

## Clarithromycin Resistance of Helicobacter pylori Infections in Gaza Strip, Palestine

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87

4  $\mu$ m

(2143A>G, 2144A>G and 2143A>C) S23

من %58.6

%3.9 (2143A>G)

%3.9

(2143A>C)

(2144A>G)

(%25.5) 13

كلمات مفتاحية: بكتيريا الملوية البوابية، مقاومة

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## Clarithromycin Resistance of Helicobacter ....

### Abstract

Clarithromycin is a key component of most treatment protocols of *Helicobacter pylori*, and clarithromycin resistance is a key factor in the failure of eradication therapy and recurrence of infection. Clarithromycin resistance rates vary throughout the world. Therefore, this cross sectional study aimed at determining clarithromycin resistance of *H. pylori* in infected patients in Gaza strip.

Biopsies were randomly collected from 87 patients subjected to gastric endoscopy for exploration based on clinical symptoms suggesting *H. pylori* infection. The biopsies were collected from different sites of the antrum and then formalin-fixed and paraffin-embedded. Four- $\mu$ m thick slides were prepared and used for fluorescent in situ hybridization detection of the 3 common mutations (2143A>G, 2144A>G and 2143A>C) in the 23S rRNA gene leading to clarithromycin resistance.

The results of the study showed that 58.6% of the examined samples were positive for *H. pylori* infection, among them 3.9% were positive for the 2143A>G mutation, 3.9% for 2144A>G while none of the samples were positive for the 2143A>C. A number of positive samples (25.5%) did not hybridize to any of the probes specific for either the wild type or the three mutations, indicating the possible existence of mutations in other nucleotides in the probes binding sites. Thus, the extent of resistance to clarithromycin may be much higher.

In conclusion, *H. pylori* infections in Gaza strip have high resistance rate for clarithromycin compared to other populations in and outside the area, probably because of the uncontrolled, abuse and misuse of antibiotics.

**Keywords:** *H. pylori*, Clarithromycin resistance, Gaza strip, Palestin.

### Introduction:

*Helicobacter pylori* as discovered in 1983 as Gram negative microaerophilic bacterium with spiral rod shape (Robin and Marshall, 1983). It infects more than half of the world population, with a prevalence ranging from 30-50% in developed countries to more than 90% in developing areas (Hunt et al, 2011). The infection is usually acquired by different routes of transmission (Vale and Vitor, 2010), and infection with multiple *H. pylori* strains is quite common (Blaser, 2012). Polyclonal infection allows DNA to be exchanged between different strains, which could promote the spread of

genes encoding important virulence factors or resistance to antibiotics (Logan and Walker, 2001).

*H. pylori* was reported to be associated with gastro-duodenal disease such as chronic active gastritis (Kreuning et al, 1994), peptic ulcer (Feldman and Peterson, 1993; Labenz and Borsch, 1994), glandular atrophy (Arikan, 2004), intestinal metaplasia (Ozdil, et al, 2010), gastric cancer (Tepes, 2009; Konturek, 2009; Bornschein et al, 2009), and mucosa-associated lymphoid tissue (MALT) lymphomas (Lehours et al, 2009; Andriani et al, 2009; Bhandari and Crowe, 2012). It was also found to be associated with some extra gastric disease such as vascular and ischemic heart disease (Pasceri et al, 1998; Pasceri et al, 2006; Wang et al, 2012), chronic immune thrombocytopenic purpura (Fujimura, 2005), iron deficiency anemia (Hershko and Ronson, 2009), hepatocellular carcinoma (Huang et al, 2004), renal resistive index (Afsar et al, 2007), insulin resistance (Polyzos et al, 2013), diabetes mellitus (Jeon et al, 2012) and respiratory system disease (Kurtaran et al, 2008).

The current treatment of *H. pylori* infection consist of a triple or quadruple regimen that include antibiotics (usually metronidazole, clarithromycin, or tetracycline) and a proton pump inhibitor such as omeprazole, lansoprazole, or pantoprazole ( Chuah et al, 2011). Although clarithromycin is a key component of most treatment recommendations to eradicate *H. pylori*, infection with a resistant strain is considered a major factor in treatment failure (Marin et al, 2013). The eradication rate when a clarithromycin resistant strain is involved decreases from 62 to 20% with clarithromycin plus ranitidine (Schütze et al, 1996) or from 92 to 50% with clarithromycin, amoxicillin and omeprazole (Wurzer et al, 1997).

