# Facing resistance of *H.pylori* infection

**Mohammad Reza Zali**

*Research Institute for Gastroenterology and Liver Disease, Shahid Beheshti University, M.C., Tehran, Iran*

## ABSTRACT

*Helicobacter pylori* is an important human pathogen and a gram-negative, spiral shaped and microaerophilic bacteria with persistence colonization in gastric mucosa, causes gastroduodenal inflammation and destruction, resulting in diseases such as duodenal ulcer disease, gastric ulcer disease. When *H.pylori* discovered by Warren and Marshall, effective therapy developed for this infection and resulted in remarkable change in management of PUD and dyspepsia. Nowadays, the increasing prevalence of drug resistance has complicated successful therapy, considering more attention for appropriate therapy. Ideally, therapy should be based on pretreatment drug susceptibility testing, and empiric use of eradication therapies should assume the presence of antimicrobial drug resistance and use increased doses for 14 days.

**Keywords**: *Helicobacter pylori,* Eradication, Resistance.
**(Gastroenterology and Hepatology From Bed to Bench 2011; 4(1): 3-11).**

## Introduction

*Helicobacter pylori* is an important human pathogen and more than two third of the world population are infected mostly in developing countries. *Helicobacter pylori* is a gram-negative, spiral shaped and microaerophilic bacteria with persistence colonization in gastric mucosa, causes gastroduodenal inflammation and destruction, resulting in diseases such as duodenal ulcer disease, gastric ulcer disease, iron and/or vitamin B12 deficiency, gastric adenocarcinoma, and primary B-cell gastric lymphoma (1-5).

When *H.pylori* discovered by Warren and Marshall, effective therapy developed for this infection and resulted in remarkable change in management of PUD and dyspepsia and also gastric mucosa-associated lymphoma. *H.pylori* therapy has been used for decades, but for the last decade the efficacy of treatment are declining and much of the decline in effectiveness is attributable to increasing antibiotic resistance due to *H.pylori* strains.

Resistance to antimicrobial drugs is a major cause of treatment failure and is largely responsible for the decline in eradication rates. Different regimens have been used so far in patients with *H.pylori* infection, including first line therapy, second line therapy, sequential therapy and concomitant therapy. There has been a decline in the effectiveness of recommended therapies resulting in unacceptably low cure rates (i.e. 80%) (6-8).

Among different regimens for *H.pylori* eradication, clarithromycin-containing triple therapy resistance is very important to treatment outcome because it dramatically reduces eradication rates. In fact, eradication rates with this

---
**Reprint or Correspondence**: Mohammad Reza Zali, MD, FACG, AGAF. Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University, M.C., Iran
**E-mail**: nnzali@hotmail.com

regimen in patients harboring a clarithromycin-resistant strain of *H.pylori* are likely to be less than 30%. Therapy for *H.pylori* infection lost its way in part because of cure rates decline (largely because of an increasing prevalence of resistant organisms). Physicians are unaware that the cure rate with triple therapy has fallen down to below 80% in most places. One reason for failure of eradication is the lack of routine post therapy evaluation and testing universally which provide an early warning of increasing antibiotic resistance. Therefore physician should know about *H.pylori* resistance strains, looking for alternative and effective therapy. Issues to be considered when selecting a regimen for treatment of an infectious disease include effectiveness, simplicity, tolerability, adverse effects, the prevalence of antibiotic resistance in the community, dose, duration, costs, and whether and how much control of gastric pH is needed (9, 10).

