COMPARISON AMONG DIFFERENT LABORATORY TESTS FOR THE DIAGNOSIS OF *Helicobacter pylori* INFECTION IN DYSPEPTIC PATIENTS IN SULAIMANI GOVERNORATE

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ABSTRACT

Background

Helicobacter pylori is a helical Gram-negative bacterium that colonizes human gastric mucosa leading to various pathologies including gastritis, gastric ulcers and gastric adenocarcinoma. Colonization is worldwide and affecting all age groups.

Objectives

To identify *H. pylori* colonization among patients with dyspepsia using different laboratory tests including serology, stool antigen and PCR, and comparing the sensitivity and specificity of these tests.

Materials and Methods

From June through December 2014, one hundred patients with dyspepsia were enrolled in this study. They were 64 females (mean age 40.2 y) and 36 males (mean age 42.1 y). Two gastric antral biopsy specimens were taken in all patients submitted to esophagogastroduodenoscopy. Blood and stool specimens were also obtained from each patient. Four diagnostic laboratory tests were used for detection of the infection. Blood samples were tested for antibodies using rapid immunochromatography assay, stool for *H. pylori* antigen with immunochromatographic method and lastly PCR amplification of *glmM* gene was used to detect the organism in both gastric tissue and stool samples.

Results

Esophagogastroduodenoscopy revealed that the majority of patients had gastritis and or duodenitis. Stool PCR for *H. pylori* was positive in 42% of the patients. Anti-*H. pylori* IgG in the blood and bacterial antigen were detected in 72% and 74% of patients respectively, while PCR test on gastric biopsy specimens was positive in 82% of the samples.

Conclusion

H. pylori infection was detected regardless of the presence or absence of visible gastroduodenal pathology. PCR examination of gastric tissue was better than stool samples. In comparison to gastric biopsy PCR, stool antigen detection was found to be the most sensitive test reaching 87.8% while stool PCR was the most specific test. No single test solely can be used to diagnose or exclude colonization.

Keywords: *Diagnosis of H. pylori, Dyspepsia, Stool antigen, Antral biopsy, PCR* .

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INTRODUCTION

Helicobacter pylori is a helix-shaped gram-negative bacterium about 3 μm long with a diameter of about 0.5 μm. It is a microaerophile that requires oxygen but at a lower concentration than found in the atmosphere [1]. *H. pylori* has a unipolar bundle of two to six sheathed flagella that enable the bacterium to move in the mucous layer of the gastric epithelium [2]. Person-to-person spread of *H. pylori* is by faecal-oral or oral-oral transmissions [3]; fecal-oral transmission is based on the studies identifying and isolating *H. pylori* in stools as well as identifying poor hygiene as a risk factor for infection [4, 5].

Helicobacter pylori expresses its pathogenicity by penetrating and colonizing the mucous layer, adhering to gastric epithelium, increasing the permeability to hydrogen ions and pepsin, penetrating in and destructing of intercellular junctions, invading gastric glands and canaliculi of parietal cells, and secreting different enzymes and cytotoxins [6]. Helicobacter flagella are essential in starting colonization process; they contain two types of flagellin proteins coded by *flaA* and *flaB* genes that are required for full motility and persistent infection of the gastric mucosa [7]. *H. pylori* adheres to receptors in the gastric epithelium by means of adhesions; several specific receptors are involved including lipids, gangliosides and sulfated carbohydrates [8]. The adherence of the bacterium to human gastric epithelial lining through helicobacter *BabA* protein is mediated by the fucosylated Lewis b (Leb) histo-blood group antigen [9].

Urease enzyme produced by *H. pylori* is critical for colonizing human gastric mucosa. Ammonia formed from metabolized urea neutralizes the microenvironment in which the bacterium resides [10]. Ammonia can also damage the gastric mucosa by disrupting the tight junctions and altering ion permeability of gastric epithelium. Moreover, urease stimulates activation of mononuclear phagocytes and production of inflammatory cytokines [11]. Among cytotoxins described for *H. pylori* is VacA (vacuolating cytotoxin A) [12]. All strains of *H. pylori* possess *vacA* gene and about 50-60% of them express a fully virulent VacA cytotoxin capable to induce acidic vacuoles in the cytoplasm of eukaryotic cells [13]. Antibodies against VacA neutralizes the cytotoxic activity [12]. The strains of *H. pylori* containing CagPAI (Cytotoxin-associated-gene Pathogenicity Island) is associated with the development of chronic active gastritis, peptic ulceration and atrophic gastritis with an increased risk of gastric cancer [14]. CagPAI is also associated with the induction of IL-8, IL-6, TNF-α and IL-1β, subsequently leading to the recruitment and activation of neutrophils and mononuclear cells [15]. Urease is also shown to be highly effective in inducing macrophages to produce reactive oxygen intermediates and pro inflammatory cytokines IL-1β, IL-6, IL-8 and TNF-α [16]. Inflammatory response to *H. pylori* infection includes mucosal infiltration by neutrophils, T cell and B cells, plasma cells and macrophages. This reaction is probably induced by *H. pylori* attachment and stimulation of cytokine release in the gastric mucosa which induce changes in gastric acid secretion and the epithelial homeostasis [17, 18]. *H. pylori* induces a predominantly Th1 lymphocyte response in humans [19]. Colonization by *H. pylori* can lead to a variety of disorders such as gastritis, peptic ulcer disease, gastric mucosa-associated lymphoid tissue lymphoma, and colonization is considered as a risk factor in the development of gastric cancer [20].

