Arch Clin Infect Dis. 2018 January; 13(1):e66693. doi: 10.5812/archcid.66693.

Published online 2018 February 2. **Research Article**

Nitazoxanide and Doxycycline Sensitivity Among Metronidazole Resistant *Helicobacter pylori* Isolates from Patients with Gastritis

Ali Baradaran Moghaddam,[1] Nour Amirmozafari,[2] Shahla Mansouri,[3] Nastaran Farzi,[4] Naser Ebrahimi-Daryani,[5] Masoud Alebouyeh,[4,*] and Mohammad Reza Zali[6]

[1]Microbiology Department, School of Medicine, Kerman University of Medical Sciences, Kerman, Iran
[2]Microbiology Department, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
[3]Microbiology Department, School of Medicine, Kerman University of Medical Sciences, Kerman, Iran
[4]Foodborne and Waterborne Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[5]Department of Gastroenterology, Imam Khomeini Hospital, Tehran University of Medical Sciences, Tehran, Iran
[6]Gastroenterology and Liver Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[*]*Corresponding author*: Masoud Alebouyeh, Foodborne and Waterborne Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran. Tel: +98-2122432518, E-mail: masoud.alebouyeh@gmail.com

Received 2016 September 29; **Revised** 2017 April 23; **Accepted** 2017 May 01.

Abstract

Background: Antibiotic therapy should be done based on resistance characteristics of *Helicobacter pylori* strains to commonly prescribed antibiotics in areas with higher resistance rates.
Objectives: This study examined antibacterial activity of nitazoxanide and doxycycline against clinical *H. pylori* isolates showing different metronidazole resistance levels.
Methods: A total of 122 patients, who underwent endoscopy were enrolled in this study from 3 hospitals of Tehran, during November 2014 to July 2015. *Helicobacter pylori* isolates were obtained from gastric biopsies of the patients after culture in specific culture medium and characterization by both biochemical and molecular methods. Antimicrobial susceptibility to metronidazole was detected using the agar dilution method and minimum inhibitory concentrations of nitazoxanide and doxycycline were determined for metronidazole resistant strains.
Results: From a total of 122 gastric biopsy specimens, 55 H. pylori strains were recovered (45%). Thirty-three of these strains (60.0%) were resistance to metronidazole. MIC_{50} and MIC_{90} values for metronidazole were 32 and 64 μg/mL, respectively. MIC_{50} and MIC_{90} values for doxycycline and nitazoxanide were measured as 4 and $\geq$8 μg/mL, and 8 and $\geq$32 μg/mL, respectively.
Conclusions: Dominance of high level metronidazole resistance *H. pylori* strains among the studied patients questioned its usefulness for first-line therapy in Iran. Nitazoxanide and doxycycline showed superior activity against *H. pylori* strains in comparison to metronidazole, which should be considered for alternative therapies.

Keywords: Antibiotic Resistance, Metronidazole, Doxycycline, Nitazoxanide, Minimum Inhibitory Concentration, *Helicobacter Pylori*

1. Background

Helicobacter pylori, spiral Gram negative and microaerophilic bacteria, are present in the gastric mucosa of most of the world's population (1). They were introduced as a class I carcinogenic agent in 1994 by the world health organization (WHO) (1). These bacteria induce chronic inflammation leading to chronic gastritis, gastric ulcer, duodenal ulcer, and ultimately cancer (2). They are rapidly becoming resistant to most antibiotics used in therapeutic regimens.

The extent of antibiotic resistance patterns varies in different geographical regions, which is a direct reflection of the antibiotic regimens used in each region (3). The most common regimen includes a bismuth salt along with 2 of the following antibiotics: metronidazole, amoxicillin, clarithromycin or tetracycline. However, antibiotic resistance is becoming very prevalent in developing countries. Antibiotic resistance is assumed to be the main reason of eradication failure in *H. pylori* infected patients (4). Since metronidazole is the most common antibiotic used, especially in developing countries, metronidazole resistance reduces treatment adequacy by 50% in these areas (5). The high level resistance to this drug has led