One way to overcome this problem is to act as the same way for other transmissible infection, to cure patients and evaluate resistance. Unfortunately practicing physicians have no knowledge about the rate of resistance in their population, failing to order follow-up testing to prove *H.pylori* eradication after a course of antimicrobial therapy. The importance of follow-up testing to confirm eradication of *H.pylori* infection in patients with ulcer disease has been highlighted in recent studies that have shown that patients who did not undergo such testing were at a greater risk of ulcer recurrence and recurrent upper gastrointestinal bleeding. All physicians are now aware of the importance of testing for and treating *H.pylori* infection in patients with a documented peptic ulcer. Therefore, all patients with an ulcer should undergo follow-up testing to prove cure. Cure of *H.pylori* infection can be confirmed by a number of different diagnostic tests. Non-endoscopic tests that can be used to reliably document eradication include the urea breath test (UBT) and the fecal

antigen test (FAT). Two versions of the UBT are commercially available, one using a small dose of radioactive 14C and another that uses the non-radioactive isotope 13C. Studies have found the monoclonal version of the FAT to be accurate in the post-treatment setting (11, 12).

Polyclonal versions of the FAT appear to be less reliable and should be avoided in the post-treatment setting. Availability of the FAT at some hospitals remains an issue. If not offered locally, most regional and national reference laboratories offer the FAT. The accuracy of both UBT and FAT can be affected adversely by the recent use of PPIs, bismuth, or antibiotics. Despite being the cheapest and most widely available diagnostic modality, *H.pylori* antibody, testing is not a reliable means of confirming eradication. *H.pylori* antibodies can remain present long after successful *H.pylori* eradication. Biopsy-based testing including the rapid urease test or histology also can be used to document *H.pylori* eradication. Because *H.pylori* infection often becomes patchy after antibiotic therapy, a minimum of 4 biopsy specimens should be obtained from the proximal and distal stomach when establishing *H.pylori* eradication with histology. Although the rapid urease test remains highly specific, its sensitivity is reduced after antibiotic or PPI therapy. *H.pylori* culture also can be performed using mucosal biopsy specimens. Such testing is highly specific and capable of providing valuable information on antibiotic resistance. Unfortunately, *H.pylori* culture is less sensitive than histology or the rapid urease test and is available in only a small number of centers. Given these practical limitations, culture should be considered only in cases in which *H.pylori* infection has persisted despite multiple courses of antibiotic treatment. Polymerase chain reaction is a DNA amplification technique that can be performed on mucosal tissue specimens. This powerful diagnostic tool is not yet available for clinical practice (11, 12).

# How difficult is *H.pylori* treatment?

The roles that the organism, gastric environment, hosts and drug regimens play in treatment failures are often inter-related. Drug regimens should be designed to overcome drug-resistant and dormant forms of the organism, a high bacterial load, the typically low gastric pH, impaired mucosal immunity and the busy lifestyle of the human host (13).

The gastric environment also influences *H.pylori* treatment success. *H.pylori* typically resides within the human stomach, and populations of organisms occupy different environments, each with its own challenges. The majority of organisms are found within the mucus layer, which is technically outside of the body. Many are found attached to surface cells and a few are found even within epithelial cells (14-16).

Most importantly, at any time, a proportion of the organisms is either not replicating or is replicating very slowly, which represents a challenge for antibiotics that require microbial replication to kill the organisms (e.g., clarithromycin and amoxicillin) (17-19).

The best method to determine which drug the infection is resistant to, is to test for antimicrobial susceptibility. As noted above, this can be done using stool specimens for clarithromycin and fluoroquinolone susceptibility which can be assessed using molecular techniques, but this is not available for the other commonly used drugs. As a general rule, when antimicrobial susceptibility testing is not feasible, one can presume that the infection is resistant to antibiotics that the patient has taken previously in which resistance is common or develops rapidly (eg, metronidazole, clarithromycin or fluoroquinolones) (20).

*H.pylori* treatment failures may also occur independently of resistance that is, treatment may fail but the organism remains susceptible to the antibiotic. This is most commonly seen with amoxicillin where failure is rarely caused by acquired resistance. This form of reversible resistance is termed phenotypical antibiotic resistance and it often due to the presence of a persistor or nonreplicating population of organisms (21-23). Bacterial populations oscillate between a non-replicating and replicating state or from intracellular to extra-cellular environments. Thus they oscillate between a phenotypically resistant and a phenotypically susceptible state during which they can be eradicated. One solution is to extend the duration of treatment such that the antibiotic will be present during at least one period of susceptibility (24). An alternative approach is to force the organism to enter the replicative state and become susceptible to the antibiotic (25-27).