The prevalence of *H. pylori* infection is 70-90% in developing countries and 25%-50% in developed countries [21, 22]. Within geographical areas, the prevalence of *H. pylori* inversely correlates with socioeconomic status and particularly to living conditions during childhood [23]. The prevalence of *H. pylori* infection among subjects in Iraq is 68.1%; *H. pylori* antibodies were detected in 79.4% of males and 57.3% of females [24]. Another study showed that 81% of the patients with gastritis were positive for serum anti- *H. pylori* IgG [25] while among dyspeptic patients, 46.01% were positive for *H. pylori* by PCR of gastric biopsy [26].

There are various invasive and non-invasive tests for the diagnosis of *H. pylori* infection [27]. In invasive tests, gastric tissue samples obtained by endoscopy are used for culture, rapid urease test, histopathological direct Gram staining or PCR. Noninvasive tests include serology, urea breath test, stool antigen test (HpSA) and stool PCR [28, 29].

Endoscopic findings of *H. pylori*-infected stomach include erythema, erosions, antral nodularity, thickened gastric folds and visible submucosal vessels. However, these findings are not a reliable method of diagnosis because of their low sensitivity and specificity [30].

The immune response against *H. pylori* typically shows a transient rise in specific IgM antibodies,

followed by a rise in IgG and IgA antibodies that persist during infection. Since IgM antibodies against *H. pylori* are detected only transiently, they have little value for the serological diagnosis of infection [31]. Diagnostic tests have been developed for the detection of *H. pylori*-specific IgG and IgA in serum, saliva or urine. Immunochromatographic assay (ICA) detection of anti-*H. pylori* antibody is based on the principle of reverse-flow immunochromatography. The sensitivity and specificity of this test for detection of anti *H. pylori* IgG in the serum are 92.4% and 83.0%, respectively, with a positive predictive value of 88.4% [32]. *H. pylori* stool antigen detection using monoclonal antibodies is a valuable noninvasive alternative when urea breath test is not available. Meta-analysis of 22 studies including 2499 patients has evaluated the monoclonal SAT before eradication therapy and showed sensitivity and specificity of 94% and 97%, respectively [33].

Polymerase chain reaction (PCR)-based techniques have been used for the detection of *H. pylori* from gastric biopsy samples, gastric juice, stool and saliva [22, 34] . The sensitivity and specificity for *H. pylori* infection using PCR-based techniques have been reported at 85% to 100% and 100% respectively when compared to other endoscopy-based tests [22, 35]. PCR-based techniques are alternatives when specimens for culture are compromised by bacterial overgrowth, or nonviable *H. pylori* strains due to unfavorable transport conditions [36]. However, PCR-based techniques have some limitations; the possible existence of Taq polymerase inhibitors can decrease the sensitivity of the reaction and the possible contamination of the specimen by exogenous *H. pylori* DNA may alters the specificity [37].

The aim of this study was to detect *H. pylori* colonization in patients with dyspepsia using different tests such as PCR, serology and stool antigen and comparing their sensitivity, specificity, positive and negative predictive values.

MATERIALS AND METHODS

This is A cross-sectional observational single center study. Laboratory specimens were collected from dyspepsic patients attending the Kurdistan Center for Gastroenterology and Hepatology (KCGH) in Sulaimani for the purpose of esophagogastroduodenoscopy (EGD). Ethical approval for the study was obtained from The Ethics Committee of the Faculty of Medical Science/School of Medicine at University of Sulaimani. Informed consent was obtained from each patient agreed to participate in the study before the samples were taken.

One hundred patients were included in the study in a period from June to December 2014. From each patient a fresh stool sample, blood sample and endoscopic gastric antral tissue biopsies were taken. The inclusion criteria were patients with symptoms of dyspepsia and no history of receiving antibiotics, Proton Pump Inhibitors (PPIs) or histamine-2 receptor blockers at least one month before taking the samples.