to elimination of many first line antibiotics (6). Different alternative regimens are now proposed for empirical first line therapy against *H. pylori* infection in high clarithromycin and metronidazole resistance areas. Nitazoxanide and doxycycline are 2 antibiotics that their use together with quinolones was suggested as a successful alternative therapy (7). Nitazoxanide, a benzamide thiazolide derivative, has been shown to be effective against many bacteria as well as helminthes and protozoa (8-10). It is believed to act through interference with pyruvate metabolism, which seems to be required for anaerobic cell energy metabolism (8). Doxycycline, which is a synthetic tetracycline derivative is absorbed more easily with food and has a much simpler dosing schedule (11). Adverse side-effects of doxycycline is lower than the numerous side-effects of tetracycline, such as tooth discoloration, gastrointestinal symptoms, candidiasis, and photosensitivity (11-14). Due to the worldwide high prevalence of metronidazole resistance *H. pylori* strains, this study sought to determine the in vitro effectiveness of nitazoxanide and doxycycline against metronidazole resistant isolates at different concentrations.

2. Objectives

This study aimed at investigating antibiotic resistance patterns of metronidazole resistant *H. pylori* strains to doxycycline and nitazoxanide.

3. Methods

3.1. Patients, Sample Collection and Culture

This cross sectional study was undertaken at 3 general hospitals of Tehran from November 2014 to July 2015. *Helicobacter pylori* strains were isolated from 122 biopsy specimens obtained from patients with gastrointestinal complaints during endoscopy. Three biopsies were obtained from antrum of stomach by a gastroenterologist, which were used for pathological analysis and examination of urease activity and culture. One of the samples was immediately transferred to the laboratory in thioglycolate medium (MERCK, Germany) for culture. The sample was subsequently processed for bacterial culture in Brucella agar medium supplemented with 10% horse blood, 10% fetal bovine serum, amphotericin B (10 mg, Sigma-Aldrich, USA), and *Campylobacter* selective supplement consisting of vancomycin 2.0 mg, polymyxin 0.05 mg, and trimethoprim 1.0 mg (Merck, Homburg, Germany). The plates were incubated at microaerophilic atmosphere (%8 N_2, %5 CO_2, %8 - 10% O_2) in a Gas Pak jar for 3 to 5 days. Exclusion criteria included treatment with proton pump inhibitor and use of antibiotics during the two last weeks prior to sample collection.

3.2. Isolation and Characterization of Helicobacter pylori Strains

Grown colonies of bacteria were examined macroscopically and biochemically. Small shiny colonies that showed positive urease, catalase, and oxidase tests were collected and preserved at -20°C for molecular identification using species specific primers. Freshly grown colonies of the bacterial isolates were subjected to QIAamp tissue DNA extraction kit, according to the manufacturer's instructions (Qiagen, Hilden, Germany). Polymerase Chain Reaction was performed by primer targeting *glmM* (*ureC*), which amplified a 296-bp fragment based on PCR conditions, as described by Shahabimehr et al. (14).

3.3. Determination of Minimum Inhibitory Concentrations

To measure the lowest inhibitory concentrations of the antibiotics for *H. pylori* strains, minimal inhibitory concentrations were determined by the agar dilution method, according to the CLSI guidelines (15). Accordingly, a suspension with a turbidity equivalent to that of a McFarland No.2 standard (approximately 6×10^8 bacteria/mL) was prepared from a 48-hour culture on agar plates. Susceptibility testing was performed on Mueller Hinton agar medium containing horse blood (10%) and appropriate dilution of metronidazole (0.5 to 64 μg/mL). Following preparation of microbial suspension, inoculation took place in plates containing different concentrations of metronidazole by spotting 10 μL of the bacterial suspension. Within a short space of time after the absorption of the samples, plates were incubated under microaerophilic conditions for 2 days at 37°C. Minimum inhibitory concentration was determined as the lowest concentration of antimicrobial agent inhibiting the total growth of bacteria. Resistance was considered when the metronidazole MIC was greater than 8 μg/mL (Eucast, version 2.0). After identification of metronidazole resistant isolates, the resistant strains were tested for determination of the MIC values of doxycycline and nitazoxanide. The same protocol was used for determination of MIC values of nitazoxanide and doxycycline, except the bacterial turbidity that was set at McFarland No.3 standard (approximately 6×10^9 bacteria/mL) for nitazoxanide. Doxycycline concentrations ranged from 0.12 to 8 μg/mL and nitazoxanide concentrations ranged from 0.03 to 32.0 μg/mL. Minimum inhibitory concentration values at which 50% and 90% of the tested strains were inhibited, were reported as MIC_{50} and MIC_{90}, respectively. Dimethyl sulfoxide was used as the solvent for providing defined concentrations of doxycycline and nitazoxanide. Metronidazole, doxycycline, and nitazoxanide were

obtained from Sigma Company (USA). Since there were no acceptable breakpoints for interpretation of nitazoxanide and doxycycline resistance for *H. pylori* strains, MIC values were reported for these two antibiotics. *Helicobacter pylori* strain RIGLD OC218 was used as a control strain in all experiments.

