**Helicobacter pylori** antibiotic resistance in Iran

Marjan Mohammadi, Delaram Doroud, Nazanin Mohajerani, Sadegh Massarrat

AIM: To examine the frequency of antibiotic resistance in Iranian Helicobacter pylori (*H pylori*) strains isolated from two major hospitals in Tehran.

METHODS: Examination of antibiotic resistance was performed on 120 strains by modified disc diffusion test and PCR-RFLP methods. In addition, in order to identify the possible causes of the therapeutic failure in Iran, we also determined the resistance of these strains to the most commonly used antibiotics (metronidazole, amoxicillin, and tetracycline) by modified disc diffusion test.

RESULTS: According to modified disc diffusion test, 1.6% of the studied strains were resistant to amoxicillin, 16.7% to clarithromycin, 57.5% to metronidazole, and there was no resistance to tetracycline. Of the clarithromycin resistant strains, 73.68% had the A2143G mutation in the 23S rRNA gene, 21.05% A2142C, and 5.26% A2142G. Of the sensitive strains were positive for any of the three point mutations. Of the metronidazole resistant strains, deletion in rdxA gene was studied and detected in only 6 (5%) of the antibiogram-based resistant strains. None of the metronidazole sensitive strains possessed rdxA gene deletion.

CONCLUSION: These data show that despite the fact that clarithromycin has not yet been introduced to the Iranian drug market as a generic drug, nearly 20% rate of resistance alerts toward the frequency of macrolide resistance strains, which may be due to the widespread prescription of erythromycin in Iran. rdxA gene inactivation, if present in Iranian *H pylori* strains, may be due to other genetic defects rather than gene deletion.

© 2005 The WJG Press and Elsevier Inc. All rights reserved.

**Key words:** *H pylori*; Clarithromycin; Metronidazole; Resistance; Iran

*Helicobacter pylori* (*H pylori*) infects the majority of the adult population in developing countries including Iran. The rate of infection in Iranian adults according to serology data is up to 80%[1]. The outcomes include gastritis, peptic ulcers and gastric adenocarcinomas, which are highly prevalent in Iran[2]. Patient compliance with the prescribed medications, presence of pre-existing resistance to the key anti-*H pylori* antibiotics (clarithromycin and metronidazole), final duration of therapy and prescribed dose of antibiotics are all the factors which influence the effectiveness of therapy[3]. Host genetic polymorphisms, i.e. IL-1-511 and CYP450 and CYP2C19, may affect the efficiency of the therapy as well[4]. Antibiotic resistance varies geographically and there is a great need for local studies.

Clarithromycin is recommended as a key component in anti-*H pylori* eradication[5]. In different geographic locations, resistance to clarithromycin differs. In countries with a higher use of macrolides, the rate of resistance to clarithromycin is reported to be proportionally higher[6]. The basis of the resistance is the presence of defined mutations in the 23S rRNA gene, which results in a decrease in the antibiotic binding to the bacterial ribosome[7-9]. Mutations in the form of A-G transition at nucleotide positions 2143, 2144, and A-C at nucleotide position 2142 have been found to confer clarithromycin resistance in *H pylori* strains[10]. Recently in *H pylori* isolates from northeast China, three new mutation points (G2224A, C2245T, and T2289C) were found to be related to the clarithromycin resistance[11]. Several different PCR-based methods have been developed for the detection of the above-mentioned mutations in order to amplify part of the V domain of 23Sr RNA gene[12]. The amplified fragments are digested by restriction enzymes, indicating mutations in the positions 2143 and 2144 which yield a high level clarithromycin resistance[10,13]. A2142C is reported to be less frequent and is detectable by PCR using 3’-mismatched specific primers[14,15].

Though most countries include clarithromycin in the prescribed anti-*H pylori* treatment regimens, this drug is infrequently prescribed in Iran due to its high cost.

