The growing importance of *Helicobacter Pylori*

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Although Warren and Marshall first definitely cultivated Helicobacter pylori (H. pylori) only 30 years ago in April 1984, and associated it with chronic gastritis and peptic ulcers, recent evidence indicates that these bacteria have colonized the human stomach for at least 58,000 years (1, 2). Since Helicobacter pylori's first cultivation, research has consistently affirmed the etiological role of these bacteria in various upper gastrointestinal diseases. Overall, H. pylori infect at least half the world's adult population and 80% to 90% of the population of developing countries (3, 4). Ten years after its discovery the World Health Organization's (WHO) International Agency for Research on Cancer (IARC) catalogued it as a well defined Group I carcinogen (5). It is now considered to be the leading cause of chronic gastritis, peptic ulcers, gastric MALTs and gastric cancer (GC) (6-8). Although it produces chronic gastritis in everyone it infects (6), most patients are asymptomatic, and a clinical entity develops in less than 20% of the people infected. These clinical entities include peptic ulcers in 15% to 18% of those infected, gastric cancer in 2% to 3% and gastric MALT in less than 0.1% (7, 8). The final consequences of the infection depend on genetic factors in the infected individual, the virulence of the strain of bacteria and environmental factors (7). Epidemiological studies have estimated that H. pylori cause at least 75% of gastric cancers (8). In other words, of the 988,000 new cases of GC that occurred in 2008 according to GLOBOCAN (9), nearly 750,000 would not have developed if H. pylori did not exist.

Since it was labeled a carcinogen by IARC, many studies have been conducted to determine if eradication can help reduce the incidence of GC. In 2008, Fukase in Japan led a multicenter study involving endoscopic resection of early GC. That study showed that H. pylori eradication reduced the incidence of GC by a third (10). Another study in an area of China with a high incidence of GC found that eradication of infection before the onset of precursor lesions such as atrophy and metaplasia significantly reduces the risk of GC (11). The most recent consensus conference concluded that eradication helps prevent GC (12). Experts from Japan, which leads the world in GC prevention strategies, have introduced H. pylori eradication into a GC removal project through monitoring high-risk patients and endoscopically treating early GC (13). Consequently, the policy of secondary prevention of GC is being reconsidered. Now primary prophylaxis through H. pylori eradication is being combined with secondary prophylaxis through screening according to endoscopic risk stratification on the basis of blood tests for H. pylori and serum pepsinogen (14). This organism is also causally related to three hematological entities: anemia from vitamin B12 deficiency, immune thrombocytopenic purpura and iron deficiency anemia (12). The association with iron deficiency anemia

has been conclusively demonstrated in adults and children (15, 16). The British Society of Gastroenterology believes that the association is so strong in patients with iron deficiency that the physician should investigate whether H. pylori is present and if it is, and endoscopy is negative for other causes of anemia, the bacteria should be eradicated (17). H. pylori can also increase the risk for adenomatous polyps and colon cancer (18-24). The association appears to be stronger for distal cancers (20). The trend for decreasing prevalence of colon cancer in industrialized countries may be related to decreasing prevalence of H. pylori infections (23). Because of these bacteria's participation in the etiologies of various clinical entities, H. pylori create a huge impact on life and on health care systems.