3.4. Statistical Analysis

Results of the demographic information and values of MICs of antibiotics were analyzed using the SPSS 18 software (SPSS, Chicago, IL, USA). Also, chi-square test and Fisher's exact test were used to analyze the data. A p value of $\leq$0.05 was considered statistically significant for differences.

4. Results

4.1. Helicobacter pylori Infection

Out of 122 gastric biopsy specimens, 55 *H. pylori* isolates were collected (45%). Identity of all the isolates was confirmed as *H. pylori* by both biochemical and molecular methods. The mean age of infected patients was 49 years (males: 49% and females: 51%). No significant difference was detected in the infection rate between males and females (54.5%, 30/55 and 45.4%, 25/55, respectively). Pathological findings showed occurrence of chronic gastritis (58%), severe active gastritis (30%), and intestinal metaplasia (12%) in these patients.

4.2. Quantitative Method

There was no significant correlation between MIC values of 3 antibiotics and pathological findings.

4.3. Antibiotic Susceptibility Testing

Results of antibiotic susceptibility testing showed a frequency of 60% (33/55) for metronidazole resistant *H.pylori* strains (MIC$>$ 8μg/mL). The MIC values of metronidazole for the *H. pylori* strains are shown in Table 1.

The MIC_{50} and MIC_{90} values for metronidazole were 32 and 64 μg/mL, respectively. In the case of doxycycline, its MIC values ranged between 1 and $\geq$8 μg/mL. The MIC_{50} and MIC_{90} values for doxycycline were measured as 4 and $\geq$8 μg/mL, respectively (Table 1). The MIC values for nitazoxanide in the metronidazole resistant *H. pylori* were in ranges of 1 to $\geq$32 μg/mL. The MIC_{50} and MIC_{90} values for nitazoxanide were determined as 8 and $\geq$32 μg/mL, respectively. Diversity of MIC values of doxycycline and nitazoxanide for all of the metronidazole resistant strains is shown in Figure 1. In general, higher activity of nitazoxanide and doxycycline was detected against metronidazole resistance *H. pylori* strains. Although *H. pylori* strains with an MIC value of $\geq$32 μg/mL for metronidazole were mainly inhibited with lower concentrations of nitazoxanide and doxycycline, this correlation was not found for the strains with lower MIC values (8 to 16 μg/mL). Accordingly, some of the metronidazole resistant strains with MIC values of $\geq$8 μg/mL were not inhibited by highest examined concentrations of these 2 antibiotics.

5. Discussion

Helicobacter pylori is an important human pathogen around the world. The prevalence of *H. pylori* infection in Iran, like other developing countries, is higher than those in the developed world (16-23). The prevalence of *H.pylori* infection in different areas of Iran is variable, ranging from 30.6% to 93.0% (19-21). In this study, the estimated infection rate was 45%, which is similar to that of other researchers reported from Tehran (24-26). Antibiotic resistance is the main reason for treatment failure and persistent infection of *H. pylori* in developing countries. The increasing resistance to antibiotics is commonly due to a lack of proper administration, inappropriate dosing, arbitrary use of drugs, and genetic mutations in *H. pylori* strains (27, 28). Metronidazole is a common antibiotic used for *H. pylori* treatment in Iran, and resistant strains to this antibiotic are becoming a major problem for therapeutic regiments (27). Resistance to metronidazole was raised among *H. pylori* strains in Iran from 73.4% in 2009 to 88.2% in 2011 (27). In the present study found high rates of metronidazole resistant strains of *H. pylori* (60%) in the studied hospitals. This resistance rate seems to be associated with historical administration of metronidazole for various parasitic and oral cavity infections, since most of the strains were isolated from patients over 40 years old. While no statistically significant change in resistance rate was detected for metronidazole in this study, a higher amount of MIC values (MIC_{50} $\geq$ 64 μg/mL vs 32 μg/mL) was found compared with previous studies from Iran (28).