# Facing with failed therapy

The increasingly low treatment success rates should have been a calling for action resulting in widespread discussion of the problem, and rigorous studies in a variety of populations using different antimicrobial agents, doses, formulations and durations, to identify new effective treatments on the results-based approach designed to be efficient and to minimize patient risk and drug exposure (28).

The fact that the most commonly recommended treatments no longer cured even 80% of infections in many populations was not appreciated by general clinicians (14).

In general, and in most countries, metronidazole resistance should be considered as ubiquitous and increased doses must be used routinely unless it has been proven that high cure rates are maintained with the standard doses and/or treatment durations shorter than 14 days. Declining treatment success has reached the point where triple regimens containing clarithromycin should be abandoned as an empiric first-line treatment.

When confronted with a patient who has failed treatment, which the issues include: (1) what

antibiotics are the infection likely to be resistant to? (2) Which drugs can the patient not take either because of an allergy or side effects? and (3) Which drugs are available locally?, an appropriate second-line treatment should be chosen. With multiple treatment failures this becomes difficult as one is choosing a 'rescue' treatment.

Besides of considerations regarding allergies and the local availability of the drugs, we recommend that clinicians use the following general rules when treating patients with a *H.pylori* infection: (1) do not use 'legacy triple therapy' consisting of a PPI, clarithromycin and amoxicillin unless it has been proven to be highly effective locally (eg, eradication >90% in per-protocol analyses); (2) use higher doses of drugs (eg, 500 mg of clarithromycin, metronidazole and tetracycline) unless it has been shown that lower doses are equally effective; (3) use 14 day duration unless a shorter duration has been shown locally to be equally effective (eg, for clarithromycin and fluoroquinolones); (4) do not use a triple regimen containing clarithromycin, if clarithromycin is commonly prescribed locally or the patient has taken clarithromycin in the past, for any indication; (5) avoid fluoroquinolones if a quinolone (eg, ciprofloxacin, levofloxacin or moxifloxacin) has been given previously, even for any indication; and (6) following treatment failure, do not reuse drugs for which resistance is likely to have developed (ie, clarithromycin and fluoroquinolones). A bismuth triple regimen consisting of a bismuth salt, metronidazole/tinidazole and tetracycline was the first successful *H.pylori* treatment; initially it provided treatment success >95% even without the use of an antisecretory drug. Resistance to bismuth does not occur, and it is probably the most underutilised agent. Amoxicillin and tetracycline resistance are usually rare in most regions. Treatment success for triple drug regimens containing amoxicillin does not always appear to be significantly lowered in the

presence of amoxicillin resistance, suggesting that resistance may often be overestimated (11), especially in areas where a relatively high prevalence (ie, >20%) is reported and where appropriate tests for resistance are not utilised. Resistance to clarithromycin, on the other hand, has increased over time (18, 19).

The fact that treatment success reliably increases as the duration of treatment increases from 3 to 14 days with amoxicillin and a PPI is probably an example of phenotypic resistance. Subsequent experience showed that eradication was reduced in the presence of metronidazole-resistant *H.pylori* and that this could be largely overcome by increasing the dose of metronidazole and duration and/or adding a PPI. As such, the dose of metronidazole (e.g., 1500 mg per day) and duration (14 days) are considered optimum especially in areas where metronidazole resistance is more than trivial (29-31).

In many populations, quadruple regimens containing a bismuth compound, metronidazole (500 mg three times a day), tetracycline (500 mg four times a day) and a PPI (standard dosing twice daily) can achieve effectiveness (18). The most commonly used salvage regimen is bismuth-based quadruple therapy. Given the potential benefits of sequential therapy in patients with clarithromycin-resistant *H.pylori* infection, one wonders about its use in patients with persistent infection. At present, there are no data available on the use of sequential therapy as a salvage regimen.