As regards to metronidazole, the basis of resistance in *H pylori* has been partly associated with inactivation of rdxA, the gene encoding an oxygen-insensitive NADPH nitroreductase may be enhanced by mutations in *frxA* gene...
encoding a NAD(P)H-flavin oxidoreductase\textsuperscript{[24]}. This study presents the first documented report from Iran on the \textit{rdxA} gene deletion. Frameshift mutations in \textit{frxA} gene were not studied as their frequent occurrence has questioned its reliability as a resistance marker\textsuperscript{[17]}. We have used modified disc diffusion test for primary analysis followed by the described molecular assays to examine resistance among Iranian \textit{H pylori} strains with particular emphasis on clarithromycin and metronidazole.

**Bacterial strains and growth conditions**

Gastric antral biopsies were taken from 120 non-\textit{H pylori} pretreated patients (52 males and 68 females, aged 42±20 years) with epigastralgia referring to gastric endoscopy (during 2001-2002) followed by rapid urease test. These patients did not undergo any prior treatment. Urease positive samples were cultured on bruccella agar (Merck) supplemented with 5% sheep blood, (6 mg/L) vancomycin (Fluka BioChemika), (5 mg/L) trimethoprim (Fluka BioChemika) and (2 mg/L) amphotericin-B (Gibco) under microaerophilic conditions (85% N\textsubscript{2}, 100 mL/L CO\textsubscript{2}, 5% O\textsubscript{2}) at 37 \degree C for 3-5 d. Single colonies of \textit{H pylori} were then isolated and confirmed for identity according to colony morphology, wet mount, microscopic observation after Gram staining and biochemical analysis (urease and catalase tests).

**Disc diffusion susceptibility test**

Bacterial resistance to clarithromycin\textsuperscript{[18]}, metronidazole\textsuperscript{[19]}, amoxicillin, and tetracycline\textsuperscript{[20]} was determined using modified Kirby-Bauer procedure as previously described\textsuperscript{[21,22]}. Suspensions of 4-d-old cultures were prepared in sterile saline to opacity of No. 4 (108 CFU/mL) McFarland standard. Muller-Hinton agar plates supplemented with 5% sheep blood were then inoculated with a swab from the prepared suspension. House-made discs containing 2 \u03bc g amoxicillin, and 30 \u03bc g tetracycline were placed separately on the culture plates for each strain. Plates were then incubated at 37 \degree C for 72 h under microaerophilic conditions generated by Gas Pak jars. The disc diffusion tests were made thrice for each strain. The inhibition zone diameters were measured in millimeters with a ruler. If similar results were observed in two experiments out of three, the isolates were considered susceptible or resistant but as mixed populations. A \textit{H pylori} control strain susceptible to metronidazole (Sydney strain, ss1) was used. The breakpoints with the Mueller-Hinton agar for the inhibition diameters are described in Table 3, briefly: 20 mm for tetracycline, 11 mm for amoxicillin, for metronidazole zones; <16 mm resistant, 16-21 mm intermediate and >21 mm susceptible (but in this study isolates in the intermediate and resistant zone were both considered as resistant), for clarithromycin development of any size zone is considered resistant\textsuperscript{[23]}.

**DNA extraction and PCR assays**

Genomic DNA was extracted as previously described\textsuperscript{[10]}. PCR primers used for this study were chosen from published reports with the implementation of the same PCR cycles\textsuperscript{[10]}.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|}
\hline
\textbf{Gene} & \textbf{Primers} & \textbf{Expected fragment (bp)} & \textbf{References} \\
\hline
\textit{23S rRNA} & CLA 18:5"-AGTCGGGAC  \\
& CTAAAGCCGCC-3" & 1 400 & 10 \\
& (set 1) CLA 21:5"-TTCCGCTTA \\
& GAGTCGGCCG-3" & 700 & 15 \\
\textit{23S rRNA} & CLA 18:5"-AGTCGGGACC \\
& TAAGCGCAGG-3" & 700 & 15 \\
& (set 2) CLA 3:5"-AGCCACCCCGG \\
& GTCTG-3" & 850 & 24 \\
\textit{RdxA} & RdxA 1:5"-AATTTGGCATG \\
& GGGCGAGA-3" & 650 & 10 \\
& (set 3) RdxA 2:5"-GAAAGCCGTTGAA  \\
& AACACCCCT-3" & 150 & 24 \\
\hline
\end{tabular}
\caption{Primers sets used in this study}
\end{table}

