East African Scholars Journal of Medicine and Surgery
Abbreviated Key Title: EAS J Med Surg
ISSN: 2663-1857 (Print) & ISSN: 2663-7332 (Online) |
Published By East African Scholars Publisher, Kenya

OPEN ACCESS

Volume-1 | Issue-2 | Mar -Apr-2019 |

Research Article

Helicobacter pylori Drug Resistence: Review on Prevalence of Drug Resistence and Role of Drug Susceptibility tests.

Smita Sharma. MD [1], Rami Abdullah Ali Al Dagrer[2], S.K.Mathur [3].

[1]MD(Biochemistry), DTCD, Clinical Biochemist,Maternity and Children Hospital, Nejran, KSA
[2]Masters in Biotechnology Director Blood Banks and Labs Nejran zone KSA Saudi Arabia
[3]DM (Endocrinology) MD (Paediatrics) Consultant Endocrinologist Max.Hospital Mohalli India

*Corresponding Author
Smita Sharma. MD

Abstract: *Helicobacter pylori* (*H. pylori)* is a Gram-negative bacillus that infects the human stomach mucosa and produces diseases of the upper gastrointestinal tract such as chronic gastritis, peptic ulcer disease, gastric marginal zone/mucosa-associated lymphoid tissue (MALT) lymphoma and gastric carcinoma (Blaser, M.J., & Atherton, J.C. 2004; Dinis-Ribeiro, M. *et al.,* 2012; Vakil, N., & Megraud, F. 2007; Fukase, K. *et al.,* 2008). *Helicobacter pylori* infection remains a very common worldwide condition with strong geographic variations and the prevalence of antibiotic resistance appears to be rapidly increasing. Antimicrobial resistance is the major factor leading to eradication failure in *H. pylori* treatment. . Knowledge of the local prevalence rate of resistance is important to define the best recommended treatment. In this review we tried to focus prevalence of drug resistence and the use of drug susceptibility tests before starting the drug regimen especially in high resistant areas.
Keywords: H.pylori,Antimicrobial resistence.

INTRODUCTION:

Helicobacter pylori infection is the most common infection and is associated with simple dyspepsia, heartburn and peptic ulcer diseases, most commonly leading to upper gastrointestinal bleeding and, ultimately, to the severe complication of gastric malignancy. Ninety percent of duodenal ulcers and 70% of gastric ulcers are associated with helicobacter pylori infections. *H. pylori* also plays a role in extradigestive diseases, including immune thrombocytopenic purpura, unexplained iron deficiency anaemia, and vitamin B12 deficiency. Eradication failure is of great importance in *Helicobacter pylori* (*H. pylori*) infection. Antibiotic resistance in *H. pylori* is widespread and increasing.

Therefore, understanding antimicrobial resistance mechanisms and detecting *H. pylori* antimicrobial susceptibility are important for guiding eradication regimens before the initiation of first-line therapy or alternative regimens for patients in who repeated eradication therapies have failed.

METHOD OF DETECTION:

The *H pylori* diagnostic tests currently available can be generally characterized as endoscopic or nonendoscopic.

Three nonendoscopic options are currently available. The first is serologic testing, which detects the presence of *H pylori*–specific antibodies in the blood. This type of testing is not recommended, as it is actually a test of *H pylori* exposure rather than a test of active infection. Patients can remain *H pylori* antibody–positive for months or even years after the infection has been eradicated.

The other 2 diagnostic tools test for active infection. One is a urea breath test (UBT), which previously used the ^{14}C radioactive isotope and now uses the ^{13}C nonradioactive isotope. A commercially available UBT is a reasonable test for both initial diagnosis and eradication testing of *H pylori* infection in adults and in children at least 3 years old. The test detects active infection and has excellent positive and negative predictive values.

Quick Response Code

Journal homepage: http://www.easpublisher.com/easms/

Article History
Received: 11.03.2019
Accepted: 25.03.2019
Published: 13.04.2019

Copyright © 2019 The Author(s): This is an open-access article distributed under the terms of the Creative Commons Attribution **4.0 International License (CC BY-NC 4.0)** which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use provided the original author and source are credited.