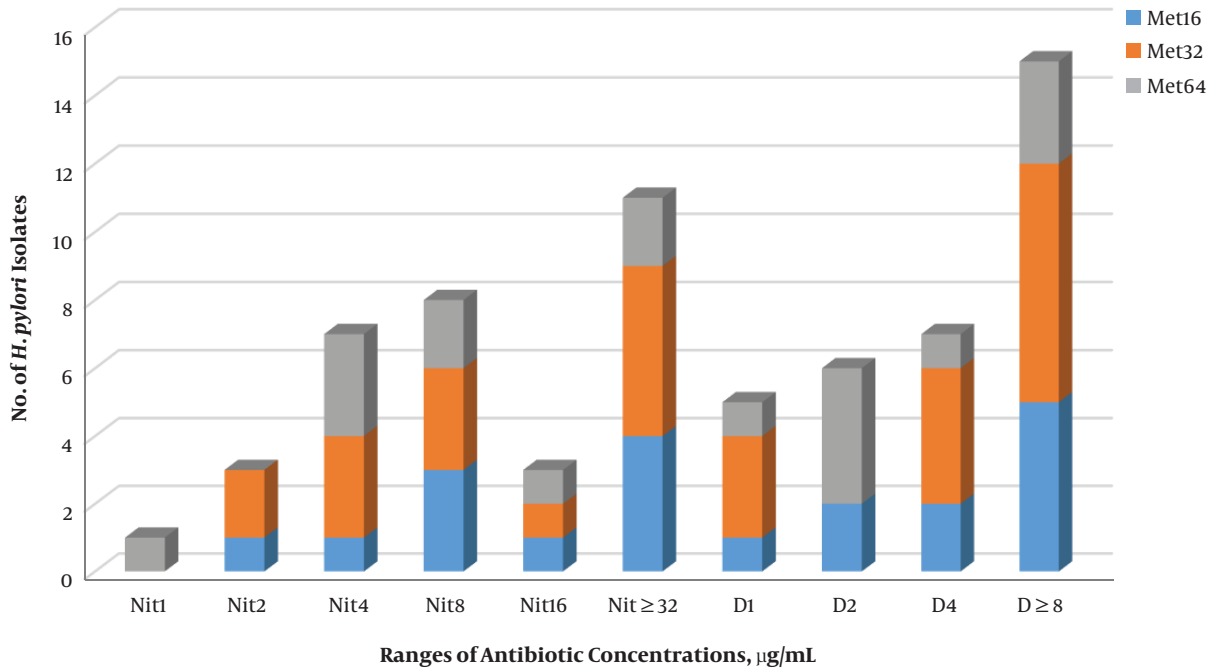
Tetracyclines are other anti-*H. pylori* antibiotics, which are used as a component of the quadruple therapy regimens. The extent of resistance towards this drug is also variable in different areas. This discrepancy may in-part be due to different sample sizes and the specific antibiogram method used (18, 19). There are many reports indicating an increasing trend of resistance towards this antibiotic; however, resistance rate to this antibiotic is lower than those described for metronidazole (29-37). Comparison of MIC_{50} for doxycycline and metronidazole (4 vs 32 μg/mL) proposed 8 times higher activity of doxycycline against metronidazole resistant *H. pylori* strains. However, nitazoxanide showed a four-fold greater anti-bacterial property compared with metronidazole, based on its MIC_{50} value. It

Table 1. Diversity of Minimum Inhibitory Concentration Values of Nitazoxanide and Doxycycline for Different Metronidazole Resistant *Helicobacter pylori* Strains[a]

Metronidazole, μg/mL	Nitazoxanide, μg/mL						Doxycycline, μg/mL				Total
	1	2	4	8	16	≥32	1	2	4	≥8	
16	0	1	1	3	1	4	1	2	2	5	10 (30.3)
32	0	2	3	3	1	5	3	0	4	7	14 (42.5)
64	1	0	3	2	1	2	1	4	1	3	9 (27.2)
Total	1 (3)	3 (9)	7 (21.3)	8 (24.4)	3 (9)	11 (33.3)	5 (12.5)	10 (25)	9 (22.5)	16 (40)	33 (100)

[a] Values are expressed as No. (%).