**Statistical analysis**

Data were analyzed by SPSS version 11.0. The Pearson \(\chi^2\) test and Fisher’s exact test were used to assess the relationships between the results of disc diffusion and PCR/ RFLP methods. Standard error of mean for each frequency was determined accordingly.

**Primary antibiotic resistance by disc diffusion**

According to the disc diffusion test, all 120 strains were sensitive to tetracycline. Of these, 2 strains (1.6%) were resistant to amoxicillin, 20 (16.7%) to clarithromycin and 69 (57.5%) to metronidazole. Five of the one hundred and twenty (4.2% of the strains) showed dual resistance to metronidazole and clarithromycin. Table 2 presents the frequency of single and multiple resistances to the tested antibiotics.

**Detection of 23S rRNA mutations via PCR-RFLP**

All the DNA samples were positive by the PCR assay for the amplification of the 1 400 bp of \textit{23S rRNA} gene. RFLP analysis pattern showed that 19 out of 120 (15.8%) strains were mutated (Table 2). In this group, 73.68% had the A2143G mutation and 5.26% the A2142G mutations. Moreover, none of the clarithromycin resistant samples showed the A2144G mutation, 21.05% of the samples were resistant[23].
of the A2142C mutation. Among the 20 clarithromycin resistant strains, only one did not show any of these mutations. None of the sensitive strains were positive for any of the mentioned mutations.

**PCR detection of rdxA gene deletion**

Among the 69 metronidazole resistant strains (based on antibiograms), only 6 (5% of total) demonstrated 200 bp deletion in the rdxA gene (Table 2).

According to the data analysis by the SPSS program, there were no relations between the 23S rRNA mutations, rdxA deletion and antibiotic resistance with age, gender, and cagA status.

### Table 2 Frequency of single and multi-drug resistance based on listed methods of detection

| Antibiotics          | Method of detection | Disc diffusion | 23S rRNA | rdxA gene deletion |
|----------------------|---------------------|----------------|----------|-------------------|
|                      | n                   | n              | n        | n                 |
| Amoxicillin only     | 1/120               | 0.8            | 14/120   | 11.7              |
| Amoxicillin and      | 1/120               | 0.8            | 12/120   | 10.0              |
| metronidazole        |                     |                |          |                   |
| Clarithromycin only  | 15/120              | 12.5           | 19/120   | 15.8              |
| Clarithromycin and   | 5/120               | 4.2            |          |                   |
| metronidazole        |                     |                |          |                   |
| Metronidazole only   | 63/120              | 52.5           | 6/120    | 5                 |
| Metronidazole and    | 1/120               | 0.8            |          |                   |
| amoxicillin          |                     |                |          |                   |
| Metronidazole and    | 5/120               | 4.2            |          |                   |
| clarithromycin       |                     |                |          |                   |
| Tetracycline         | 0/120               | 0              |          |                   |

