Retrospective Study

Five-year sequential changes in secondary antibiotic resistance of Helicobacter pylori in Taiwan

I-Ting Wu, Seng-Kee Chuah, Chen-Hsiang Lee, Chih-Ming Liang, Lung-Sheng Lu, Yuan-Hung Kuo, Yi-Hao Yen, Ming-Luen Hu, Yeh-Pin Chou, Shih-Cheng Yang, Chung-Mou Kuo, Chung-Huang Kuo, Chun-Chih Chien, Yu-Shao Chiang, Shue-Shian Chiou, Tsung-Hui Hu, Wei-Chen Tai

Abstract

AIM: To determine changes in the antibiotic resistance of Helicobacter pylori (H. pylori) in southern Taiwan after failure of first-line standard triple therapy.

METHODS: We analyzed 137 H. pylori-infected isolates from patients who experienced eradication failure after standard first-line triple therapy from January 2010 to December 2014. The H. pylori strains were tested for susceptibility to amoxicillin, clarithromycin, levofloxacin, metronidazole and tetracycline using the E-test method. The minimal inhibitory concentration (MIC) was determined by the agar dilution test.

Institutional review board statement: This retrospective chart review study was approved by both the Institutional Review Board and the Ethics Committee of Chang Gung Memorial Hospital, Taiwan (IRB-104-1245B).

Informed consent statement: The Ethics Committee waived the requirement for informed consent, and each patient’s medical records were anonymized and redacted prior to access.

Conflict-of-interest statement: The authors declare no conflicts of interest.

Data sharing statement: No additional data are available.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/

Correspondence to: Wei-Chen Tai, MD, Division of Hepatogastroenterology, Department of Internal Medicine, Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine, 123, Ta-Pei Road, Niao-Sung Hsiang, Kaohsiung 833, Taiwan. luketai1019@gmail.com
Telephone: +886-7-7317123-8301
Fax: +886-7-7322402

Received: July 5, 2015
Peer-review started: July 7, 2015
First decision: July 19, 2015
Revised: August 8, 2015
Accepted: September 2, 2015
Article in press: September 2, 2015
Published online: October 7, 2015
MIC values of $\geq 0.5$, $\geq 1$, $\geq 4$ and $\geq 8$ mg/L were considered to be the resistance breakpoints for amoxicillin, clarithromycin, levofloxacin, tetracycline and metronidazole, respectively.

RESULTS: A high resistance rate was found for clarithromycin (65%-75%) and metronidazole (30%-40%) among patients who failed first-line standard therapy. The resistance levels to amoxicillin and tetracycline remained very low; however, levofloxacin resistance was as high as 37.5% in 2010 but did not increase any further during the past 5 years. The rates of resistance to these antibiotics did not show a statistically significant upward or downward trend.

CONCLUSION: Antibiotic resistance of H. pylori remains a problem for the effective eradication of this pathogen and its associated diseases in Taiwan. High clarithromycin resistance indicated that this antibiotic should not be prescribed as a second-line H. pylori eradication therapy. Moreover, levofloxacin-based second-line therapy should be used cautiously, and the local resistance rates should be carefully monitored.

Key words: Helicobacter pylori; Antibiotic resistance; Five-year sequential changes; Failed first-line therapy; Southern Taiwan

© The Author(s) 2015. Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: Antibiotic resistance of Helicobacter pylori (H. pylori) is one of the major causes of eradication therapy failure. This study was designed to assess the 5-year sequential changes in antibiotic resistance of H. pylori in Southern Taiwan after the failure of first-line standard triple therapy. The rates of resistance to antibiotics did not show a statistically significant upward or downward trend. Antibiotic resistance of H. pylori has remained a problem in the effective eradication of this type of bacteria in Taiwan. High clarithromycin resistance indicated that this antibiotic should not be prescribed as a second-line therapy for H. pylori eradication. Therefore, levofloxacin-based second-line therapy should be used cautiously, and the local resistance rates should be carefully monitored.