Figure 1. Correlation of Minimal Inhibitory Concentrations of Metronidazole, Doxycycline, and Nitazoxanide in Metronidazole Resistant *Helicobacter pylori* Stains

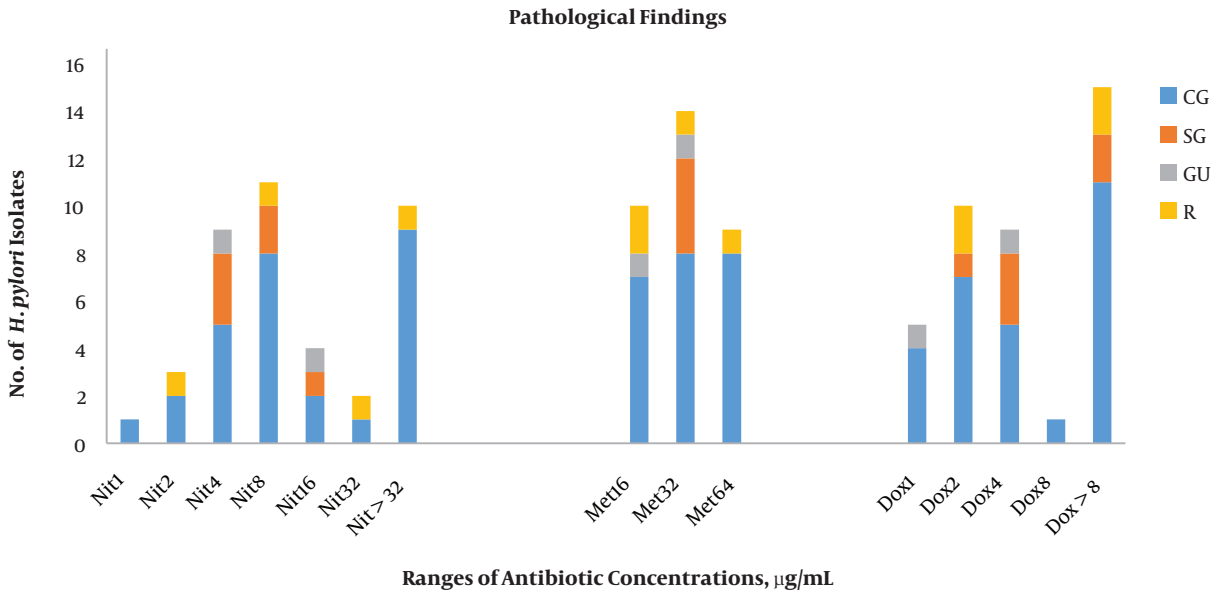


Met, Metronidazole; Nit, Nitazoxanide; D, Doxycycline.

could therefore be suggested that these 2 drugs were superior in their ability to inhibit growth of *H. pylori* in comparison to metronidazole. There are a few studies on the activity of doxycycline and nitazoxanide against metronidazole resistant strains. In a study by Cammarota et al. in Italy, superior effects of doxycycline-based quadruple regimen for eradication of metronidazole resistant *H. pylori* infection, with MIC range of 0.056 to 1 μg/mL and MIC_{50} and MIC_{90} of 0.125 and 0.5 μg/mL, was presented (38). The MIC range of 0.25 to 8.0 μg/mL for doxycycline and MIC_{50} and MIC90 of 0.5 and 2 was also reported for clinical isolates of *H. pylori* in Canada (39). Results of the current study showed higher MIC ranges for this antibiotic (MIC range of 1 to ≥8.0 μg/mL and MIC_{50} and MIC_{90} of 4 and ≥8), which suggests greater consideration for its administration to prevent emergence of more resistant strains in Iran. In case of nitazoxanide, Megraud F. et al reported MIC range of 0.25 to 8 μg/mL, with MIC_{50} and MIC_{90} of 1 and 4, among metronidazole resistant and susceptible strains (0.25- >32, MIC_{50} and MIC_{90} 2 and >32 μg/mL) (40). In a study from Australia, MIC range of nitazoxanide varied between 0.06 and 4 μg/mL (41). Likewise, these MIC ranges were lower than those detected in Iran. However, they observed no significant change in MIC levels after nitazoxanide administration compared with metronidazole, and its higher activity against *H. pylori* strains suggests its preference for clinical usage.

Lack of association between MIC values of metronidazole and those from nitazoxanide or doxycycline could be explained by diversity of resistance mechanisms to these drugs (35). Although the current results provide some evidence about preferred activity of nitazoxanide and doxycycline, further studies are needed to investigate their usage

Figure 2. Correlation Between Minimum Inhibitory Concentration Values of Antibiotics and Pathological Findings



p value, 0.395 for metronidazole; P value, 0.573 for doxycycline; P value, 0.486 for nitazoxanide. CG, Chronic Gastritis; SAG, Severe Active Gastritis; GU, Gastric Ulcer; R, Reflux.

against metronidazole resistance *H. pylori* strains.

