

# ISOLATION AND DETECTION OF *HELICOBACTER PYLORI* FROM PATIENTS SUFFERING FROM PEPTIC ULCER USING BIOCHEMICAL TESTS AND MOLECULAR TECHNIQUES

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## ABSTRACT

*Helicobacter pylori* is the causative agent of most cases of gastritis. There is no established gold standard for the diagnosis of *H. pylori* infection. A reliable diagnosis is crucial to confirm that eradication therapy has been successful. Eighty gastric biopsy and blood samples were obtained from fasting Jordanian patients with Esophago-Gastro-Doudenoscopy (EGD). Several diagnosis tests for *H. pylori* infections were used and compared including: Culture, microscopic examination, histopathology, Rapid Urease Test (RUT), serology, biochemical tests, antibiotic susceptibility test and molecular method. Forty two patients were considered *H. pylori* positive in both histopathology examination and RUT test. On the other hand, 57 patient were detected to have anti-IgA, IgG *H. pylori* antibody positive by ELISA test. Ten patients had equivocal results but not in both tests. A total of 19 biopsy samples were positive for *H. pylori* according to culture test. This result was confirmed by endoscopic examination, urease, catalase and oxidase. A high percentages of resistance to vancomycin, polymyxin B and amoxicillin was observed (100, 100 and 94.7%, respectively) with various degree of sensitivity to all of the first line of antibiotics. Molecular technique (PCR) was used to detect CagA gene which appeared positive in 14 patients. We conclude that the histopathology and RUT tests are reliable invasive diagnosis for *H. pylori*. However, culture test appear to be the most important (if the therapy failed) to detect antibiotic susceptibility to *H. pylori* strains.

**Keywords:** *Helicobacter Pylori*, Biochemical Diagnosis, ELISA, Antibiotic Sensitivity, Molecular Detection

## 1. INTRODUCTION

The discovery of *Helicobacter pylori* in 1982 was the starting point of a revolution concerning the concepts and management of gastroduodenal diseases (Megraud and Lehours, 2007). *Helicobacter pylori*, originally classified as *Campylobacter pylori* (Al-Sulami *et al.*, 2010). Self-ingestion experiments and experiments with volunteers demonstrated that these bacteria can colonize the human stomach, thereby inducing inflammation of the gastric

mucosa (Morris *et al.*, 1991). These initial data stimulated for further study, which showed that gastric colonization with *H. pylori* can lead to upper gastrointestinal disorders, such as chronic gastritis, peptic ulcer disease, gastric mucosaassociated lymphoid tissue lymphoma and gastric cancer. The severity of the inflammation is likely to underlie *H. pylori*-related diseases (Abdalsadeg *et al.*, 2012). Although most individuals do not develop adverse clinical consequences from *H. pylori* colonization, between 10 and 15% will experience some form of symptomatology

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and the clinical result of the infection will be determined by complex interactions between host and bacterial factors. While the host factors remain unknown, the identification of specific bacterial factors is continuously advancing (Cogo *et al.*, 2011).

*H. pylori* is a gram negative bacterium typically appearing as a curved rod or short spiral. It is believed to be one of the more common pathogenic infections of man, with prevalence rates reaching 30-60% in developed countries, depending on age and socioeconomic status (Vinetto *et al.*, 2002). The mode of transmission and other aspects of the epidemiology of *H. pylori* infection still remain unclear (Tiwari *et al.*, 2005). Several virulence factors that aid *H. pylori* in colonization of the host and contribute to disease development have been identified by a variety of methods. One of these factors is the urease enzyme that *H. pylori* needs to survive in the low-pH gastric lumen as it makes its way to the gastric mucosa, which has a more neutral pH (Montecucco and Rappuoli, 2001). *H. pylori* also requires several motility and chemotaxis genes for colonization, presumably so that it can locate and move to its preferred site of infection and remain there (Ottemann and Lowenthal, 2002).

Diagnosing *H. pylori* infection is sometimes difficult (Tiwari *et al.*, 2005). Accurate diagnosis is essential for the effective treatment and management of infections caused by this organism (Abdalsadeg *et al.*, 2012). *H. pylori* infection can be diagnosed by invasive techniques (endoscopy with biopsies for histology, culture and a rapid urease test) (Vaira and Vakil, 2001). Culture and identifying *H. pylori* in gastric biopsy require experience and dexterity, as identification and culturing are sometimes difficult. Microscopy and rapid urease test can be highly specific if strictly performed, but they are based on biopsy specimens and thus are theoretically prone to sampling error, as in the case of culture (Yoshida *et al.*, 1998). Since invasive methods are expensive, less invasive methods such as serological examination of blood and the urea breath test have become more popular (Zagari *et al.*, 1999). However, positive results by blood serology do not necessarily allow delineation of active *H. pylori* infection. Urea breath tests require expensive specialized equipment and reagents and sometimes become apparently positive in culture negative patients (Bazzoli *et al.*, 1997).