Various issues related to H. pylori still need to be investigated. These include virulence factors, interactions with hosts, immune response, antibiotic eradication strategies, and prevention through the development of therapeutic vaccines (which may also protect against the GC) (25). Since H. pylori produce considerable morbidity and mortality, diagnosis and treatment are of utmost importance. Diagnosis can be indirect or direct (26). Some indirect detection techniques determine the activity of enzymes. They include the rapid urease test of a gastric biopsy (sensitivity 96%), labeled urea breath test (sensitivity and specificity > 95%). Other indirect detection techniques use serology to identify anti H. pylori IgG (sensitivity 84%), anti CagA, or antibodies against bacterial antigens expelled in the stool (12, 26). Direct techniques demonstrate the presence of the bacteria in the gastric mucosa through histology, polymerase chain reactions (PCR), or cultures. Histology, with a sensitivity of 98%, is considered to be the gold standard of direct diagnosis (12, 26, 27). The conventional Giemsa method is most widely used (27), although immuno-histopathology increases both specificity and sensitivity (27). Another method is direct DNA detection by PCR in fecal matter which has a sensitivity of 94% (26). Of all the above, culturing is considered to be the most specific, and moreover it has various other uses such as microorganism genotypic classification, diagnostic testing, evaluation of toxicity and virulence, determination and monitoring of resistance to antibiotics, production of antigens, determination of virulence factors and - very importantly - the strain identified can be preserved for future studies (28, 29). Nevertheless, culturing gastric biopsies is not very popular among clinical laboratories because it is expensive and time consuming. H. pylori are gram-negative, microaerophilic bacteria which are difficult to culture. Culturing H. pylori requires skilled personnel with "enthusiasm." H. pylori culturing is demanding because it requires special conditions for transport and rapid processing, requires costly and complicated culture media, and has very specific incubation conditions (26-30). Sensitivity ranges from 68% to 98% with specificity of 100% depending on expertise and the culture media used (31). There is no consensus on general culturing principles such as whether a medium should contain blood or serum, correct microaerophilic atmospheres, CO_2 concentrations of 10%, high humidity, temperature of 37°C and incubation periods ranging from 4 to 10 days (26, 30). Recent publications have provided detailed descriptions of techniques successfully used by laboratories which are expert in the culturing this organism (32). They have also published specific protocols and techniques for the investigation of this bacterium in the laboratory (33). Despite the disadvantages of culturing already mentioned, it is imperative that sufficient expertise in culturing be acquired here in Colombia since there are clinical situations that make this technique essential. In this issue of our review, Bayonne MA (34), has published a general narrative review about culturing H. pylori. Although it does not detail experience of the group, it is a commendable effort to draw attention to the importance of culturing H. pylori which mentions national research groups with more experience in this field. They deserve to be recognized for the substantial contribution to our knowledge of this organism in our environment (35-47).

Given the importance of our ability to identify H. pylori, it is necessary to highlight a few issues. H. pylori is a pathogen that selectively colonizes the gastric epithelium, specifically the receptor for its ligand (48). Consequently, the bacteria's only natural reservoir is the stomach (49). For this reason samples for cultivation must be obtained from biopsies from the mucosa of stomach (26, 27, 30, 32), the gastric juice, continuous parts of the gastric epithelium, and pieces peeling off of the gastric epithelium (26). Since peristaltic movements of the small intestine reach the colon, samples can occasionally be cultivated from the feces. The presence of H. pylori can also be determined by immunological techniques that detect antigens of the bacterium and which today are the beginning of antigen testing (26, 31). All of this implies that it is incorrect to think that the bacteria can be cultured from the bladder, rectum or esophagus. The exception would be when there is ectopic gastric mucosa in these epithelia (50), and a physician or researcher desires out of curiosity to investigate the infection in those locations. Successful cultivation of the bacteria is obtained from stomach samples but rarely from stool samples. Dental plaque, which is frequently used for identification of DNA by PCR, is rarely cultured since bacterial presence in the plaque is probably temporary and not permanent (51). It is imperative to establish whether H. pylori infection is present in patients with bleeding peptic ulcers since eradication decreases recurrent bleeding in patients by between 2.9% and 20% compared to patients who do not have the bacteria eradicated and who continue

treatment with PPIs (52). Eradication of infection without maintenance therapy is sufficient to prevent recurrence of an ulcer caused by H. pylori (52, 53). Bleeding decreases the sensitivity of the rapid urease test and culturing but decreases the performance of histology (54). Detection of DNA by PCR appears to be superior (55), but serology probably provides the greatest diagnostic accuracy in this specific situation (56). When it is necessary to determine whether there is an H. pylori infection, the urea breath test can be used. Because of the benefits provided by eradication, whatever means are necessary should be used to determine whether or not these bacteria are present (57). When gastric samples are required for identification, they should not be taken from bleeding ulcers but from traditional sites such as the antrum and corpus.

We believe that the largest possible number of laboratories should begin culturing H. pylori because it provides irreplaceable information for daily practice. All experts agree about the limitations already mentioned about this excellent resource including the facts that H. pylori are fastidious, culturing is expensive, and culturing requires highly trained personnel. Nevertheless, these drawbacks can be overcome with interest and perseverance. In daily practice, the most useful applications of H. pylori cultures would be:

1. To determine pretreatment levels of resistance through studies of treatment. This would allow researchers to gauge the impact of resistance on the success of the treatment regimen and thus help plan future therapeutic strategies (38).
2. To determine local patterns of prevalence of resistance to different antibiotics in order to investigate more rational treatment schemes. For example clarithromycin and quinolones have good correlations between in vitro resistance and eradication rates after treatment, but this is not the case for metronidazole for which it has been shown that in vitro resistance can be overcome by increasing the dosage and duration of treatment (58).
3. To determine H. pylori persistence after failed treatment and whether a patient needs an endoscopy after a second treatment failure (12). Molecular testing may be a lower cost and faster alternative to culturing for determining antimicrobial resistance (59).

