 6, pp. 364–369, 2011.

[21] P. Bogaerts, C. Berhin, H. Nizet, and Y. Glupczynski, "Prevalence and mechanisms of resistance to fluoroquinolones in *Helicobacter pylori* strains from patients living in Belgium," *Helicobacter*, vol. 11, no. 5, pp. 441–445, 2006.

[22] F. Mégraud, N. Lehn, T. Lind et al., "Antimicrobial susceptibility testing of *Helicobacter pylori* in a large multicenter trial: the MACH 2 study," *Antimicrobial Agents and Chemotherapy*, vol. 43, no. 11, pp. 2747–2752, 1999.

[23] F. Mégraud, "H pylori antibiotic resistance: prevalence, importance, and advances in testing," *Gut*, vol. 53, no. 9, pp. 1374–1384, 2004.

[24] A. Cuadrado-Lavín, J. R. Salcines-Caviedes, M. F. Carrascosa et al., "Antimicrobial susceptibility of Helicobacter pylori to six antibiotics currently used in Spain," *Journal of Antimicrobial Chemotherapy*, vol. 67, no. 1, pp. 170–173, 2012.

[25] D. Dzierżanowska, *Antybiotykoterapia Praktyczna*, chapter 2.1, Alfa Medica Press, Bielsko-Biała, Poland, 2008.

[26] K. R. Jones, J. H. Cha, and D. S. Merrell, "Who's winning the war? Molecular mechanisms of antibiotic resistance in *Helicobacter pylori*," *Current Drug Therapy*, vol. 3, no. 3, pp. 190–203, 2008.

[27] V. Y. Miendje Deyi, P. Bontems, J. Vanderpas et al., "Multicenter survey of routine determinations of resistance of *Helicobacter pylori* to antimicrobials over the last 20 years (1990 to 2009) in Belgium," *Journal of Clinical Microbiology*, vol. 49, no. 6, pp. 2200–2209, 2011.

[28] J. Cabrita, M. Oleastro, R. Matos et al., "Features and trends in *Helicobacter pylori* antibiotic resistance in Lisbon area, Portugal (1990–1999)," *Journal of Antimicrobial Chemotherapy*, vol. 46, no. 6, pp. 1029–1031, 2000.

[29] K. H. Hung, B. S. Sheu, W. L. Chang, H. M. Wu, C. C. Liu, and J. J. Wu, "Prevalence of primary fluoroquinolone resistance among clinical isolates of *Helicobacter pylori* at a University Hospital in Southern Taiwan," *Helicobacter*, vol. 14, no. 1, pp. 61–65, 2009.

[30] J.-T. Lin, Successful treatment of *Helicobacter pylori* infections with levofloxacin: a case report from Taiwan and a review of the literature, 2008, http://www.infectweb.com/only/artsrv2008_4.pdf.

[31] J. P. Gisbert, M. Castro-Fernández, F. Bermejo et al., "Third-line rescue therapy with levofloxacin after two H. pylori treatment failures," *American Journal of Gastroenterology*, vol. 101, no. 2, pp. 243–247, 2006.

[32] L. Marzio, D. Coraggio, S. Capodicasa, L. Grossi, and G. Cappello, "Role of the preliminary susceptibility testing for initial and after failed therapy of *Helicobacter pylori* infection with levofloxacin, amoxicillin, and esomeprazole," *Helicobacter*, vol. 11, no. 4, pp. 237–242, 2006.

[33] R. Cianci, M. Montalto, F. Pandolfi, G. B. Gasbarrini, and G. Cammarota, "Third-line rescue therapy for *Helicobacter pylori* infection," *World Journal of Gastroenterology*, vol. 12, no. 15, pp. 2313–2319, 2006.

[34] L. Gatta, A. Zullo, F. Perna et al., "A 10-day levofloxacin-based triple therapy in patients who have failed two eradication

courses," *Alimentary Pharmacology and Therapeutics*, vol. 22, no. 1, pp. 45–49, 2005.

[35] M. Castro-Fernández, E. Lamas, A. Pérez-Pastor et al., "Efficacy of triple therapy with a proton pump inhibitor, levofloxacin, and amoxicillin as first-line treatment to eradicate *Helicobacter pylori*," *Revista Espanola de Enfermedades Digestivas*, vol. 101, no. 6, pp. 399–402, 2009.

[36] J. P. Gisbert, A. Pérez-Aisa, M. Castro-Fernández et al., "*Helicobacter pylori* first-line treatment and rescue option containing levofloxacin in patients allergic to penicillin," *Digestive and Liver Disease*, vol. 42, no. 4, pp. 287–290, 2010.

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Review Article

Rescue Therapy for *Helicobacter pylori* Infection 2012

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Helicobacter pylori infection is the main cause of gastritis, gastroduodenal ulcer disease, and gastric cancer. After 30 years of experience in *H. pylori* treatment, however, the ideal regimen to treat this infection has still to be found. Nowadays, apart from having to know well first-line eradication regimens, we must also be prepared to face treatment failures. In designing a treatment strategy, we should not only focus on the results of primary therapy alone but also on the final—overall—eradication rate. The choice of a "rescue" treatment depends on which treatment is used initially. If a first-line clarithromycin-based regimen was used, a second-line metronidazole-based treatment (quadruple therapy) may be used afterwards, and then a levofloxacin-based combination would be a third-line "rescue" option. Alternatively, it has recently been suggested that levofloxacin-based "rescue" therapy constitutes an encouraging 2nd-line strategy, representing an alternative to quadruple therapy in patients with previous PPI-clarithromycin-amoxicillin failure, with the advantage of efficacy, simplicity and safety. In this case, quadruple regimen may be reserved as a 3rd-line "rescue" option. Even after two consecutive failures, several studies have demonstrated that *H. pylori* eradication can finally be achieved in almost all patients if several "rescue" therapies are consecutively given.

1. Introduction

Helicobacter pylori infection is the main known cause of gastritis, gastroduodenal ulcer disease, and gastric cancer. After 30 years of experience in *H. pylori* treatment, however, the ideal regimen to treat this infection has still to be found [1–3]. Consensus conferences have recommended therapeutic regimens that achieve *H. pylori* cure rates higher than 80% on an intention-to-treat basis [4–7]. However, several large clinical trials and meta-analyses have shown that the most commonly used first-line therapies, including proton pump inhibitors (PPIs) plus two antibiotics, may fail in ≥20% of patients [8, 9], and in the clinical routine setting, the treatment failure rate might be even higher [10, 11]. Moreover, during the last few years, the efficacy of PPI-based regimens seems to be decreasing, and several studies have reported intention-to-treat eradication rates lower than 75% and even lower than 50% [12–15]. Antibiotic resistance to clarithromycin has been identified as one of the major factors affecting our ability to cure *H. pylori* infection, and the rate of resistance to this antibiotic seems to be increasing in many geographical areas [16–19].

Several "rescue" therapies have been recommended, but they still fail to eradicate *H. pylori* in more than 20% of the cases, and these patients constitute a therapeutic dilemma [20–22]. Patients who are not cured with two consecutive treatments including clarithromycin and metronidazole will have at least single, and usually double, resistance [17, 23]. Furthermore, bismuth salts are not available worldwide anymore; therefore, management of first-line eradication failures is becoming challenging. Currently, a standard third-line therapy is lacking, and European guidelines recommend culture in these patients to select a third-line treatment according to microbial sensitivity to antibiotics [5, 6]. However, cultures are often carried out only in research centers, and the use of this procedure as "routine practice" in patients who failed several treatments seems not to be feasible [20, 21, 24–26]. Therefore, the evaluation of drugs without cross-resistance to nitroimidazole or macrolides as components of retreatment combination therapies would be worthwhile [27, 28].

All these issues are important at the present time, but they will be even more relevant in the near future, as

therapy for *H. pylori* infection is becoming more and more frequently prescribed. Therefore, the evaluation of second or third "rescue" regimens for these problematic cases seems to be worthwhile [29]. In designing a treatment strategy, we should not focus on the results of primary therapy alone; an adequate strategy for treating this infection should use several therapies which, if consecutively prescribed, come as close to the 100% cure rate as possible [20, 21, 25, 26, 30, 31].

The aim of the present paper will be to review the experience dealing with "nonresponders" to *H. pylori* eradication therapy, and specifically with *H. pylori* "rescue" therapies after failure of the first-line eradication regimen. As, at present, the current most prescribed first-line regimens include a combination of PPI plus two antibiotics, the present paper will focus on "rescue" regimen when these triple combinations fail. Bibliographical searches were performed in the PubMed (Internet) database including studies available until October 2011, looking for the following words (all fields): pylori AND (retreatment OR re-treatment OR rescue OR failure OR salvage OR second-line).

2. Is It Necessary to Perform Culture After Failure of the First Eradication Treatment?

Pretreatment antibiotic resistance is the most important factor in nonresponse to initial treatment [32]. Thus, the choice of a second-line treatment depends on which treatment was used initially, as it would appear that retreatment with the same regimen cannot be recommended [33]. If a clarithromycin-based regimen was used, a metronidazole-based treatment (or at least a clarithromycin-free regimen) should be used afterwards, and *vice versa* [34]. This recommendation is based on the observation that acquired bacterial resistance to metronidazole or clarithromycin results primarily from the previous treatment failure [32], and therefore "rescue" therapies should avoid these antibiotics and use different combinations.

An antimicrobial susceptibility test for *H. pylori* before second-line treatment is sometimes performed, although whether the test is truly necessary remains unknown. Some authors have evaluated the efficacy of susceptibility-guided versus empiric retreatment for *H. pylori* after a treatment failure. In the study by Yahav et al. [35], patients in whom at least one treatment regimen for *H. pylori* eradication had failed underwent gastric biopsy and culture and were retreated according to the *in vitro* susceptibility results. Findings were compared with those for control patients (where culture was unavailable). Susceptibility-guided retreatment was associated with better eradication rates (86%) than empiric treatment (63%). However, several methodological drawbacks exist in this study. Firstly, more than 50% of the patients received first-line eradication treatment with both clarithromycin and metronidazole (instead of including clarithromycin and amoxicillin), which is not the generally recommended combination; consequently, no logical empirical treatment remained afterwards (levofloxacin-based regimens were not available at that time). In this respect, when only the eradication rates in control (culture unavailable) patients treated with a first regimen of PPI-amoxicillin-clarithromycin followed by a second *empiric* quadruple regimen were considered (the generally recommended first- and second-line strategies), the success figures were not significantly different from those reported in patients receiving susceptibility-guided retreatment. Secondly, because this study was nonrandomized, there might have been heterogeneity among the two groups with respect to the treatment regimens prescribed by the treating physicians. Finally, this study was limited by the lack of susceptibility data for the controls, which restricted the ability to analyze the reasons why empiric therapy did not work as well as the susceptibility-guided protocol.