DOI: 10.36349/easjms.2019.v01i02.012

The other option is a stool antigen test. This test is also very accurate, but it requires stool collection and transporting the sample on ice. However, despite the more cumbersome nature of the stool test, its accuracy is comparable to that of the breath test.

INVASIVE METHODS:

Gastroduodenoscopy is very essential in symptomatic individuals who are not responding to therapy and those aged older than 45 years according to European guidelines (Mentis, A. *et al.,* 2015). It is not only diagnostic but also therapeutic in upper gastrointestinal bleeding (UGIB) cases. Additionally, it is a very important procedure in taking biopsies to diagnose H. pylori infection using RUT, a histopathological evaluation, culture and sensitivity.

Histology:

Histology remains the gold standard for diagnosis of HP gastritis and detection of H. pylori organisms, with a sensitivity and specificity >95% (Hunt, R. H. *et al.,* 2011). Giemsa staining is a widely used technique, and immunostaining would increase the sensitivity and specificity to 100% and 98-99%, respectively (Tonkic, A. *et al.,* 2012; Malfertheiner, P. *et al.,* 2012; Braden, B. 2012; Lee, H. S. 2016).

Treatment Guidelines:

Clarithromycin triple therapy consisting of a PPI, clarithromycin, and amoxicillin or metronidazole for 14 days remains a recommended treatment in regions where H. pylori clarithromycin resistance is known to be<15%. Bismuth quadruple therapy consisting of a PPI, bismuth, tetracycline, and a nitroimidazole for 10– 14 days is a recommended first-line treatment option. Bismuth quadruple therapy is particularly attractive in patients with any previous macrolide exposure or who are allergic to penicillin (Chey, W. D. *et al.,* 2017).

Concomitant therapy consisting of a PPI, clarithromycin, amoxicillin and a nitroimidazole for 10– 14 days is a recommended first-line treatment option. Sequential therapy consisting of a PPI and amoxicillin for 5–7 days followed by a PPI, clarithromycin, and a nitroimidazole for 5–7 days is a suggested first-line treatment option (Chey, W. D. *et al.,* 2017).

Hybrid therapy consisting of a PPI and amoxicillin for 7 days followed by a PPI, amoxicillin, clarithromycin and a nitroimidazole for 7 days is a suggested first-line treatment option. Levofloxacin triple therapy consisting of a PPI, levofloxacin, and amoxicillin for 10–14 days is a suggested first-line treatment option. Fluoroquinolone sequential therapy consisting of a PPI and amoxicillin for 5–7 days followed by a PPI, fluoroquinolone, and nitroimidazole for 5–7 days is a suggested first-line treatment option (Chey, W. D. *et al.,* 2017).

Whenever H. pylori infection is identified and treated, testing to prove eradication should be performed using a urea breath test, fecal antigen test or biopsy-based testing at least 4 weeks after the completion of antibiotic therapy and after PPI therapy has been withheld for 1–2 week.

Options for Salvage Therapy When First-Line Therapy Fails:

Bismuth quadruple therapy or levofloxacin salvage regimens are the preferred treatment options if a patient received a first-line treatment containing clarithromycin. Selection of best salvage regimen should be directed by local antimicrobial resistance data and the patient's previous exposure to antibiotics.

Mechanism of Drug Resistence and Prevalence of Drug Resistence:

Clarithromycin is a bacteriostatic antibiotic that inhibits bacterial protein synthesis by reversibly binding to the 50S ribosomal subunits. The 50S ribosomal subunit is itself composed of 23S ribosomal RNA, 5S ribosomal RNA, and RNA binding proteins. The peptidyl transferase loop of the V domain of 23S ribosomal RNA molecule is the target site of clarithromycin. Resistance to clarithromycin is generally caused by point mutations in the 23S rRNA gene, the most frequent is A2143G (69.8%), followed by A2142G (11.7%) and A2142C (2.6%) (Megraud, F. 2004). Current Maastricht consensus guidelines recommend abandoning clarithromycin-containing triple therapy without previous susceptibility testing when the local clarithromycin resistance rate is higher than 15%.