Currently H. pylori eradication is very complicated everywhere on our planet due to resistance to antibiotics. Standard triple therapy combining a PPI with clarithromycin and amoxicillin is still recommended as first-line therapy if clarithromycin resistance is less than 20%. If the scheme fails, it can be empirically continued with a second regimen containing levofloxacin (12). Our environment has shown a high rate of resistance to clarithromycin (16%), and the triple therapies recommended by Maastricht IV (12) have eradication rates of only 74% and 79% respectively (41). With sequential therapy we have achieved success in 62% of cases (60). If resistance to clarithromycin is greater than 20% and resistance to metronidazole exceeds 40%, the first line treatment may be quadruple therapy with bismuth or sequential therapy. If bismuth is not available, concomitant therapies (quadruple therapy without bismuth) may be necessary (12). Recently, here in Colombia, Sierra et al. tested an empirical therapy which they called "miscellaneous therapy." It is complex therapy for 15 days that includes lansoprazole twice daily combined with ascending doses of metronidazole, amoxicillin and clarithromycin. The success rate was 91% measured by intention to treat (61). In any case, since our aim is to eradicate H. pylori, schemes that have demonstrated the best efficacy locally (> 90 or 95%) should be used regardless of the guides and the consensus (62, 63). In our opinion it is most important to keep in mind that if your first treatment scheme fails, treatment should continue with a second scheme since to date no treatment scheme has been 100% effective. Successive schemes can achieve a cumulatively efficient success rate of close to 100% (57, 64, 65). Ideally, you should use the scheme with the highest success rate initially to expose patients less often to the antibiotics used in the second scheme. In addition, the second scheme should use antibiotics not used in the first scheme. Although no studies on the cumulative effectiveness of empirical schemes have been conducted in our environment, we recommend that after use of a traditional triple therapy with an eradication rate below 80%, the next scheme should be seven day concomitant therapy with 500 mg of levofloxacin once daily, 500 mg nitazoxanide twice daily, 100 mg doxycycline twice daily, plus 40 mg esomeprazole twice daily. Using this scheme, but with lower doses, a recent study found an eradication rate of 89% (66). Another possible scheme is 14 days of treatment with a PPI twice daily, 1g amoxicillin twice daily, 500 mg clarithromycin twice daily, and 500 mg nitroimidazole twice daily. This scheme has shown 92% efficiency by intention to treat (67). Another possibility is a hybrid scheme consisting of a PPI twice daily and 1g amoxicillin twice a day for 14 days. Then, for seven additional days, 500 mg clarithromycin twice daily and 500 mg nitroimidazole twice daily are added. If this fails, you need a culture with an antibiogram!

REFERENCES

1. Linz B, Balloux F, Moodley Y, Manic A, A, Liu H, Roumagnac P, et al. An African origin for the intimate association between humans and *Helicobacter pylori*. Nature 2007; 445: 915-8.

2. Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet 1984; 1: 1311-5.
3. Dunn Be, Cohen H, Blaser MJ. Helicobacter pylori. Clin Microbiol Rev 1997; 10: 720-41.
4. Suerbaum S, Michetti P. *Helicobacter pylori* infection. N Engl J Med 2002; 347: 1175-86.
5. International Agency for research on Cancer. Schistosomes, liver flukes and *Helicobacter pylori.* IARC Monographs on the Evaluation of carcinogenics risks to humans. Vol. 61. Lyon: IARC 1994.
6. Otero W, Trespalacios AA, Otero E. *Helicobacter pylori:* Tratamiento actual. Un importante reto en gastroenterología. Rev Col Gastroenterol 2009; 24: 279-292.
7. Otero W, Gómez M, Castro D. Carcinogénesis gástrica. Rev Col Gastroenterol 2009; 24: 314-329.
8. Herrera V, Parsonnet J. *Helicobacter pylori* and gastric adenocarcinoma. Clin Microbiol Infect 2009; 15: 971-6.
9. Ferlay J, Shin HR, Bray F. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2010; 127: 2893-17.
10. Fukase K, Kato M, Kikuchi S. Effect of metachronous gastric carcinoma after endoscopic resection of early gastric cancer: an open-label, randomized controlled trial. Lancet 2008; 372: 392-7.
11. Wong BC, Brown LM, Zhang L, *Helicobacter pylori* eradication to prevent gastric cancer in a high-risk region of China: a randomized controlled trial. JAMA 2004; 291: 187-94.
12. Malfertheiner P, Megraud F, O'Morain CA, et al. Management of *Helicobacter pylori* infection: the Maastricht IV/Florence Consensus Report. Gut 2012; 61: 646-664.
13. Asaka A. A new approach for elimination of gastric cancer deaths in Japan. In J Cancer 2013; 132: 1272-6.
14. Kato M, Asaka M. Recent development of gastric cancer prevention. Jpn J Clin Oncol 2012; 42: 987-94.
15. Qu XH, Huang XL, Xiong P, et al. Does *Helicobacter pylori* infection play a role in iron deficiency anemia? A meta-analysis. World J Gastroenterol 2010; 16: 886-96.
16. Muhsen K, Cohen D. *Helicobacter pylori* infection and iron stores: a systematic review and meta-analysis. Helicobacter 2008; 13: 323-40.
17. Goddard AF, James MW, McIntyre AS, Scott BB. British Society of Gastroenterology. Guidelines for the management of iron deficiency anaemia. Gut 2011; 60: 1309-16.
18. Zumkeller N, Brenner H, Zwahlen M, Rothenbacher D. Helicobacter pylori infection and colorectal cancer risk: a meta-analysis. Helicobacter 2006; 11: 75-80.
19. Zhao YS, Wang F, Chang D, et al. Meta-analysis of different test indicators: *Helicobacter pylori* infection and the risk of colorectal cancer. Int J Colorectal Dis 2008; 23: 875-882.
20. Zhang Y, Hoffmeister M, Weck MN, et al. *Helicobacter pylori* infection and colorectal cancer risk: evidence from a large population-based case-control study in Germany. Am J Epidemiol 2012; 175: 441-450.
21. Inoue I, Mukoubayashi C, Yoshimura N, et al. Elevated risk of colorectal adenoma with *Helicobacter pylori*-related chronic gastritis: a population based case-control study. Int J Cancer 2011; 129: 2704-2711.