INTRODUCTION
The prevalence of antibiotic resistance of Helicobacter pylori (H. pylori) varies among countries and may be partly determined by geographical factors. H. pylori infects approximately 50% of the global population; its prevalence is approximately 70% in developing nations and approximately 20%-30% in industrialized nations[1]. In Taiwan, the mean seroprevalence rate is approximately 54.5%[2]. The eradication of H. pylori is an important issue in the field of preventive medicine. The Maastricht IV/Florence-Consensus Report has recommended that the standard triple therapy should now be avoided in areas where clarithromycin (CAM) resistance is high (> 15%)[3]. Over time, the reported local primary resistance rate to CAM in Taiwan has ranged from 6%-18%[4,5]. In fact, the primary resistance rate to CAM was reported to be as high as 22.7% in patients who lived in rural areas in eastern Taiwan[6]. If the resistance rates continue to rise, the use of first-line H. pylori eradication with standard triple therapy, which consists of a proton pump inhibitor (PPI), clarithromycin and amoxicillin, might lead to a poor outcome (< 80%)[7]. Ten years ago, Hsu et al[8] reported a > 90% eradication rate of H. pylori with the use of first-line standard triple therapy for 7 d in Taiwan.

When first-line therapy fails, the Maastricht IV Consensus Report recommends that bismuth-containing quadruple therapy be a choice for second-line therapy[3]. However, in areas where bismuth is not available, a levofloxacin-containing triple therapy is recommended in areas of both high and low CAM resistance. Unfortunately, growing primary resistance to levofloxacin has been reported worldwide, including in the reports from 1998-2007 in Taiwan[9]. Due to the rapid development of quinolone resistance, the issue of empirical second-line quinolone-based therapy should be further examined. Clearly, antibiotic resistance determines the success of eradication. Our study aimed to investigate the 5-year sequential changes in antibiotic susceptibility of H. pylori among patients who failed first-line therapy in southern Taiwan.

MATERIALS AND METHODS
Patients
A retrospective study was conducted on H. pylori-infected patients who had failed standard first-line triple therapy (PPI twice daily, 500 mg clarithromycin twice daily, and 1 g amoxicillin twice daily for 7 d) between January 2010 and December 2014 at outpatient clinics at Kaohsiung Chang Gung Memorial Hospital, Taiwan. H. pylori eradication failure was defined as a positive 13C-UBT or any two positive rapid urease tests, as well as positive histology and culture after treatment with first-line eradication therapy. However, we only recruited those patients with positive H. pylori cultures. Among them, a total of 137 H. pylori isolates were obtained from gastric biopsy specimens of patients. All isolates from patients who had been previously treated for H. pylori infection or who had
Demonstrated a positive result. The resistance rates failed first-line therapy were cultured for all isolates from 137 strains. The antibiotic resistance was evaluated using the E-test method (AB BIODISK, Solna, Sweden). The minimal inhibitory concentration (MIC) was determined by the agar dilution test. The H. pylori strains were tested for susceptibility to amoxicillin (AMX), clarithromycin (CAM), levofloxacin (LEV), metronidazole (MET) and tetracycline (TET) using the E-test method (AB BIODISK, Solna, Sweden). H. pylori strains had MIC values of ≥ 0.5, ≥ 1, ≥ 1, ≥ 4 and ≥ 8 mg/L, which were considered to be the resistance breakpoints for AMX, CAM, LEV, TET and MET, respectively.

Statistical analysis
A χ² test for linear trends was used to assess the trend of antibiotic resistance over time from 2010-2014. A P value < 0.05 was considered statistically significant.