Metronidazole resistance occurs mainly by mutations in the rdxA gene of H. pylori, which encodes an enzyme that reduces metronidazole to active metabolites. Different mutations involving the rdxA gene, which encodes an oxygen insensitive NADPH nitroreductase, have been identified in metronidazole-resistant strains. Moreover, other genes such as frxA also seemed to be involved.Mutations in the frxA gene, encoding an NAD (P) H flavin reductase showing high homology with the rdxA product, can also affect metronidazole susceptibility (Goodwin, A. *et al.,* 1998; Jenks, P.J., & Edwards, D.I. 2002; Mirzaei, N. *et al.,* 2014).

H.pylori mutation conferring resistance to tetracycline had two base pair mutation AGC926-928 as observed in Congo Brazzaville, similar mutation was obtained in other parts of the world, in America (Toledo, H., & López-Solís, R. 2009; Ribeiro, M. L. *et al.,* 2004), Europe and Asia (Gerrits, M. M. *et al.,* 2003; Dadashzadeh, K. *et al.,* 2014).

The European Multicentre Study Group included 2204 patients from 2008 to 2009, spanning 18 European countries and demonstrated *H. pylori* resistance rates to clarithromycin, metronidazole and levofloxacin at 17.5%, 34.9% and 14.1% respectively. (Megraud, F. *et al.,* 2013) countries with higher rates of *H. pylori* seropositivity are associated with dramatically increasing rates of clarithromycin resistance. For example, Horiki *et al.,* (2009) demonstrated that the prevalence of clarithromycin resistance has increased considerably from 1.8% in 1996 to 27.1% in 2008 in the Japanese population (Horiki, N. *et al.,* 2009). Okamura *et al.,*. described an overall resistance rate of 31.1% in patients studied between 2000 and 2013 (Okamura, T. *et al.,* 2014). The prevalence of *H. pylori* seropositivity over this time period has increased from approximately 40% to 55% (Hunt, R. H. *et al.,* 2011; Fujisawa, T. *et al.,* 1999; Horiki, N. *et al.,* 2009). China has experienced an increase in clarithromycin resistance from 14.8% in 2000 to 52.6% in 2014 with an increase in seropositivity rates from approximately 65% to 83% (Zhang, M. *et al.,* 2014; Rahman, R. *et al.,* 2014; Ma, J. L. *et al.,* 1998; Gao, W. *et al.,* 2010; De Francesco, V. *et al.,* 2010). In addition, a marked increase in prevalence of clarithromycin resistance was seen in Korea from 11% in 2005 to 60% in 2009 (Lee, J. H. *et al.,* 2005). Fayaz Ahmad Wani *et al.,* (2018) demonstrated maximum resistance to metronidazole (81.66%) followed by clarithromycin (45%) and quinolones (3.33%).

Maastricht consensus guidelines recommend abandoning clarithromycin-containing triple therapy without previous susceptibility testing when the local clarithromycin resistance rate is higher than 15%.

The worldwide prevalence of metronidazole resistance has been found to range from is 31% - 53% in Europe and South America, and between 64% and 80% in Iran and Saudi Arabia (Ghotaslou, R. *et al.,* 2015; Khademi, F. *et al.,* 2015). in Africa the observed tetracycline resistance (49.8%) was comparable to that in Asia which was found to range from 0.01% in Japan to 53.8% in India (Ghotaslou, R. *et al.,* 2015).

The rate of quinolone resistance observed in Africa is almost similar to that documented in South America (21%), Asia (25.3%) and North America (19%) but higher than that in Europe (Metronidazole mutations 36,37,38] and quinolones mutations (Butlop, T. *et al.,* 2016; Kwon, D. H. *et al.,* 2000; Marais, A. *et al.,* 2003) observed in Africa were similar to that observed in Europe, in Asia and America.14.2%) (Ghotaslou, R. *et al.,* 2015).