22. Hong SN, Lee SM, Kim JH, et al. *Helicobacter pylori* infection increases the risk of colorectal adenomas: cross-sectional study and meta-analysis. Dig Dis Sci 2012; 57: 2184-2194.
23. Sonnenberg A, Genta R. *Helicobacter pylori* is a risk for colonic neoplasms. Am J Gastroenterol 2013; 108: 208-15.
24. Wu Q, Yang ZP, Xu P, Gao LC, Fan DM. Association between *Helicobacter pylori* infection and the risk of colorectal neoplasia: a systematic review and meta-analysis. Colorectal Dis 2013 May 15 (Epub ahead).
25. Sutton P, Chionh YT. Why can't we make an effective vaccine against *Helicobacter pylori?* Expert Rev Vaccines. 2013; 12: 433-41.
26. Mohammadi M, Hashani SS, Garoosi Y, Talebkhan T, Jahangiti S. In vivo measurement of *Helicobacter pylori*. Meth Mol Biol 2012; 921: 239-49.
27. Tonkic A, Tonkic M Lehours P, Mefraud F. Epidemiology and diagnosis of *Helicobacter pylori* infection. Helicobacter 2012; 17 (Suppl. 1): 1-8.
28. Hutton ML, Kaparakis-Liaskos M, Ferrero RL: The use of AlbuMAXII as a blood or serum alternative for the culture of *Helicobacter pylori*. Helicobacter 2011; 17: 68-76.
29. Asaka M, Kato M, Takahashi S, Fukuda Y, Sugyyama T, Ota H, et al. Guidelines for the management of *Helicobacter pylori* infection in Japan: 2009 revised edition. Helicobacter 2010; 15: 1-20.
30. Cover TL. Perspectives on methodology for in vitro culture of *Helicobacter pylori.* Methods Mol Biol 2012; 921: 11-15.
31. Cutler AF. Diagnostic tests for *Helicobacter pylori* infection. Gastroenterologist 1997; 5: 202-12.
32. Whitmire JM, Merrell DS. Successful culture techniques for Helicobacter Species: general culture techniques for *Helicobacter pylori*. Meth Mol Biol 2012; 921:17-27.
33. Houghton JM (Edit). Helicobacter Species Methods and Protocols Springer Science Business Media, 2012.
34. Bayona MA. Condiciones microbiológicas para el cultivo de *Helicobacter pylori.* Rev Col Gastroenterol 2013; 28: 94-99.
35. Henao S, Otero W, Martìnez J, et al. Resistencia primaria de *Helicobacter pylori* a metronidazol en Colombia. Rev Col Gastroenterol 2009; 24: 10-15.
36. Henao SC, Quiroga A, Martinez JD, Otero W. Resistencia primaria a claritromicina en aislamientos de *Helicobacter pylori*. Rev Col Gastroenterol 2009; 24: 110-115.
37. Ávila JM, Rey M, Mercado MM; Villamizar OR, Otero W, Trespalacios AA. Comparación de las pruebas de dilución en agar y PCR para determinación de susceptibilidad antimicrobiana de *Helicobacter pylori*. Rev sistemática de la literatura. Rev Col Gastroenterol 2009; 24: 116-27.
38. Trespalacios AA, Otero W, Mercado Marcela. Resistencia de *Helicobacter pylori* a claritromicina, amoxicilina y metronidazol en pacientes colombianos. Rev Col Gastroenterol 2010; 25: 31-8.
39. Trespalacios AA, A. Arévalo, W Otero M, Mercado, E. Caminos, R. Potou, D. Graham, E. Rimbara. Primary Levofloxacin resistance among *H. pylori* in Colombia. Helicobacter 2010; 15: 287-8 (Abstract).