The resistance of *H. pylori* to clarithromycin has been shown to be due to point mutations at the domain V loop of the 23S rRNA gene (Taylor, 2000). *H. pylori* has two copies of that gene. A mutation in one of the two 23S rRNA copies was found enough to cause high resistance in a dominant fashion (Hulten et al, 1997). The mechanism of resistance to clarithromycin appears to involve the decreased ribosome binding of the macrolide so that it fails to act by interrupting protein biosynthesis (Owen, 2002). Among these mutations, the three most common mutations worldwide are 2143A>G, 2144A>G and 2143A>C (Xiong et al, 2013).

In Gaza, *H. pylori* is not routinely cultured and therefore no data of its susceptibility to antibiotics is available. Moreover, drug resistance was

## **Clarithromycin Resistance of Helicobacter ....**

documented in many other bacteria and viruses circulating in Gaza strip, probably because of the irrationalized administration of antibiotics (Elmanama et al, 2006; Abu Elamreen et al, 2008; Al Jarousha et al, 2009; Elmanama et al, 2013). The aim of this study is to determine the extent of clarithromycin resistance resulting from the common (2143A>G, 2144A>G and 2143A>C) mutations among *H. pylori* patients in Gaza strip, Palestine.

### **Materials and Method:**

#### **Study design and study population:**

This study is a cross sectional research aiming at the determination of clarithromycin resistance of *H. pylori* infected patients in Gaza strip. The study population is composed of 87 randomly selected patients subjected to gastric endoscopy for exploration based on clinical symptoms suggesting *H. pylori* infection. There was no specific age or sex limitation (74.5 % were males and 25.5 % females).

Samples were collected from 3 hospitals across Gaza strip, (32 from Balsam-hospital, 24 from Al-karamah hospital and 1 from European Gaza-hospital). The patients included in this study were from different parts of Gaza strip (33 from the North of Gaza Strip, 21 from Gaza city, 11 from the mid region and 22 from the southern region). The mean age was ( $36.1 \pm 12.2$  years). The minimum age was 16 years and the maximum age was 70 years. The mean male age ( $36.0 \pm 11.1$ years) and the female mean age was ( $36.3 \pm 14.7$  years).

#### **Ethical consideration:**

The procedure of the study was approved by the local Helsinki committee according to the World Medical Association Declaration of Helsinki (WMA Declaration of Helsinki, 2008). Verbal consents were taken from the patients to participate in the study after being informed about the purpose of this research.

#### **Sample collection and processing:**

A gastric biopsy was collected from the antrum by specialized physicians, using standard endoscopy biopsy forcipes. The specimen was fixed immediately in a tube containing 5 ml of freshly prepared 10% formalin for 24-48 hours according to (Trebesius et al, 2000), and stored at 4°C from collection to processing. The specimen was then paraffin-embedded and sectioned into seven 4µm-thick sections, spotted onto glass

slides and fixed by incubation overnight at 55°C (Russmann et al, 2003). The slides were stored at 4°C until processing. The tissue sections were numbered and processed by Fluorescent in situ hybridization (FISH) as indicated in (Table 1).

**Table 1. Hybridization plan.**

Slide No.	Probe	Purpose
1	FISH/ 16S rRNA	Identification of H. pylori infection
2	FISH/23S rRNA	Detection the wild type strain.
3	FISH/23S rRNA	Detection the 2143A>G mutation.
4	FISH/23S rRNA	Detection the 2144A>G mutation.
5	FISH/23S rRNA	Detection the 2143A>C mutation.

**FISH procedure:**

The oligonucleotide probes used for FISH listed in table 2 (ClaR1, ClaR2 and ClaR3) were 5' labeled with the fluorochromes Cy3 red signal while (Hpy-1 and ClaWT) were labeled with fluorescein isothiocyanate green signal (Integrated DNA Technologies, USA). The procedure was carried out according to (Russmann et al, 2001).

Slide no. 1 from each biopsy was initially hybridized with Hpy-1 probe, which targets the 16S rRNA gene for the identification of H. pylori. If it was positive, the slides no. 2, 3, 4 and 5 were tested for the ClaWT ClaR1 ClaR2 ClaR3 respectively to determine the clarithromycin resistance mutation.