Acknowledgments

The authors thank Prof. Naser Ebrahimi-Daryani and Prof. Mohammad Reza Zali for providing the endoscopic biopsies. The authors of this article appreciate all staff of Medical Microbiology department of Iran University of Medical Sciences and foodborne and waterborne diseases research center of Shahid Beheshti University of Medical Sciences.

Footnotes

Authors' Contribution: Ali Baradaran Moghaddam performed the main laboratory practices for antimicrobial susceptibility testing and wrote the article; Nastaran Farzi cultured the biopsy samples, Nour Amirmozafari directed the study and revised the article; Shahla Mansouri directed the study; Naser Ebrahimi-Daryani and Mohammad Reza Zali performed the endoscopic examination; Masoud Alebouyeh designed the study and revised the paper. Masoud Alebouyeh and Nour Amirmozafari contributed equally to this work.

Funding/Support: This research was supported financially by grant No. 26419-30-04-94 from Iran University of Medical Sciences, Tehran, Iran. This study was also supported in part by Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

References

1. Haag S, Talley NJ, Holtmann G. Symptom patterns in functional dyspepsia and irritable bowel syndrome: relationship to disturbances in gastric emptying and response to a nutrient challenge in consulters and non-consulters. *Gut*. 2004;**53**(10):1445–51. doi: 10.1136/gut.2003.030049. [PubMed: 15361493].
2. Wotherspoon AC. Gastric lymphoma of mucosa-associated lymphoid tissue and Helicobacter pylori. *Annu Rev Med*. 1998;**49**:289–99. doi: 10.1146/annurev.med.49.1.289. [PubMed: 9509264].
3. Covacci A, Telford JL, Del Giudice G, Parsonnet J, Rappuoli R. Helicobacter pylori virulence and genetic geography. *Science*. 1999;**284**(5418):1328–33. doi: 10.1126/science.284.5418.1328. [PubMed: 10334982].
4. Öztürk E. Diagnostic methods of Helicobacter pylori infection. *Gulhane Med J*. 2008;**50**(1):60–4.
5. Wu H, Shi XD, Wang HT, Liu JX. Resistance of helicobacter pylori to metronidazole, tetracycline and amoxycillin. *J Antimicrob Chemother*. 2000;**46**(1):121–3. doi: 10.1093/jac/46.1.121. [PubMed: 10882700].
6. Zullo A, De Francesco V, Hassan C, Morini S, Vaira D. The sequential therapy regimen for Helicobacter pylori eradication: a pooled-data analysis. *Gut*. 2007;**56**(10):1353–7. doi: 10.1136/gut.2007.125658. [PubMed: 17566020].
7. Basu PP, Rayapudi K, Pacana T, Shah NJ, Krishnaswamy N, Flynn M. A randomized study comparing levofloxacin, omeprazole, nitazoxanide, and doxycycline versus triple therapy for the eradication of Helicobacter pylori. *Am J Gastroenterol*. 2011;**106**(11):1970–5. doi: 10.1038/ajg.2011.306. [PubMed: 21989146].