In a French multicenter study [36], patients, in whom one previous *H. pylori* eradication therapy (mainly with PPI-amoxicillin-clarithromycin) has failed, were randomized to receive one of three empirical triple-therapy regimens or a strategy based on antibiotic susceptibility. The empirical regimens were PPI-amoxicillin-clarithromycin (for 7 or 14 days) or PPI-amoxicillin-metronidazole (for 14 days). In the susceptibility-based strategy, patients with clarithromycin-susceptible strains received PPI-amoxicillin-clarithromycin, whilst the others received PPI-amoxicillin-metronidazole. The eradication rates for empirical therapies were low, while the cure rate was higher (74%) for the susceptibility-based treatment. If the *H. pylori* strain was clarithromycin-susceptible (which occurred in approximately 1/3 of the cases), a high-success rate was obtained with the PPI-clarithromycin-amoxicillin "rescue" regimen. The study, however, was done in France, where bismuth is banned, so that the use of quadruple therapy with a PPI, bismuth, tetracycline, and metronidazole as recommended by the updated Maastricht Consensus Report [6], was not tested. In fact, as it will be reviewed later, several studies have obtained relatively good results with this quadruple regimen empirically prescribed, with mean eradication rate of 77% (i.e., a similar figure than the 74% achieved for the susceptibility-based treatment in the present study). Thus, in this study, instead of not readministering any of the antibiotics against which *H. pylori* has probably become resistant, the authors insist on prescribing again clarithromycin (or metronidazole) for the second-line treatment. Furthermore, statistically significant differences were not demonstrated when comparing the efficacy of the empirical PPI-amoxicillin-metronidazole and the susceptibility-based strategy, suggesting that the metronidazole-based combination may be an effective empirical alternative after failure of a clarithromycin-based combination.

In the updated Maastricht Consensus Report [6], it was recommended that culture and antimicrobial sensitivity testing should be routinely performed only after two treatment failures with different antibiotics. According to this statement, some studies have suggested that an antimicrobial susceptibility test for *H. pylori* before administering second-line treatment is not necessary. In this respect, in the study by Avidan et al. [37], after failure of first-line eradication treatment, half of the patients were randomly assigned to treatment with a different PPI-based triple regimen regardless of the culture obtained, and the other half were assigned

to treatment with PPI and two antibacterial agents chosen according to a susceptibility test; the authors found that the culture results did not influence the treatment protocol employed. Similarly, in the study by Miwa et al [38], patients with *H. pylori* infection for whom first-line treatment with a PPI-amoxicillin-clarithromycin regimen had failed were randomly assigned to two groups: those having or not having the susceptibility test before retreatment. For those patients in the susceptibility-test group, the authors used what they considered the best regimen based on susceptibility testing; while for those patients in the group with no susceptibility testing, PPI-amoxicillin-metronidazole was prescribed. The cure rates in the groups with and without susceptibility testing were not different.

3. Second-Line *H. pylori* "Rescue" Therapy after Failure of One Eradication Treatment

3.1. "Rescue" Regimen after PPI-Clarithromycin-Amoxicillin Failure

3.1.1. PPI, Amoxicillin, and Metronidazole. After failure of a combination of PPI, amoxicillin, and clarithromycin, a theoretically correct alternative would be the use, as second option, of other PPI-based triple therapy including amoxicillin (that does not induce resistance) and metronidazole (an antibiotic not used in the first trial), and several authors have reported encouraging results with this strategy [38–45]. However, in our experience, when this therapy has been administered twice daily for one week, eradication rates lower than 50% have been obtained [46]; the subsequently use of higher (three times per day) antibiotic doses was followed only by a mild increase in eradication rate (58%), which was still unacceptable [46]. Nagahara et al. [47] studied a group of patients who, after failure of first-line PPI-clarithromycin-amoxicillin therapy, had received second-line therapy with the same regimen (for 14 days) or had received PPI-amoxicillin-metronidazole (for 10 days). The eradication rate for second-line therapy with the same regimen (thus readministering clarithromycin) was of only 53%, while it was of 81% with PPI-amoxicillin-metronidazole. These observations underlie the idea that antibiotics, and specifically clarithromycin, should not be readministered in successive treatments.

3.1.2. Quadruple Therapy. Another alternative, the use of a quadruple regimen (i.e., PPI, bismuth, tetracycline, and metronidazole), has been generally used as the optimal second-line therapy after PPI-clarithromycin-amoxicillin failure and has been the recommended "rescue" regimen in several guidelines [6, 48, 49]. Several studies have obtained relatively good results with this quadruple regimen, the results are summarized in Table 1 [46, 50–64]. Thus, the weighted mean eradication rate with this "rescue" therapy, calculated from the studies included in the table, is of 77%. In this combination regimen, PPI should be prescribed in the usual dose and twice a day, colloidal bismuth subcitrate 120 mg four times per day, tetracycline 500 mg four times per day, and metronidazole is probably best prescribed at high doses (i.e., 500 mg three times per day). Precisely, the study with the lowest efficacy [56] administered metronidazole at low doses (250 mg four times per day). Although PPIs are generally prescribed as the antisecretors in quadruple therapy, some authors have shown, in a randomized study, that omeprazole 20 mg b.i.d. and ranitidine 300 mg b.i.d. were equally effective as antisecretory agents combined in a second-line quadruple eradication regimen after failure with previous regimens without metronidazole (although the power of the study to find statistically significant differences was limited) [61]. Nevertheless, these regimens were administered during 14 days; therefore, it remains to be

Table 1: Eradication rates with quadruple therapy (proton pump inhibitor, bismuth, tetracycline, and a nitroimidazole) as "rescue" therapy for proton pump inhibitor-clarithromycin-amoxicillin failure.

Author	Number of patients	Duration (days)	Eradication rate (%)
Baena et al. [50]	31	14	90
Bilardi et al. [51]	46	7	37
Elizalde et al. [52]	31	7	87
Choung et al. [53]	56	7	77
Choung et al. [53]	99	14	88
Su et al. [54]	87	7	84
Chung et al. [64]	90	7	82
Chung et al. [64]	101	14	85
Gasbarrini et al. [55]	9	7	88
Gisbert et al. [56]	30	7	57
Gisbert et al. [46]	9	7	78
Gomollón et al. [57]	21	7	95
Lee et al. [58]	20	7	68
Lee et al. [59]	63	7	75
Lee et al. [153]	112	7	64
Lee et al. [153]	115	10	83
Marko et al. [60]	27	7	63
Michopoulos et al. [61]	38	14	76
Navarro-Jarabo et al. [62]	54	7	70
Nista et al. [63]	70	7	63
Nista et al. [63]	70	14	68
Orsi et al. [93]	50	12	88
Perri et al. [154]	45	10	67
Perri et al. [92]	60	7	83
Sicilia et al. [155]	21	10	83
Usta et al. [156]	89	14	67
Uygun et al. [157]	100	14	82
Wong et al. [94]	53	7	91
Wu et al. [158]	47	7	77
Wu et al. [159]	62	7	81

Eradication rates by intention-to-treat analysis when available. *H. pylori* eradication rate (weighted mean) with quadruple therapy: 77%.

demonstrated if the equivalence between both antisecretors—PPIs and H_2-blockers—is also observable with 7-day or 10-day regimens.

The question may be suggested whether treatment with PPI-clarithromycin-amoxicillin followed by "rescue" with quadruple therapy if failure is preferable to the inverse strategy. To analyze this interesting aspect, Gomollón et al. [65] randomized consecutive patients to one of two strategies: (a) treatment during 7 days with quadruple therapy, and if failure second-line treatment with omeprazole-clarithromycin-amoxicillin during 7 days, (b) initial treatment with omeprazole-clarithromycin-amoxicillin, and if failure treatment with quadruple therapy. Direct and indirect costs were estimated, and a cost-effectiveness analysis using a decision-tree model was undertaken after real clinical data. Eradication was obtained (intention-to-treat) in 73% with the first strategy, *versus* 92% with the second one. Furthermore, cost per case eradicated was lower in the second group (320 *versus* 296 Euros). However, in a similar but more recent study, Marko et al. [60] assessed the usefulness and the cost-effectiveness of these two treatment strategies, performing a decision analysis. The effectiveness of "triple first" and "quadruple first" strategies was similar, although the latter seemed slightly more cost-effective.

3.1.3. PPI, Amoxicillin, and Levofloxacin. As previously mentioned, after failure of a combination of a PPI-based triple regimen, the use of the quadruple therapy has been generally recommended as the optimal second-line therapy based on the relatively good results reported by several authors. However, this quadruple regimen requires the administration of 4 drugs with a complex scheme (bismuth and tetracycline usually prescribed every 6 hours, and metronidazole every 8 hours) and is associated with a relatively high incidence of adverse effects [20]; however, this drawback may be overcome, thanks to a novel single capsule containing bismuth, metronidazole, and tetracycline that has recently become available [66, 67]. Nevertheless, this quadruple regimen still fails to eradicate *H. pylori* in approximately 20 to 30% of the patients, and these cases constitute a therapeutic dilemma, as patients who are not cured with two consecutive treatments including clarithromycin and metronidazole will usually have double resistance [20].