Methods for detection of H.pylori Drug Resistence:

In vitro susceptibility testing of *H. pylori* using agar dilution method are practical for testing large numbers of strains; it is not suitable for the testing of small numbers of strains on an ongoing basis (Grignon, B. *et al.,* 2002; NCCLS. 2000). The Epsilometer test (*E*-test) method involves the use of test strips applied to an inoculated agar plate in order to determine the antibiotic's minimum inhibitory concentration (Hachem, C. Y. *et al.,* 1996). One study found the *E*-test produced reproducible results in determining the sensitivity of *H. pylori* isolates to ampicillin, clarithromycin and metronidazole (Hachem, C. Y. *et al.,* 1996; Destura, R. V. *et al.,* 2004). From an international perspective, *E*-test appears to be a suitable method for determining *H. pylori* antibiotic sensitivity (Destura, R. V. *et al.,* 2004; Yilmaz, Ö., & Demiray, E. 2007). However, the availability of the *E*-test strips for one of the key antibiotics of interest, clarithromycin, is currently not globally available for clinical use.

One of the main drawbacks of both the agar dilution and *E*-test is that they only test a single *H. pylori* strain. In areas of high *H. pylori* prevalence and increased likelihood of patients being infected with multiple *H. pylori* strains, these two testing modalities may fail to provide complete antimicrobial resistance data.

Molecular Techniques:

The gold standard methods of antibiotic resistance are based on phenotypic methods performed by the agar dilution method (Grignon, B. *et al.,* 2002; Burucoa, C. *et al.,* 2008). These methods, however, can take up to 2 weeks to be completed.Molecular testing for *H. pylori* offers an attractive alternative to culture and allows for molecular genetic identification of *H. pylori* and antibiotic resistance directly from biopsy samples. As such, it provides the opportunity for rapid analysis, enabling same-day diagnosis.. Molecular methods should be considered a useful approach for monitoring the prevalence of *H. pylori* clarithromycin resistance nationally as well as a means for tailoring individual patient therapy. In addition, molecular techniques can often use either fresh or formalin-fixed samples.

Real-time PCR has been used to successfully determine *H. pylori* susceptibility to clarithromycin (van Doorn, L. J. *et al.,* 2001; Schabereiter-Gurtner, C. *et al.,* 2004). Additionally, PCR using formalin-fixed paraffin-embedded samples has been shown to reliably detect the *H. pylori* 23S rRNA mutations associated with clarithromycin resistance (Mitui, M. *et al.,* 2014). Another advantage of PCR is the potential to gather complete antimicrobial resistance data in patients infected with multiple strains of *H. pylori*. Although the use of PCR-based methods provides rapid detection of micro-organisms, these techniques can be affected by DNA contamination or degradation since the high

sensitivity of these methods often result in the detection of dead or nonculturable microorganisms (Mégraud, F., & Lehours, P. 2007).

Fluorescence in situ hybridisation (FISH) is a time-saving, accurate and cost-effective method for the detection of antibiotic resistance in cultured *H. pylori* colonies. This method can be used directly on biopsy specimens procured for histopathological and microbiological examination, allowing for rapid detection of *H. pylori* resistance without requiring DNA preparation (Rüssmann, H. *et al.,* 2001 Yilmaz, Ö., & Demiray, E. 2007). The results can theoretically be available within 3 hours after an endoscopy by utilising frozen tissue sections (Mégraud, F., & Lehours, P. 2007). The limitations of this method include the degradation of the probe by proteases and nucleases present in the sample and poor accessibility of the microbial cell wall for the probes.

Recently, peptide nucleic acid (PNA) probes using FISH have been used for the detection of several bacteria in lieu of the typical DNA molecular probes (Perry-O'Keefe, H. *et al.,* 2001). PNA molecules are DNA mimics with high affinity for DNA or RNA complementary sequences (Stender, H. *et al.,* 2002; Cerqueira, L., *et al.,* 2008). PNA probes are normally relatively small (13–18 nucleotides), increasing their ability to penetrate the bacterial cell wall. Moreover, the PNA molecules are more resistant to nucleases and proteases than DNA molecules.

CONCLUSION:

As antibiotic resistance is a constantly evolving process, an on-going effort to monitor antibiotic resistance rates, using both culture and molecular-based methods, should be done to monitor the prevalence of resistance.

Conflicts of Interests:

There is no conflict of interest.

