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# Bacteriological Investigation of *Helicobacter pylori* Infections in Patients with Gastric and Duodenal Ulcer

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**A B S T R A C T**

*H. pylori* colonize all human populations worldwide. The risk of being colonized by *H. pylori* depends on geographic area, socioeconomic status and age, and initial colonization is thought to occur during early childhood. In developing countries the infection can be almost ubiquitous, whereas in industrialized countries *H. pylori* infect 30–50% of adults. *H pylori* response to both Clarithromycin and Metronidazole antibiotic sensitivity was giving the same figures. In respect to gender, 90.9 % of males and 88.2 % of females were sensitive to both antibiotics. Tetracycline antibiotic sensitivity was showing that 81.8 % of the males and 88.2 % of the females were sensitive to Tetracycline antibiotics. Amoxicillin antibiotic sensitivity was showing that, 81.8 % of the males and 70.6 % of the females were sensitive to Amoxicillin antibiotics. Finally Furazolidone antibiotic sensitivity was showing that, 100 % of the males and 76.5 % of the females were sensitive to Furazolidone antibiotics. Finally, females were more susceptible to *H pylori* infection than male. Stool antigen analysis was more sensitive than either gram stain or urease test but still not reaching 100 percent as biopsy culture do. *H pylori* are more sensitive to Clarithromycin and Metronidazole while, Amoxicillin show less sensitivity in response to *H pylori* infection. From the study we may recommend to detect *H pylori* infection using molecular biology technique for detection of *H pylori* gene to increase the sensitivity to *H pylori* infection diagnosis.

## Introduction

*Helicobacter pylori* are gram-negative spiral bacterium. Over half the world's population is infected with it, mainly during childhood, it is not certain as to how the disease is transmitted. It colonizes the gastrointestinal system, predominantly the stomach. The bacterium has specific survival conditions that the gastric microenvironment: it is both capnophilic and microaerophilic. *H. pylori* also exhibit a tropism for gastric epithelial lining and the gastric mucosal layer about it. Gastric colonization of this bacterium triggers a robust immune response leading to moderate to severe inflammation. This inflammatory response triggers a cascade of mucosal changes that can persist from chronic gastritis, duodenal cancer, metaplasia, dysplasia, carcinoma, to mucosal associated

lymphoid tissue lymphoma (MALT lymphoma) (O'Connor *et al.,* 2017).

Many individuals go through life without realizing they are infected because they were exposed young and their body sees it as normal flora. However, signs and symptoms are gastritis, burning abdominal pain, weight loss, appetite loss, bloating, burping, nausea, bloody vomit, and black tarry stools. Infection is easy enough to detect: GI X-rays, endoscopy, blood tests for anti-Helicobacter antibodies, a stool test, and a urease breath test (which is a by-product of the bacteria). If caught soon enough, it can be treated with three doses of different proton pump inhibitors as well as two antibiotics, taking about a week to cure. If not caught soon enough, surgery may be required (Goering, 2014; Wier *et al.,* 2015).

## Acute infection by *Helicobacter pylori*

*H. pylori* can survive in the superficial mucous layer by its urease activity causing a livable pH in its vicinity; the mechanisms by which *H. pylori* can survive and proliferate when infecting a normal stomach are not so well understood. Nevertheless, the two known voluntary infections were successful only after inhibition of gastric acidity, suggesting the role of gastric juice in the defense against the initial infection. Both these subjects developed self-limited symptoms from the epigastric area with fullness, nausea and vomiting some days after the infection and lasting for about a week (Ozbek *et al.,* 2010).

*H. pylori* have retrospectively been presumed to be the causative agent. Also in these subjects, *H. pylori* may have been introduced to the stomach without gastric juice, which was continuously aspirated or as part of studying meal-stimulated acid secretion where the luminal content *in vivo* was titrated to pH 5.0. Thus, during infections, as well as the transmission by the nasogastric tube, *H. pylori* entered a stomach without acid, allowing the bacterium to bury into the mucous layer before normal gastric acidity was reestablished. In most cases of chronic *H. pylori* gastritis, there is no information of any symptomatic episode (Martínez *et al.,* 2016).