Several studies comparing the different susceptibility techniques for *H pylori* have been published, but the results are controversial, as *H pylori* is a very slow growing bacterium with particular growth needs. The oxygen-dependent metabolism of metronidazole in *H pylori* via several nitroreductases possibly results in great difference in MIC when isolates are tested repeatedly. Nevertheless, strains continue to be classified as resistant or susceptible. As regards to culture-based antimicrobial susceptibility testing (especially to metronidazole), routine methods of agar dilution, disc diffusion, and E-test seem to be poorly standardized. Though time consuming agar dilution is accepted as the gold standard and is reported to be highly reproducible in several studies, nowadays, E-test is used most frequently as a substitute as it is easier to perform and relatively reproducible. Finally, a cheap and easy way to perform susceptibility testing is via disc diffusion, but no MIC value can be obtained. Recently in UK, susceptibility testing to metronidazole, tetracycline, macrolide, and amoxicillin is performed by the modified disc diffusion method and the results are adequate to determine the resistance rates in a large cohort of patients from a single clinical center whom resistance patterns have been evaluated over time. Then again in a study in France, reproducibility study on randomly selected strains declared that disc diffusion is more reproducible than E-test for both clarithromycin and erythromycin. Due to these observations and high cost of E-test, modified disc diffusion test was chosen for this study.

Worldwide antibiotic resistance has been carefully reviewed by Megraud, and our resistance rate to clarithromycin and metronidazole is comparable to the resistance rate of 50% to metronidazole and 8% to clarithromycin in China.

Current regimens for the eradication of *H pylori* in Iran consist of a proton pump inhibitor (omeprazole) or an H2 receptor blocker (ranitidine), a bismuth salt plus two antibacterial agents, such as amoxicillin, furazolidone/metronidazole or recently clarithromycin. Treatment regimens of 4 or 7 d are unacceptable for *H pylori* infection in Iran, even in the presence of a favorable sensitivity profile. Although the presented data do not support the association with the failure of eradication in our country, according to our results, such rates of resistance to metronidazole and clarithromycin could be the major cause of eradication failure in Iran. For developing countries, the standard triple therapy remains as the best option for the eradication regimen because of its low cost. However, in Iran bismuth triple therapy in the presence of high prevalence of metronidazole resistance has a poor efficacy unless higher doses of metronidazole are prescribed to increase the cure rate of therapy.

Our results indicate that making clarithromycin available may not be an effective strategy to improve the eradication rates in Iran, as the level of resistance is already significant. There is a worldwide need for simple and cost effective anti-*H pylori* therapies. Since the effectiveness relates to the level of antibiotic resistance, knowledge of the resistance pattern is essential for choosing empiric therapy. Metronidazole is widely used in Iran because of the prescription in most periodontal and parasitic diseases. Resistance to metronidazole among Iranian patients, as most of the developing countries, is relatively high and this is the major cause of eradication failure in these countries. Thus, the need for a suitable alternative for this drug is highly perceptible and crucial to plan a more effective therapeutic strategy. (OABC) Omeprazole, Amoxicillin, Bismuth, Clarithromycin and (OABF)Omeprazole, Amoxicillin, Bismuth, Furazolidone are both effective in eradicating *H pylori* in countries where metronidazole resistance is a problem. OABF is a good alternative in the face of growing resistance to clarithromycin in developed countries, and is attractive for developing countries where clarithromycin is not readily available and is particularly recommended for Iran.
In recent years, rdxA gene analysis of the fresh samples showed that the metronidazole resistance is mainly attributed to the mutations in this gene including gene deletion [24]. Antibiogram analysis of our strains demonstrated 57% metronidazole resistance; however, the majority of the metronidazole resistant strains did not show the 200 bp deletion in the rdxA gene. One obvious explanation is that the deletion in this gene is not the only defect causing gene inactivation and is not an informative indication of metronidazole resistance in Iranian H. pylori strains (with ~60% metronidazole resistance). The data are supported by data from Germany [30] and France [10] (with 40-50% metronidazole resistance) and contradicted by data from China [41] and Taiwan [42] (with 50-60% resistance). Due to the reported disparity, it seems that rdxA gene mutation is not a suitable marker for molecular prediction of metronidazole resistance. Although rdxA gene deletion is not a good resistance indicator, it is only detected in resistant strains and all of the strains with rdxA deletion clustered in specific rapedemes (analyzed by RAPD-PCR), suggesting that they are genetically similar (data not shown). Unlike clarithromycin, metronidazole resistance in vitro does not reflect in vivo resistance. Other existing genetic defects causing metronidazole resistant phenotypes and the high incurring cost of molecular diagnostic assays lead us to the conclusion that standard culture-based techniques (simple and inexpensive disc diffusion method) remain the recommended method of choice in Iran.