Molecular methods like Polymerase Chain Reaction (PCR) have been used extensively for the diagnosis of *H. pylori* from gastric biopsy specimens, saliva, faeces and archival specimens, as well as for detecting clarithromycin resistance (Zsikla *et al.*, 2006). PCR yields information on the presence of potential virulence markers in the strain, which might have implications for the development of severe disease or efficacy of

eradication (Ricci *et al.*, 2007). Different primers have been utilized and some have been developed into commercial kits. Different loci have been used as the target for the amplification: 16S rRNA; A-B-and C-urease; *flaA*; *CagA*; *vacA* and heat-shock protein (*hsp*). Real-time results can be obtained using light-cycle technology (Ricci *et al.*, 2007).

The present study was therefore carried out to test some feasible non-invasive and invasive methods for the rapid diagnosis of *H. pylori* among infected patients suffering from various gastric maladies using biochemical tests and molecular techniques.

## 2. MATERIALS AND METHODS

### 2.1. Collection of Samples

#### 2.1.1. Biopsy Samples

A total of 80 biopsy patients (30 males and 50 females) aged from 18-81 years were collected from two different local hospitals. The patients were suffering from dyspepsia and advised by the doctors to have clinical indications for an endoscopy in the gastrointestinal clinic of the hospitals. All the patients with dyspepsia undergoing endoscopy were asked to fill the inclusion questionnaire. Biopsies of gastric tissue were collected from the corpus or the antrum or corpus and antrum of the patient's stomach. Three biopsies were taken from each patient. Then, specimens were sent for histopathologic study, gram staining, sensitivity and rapid urease test.

The taken biopsies were classified according to the performed test. The biopsy used for histopathology examination was transported to the histopathology laboratory with 10% buffered formalin for at least 24 h. The biopsy used for culture was transported to the laboratory with 2-2.5 mL<sup>-1</sup> Tryptical Soy Broth (TSB) as a transport medium. The biopsy which used for RUT was tested immediately at the department of gastroenterology and the result examined within 1 h.

#### 2.1.2. Blood Samples

Blood samples (3-5 mL<sup>-1</sup>) were taken before each patient's enter to endoscopic unit and before initiation of any therapy. The blood was kept at room temperature for 1 h; the clot was removed by centrifuging at 1,000-2,000xg for 10 min in the centrifuge. The resulting supernatant was designated serum. Following centrifugation, the samples were immediately transferred into a clean, sterile eppendorf tube using a Pasteur pipette. The samples were maintained at 2-8°C while handling. Then the serum was stored at -20°C until using for Enzyme-Linked Immunosorbent Assay (ELISA) test.

Samples that are hemolyzed, icteric or lipemic were excluded from the study.

## 2.2. Histopathologic Examination

This test was performed by the laboratory technical in the hospital and read by the doctor following standard procedure (Dixon *et al.*, 1996). The gastric biopsies were fixed in 10% buffered formalin for at least 24 h and then embedded in paraffin. In each case, three sections of the tissues were cut at 0.3 micron, de-paraffinized and hydrated in descending grades of alcohol, cut in sequential 4- $\mu$ m sections. One section was stained with routine haematoxylin and eosin stain. The second section was stained with modified Giemsa stain to demonstrate the presence of *H. pylori*. The *H. pylori* were identified as curved rods on the luminal surface of the gastric epithelial cells. The third section was stained with alcian blue/Periodic Acid Schiff's stain to demonstrate the presence of intestinal metaplasia.

## 2.3. Biochemical Tests

### 2.3.1. Culture of *H. Pylori* from Biopsy Sample

The antral biopsy specimens were transported to the microbiology laboratory immediately within less than one hour in 2-2.5 mL tryptic soy broth as a transport medium. The biopsy specimens were first ground in a sterile mortar with the aid of a sterile fine glass rod until the formation of homogenate. The biopsies were inoculated at Columbia Agar (CA) plates supplemented with 8% sheep blood, vancomycin (10 mg mL<sup>-1</sup>), trimethoprim lactate (5 mg L<sup>-1</sup>), amphotericin-B (5 mg mL<sup>-1</sup>), polymyxin-B (2500 units mL<sup>-1</sup>) and incubated in a microaerobic atmosphere (10 CO<sub>2</sub>, 85 N<sub>2</sub>, 5% O<sub>2</sub>) (Sigma, Aldrich) at 37°C for 5-7 days. The bacteria were identified as *H. pylori* by Gram staining, colony morphology, positive oxidase, catalase and urease reactions (Balows *et al.*, 1991). Bacteria were sub-cultured using the same conditions into two different media: Columbia agar with supplements and brain heart infusion broth.