This may indicate that most of the acute infections are asymptomatic, or alternatively, gastroenteritis due to *H. pylori* infection has been misdiagnosed as viral. Moreover, childhood *H. pylori* infection seems to be prevalent, which may be explained by reduced gastric acidity during early life Alternatively, gastroenteritis by other causes may make the gastric content hypoacidic and thus give time and possibility for *H. pylori* to proliferate and infect the stomach. The higher frequency of *H. pylori* infection in underdeveloped countries may perhaps be explained by a higher frequency of gastroenteritis in these countries. The initial infection affects both oxyntic and antral mucosa the mechanisms behind the resolution of the initial infection resulting in loss of symptoms and restoration of acid secretion Nevertheless, it seems that the infection persists in all infected subjects, with some having the ability to limit the infection to the antral mucosa whereas others develop a chronic pan-gastric infection at an early stage (Helge *et al.,* 2016).

## Incidence of *H. pylori*

Gastric mucosa-associated lymphoid tissue (MALT) lymphoma is a rare type of non-Hodgkin lymphoma (NHL) that represents ~12–18% of extra-nodal NHL, with an annual incidence of 1 per 100,000 population. Nearly all patients with gastric MALT lymphoma have confirmed *H. pylori* infection, and the disease can be cured by eradicating the bacterium. *H. pylori* are also implicated in the majority of cases of peptic ulcer disease,

particularly duodenal ulcer. Eradication therapy is both effective in healing peptic ulcer disease and in preventing relapse, and is cost-effective (Moleiro *et al.,* 2016).*H. pylori* infection has also been associated with weight loss and failure to thrive among older adults. With respect to non-gastrointestinal diseases, clear associations between *H. pylori* and iron deficiency anaemia and idiopathic thrombocytopaenic purpura have been demonstrated, although proposed associations with a multitude of other conditions, includ-ing atherosclerotic vascular disease and neurological syndromes remain contentious (Yuan *et al.,* 2010).

*H. pylori* colonize all human populations worldwide. The risk of being colonized by *H. pylori* depends on geographic area, socioeconomic status and age, and initial colonization is thought to occur during early childhood. In developing countries the infection can be almost ubiquitous, whereas in industrialized countries *H. pylori* infect 30–50% of adults. The decline in *H. pylori* infection incidence that relates to industrialization and improvements in socioeconomic levels may be explained by the frequent use of antibiotics, improvements in sanitation, and reduced crowding. The higher prevalence of *H. pylori* in individuals over 40 years of age is considered to be due to a birth cohort effect rather than a continuous risk of being infected, i.e. the incidence of infection was higher in the past (Alberts *et al.,* 2002).

Transmission may be related to the ability of *H. pylori* to form non-cultivable coccoid forms when exposed to unfavorable environmental conditions. However, controversy exists as to whether these coccoids are alive and important for transmission .There is also a high probability that no significant reservoirs exist outside the human stomach, since *H. pylori* has a rather small genome which does not support all necessary metabolic pathways for a nonparasitic life-style (Alm *et al.,* 2000).

Thus, person-to person contact involving ingestion of *H. pylori* from saliva, vomits, feces or recently contaminated foods or beverages would be the most likely modes of transmission. By use of serological and DNA fingerprinting analyses, several studies have suggested that person-to-person transmission occurs mainly within families i.e. vertical transmission instead of horizontal (epidemic) transmission, which is the most common for infectious diseases (Amieva *et al.,* 2003).

## *H. pylori* adhesion and invasion mechanism

The pathogenicity of *H. pylori* and the mechanism by which it colonizes the gastricmucosa have been investigated in many studies reviewed by Huang *et al.,* (2016). An increasing number of *in vivo* and *in vitro* studies demonstrate that *H. pylori* can invade and proliferate in epithelial cells, suggesting that this process might play an important role in disease induction, immune escape and chronic infection. Therefore, to explore the process and mechanism of adhesion and invasion of gastric mucosa epithelial cells by *H. pylori* is particularly important.