8. Ramos-Soriano AG, Black J. Nitazoxanide Use as Part of an Empiric Multi-Drug Regimen in Treating Children with Suspected Helicobacter pylori Infection. *Case Rep Gastroenterol*. 2015;**9**(1):36–42. doi: 10.1159/000375116. [PubMed: 25759631].
9. Doumbo O, Rossignol JF, Pichard E, Traore H, Dembele MM, Diakite M, et al. Nitazoxanide in the treatment of cryptosporidiosis in 24 AIDS patients with chronic diarrhea in Mali. *Am J Trop Med Hyg*. 1997;**26**(56):637–9.
10. Dubreuil L, Houcke I, Mouton Y, Rossignol JF. In vitro evaluation of activities of nitazoxanide and tizoxanide against anaerobes and aerobic organisms. *Antimicrob Agents Chemother*. 1996;**40**(10):2266–70. [PubMed: 8891127].
11. Barza M, Schiefe RT. Antimicrobial spectrum, pharmacology and therapeutic use of antibiotics. Part 1: tetracyclines. *Am J Hosp Pharm*. 1977;**34**(1):49–57. [PubMed: 318799].
12. Meier J. Doxycycline in respiratory tract infections. A review of a multi-centre trial. *Chemotherapy*. 1975;**21 Suppl 1**:130–5. doi: 10.1159/000221899. [PubMed: 239837].
13. Smith K, Leyden JJ. Safety of doxycycline and minocycline: a systematic review. *Clin Ther*. 2005;**27**(9):1329–42. doi: 10.1016/j.clinthera.2005.09.005. [PubMed: 16291409].
14. Shahabimehr M, Alebouyeh M, Farzi N, Mahboubi A, Taslimi R, Zali MR. Resistance rate and minimum inhibitory concentration of metronidazole among Helicobacter pylori strains in Tehran, Iran. *Arch Clin Infect Dis*. 2016;**11**(2). doi: 10.5812/archcid.34478.
15. Clinical and Laboratory Standard Institute . *Performance standards for antimicrobial susceptibility testing; 17th informational supplement*. Wayne PA , editor. 2007.
16. Hunt RH, Xiao SD, Megraud F, Leon-Barua R, Bazzoli F, van der Merwe S, et al. Helicobacter pylori in developing countries. World Gastroenterology Organisation Global Guideline. *J Gastrointestin Liver Dis*. 2011;**20**(3):299–304. [PubMed: 21961099].
17. Novis BH, Gabay G, Naftali T. Helicobacter pylori: the Middle East scenario. *Yale J Biol Med*. 1998;**71**(2):135–41. [PubMed: 10378359].
18. Savari M, Abdollahi H, Zahedi MJ, Darvish Moghadam S, Hayat Bakhsh Abasi M. Antibiotic-resistance patterns of Helicobacter pylori isolates obtained from patients in Kerman-2009 [In Persian]. *J Kerman Univ Med Sci*. 2011;**18**(1):73–82.
19. Khedmat H, Karbasi-Afshar R, Agah S, Taheri S. Helicobacter pylori Infection in the general population: A Middle Eastern perspective. *Caspian J Intern Med*. 2013;**4**(4):745–53. [PubMed: 24294467].
20. Moradi A, Rashidipour A. Seroepidemiology of Helicobacter pylori infection in Semnan [In Persian]. *J Koomesh*. 2000;**1**(3):53–7.
21. Mokhtari M. Evaluation of antibody of Helicobacter pylori infection in preschool children in Isfahan. *Iran J Gastroenterol*. 2002;(36):6.
22. Kargar M, Souod N, Doosti A, Ghorbani-Dalini S. Prevalence of Helicobacter pylori vacuolating cytotoxin A gene as a predictive marker for different gastrodoudenal diseases. *Arch Clin Infect Dis*. 2011;**6**(2):85–9.
23. Sheikholeslami H, Ghasemibarghi R, Moosavi H. Comparison of prevalence of Helicobacter pylori infection in urban and rural areas of Qazvin (2002) [In Persian]. *J Qazvin Univ Med Sci*. 2004;**8**(3):47–51.
24. Vaziri F, Najar Peerayeh S, Alebouyeh M, Molaei M, Maghsoudi N, Zali MR. Determination of Helicobacter pylori CagA EPIYA types in Iranian isolates with different gastroduodenal disorders. *Infect Genet Evol*. 2013;**17**:101–5. doi: 10.1016/j.meegid.2013.03.048. [PubMed: 23567822].
25. Yadegar A, Alebouyeh M, Zali MR. Analysis of the intactness of Helicobacter pylori cag pathogenicity island in Iranian strains by a new PCR-based strategy and its relationship with virulence genotypes and EPIYA motifs. *Infect Genet Evol*. 2015;**35**:19–26. doi: 10.1016/j.meegid.2015.07.026. [PubMed: 26205689].