Several other antibiotics have been used in salvage regimens including levofloxacin, rifabutin, and furazolidone. A suitable alternative to quadruple therapy is levofloxacin based triple therapy consisting of levofloxacin 500 mg once daily, amoxicillin 1 g twice daily, and a PPI twice daily for 10 days. Furazolidone is a synthetic derivative of the nitrofuran class of antimicrobials, which also has been evaluated in a number of studies with eradication rates ranging from 52% to

90% in the treatment of persistent *H.pylori*. It is difficult to assess the efficacy of furazolidone-based salvage therapy owing to the variability in treatment regimens. In these studies, the daily dose of furazolidone varied from 200 to 600 mg. Furthermore, a variety of triple- and quadruple-drug regimens were used with treatment durations ranging from 7 to 14 days. Side effects can be substantial in these furazolidone-based therapies, occurring in as many as 35% of patients, with vomiting, nausea, and dizziness being reported most commonly. Furazolidone also may interact with a variety of foods and other drugs given its effect on monoamine oxidase as well as glucose-6-phosphate dehydrogenase activity and its potential disulfiram like reaction with alcohol. Furazolidone has fallen out of favor related to its adverse event profile and limited availability (31-33).

## Persistance *H.pylori* infection

There is less consensus regarding treatment regimens for persistent *H.pylori* infection despite 2 or more previous courses of antibiotics. Bismuth-based quadruple therapy, levofloxacin-based triple therapy, rifabutin-based triple therapy, and furazolidone-based regimens have been investigated. A 14-day course of bismuth quadruple therapy yielded an eradication rate of 95% (95% CI, 85%–99%) in 42 patients whereas another study in which 18 patients received a 7-day course of therapy reported an eradication rate of only 33%. Levofloxacin triple therapy was found to eradicate *H.pylori* in 86%, whereas a more recent study showed an eradication rate of only 60% (95% CI, 50%–70%) in 100 patients who had failed 2 previous courses of therapy. Concomitant therapy with non-bismuth containing, four-drug, three-antibiotic therapy, was introduced before sequential therapy and there is experience of its use in about 1,000 patients (34-42).

It consists of four drugs- a PPI, clarithromycin, metronidazole or tinidazole, and amoxicillin- all given twice daily. The duration of concomitant therapy has ranged from 3 days to 7 days and it has produced Grade B results, similar to those obtained with sequential therapy (34-42). Studies are needed to test whether extending the duration of therapy will improve these results. As with sequential therapy, concomitant therapy would probably be a poor choice in the presence of *H.pylori* that are resistant to clarithromycin and metronidazole, or for treatment after multiple drug regimes have failed, as those patients can be expected to have multidrug resistant infections. Importantly, however, traditional triple therapy can easily be converted to concomitant therapy by the addition of 500 mg of metronidazole or tinidazole twice daily (43-56).

Thus, we recommend including at least one antibiotic the patient has not previously taken. Sequential and concomitant treatments expose the patient to both clarithromycin and metronidazole/tinidazole, and therefore we do not recommend that both drugs be used in the rescue treatment as well. One recent approach has been to use a fluoroquinolone containing triple or quadruple regimen. However, the worldwide rapid increase in fluoroquinolone resistance has undermined this approach in many areas and one must fall back on the 'only use what works locally' rule (33, 57, 58).

In addition, one should generally expect the same treatment success rate for first, second and even third treatment regimens if the organisms are susceptible. Thus, if one initially used a clarithromycin containing concomitant or sequential regimen, one of the reasonable second choices would be 14 day bismuth quadruple regimen. If this bismuth quadruple rescue treatment is ineffective in the local population, or when metronidazole is not a viable drug option, metronidazole can be replaced by furazolidone (100 mg).

As previously mentioned, PPI plus amoxicillin dual treatment can be improved by increasing the frequency and dose of the PPI and extending treatment duration. With higher doses and longer durations, we recommend adding a third drug (not previously used by the patient) to a 14 day PPI plus amoxicillin regimen. A fluoroquinolone (once a day) is a good choice for the third drug, provided that resistance is not present. The other option that is commonly used is rifabutin (150 mg twice a day) (52-56).