Levofloxacin is a fluoroquinolone antibacterial agent with a broad spectrum of activity against Gram-positive and Gram-negative bacteria and atypical respiratory pathogens [68]. Recently, some studies have evaluated the efficacy of new fluoroquinolones, such as levofloxacin, that could prove to be a valid alternative to standard antibiotics not only as first-line therapies but, more interesting, as second-line regimens [21, 69–71]. In this respect, levofloxacin-based second-line therapies represent an encouraging strategy for eradication failures, as some studies have demonstrated that levofloxacin has, *in vitro*, remarkable activity against *H. pylori* [72], and that primary resistances to such antibiotic in several countries are (still) relatively infrequent (when compared with metronidazole or clarithromycin) [73–80]. A recent *in vitro* study also showed a synergistic effect of quinolone antimicrobial agents and PPIs on strains of *H. pylori* [81]. Furthermore, it has been shown *in vitro* that levofloxacin retains its activity when *H. pylori* strains are resistant to clarithromycin and metronidazole [76, 82, 83]. These favorable results have been confirmed *in vivo*, indicating that most of the patients with both metronidazole and clarithromycin resistance are cured with the levofloxacin-based regimen [51, 75, 84, 85].

A combination of a PPI, amoxicillin and levofloxacin, as first-line regimen, has been associated with favorable results, with mean eradication rates of about 90% [76, 86–91]. Later, other authors studied this same regimen in patients with one previous eradication failure, also reporting exciting results, with *H. pylori* cures rates ranging from 60% to 94%% [51, 63, 82, 84, 91–104]. A recent systematic paper showed a mean eradication rate with levofloxacin-based "rescue" regimens (combined with amoxicillin and a PPI in most studies) of 80%, which represents a relatively high figure when considering that this regimen was evaluated as a "rescue" therapy [70]. This systematic paper found, in agreement with recent randomized clinical trials [105], higher *H. pylori* cure rates with 10-day than with 7-day regimens, both in general (81% versus 73%) and also with the levofloxacin-amoxicillin-PPI combination in particular (80% versus 68%), suggesting that the longer (10-day) therapeutic scheme should be chosen.

Furthermore, three recent meta-analyses have suggested that after *H. pylori* eradication failure, levofloxacin-based "rescue" regimen is more effective than the generally recommended quadruple therapy [69, 70, 106]. In one of these meta-analyses [70], higher *H. pylori* cure rates with the levofloxacin-based triple regimens than with the quadruple combinations were found (81% versus 70%), but with borderline statistical significance (Figure 1). Nevertheless, results were heterogeneous, mainly due to the discordant results of the study by Perri et al. [92], who reported a cure rate of only 63% with the levofloxacin-regimen, the lowest reported in the literature, a figure that contrasts with the mean eradication rate of 80% calculated in a systematic paper [70]. Nevertheless, when that single outlier study [92] was excluded from the meta-analysis, the difference between cure rates with both regimens reached statistical significance and heterogeneity markedly decreased. Furthermore, when only high-quality studies were considered, the advantage of the levofloxacin regimen over the quadruple regimen increased (88% versus 64%), also achieving statistical significance, and heterogeneity among studies almost disappeared [70]. Nevertheless, the benefit of the levofloxacin-based "rescue" regimen seems to be less clear in Asia, as two studies from Taiwan and Hong Kong showed that levofloxacin-based triple therapies were at most comparable to quadruple therapy [95, 102].

As previously mentioned, the quadruple regimen requires the administration of a complex scheme [20]. On the contrary, levofloxacin-based regimens (with amoxicillin and PPIs administered twice daily, and levofloxacin every 12 or 24 hours) represent an encouraging alternative to quadruple therapy, with the advantage of simplicity. Furthermore, the quadruple regimen is associated with a relatively high incidence of adverse effects [20]. In contrast, levofloxacin

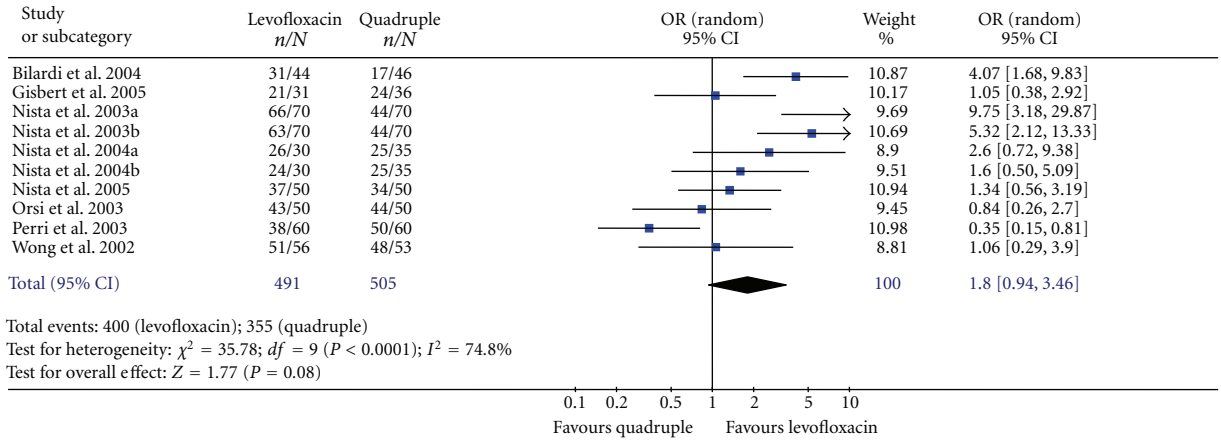

Figure 1: Meta-analysis comparing *H. pylori* eradication efficacy with levofloxacin-based triple regimens *versus* quadruple therapy, as second-line "rescue" regimen after failure of a proton pump inhibitor-amoxicillin-clarithromycin.

is generally well tolerated, and most adverse events associated with its use are mild to moderate in severity and transient [68]. The most frequent adverse effects affect the gastrointestinal tract [68]. Occasional cases of tendinitis and tendon rupture have been reported in the literature with levofloxacin therapy [51, 68]. However, data derived from more than 15 million prescriptions in the US indicated the rate is fewer than 4 per million prescriptions [107]. *Clostridium difficile* infection may be associated with the use of this broad spectrum activity antibiotic [68]. In the aforementioned systematic review [70], adverse effects were reported, overall, by 18% of the patients treated with levofloxacin-based therapies, and these adverse effects were severe (defined so by the authors or explaining treatment discontinuation) in only 3% of the cases. Furthermore, the incidence of adverse effects was not different when levofloxacin-amoxicillin-PPI was administered for 7 or 10 days, supporting the aforementioned recommendation of prescribing the more effective 10-day regimen. Moreover, two meta-analyses have demonstrated a lower incidence of adverse effects with levofloxacin-based treatments than with the quadruple combinations [69, 70]. Finally, it has recently been demonstrated that moxifloxacin-containing triple regimen is more effective and better tolerated than the bismuth-containing quadruple therapy in the second-line treatment of *H. pylori* infection [106].

Unfortunately, it has been shown that resistance to quinolones is easily acquired, and in countries with a high consumption of these drugs, the resistance rate is increasing and is already relatively high [75, 88, 95, 108–125]. More importantly, it has been demonstrated that the presence of levofloxacin resistance significantly reduce the eradication rate following a therapy with this antibiotic [75, 88, 118, 126, 127]. Therefore, it has been suggested to reserve levofloxacin for "rescue" treatment to avoid the increase of the resistance phenomenon [128].

3.2. "Rescue" Regimen after PPI-Amoxicillin-Nitroimidazole Failure. After PPI-amoxicillin-nitroimidazole failure, retreatment with PPI-amoxicillin-clarithromycin has proved to be very effective, and it seems to be a logical strategy, as while amoxicillin is maintained (which does not induce resistance), clarithromycin is substituted for metronidazole. Furthermore, the absence of cross-resistance among nitroimidazoles and clarithromycin favors this position. With this therapy, some authors [46] have achieved *H. pylori* eradication in 85% of cases, while others have reported success rates of 86% [129] or even 100%% [130]. In favor of this strategy is the study by Magaret et al. [131] who studied a group of 48 patients after failure of previous *H. pylori* therapy with a metronidazole-containing regimen and randomized them to either lansoprazole, amoxicillin, and clarithromycin twice daily for 14 days (i.e., the logical approach with triple therapy not repeating metronidazole) or to lansoprazole, bismuth, metronidazole and tetracycline for 14 days (i.e., the quadruple therapy repeating metronidazole). Intention-to-treat efficacies were 75% for triple regimen and 71% for quadruple. Although this difference did not reach statistical significance, the small sample size of this study does not preclude the possibility of a small but clinically significant difference in efficacy between the regimens.

3.3. "Rescue" Regimen after PPI-Clarithromycin-Nitroimidazole Failure. As previously mentioned, acquired bacterial resistance to metronidazole or clarithromycin results primarily from the previous treatment failure [132], and therefore the first choice probably should not be a regimen that combines these two antibiotics in the same regimen [30, 31, 133]. Although this regimen is very effective [8], patients who are not cured will probably have double resistance [134, 135], and no logical empirical treatment remains afterwards (although, more recently, the levofloxacin-based regimens may represent an option). Thus, some authors have demonstrated that initial regimens containing both clarithromycin and nitroimidazole are associated with significantly worse results *overall*, with lower eradication rates after logically chosen second-line therapy and sensitivity-directed third-line therapy; these poor results were due to the emergence of multiply resistant strains as evidenced by the results of culture testing after the second failed course

[136]. In summary, due to problems with resistance it could be suggested that both key antibiotics—clarithromycin and metronidazole—should not be used together until a valid empirical back up regimen is available [30].

Nevertheless, if culture is not performed after failure of PPI-clarithromycin-metronidazole, and hence antibiotic susceptibility is unknown, several "rescue" options may be suggested. Firstly, omeprazole plus amoxicillin, with a high dose of both the antibiotic and the antisecretor, could, in theory, be recommended [133, 137]; however, we must remember that this "old-fashioned" dual combination has achieved disappointing results in many countries [138]. Therefore, a second antibiotic should be added, and at this point a difficult decision appears, as both antibiotics used in the first trial (clarithromycin and metronidazole) are capable of inducing secondary resistance to *H. pylori*, playing a negative role in future efficacy [134, 139–144]. Nevertheless, the following possibilities exist.

3.3.1. Readministering Metronidazole. Due to the fact that metronidazole resistance is frequent and clinically relevant [134, 139–141], if this antibiotic is readministered, it should be used within bismuth-based quadruple regimen (thus PPI might reduce the negative effect of metronidazole resistance [57, 141, 145]). With this regimen, eradication rates up to 80% have been achieved [46].