In conclusion, the discovery of such a high rate of resistance to clarithromycin, which is most likely due to the fast consumption of erythromycin in cases of upper respiratory infections, calls for an effective eradication program and disqualifies clarithromycin as an alternative to metronidazole. These results further confirm the essence of continuous monitoring of antibiotic resistance patterns in order to reduce the rate of eradication failure in Iran or any other target population.

The authors would like to thank Dr. David Graham, Digestive Diseases Section, Veterans Affairs Medical Center, Houston, TX, USA, for his kind review of this manuscript.

1. Massarrat S, Saberi-Firooz M, Soleimani M, Himmelmann GW, Hitzges M, Koshavarz H. Peptic ulcer disease, irritable bowel syndrome and constipation in two populations in Iran. Eur J Gastroenterol Hepatol 1995; 7: 427-433
2. Sadjadi A, Malekzadeh R, Derakhshan MH, Sepehr A, Nouraei M, Sotoudeh M, Yazdanbod A, Shokooohi B, Mashayekhi A, Arshi S, Majdpour A, Babaei M, Mosavi A, Mohagheghi MA. Cancer occurrence in Ardabil: results of a population-based cancer registry from Iran. Int J Cancer 2003; 107: 113-118
3. Perri F, Villani MR, Festa V, Quittadamo M, Andriulli A. Predictors of failure of Helicobacter pylori eradication with the standard "Maastricht triple therapy. Aliment Pharmacol Ther 2001; 15: 1023-1029
4. Furuta T, Shirai N, Xiao F, El-Omar EM, Rabkin CS, Sugimura H, Ishizaki T, Ohashi K. Polyposis of interluekin-IBeta affect the eradication rates of Helicobacter pylori by triple therapy. Clin Gastroenterol Hepatol 2004; 2: 22-30
5. Gisbert JP, Pajares JM. Helicobacter pylori therapy: first-line options and rescue regimen. Dig Dis 2001; 19: 134-143
6. Grove DI, Koutsouridou G. Increasing resistance of Helicobacter pylori to clarithromycin: is the horse bolting? Pathology 2002; 34: 71-73
7. Piana A, Are BM, Maida I, Gore MP, Sotgiu G, Realdi G, Mura I. Genotypic characterization of clarithromycin-resistant Helicobacter pylori strains. New Microbiol 2002; 25: 123-130
8. Perri F, Qasim A, Manns L, O’Morain C. Treatment of Helicobacter pylori infection. Helicobacter 2003; 8: 53-60
9. Taylor DE, Ge Z, Purzych D, Lo T, Hiratsuka K. Cloning and sequence analysis of two copies of a 23S rRNA gene from Helicobacter pylori and association of clarithromycin resistance with 23S rRNA mutations. Antimicrob Agents Chemother 1997; 41: 2621-2628
10. Versalovic JSD, Kibler K, Griffy MV, Beyer J, Flamm RK, Tanaka SK, Graham DY, Go MF. Mutations in 23S rRNA are associated with clarithromycin resistance in Helicobacter pylori. Antimicrob Agents Chemother 1996; 40: 477-480
11. Hao Q, Li Y, Zhang ZJ, Liu Y, Gao H. New mutation points in 23S rRNA gene associated with Helicobacter pylori resistance to clarithromycin in northeast China. World J Gastroenterol 2004; 1: 1075-1077
12. Chisholm SA, Owen RJ, Teare EL, Savarymuthu S. PCR-based diagnosis of Helicobacter pylori infection and real-time determination of clarithromycin resistance directly from human gastric biopsy samples. J Clin Microbiol 2001; 39: 1217-1220