26. Bohr UR, Primus A, Zagoura A, Glasbrenner B, Wex T, Malfertheiner P. A group-specific PCR assay for the detection of Helicobacteraceae in human gut. *Helicobacter*. 2002;**7**(6):378–83. doi: 10.1046/j.1523-5378.2002.00113.x. [PubMed: 12485125].
27. 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**(45):8168–80. doi: 10.3748/wjg.v19.i45.8168. [PubMed: 24363506].
28. Shokrzadeh L, Alebouyeh M, Mirzaei T, Farzi N, Zali MR. Prevalence of multiple drug-resistant Helicobacter pylori strains among patients with different gastric disorders in Iran. *Microb Drug Resist*. 2015;**21**(1):105–10. doi: 10.1089/mdr.2014.0081. [PubMed: 25303151].
29. Rafeey M, Ghotaslou R, Nikvash S, Hafez AA. Primary resistance in Helicobacter pylori isolated in children from Iran. *J Infect Chemother*. 2007;**13**(5):291–5. doi: 10.1007/s10156-007-0543-6. [PubMed: 17982716].
30. Haghi Tomatari F, Mobarez A, Amini M, Hosseini D, Abadi A. Helicobacter pylori resistance to metronidazole and clarithromycin in dyspeptic patients in Iran. *Iran Red Crescent Med J*. 2010;**12**(4):409–12.
31. Mohammadi M, Doroud D, Mohajerani N, Massarrat S. Helicobacter pylori antibiotic resistance in Iran. *World J Gastroenterol*. 2005;**11**(38):6009–13. doi: 10.3748/wjg.v11.i38.6009. [PubMed: 16273615].
32. Falsafi T, Mobasheri F, Nariman F, Najafi M. Susceptibilities to different antibiotics of Helicobacter pylori strains isolated from patients at the pediatric medical center of Tehran, Iran. *J Clin Microbiol*. 2004;**42**(1):387–9. doi: 10.1128/JCM.42.1.387-389.2004. [PubMed: 14715786].
33. Siavoshi F, Saniee P, Latifi-Navid S, Massarrat S, Sheykholeslami A. Increase in resistance rates of H. pylori isolates to metronidazole and tetracycline–comparison of three 3-year studies. *Arch Iran Med*. 2010;**13**(3):177–87. [PubMed: 20433221].
34. Mirzaei N, Poursina F, Faghri J, Talebi M, Khataminezhad MR, Hasanzadeh A, et al. Prevalence of resistance of Helicobacter pylori strains to selected antibiotics in Isfahan, Iran. *Jundishapur J Microbiol*. 2013;**6**(5). doi: 10.5812/jjm.6342.
35. Francesco VD, Zullo A, Hassan C, Giorgio F, Rosania R, Ierardi E. Mechanisms of Helicobacter pylori antibiotic resistance: An updated appraisal. *World J Gastrointest Pathophysiol*. 2011;**2**(3):35–41. doi: 10.4291/wjgp.v2.i3.35. [PubMed: 21860834].
36. Talebi Bezmin Abadi A, Mobarez AM, Taghvaei T, Wolfram L. Antibiotic resistance of Helicobacter pylori in Mazandaran, North of Iran. *Helicobacter*. 2010;**15**(6):505–9. doi: 10.1111/j.1523-5378.2010.00795.x. [PubMed: 21073606].
37. Wu JY, Kim JJ, Reddy R, Wang WM, Graham DY, Kwon DH. Tetracycline-resistant clinical Helicobacter pylori isolates with and without mutations in 16S rRNA-encoding genes. *Antimicrob Agents Chemother*. 2005;**49**(2):578–83. doi: 10.1128/AAC.49.2.578-583.2005. [PubMed: 15673736].
38. Cammarota G, Martino A, Pirozzi G, Cianci R, Branca G, Nista EC, 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. *Aliment Pharmacol Ther*. 2004;**19**(7):789–95. doi: 10.1111/j.1365-2036.2004.01910.x. [PubMed: 15043520].
39. Loo VG, Sherman P, Matlow AG. Helicobacter pylori infection in a pediatric population: in vitro susceptibilities to omeprazole and eight antimicrobial agents. *Antimicrob Agents Chemother*. 1992;**36**(5):1133–5. doi: 10.1128/AAC.36.5.1133. [PubMed: 1510406].
40. Megraud F, Occhialini A, Rossignol JF. Nitazoxanide, a potential drug for eradication of Helicobacter pylori with no cross-resistance to metronidazole. *Antimicrob Agents Chemother*. 1998;**42**(11):2836–40. [PubMed: 9797212].
41. Guttner Y, Windsor HM, Viiala CH, Dusci L, Marshall BJ. Nitazoxanide in treatment of Helicobacter pylori: a clinical and in vitro study. *Antimicrob Agents Chemother*. 2003;**47**(12):3780–3. doi: 10.1128/AAC.47.12.3780-3783.2003. [PubMed: 14638482].