RESULTS
All isolates from 137 H. pylori-infected patients who failed first-line therapy were cultured for H. pylori and demonstrated a positive result. The resistance rates to AMX, CAM, LEV, TET and MET were 0%, 70.8%, 37.5%, 0% and 37.5%, respectively, in 2010, 25%, 65.8%, 0%, 34.4%, and 0%, respectively, in 2011 and 30%, 65%, 10%, 30%, and 0%, respectively, in 2012. The resistance rates to AMX, CAM, LEV, TET and MET were 30%, 75%, 0%, 35% and 0%, respectively, in 2013 and 0%, 70.7%, 26.8%, 2.4% and 43.9%, respectively, in 2014 (Table 1 and Figure 1). However, none of the isolates showed a statistically significant upward or downward trend in terms of resistance rates when they were analyzed by χ² test for linear trends (Table 1).

DISCUSSION
In our study, the annual changes in the antibiotic resistance rates of H. pylori after failure of standard first-line treatment in southern Taiwan from 2010 to 2014 revealed a high resistance to CAM (up to 70%) and MET (43.9%) and a low resistance to both AMX (0%) and TET (0%-10%). The resistance to LEV was as high as 37.5% in 2010 but was only 26.5% in 2014.

The cause of CAM resistance may be point mutations, which occur at A2143G, A2142G and A2142C, in the 23S rRNA component of ribosomes. The second-line resistance rate to CAM was as high as 65%-75%. Therefore, CAM should no longer be used as a second-line regimen for eradication therapy because high CAM resistance decreased the efficacy of CAM-based therapy by 66%-77% according to previous studies. The high rate of resistance to CAM in Taiwan was one of the major reasons for the failure of first-line standard triple therapy. In fact, the eradication rate after first-line therapy in Taiwan dropped from 95% in 2005 to 79.3% in 2014. This result indicated that the standard first-line therapy may need to be substituted with alternative combination treatments such as non-bismuth quadruple (concomitant, sequential or hybrid therapy).
therapy in Taiwan.

MET resistance in cases of 
H. pylori
infection is complex and is primarily associated with mutational inactivation of several redox-related genes (frxA, rdxA)\(^{15}\). Fortunately, in contrast to CAM resistance, MET resistance had less impact on the eradication rate and could be overcome via an increase in the dose\(^{16,17}\). Therefore, MET continues to be a widely used drug for eradication therapy, despite a relatively high resistance rate. Some studies have shown that MET resistance is not a major determinant of the failure rate because MET-resistant 
H. pylori
isolates reduce the efficacy of MET-containing regimens but do not render them completely ineffective\(^{18,19}\). This discrepancy between \textit{in vitro} MET resistance and treatment outcomes may be partially explained by changes in the oxygen pressure in the gastric environment, as MET-resistant 
H. pylori
isolates can become susceptible to MET under low oxygen conditions \textit{in vitro}. These combined results suggested that treatment with MET could overcome MET resistance to some degree\(^{20}\). However, MET, which is an important antibiotic that is used in quadruple therapy, demonstrated a high, continuous level of resistance (25%-43.9%). This is similar to what has been observed in other Asian countries such as Malaysia and South Korea\(^{21,22}\).

Nevertheless, it is still recommended that bismuth-based quadruple therapy or levofloxacin-containing triple therapy be used as a second-line therapy\(^{23,24}\). However, bismuth is not available in many countries. Alternatively, the Maastricht IV Consensus Report has also recommended LEV-based therapy as a second-line rescue treatment after failure of first-line therapy. Unfortunately, the growing resistance to LEV has become a global problem\(^{25}\). Quinolone resistance is determined in 
H. pylori (N87 and D91) in the quinolone resistance-determining region of the gyrA gene of 
H. pylori\(^{24}\). In the current study, the prevalence of LEV resistance was 37.5% in 2010, and fortunately, it has not increased since then. This might be because the bureau of our hospital, via an electronic audit program, issued a reimbursement regulation that restricts the use of any antibiotics in patients throughout the hospital. Another reason might be due to the practice of tailored therapy for patients with documented LEV-susceptibility strains whenever 
H. pylori culture was available\(^{25}\). However, additional follow-up reports on LEV resistance are needed in the next couple of years. Although the current 5-year report showed no statistically significant upward or downward trend, the resistance rate reported in the current study was still much higher than the 11.8% that was reported in 2007 and the 26.5% in 2014\(^{26}\).