**Table 2. Sequence of FISH probes.**

Probe I.D.	Sequence (5' to 3') <sup>*</sup>	5'-label <sup>+</sup>	Target
Hpy-1	CACACCTGACTGACTAT CCGG	FITC	16S rRNA
ClaR1	CGG GGT CTT <u>CCC</u> GTC TT	Cy3	2143A>G
ClaR2	CGG GGT <u>CTC</u> TCC GTC TT	Cy3	2144A>G
ClaR3	CGG GGT CTT <u>GCC</u> GTC TT	Cy3	2143A>C
ClaWT	CGG GGT CTT TCC GTC TT	FITC	wild type

\* The underlined bold nucleotides are the site of point mutation.

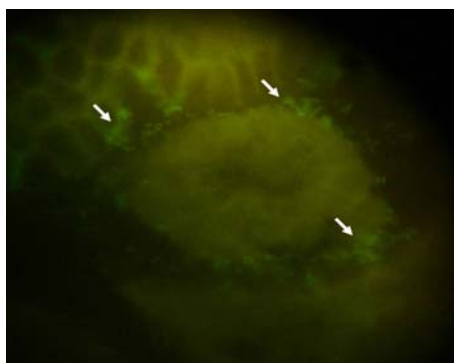
+ FITC is Fluorescein isothiocyanate.

Hpy-1, ClaR1, ClaR2, and ClaR3 are from (Chattopadhyay et al, 2004); and ClaWT from (Trebesius et al, 2000)

## Clarithromycin Resistance of Helicobacter ....

### Results:

The samples were first examined by the Hyp-1 probe for presence of *H. pylori*. A total of 51 samples (58.6%) were found positive (figure 1) and 36 were negative.



**Figure 1.** A representative micrograph showing green signals (white arrows) obtained by hybridization of the FITC-labeled Hyp-1 probe to 16S rRNA of *H. pylori*.

The positive samples were further analyzed for the presence of clarithromycin resistance (table 3). Two of the positive samples (3.9%) were positive for the 2143A>G mutation, two samples (3.9%) for 2144A>G mutation while the 2143A>C mutation was not detected in any of the samples. Thirteen samples (25.5%) were negative for the wild type probe as well as for the 3 mutation specific probes (non-typable).

**Table 3. Clarithromycin resistance in *H. pylori* positive samples.**

	Description	Count	%
Positive	wild type	34	66.7
	2143A>G	2	3.9
	2144A>G	2	3.9
	2143A>C	0	0
	Nontypable	13	25.5
	Total	51	100

### Discussion:

*Helicobacter pylori* is found in half the population of the world. Its prevalence is highly variable in relation to geography, ethnicity, age, and socioeconomic factors—high in developing countries and lower in the

developed world. It is associated with different types of diseases ranging from gastric to non-gastric diseases. Clarithromycin is a key component in the treatment of the infection and, treatment failure is frequently associated with clarithromycin resistance.

In this study, sensitivity of *H. pylori* to clarithromycin was evaluated by detecting the three most common mutations associated with clarithromycin resistance in *H. pylori* positive biopsies. A mutation in one copy of the 23S rRNA is enough to confer a stable and high level of clarithromycin resistance in *H. pylori* (Taylor et al, 1997; Hulten et al, 1997; Versalovic et al, 1997).

In total, 4 samples (7.8%) were found resistant to clarithromycin (2 had the 2143 A>G transition mutation and the other 2 had the 2144 A>G mutation). Importantly, additional 13 samples (25.5%) positively hybridized to the generic probe, specific for the 16S rRNA (Hpy-1) but consistently did not hybridize to any of the 4 probes specific for the 23S rRNA (ClaR1 for 2143A>G, ClaR2 for 2144A>G, ClaR3 for 2143A>C and ClaWT for the wild-type sequences). This may have resulted from the existence of other point mutations or multiple mutations in the binding site of these probes. Such mutations were previously documented in other studies (e.g., 2142G>A, 2144A>C, 2147C>G and 2143A>T) and were found to be associated with resistance to clarithromycin (Stone et al, 1996; Hultén et al, 1997; Ende et al, 2001; Garrido and Toledo, 2007). Therefore, based on our results, we may conclude that the proportions of clarithromycin resistance are much higher in Gaza strip. The extended use of clarithromycin for the treatment of *H. pylori* may give rise to such high frequency of resistance to macrolides, including cross-resistance within the macrolides group of antibacterial agents (Hultén et al, 1997; Samra et al, 2002; McMahan et al, 2003).