Collected stool samples were tested for *H. pylori* antigen and stool bacterial DNA extraction. Fifty grams of stool was mixed with 700 µL absolute ethanol in a microcentrifuge tube and stored in an ice-box until DNA extraction within 4 hours. Two milliliters of venous blood taken from each patient was put into a sterile plain test tube. The serum was separated and stored at 2-8°C until ICA was done. Through using an Olympus CV-260 endoscope, endoscopic findings were recorded and two gastric antral biopsy specimens were obtained and put in a sterile container containing 1 mL of normal saline solution. The tissue samples were stored at 2-8°C until DNA extracted within 4 hr. A rapid serology test to detect serum anti *H. pylori* IgG (R0191C -BIOTECH, USA) and *H. pylori* stool antigen detection (CerTest ™ kit, Spain) were carried out according to the manufacturer's instructions.

Genomic DNA mini kit (GT300 Geneaid, Taiwan) and AccuPrep® stool DNA extraction K-3036 kit (BIONEER, South Korea) were used to extract genomic DNA from both gastric biopsies and stool samples respectively and according to manufacturer's recommendations. Extracted DNA was quantitated with Genova 3 in 1 Spectrophometer then stored at -60ºC until PCR was performed. For PCR, primers targeting *glmM* gene (F: 5′-GGATAAGCTTTTAGGGGTGTTAGGGG-3′ and R:5′GCTTACTTTCTAACACTAACGCGC-3′) were used to yield a 294 bp fragment [38]. The same primers were used on tissue and stool samples.

We used PCR kit Prime Taq Premix 2X (GENETBIO, Korea) where primers and the reaction mixture were cycled using a thermal cycler (TECHNE 3 Prime, UK) for an initial denaturation of 5 min at 94ºC, then 35 cycles each for 30 sec for denaturation at 94°C, annealing at 64ºC and extension at 72ºC, then a final extension at 72ºC for 5 min. PCR products were resolved with 1.5% agarose gel which was electrophoresed at 85 Volts for 1 hr where ethidium bromide stained DNA band size was

evaluated under UV light of (MultiDoc-It TM Digital Imaging System) using DNA ladder (GENETBIO, Korea).

Sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) were analyzed. The criterion for a true *H. pylori* colonization or infection was considered to as having a positive result of gastric biopsy PCR [39, 40].

RESULTS

The study included one hundred patients complaining of dyspepsia referred to undergo upper endoscopic evaluation at KCGH. Table 1 summarizes the age and symptoms of the patients with their EGD findings. Gastritis alone was the most common finding and was observed in 40 patients (40%), followed by gastritis and duodenitis in 18 patients (18%) and lastly duodenitis in 14 patients (14%). Normal mucosa was found in 6 patients (6%).

Table 1. Age, symptoms, EGD diagnosis in relation to the sex of the participants.

Parameter	Male n=36	Female n=64	Total
Mean age in years (SD)	42.1 (15.85)	40.2 (13.62)	
Age range in years	17-75	15-70	
Symptoms			
Epigastric pain	36	64	100
Vomiting	0	0	0
Yes	12	32	44
No	24	32	56
Bleeding	0	0	0
EGD diagnosis			
Gastritis	18	22	40
Gastritis and Duodenitis	4	14	18
Duodenitis	6	8	14
Gastric ulcer	2	8	10
Duodenal ulcer	2	6	8
Gastric cancer	0	4	4
Normal mucosa	4	2	6

Anti-*H. pylori* IgG was found in serum of 72 patients (72%) while *H. pylori* antigen was detected in 74 (74%) stool samples. The results of these tests and PCR tests in relation to EGD diagnosis are shown in Table 2. Gastric biopsy PCR was found to have the highest diagnostic yield (82%) with predominance of females (60.98%) when compared with infected males (39.02%). PCR amplification of *glmM* gene fragment of *H. pylori* from stool extracted DNA showed 294-bp band in 42 samples (42%) while PCR was positive in 82 (82%) DNA samples extract from gastric biopsy showing the same band size of *glmM* gene fragment (Table 2; Figures 1 and 2).

Gastric biopsy PCR was able to detect the highest number of infections (82%) in comparison with others tests. Infection was detected among 32 males (88.8%) and 50 (78.1%) females, the correlation of sex to infection was not statistically significant using chi square test ($p = 0.17868$), table 3.

Table 4 shows that the most sensitive test to detect the infection was stool antigen test (87.8%) while stool PCR test specificity was almost 100%. The diagnostic accuracy of each test was also calculated showing the predominance of stool antigen test again (82%) in comparison to other tests. The statistical difference between the male and female results of the tests using chi square test were all non-significant (p value > 0.05) except for serology where p value was 0.006.

Table 2. Detection of *Helicobacter pylori* by four tests in relation EGD diagnosis among hundred patients with dyspepsia.