### 2.3.2. Rapid Urease Test

Biopsy specimens were tested for urease activity by using the "HelicotecUT@Plus" test.

### 2.3.3. Urease Test

The urease test was applied according to MacFaddin (2000). The pure isolates were inoculated heavily on the entire surface of urea agar and stab with loop wire. The tubes were inoculated at 37°C in the incubator. The formation of purple color was examined after 4 h.

### 2.3.4. Oxidase Test

The isolates were tested for oxidase activity by using oxidase test strip.

### 2.3.5. Catalase Test

The isolates were tested for catalase activity by using slide (drop) method in which the sterile wire loop is stacked on the surface of the pure colony and transferred to a microscope slide and one or two drop of 3% H<sub>2</sub>O<sub>2</sub> were added onto the organism on the slide and observed for immediate oxygen bubble formation.

## 2.4. Antibiotic Susceptibility Test

The used antibiotics were: Clarithromycin (15  $\mu$ g<sup>-1</sup>), tetracycline (10  $\mu$ g<sup>-1</sup>), amoxicillin (25 mcg), polymyxin B300 UNITS, vancomycin (5  $\mu$ g<sup>-1</sup>), trimethoprim (2.5), kanamycin (30mcg) and rifampin (5mcg) (MASTDISCS<sup>TM</sup>, Bioanalyse®). Muller Hinton (MH) (Oxoid, England) containing 10% human blood and *H. pylori*-selective supplement (Oxoid, England) was used for the disk diffusion (Kirby-Bauer) technique. Using the published CLSI guidelines, the susceptibility, or resistance of the *H. pylori* to each antibiotic was determined disk diffusion method for Antimicrobial Susceptibility Testing (EUCAST).

## 2.5. Serology Test

### 2.5.1. Serum Enzyme-Linked Immunosorbent Assay Testing (Elisa)

Serum samples were tested for anti-*H. pylori* antibodies IgA/IgG by using the NovaLisa<sup>TM</sup> Enzyme-Linked ImmunoSorbent Assay (ELISA) kit (NovaTec Immundiagnostica GmbH) according to manufacturer directions. Value of <15 Nephelometric Turbidity Units (NTU)/mL were negative, >20 NTU/mL were positive, between 15 and 20 NTU/mL were equivocal.

## 2.6. Molecular Techniques

### 2.6.1. DNA Extraction from Biopsy Samples

Specimens of gastric biopsy were collected in a sterile container containing digestion buffer (100 mM NaCl, 10 mM Tris-HCl (pH 8.0) 0.5% SDS) and frozen at -70°C until using. Genomic DNA was isolated from all samples by the Cetyltrimethyl Ammonium Bromide (CTAB) method with an additional initial digestion with lysozyme buffer and incubation with the buffer at 37°C for 24 h, then according to the standard protocol. Extensive care was taken to avoid contamination during all steps of collection and preparation of the samples.

### 2.6.2. PCR Amplification

Two oligonucleotide primers designated *CagA-F* (5'-AATACACCAACGCCTCCAAG-3') and *CagA-R* (5'-TTGTTGCCGCTTGCTCTC-3') was used. The amplified product of these two primers with DNAs was a 395 bp fragment. The template DNA [2 μL<sup>-1</sup>] was added to 18 μL<sup>-1</sup> of the reaction mixture containing 1X PCR buffer [50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5% (v/v) Triton X-100], 1.5 mM MgCl<sub>2</sub>, 200 μM concentrations of each dNTPs, 10 pmol of each primer and 1U of *Taq* polymerase. PCR amplification was performed according to the following program: Initial denaturation at 96°C for 5 min 40 cycles with each cycle consisting of 94°C for 1 min, 62°C for 1 min and 72°C for 2 min. The final cycle included a 10 min extension step to ensure full extension of the PCR products. In each batch of PCR assays the negative control consisted of all the reagents of the master mix except the template DNA. The PCR-amplified products were analyzed by agarose gel electrophoresis. The gel was stained with ethidium bromide and examined under UV transilluminator for the presence of the amplified DNA.