3.3.2. Readministering Clarithromycin. Several studies have underlined the relevance of clarithromycin resistance [134, 139, 140, 142], which advise against readministering this antibiotic. Therefore, a further option which has been proposed is to add (e.g., to PPI-amoxicillin-clarithromycin) a fourth medication (as bismuth [146, 147]) with bactericidal effect against *H. pylori*, with which 70% eradication rate has been achieved [46].

3.3.3. Readministering No Antibiotic. A final alternative, obviously, consists of no readministering either metronidazole or clarithromycin. Although only published in abstract form, one study has prescribed ranitidine bismuth citrate, tetracycline, and amoxicillin for 2 weeks and has reported the eradication in 89% of the cases who had previously failed PPI, clarithromycin, and tinidazole [148]. These encouraging results may be due, at least in part, to the use of ranitidine bismuth citrate instead of bismuth in this regimen, as "classic" triple therapy with bismuth, tetracycline, and amoxicillin has been previously considered relatively ineffective. Finally, although not specifically evaluated in PPI-clarithromycin-metronidazole failures, rifabutin, or levofloxacin-based regimens (e.g., PPI, amoxicillin and either levofloxacin or rifabutin) could play a role in this difficult situation. However, several concerns remain regarding rifabutin treatment [149]. Firstly, this drug is very expensive. Secondly, severe leucopoenia and thrombocytopenia have been reported in some patients treated with rifabutin. Finally, there is some concern about a wide-spread use of rifabutin, a member of a class of established antimycobacterial drugs, in patients with *H. pylori* infection. Because multiresistant strains of *Mycobacterium tuberculosis* increase in numbers, indications for these drugs should be chosen very carefully to avoid further acceleration of development of resistance.

3.4. "Rescue" Regimen after Nonbismuth Quadruple "Sequential" and "Concomitant" Treatment Failure. As previously mentioned, the most widely recommended treatment for the eradication of *H. pylori* is the standard, or PPI-based triple therapy, which combines 2 antibiotics (clarithromycin plus amoxicillin or metronidazole). However, one recent innovation, postulated as an alternative to standard triple therapy, is sequential treatment, which involves a simple dual regimen including a PPI plus amoxicillin for the first 5 days followed by a triple regimen including a PPI, clarithromycin, and tinidazole for the following 5 days [2]. On the other hand, the concept of a nonbismuth quadruple regimen or "concomitant" regimen has recently resurfaced. Traditional standard triple therapy (PPI-clarithromycin-amoxicillin) can easily be converted to "concomitant" therapy by the addition of 500 mg of metronidazole or tinidazole twice daily [3].

It remains unclear how failure of non-bismuth quadruple "sequential" or "concomitant" therapy should be managed. One potential disadvantage of these therapies is that patients with failed eradication would have limited options for further treatment, because they would already have received 3 different antibiotics: amoxicillin, clarithromycin, and nitroimidazole. However, the recent appearance of levofloxacin may overcome this problem. Thus, Zullo et al. [150] recently performed a pilot study on patients who failed sequential therapy; following 10-day triple therapy with a PPI, levofloxacin, and amoxicillin, *H. pylori* infection was successfully cured in 86% of cases. In another study, Perna et al. [118] prescribed a 10-day triple regimen with a PPI, levofloxacin, and amoxicillin in patients in whom first treatment with either standard 10-day triple or sequential therapy (only 10 patients) had failed. *H. pylori* was eradicated in 73% of cases, although the authors do not provide separate efficacy rates depending on the first (failure) treatment. These data seem to indicate that a triple regimen (PPI-levofloxacin-amoxicillin) is a suitable approach for second-line treatment in patients whose sequential—and probably also concomitant—therapy fails.

4. Conclusion

Even with the current most effective treatment regimens, ≥20% of patients will fail to eradicate *H. pylori* infection. This paper seems important at the present time, as therapy for *H. pylori* infection is becoming more and more frequently prescribed. Nowadays, apart from having to know well first-line eradication regimens, we must also be prepared to face treatment failures. Therefore, in designing a treatment strategy we should not only focus on the results of primary therapy alone, but also on the final—overall—eradication rate.

The choice of a "rescue" treatment depends on which treatment is used initially. If a first-line clarithromycin-based regimen was used, a second-line metronidazole-based

treatment (such as the quadruple therapy) may be used afterwards, and then a levofloxacin-based combination would be a third-line "rescue" option. Alternatively, it has recently been suggested that levofloxacin-based "rescue" therapy constitutes an encouraging 2nd-line strategy, representing an alternative to quadruple therapy in patients with previous PPI-clarithromycin-amoxicillin failure, with the advantage of efficacy, simplicity, and safety. In this case, quadruple regimen may be reserved as a 3rd-line "rescue" option.

Even after two consecutive failures, several studies have demonstrated that *H. pylori* eradication can finally be achieved in almost all patients if several "rescue" therapies are consecutively given [22, 151]. As a final conclusion, therefore, the attitude in *H. pylori* eradication therapy failure, even after two or more unsuccessful attempts, should be to fight and not to surrender [152].

Abbreviations

H. pylori: *Helicobacter pylori*
PPI: Proton pump inhibitor.

References

[1] N. Vakil, "*H. pylori* treatment: new wine in old bottles," *American Journal of Gastroenterology*, vol. 104, no. 1, pp. 26–30, 2009.

[2] J. P. Gisbert, X. Calvet, A. O'Connor, F. Mégraud, and C. A. O'Morain, "Sequential therapy for *Helicobacter pylori* eradication: a critical review," *Journal of Clinical Gastroenterology*, vol. 44, no. 5, pp. 313–325, 2010.

[3] J. P. Gisbert and X. Calvet, "Review article: non-bismuth quadruple (concomitant) therapy for eradication of Helicobater pylori," *Alimentary Pharmacology and Therapeutics*, vol. 34, no. 6, pp. 604–617, 2011.

[4] C. W. Howden and R. H. Hunt, "Guidelines for the management of *Helicobacter pylori* infection," *American Journal of Gastroenterology*, vol. 93, no. 12, pp. 2330–2338, 1998.

[5] P. Malfertheiner, F. Mégraud, C. O'Morain et al., "Current concepts in the management of *Helicobacter pylori* infection—the Maastricht 2-2000 Consensus Report," *Alimentary Pharmacology and Therapeutics*, vol. 16, no. 2, pp. 167–180, 2002.

[6] P. Malfertheiner, F. Megraud, C. O'Morain et al., "Current concepts in the management of *Helicobacter pylori* infection: the Maastricht III Consensus Report," *Gut*, vol. 56, no. 6, pp. 772–781, 2007.

[7] M. Selgrad, J. Bornschein, and P. Malfertheiner, "Guidelines for treatment of *Helicobacter pylori* in the East and West," *Expert Review of Anti-Infective Therapy*, vol. 9, no. 8, pp. 581–588, 2011.

[8] J. P. Gisbert, L. González, X. Calvet et al., "Proton pump inhibitor, clarithromycin and either amoxycillin or nitroimidazole: a meta-analysis of eradication of *Helicobacter pylori*," *Alimentary Pharmacology and Therapeutics*, vol. 14, no. 10, pp. 1319–1328, 2000.

[9] J. P. Gisbert, R. Pajares, and J. M. Pajares, "Evolution of *Helicobacter pylori* therapy from a meta-analytical perspective," *Helicobacter*, vol. 12, no. 2, pp. 50–58, 2007.

[10] D. Y. Graham and L. Fischbach, "*Helicobacter pylori* treatment in the era of increasing antibiotic resistance," *Gut*, vol. 59, no. 8, pp. 1143–1153, 2010.

[11] D. Y. Graham and L. Fischbach, "Sequential therapy for *Helicobacter pylori* infection: ethical considerations revisited," *Journal of Clinical Gastroenterology*, vol. 44, no. 7, p. 523, 2010.

[12] A. O'Connor, J. P. Gisbert, D. McNamara, and C. O'Morain, "Treatment of *Helicobacter pylori* Infection 2010," *Helicobacter*, vol. 15, no. 1, pp. 46–52, 2010.

[13] A. O'Connor, J. P. Gisbert, D. Mcnamara, and C. O'Morain, "Treatment of *Helicobacter pylori* infection 2011," *Helicobacter*, vol. 16, supplement 1, pp. 53–58, 2011.

[14] J. P. Gisbert and X. Calvet, "Review article: the effectiveness of standard triple therapy for *Helicobacter pylori* has not changed over the last decade, but it is not good enough," *Alimentary Pharmacology and Therapeutics*, vol. 34, no. 11-12, pp. 1255–1268, 2011.

[15] J. P. Gisbert and A. G. McNicholl, "Maintenance of *Helicobacter pylori* eradication rates with triple therapy over 12 years in a Spanish hospital," *Helicobacter*. In press.

[16] N. Vakil, B. Hahn, and D. McSorley, "Clarithromycin-resistant *Helicobacter pylori* in patients with duodenal ulcer in the United States," *American Journal of Gastroenterology*, vol. 93, no. 9, pp. 1432–1435, 1998.

[17] F. Mégraud, "H pylori antibiotic resistance: prevalence, importance, and advances in testing," *Gut*, vol. 53, no. 9, pp. 1374–1384, 2004.

[18] B. J. Egan, L. Marzio, H. O'Connor, and C. O'Morain, "Treatment of *Helicobacter pylori* infection," *Helicobacter*, vol. 13, no. 1, pp. 35–40, 2008.

[19] J. Yakoob, S. Abid, Z. Abbas, and S. N.W. Jafri, "Antibiotic susceptibility patterns of *Helicobacter pylori* and triple therapy in a high-prevalence area," *British Journal of Biomedical Science*, vol. 67, no. 4, pp. 197–201, 2010.

[20] J. P. Gisbert and J. M. Pajares, "Review article: *Helicobacter pylori* 'rescue' regimen when proton pump inhibitor-based triple therapies fail," *Alimentary Pharmacology and Therapeutics*, vol. 16, no. 6, pp. 1047–1057, 2002.

[21] J. P. Gisbert and J. M. Pajares, "*Helicobacter pylori* "rescue" therapy after failure of two eradication treatments," *Helicobacter*, vol. 10, no. 5, pp. 363–372, 2005.