Diagnostic test		Gastritis n=40, N (%)	Gastritis and duodenitis n=18 N (%)	Duodenitis n=14 N (%)	Gastric ulcer n=10 N (%)	Duodenal ulcer n=8 N (%)	Gastric cancer n=4 N (%)	Normal mucosa n=6 N (%)
Anti-H. pylori IgG	**Positive**	30 (75)	13 (72.2)	8 (57)	8 (80)	7 (87.5)	2 (50)	4 (66.6)
	Negative	10 (25)	5 (27.8)	6 (43)	2 (20)	1 (12.5)	2 (50)	2 (33.4)
Stool antigen	**Positive**	32 (80)	16 (88.8)	10 (71.4)	6 (60)	6 (75)	2 (50)	2 (33.3)
	Negative	8 (20)	2 (11.2)	4 (28.6)	4 (40)	2 (25)	2 (50)	4 (66.6)
Stool PCR	**Positive**	18 (45)	8 (44.4)	6 (42.8)	4 (40)	4 (50)	1 (25)	1 (16.6)
	Negative	22 (55)	10 (55.6)	8 (57.2)	6 (60)	4 (50)	3 (75)	5 (83.4)
Gastric Biopsy PCR	**Positive**	36 (90)	14 (77.7)	12 (85.7)	8 (80)	6 (75)	2 (50)	4 (66.6)
	Negative	4 (10)	4 (22.2)	2 (13.3)	2 (20)	2 (25)	2 (50)	2 (33.3)

Table 3. The number and percentage of *Helicobacter pylori* positive PCR of gastric biopsy in relation to the sex of the dyspepsic patients.

Sex	Positive No. (%)	Negative No. (%)	Total No. (%)
Males	32 (88.8)	4 (11.2)	36
Females	50 (78.1)	14 (21.9)	64
Total	82 (82)	18 (18)	100

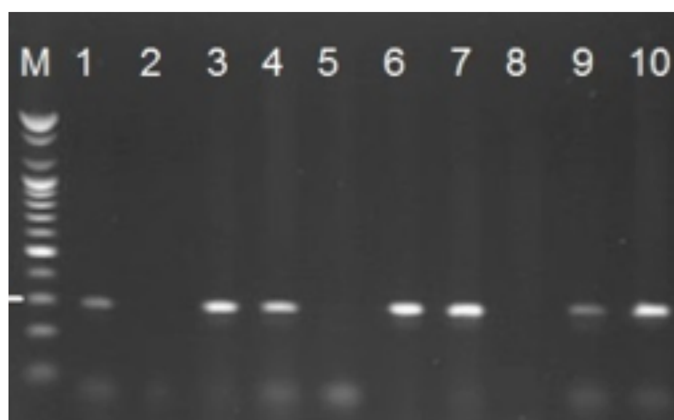

Figure 1. Agarose gel electrophoresis (1.5 %) of PCR products showing amplification of 294-bp DNA fragment of *glmM* extracted from *H. pylori* in stool. M 100 bp DNA marker ladder; lane 1 positive control; lane 2 negative control; lanes 3, 4, 6, 7, 9, 10 and 11 positive results; lanes 5 and 8 negative results.

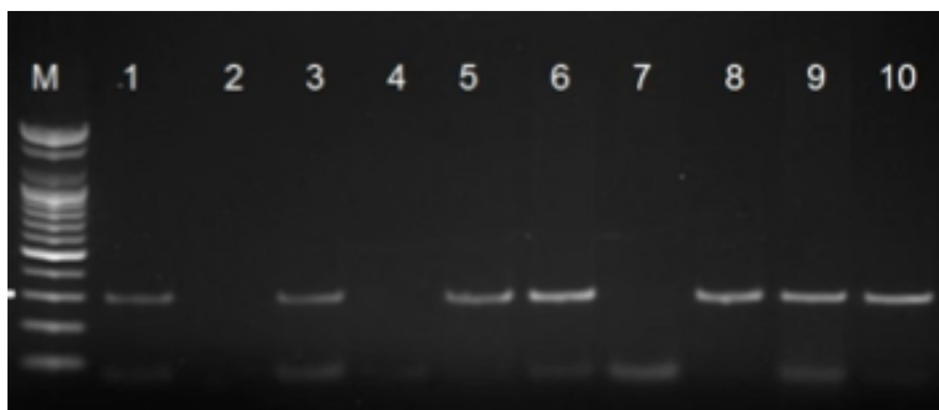

Figure 4 . Agarose gel electrophoresis (1.5%) of PCR products of DNA extracted from gastric biopsy showing amplification of 294-bp fragment of *glmM* gene of *H. pylori*. M, 100 bp DNA marker ladder; lane 1 positive control; lane 2 negative control; lanes 3, 5, 6, 8, 9 and 10 positive results; lanes 4 and 7 negative results.

Table 4. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of each test.