REFERENCES:

1. Blaser, M.J., & Atherton, J.C. (2004). *Helicobacter pylori* persistence: biology and disease. *J Clin Invest*, 113, 321–33.
2. Dinis-Ribeiro, M., Areia, M., De Vries, A. C., Marcos-Pinto, R., Monteiro-Soares, M., O'connor, A., ... & Dumonceau, J. M. (2012). Management of precancerous conditions and lesions in the stomach (MAPS): guideline from the European Society of Gastrointestinal Endoscopy (ESGE), European Helicobacter Study Group (EHSG), European Society of Pathology (ESP), and the Sociedade Portuguesa de Endoscopia Digestiva (SPED). *Endoscopy*, *44*(01), 74-94.
3. Vakil, N., & Megraud, F. (2007). Eradication therapy for *Helicobacter pylori*. *Gastroenterology*, 133, 985–1001.
4. Fukase, K., Kato, M., Kikuchi, S., Inoue, K., Uemura, N., Okamoto, S., ... & Japan Gast Study Group. (2008). Effect of eradication of Helicobacter pylori on incidence of metachronous gastric carcinoma after endoscopic resection of early gastric cancer: an open-label, randomised controlled trial. *The Lancet*, *372*(9636), 392-397.
5. Mentis, A., Lehours, P., & Mégraud, F. (2015). Epidemiology and Diagnosis of Helicobacter pylori infection. *Helicobacter,* 20, 1-7.
6. Hunt, R. H., Xiao, S. D., Megraud, F., Leon-Barua, R., Bazzoli, F., Van der Merwe, S., ... & Malfertheiner, P. (2011). Helicobacter pylori in developing countries. World gastroenterology organisation global guideline. *J Gastrointestin Liver Dis*, *20*(3), 299-304.
7. Lario, S., Ramírez-Lázaro, M. J., Montserrat, A., Quílez, M. E., Junquera, F., Martínez-Bauer, E., ... & Calvet, X. (2016). Diagnostic accuracy of three monoclonal stool tests in a large series of untreated Helicobacter pylori infected patients. *Clinical biochemistry*, *49*(9), 682-687.
8. Tonkic, A., Tonkic, M., Lehours, P., & Mégraud, F. (2012). Epidemiology and diagnosis of Helicobacter pylori infection. *Helicobacter,* 17, 1-8.
9. Malfertheiner, P., Megraud, F., O'Morain, C. A., Atherton, J., Axon, A. T., Bazzoli, F., ... & El-Omar, E. M. (2012). Management of Helicobacter pylori infection—the Maastricht IV/Florence consensus report. *Gut*, *61*(5), 646-664.
10. Braden, B. (2012). Diagnosis of Helicobacter pylori infection. *BMJ,* 344, e828.
11. Lee, H. S. (2016). Histopathologic Diagnosis of H. pylori Infection and Associated Gastric Diseases. In *Helicobacter pylori* (pp. 119-127). Springer, Singapore.
12. Chey, W. D., Leontiadis, G. I., Howden, C. W., & Moss, S. F. (2017). ACG clinical guideline: treatment of Helicobacter pylori infection. *The American journal of gastroenterology*, *112*(2), 212.
13. Megraud, F. (2004). H pylori antibiotic resistance: prevalence, importance, and advances in testing. *Gut*, *53*(9), 1374-1384.
14. Goodwin, A., Kersulyte, D., Sisson, G., Veldhuyzen van Zanten, S. J., Berg, D. E., & Hoffman, P. S. (1998). Metronidazole resistance in Helicobacter pylori is due to null mutations in a gene (rdxA) that encodes an oxygen‐insensitive NADPH nitroreductase. *Molecular microbiology*, *28*(2), 383-393.
15. Jenks, P.J., & Edwards, D.I. (2002). Metronidazole resistance in *Helicobacter pylori*. *Int J Antimicrob Agents*, 19, 1–7.
16. Mirzaei, N., Poursina, F., Moghim, S., Rahimi, E., & Safaei, H. G. The mutation of the rdxA gene in metronidazole-resistant Helicobacter pylori clinical isolates. Adv Biomed Res. 2014; 3: 90.
17. Toledo, H., & López-Solís, R. (2009). Tetracycline resistance in Chilean clinical isolates of Helicobacter pylori. *Journal of antimicrobial chemotherapy*, *65*(3), 470-473.
18. Ribeiro, M. L., Gerrits, M. M., Benvengo, Y. H., Berning, M., Godoy, A. P., Kuipers, E. J., ... & Kusters, J. G. (2004). Detection of high-level tetracycline resistance in clinical isolates of

Helicobacter pylori using PCR-RFLP. *FEMS Immunology & Medical Microbiology*, *40*(1), 57-61.