[22] J. P. Gisbert, J. L. Gisbert, S. Marcos, I. Jimenez-Alonso, R. Moreno-Otero, and J. M. Pajares, "Empirical rescue therapy after *Helicobacter pylori* treatment failure: a 10-year single-centre study of 500 patients," *Alimentary Pharmacology and Therapeutics*, vol. 27, no. 4, pp. 346–354, 2008.

[23] M. Romano, M. R. Iovene, M. I. Russo et al., "Failure of first-line eradication treatment significantly increases prevalence of antimicrobial-resistant *Helicobacter pylori* clinical isolates," *Journal of Clinical Pathology*, vol. 61, no. 10, pp. 1112–1115, 2008.

[24] A. Zullo, C. Hassan, R. Lorenzetti, S. Winn, and S. Morini, "A clinical practice viewpoint: to culture or not to culture *Helicobacter pylori*?" *Digestive and Liver Disease*, vol. 35, no. 5, pp. 357–361, 2003.

[25] J. P. Gisbert, ""Rescue" regimens after *Helicobacter pylori* treatment failure," *World Journal of Gastroenterology*, vol. 14, no. 35, pp. 5385–5402, 2008.

[26] J. P. Gisbert, "Rescue therapy after *Helicobacter pylori* eradication failure," *Gastroenterologia y Hepatologia*, vol. 34, no. 2, pp. 89–99, 2011.

[27] D. Y. Graham, "Efficient identification and evaluation of effective *Helicobacter pylori* therapies," *Clinical Gastroenterology and Hepatology*, vol. 7, no. 2, pp. 145–148, 2009.

[28] X. Calvet, J. P. Gisbert, and D. Suarez, "Key points for designing and reporting *Helicobacter pylori* therapeutic trials:

a personal view," *Helicobacter*, vol. 16, no. 5, pp. 346–355, 2011.

[29] J. Luther, W. D. Chey, and R. J. Saad, "A clinician's guide to salvage therapy for persistent *Helicobacter pylori* infection," *Hospital Practice*, vol. 39, no. 1, pp. 133–140, 2011.

[30] W. A. de Boer and G. N. Tytgat, "Regular review: treatment of Helicobacter pylori infection," *British Medical Journal*, vol. 320, no. 7226, pp. 31–34, 2000.

[31] X. Calvet, E. Gené, and I. Sanfeliu, "Therapeutic strategies in *Helicobacter pylori* infection," *Medicina Clinica*, vol. 116, no. 6, p. 239, 2001.

[32] F. Megraud, "*Helicobacter pylori* and antibiotic resistance," *Gut*, vol. 56, no. 11, p. 1502, 2007.

[33] F. Parente, C. Cucino, and G. Bianchi Porro, "Treatment options for patients with *Helicobacter pylori* infection resistant to one or more eradication attempts," *Digestive and Liver Disease*, vol. 35, no. 8, pp. 523–528, 2003.

[34] G. Battaglia, F. Di Mario, S. Vigneri et al., "Strategy for the retreatment of failed *Helicobacter pylori* eradication therapy: a case series," *Italian Journal of Gastroenterology and Hepatology*, vol. 30, no. 4, pp. 370–374, 1998.

[35] J. Yahav, Z. Samra, Y. Niv et al., "Susceptibility-guided vs. empiric retreatment of *Helicobacter pylori* infection after treatment failure," *Digestive Diseases and Sciences*, vol. 51, no. 12, pp. 2316–2321, 2006.

[36] H. Lamouliatte, F. Mégraud, J.-C. Delchier et al., "Second-line treatment for failure to eradicate *Helicobacter pylori*: a randomized trial comparing four treatment strategies," *Alimentary Pharmacology and Therapeutics*, vol. 18, no. 8, pp. 791–797, 2003.

[37] B. Avidan, E. Melzer, N. Keller, and S. Bar-Meir, "The effect of culture results for *Helicobacter pylori* on the choice of treatment following failure of initial eradication," *Israel Medical Association Journal*, vol. 3, no. 3, pp. 163–165, 2001.

[38] H. Miwa, A. Nacahara, A. Kurosawa et al., "Is antimicrobial susceptibility testing necessary before second-line treatment for *Helicobacter pylori* infection?" *Alimentary Pharmacology and Therapeutics*, vol. 17, no. 12, pp. 1545–1551, 2003.

[39] H. Isomoto, K. Inoue, H. Furusu et al., "High-dose rabeprazole-amoxicillin versus rabeprazole-amoxicillin-metronidazole as second-line treatment after failure of the Japanese standard regimen for *Helicobacter pylori* infection," *Alimentary Pharmacology and Therapeutics*, vol. 18, no. 1, pp. 101–107, 2003.

[40] A. Nagahara, H. Miwa, M. Kawabe et al., "Second-line treatment for *Helicobacter pylori* infection in Japan: proton pump inhibitor-based amoxicillin and metronidazole regimen," *Journal of Gastroenterology*, vol. 39, no. 11, pp. 1051–1055, 2004.

[41] T. Shimoyama, S. Fukuda, T. Mikami, M. Fukushi, and A. Munakata, "Efficacy of metronidazole for the treatment of clarithromycin-resistant *Helicobacter pylori* infection in a Japanese population," *Journal of Gastroenterology*, vol. 39, no. 10, pp. 927–930, 2004.

[42] K. Murakami, T. Okimoto, M. Kodama et al., "Comparison of amoxicillin-metronidazole plus famotidine or lansoprazole for amoxicillin-clarithromycin-proton pump inhibitor treatment failures for *Helicobacter pylori* infection," *Helicobacter*, vol. 11, no. 5, pp. 436–440, 2006.

[43] K. Murakami, T. Okimoto, M. Kodama, R. Sato, K. Watanabe, and T. Fujioka, "Evaluation of three different proton pump inhibitors with amoxicillin and metronidazole in retreatment for *Helicobacter pylori* infection," *Journal of Clinical Gastroenterology*, vol. 42, no. 2, pp. 139–142, 2008.

[44] N. Ueki, K. Miyake, M. Kusunoki et al., "Impact of quadruple regimen of clarithromycin added to metronidazole-containing triple therapy against *Helicobacter pylori* infection following clarithromycin-containing triple-therapy failure," *Helicobacter*, vol. 14, no. 2, pp. 91–99, 2009.

[45] K. Hori, H. Miwa, and T. Matsumoto, "Efficacy of 2-week, second-line *Helicobacter pylori* eradication therapy using rabeprazole, amoxicillin, and metronidazole for the Japanese population," *Helicobacter*, vol. 16, no. 3, pp. 234–240, 2011.

[46] J. P. Gisbert, D. Boixeda, F. Bermejo et al., "Re-treatment after *Helicobacter pylori* eradication failure," *European Journal of Gastroenterology and Hepatology*, vol. 11, no. 9, pp. 1049–1054, 1999.

[47] A. Nagahara, H. Miwa, R. Ohkura et al., "Strategy for retreatment of therapeutic failure of eradication of *Helicobacter pylori* infection," *Journal of Gastroenterology and Hepatology*, vol. 16, no. 6, pp. 613–618, 2001.

[48] S. K. Lam and N. J. Talley, "Report of the 1997 Asia Pacific Consensus Conference on the management of *Helicobacter pylori* infection," *Journal of Gastroenterology and Hepatology*, vol. 13, no. 1, pp. 1–12, 1998.

[49] J. P. Gisbert, X. Calvet, F. Gomollon, and R. Sainz, "Treatment for the eradication of *Helicobacter pylori*. Recommendations of the Spanish Consensus Conference," *Medicina Clínica*, vol. 114, no. 5, pp. 185–195, 2000.

[50] J. M. Baena Díez, C. López Mompó, F. Rams Rams, M. García Lareo, M. R. Hernández Ibáñez, and J. Teruel Gila, "Efficacy of a multistep strategy for *Helicobacter pylori* radication: quadruple therapy with omeprazole, metronidazole, tetracycline and bismuth after failure of combination of omeprazole, clarithromycin and amoxycillin," *Medicina Clinica*, vol. 115, no. 16, pp. 617–619, 2000.

[51] C. Bilardi, P. Dulbecco, P. Zentilin et al., "A 10-day levofloxacin-based therapy in patients with resistant *Helicobacter pylori* infection: a controlled trial," *Clinical Gastroenterology and Hepatology*, vol. 2, no. 11, pp. 997–1002, 2004.

[52] I. R. Elizalde, F. Borda, C. Jara, and A. Martínez, "Eficacia de dos tratamientos consecutivos en la erradicación de *Helicobacter pylori*," *Anales del Sistema Sanitario de Navarra*, vol. 21, supplement 2, pp. 83–88, 1998.

[53] R. S. Choung, S. W. Lee, S. W. Jung et al., "Comparison of the effectiveness of quadruple salvage regimen for *Helicobacter pylori* infection according to the duration of treatment," *The Korean Journal of Gastroenterology*, vol. 47, no. 2, pp. 131–135, 2006.

[54] J. C. Su, H. L. Dong, N. Kim et al., "Eradication rates of *Helicobacter pylori* infection with second-line treatment: non-ulcer dyspepsia compared to peptic ulcer disease," *Hepato-Gastroenterology*, vol. 54, no. 76, pp. 1293–1296, 2007.

[55] A. Gasbarrini, V. Ojetti, D. Pitocco et al., "Efficacy of different *Helicobacter pylori* eradication regimens in patients affected by insulin-dependent diabetes mellitus," *Scandinavian Journal of Gastroenterology*, vol. 35, no. 3, pp. 260–263, 2000.

[56] J. P. Gisbert, J. L. Gisbert, S. Marcos, R. García Grávalos, D. Carpio, and J. M. Pajares, "Seven-day "rescue" therapy after *Helicobacter pylori* treatment failure: omeprazole, bismuth, tetracycline and metronidazole vs. ranitidine bismuth citrate, tetracycline and metronidazole," *Alimentary Pharmacology and Therapeutics*, vol. 13, no. 10, pp. 1311–1316, 1999.

[57] F. Gomollón, J. A. Ducóns, M. Ferrero et al., "Quadruple therapy is effective for eradicating *Helicobacter pylori* after failure of triple proton-pump inhibitor-based therapy:

a detailed, prospective analysis of 21 consecutive cases," *Helicobacter*, vol. 4, no. 4, pp. 222–225, 1999.