### 2.7. Statistical Analysis

Standard method was used to calculate sensitivity and specificity for the result of diagnostic test. Sensitivity relates to the test's ability to identify positive results. The sensitivity of a test is the proportion of people that are known to have the disease who test positive for it. This can also be written as:

$$\text{Sensitivity} = \frac{\text{number of true positive}}{\text{true positive} + \text{false negative}}$$

$$\text{Specificity} = \frac{\text{number of true negative}}{\text{true negative} + \text{false positive}}$$

## 3. RESULTS

### 3.1. Data Collection Analysis

A total of 240 biopsy samples were collected from 80 Jordanian patients. The objectives of this study were described for the patients and they agreed to be in the population of the study. They filled the required case history data which included civilian information about the patients (sex, age,), the symptoms they suffering from (epigastric pain, nausea, vomiting....) and their medical history (other diseases and medications).

The results of the case history data indicated that there were 38.75% (31 out of 80) males and 61.25% (49 out of 80) females with age range 18-81 years and mean

age 42.55 years. 96.25% (77 out of 80) of the patients were not on any medications while 3.75% (3 out of 80) used aspirin, famodar, NAISD before presentation to the outpatients, but not in a regular way. Forty four patients were suffering from epigastric pain for 6-12 months and 67 suffering from heartburn and 55 suffering from the symptoms after eating.

### 3.2. Serum Enzyme-Linked Immunosorbent Assay Test (ELISA)

The 80 patients consented to be randomly tested against *H. pylori* serum antibody (IgG and IgA). Anti- *H. pylori* IgG antibodies were present in 56 (70%), while anti-*H. pylori* IgA antibodies present in 47(58.75%) (Table 1). The test depends on the optical density for the formed colored because of interaction between antibodies (if they present in serum) with the substrate solution form yellow color. The results of the absorbance and concentrations of Anti-*H. pylori*, IgG and IgA antibodies for the 80 samples are presented as standard curve in (Fig. 1).

### 3.3. Histopathologic and Endoscopic Examination with RUT Test

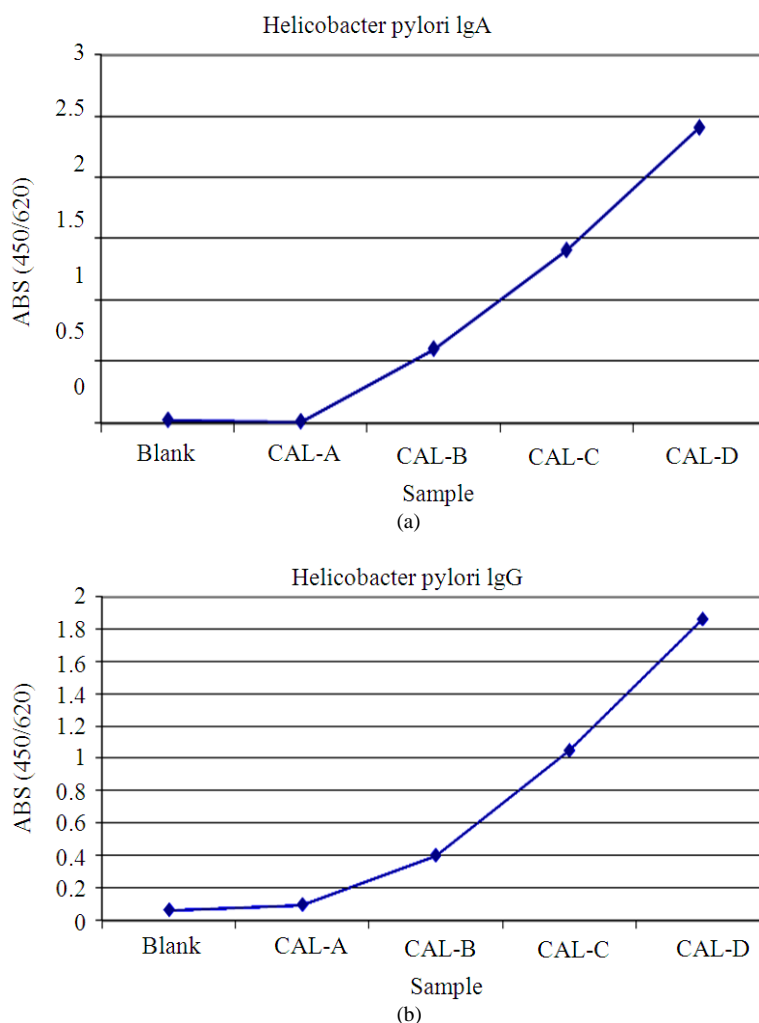
To confirm the infection of 80 patients with *H. pylori*, histopathology and endoscopy examination and RUT test were used. The result indicated that 43 (53.75%) patients were RUT positive and 37 (46.25%) were RUT negative. Forty (50%) were histology positive and 40 (50%) were negative (Fig. 2). The main endoscopic findings were gastritis 52 (65%), gastroesophageal reflux disease 13 (16.25%), duodenitis 8 (10%) and the ulceration 10 (12.5%) (Table 2).