Furthermore, the impact of the increase in levofloxacin resistance might be crucial in the eradication of 
H. pylori. Perna et al\(^{27}\) reported that the eradication rate was lower in patients with LEV-resistant strains compared with those with susceptible strains (33.3% vs 75%). In addition, Tai et al\(^{25}\) noted that the eradication rate dropped from 92.8% to 14.3% in patients with levofloxacin-resistance strains who received 10-d LEV triple therapy. These results imply that treatment with levofloxacin-based therapy for 
H. pylori should be limited to patients with susceptible strains in order to avoid a potential resistance problem.

Importantly, the global increase in the prescription of quinolones is responsible for the rapid rise in the resistance of 
H. pylori, but it has also impacted other types of bacteria\(^{28}\). For example, \textit{Mycobacterium tuberculosis} is highly prevalent in Taiwan. One study reported that patients who were recently exposed to quinolone for 5 d or more were less likely to be smear-positive (OR = 0.27, 95%CI: 0.11-0.63) and were more likely to experience a delay in the receipt of proper treatment for tuberculosis (time ratio 2.02, 95%CI: 1.19-3.44)\(^{29}\). Moreover, quinolone exposure for > 10 d that occurred > 60 d before a diagnosis of tuberculosis was associated with the highest risk of quinolone resistance (OR = 17.0, 95%CI: 5.1-56.8) compared with no exposure\(^{30}\). Importantly, the current report reminds us of the potential impact of the empiric use of LEV as a second-line eradication therapy, which is recommended by the Maastricht IV Consensus Report, and that the use of this antibiotic should be carefully monitored.

Our study showed that 
H. pylori isolates were highly susceptible to AMX and TET (0% throughout the 5-year period except for 10% in TET strains that were resistant in 2012). This is similar to what was found in previous reports by Hung et al\(^{26}\) and Mégraud\(^{31}\). AMX resistance is associated with alterations in penicillin-binding proteins. For time-dependent antibiotics such as AMX, it is more important to prolong the time so that the plasma concentration is higher than the minimal inhibitory concentration (MIC) to achieve higher plasma levels of the drug\(^{32}\). On the contrary, the bactericidal activity of TET is a result of the drug’ s ability to prevent the synthesis of nascent peptide chains by binding to the 30S ribosomal subunit as well as by blocking the binding of aminoacyl-tRNA\(^{33}\). The low resistance rates to both AMX and TET imply that they might be good candidate antibiotics for use as second-line therapies for 
H. pylori eradication when given in combination because the prevention of antibiotic resistance is a key factor for success. Unfortunately, the reported success rates when these two antibiotics were given in combination were unacceptable in two previous publications from Taiwan (62%-75% in an intention-to-treat analysis and 64%-80% in a per-protocol analysis)\(^{34,35}\). The reason for the disappointing \textit{in vivo} 
H. pylori eradication rates might be related to a drug-drug interaction between amoxicillin and tetracycline, both of which exhibit low antibiotic resistance \textit{in vitro}. Sorice et al\(^{36}\) proposed that bacteriostatic drugs such as tetracycline might interfere with the bactericidal action of penicillin. The bactericidal action of penicillin involves the inhibition
of cell wall formation, which is dependent on how fast the bacteria multiply. Bacteriostatic antibiotics such as TET may reduce the effectiveness of penicillin via the inhibition of the cellular protein synthesis that is required for cell division[9].