The prevalence of clarithromycin resistance in *H. pylori* infections is variable worldwide. It varies from 4% in Egypt, 2.46-19.5% in Brazil, 13.2% in Ireland, 8.3% in England, 9.5% in Ecuador, 29.1% in Spain, 8.2 to as high as 46.4% in "Israel" (Samra et al, 2002; Sherif et al, 2004; Chisholm et al, 2007; O'connor et al, 2010; Suzuki et al, 2013; Ogata et al, 2013).

Clarithromycin resistance is associated with a greater risk for failure with clarithromycin-based treatments (McMahan et al, 2003; Marin et al, 2013). The eradication rate may be reduced by as much as 40% in resistant

## Clarithromycin Resistance of Helicobacter ....

compared to clarithromycin sensitive strains (Matsumura et al, 2001; Kato et al, 2002).

In conclusion, if we consider the non-typable samples as potentially resistant ones in addition to the two 2143A>G and two 2143 A>C mutant samples, then the resistance rate of *H. pylori* to clarithromycin in Gaza strip would be as high as 33%. This proposed high resistance rate concords well with the uncontrolled and abused administration of antibiotics in general and the macrolides in particular. Further studies should be performed to identify the other types of mutations present in Gaza strips and their correlation to drug resistance.

### References:

1. Abu Elamreen FH., Sharif FA., Deeb JE. 2008: Isolation and antibiotic susceptibility of Salmonella and Shigella strains isolated from children in Gaza, Palestine from 1999 to 2006, *J Gastroenterology Hepatol*, 23(8 Pt 2), e330-333.
2. Afsar B., Ozdemir FN., Elsurer R., Sezer S. 2007: Helicobacter pylori infection may increase renal resistive index, *Med Hypotheses*, 69, 956-957.
3. Al Jarousha AM., El Jadba AH., Al Afifi AS., El Qouqa IA. 2009: Nosocomial multidrug-resistant Acinetobacter baumannii in the neonatal intensive care unit in Gaza City, Palestine, *Int J Infect Dis*, 13(5), 623-628.
4. Andriani A., Miedico A., Tedeschi L., Patti C., Di Raimondo F., Leone M., Schinocca L., Romanelli A., Bonanno G., Linea C., Giustini M., Hassan C., Cottone M., Zullo A. 2009: Management and long-term follow-up of early stage *H. pylori*-associated gastric MALT-lymphoma in clinical practice: an Italian, multicentre study, *Dig Liver Dis*, 41, 467-473.
5. Arikan C., F OZ., Turgor G., Doganavsargil B., Aydogdu S., Yagci RV. 2004: Antral glandular atrophy and intestinal metaplasia in children with Helicobacter pylori infection, *J Pediatr Gastroenterol Nutr*, 38, 361-362.
6. Bhandari A. and Crowe SE. 2012: Helicobacter pylori in Gastric Malignancies, *Curr Gastroenterol Rep*, 14, 489-496.
7. Blaser MJ. 2012: Heterogeneity of Helicobacter pylori, *Eur J Gastroenterol Hepatol*, 9 (Suppl 1), S3-7.
8. Bornschein J., Rokkas T., Selgrad M., Malfertheiner P. 2009: Helicobacter pylori and clinical aspects of gastric cancer, *Helicobacter*, 14 (Suppl 1), 41-45.