**Table 1.** Anti- *H. pylori* antibody IgG and IgA ELISA test (n = 80)

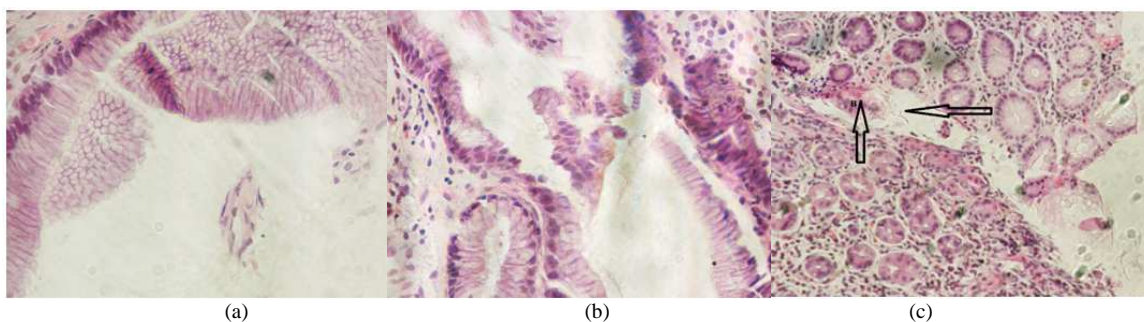
Samples	Anti- <i>H. pylori</i> IgA (%)	Anti- <i>H. pylori</i> IgG (%)
Positive	47 (58.75)	56 (70)
Negative	33 (41.25)	24(30)

**Table 2.** Rapid Urease Test (RUT), endoscopic and histopathology results of the patients (n = 80)

	The test	Number of patients (%)
Endoscopic finding	Gastritis	52(65)
	Gastroesophagea l reflux	13(16.25)
	Duodenitis	8(10)
	Peptic and duodenal ulceration	10(12.5)
Histopathology	Positive	40(50)
	Negative	40(50)
RUT test	Positive	43(53.75)
	Negative	37(46.25)



**Fig. 1.** The standard curve for the ELISA standard and blank solutions anti-H. pylori IgG and IgA antibody



**Fig. 2.** Histopathology examination results (A, B) Negative hestopathological result the lumen is clear there is no bacteria and no inflammatory response (C). Positive hestopathological result showed inflammatory cells and the presence of H. pylori. The horizontal arrow indicates the presence of spiral shaped H. pylori colonization at the lumen of the gastric tissue, while the vertical arrow showed the inflammation at the tissue (C).

### 3.4. Microscopic Examination, Oxidase, Urease and Catalase Tests

Nineteen *Helicobacter pylori* isolates were obtained from the whole 80 biopsy specimen samples which cultured from the transport media on Columbia agar supplemented with selective antibiotic and 8% sheep blood, in a microaeriphilic jar for 5-7 days. The isolations were identified firstly according to colony morphology that appeared transparent almost with poor growth. Secondly, they were microscopically examined after stained with Gram stain; the isolates were Gram negative, long and spiral shape. Biochemical examinations with catalase, urease and oxidase activity were applied to all of the 19 detected bacterial colonies that are positive for *H. pylori* (Table 3). The results indicated that all of the 19 isolates were oxidase positive, transparent colonies and appeared spiral shaped under the microscopic examination.

### 3.5. Antibiotic Susceptibility Test

The 19 *H. pylori* positive isolates (according to the biochemical tests and gram stain) were tested for their susceptibility to 8 different types of antibiotics. The results indicated that the isolates were 100% VANcomycin (VA) and Polymyxins B (PB) resistant, 94, 84.2 and 47.4% were resistant to Amoxicillin (AX), Kanamycin (K), Claithromycin (CLA) respectively and 84.2, 89.5 and

94.7% were sensitive to Tetracycline (TE), TriMethoprim (TM) and Rifampin (RA) respectively (Table 4).