Test	True positive	True negative	False positive	False negative	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Anti-H. pylori IgG	66	12	6	16	80.4	66.6	91.6	42.8	78
Stool antigen	70	14	4	12	85.3	77.7	94.5	53.8	82
Stool PCR	42	18	0	40	51.2	100	100	31	81

DISCUSSION

Since identifying the role of *H. pylori* in the pathogenesis of upper gastrointestinal disorders, many procedures and laboratory tests are used to identify the infection [41]. In this study we used four laboratory tests to identify *H. pylori* infection in a group of patients suffering from dyspepsia who have been referred to undergo upper endoscopy. Finding anti *H. pylori* antibodies is useful for screening purposes [42] but has limitations because it cannot confirm true infection and the antibody titer gradually decreases following antimicrobial treatment [43]. Immunological methods are also used to detect *H. pylori* antigens as they pass in the stool [5]. These tests are easy and convenient for the patients and can be used also as screening tools [44]. Nucleic acid amplification by PCR can be used on gastric tissue or stool samples to identify several *H. pylori* genes such as *glmM* gene or other virulence genes [5, 45].

All participants in this study were complaining of dyspepsia. Despite of a larger number of dyspeptic patients referred to undergo upper endoscopy, only 100 of them agreed to enter this study and a fresh stool and blood samples were taken from each case.

Although a local study has found a higher prevalence of dyspepsia among male patients [25], in our study females were more and constituting 64% of the participants predominantly in fourth decades of their life (age range 15 to 75 years).

Gastric biopsy PCR was positive in 88.8% of males and 78.1% of females with no statistical difference ($p > 0.05$) which is agreed with results obtained previously

[46, 47]. Patients of all ages gave positive test in the range of 75% in those above 60 years to 87% in 30-39 year age group indicating that *H. pylori* infection affects all ages. Previous studies have shown variable effects of gender on *H. pylori* infection. Some studies have shown that the prevalence is independent on sex of the infected patients [48]. There are reports of a higher prevalence in both females [49] and in males [50].

The main pathological finding reported during EGD was gastritis and/or doudenitis. Other endoscopic findings such as ulcers and cancers were also reported in addition to normal endoscopic findings. Studies showed that *H. pylori* has an etiological role in gastritis as well as peptic ulcer disease and associated with duodenal ulcer relapse [51] and there are an increasing evidences supporting the role of *H. pylori* infection in the development of gastric cancer [52].

Using rapid ICA 72% of the patients were found to have anti *H. pylori* antibodies. ICA had a high sensitivity and specificity for the detection of *H. pylori* IgG antibody in banked sera. The results of the current study were compatible with observations recorded by Cognein *et al.* [53]. False positive result occurs due to past infection; and the test may remain positive for over 6 months from clearance of the disease [54] but in our study this possibility has been ruled out during the history taking before admission of each patient.

H. pylori stool antigen (HPSA) was detected in 74% of the patients. This finding was higher than that observed by Baqai *et al.* and Li *et al.* in which they detected *H. pylori* stool antigen in 48% and 50% of their patients respectively [55, 56]. This may be explained by that HPSA test detects active infection and many of our patients were symptomatic. As a noninvasive test, the present study has shown that HPSA test has a promising result and can be used for screening purpose.

PCR of gastric biopsy produced most positive results (82%). Similar results ranging between 74.7 to 81.5 % were reported in Iraqi patients has been found by Al-Yas in 2006 (81.5%), while lower results were reported by others 65.7% [57, 58] and 74.7% [46]. Various factors have been attributed to yield different results. These include prevalence of the infection, gastric sampling location, specimen preparation, the choice of primers and target DNA, bacterial density and technical errors during DNA extraction [21]. The *glmM* gene (also called *ureC*) has been extensively used for confirming the presence of *H. pylori* and is the most sensitive and specific way for the detection of *H. pylori* in gastric biopsy [59].

In this study, gastric biopsy PCR assay was a sensitive method for the diagnosis of gastric infection by *H. pylori* and can be useful during and after eradication treatment. PCR of stool samples with the same primers produced the least positive results (42%) among the same patients; reasons for low rate recovery of *H. pylori* may be low bacterial numbers in the stool, low yield of the DNA extract compared to DNA extract from gastric biopsy, *H. pylori* degradation, and presence of inhibitors such as complex polysaccharides [22].

Cultivation of biopsy samples is regarded as the golden method for diagnosis [60] but it needs sophisticated incubation conditions and time. Due to lack of culture techniques in this study, the results of gastric biopsy PCR was used as a predictor for true infection with *H. pylori* and according to PCR 82% positive biopsies for *H. pylori* was found among the enrolled patients.

In our study we used the results of gastric biopsy PCR as a true positive result to compare with other tests; HpSA was the most sensitive method reaching 87.8%. Therefore, stool test may be used as an alternative to diagnose the infection in adult outpatients and to monitor the success of eradication treatment. The stool PCR produced a value of 100% specificity while its sensitivity was 51.2%, a slightly higher figure (62.5%) has been reported previously [28]. Stool PCR test is a valuable method in the pre-eradication assessment of infection and it can be used in epidemiological studies to determine the prevalence of *H. pylori* infection in symptomatic and asymptomatic subjects.