19. Gerrits, M. M., Berning, M., Van Vliet, A. H., Kuipers, E. J., & Kusters, J. G. (2003). Effects of 16S rRNA gene mutations on tetracycline resistance in Helicobacter pylori. *Antimicrobial agents and chemotherapy*, *47*(9), 2984-2986.
20. Dadashzadeh, K., Milani, M., Rahmati, M., & Akbarzadeh, A. (2014). Real-time PCR detection of 16S rRNA novel mutations associated with Helicobacter pylori tetracycline resistance in Iran. *Asian Pac J Cancer Prev*, *15*(20), 8883-8886.
21. Megraud, F., Coenen, S., Versporten, A., Kist, M., Lopez-Brea, M., Hirschl, A. M., ... & Glupczynski, Y. (2013). Helicobacter pylori resistance to antibiotics in Europe and its relationship to antibiotic consumption. *Gut*, *62*(1), 34-42.
22. Horiki, N., Omata, F., Uemura, M., Suzuki, S., Ishii, N., Iizuka, Y., ... & Cesar, G. E. (2009). Annual change of primary resistance to clarithromycin among Helicobacter pylori isolates from 1996 through 2008 in Japan. *Helicobacter*, *14*(5), 438-442.
23. Okamura, T., Suga, T., Nagaya, T., Arakura, N., Matsumoto, T., Nakayama, Y., & Tanaka, E. (2014). Antimicrobial Resistance and Characteristics of Eradication Therapy of H elicobacter pylori in J apan: A Multi-Generational Comparison. *Helicobacter*, *19*(3), 214-220.
24. Hunt, R. H., Xiao, S. D., Megraud, F., Leon-Barua, R., Bazzoli, F., Van der Merwe, S., ... & Malfertheiner, P. (2011). Helicobacter pylori in developing countries. World gastroenterology organisation global guideline. *J Gastrointestin Liver Dis*, *20*(3), 299-304.
25. Fujisawa, T., Kumagai, T., Akamatsu, T., Kiyosawa, K., & Matsunaga, Y. (1999). Changes in seroepidemiological pattern of Helicobacter pylori and hepatitis A virus over the last 20 years in Japan. *The American journal of gastroenterology*, *94*(8), 2094.
26. Zhang, M., Zhou, Y. Z., Li, X. Y., Tang, Z., Zhu, H. M., Yang, Y., & Chhetri, J. K. (2014). Seroepidemiology of Helicobacter pylori infection in elderly people in the Beijing region, China. *World journal of gastroenterology: WJG*, *20*(13), 3635.
27. Rahman, R., Asombang, A. W., & Ibdah, J. A. (2014). Characteristics of gastric cancer in Asia. *World journal of gastroenterology: WJG*, *20*(16), 4483.
28. Ma, J. L., You, W. C., Gail, M. H., Zhang, L., Blot, W. J., Chang, Y. S., ... & Xu, G. W. (1998). Helicobacter pylori infection and mode of transmission in a population at high risk of stomach cancer. *International journal of epidemiology*, *27*(4), 570-573.
29. Gao, W., Cheng, H., Hu, F., Li, J., Wang, L., Yang, G., ... & Zheng, X. (2010). The evolution of Helicobacter pylori antibiotics resistance over 10 years in Beijing, China. *Helicobacter*, *15*(5), 460-466.
30. De Francesco, V., Giorgio, F., Hassan, C., Manes, G., Vannella, L., Panella, C., ... & Zullo, A. (2010). Worldwide H. pylori antibiotic resistance: a systematic review. *Journal of Gastrointestinal & Liver Diseases*, *19*(4).