## Conclusion

*H.pylori* causes a serious, transmissible, infectious disease. The increasing prevalence of drug resistance has complicated successful therapy. Therefore we must consider and look for appropriate therapy.

Ideally, therapy should be based on pretreatment drug susceptibility testing, and empiric use of eradication therapies should assume the presence of antimicrobial drug resistance and use increased doses for 14 days. Clarithromycin-containing triple therapies now typically produce ≤80% ITT cure rates and are thus no longer acceptable as empiric therapy. Current options for initial treatment include sequential therapy, concomitant therapy, and bismuth-containing quadruple therapy. Advances have shown that phenotypic and genetic resistance to therapy can be successfully dealt with, and what still needs to be done is now clearly identifiable. An improved appreciation of the role of gastric pH in phenotypic resistance has resulted in high cure rates with high-dose PPI plus amoxicillin dual therapy, although studies are still needed to devise improved dual-therapy based multidrug regimes. Selection of appropriate antimicrobial drugs following treatment failure is best approached by drug-susceptibility testing. If such testing is not available, we recommended a bismuth-containing quadruple therapy, with substitution of a new drug

for the metronidazole or tinidazole and/or the clarithromycin if these agents have been used previously. One alternative approach would be to use 14 days of treatment with a high-dose PPI and amoxicillin-containing triple therapy with rifabutin, a fluoroquinolone, or furazolidone. The time is long overdue for us, the *H.pylori* investigators, to speak up and communicate to clinicians that the treatments they are using are often no longer effective, recommend superior alternative treatments and seek out new highly efficacious treatments.

## References

1. Graham DY, Sung JY. *Helicobacter pylori*. In: Feldman M, Friedman LS, Brandt LJ, eds. Sleisenger and Fordtran's gastrointestinal and liver disease. pathophysiology, diagnosis, management. 7th ed. Philadelphia: WB Saunders Co; 2006. P.1049–66.

2. Cardenas VM, Mulla ZD, Ortiz M, Graham DY. Iron deficiency and *Helicobacter pylori* infection in the United States. Am J Epidemiol 2006; 163: 127–34.

3. Dholakia KR, Dharmarajan TS, Yadav D, Oiseth S, Norkus EP, Pitchumoni CS. Vitamin B12 deficiency and gastric histopathology in older patients. World J Gastroenterol 2005; 11: 7078–83.

4. DuBois S, Kearney DJ. Iron-deficiency anemia and Helicobacter pylori infection: a review of the evidence. Am J Gastroenterol 2005; 100: 453–59.

5. Alizadeh AH, Ansari S, Ranjbar M, Shalmani HM, Habibi I, Firouzi M, et al. Seroprevalence of Helicobacter pylori in Nahavand: a population-based study. East Mediterr Health J. 2009 Jan-Feb;15(1):129-35.

6. Kaboli SA, Zojaji H, Mirsattari D, Talaie R, Derakhshan F, Zali MR, et al. Effect of addition of vitamin C to clarithromycin-amoxicillin-omeprazol triple regimen on Helicobacter pylori eradication. Acta Gastroenterol Belg 2009; 72: 222-24.

7. Zojaji H, Talaie R, Mirsattari D, Haghazali M, Molaei M, Mohsenian N, et al. The efficacy of *Helicobacter pylori* eradication regimen with and without vitamin C supplementation. Dig Liver Dis 2009; 41: 644-47.

8. Chey WD, Wong BC. American College of Gastroenterology guideline on the management of

*Helicobacter pylori* infection. Am J Gastroenterol 2007; 102: 1808–25.

9. Jafri NS, Hornung CA, Howden CW. Meta-analysis: sequential therapy appears superior to standard therapy for *Helicobacter pylori* infection in patients naive to treatment. Ann Intern Med 2008; 148: 923–31.

10. Graham DY, Shiotani A. New concepts of resistance in the treatment of *Helicobacter pylori* infections. Nat Clin Pract Gastroenterol Hepatol 2008; 5: 331.