9. Chattopadhyay S., Patra R., Ramamurthy T., Chowdhury A., Santra A., Dhali GK., Bhattacharya SK., Berg DE., Nair GB., Mukhopadhyay AK. 2004: Multiplex PCR assay for rapid detection and genotyping of *Helicobacter pylori* directly from biopsy specimens, *J Clin Microbiol*, 42, 2821-2824.
10. Chisholm SA., Teare EL., Davies K., Owen RJ. 2007: Surveillance of primary antibiotic resistance of *Helicobacter pylori* at centres in England and Wales over a six-year period (2000-2005), *Euro Surveill*, 12(7),E3-4.
11. Chuah SK., Tsay FW., Hsu PI., Wu DC. 2011: A new look at anti-*Helicobacter pylori* therapy, *World J Gastroenterol*, 17(35), 3971-3975.
12. Elmanama AA., Elaiwa NM., El-Ottol AE., Abu-Elamreen FH. 2006: Antibiotic resistance of uropathogens isolated from Al-Shifa hospital in Gaza Strip in 2002, *J Chemother*, 18(3), 298-302.
13. Elmanama AA., Laham NA., Tayh GA. 2013: Antimicrobial susceptibility of bacterial isolates from burn units in Gaza, *Burns*, 39(8), 1612-1618.
14. Ende A., Van Doorn L., Rooijackers S., Feller M., Tytgat G., Dankert J. 2001: Clarithromycin-Susceptible and -Resistant *Helicobacter pylori* Isolates with Identical Randomly Amplified Polymorphic DNA-PCR Genotypes Cultured from Single Gastric Biopsy Specimens Prior to Antibiotic Therapy, *Journal of Clinical Microbiology*, 39(7), 2648-2651.
15. Feldman M. and Peterson WL. 1993: *Helicobacter pylori* and peptic ulcer disease, *West J Med*, 159, 555-559.
16. Fujimura K. 2005: *Helicobacter pylori* infection and idiopathic thrombocytopenic purpura, *Int J Hematol*, 81, 113-118.
17. Garrido L. and Toledo H. 2007: Novel genotypes in *Helicobacter pylori* involving domain V of the 23S rRNA gene, *Helicobacter*, 12(5), 505-509.
18. Hershko C. and Ronson A. 2009: Iron deficiency, *Helicobacter* infection and gastritis, *Acta Haematol*, 122, 97-102.
19. Huang Y., Fan XG., Wang ZM., Zhou JH., Tian XF., Li N. 2004: Identification of *Helicobacter* species in human liver samples from patients with primary hepatocellular carcinoma, *J Clin Pathol*, 57, 1273-1277.
20. Hultén K., Gibreel A., Sköld O., Engstrand L. 1997: Macrolide resistance in *Helicobacter pylori*: mechanism and stability in strains from clarithromycin-treated patients, *Antimicrob Agents Chemother*, 41(11), 2550-2553.

## Clarithromycin Resistance of Helicobacter ....

21. Hunt RH., Xiao SD., Megraud F., Leon-Barua R., Bazzoli F., van der Merwe S., Vaz Coelho LG., Fock M., Fedail S., Cohen H., Malfertheiner P., Vakil N., Hamid S., Goh KL., Wong BC., Krabshuis J., Le Mair A. 2011: Helicobacter pylori in developing countries. World Gastroenterology Organisation Global Guideline, J Gastrointestin Liver Dis, 20, 299-304.
22. Jeon CY., Haan MN., Cheng C., Clayton ER., Mayeda ER., Miller JW., Aiello AE. 2012: Helicobacter pylori infection is associated with an increased rate of diabetes, Diabetes Care, 35, 520-525.
23. Kato S., Fujimura S., Udagawa H., Shimizu T., Maisawa S., Ozawa K., Inuma K. 2002: Antibiotic Resistance of Helicobacter pylori Strains in Japanese Children, Journal of Clinical Microbiology, 40(2), 649-653.
24. Konturek PC., Konturek SJ., Brzozowski T. 2009: Helicobacter pylori infection in gastric cancerogenesis, J Physiol Pharmacol, 60, 3-21.
25. Kreuning J., Lindeman J., Biemond I., Lamers CB. 1994: Relation between IgG and IgA antibody titres against Helicobacter pylori in serum and severity of gastritis in asymptomatic subjects, J Clin Pathol, 47, 227-231.
26. Kurtaran H., Uyar ME., Kasapoglu B., Turkay C., Yilmaz T., Akcay A., Kanbay M. 2008: Role of Helicobacter pylori in pathogenesis of upper respiratory system diseases, J Natl Med Assoc, 100, 1224-1230.
27. Labenz J. and Borsch G. 1994: Evidence for the essential role of Helicobacter pylori in gastric ulcer disease, Gut, 35, 19-22.
28. Lehours P., Zheng Z., Skoglund A., Megraud F., Engstrand L. 2009: Is there a link between the lipopolysaccharide of Helicobacter pylori gastric MALT lymphoma associated strains and lymphoma pathogenesis?, PLoS One, 4, e7297.
29. Logan RPH. and Walker MM. 2001: Epidemiology and diagnosis of Helicobacter pylori infection, BMJ, 323, 920-922.
30. Marin AC., McNicholl AG., Gisbert JP. 2013: A review of rescue regimens after clarithromycin-containing triple therapy failure (for Helicobacter pylori eradication), Expert Opin Pharmacother, 14(7), 843-861.
31. Matsumura M., Hikiba Y., Ogura K., Togo G., Tsukuda I., Ushikawa K., Shiratori Y., Omata M. 2001: Rapid Detection of Mutations in the 23S rRNA Gene of Helicobacter pylori That Confers Resistance to