31. Lee, J. H., Shin, J. H., Roe, I. H., Sohn, S. G., Lee, J. H., Kang, G. H., ... & Lee, S. H. (2005). Impact of clarithromycin resistance on eradication of Helicobacter pylori in infected adults. *Antimicrobial agents and chemotherapy*, *49*(4), 1600-1603.
32. Wani, F. A., Bashir, G., Khan, M. A., Zargar, S. A., Rasool, Z., & Qadri, Q. (2018). Antibiotic resistance in Helicobacter pylori: a mutational analysis from a tertiary care hospital in Kashmir, India. *Indian journal of medical microbiology*, *36*(2), 265.
33. Ghotaslou, R., Leylabadlo, H. E., & Asl, Y. M. (2015). Prevalence of antibiotic resistance in Helicobacter pylori: A recent literature review. *World journal of methodology*, *5*(3), 164.
34. Khademi, F., Poursina, F., Hosseini, E., Akbari, M., & Safaei, H. G. (2015). Helicobacter pylori in Iran: A systematic review on the antibiotic resistance. *Iranian journal of basic medical sciences*, *18*(1), 2.
35. Hunt, R. H., Xiao, S. D., Megraud, F., Leon-Barua, R., Bazzoli, F., Van der Merwe, S., ... & Malfertheiner, P. (2011). Helicobacter pylori in developing countries. World gastroenterology organisation global guideline. *J Gastrointestin Liver Dis*, *20*(3), 299-304.
36. Fathi, M. S., El-Folly, R. F., Hassan, R. A., & El-Arab, M. E. (2013). Genotypic and phenotypic patterns of antimicrobial susceptibility of Helicobacter pylori strains among Egyptian patients. *Egyptian Journal of Medical Human Genetics*, *14*(3), 235-246.
37. Secka, O., Berg, D. E., Antonio, M., Corrah, T., Tapgun, M., Walton, R., ... & Thomas, J. E. (2013). Antimicrobial susceptibility and resistance patterns among Helicobacter pylori strains from The Gambia, West Africa. *Antimicrobial agents and chemotherapy*, *57*(3), 1231-1237.
38. Tanih, N. F., Ndip, L. M., & Ndip, R. N. (2011). Characterisation of the genes encoding resistance to metronidazole (rdxA and frxA) and clarithromycin (the 23S-rRNA genes) in South African isolates of Helicobacter pylori. *Annals of Tropical Medicine & Parasitology*, *105*(3), 251-259.
39. Butlop, T., Mungkote, N., & Chaichanawongsaroj, N. (2016). Analysis of allelic variants of rdxA associated with metronidazole resistance in Helicobacter pylori: detection of common genotypes in rdxA by multiplex allele-specific polymerase chain reaction. *Genet Mol Res*, *15*(3).
40. Kwon, D. H., El-Zaatari, F. A., Kato, M., Osato, M. S., Reddy, R., Yamaoka, Y., & Graham, D. Y. (2000). Analysis of rdxA and involvement of additional genes encoding NAD (P) H flavin oxidoreductase (FrxA) and ferredoxin-like protein (FdxB) in metronidazole resistance of Helicobacter pylori. *Antimicrobial agents and chemotherapy*, *44*(8), 2133-2142.
41. Marais, A., Bilardi, C., Cantet, F., Mendz, G. L., & Mégraud, F. (2003). Characterization of the genes rdxA and frxA involved in metronidazole resistance in