40. Arévalo A, Trespalacios AA, Otero W, Mercado M, Potou R. Prevalence of cagA, vacA, babA2 and iceA Genes in *H. pylori* Strains Isolated from Colombian Patients with Functional Dyspepsia. Polish Journal of Microbiology 2012; 61: 33-40.
41. Trespalacios AA, Otero W, Mercado M, et al. Impacto de la resistencia de *Helicobacter pylori* a los antimicrobianos en la eficacia de la terapia triple estándar y en dos triples terapias con levofloxacina en pacientes colombianos. Gastroenterol Latinoam 2012; 23: S35.
42. Mercado M, Trespalacios AA, Otero W. Sensibilidad de *Helicobacter pylori* a claritromicina y a levofloxacina y el éxito terapéutico. Gastroenterol Latinoam 2012; 23: S35.
43. Duque R, Arévalo A, Poutou R, Trepalacios AA. Sequential statistical improvement of the liquid cultivation of *Helicobacer pylori*. Helicobacter 2010; 15: 303-12.
44. Cittelly D, Henao S, Orozco O, Martínez JD. Detección de *Helicobacter pylori* en Colombia: diferentes metodologías aplicadas a su estudio en una población de alto riesgo de cáncer gástrico. Rev Col Gastroenterol 1999; 14: 164-169.
45. Trespalacios AA, Mercado M, Otero W, et al. Prevalencia de la resistencia de *Helicobacter pylori* a los antimicrobianos utilizados en las terapias de primera línea y de rescate en Colombia. Gastroenterol Latinoam 2012; 23: S36.
46. Quiroga, A, Citelly, D, Bravo, M. Frecuencia de los genotipos babA2, oipA y cagE de *H. pylori* en pacientes colombianos con enfermedades gastroduodenales. Biomédica 2005; 25: 325-334.
47. Navarro J, Perea A, Pineda J, Díez O, Mercado M, Trespalacios A. Evaluación de la productividad de tres medios de cultivo para la recuperación de *H. pylori*. Universita Scientiarum 2007; 12: 79-86.
48. Basset C, Holton J, Gatta L, Ricci C, Bernavucci V, Liuzzi G, Vaira A. *Helicobacter pylori* infection: anything new should we know? Aliment Pharmacol Ther 2004; 20 (Suppl. 2): 31-41.
49. Simoot DT, Resau JH, Naab T, et al. Adherence of *Helicobacter pylori* to culture human gastric epithelial cells. Infect Immun 1993; 61: 350-5.
50. Carrigan MA, Shields CJ, Keohane C, Kirwan WO. The immunohistochemical demonstration of *Helicobacter pylori* in rectal ectopic. Surg Laparosc Endosc Percutan Tech 2009; 19: 146-8.
51. Al-Ahmad A, Kürschner A, Weckesserv S, Wittmer A, Rauberger H, et al. Is *Helicobacter pylori* resident o transient in the human oral cavity? J Med Microbiol 2012; 61: 1146-52.
52. Galindo JV, Otero W, Gómez M. Úlcera duodenal no complicada y complicada con sangrado: ¿cuál es la importancia del tratamiento contra *Helicobacter pylori*? Rev Col Gastroenterol 2010; 25: 295-300.
53. Lain L, Jensen DM Management of patients with ulcer bleeding. Am J Gastroenterol 2012; 107: 345-360.
54. Choi YJ, Kim N, Lim J, Jo SY, Shin CM, Lee HS, et al. Accuracy of diagnostic tests for *Helicobacter pylori* in patients with peptic ulcer peptic bleeding. Helicobacter 2012; 17: 77-85.
55. Saez J, Belda S, Santibañez M, Rodriguez JC, Sola-Vera J, Galiana A, et al. Real-Time PCR for diagnosing *Helicobacter pylori* infection in patients with upper gastrointestinal bleeding: a comparison with other classical diagnosis methods. J Clin Microbiol 2012; 50: 3233-7.
56. Stenstrom, B., Mendis, A. Marshall, B. *Helicobacter pylori*—the latest in diagnosis and treatment. Aust. Fam. Physician 2008; 37: 608-612.
57. Barkun A, Bardou M, Kuipers E, Sung J, Hunt R, Martel M, et al. International Consensus Recommendations on the Management of Patients With Nonvariceal Upper Gastrointestinal Bleeding. Ann Intern Med 2010; 152: 101-113.
58. Fischbach L, Evans EL. Metanalysis: the effect of antibiotic resistance status on the efficacy of triple therapy and quadruple first-line therapies for *Helicobacter pylori*. Aliment Pharmacol ther 2007; 26: 343-57.
59. Trespalacios AA, Rimbara E, Otero W, Mercado M, Caminos JE, Reddy R, Graham D. PCR Alelo-Específica para detección de resistencia de *Helicobacter pylori* a claritromicina y fluoroquinolonas en biopsias gástricas de Colombia incluyendo la identificación de una nueva mutación en gyrA; manuscrito en preparación.
60. Gómez M, Otero W, Melgar C. Comparación de la Terapia secuencial con la triple terapia estándar en la erradicación de *Helicobacter pylori.* Rev Col Gastroenterol 2011; 26: 171-7.
61. Sierra F, Forero JD, Botero ML, Cárdenas AC. Pilot study: miscellaneous therapy is highly successful for *Helicobacter pylori* eradication. Aliment Pharmacol Ther 2013; Aliment Pharmacol Ther 2013; 37: 1165-1171.
62. Graham DY, Fischbach L. *Helicobacter pylori* treatment in the era of increasing antibiotic resistance. Gut 2010; 59: 1143-53.
63. Graham DY, Calvet X. Guide Regarding choice of second-line therapy to obtain a high cumulative cure rate. Helicobacter 2012; 17: 243-5.
64. Manfredi M, Bazzarri B, D'Angelis GL. *Helicobacter pylori* infection: sequential therapy followed by levofloxacin-containing triple therapy provides a good cumulative eradication rate. Helicobacter 2012; 17: 246-263.
65. Gisbert JP, Gisbert JL, Marcos S, Jimenez-Alonso I, Moreno-Otero I. Empirical rescue therapy after *Helicobacter pylori* treatment failure: a 10-year single-centre study of 500 patients. Aliment Pharmacol Ther 2008; 27: 346-354.
66. Basu PP, Rayapudi K, Pacana T, Shah NJ, Krishnaswamy N.Flyn M, et al A Randomized Study comparing Levofloxacin, Omeprazole, Nitazoxanide, and Doxycycline versus Triple Therapy for the Eradication of *Helicobacter pylori* . Am J Gastroenterol 2011; 106: 1970-75.
67. Molina Infante J, Romano M, Fernández-Bermejo M, Federico A, Gravina AG, Pozzati L, et al. Optimized non bismuth quadruple therapies cure most patients with *Helicobacter pylori* infection in populations with high rates of antibiotic resistance. Gastroenterology 2013 online may 16.