13. Occhialini AM, Urdaci M, Doucet-Populaire F, Bebear CM, Lamouliatte H, Megraud F. Macrolide resistance in Helicobacter pylori: rapid detection of point mutations and assays of macrolide binding to ribosomes. Antimicrob Agents Chemother 1997; 41: 2724-2728
14. Menard A, Santos A, Megraud F, Oleastro M. PCR-restriction fragment length polymorphism can also detect point mutation A2142C in the 23S rRNA gene, associated with Helicobacter pylori pylori resistance to clarithromycin. Antimicrob Agents Chemother 2002; 46: 1156-1157
15. Alarcon T, Domingo D, Prieto N, Lopez-Brea M. PCR using 3’-mismatched primers to detect A2142C mutation in 23S rRNA conferring resistance to clarithromycin in Helicobacter pylori clinical isolates. J Clin Microbiol 2000; 38: 923-925
16. Marais A, Bilardi C, Cantet F, Mendz GL, Megraud F. Characterization of the genes rdxA and fpxA involved in metronidazole resistance in Helicobacter pylori. Res Microbiol 2003; 154: 137-144
17. Chisholm SA, Owen RJ. Frame-shift mutations in fpxA occur frequently and do not provide a reliable marker for metronidazole resistance in UK isolates of Helicobacter pylori. J Med Microbiol 2004; 53: 135-140
18. Grignon B, Tankovic J, Megraud F, Glupczynski Y, Husson MO, Conroy MC, Emond JP, Lougerie J, Raymond J, Fauchere JL. Validation of diffusion methods for macrolide susceptibility testing of Helicobacter pylori. Microb Drug Resist 2002; 8: 61-66
19. Midolo PD, Turnidge J, Lambert JR. Validation of a modified Kirby-Bauer disk diffusion method for metronidazole susceptibility testing of Helicobacter pylori. Diagn Micro Microbiol Infect Dis 1995; 21: 135-140
20. Eltahawy AW. Prevalence of primary Helicobacter pylori resistance to several antimicrobials in a Saudi Teaching Hospital. Med Princ Pract 2002; 11: 65-68
21. McNulty C, Owen R, Tompkins D, Hawtin P, McColl K, Price A, Smith G, Teare L. Helicobacter pylori susceptibility testing by disc diffusion. J Antimicrob Chemother 2002; 49: 601-609
22. Debets-Ossenkopp YJ, Herscheid AJ, Pot RG, Kuipers EJ, Kusters JG, Vandenbroucke-Grauls CM. Prevalence of Helicobacter pylori resistance to metronidazole, clarithromycin, amoxicillin, tetracycline and trovafloxacin in The Netherlands. J Antimicrob Chemother 1999; 43: 511-515
23. Chaves S, Gadano M, Tenreiro R, Cabrita J. Assessment of metronidazole susceptibility in Helicobacter pylori: statistical validation and error rate analysis of breakpoints determined by the disk diffusion test. J Clin Microbiol 1999; 37: 1628-1631
Hachem CY, Edwards DI, Weiss K, DeCross AJ, Knapp CC, Glupczynski Y, Debets-Ossenkopp YJ, Piccolomini R, DeWoods RJ, Kleibeuker JH, van Zweit AA. Subpopulations of Helicobacter pylori are responsible for discrepancies in the outcome of nitroimidazole susceptibility testing. Antimicrob Agents Chemother 1999; 43: 1448-1456

26 Piccolomini R, Di Bonaventura G, Catamo G. Comparative evaluation of the E-test, agar dilution, and broth microdilution for testing susceptibilities of Helicobacter pylori strains to 20 antimicrobial agents. J Clin Microbiol 1997; 35: 2397-2398

27 DeCross AJ, Marshall BJ, McCallum RW, Hoffman SR, Barrett LJ, Guerrat RL. Metronidazole susceptibility testing for Helicobacter pylori: comparison of disk, broth, and agar dilution methods and their clinical relevance. J Clin Microbiol 1993; 31: 1971-1974