Helicobacter pylori. *Research in microbiology*, *154*(2), 137-144.

42. Grignon, B., Tankovic, J., Megraud, F., Glupczynski, Y., Husson, M. O., Conroy, M. C., ... & Fauchere, J. L. (2002). Validation of diffusion methods for macrolide susceptibility testing of Helicobacter pylori. *Microbial Drug Resistance*, *8*(1), 61-66.
43. Hartzen, S. H., Andersen, L. P., Bremmelgaard, A., Colding, H., Arpi, M., Kristiansen, J., ... & Bonnevie, O. (1997). Antimicrobial susceptibility testing of 230 Helicobacter pylori strains: importance of medium, inoculum, and incubation time. *Antimicrobial agents and chemotherapy*, *41*(12), 2634-2639.
44. Henriksen, T. H., Lia, A., Schøyen, R., Thoresen, T., & Berstad, A. (2000). Assessment of optimal atmospheric conditions for growth of Helicobacter pylori. *European Journal of Clinical Microbiology and Infectious Diseases*, *19*(9), 718-720.
45. National Committee for Clinical Laboratory Standards. (2000). *Performance Standards for Antimicrobial Susceptibility Testing and Approved Standard M7-A5. Informational Supplement M100-S10*. Wayne, PA: National Committee for Clinical Laboratory Standards.
46. Hachem, C. Y., Clarridge, J. E., Reddy, R., Flamm, R., Evans, D. G., Tanaka, S. K., & Graham, D. Y. (1996). Antimicrobial susceptibility testing of Helicobacter pylori comparison of E-test, broth microdilution, and disk diffusion for ampicillin, clarithromycin, and metronidazole. *Diagnostic microbiology and infectious disease*, *24*(1), 37-41.
47. Destura, R. V., Labio, E. D., Barrett, L. J., Alcantara, C. S., Gloria, V. I., Daez, M. L. O., & Guerrant, R. L. (2004). Laboratory diagnosis and susceptibility profile of Helicobacter pylori infection in the Philippines. *Annals of clinical microbiology and antimicrobials*, *3*(1), 25.
48. Glupczynski, Y., Broutet, N., Cantagrel, A., Andersen, L., Alarcon, T., Lopez-Brea, M., & Megraud, F. (2002). Comparison of the E test and agar dilution method for antimicrobial suceptibility testing of Helicobacter pylori. *European Journal of Clinical Microbiology and Infectious Diseases*, *21*(7), 549-552.
49. Yilmaz, Ö., & Demiray, E. (2007). Clinical role and importance of fluorescence in situ hybridization method in diagnosis of H pylori infection and determination of clarithromycin resistance in H pylori eradication therapy. *World journal of gastroenterology: WJG*, *13*(5), 671.
50. Burucoa, C., Garnier, M., Silvain, C., & Fauchère, J. L. (2008). Quadruplex real-time PCR assay using allele-specific scorpion primers for detection of mutations conferring clarithromycin resistance to Helicobacter pylori. *Journal of clinical microbiology*, *46*(7), 2320-2326.
51. van Doorn, L. J., Glupczynski, Y., Kusters, J. G., Mégraud, F., Midolo, P., Maggi-Solcà, N., ... & Quint, W. G. (2001). Accurate prediction of macrolide resistance in Helicobacter pylori by a PCR line probe assay for detection of mutations in the 23S rRNA gene: multicenter validation study. *Antimicrobial agents and chemotherapy*, *45*(5), 1500-1504.
52. Schabereiter-Gurtner, C., Hirschl, A. M., Dragosics, B., Hufnagl, P., Puz, S., Kovách, Z., ... & Makristathis, A. (2004). Novel real-time PCR assay for detection of Helicobacter pylori infection and simultaneous clarithromycin susceptibility testing of stool and biopsy specimens. *Journal of clinical microbiology*, *42*(10), 4512-4518.
53. Mitui, M., Patel, A., Leos, N. K., Doern, C. D., & Park, J. Y. (2014). Novel Helicobacter pylori sequencing test identifies high rate of clarithromycin resistance. *Journal of pediatric gastroenterology and nutrition*, *59*(1), 6-9.
54. Mégraud, F., & Lehours, P. (2007). Helicobacter pylori detection and antimicrobial susceptibility testing. *Clinical microbiology reviews*, *20*(2), 280-322.
55. Rüssmann, 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. *Journal of clinical microbiology*, *39*(11), 4142-4144.
56. Perry-O'Keefe, H., Stender, H., Broomer, A., Oliveira, K., Coull, J., & Hyldig-Nielsen, J. J. (2001). Filter-based PNA in situ hybridization for rapid detection, identification and enumeration of specific micro-organisms. *Journal of applied microbiology*, *90*(2), 180-189.
57. Stender, H., Fiandaca, M., Hyldig-Nielsen, J. J., & Coull, J. (2002). PNA for rapid microbiology. *Journal of Microbiological Methods*, *48*(1), 1-17.
58. Cerqueira, L., Azevedo, N., Almeida, C., Jardim, T., Keevil, C., & Vieira, M. (2008). DNA mimics for the rapid identification of microorganisms by fluorescence in situ hybridization (FISH). *International Journal of Molecular Sciences*, *9*(10), 1944-1960.