The current study has some limitations. First, this was a single-center report, and multicenter data would be more convincing with respect to this issue. Second, it is a retrospective study with a relatively small sample size, and thus, bias may exist. Third, we were unable to provide any further genetic data for these resistant strains. As is already known, antibiotic resistance is conferred by point mutations in H. pylori DNA[8]. Resistance is currently detected by culture-based and molecular methods. Molecular techniques could predict the antibiotic resistance by the detection of point mutations. In reality, these techniques are not always feasible and are still difficult to apply in clinical practice due to the high costs that are associated with routine use[9].

In conclusion, antibiotic resistance of H. pylori remains a hindrance to the effective eradication of H. pylori infections in Taiwan. High CAM resistance indicates that it should not be prescribed as a second-line therapy for H. pylori eradication. Levofloxacin-based second-line therapy should be used cautiously, and the local resistance rates should be carefully monitored.

COMMENTS

Background
The prevalence of antibiotic resistance of Helicobacter pylori (H. pylori) varies among countries and may be partly determined by geographical factors. H. pylori infects approximately 50% of the global population; its prevalence is approximately 70% in developing nations and approximately 20%-30% in industrialized nations. Antibiotic resistance of H. pylori is one of the major causes of failure of eradication therapy.

Research frontiers
The growing primary resistance to levofloxacin has been reported worldwide, including in the studies from Taiwan that were conducted between 1998 and 2007. Due to the rapid development of quinolone resistance, the issue of empirical second-line quinolone-based therapy should be further examined. Clearly, antibiotic resistance determines the success of eradication therapies. We investigated the 5-year sequential change in the antibiotic susceptibility of H. pylori among patients who failed first-line therapy in southern Taiwan.

Innovations and breakthroughs
The result of the present study showed high resistance rates to clarithromycin (65%-75%) and metronidazole (30%-40%) among patients who failed first-line standard therapy. The resistance to amoxicillin and tetracycline remained very low, but the resistance to levofloxacin was as high as 37.5% in 2010; however, this value did not increase during the past 5 years. The resistance rates to these antibiotics did not show a statistically significant upward or downward trend.

Applications
Antibiotic resistance of H. pylori has remained a problem in the effective eradication of H. pylori infection in Taiwan. High clarithromycin resistance indicated that this antibiotic should not be prescribed as a second-line therapy for H. pylori eradication. A levofloxacin-based second-line therapy should be used cautiously, and the local resistance rates should be carefully monitored.

Terminology
The H. pylori strains were tested for susceptibility to amoxicillin (AMX), CAM, levofloxacin (LEV), metronidazole (MET) and tetracycline (TET) using the E-test method (AB BIODISK, Solna, Sweden). H. pylori strains had minimal inhibitory concentration values ≥ 0.5, ≥ 1, ≥ 1, ≥ 4 and ≥ 8 mg/L, which were considered to be the resistance breakpoints for AMX, CAM, LEV, TET and MET, respectively.

Peer-review
The study is well written and organized. The issue is very interesting.