### 3.6. Molecular Detection of *H. pylori* Isolates

To confirm the results of the conventional methods that mentioned above for diagnosis of *H. pylori* (ELISA, RUT test, histopathology, culture and biochemical tests), PCR technique was used. Genomic DNA was extracted from the 19 isolates (Fig. 3) and the concentration was measured by nanodrop instrument. The extracted genomic DNA was tested to detect the presence of CagA gene in the *H. pylori* isolates. The CagA gene was amplified with a specific primers (5'-AATACACCAACGCCTCCAAG-3') and (5'-TTGTTGCCGCTTGCTCTC-3'). The amplified genomic DNA of the isolates produced a single PCR band of about 395 bp in size (Fig. 4). The diagnosis test results and their histopathology examination for the 19 patients are presented in Table 5.

### 3.7. Statistical Analysis

Using the statistical standard statistical equation, the sensitivity and specificity values for the *H. pylori* diagnosis test were calculated as shown in Table 6. All the tests have a high sensitivity for detection of *H. pylori* except the culture test which produced about (47.5%) sensitivity values, where histopathology and culture tests shows 100% specificity.

**Table 3.** Gram stain, morphological examination and biochemical test result (n = 80)

Tests	Transparent colonies	Gram negative	Long and spiral shaped	Oxidase positive	Catalase positive	Urease positive
Results	19 (23.75%)	30 (37.5%)	19 (23.75%)	19 (23.75)	40 (50%)	43 (53.75)



**Fig. 3.** Genomic DNA extraction from biopsy specimens of 19 gastric maladies patients Agarose gel (1%) electrophoresis of genomic DNA extracted from 19 gastric biopsies, lane M: For 1 kb DNA ladder marker



**Fig. 4.** The amplified CagA gene sequence for biopsy specimens. Agarose gel (1%) electrophoresis of PCR amplification of CagA gene fragment. Lane M: For 1 kb DNA ladder marker

**Table 4.** Antibiotic susceptibility test result (n = 19)

Antibiotics	Resistance (%)	Intermediate (%)	Sensitive (%)
Vancomycin, VA (5 µg)	19 (100)	-----	-----
Polymyxins, PB (300 U)	19 (100)	-----	-----
Amoxicillin, AX (25 mcg)	18 (94.7)	1 (5.3)	-----
Kanamycin, K (30 mcg)	16 (84.2)	-----	3 (15.8)
Claithromycin, CLA (15 µg)	9 (47.4)	1 (5.3)	9 (47.4)
Tetracycline, TE (10 µg)	3 (15.8)	-----	16 (84.2)
Trimethoprim, TM (2.5 µg)	2 (10.5)	-----	17 (89.5)
Rifampin, RA (5 mcg)	1 (5.30)	-----	18 (94.7)

**Table 5.** Patients data and test results (n = 19)

No.	Sex	Age	Biochemical test					Histo.	RUT	ELISA*		Endoscopic examination
			Culture	Oxidase	catalase	urease	IgA NTU/mL			IgG NTU/mL		
13	M	40	+	+	+	+	+	+	24.5 +	105.8 +	<i>H. pylori</i> gastritis	
15	M	30	+	+	+	+	+	+	22.4 +	101.7 +	<i>H. pylori</i> gastritis	
20	F	72	+	+	+	+	+	+	31.1 +	60.8 +	<i>H. pylori</i> gastritis	
22	M	29	+	+	+	+	+	+	26.2 +	101.3 +	acute duodenal ulcer	
23	F	37	+	+	+	+	+	+	33.1 +	62.0 +	acute duodenal ulcer	
24	F	34	+	+	+	+	+	+	41.7 +	143.1 +	Gastric ulcer	
25	M	40	+	+	+	+	+	+	21.8 +	92.0 +	Gastric ulcer	
28	M	41	+	+	+	+	+	+	23.2 +	99.2 +	Gastric ulcer	
29	F	30	+	+	+	+	+	+	27.1 +	25.7 +	acute duodenal ulcer	
32	F	46	+	+	+	+	+	+	32.4 +	99.0 +	Gastric ulcer	
39	F	52	+	+	+	+	+	+	27.8 +	130.0 +	<i>H. pylori</i> gastritis	
40	M	60	+	+	+	+	+	+	26.8 +	72.1 +	<i>H. pylori</i> gastritis	
45	F	44	+	+	+	+	+	+	64.7 +	62.3 +	Gastric ulcer	
48	F	48	+	+	+	+	+	+	16.6 +	46.0 +	<i>H. pylori</i> gastritis	
51	F	30	+	+	+	+	+	+	21.9 +	98.7 +	acute duodenal ulcer	
57	F	18	+	+	+	+	+	+	25.3 +	118.0 +	acute duodenal ulcer	
60	F	45	+	+	+	+	+	+	41.8 +	25.7 +	Gastric ulcer	
79	F	30	+	+	+	+	+	+	30.2 +	120.1 +	Gastric ulcer	
80	M	39	+	+	+	+	+	+	21.7 +	36.2 +	<i>H. pylori</i> gastritis	