11. Vaira D, Vakil N, Menagatti M, van't Hoff B, Ricci C, Gatta L et al. The stool antigen test for the detection of *Helicobacter pylori* after eradication therapy. Ann Intern Med 2002; 136: 280–87.

12. Gisbert JP, de la Morena F, Abraira V. Accuracy of monoclonal stool antigen test for the diagnosis of H. pylori infection: a systematic review and meta-analysis. Am J Gastroenterol 2006; 101: 1921–30.

13. Baghaei K, Shokrzadeh L, Jafari F, Dabiri H, Yamaoka Y, Bolfion M, et al. Determination of Helicobacter pylori virulence by analysis of the cag pathogenicity island isolated from Iranian patients. Dig Liver Dis 2009; 41: 634-38.

14. Dubois A, Boren T. *Helicobacter pylori* is invasive and it may be a facultative intracellular organism. Cell Microbiol 2007; 9: 1108-16.

15. Necchi V, Candusso ME, Tava F, Luinetti O, Ventura U, Fiocca R, et al. Intracellular, intercellular, and stromal invasion of gastric mucosa, preneoplastic lesions, and cancer by *Helicobacter pylori*. Gastroenterology 2007; 132: 1009-23.

16. Ito T, Kobayashi D, Uchida K, Takemura T, Nagaoka S, Kobayashi I, et al. *Helicobacter pylori* invades the gastric mucosa and translocates to the gastric lymph nodes. Lab Invest 2008; 88: 664-81.

17. Graham DY, Lu H, Yamaoka Y. A report card to grade *Helicobacter pylori* therapy. Helicobacter 2007; 12: 275-78.

18. Fischbach LA, van Zanten S, Dickason J. Meta-analysis: the efficacy, adverse events, and adherence related to first-line anti- Helicobacter pylori quadruple therapies. Aliment Pharmacol Ther 2004; 20: 1071-82.

19. Graham DY. Antibiotic resistance in *Helicobacter pylori*: implications for therapy. Gastroenterology 1998; 115: 1272-77.

20. Kawai T, Yamagishi T, Yagi K, Kataoka M, Kawakami K, Sofuni A, et al. Tailored eradication therapy based on fecal *Helicobacter pylori*

clarithromycin sensitivities. J Gastroenterol Hepatol 2008; 23 (Suppl 2): S171-74.

21. Keren I, Kaldalu N, Spoering A, Wang Y, Lewis K. Persister cells and tolerance to antimicrobials. FEMS Microbiol Lett 2004; 230: 13-18.

22. Lewis K. Persister cells, dormancy and infectious disease. Nat Rev Microbiol 2007; 5: 48-56.

23. Connolly LE, Edelstein PH, Ramakrishnan L. Why is long-term therapy required to cure tuberculosis? PLoS Med 2007; 4: 120.

24. Tanimura H, Kawano S, Kubo M, Abe T, Goto M, Tanabe J, et al. Does Helicobacter pylori eradication depend on the period of amoxicillin treatment? A retrospective study. J Gastroenterol 1998; 33: 23-26.

25. Bigger JW. Treatment of staphylococcal infections with penicillin by intermittent sterilization. Lancet 1944; 247: 497-500.

26. Graham DY, Shiotani A. New concepts of resistance in the treatment of Helicobacter pylori infections. Nat Clin Pract Gastroenterol Hepatol 2008; 5: 321-31.

27. de Boer WA, Tytgat GN. The best therapy for Helicobacter pylori infection: should efficacy or side-effect profile determine our choice? Scand J Gastroenterol 1995; 30:401-407.

28. Choi WH, Park DI, Oh SJ, Baek YH, Hong CH, Hong EJ, et al. Effectiveness of 10 days-sequential therapy for *Helicobacter pylori* eradication in Korea. Korean J Gastroenterol 2008; 51: 280-84.

29. Fischbach L, Evans EL. Meta-analysis: the effect of antibiotic resistance status on the efficacy of triple and quadruple first-line therapies for Helicobacter pylori. Aliment Pharmacol Ther 2007; 26: 343-57.