[58] J. M. Lee, N. P. Breslin, D. K. Hyde, M. J. Buckley, and C. A. O'Morain, "Treatment options for *Helicobacter pylori* infection when proton pump inhibitor-based triple therapy fails in clinical practice," *Alimentary Pharmacology and Therapeutics*, vol. 13, no. 4, pp. 489–496, 1999.

[59] J. H. Lee, J. H. Cheon, M. J. Park et al., "The trend of eradication rates of second-line quadruple therapy containing metronidazole for *Helicobacter pylori* infection: an analysis of recent eight years," *The Korean Journal of Gastroenterology*, vol. 46, no. 2, pp. 94–98, 2005.

[60] D. Marko, X. Calvet, J. Ducons, J. Guardiola, L. Tito, and F. Bory, "Comparison of two management strategies for *Helicobacter pylori* treatment: clinical study and cost-effectiveness analysis," *Helicobacter*, vol. 10, no. 1, pp. 22–32, 2005.

[61] S. Michopoulos, P. Tsibouris, H. Bouzakis et al., "Randomized study comparing omeprazole with ranitidine as anti-secretory agents combined in quadruple second-Iine *Helicobacter pylori* eradication regimens," *Alimentary Pharmacology and Therapeutics*, vol. 14, no. 6, pp. 737–744, 2000.

[62] J. M. Navarro-Jarabo, N. Fernández, F. L. Sousa et al., "Efficacy of rifabutin-based triple therapy as second-line treatment to eradicate *Helicobacter pylori* infection," *BMC Gastroenterology*, vol. 7, article 31, 2007.

[63] E. C. Nista, M. Candelli, F. Cremonini et al., "Levofloxacin-based triple therapy vs. quadruple therapy in second-line *Helicobacter pylori* treatment: a randomized trial," *Alimentary Pharmacology and Therapeutics*, vol. 18, no. 6, pp. 627–633, 2003.

[64] J.-W. Chung, J. H. Lee, H.-Y. Jung et al., "Second-line *Helicobacter pylori* eradication: a randomized comparison of 1-week or 2-week bismuth-containing quadruple therapy," *Helicobacter*, vol. 16, no. 4, pp. 289–294, 2011.

[65] F. Gomollón, J. Valdepérez, R. Garuz et al., "Cost/effectiveness analysis of two strategies for *Helicobacter pylori* eradication: results of a prospective and randomized study in primary care setting," *Medicina Clinica*, vol. 115, no. 1, pp. 1–6, 2000.

[66] P. Malfertheiner, F. Bazzoli, J.-C. Delchier et al., "*Helicobacter pylori* eradication with a capsule containing bismuth subcitrate potassium, metronidazole, and tetracycline given with omeprazole versus clarithromycin-based triple therapy: a randomised, open-label, non-inferiority, phase 3 trial," *The Lancet*, vol. 377, no. 9769, pp. 905–913, 2011.

[67] J. P. Gisbert, "*Helicobacter pylori* eradication: a new, single-capsule bismuth-containing quadruple therapy," *Nature Reviews Gastroenterology and Hepatology*, vol. 8, no. 6, pp. 307–309, 2011.

[68] K. F. Croom and K. L. Goa, "Levofloxacin: a review of its use in the treatment of bacterial infections in the united states," *Drugs*, vol. 63, no. 24, pp. 2769–2802, 2003.

[69] R. J. Saad, P. Schoenfeld, M. K. Hyungjin, and W. D. Chey, "Levofloxacin-based triple therapy versus bismuth-based quadruple therapy for persistent *Helicobacter pylori* infection: a meta-analysis," *American Journal of Gastroenterology*, vol. 101, no. 3, pp. 488–496, 2006.

[70] J. P. Gisbert and F. De La Morena, "Systematic review and meta-analysis: levofloxacin-based rescue regimens after *Helicobacter pylori* treatment failure," *Alimentary Pharmacology and Therapeutics*, vol. 23, no. 1, pp. 35–44, 2006.

[71] D. Vaira, C. Ricci, A. Lanzini, F. Perna, A. Romano, and R. Corinaldesi, "How to proceed in *Helicobacter pylori*-positive chronic gastritis refractory to first- and second-line eradication therapy," *Digestive Diseases*, vol. 25, no. 3, pp. 203–205, 2007.

[72] J. E. García Sánchez, N. García Sáenz, M. Rodríguez Rincón, I. Trujillano Martín, E. García Sánchez, and M. J. Fresnadillo Martínez, "Susceptibility of *Helicobacter pylori* to mupirocin, oxazolidionones, quinupristin/dalfopristin and new quinolones," *Journal of Antimicrobial Chemotherapy*, vol. 46, no. 2, pp. 283–285, 2000.

[73] H. H. X. Xia, B. C. Y. Wong, N. J. Talley, and S. K. Lam, "Alternative and rescue treatment regimens for *Helicobacter pylori* eradication," *Expert Opinion on Pharmacotherapy*, vol. 3, no. 9, pp. 1301–1311, 2002.

[74] J. Zou, Z. X. Yang, and Z. M. Qin, "Laboratory and clinical study of levofloxacin against *Helicobacter pylori*," *Zhonghua Yi Xue Za Zhi*, vol. 83, no. 20, pp. 1778–1781, 2003.

[75] L. Gatta, A. Zullo, F. Perna et al., "A 10-day levofloxacin-based triple therapy in patients who have failed two eradication courses," *Alimentary Pharmacology and Therapeutics*, vol. 22, no. 1, pp. 45–49, 2005.

[76] D. Antos, W. Schneider-Brachert, E. Bästlein et al., "7-Day triple therapy of *Helicobacter pylori* infection with levofloxacin, amoxicillin, and high-dose esomeprazole in patients with known antimicrobial sensitivity," *Helicobacter*, vol. 11, no. 1, pp. 39–45, 2006.

[77] W. Kumala and A. Rani, "Patterns of *Helicobacter pylori* isolate resistance to fluoroquinolones, amoxicillin, clarithromycin and metronidazoles," *Southeast Asian Journal of Tropical Medicine and Public Health*, vol. 37, no. 5, pp. 970–974, 2006.

[78] W. L. Chang, B. S. Sheu, H. C. Cheng, Y. J. Yang, H. B. Yang, and J. J. Wu, "Resistance to metronidazole, clarithromycin and levofloxacin of *Helicobacter pylori* before and after clarithromycin-based therapy in Taiwan," *Journal of Gastroenterology and Hepatology*, vol. 24, no. 7, pp. 1230–1235, 2009.

[79] S. A. Chisholm and R. J. Owen, "Frequency and molecular characteristics of ciprofloxacin- and rifampicin-resistant *Helicobacter pylori* from gastric infections in the UK," *Journal of Medical Microbiology*, vol. 58, no. 10, pp. 1322–1328, 2009.

[80] E. Karczewska, I. Wojtas-Bonior, E. Sito, M. Zwoliñska-Wcisło, and A. Budak, "Primary and secondary clarithromycin, metronidazole, amoxicillin and levofloxacin resistance to *Helicobacter pylori* in southern Poland," *Pharmacological Reports*, vol. 63, no. 3, pp. 799–807, 2011.

[81] M. Tanaka, E. Isogai, H. Isogai et al., "Synergic effect of quinolone antibacterial agents and proton pump inhibitors on *Helicobacter pylori*," *Journal of Antimicrobial Chemotherapy*, vol. 49, no. 6, pp. 1039–1040, 2002.

[82] Y. Watanabe, N. Aoyama, D. Shirasaka et al., "Levofloxacin based triple therapy as a second-line treatment after failure of *Helicobacter pylori* eradication with standard triple therapy," *Digestive and Liver Disease*, vol. 35, no. 10, pp. 711–715, 2003.

[83] J. Yahav, H. Shmuely, Y. Niv, J. Bechor, and Z. Samra, "In vitro activity of levofloxacin against *Helicobacter pylori* isolates from patients after treatment failure," *Diagnostic Microbiology and Infectious Disease*, vol. 55, no. 1, pp. 81–83, 2006.

[84] Y. Matsumoto, I. Miki, N. Aoyama et al., "Levofloxacin- versus metronidazole-based rescue therapy for *H. pylori* infection in Japan," *Digestive and Liver Disease*, vol. 37, no. 11, pp. 821–825, 2005.

[85] Y. Matsumoto, A. Nobuo, M. Iyuka et al., "A High Dosage of Levofloxacin- versus Metronidazole-based triple theraphy as a second-line treatment after failure of *Helicobacter*

pylori eradication with standard triple therapy in Japan," *Gastroentorology*, vol. 128, no. 2, article T963, 2005.

[86] G. Cammarota, R. Cianci, O. Cannizzaro et al., "Efficacy of two one-week rabeprazole/levofloxacin-based triple therapies for *Helicobacter pylori* infection," *Alimentary Pharmacology and Therapeutics*, vol. 14, no. 10, pp. 1339–1343, 2000.

[87] S. Di Caro, M. A. Zocco, F. Cremonini et al., "Levofloxacin based regimens for the eradication of *Helicobacter pylori*," *European Journal of Gastroenterology and Hepatology*, vol. 14, no. 12, pp. 1309–1312, 2002.

[88] L. Marzio, D. Coraggio, S. Capodicasa, L. Grossi, and G. Cappello, "Role of the preliminary susceptibility testing for initial and after failed therapy of *Helicobacter pylori* infection with levofloxacin, amoxicillin, and esomeprazole," *Helicobacter*, vol. 11, no. 4, pp. 237–242, 2006.

[89] A. Rispo, E. Di Girolamo, A. Cozzolino, R. Bozzi, A. Morante, and L. Pasquale, "Levofloxacin in first-line treatment of *Helicobacter pylori* infection," *Helicobacter*, vol. 12, no. 4, pp. 364–365, 2007.

[90] J. P. Gisbert, M. Fernández-Bermejo, J. Molina-Infante et al., "First-line triple therapy with levofloxacin for *Helicobacter pylori* eradication," *Alimentary Pharmacology and Therapeutics*, vol. 26, no. 3, pp. 495–500, 2007.