Diagnostic tests such as PCR, HpSA, ICA and stool PCR were independently sufficient in making an etiological diagnosis of *H. pylori*. However, more than one method may be required for the definitive diagnosis of *H. pylori* infection. Our findings and many previous studies recommend multiple diagnostic tests for accurate diagnose *H. pylori* infection [25, 61].

In conclusion, *H. pylori* colonization was detected regardless of the presence or absence of visible gastroduodenal pathology. Gastric biopsy PCR was better than stool PCR. Stool antigen detection test was the most sensitive method while stool PCR test was the most specific one. More than one test is needed to indicate or exclude this bacterial colonization.

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REFERENCES

1. Andersen LP, Wadström T. Basic Bacteriology and Culture. In: Mobley HLT, Mendz GL, Hazell SL, editors. Helicobacter pylori: Physiology and Genetics. Washington (DC): ASM Press; 2001. Chapter 4. Available from: https://www.ncbi.nlm.nih.gov/books/NBK2444/.

2. Suerbaum S. The complex flagella of gastric Helicobacter species. Trends in microbiology. 1995;3(5):168-70; discussion 70-1.

3. Allaker RP, Young KA, Hardie JM, Domizio P, Meadows NJ. Prevalence of helicobacter pylori at oral and gastrointestinal sites in children: evidence for possible oral-to-oral transmission. Journal of medical microbiology. 2002;51(4):312-7.

4. Peach HG, Pearce DC, Farish SJ. Helicobacter pylori infection in an Australian regional city: prevalence and risk factors. The Medical journal of Australia. 1997;167(6):310-3.

5. Makristathis A, Pasching E, Schutze K, Wimmer M, Rotter ML, Hirschl AM. Detection of Helicobacter pylori in stool specimens by PCR and antigen enzyme immunoassay. Journal of clinical microbiology. 1998;36(9):2772-4.

6. Covacci A, Telford JL, Del Giudice G, Parsonnet J, Rappuoli R. Helicobacter pylori virulence and genetic geography. Science. 1999;284(5418):1328-33.

7. Schmitz A, Josenhans C, Suerbaum S. Cloning and characterization of the Helicobacter pylori flbA gene, which codes for a membrane protein involved in coordinated expression of flagellar genes. Journal of bacteriology. 1997;179(4):987-97.

8. Simon PM, Goode PL, Mobasseri A, Zopf D. Inhibition of Helicobacter pylori binding to gastrointestinal epithelial cells by sialic acid-containing oligosaccharides. Infection and immunity. 1997;65(2):750-7.

9. Ilver D, Arnqvist A, Ogren J, Frick IM, Kersulyte D, Incecik ET, et al. Helicobacter pylori adhesin binding fucosylated histo-blood group antigens revealed by retagging. Science. 1998;279(5349):373-7.

10. Bode G, Mauch F, Malfertheiner P. The coccoid forms of Helicobacter pylori. Criteria for their viability. Epidemiology and infection. 1993;111(3):483-90.

11. Harris PR, Mobley HL, Perez-Perez GI, Blaser MJ, Smith PD. Helicobacter pylori urease is a potent stimulus of mononuclear phagocyte activation and inflammatory cytokine production. Gastroenterology. 1996;111(2):419-25.

12. Cover TL, Blaser MJ. Purification and characterization of the vacuolating toxin from Helicobacter pylori. The Journal of biological chemistry. 1992;267(15):10570-5.

13. Ghiara P, Marchetti M, Blaser MJ, Tummuru MK, Cover TL, Segal ED, et al. Role of the Helicobacter pylori virulence factors vacuolating cytotoxin, CagA, and urease in a mouse model of disease. Infection and immunity. 1995;63(10):4154-60.

14. Walker MM, Crabtree JE. Helicobacter pylori infection and the pathogenesis of duodenal ulceration. Annals of the New York Academy of Sciences. 1998;859:96-111.

15. Crabtree JE, Kersulyte D, Li SD, Lindley IJ, Berg DE. Modulation of Helicobacter pylori induced interleukin-8 synthesis in gastric epithelial cells mediated by cag PAI encoded VirD4 homologue. Journal of clinical pathology. 1999;52(9):653-7.

16. Salzman A, Denenberg AG, Ueta I, O'Connor M, Linn SC, Szabo C. Induction and activity of nitric oxide synthase in cultured human intestinal epithelial monolayers. The American journal of physiology. 1996;270(4 Pt 1):G565-73.

17. Andrutis KA, Fox JG, Schauer DB, Marini RP, Murphy JC, Yan L, et al. Inability of an isogenic urease-negative mutant stain of Helicobacter mustelae to colonize the ferret stomach. Infection and immunity. 1995;63(9):3722-5.

18. Eaton KA, Krakowka S. Effect of gastric pH on urease-dependent colonization of gnotobiotic piglets by Helicobacter pylori. Infection and immunity. 1994;62(9):3604-7.