REFERENCES

1 Fox JG, Wang TC. Inflammation, atrophy, and gastric cancer. J Clin Invest 2007; 117: 60-69 [PMID: 17200707 DOI: 10.1172/JCI30111]
2 Teh BH, Lin JT, Pan WH, Lin SH, Wang LY, Lee TK, Chen CJ. Seroprevalence and associated risk factors of Helicobacter pylori infection in Taiwan. Anticancer Res 1994; 14: 1389-1392 [PMID: 8067711]
3 Malferttheiner P, Megraud F, O’Morain CA, Atherton J, Axon AT, Bazzoli F, Gensini GF, Gisbert JP, Graham DY, Rockkas T, El-Omar EM, Kuipers EJ. Management of Helicobacter pylori infection—the Maastricht IV/ Florence Consensus Report. Gut 2012; 61: 646-664 [PMID: 22491499 DOI: 10.1136/gutjnl-2012-302084]
4 Chang WL, Sheu BS, Cheng HC, Yang YJ, Yang HB, Wu JJ. Resistance to metronidazole, clarithromycin and levofloxacin of Helicobacter pylori before and after clarithromycin-based therapy in Taiwan. J Gastroenterol Hepatol 2009; 24: 1230-1235 [PMID: 19476562 DOI: 10.1111/j.1440-1746.2009.05829.x]
5 Hu CT, Wu CC, Lin CY, Cheng CC, Su SC, Tseng YH, Lin NT. Resistance rate to antibiotics of Helicobacter pylori isolates in eastern Taiwan. J Gastroenterol Hepatol 2007; 22: 720-723 [PMID: 17444862 DOI: 10.1111/j.1440-1746.2006.04743.x]
6 Chen MC, Lei WY, Lin JS, Yi CH, Wu DC, Hu CT. Levofloxacin-amoxicillin-clavulanate-rabeprazole versus a standard seven-day triple therapy for eradication of Helicobacter pylori infection. Biomed Res Int 2014; 2014: 158520 [PMID: 24995271 DOI: 10.1155/2014/158520]
7 Tai WC, Liang CM, Lee CH, Chu CH, Hu ML, Lu LS, Kuo YH, Kuo YM, Yen YH, Kuo CH, Chiu SS, Wu KL, Chiu YC, Hu TH, Chuah SK. Seven-Day Nonbismuth Containing Quadruple Therapy Could Achieve a Grade “A” Success Rate for First-Line Helicobacter pylori Eradication. Biomed Res Int 2015; 2015: 623732 [PMID: 26090428 DOI: 10.1155/2015/623732]
8 Huo PI, Lai KH, Lin CK, Chen WC, Yu HC, Cheng JS, Tsay FW, Wu CJ, Lo CC, Tseng HH, Yamaoka Y, Chen JL, Lo GH. A prospective randomized trial of esomeprazole- versus pantoprazole-based triple therapy for Helicobacter pylori eradication. Am J Gastroenterol 2005; 100: 2387-2392 [PMID: 16279889 DOI: 10.1111/j.1572-0241.2005.0024x.x]
9 Chuah SK, Tai WC, Lee CH, Liang CM, Hu TH. Quinolone-containing therapies in the eradication of Helicobacter pylori. Biomed Res Int 2014; 2014: 151543 [PMID: 25243116 DOI: 10.1155/2014/151543]
10 Huo PI, Hwang IR, Cittelly D, Lai KH, El-Zimaity HM, Gutierrez O, Kim JG, Osato MS, Graham DY, Yamaoka Y. Clinical presentation in relation to diversity within the Helicobacter pylori cag pathogenicity island. Am J Gastroenterol 2002; 97: 2231-2238 [PMID: 12385238 DOI: 10.1111/j.1572-0241.2002.05977.x]
11 Chuah SK, Tai WC, Hu PI, Wu DC, Wu KL, Kuo CM, Chiu YC, Hu ML, Chou YP, Kuo YH, Liang CM, Chiu KW, Hu TH. The efficacy of second-line anti-Helicobacter pylori therapy using an extended 14-day levofloxacin/amoxicillin/proton-pump inhibitor treatment—a pilot study. Helicobacter 2012; 17: 374-381 [PMID: 22967121 DOI: 10.1111/j.1523-5378.2012.00960.x]
Sequential changes in secondary antibiotic resistance of H. pylori.

12 Ierardi E, Giorgio F, Losurdo G, Di Leo A, Principi M. How antibiotic resistances could change Helicobacter pylori treatment: A matter of geography? World J Gastroenterol 2013; 19: 8168-8180 [PMID: 23635606 DOI: 10.3748/wjg.v19.i45.8168]

13 Venerito M, Krieger T, Ecker T, Leandro G, Malfertheiner P. Meta-analysis of bismuth quadruple therapy versus clarithromycin triple therapy for empiric primary treatment of Helicobacter pylori infection. Digestion 2013; 88: 33-45 [PMID: 23880479 DOI: 10.1159/000350719]