**Table 6.** Sensitivity and specificity values for diagnostic tests of *H. pylori*

The test	Sensitivity (%)	Specificity (%)
ELISA IgG antibody	100.0	60.0
ELISA IgA antibody	100.0	82.0
Histopathology	100.0	100.0
RUT test	100.0	93.5
Culture	47.5	100.0

#### 4. DISSCUSION

The prevalence of *Helicobacter pylori* differs both between and within countries, with high rates of infection being associated with low socioeconomic status and high densities of living (Goodman and Cockburn, 2001; Hazel and Francis, 2002). Approximately, 40 and 80% of adult individuals in developed and developing countries are infected respectively (Timothy and Martin, 1995). However, the percentage of infected people increases with age, since 50% of infected people were those over the age of 60 compared with around 10% between 18 and 30 years (Brown, 2000). This was not the case in this study, since the highest percentage of patients was among young people ranging from 40-60 years. In a large French cross-sectional study, a significantly lower prevalence of *H. pylori* infection was observed in females as compared with males (Broutet *et al.*, 2001). However, in this study a highest range of infection was found among females.

The results of this study indicated that 53.75% of symptomatic patients were infected with *H. pylori*. The infection was associated with variable gastrointestinal illness, chronic gastritis, intestinal metaplasia and ulceration disease. This was in agreement with Pilotto *et al.* (1998) who reported that chronic superficial gastritis associated with *H. pylori* infection is a significant predisposing factor for the development of peptic ulcer, atrophic gastritis, gastric lymphoma and gastric adenocarcinoma.

Many tests are available for diagnosis of *H. pylori* infection. Invasive tests, such as culture, histopathology and biopsy urease test that are required endoscopic biopsy of gastric tissue. Non-invasive tests, such as antibody that was detected in the serum also have been used. Patients with alarming symptoms should undergo endoscopy for the diagnosis of *H. pylori* infection. When endoscopy is clinically indicated, the test of first choice is the urease test on an antral-biopsy specimen. It permits cheap and rapid detection of urease activity in the biopsy material, with a sensitivity of 79 to 100% and a specificity of 92 to 100%. Sensitivity can be improved by additional biopsies, but false negative results are observed in patients with active or recent

bleeding and in patients taking antibiotics or antisecretory compounds (Suerbaum and Michetti, 2002). If the urease test is negative, additional biopsy specimens stored in fixative can be sent for histological examination. Rapid urease test has been shown to be as reliable as histological examination. There were only minor differences in accuracy between the RUT and histopathology invasive tests for *H. pylori* infection in this population. RUT may be recommended as the first choice since a result is obtained within one hour (Suerbaum and Michetti, 2002).

The present study was aimed to compare the results of mostly used three invasive methods: Culture test, rapid urease test and histopathological stained based detection of *H. pylori* infection. The results of histopathology and RUT kit were almost similar, 43 (53.75%) patients were RUT positive and 40 (50%) were histology positive. The sensitivity values for these two tests were 100% to *H. pylori*, while the specificity value was found to be 93.5 for RUT and 100% for histopathology. The result of RUT is read after 60 min but the median positive reaction time was only 1 min. The results of this study were in agreement with (Ricci *et al.*, 2007) who mentioned that the sensitivity, specificity, positive predictive value, negative predictive value and accuracy were 98.2, 99, 99, 97.9 and 98.5%, respectively. The sensitivity and specificity of histology for the diagnosis of *H. pylori* varies from 53 to 90%, depending partly on the clinical setting, partly on the density of colonization and partly on the experience of the histopathologist.

*H. pylori* serologic test is cheap and widely used for the diagnosis of *H. pylori* infection in patients before treatment. Although approved laboratory-based tests have sensitivities and specificities of the commercial *H. pylori*, antibody tests seemed to vary between 60 and 100% (Salomaa *et al.*, 2004). In this study, the result of serological test indicated that Anti-*H. pylori* IgG antibodies were present in 56 (70%) of the samples while 47(58.75%) have IgA anti-*H. pylori* antibodies. Specificity and sensitivity for IgG were 60 and 100 and were 80 and 100% for IgA, respectively. The results of this study indicated that the accuracy of the *H. pylori* IgG and IgA tests were in agreement with the previous reference. The accuracy of serological tests is strongly dependent on the prevalence of *H. pylori* infection. Although it has been recommended that antibody assays be evaluated locally, this has rarely been carried out for different age groups. To avoid any misjudgment in the validation of serological tests for *H. pylori* antibodies in adult subjects, the validation should be carried out



separately for different age groups with special emphasis not only on the known *H. pylori* status but also on the presence of atrophic gastritis (Salomaa *et al.*, 2004).