30. Bardhan K, Bayerdorffer E, Veldhuyzen van Zanten SJ, Lind T, Mégraud F, Delchier JC, et al. The HOMER Study: the effect of increasing the dose of metronidazole when given with omeprazole and amoxicillin to cure *Helicobacter pylori* infection. Helicobacter 2000; 5: 196-201.

31. de Boer WA. Bismuth triple therapy: still a very important drug regimen for curing *Helicobacter pylori* infection. Eur J Gastroenterol Hepatol 1999; 11: 697-700.

32. Glupczunski Y, Bourdeaux L, De Prez C, De Vos D, Devreker T, Balegamire B, et al. Prevalence of *Helicobacter pylori* in rural Kivu, eastern Zaire: a prospective endoscopic study. Eur J Gastroenterol Hepatol 1991; 3: 449-55.

33. Hung IF, Chan P, Leung S, Chan FS, Hsu A, But D, et al. Clarithromycine amoxycillin containing triple

therapy: a valid empirical first-line treatment for *Helicobacter pylori* eradication in Hong Kong? Helicobacter 2009;14:505-11

34. Furuta T, Sagehashi Y, Shirai N, Sugimoto M, Nakamura A, Kodaira M, et al. Influence of CYP2C19 polymorphism and *Helicobacter pylori* genotype determined from gastric tissue samples on response to triple therapy for *H.pylori* infection. Clin Gastroenterol Hepatol 2005; 3: 564-73.

35. Hsu PI, Lai KH, Lin CK, Chen WC, Yu HC, Cheng JS, et al. A prospective randomized trial of esomeprazole- versus pantoprazole-based triple therapy for *Helicobacter pylori* eradication. Am J Gastroenterol 2005; 100: 2387-92.

36. Park SH, Park DI, Kim SH, Kim HJ, Cho YK, Sung IK, et al. Effect of high-dose aspirin on *Helicobacter pylori* eradication. Dig Dis Sci 2005; 50: 626-29.

37. Zullo A, Gatta L, De Francesco V, Hassan C, Ricci C, Bernabucci V, et al. High rate of *Helicobacter pylori* eradication with sequential therapy in elderly patients with peptic ulcer: a prospective controlled study. Aliment Pharmacol Ther 2005; 21: 1419-24.

38. Altintas E, Sezgin O, Ulu O, Aydin O, Camdeviren H. Maastricht II treatment scheme and efficacy of different proton pump inhibitors in eradicating *Helicobacter pylori*. World J Gastroenterol 2004; 10: 1656-58.

39. De Francesco V, Zullo A, Margiotta M, Marangi S, Burattini O, Berloco P, et al. Sequential treatment for *Helicobacter pylori* does not share the risk factors of triple therapy failure. Aliment Pharmacol Ther 2004; 19: 407-14.

40. Ghazzawi IM, Obeidat WA, Zuriekat FA. Triple therapy with pantoprazole, clarithromycin and amoxicillin for eradication in patients with Helicobacter pylori positive duodenal ulcers. Saudi Med J 2004; 25: 1006-1009.

41. He XX, Zhao YH, Hao YT. Effect of CYP2C19 genetic polymorphism on treatment efficacy of Helicobacter pylori infection with rabeprazole-based triple therapy in Chinese. Zhonghua Nei Ke Za Zhi 2004; 43: 13-15.

42. Vakil N, Lanza F, Schwartz H, Barth J. Seven-day therapy for *Helicobacter pylori* in the United States. Aliment Pharmacol Ther 2004; 20: 99-107.

43. Noguchi N, Rimbara E, Kato A, Tanaka A, Tokunaga K, Kawai T, et al. Detection of mixed clarithromycin-resistant and -susceptible *Helicobacter pylori* using nested PCR and direct sequencing of

DNA extracted from faeces. J Med Microbiol 2007; 56: 1174-80.

44. Furuta T, Shirai N, Kodaira M, Sugimoto M, Nogaki A, Kuriyama S, et al. Pharmacogenomics-based tailored versus standard therapeutic regimen for eradication of *H.pylori*. Clin Pharmacol Ther 2007; 81: 521-28.