14 Yang JC, Lin CJ, Wang HL, Chen JD, Kao JY, Shun CT, Lu CW, Lin BR, Shieh MJ, Chang MC, Chang YT, Wei SC, Lin LC, Yeh WC, Kuo JS, Tung CC, Leong YL, Wang WH, Tong JM. High-dose dual therapy is superior to standard first-line or rescue therapy for Helicobacter pylori infection. Clin Gastroenterol Hepatol 2015; 13: 895-905.e5 [PMID: 25460556 DOI: 10.1016/j.cgh.2014.10.036]

15 Tsugawa H, Suzuki H, Satoh K, Hirata K, Matsuzaki J, Saito Y, Suematsu M, Hibi T. Two amino acids mutation of ferric uptake regulator determines Helicobacter pylori resistance to metronidazole. Antimicrob Res Comb 2011; 14: 23-25 [PMID: 20518707 DOI: 10.1089/arcs.2010.3146]

16 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 Pharmocol Ther 2007; 26: 343-357 [PMID: 17635369 DOI: 10.1111/j.1365-2036.2007.03386.x]

17 Laine L, Hunt R, El-Zimati H, Nguyen B, Osato M, Spérandio J. Bismuth-based quadruple therapy using a single capsule of bismuth biskalicate, 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. Am J Gastroenterol 2003; 98: 562-567 [PMID: 12650788]

18 Nista EC, Candelli M, Cremonini F, Cazzato IA, Carothers JJ, Parrella MM, Carothers J, Vrbic R, Visalli F, Aasheim O, Kaspersen J. Regulator determines Helicobacter pylori resistance to metronidazole. J Med Microbiol 2004; 53: 1123-1128 [PMID: 15496391]

19 Goh KL, Naravatnam P. High Helicobacter pylori resistance to metronidazole but zero or low resistance to clarithromycin, levofloxacin, and other antibiotics in Malaysia. Helicobacter 2011; 16: 241-245 [PMID: 21585611 DOI: 10.1111/j.1537-5378.2011.00841.x]

20 Lee JW, Kim GH, You HS, Lee BE, Ryu DY, Cheong JH, Jung JM, Jeon CY, Kim JY, Shin SS. Prevalence of primary and secondary antimicrobial resistance of Helicobacter pylori in Korea from 2003 through 2012. Helicobacter 2013; 18: 206-214 [PMID: 23241101 DOI: 10.1111/hel.12031]

21 Song M, Ang TL. Second and third line treatment options for Helicobacter pylori eradication. World J Gastroenterol 2014; 20: 1517-1528 [PMID: 24587627 DOI: 10.3748/wjg.v20.i6.1517]

22 Boyanova L. Prevalence of multidrug-resistant Helicobacter pylori in Bulgaria. J Med Microbiol 2009; 58: 930-935 [PMID: 19502370 DOI: 10.1099/jmm.0.009930-0]

23 Tai WC, Lee CH, Chiu SS, Kuo CM, Kuo CH, Liang CM, Lu LS, Chiu CH, Wu KL, Chiu YC, Hu TH, Chuah SK. The clinical and bacteriological factors for optimal levofloxacin-containing triple therapy in second-line Helicobacter pylori eradication. PLoS One 2014; 9: e105822 [PMID: 25141137]

24 Hung KH, Shiu BS, Chang WL, Wu HM, Liu CC, Wu JJ. Prevalence of primary fluoroquinolone resistance among clinical isolates of Helicobacter pylori at a University Hospital in Southern Taiwan. Helicobacter 2009; 14: 61-65 [PMID: 19191989]

25 Perna F, Zullo A, Ricci C, Hassan C, Morini S, Vaira D. Levofloxacin-based triple therapy for Helicobacter pylori re-treatment: role of bacterial resistance. Dig Liver Dis 2007; 39: 1001-1005 [PMID: 17886267 DOI: 10.1016/j.dld.2007.06.016]