- Clarithromycin Treatment to the Bacterium, *Journal of Clinical Microbiology*, 39(2), 691-695.
32. McMahon B., Hennessy T. W., Bensler J., Bruden D., Parkinson A., Morris JM., Reasonover AL., Hurlburt DA., Bruce MG., Sacco F., Butler JC. 2003: The Relationship among Previous Antimicrobial Use Antimicrobial Resistance and Treatment Outcomes for *Helicobacter pylori* Infections, *Annals of Internal Medicine*, 139, 463-469.
  33. O'connor A., Taneike I., Nami A., Fitzgerald N., Murphy P., Ryan B., O'connor H., Qasim A., Breslin N., O'moráin C. 2010: *Helicobacter pylori* resistance to metronidazole and clarithromycin in Ireland, *Eur J Gastroenterol Hepatol*, 22(9),1123-1127.
  34. Ogata SK., Godoy AP., da Silva Patricio FR., Kawakami E. 2013: High *Helicobacter pylori* resistance to metronidazole and clarithromycin in Brazilian children and adolescents, *J Pediatr Gastroenterol Nutr*, 56(6), 645-648.
  35. Owen R. 2002: Molecular testing for antibiotic resistance in *Helicobacter pylori*, *Gut*, 50, 285-289.
  36. Ozdil K., Sahin A., Kahraman R., Yuzbasioglu B., Demirdag H., Calhan T., Yilmaz MS., Sokmen HM. 2010: Current prevalence of intestinal metaplasia and *Helicobacter pylori* infection in dyspeptic adult patients from Turkey, *Hepatogastroenterology*, 57, 1563-1566.
  37. Pasceri V., Cammarota G., Patti G., Cuoco L., Gasbarrini A., Grillo RL., Fedeli G., Gasbarrini G., Maseri A. 1998: Association of virulent *Helicobacter pylori* strains with ischemic heart disease, *Circulation*, 97, 1675-1679.
  38. Pasceri V., Patti G., Cammarota G., Pristipino C., Richichi G., Di Sciascio G. 2006: Virulent strains of *Helicobacter pylori* and vascular diseases: a meta-analysis, *Am Heart J*, 151, 1215-1222.
  39. Polyzos SA., Kountouras J., Zavos C., Deretzi G. 2013: *Helicobacter pylori* Infection and Insulin Resistance, *Helicobacter*, 18(2), 165-166.
  40. Robin Warren J. and Marshall B. 1983: Unidentified curved bacilli on gastric epithelium in active chronic gastritis, *The Lancet*, 321, 1273-1275.
  41. Russmann H., Adler K., Haas R., Gebert B., Koletzko S., Heesemann J. 2001: Rapid and accurate determination of genotypic clarithromycin resistance in cultured *Helicobacter pylori* by fluorescent in situ hybridization, *J Clin Microbiol*, 39, 4142-4144.