Primary isolation of *H. pylori* from a biopsy specimen is a difficult process. Several factors, which are difficult to control, cause difficulty with the culturing of this bacterium. Another study showed that the percentage of culture positive specimen was 31.94% (Kagar *et al.*, 2011). Patchy distribution of the organism on the gastric mucosa, contamination of biopsy forceps, presence of oropharyngeal flora, loss of viability of the organism during transportation, all these may be responsible for a poor negative predictive value associated with culture of *H. pylori* (Meunier *et al.*, 1997). Culture of *H. pylori* with antibiotic-sensitivity testing is not routinely performed for the initial diagnosis of *H. pylori* infection, but it is recommended after the failure of second-line therapy (Bazzoli, 2001). Guidelines for performing antibiotic-susceptibility tests of *H. pylori* have been developed by the National Committee for Clinical Laboratory Standards (Performance standards for antimicrobial susceptibility testing, 1999). In this study, the rate of isolation of *H. pylori* from the antral biopsy specimens were 47.5% and the sensitivity and specificity of this test were 47.5, 100% respectively. This is in agreement with Ricci *et al.* (2007) who indicated that culture test has a high specificity (100%) and the sensitivity is often lower.

Bacterial contamination of the medium was frequent. The contaminant bacteria were *Pseudomonas* spp., *Proteus* spp. and *Klebsiella* spp., the source of which could be contaminated biopsy forceps and contamination during obtaining, transporting and preparing of the defibrinated sheep blood added to the classic Columbia agar. The growth rate of *H. pylori* on this medium was slow, as 5 to 7 days were needed for the colonies to appear. Columbia blood agar is a nonselective medium used for many years alone or in combination with other non-selective and selective media for culturing *H. pylori* from antral biopsy specimens taken from peptic ulcer patients during upper gastrointestinal endoscopy (Fresnadillo Martinez *et al.*, 1997). The isolation rate of *H. pylori* using this medium alone is very variable. Results as low as only 28.5% total isolation rate were reported by some authors (Piccolomini *et al.*, 1997).

PCR has been used extensively for the diagnosis of *H. pylori* from gastric biopsy specimens, saliva, faeces and archival specimens, as well as for detecting clarithromycin resistance (Ricci *et al.*, 2007). The targets of these PCR methods included urease A (ureA) gene cag A gene, phosphosamine mutase (glmM) gene and 23S rRNA gene (Tomatari *et al.*, 2010). In this study,

PCR technique was used to determine the virulence factor CagA in *H. pylori*; 73.6% of the biopsy specimens were CagA positive. Smith *et al.* (2011) and his colleagues detected *H. pylori* in 35% of their patients using a PCR method in Nigeria.

The disadvantages of PCR as a routine test are that it is a technically demanding and expensive test compared to culture, histology and the rapid urease tests. It requires special laboratory conditions with separate facilities for each stage of the technique and as it is highly sensitive, it is subject to false-positive results by contamination. A positive result detected by any of the molecular techniques does not only indicate current infection but will also detect the DNA or dead organisms (Lisby, 1999). Molecular biology is useful in the study of pathogenicity and immunization vis-à-vis *Helicobacter pylori*. The recent description of the genome of *H. pylori* causes molecular biology at the forefront in research on *H. pylori*. PCR is considered the most sensitive method for the detection of microorganisms. This would make the test particularly useful in the evaluation of eradication. Other indications include the detection of *H. pylori* outside the stomach, although *H. pylori* is present in small numbers among many other microorganisms causing false positive in other tests based on urease and culture (Hassina and Ahmed, 2013).

Antibiotic resistance has increasingly been recognized as a major cause of treatment failure for *H. pylori* infection. Primary antimicrobial resistance against clarithromycin and metronidazole is now commonplace in several countries (Poon *et al.*, 2002). Regional variations in susceptibility and resistance patterns may be ascribed to differences in local antibiotic prescription practices, antibiotic usage in the community and mass eradication programs for *H. pylori* infection as part of gastric cancer prevention strategies. These factors may well be expected to influence success of eradication therapy (Wong *et al.*, 2002). In this study, all the 19 isolated strains showed various degree of sensitivity to all the first line of antibiotics namely trimethoprim, rifampin, clarithromycin, kanamycin and tetracycline. The results indicated that the isolates were 100% vancomycin and polymyxins B resistant and 94%, amoxicillin resistant.

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