Although the detailed transmission route of *H. pylori* remains uncertain, an oral-oral or fecal-oral route during childhood is thought to be the most plausible method of human-to-human transmission (Goh *et al.,* 2011). Once established, *H. pylori* have no significant bacterial competitors (Peek and Blaser, 2002).

The prevalence of *H. pylori* infection varies widely by geographic area, age, race, and socioeconomic status (SES), and developing appear to have higher infection rates than developed countries (Hosseini *et al.,* 2012).

*H. pylori* is unique in that the bacterium can persist for decades in the harsh stomach environment, where it damages the gastric mucosa and alters the pattern of gastric hormone release, there by affecting gastric physiology. The slow development of cancer known as Correa'scascade include a series of inter mediate stages (precancerous lesions) before malignancy per se occurs. These precancerous lesions occur in the following order: gastritis, atrophy, intestinal metaplasia (IM), and eventually dysplasia. *H. pylori* represent the most significant risk factor for gastric malignant tumors (Wang *et al.,* 2014). Gastric cancer (GC) is an insidious disease, with symptoms that often manifest at an advanced stage, a time when the few remaining therapeutic options have low efficiency (Boreiri *et al.,* 2013). Approximately 10% of infected individuals develop severe gastric lesions, such as those in peptic ulcer disease; 1–3% progress to GC, with a low 5-year survival rate, and 0–1% develop mucosa-associated lymphoid tissue (MALT) lymphoma (Parreira *et al.,* 2013; Wang *et al.,* 2014).

Compared with uninfected individuals, individuals infected with *H. pylori* are estimated to have a 2–8-fold increased risk of developing GC, and the International Agency for Research on Cancer (IARC) has classified *H. pylori* as a class I carcinogen. However, the inability of the immune system to clear *H. pylori* infection is not well described. Furthermore, the mechanisms controlling the induction and maintenance of *H. pylori*-induced chronic inflammation are only partly understood (Huang *et al.,* 2016).

A phagosome forms after *H. pylori* invades gastric epithelial cells; the bacterium then exits cells to colonize again while conditions are suitable and repeatedly infects cells. These findings suggest that invasion might play an important role in disease induction, immune escape, and chronic infection (Tan *et al.,* 2009; Chu *et al.,* 2010; Jang *et al.,* 2013). In this "cellular internalization" process, a bacterium specifically binds to a host cell receptor and enters the cytoplasm via phagocytic vacuoles formed through cell membrane invagination. The pathogenic mechanism of *H. pylori* invasion and adhesion has been reviewed, (Huang *et al.,* 2016).

## Aim of the work

The work of the present study objective is to investigate the *Helicobacter pylori* infections bacteriological in patients with gastric ulcer in Gastroenterology center, Mansoura University.

This aim will be achieved through different aims:

1. Isolation and Identification of *Helicobacter pylori* from Biopsy specimen from patients with gastric and peptic ulcers.
2. Identification by rapid detection of *H. pylori* by Urease and Gimsa Stain.
3. Detect of *Helicobacter pylori* antigen in the stool.
4. Antibiotic sensitivity pattern and resistance.

## Materials and Methods

### Subjects

This study comprised fifty gastritis peptic ulcers patients, 24 of them were males and 26 were females. The mean age and standard error were 39.7 ± 2.1 (range: 16- 73). These patients were attending the gastroscope clinic at Gastroenterology center (GEC), Mansoura University Hospitals (MUHs), over 6 months, during the period from May 2015 to April

2016, with symptoms and signs suggesting gastritis.

None of the examined patient had recent active gastro-inteslinal bleeding. Informed consent was obtained from each patient. All patients were subjected to:

### Medical examination

Confirm the gastritis peptic ulcers using endoscopy. With biopsy specimens were taken for *H. pylori* culture.

### Patients in this study were advised to stop at least 4 weeks before endoscopy

Treatment for *H. pylori*.
Antibiotics treatment.
Non steroidal anti-inflammatory drugs.
Antacid treatment at least 2 weeks before endoscopy.