28 Weiss K, Laverdiere M, Restieri C. Comparison of activity to 10 antibiotics against clinical strains of Helicobacter pylori by three different techniques. Can J Gastroenterol 1998; 12: 181-185

29 Edwards DL. Nitroimidazole drugs-action and resistance mechanisms. I. Mechanisms of resistance. J Antimicrob Chemother 1993; 31: 9-20

30 Knapp CC, Ludwig MD, W JA. In vitro activity of metronidazole against Helicobacter pylori as determined by agar dilution and agar diffusion. Antimicrob Agents Chemother 1991; 35: 1230-1231

31 Hachem CY, Claridge JE, Reddy R, Flamm R, Evans DG, Tanaka SK, Graham DY. Antimicrobial susceptibility testing of Helicobacter pylori. Comparison of E-test, broth microdilution, and disk diffusion for ampicillin, clarithromycin, and metronidazole. Diagn Microbiol Infect Dis 1996; 24: 37-41

32 Glupczynski Y, Brouet N, Cantagrel A, Andersen LP, Alarcon T, Lopez-Brea M, Megraud F. Comparison of the E-test and agar dilution method for antimicrobial susceptibility testing of Helicobacter pylori. Eur J Clin Microbiol Infect Dis 2002; 21: 549-552

33 Parsons HK, Carter MJ, Sanders DS, Winstanley T, Lobo AJ. Helicobacter pylori antimicrobial resistance in the United Kingdom: the effect of age, sex and socio-economic status. Aliment Pharmacol Ther 2001; 15: 1473-1478

34 Grignon B, Tankovic J, Megraud F, Glupczynski Y, Husson MO, Conroy MC, Emond JP, Loulergue J, Raymond J, Fauchere JL. Validation of diffusion methods for macrolide susceptibility testing of Helicobacter pylori. Microb Drug Resist 2002; 8: 61-66

35 Megraud F. H pylori antibiotic resistance: prevalence, importance, and advances in testing. Gut 2004; 53: 1374-1384

36 Yakoob J, Fan X, Hu G, Liu L, Zhang Z. Antibiotic susceptibility of Helicobacter pylori in the Chinese population. J Gastroenterol Hepatol 2001; 16: 981-985

37 Fakheri H, Malekzadeh RKM, Merat S, Fazel A, Alizadeh BZ, Massarrat S. Clarithromycin vs furazolidone in quadruple therapy regimens for the treatment of Helicobacter pylori in a population with a high metronidazole resistance rate. Aliment Pharmacol Ther 2001; 15: 411-416

38 Malekzadeh R, Merat S, Derakhshan MH, Siavoshi F, Yazdanbod A, Mikaeli J, Sotudehmanesh R, Sotude M, Farahvash MJ, Nasser-Moghadam S, Pursham S, Dolatshahi S, Abedi B, Babaei M, Arashi S, Majidpour A. Low Helicobacter pylori eradication rate with 4-7 d regimen in an Iranian population. J Gastroenterol Hepatol 2003; 18: 13-17

39 Roghani HS, Massarrat S, Pahlawanzadeh MR, Dashni M. Effect of two different doses of metronidazole and tetracycline in bismuth triple therapy on eradication of Helicobacter pylori and its resistant strains. Eur J Gastroenterol Hepatol 1999; 11: 709-712

40 Bereswill S, Krainick C, Stahler F, Herrmann L, Kist M. 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 Immunol Med Microbiol 2003; 36: 193-198

41 Dai N, Zhou C, Yan J. Correlation of rdxA gene mutation and metronidazole resistance of Helicobacter pylori. Zhejiang Daxue Xuebao YiXueBan 2003; 32: 37-40

42 Yang YJ, Wu JJ, Sheu BS, Kao AW, Huang AH. The rdxA gene plays a more major role than frxA gene mutation in high-level metronidazole resistance of Helicobacter pylori in Taiwan. Helicobacter 2004; 9: 400-407