[91] J. H. Lee, S. P. Hong, C. I. Kwon et al., "The efficacy of levofloxacin based triple therapy for *Helicobacter pylori* eradication," *The Korean Journal of Gastroenterology*, vol. 48, no. 1, pp. 19–24, 2006.

[92] F. Perri, V. Festa, A. Merla, F. Barberani, A. Pilotto, and A. Andriulli, "Randomized study of different "second-line" therapies for *Helicobacter pylori* infection after failure of the standard "Maastricht triple therapy"," *Alimentary Pharmacology and Therapeutics*, vol. 18, no. 8, pp. 815–820, 2003.

[93] P. Orsi, O. Pinazzi, G. Aragona, and F. Di Mario, "Rabeprazole/Levofloxacin based triple therapy as a Salvatage Treatment after Failure of *H. pylori* eradication with standard regimens," *Helicobacter*, vol. 8, pp. 339–493, 2003.

[94] W. M. Wong, O. Gu, S. K. Lam et al., "Randomized controlled study of rabeprazole, levofloxacin and rifabutin triple therapy vs. quadruple therapy as second-line treatment for *Helicobacter pylori* infection," *Alimentary Pharmacology and Therapeutics*, vol. 17, no. 4, pp. 553–560, 2003.

[95] W. M. Wong, Q. Gu, K. M. Chu et al., "Lansoprazole, levofloxacin and amoxicillin triple therapy vs. quadruple therapy as second-line treatment of resistant *Helicobacter pylori* infection," *Alimentary Pharmacology and Therapeutics*, vol. 23, no. 3, pp. 421–427, 2006.

[96] J. P. Gisbert, F. Bermejo, M. Castro-Fernández et al., "Second-line rescue therapy with levofloxacin after *H. pylori* treatment failure: a Spanish multicenter study of 300 patients," *American Journal of Gastroenterology*, vol. 103, no. 1, pp. 71–76, 2008.

[97] E. G. Giannini, C. Bilardi, P. Dulbecco et al., "A study of 4- and 7-day triple therapy with rabeprazole, high-dose levofloxacin and tinidazole rescue treatment for *Helicobacter pylori* eradication," *Alimentary Pharmacology and Therapeutics*, vol. 23, no. 2, pp. 281–287, 2006.

[98] R. L. Page, D. Ferguson, and M. Cantu, "An alternative salvage regimen for *Helicobacter pylori*-resistant patients with heart failure," *Cardiology*, vol. 110, no. 2, pp. 112–115, 2008.

[99] Y. C. Lee, H. M. Wu, T. H. H. Chen et al., "A community-based study of *Helicobacter pylori* therapy using the strategy of test, treat, retest, and re-treat initial treatment failures," *Helicobacter*, vol. 11, no. 5, pp. 418–424, 2006.

[100] S. Di Caro, F. Franceschi, A. Mariani et al., "Second-line levofloxacin-based triple schemes for *Helicobacter pylori* eradication," *Digestive and Liver Disease*, vol. 41, no. 7, pp. 480–485, 2009.

[101] J. N. Eisig, F. M. Silva, R. C. Barbuti et al., "Efficacy of a 7-day course of furazolidone, levofloxacin, and lansoprazole after failed *Helicobacter pylori* eradication," *BMC Gastroenterology*, vol. 9, article 38, 2009.

[102] C.-H. Kuo, H.-M. Hu, F.-C. Kuo et al., "Efficacy of levofloxacin-based rescue therapy for *Helicobacter pylori* infection after standard triple therapy: a randomized controlled trial," *Journal of Antimicrobial Chemotherapy*, vol. 63, no. 5, pp. 1017–1024, 2009.

[103] R. W. M. Schrauwen, M. J. R. Janssen, and W. A. de Boer, "Seven-day PPI-triple therapy with levofloxacin is very effective for *Helicobacter pylori* eradication," *Netherlands Journal of Medicine*, vol. 67, no. 3, pp. 96–101, 2009.

[104] L. W. Chen, R. N. Chien, J. J. Chang, K. M. Fang, and L. C. Chang, "Comparison of the once-daily levofloxacin-containing triple therapy with the twice-daily standard triple therapy for first-line *Helicobacter pylori* eradication: a prospective randomised study," *International Journal of Clinical Practice*, vol. 64, no. 11, pp. 1530–1534, 2010.

[105] C. N. Erçin, A. Uygun, A. B. Toros et al., "Comparison of 7- and 14-day first-line therapies including levofloxacin in patients with *Helicobacter pylori* positive non-ulcer dyspepsia," *Turkish Journal of Gastroenterology*, vol. 21, no. 1, pp. 12–16, 2010.

[106] Y. Li, X. Huang, L. Yao, R. Shi, and G. Zhang, "Advantages of Moxifloxacin and Levofloxacin-based triple therapy for second-line treatments of persistent *Helicobacter pylori* infection: a meta analysis," *Wiener Klinische Wochenschrift*, vol. 122, no. 13-14, pp. 413–422, 2010.

[107] J. B. Kahn, "Latest industry information on the safety profile of levofloxacin in the US," *Chemotherapy*, vol. 47, supplement 3, pp. 32–37, 2001.

[108] J. Cabrita, M. Oleastro, R. Matos et al., "Features and trends in *Helicobacter pylori* antibiotic resistance in Lisbon area, Portugal (1990–1999)," *Journal of Antimicrobial Chemotherapy*, vol. 46, no. 6, pp. 1029–1031, 2000.

[109] G. Cammarota, R. Cianci, O. Cannizzaro et al., "High-dose versus low-dose clarithromycin in 1-week triple therapy, including rabeprazole and levofloxacin, for *Helicobacter pylori* eradication," *Journal of Clinical Gastroenterology*, vol. 38, no. 2, pp. 110–114, 2004.

[110] L. G.V. Coelho, L. D. Moretzsohn, W. L.S. Vieira et al., "New once-daily, highly effective rescue triple therapy after multiple Helicobacter pylori treatment failures: a pilot study," *Alimentary Pharmacology and Therapeutics*, vol. 21, no. 6, pp. 783–787, 2005.

[111] P. Bogaerts, C. Berhin, H. Nizet, and Y. Glupczynski, "Prevalence and mechanisms of resistance to fluoroquinolones in *Helicobacter pylori* strains from patients living in Belgium," *Helicobacter*, vol. 11, no. 5, pp. 441–445, 2006.

[112] J. M. Kim, "Antibiotic resistance of *Helicobacter pylori* isolated from Korean patients," *The Korean Journal of Gastroenterology*, vol. 47, no. 5, pp. 337–349, 2006.

[113] J. M. Kim, J. S. Kim, N. Kim, S. G. Kim, H. C. Jung, and I. S. Song, "Comparison of primary and secondary antimicrobial minimum inhibitory concentrations for *Helicobacter pylori* isolated from Korean patients," *International Journal of Antimicrobial Agents*, vol. 28, no. 1, pp. 6–13, 2006.

[114] H. Miyachi, I. Miki, N. Aoyama et al., "Primary levofloxacin resistance and gyrA/B mutations among *Helicobacter pylori* in Japan," *Helicobacter*, vol. 11, no. 4, pp. 243–249, 2006.

[115] J. J. Carothers, M. G. Bruce, T. W. Hennessy et al., "The relationship between previous fluoroquinolone use and levofloxacin resistance in *Helicobacter pylori* infection," *Clinical Infectious Diseases*, vol. 44, no. 2, pp. e5–e8, 2007.

[116] V. Cattoir, J. Nectoux, C. Lascols et al., "Update on fluoroquinolone resistance in *Helicobacter pylori*: new mutations leading to resistance and first description of a gyrA polymorphism associated with hypersusceptibility," *International Journal of Antimicrobial Agents*, vol. 29, no. 4, pp. 389–396, 2007.

[117] E. Glocker, H.-P. Stueger, and M. Kist, "Quinolone resistance in *Helicobacter pylori* isolates in Germany," *Antimicrobial Agents and Chemotherapy*, vol. 51, no. 1, pp. 346–349, 2007.

[118] F. Perna, A. Zullo, C. Ricci, C. Hassan, S. Morini, and D. Vaira, "Levofloxacin-based triple therapy for *Helicobacter pylori* re-treatment: role of bacterial resistance," *Digestive and Liver Disease*, vol. 39, no. 11, pp. 1001–1005, 2007.

[119] A. Zullo, F. Perna, C. Hassan et al., "Primary antibiotic resistance in *Helicobacter pylori* strains isolated in northern and central Italy," *Alimentary Pharmacology and Therapeutics*, vol. 25, no. 12, pp. 1429–1434, 2007.

[120] W. Gao, H. Cheng, F. Hu et al., "The evolution of *Helicobacter pylori* antibiotics resistance over 10 years in Beijing, China," *Helicobacter*, vol. 15, no. 5, pp. 460–466, 2010.

[121] J. Raymond, D. Lamarque, N. Kalach, S. Chaussade, and C. Burucoa, "High level of antimicrobial resistance in French *Helicobacter pylori* isolates," *Helicobacter*, vol. 15, no. 1, pp. 21–27, 2010.

[122] K. H. Hung, B. S. Sheu, W. L. Chang, H. M. Wu, C. C. Liu, and J. J. Wu, "Prevalence of primary fluoroquinolone resistance among clinical isolates of *Helicobacter pylori* at a University Hospital in Southern Taiwan," *Helicobacter*, vol. 14, no. 1, pp. 61–65, 2009.

[123] L. Boyanova and I. Mitov, "Geographic map and evolution of primary *Helicobacter pylori* resistance to antibacterial agents," *Expert Review of Anti-Infective Therapy*, vol. 8, no. 1, pp. 59–70, 2010.

[124] F. Ermis, F. Akyuz, A. Uyanikoglu et al., "Second-line levofloxacin-based triple therapy's efficiency for *Helicobacter pylori* eradication in patients with peptic ulcer," *Southern Medical Journal*, vol. 104, no. 8, pp. 579–583, 2011.

[125] M. Yamade, M. Sugimoto, T. Uotani, M. Nishino, C. Kodaira, and T. Furuta, "Resistance of *Helicobacter pylori* to quinolones and clarithromycin assessed by genetic testing in Japan," *Journal of Gastroenterology and Hepatology*, vol. 26, no. 9, pp. 1457–1461, 2011.