26 Carothers JJ, MG, Hennessy TW, Bensler M, Morris JM, Reasonover AL, Hurlbut DA, Parkinson AJ, Coleman JM, McMahon BJ. The relationship between previous fluoroquinolone use and levofloxacin resistance in Helicobacter pylori infection. Clin Infect Dis 2007; 44: e5-8 [PMID: 17173210 DOI: 10.10865.00704]

27 Leon CV, Calver AD, Victor TC, Warren RM, Shin SS, Murray MB. Use of fluoroquinolone antibiotics leads to tuberculosis treatment delay in a South African gold mining community. Int J Tuberc Lung Dis 2011; 15: 77-83 [PMID: 21276301]

28 Devasia RA, Blackman A, Gebretsadik T, Griffin M, Shintani A, May C, Smith T, Hooper N, Maruri F, Warkentin J, Mitchel E, Sterling TR. Fluoroquinolone resistance in Mycobacterium tuberculosis: the effect of duration and timing of fluoroquinolone exposure. Am J Respir Crit Care Med 2009; 180: 365-370 [PMID: 19483111 DOI: 10.1164/rccm.200901-0166OC]

29 Mégraud F. Current recommendations for Helicobacter pylori therapies in a world of evolving resistance. Gut Microbes 2013; 4: 541-548 [PMID: 23929066 DOI: 10.4161/gmic.25930]

30 Yang JC, Lu CW, Lin CJ. Treatment of Helicobacter pylori infection: current status and future concepts. World J Gastroenterol 2014; 20: 5283-5293 [PMID: 24833858 DOI: 10.3748/wjg.v20.i.815283]

31 Chopra I, Roberts M. Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. Microbiol Mol Biol Rev 2001; 65: 232-260; second page, table of contents [PMID: 11381101 DOI: 10.1128/MMBR.65.2.232-260.2001]

32 Chuaah SK, Hsu PI, Chang KC, Chiu YC, Wu KL, Chou YP, Hu ML, Tai WC, Chiu KW, Chiu SS, Wu DC, Hu TH. Randomized comparison of two non-bismuth-containing second-line rescue therapies for Helicobacter pylori. Helicobacter 2012; 17: 216-223 [PMID: 22515360 DOI: 10.1111/j.1537-5378.2012.00937.x]

33 Wu DC, Hsu PI, Tseng HH, Tsay FW, Lai KH, Kuo CH, Wang SW, Chen A. Helicobacter pylori infection: a randomized, controlled study comparing 2 rescue therapies after failure of standard triple therapies. Medicine (Baltimore) 2011; 90: 180-185 [PMID: 21512411 DOI: 10.1097/MD.0b013e3182d94dc1]

34 Sorice F, Ortona L, Pizzigallo E. Further aspects of combination antibiotic therapy. Critical review and personal case studies. Minerva Med 1975; 66: 2805-2822 [PMID: 1161172]

35 Pavlic MJ, Naravac F, Verbom T, van Winkelhoff AJ, De Graaff J. In vitro susceptibility of Helicobacter pylori to several antimicrobial combinations. Antimicrob Agents Chemother 1993; 37: 1184-1186 [PMID: 8517712 DOI: 10.1128/AAC.37.5.1184]

36 Mégraud F, Lehours P. Helicobacter pylori detection and antimicrobial susceptibility testing. Clin Microbiol Rev 2007; 20: 280-322 [PMID: 17428887 DOI: 10.1128/CMR.00033-06]

37 Graham DY, Lu H, Yamaoka Y. A report card to grade Helicobacter pylori therapy. Helicobacter 2007; 12: 275-278 [PMID: 17669098 DOI: 10.1111/j.1537-5378.2007.00518.x]

P- Reviewer: Abdel-Salam OME, Carreira H E- Editor: Yu J L- Editor: Wang TQ E- Editor: Wang CH