45. McMahon BJ, Hennessy TW, Bensler JM, Bruden DL, Parkinson AJ, Morris JM, et al. The relationship among previous antimicrobial use, antimicrobial resistance, and treatment outcomes for *Helicobacter pylori* infections. Ann Intern Med 2003; 139: 463-69.

46. Okada M, Oki K, Shirotani T, Seo M, Okabe N, Maeda K, et al. A new quadruple therapy for the eradication of *Helicobacter pylori*. Effect of pretreatment with omeprazole on the cure rate. J Gastroenterol 1998; 33: 640-45.

47. Treiber G, Ammon S, Schneider E, Klotz U. Amoxicillin/ metronidazole/ omeprazole/ clarithromycin: a new, short quadruple therapy for *Helicobacter pylori* eradication. Helicobacter 1998; 3: 54-58.

48. Okada M, Nishimura H, Kawashima M, Okabe N, Maeda K, Seo M, et al. A new quadruple therapy for Helicobacter pylori: influence of resistant strains on treatment outcome. Aliment Pharmacol Ther 1999; 13: 769-74.

49. Treiber G, Wittig J, Ammon S, Walker S, van Doorn LJ, Klotz U. Clinical outcome and influencing factors of a new short-term quadruple therapy for *Helicobacter pylori* eradication: a randomized controlled trial (MACLOR study). Arch Intern Med 2002; 162: 153-60.

50. Wu DC, Hsu PI, Wu JY, Opekun AR, Kuo CH, Wu IC, et al. Sequential and concomitant therapy with 4 drugs are equally effective for eradication of *H. pylori* infection. Clin Gastroenterol Hepatol 2010; 8: 36-41.

51. Zullo A, Rinaldi V, Winn S, Meddi P, Lionetti R, Hassan C, et al. A new highly effective shortterm therapy schedule for *Helicobacter pylori* eradication. Aliment Pharmacol Ther 2000; 14: 715-18.

52. De Francesco V, Zullo A, Hassan C, Faleo D, Ierardi E, Panella C, et al. Two new treatment regimens for *Helicobacter pylori* eradication: a randomised study. Dig Liver Dis 2001; 33: 676-79.

53. Hassan C, De Francesco V, Zullo A, Scaccianoce G, Piglionica D, Ierardi E, et al. Sequential treatment for *Helicobacter pylori* eradication in duodenal ulcer patients: improving the cost of pharmacotherapy. Aliment Pharmacol Ther 2003; 18: 641-46.

54. De Francesco V, Della VN, Stoppino V, Amoruso A, Muscatiello N, Panella C, et al. Effectiveness and pharmaceutical cost of sequential treatment for *Helicobacter pylori* in patients with non-ulcer dyspepsia. Aliment Pharmacol Ther 2004; 19: 993-98.

55. De Francesco V, Zullo A, Hassan C, Della Valle N, Pietrini L, Minenna MF, et al. The prolongation of triple therapy for *Helicobacter pylori* does not allow reaching therapeutic outcome of sequential scheme: a prospective, randomised study. Dig Liver Dis 2004; 36: 322-26.

56. Sanchez-Delgado J, Calvet X, Bujanda L, Gisbert JP, Tító L, Castro M. Ten-day sequential treatment for *Helicobacter pylori* eradication in clinical practice. Am J Gastroenterol 2008; 103: 2220-23.

57. Kim JM, Kim JS, Kim N, Jung HC, Song IS. Distribution of fluoroquinolone MICs in *Helicobacter pylori* strains from Korean patients. J Antimicrob Chemother 2005; 56: 965-67.

58. Graham DY, Abudayyeh S, El-Zimaity HM, Hoffman J, Reddy R, Opekun AR. Sequential therapy using high-dose esomeprazoleeamoxicillin followed by gatifloxacin for *Helicobacter pylori* infection. Aliment Pharmacol Ther 2006; 24: 845-50.