[126] S. Krakowka, S. S. Ringler, K. A. Eaton, W. B. Green, and R. Leunk, "Manifestations of the local gastric immune response in gnotobiotic piglets infected with *Helicobacter pylori*," *Veterinary Immunology and Immunopathology*, vol. 52, no. 3, pp. 159–173, 1996.

[127] J. W. Lee, N. Kim, R. H. Nam et al., "Mutations of *Helicobacter pylori* associated with fluoroquinolone resistance in Korea," *Helicobacter*, vol. 16, no. 4, pp. 301–310, 2011.

[128] A. Zullo, V. de Francesco, G. Manes, G. Scaccianoce, F. Cristofari, and C. Hassan, "Second-line and rescue therapies for *Helicobacter pylori* eradication in clinical practice," *Journal of Gastrointestinal and Liver Diseases*, vol. 19, no. 2, pp. 131–134, 2010.

[129] T. G. Reilly, R. C.S. Ayres, V. Poxon, and R. P. Walt, "*Helicobacter pylori* eradication in a clinical setting: success rates and the effect on the quality of life in peptic ulcer," *Alimentary Pharmacology and Therapeutics*, vol. 9, no. 5, pp. 483–490, 1995.

[130] F. Lerang, B. Moum, J. B. Haug et al., "Highly effective second-line anti-*Helicobacter pylori* therapy in patients with previously failed metronidazole-based therapy," *Scandinavian Journal of Gastroenterology*, vol. 32, no. 12, pp. 1209–1214, 1997.

[131] N. Magaret, M. Burm, D. Faigel, C. Kelly, W. Peterson, and M. B. Fennerty, "A randomized trial of lansoprazole, amoxycillin, and clarithromycin versus lansoprazole, bismuth, metronidazole and tetracycline in the retreatment of patients failing initial *Helicobacter pylori* therapy," *Digestive Diseases*, vol. 19, no. 2, pp. 174–178, 2001.

[132] M. Deltenre, R. Ntounda, C. Jonas, and E. De Koster, "Eradication of *Helicobacter pylori*: why does it fail?" *Italian Journal of Gastroenterology and Hepatology*, vol. 30, supplement 3, pp. S326–S328, 1998.

[133] A. T. R. Axon, "Treatment of *Helicobacter pylori*: an overview," *Alimentary Pharmacology and Therapeutics*, vol. 14, no. 3, pp. 1–6, 2000.

[134] U. Peitz, A. Hackelsberger, and P. Malfertheiner, "A practical approach to patients with refractory *Helicobacter pylori* infection, or who are re-infected after standard therapy," *Drugs*, vol. 57, no. 6, pp. 905–920, 1999.

[135] U. Peitz, M. Sulliga, K. Wolle et al., "High rate of post-therapeutic resistance after failure of macrolide-nitroimidazole triple therapy to cure *Helicobacter pylori* infection: impact of two second-line therapies in a randomized study," *Alimentary Pharmacology and Therapeutics*, vol. 16, no. 2, pp. 315–324, 2002.

[136] I. L. P. Beales, "Efficacy of *Helicobacter pylori* eradication therapies: a single centre observational study," *BMC Gastroenterology*, vol. 1, article 7, 2001.

[137] S. Miehlke, K. Hansky, W. Schneider-Brachert et al., "Randomized trial of rifabutin-based triple therapy and high-dose dual therapy for rescue treatment of *Helicobacter pylori* resistant to both metronidazole and clarithromycin," *Alimentary Pharmacology and Therapeutics*, vol. 24, no. 2, pp. 395–403, 2006.

[138] J. P. Gisbert, M. Mur, S. Sainz et al., "Is the combination omeprazole plus amoxycillin useful in eradicating Helicobacter playori in Spain?" *Revista Espanola de Enfermedades Digestivas*, vol. 88, no. 2, pp. 99–106, 1996.

[139] M. H. M. G. Houben, D. Van De Beek, E. F. Hensen, A. J. M. De Craen, E. A. J. Rauws, and G. N. J. Tytgat, "A systematic review of *Helicobacter pylori* eradication therapy—the impact of antimicrobial resistance on eradication rates," *Alimentary Pharmacology and Therapeutics*, vol. 13, no. 8, pp. 1047–1055, 1999.

[140] M. P. Dore, G. Leandro, G. Realdi, A. R. Sepulveda, and D. Y. Graham, "Effect of pretreatment antibiotic resistance to metronidazole and clarithromycin on outcome of *Helicobacter pylori* therapy: a meta-analytical approach," *Digestive Diseases and Sciences*, vol. 45, no. 1, pp. 68–76, 2000.

[141] E. J. Van Der Wouden, J. C. Thijs, A. A. Van Zwet, W. J. Sluiter, and J. H. Kleibeuker, "The influence of in vitro nitroimidazole resistance on the efficacy of nitroimidazole-containing anti-*Helicobacter pylori* regimens: a meta-analysis," *American Journal of Gastroenterology*, vol. 94, no. 7, pp. 1751–1759, 1999.

[142] D. S. Tompkins, J. Perkin, and C. Smith, "Failed treatment of *Helicobacter pylori* infection associated with resistance to clarithromycin," *Helicobacter*, vol. 2, no. 4, pp. 185–187, 1997.

[143] A. Pilotto, M. Franceschi, M. Rassu et al., "Incidence of secondary *Helicobacter pylori* resistance to antibiotics in treatment failures after 1-week proton pump inhibitor-based triple therapies: a prospective study," *Digestive and Liver Disease*, vol. 32, no. 8, pp. 667–672, 2000.

[144] R. J. Saad and W. D. Chey, "Persistent *Helicobacter pylori* infection after a course of antimicrobial therapy-what's next?" *Clinical Gastroenterology and Hepatology*, vol. 6, no. 10, pp. 1086–1090, 2008.

[145] W. De Boer, W. Driessen, A. Jansz, and G. Tytgat, "Effect of acid suppression on efficacy of treatment for *Helicobacter pylori* infection," *The Lancet*, vol. 345, no. 8953, pp. 806–820, 1995.

[146] Q. Sun, X. Liang, Q. Zheng et al., "High efficacy of 14-day triple therapy-based, bismuth-containing quadruple therapy for initial *Helicobacter pylori* eradication," *Helicobacter*, vol. 15, no. 3, pp. 233–238, 2010.

[147] M. Minakari, A. H. Davarpanah Jazi, A. Shavakhi, N. Moghareabed, and F. Fatahi, "A randomized controlled trial: efficacy and safety of azithromycin, ofloxacin, bismuth, and omeprazole compared with amoxicillin, clarithromycin, bismuth, and omeprazole as second-line therapy in patients with *Helicobacter pylori* infection," *Helicobacter*, vol. 15, no. 2, pp. 154–159, 2010.

[148] B. Cudia, M. Romano, F. P. Gioè, N. Barbera, D. Lo Gerfo, and R. Montalto, "'Rescue' therapy including ranitidine bismuth citrate + minocicline + amoxicillin for eradication of *Helicobacter pylori* in previous H.P. treatment failure," *Gut*, vol. 41, supplement 1, article A103, 1997.

[149] J. P. Gisbert and X. Calvet, "Review article: rifabutin in the treatment of refractory *Helicobacter pylori* infection," *Alimentary Pharmacology and Therapeutics*, vol. 35, no. 2, pp. 209–221, 2012.

[150] A. Zullo, V. De Francesco, C. Hassan, C. Panella, S. Morini, and E. Ierardi, "Second-line treatment for *Helicobacter pylori* eradication after sequential therapy failure: a pilot study," *Therapy*, vol. 3, no. 2, pp. 251–254, 2006.

[151] T. Rokkas, P. Sechopoulos, I. Robotis, G. Margantinis, and D. Pistiolas, "Cumulative *H. pylori* eradication rates in clinical practice by adopting first and second-line regimens proposed by the maastricht III consensus and a third-line empirical regimen," *American Journal of Gastroenterology*, vol. 104, no. 1, pp. 21–25, 2009.

[152] J. P. Gisbert, "Attitude in *Helicobacter pylori* eradication therapy failure: surrender or combat?" *Medicina Clinica*, vol. 111, no. 20, pp. 778–782, 1998.

[153] B. H. Lee, N. Kim, T. J. Hwang et al., "Bismuth-containing quadruple therapy as second-line treatment for *Helicobacter pylori* infection: effect of treatment duration and antibiotic resistance on the eradication rate in Korea," *Helicobacter*, vol. 15, no. 1, pp. 38–45, 2010.

[154] F. Perri, V. Festa, R. Clemente et al., "Randomized study of two "rescue" therapies for *Helicobacter pylori*-infected patients after failure of standard triple therapies," *American Journal of Gastroenterology*, vol. 96, no. 1, pp. 58–62, 2001.

[155] B. Sicilia, E. Sierra, A. Lago, M. Villar, S. García, and F. Gomollón, "High eradication rates in *Helicobacter pylori* infection in patients with duodenal ulcer who failed previous eradication therapy," *Medicina Clinica*, vol. 115, no. 17, pp. 641–643, 2000.

[156] Y. Usta, I. N. Saltik-Temizel, H. Demir et al., "Comparison of short- and long-term treatment protocols and the results of second-line quadruple therapy in children with *Helicobacter pylori* infection," *Journal of Gastroenterology*, vol. 43, no. 6, pp. 429–433, 2008.

[157] A. Uygun, A. M. Ozel, O. Yildiz et al., "Comparison of three different second-line quadruple therapies including bismuth subcitrate in Turkish patients with non-ulcer dyspepsia who failed to eradicate *Helicobacter pylori* with a 14-day standard first-line therapy," *Journal of Gastroenterology and Hepatology*, vol. 23, no. 1, pp. 42–45, 2008.

[158] D. C. Wu, P. I. Hsu, A. Chen et al., "Randomized comparison of two rescue therapies for *Helicobacter pylori* infection," *European Journal of Clinical Investigation*, vol. 36, no. 11, pp. 803–809, 2006.

[159] D.-C. Wu, P.-I. Hsu, H.-H. Tseng et al., "*Helicobacter pylori* infection: a randomized, controlled study comparing 2 rescue therapies after failure of standard triple therapies," *Medicine*, vol. 90, no. 3, pp. 180–185, 2011.