19. Harris PR, Smythies LE, Smith PD, Dubois A. Inflammatory cytokine mRNA expression during early and persistent Helicobacter pylori infection in nonhuman primates. The Journal of infectious diseases. 2000;181(2):783-6.

20. Blaser MJ, Berg DE. Helicobacter pylori genetic diversity and risk of human disease. The Journal of clinical investigation. 2001;107(7):767-73.

21. Dunn BE, Cohen H, Blaser MJ. Helicobacter pylori. Clinical microbiology reviews. 1997;10(4):720-41.

22. Kabir S. Detection of Helicobacter pylori in faeces by culture, PCR and enzyme immunoassay. Journal of medical microbiology. 2001;50(12):1021-9.

23. Malaty HM, Graham DY. Importance of childhood socioeconomic status on the current prevalence of Helicobacter pylori infection. Gut. 1994;35(6):742-5.

24. Ibrahim HAE, Al-Dhahir HAR, Mohammed MO. Effect of age, gender, blood group and social state on the sero-prevalence of Helicobacter pylori Infection among asymptomatic subjects in Sulaimani. Journal of Sulaimani Medical College. 2014;5(1).

25. Alsaimary I, Al-Sadoon M, Jassim A, Hamadi S. Clinical findings and Prevalence of Helicobacter pylori in Patients with Gastritis B in Al-basrah governorate. Oman medical journal. 2009;24(3):208-11.

26. Kalaf EA, Yassen NY, AL-Khafaji ZM, A'Rif GL, Abass BA. Multiplex PCR assay for detection of Helicobacter pylori isolated from Iraqi dyspeptic patients. Iraqi Journal of Cancer and Medical Genetics. 2013;6(1):49-54.

27. Megraud F, Lehours P. Helicobacter pylori detection and antimicrobial susceptibility testing. Clinical microbiology reviews. 2007;20(2):280-322.

28. Falsafi T, Favaedi R, Mahjoub F, Najafi M. Application of stool-PCR test for diagnosis of Helicobacter pylori infection in children. World journal of gastroenterology : WJG. 2009;15(4):484-8.

29. Megraud F, Broutet N. Review article: have we found the source of Helicobacter pylori? Alimentary pharmacology & therapeutics. 2000;14 Suppl 3:7-12.

30. Redeen S, Petersson F, Jonsson KA, Borch K. Relationship of gastroscopic features to histological findings in gastritis and Helicobacter pylori infection in a general population sample. Endoscopy. 2003;35(11):946-50.

31. Herbrink P, van Doorn LJ. Serological methods for diagnosis of Helicobacter pylori infection and monitoring of eradication therapy. European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology. 2000;19(3):164-73.

32. Anderson JC, Cheng E, Roeske M, Marchildon P, Peacock J, Shaw RD. Detection of serum antibodies to Helicobacter pylori by an immunochromatographic method. The American journal of gastroenterology. 1997;92(7):1135-9.

33. Gisbert JP, de la Morena F, Abraira V. Accuracy of monoclonal stool antigen test for the diagnosis of H. pylori infection: a systematic review and meta-analysis. The American journal of gastroenterology. 2006;101(8):1921-30.

34. Simala-Grant JL, Taylor DE. Molecular biology methods for the characterization of Helicobacter pylori infections and their diagnosis. APMIS : acta pathologica, microbiologica, et immunologica Scandinavica. 2004;112(11-12):886-97.

35. Kisa O, Albay A, Mas MR, Celasun B, Doganci L. The evaluation of diagnostic methods for the detection of Helicobacter pylori in gastric biopsy specimens. Diagnostic microbiology and infectious disease. 2002;43(4):251-5.

36. Chisholm SA, Owen RJ. Application of polymerase chain reaction-based assays for rapid identification and antibiotic resistance screening of Helicobacter pylori in gastric biopsies. Diagnostic microbiology and infectious disease. 2008;61(1):67-71.

37. Thoreson AC, Borre M, Andersen LP, Jorgensen F, Kiilerich S, Scheibel J, et al. Helicobacter pylori detection in human biopsies: a competitive PCR assay with internal control reveals false results. FEMS immunology and medical microbiology. 1999;24(2):201-8.

38. Kansau I, Raymond J, Bingen E, Courcoux P, Kalach N, Bergeret M, et al. Genotyping of Helicobacter pylori isolates by sequencing of PCR products and comparison with the RAPD technique. Research in microbiology. 1996;147(8):661-9.

39. Pajares-Garcia JM. Diagnosis of Helicobacter pylori: invasive methods. Italian journal of gastroenterology and hepatology. 1998;30 Suppl 3:S320-3.