## Clarithromycin Resistance of Helicobacter ....

42. Russmann H., Feydt-Schmidt A., Adler K., Aust D., Fischer A., Koletzko S. 2003: Detection of *Helicobacter pylori* in paraffin-embedded and in shock-frozen gastric biopsy samples by fluorescent in situ hybridization, *J Clin Microbiol*, 41, 813-815.
43. Samra Z., Shmuely H., Niv Y., Dinari G., Passaro D., Geler A., Gal E., Fishman M., Bachor J., Yahav J. 2002: Resistance of *Helicobacter pylori* isolated in Israel to metronidazole, clarithromycin, tetracycline, amoxicillin and cefixime, *Journal of Antimicrobial Chemotherapy*, 49, 1023-1026.
44. Schütze K., Hentschel E., Hirschl. 1996: Clarithromycin or amoxicillin plus high-dose ranitidine in the treatment of *Helicobacter pylori*-positive functional dyspepsia, *AM. Eur J Gastroenterol Hepatol*, 8(1), 41-46.
45. Sherif M., Mohran Z., Fathy H., Rockabrand D., Rozmajzl P., Frenck RW. 2004: Universal High-Level Primary Metronidazole Resistance in *Helicobacter pylori* Isolated from Children in Egypt, *Journal of Clinical Microbiology*, 42(10), 4832-4834.
46. Stone GG., Shortridge D., Flamm RK., Versalovic J., Beyer J., Idler K., Zulawinski L., Tanaka SK. 1996: Identification of a 23S rRNA gene mutation in clarithromycin-resistant *Helicobacter pylori* *Helicobacter*, 1(4), 227-228.
47. Suzuki RB., Lopes RA., da Câmara Lopes GA., Hung Ho T., Sperança MA. 2013: Low *Helicobacter pylori* primary resistance to clarithromycin in gastric biopsy specimens from dyspeptic patients of a city in the interior of São Paulo, Brazil, *BMC Gastroenterol*, 3, 164.
48. Taylor DE. 2000: Pathophysiology of antibiotic resistance: clarithromycin, *Can J Gastroenterol*, 14(10), 891-894.
49. Taylor DE., Ge Z., Purych D., Lo T., Hiratsuka K. 1997: 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*, 41(12), 2621-2628.
50. Tepes B. 2009: Can gastric cancer be prevented, *J Physiol Pharmacol*, 60 (Suppl 7), 71-77.
51. Trebesius K., Panthel K., Strobel S., Vogt K., Faller G., Kirchner T., Kist M., Heesemann J., Haas R. 2000: Rapid and specific detection of *Helicobacter pylori* macrolide resistance in gastric tissue by fluorescent in situ hybridization, *Gut*, 46, 608-614.

52. Trebesius K., Panthel K., Strobel S., Vogt K., Faller G., Kirchner T., Kist M., Heesemann J., Haas R. 2000: Rapid and specific detection of *Helicobacter pylori* macrolide resistance in gastric tissue by fluorescent in situ hybridization, *Gut*, 46, 608-614.
53. Vale FF. and Vitor JM. 2010: Transmission pathway of *Helicobacter pylori*: does food play a role in rural and urban areas, *Int J Food Microbiol*, 138, 1-12.
54. Versalovic J., Shortridge D., Kibler K., Griffy MV., Beyer J., Flamm RK., Tanaka SK., Graham DY., Go MF. 1996: Mutations in 23S rRNA are associated with clarithromycin resistance in *Helicobacter pylori*, *Antimicrob Agents Chemother*, 40(2), 477-480.
55. Wang ZW., Li Y., Huang LY., Guan QK., Xu DW., Zhou WK., Zhang XZ. 2012: *Helicobacter pylori* infection contributes to high risk of ischemic stroke: evidence from a meta-analysis, *J Neurol*, 259(12), 2527-2537
56. WMA Declaration of Helsinki -Ethical Principles for Medical Research Involving Human Subjects  
[<http://www.wma.net/en/30publications/10policies/b3/>], last accessed on January, 30, 2013.
57. Wurzer H., Rodrigo L., Stamler D., Archambault A., Rokkas T., Skandalis N., Fedorak R., Bazzoli F., Hentschel E., Mora P., Archimandritis A., Megraud F.1997: Short-course therapy with amoxicillin-clarithromycin triple therapy for 10 days (ACT-10) eradicates *Helicobacter pylori* and heals duodenal ulcer. ACT-10 Study Group, *Aliment Pharmacol Ther*, 11(5), 943-952.
58. Xiong LJ., Tong Y., Wang Z., Mao M. 2013: Detection of clarithromycin-resistant *Helicobacter pylori* by stool PCR in children: a comprehensive review of literature, *Helicobacter*, 18(2), 89-101.