40. Liao CC, Lee CL, Lai YC, Huang SH, Lee SC, Wu CH, et al. Accuracy of three diagnostic tests used alone and in combination for detecting Helicobacter pylori infection in patients with bleeding gastric ulcers. Chinese medical journal. 2003;116(12):1821-6.

41. Hino B, Eliakim R, Levine A, Sprecher H, Berkowitz D, Hartman C, et al. Comparison of invasive and non-invasive tests diagnosis and monitoring of Helicobacter pylori infection in children. Journal of pediatric gastroenterology and nutrition. 2004;39(5):519-23.

42. McNulty CA, Nair P, Watson BE, Uff JS, Valori RM. A comparison of six commercial kits for Helicobacter pylori detection. Communicable disease and public health / PHLS. 1999;2(1):59-63.

43. Feldman M, Cryer B, Lee E, Peterson WL. Role of seroconversion in confirming cure of Helicobacter pylori infection. Jama. 1998;280(4):363-5.

44. Andrews J, Marsden B, Brown D, Wong VS, Wood E, Kelsey M. Comparison of three stool antigen tests for Helicobacter pylori detection. Journal of clinical pathology. 2003;56(10):769-71.

45. Brooks HJ, Ahmed D, McConnell MA, Barbezat GO. Diagnosis of helicobacter pylori infection by polymerase chain reaction: is it worth it? Diagnostic microbiology and infectious disease. 2004;50(1):1-5.

46. Al-Dhaher Z. Study of Some Bacteriological and Immunological Aspects of H. pylori Al-Mustansiriya University; 2001.

47. Mohammed O. Mohammed. Correlation of Endoscopic Findings with Various Helicobacter pylori Tests among Dyspeptic Patients. International Journal of Clinical Medicine. 2014;5:1180-8

48. Macarthur C, Saunders N, Feldman W. Helicobacter pylori, gastroduodenal disease, and recurrent abdominal pain in children. Jama. 1995;273(9):729-34.

49. Klein PD, Gilman RH, Leon-Barua R, Diaz F, Smith EO, Graham DY. The epidemiology of Helicobacter pylori in Peruvian children between 6 and 30 months of age. The American journal of gastroenterology. 1994;89(12):2196-200.

50. Ma JL, You WC, Gail MH, Zhang L, Blot WJ, Chang YS, et al. Helicobacter pylori infection and mode of transmission in a population at high risk of stomach cancer. International journal of epidemiology. 1998;27(4):570-3.

51. Rauws EA, Tytgat GN. Cure of duodenal ulcer associated with eradication of Helicobacter pylori. Lancet. 1990;335(8700):1233-5.

52. Wroblewski LE, Peek RM, Jr., Wilson KT. Helicobacter pylori and gastric cancer: factors that modulate disease risk. Clinical microbiology reviews. 2010;23(4):713-39.

53. Cognein P, Costa A, Giacosa A. Serodiagnosis of Helicobacter pylori: evaluation of a rapid, miniaturized immunochromatographic test. European journal of cancer prevention : the official journal of the European Cancer Prevention Organisation. 1994;3(6):457-63.

54. Eugenia M, Quintana-Guzman, Schosinky-Nevermann K, Arias-Echandi ML, Davidovich-Rose H. Comparative study of urease tests for Helicobacter pylori detection In gastric biopsies. Rev Biomed. 1999;10:145-51.

55. Baqai R, Qureshi H, Arian G, Mehdi I. Diagnostic efficacy of stool antigen test (HPSA), CLO test and serology for the detection of Helicobacter pylori infection. Journal of Ayub Medical College, Abbottabad : JAMC. 2003;15(4):34-6.

56. Li YH, Guo H, Zhang PB, Zhao XY, Da SP. Clinical value of Helicobacter pylori stool antigen test, ImmunoCard STAT HpSA, for detecting H pylori infection. World journal of gastroenterology : WJG. 2004;10(6):913-4.

57. Al-Yas M. Comparative Study Between H. pylori Isolated from Human and Some Domestic Animals: Al-Nahrain University; 2006.

58. Twaij A. Invasive and Non Invasive Methods for Detection of H. pylori With Some Molecular Aspects of its Pathogenesis [PhD Thesis]: Al-Nahrain University; 2006.

59. Lu JJ, Perng CL, Shyu RY, Chen CH, Lou Q, Chong SK, et al. Comparison of five PCR methods for detection of Helicobacter pylori DNA in gastric tissues. Journal of clinical microbiology. 1999;37(3):772-4.

60. Patel SK, Pratap CB, Jain AK, Gulati AK, Nath G. Diagnosis of Helicobacter pylori: what should be the gold standard? World journal of gastroenterology : WJG. 2014;20(36):12847-59.

61. Mishra S, Singh V, Rao GR, Jain AK, Dixit VK, Gulati AK, et al. Detection of Helicobacter pylori in stool specimens: comparative evaluation of nested PCR and antigen detection. Journal of infection in developing countries. 2008;2(3):206-10.