### Experimental study design

Biopsy samples were cultured in specific media for *H. pylori* growth. Positive culture were further confirmed by gram stain and urease test as well as stool antigen analysis from stool samples which taken in the same day of the biopsy.

Positive culture was also exposed to sensitivity test using different antibiotics. The antibiotics used were: Clarithromycin, Metronidazole, Tetracycline, Amoxicillin and Furazolidone.

### Gastric biopsy specimens

During endoscopy using Olympus gastroscope (Q20 or Q200, Olympus, Tokyo), six antral biopsy specimens were obtained from adjacent areas of the gastric antrum with Olympus biopsy forceps FB- 24KR (Cap size, 6 mm.). Two specimens directly dipped in 1 ml brain heart infusion broth in a sterile screw capped bottle for microbiological study.

### Media for isolation of *H. pylori*

Composition: Dent's medium (Cover, 2012)

a. Clumbia agar base (Oxoid).
b. *Helicobacter pylori* selective supplement (Oxoid).

### Processing of gastric biopsy specimens

Gastric biopsy specimens in 0.5 ml brain heart infusion broth were homogenized using tissue homogenizer (Kontes, Vineland, New Jersey) (Morgner *et al.,* 2000). Then used for:

### Culture

Two drops of homogenate were inoculated onto agar plate of Dent's medium (Selective medium) and another 2 drops were inoculated onto an agar plate of Chocolate medium (non selective medium), incubated at 37°C under microaerophilic conditions (Campy pale systems, BBL, Cockeysville, Maryland, USA) for up to 5 days, (Kist., 2006).

### Identification of isolated organism

The growth was identified by

- Colonial Morphology
- Gram Stained Film.
- Biochemical Reaction.
- Immunological Reaction

### Colonial morphology

Colonies of *H. pylori* were pin point colonies (less than 2 mm. in diameter), gray, translucent and non hemolytic(Cover et al, 2012).

### Gram stained film from culture

*H. pylori* are Gram negative short curved rods

or spiral bacteria. Direct smear was prepared by vigorously rubbing the suspended specimen on a sterile glass slide with a sterile loop. After air drying the smear was fixed by heating and stained with Gram's stain, (Morgner et al, 2000).

### Biochemical reactions

### Urease test

Urease test was done by inoculation of a loop full of suspected organism onto Clrristensen's urea agar slant and incubated microaerophilically for 24 hours at 37°C. Change the color from yellow to pink indicates positive urease test (Tseng et at., 2005).

### Immunological reaction

Patients were asked to give a stool sample in sterile containers, which were transported to the microbiology laboratory and immediately frozen at -20°C until they were tested (Ashgar, 2013).

### Sample preparation

- The samples were removed from the freezer and thawed.
- Samples thoroughly mixed so that the probable antigens could locate all over the stool sample constantly.
- Stool sample approximately the size of a pea was added to 200µl of the triple buffers, i.e., 0.05 M saline phosphate buffer, saline phosphate buffer containing 0.1% triton X-100, and 1.5 M glycine buffer with pH = 7.2 which was used as a diluents.
- It was mixed thoroughly using a vortex mixer.
- Subsequently, the samples were centrifuged at 5000 rpm for 10 minutes.
- The supernatant was transferred to a 1.5-ml Eppendorf tube that was then used for the ELISA test.

### Test procedures

- For the monoclonal antibody (mAb)-based Amplified IDEIA HpStAR test (Oxoid, UK).
- The supernatant of stool suspension and peroxidase conjugated mAbs were pipetted into the wells.
- After washing, substrate was added, and the results were read by spectrophotometry.
- According to manufacturer's instructions, OD values ≥0.190 (450 nm) were assessed as positive and <0.190 as negative.

## Results and Discussion

### General population

This study aimed to assess the incidence of *H. pylori* infection among gastritis with peptic ulcers patients. The patients' selection was confirmed by biopsy specimens. The work was recruited fifty gastritis peptic ulcers patients, 24 of them were males (48%) and 26 were females (52%) illustrated in**).** The mean age and standard error were 39.7 ± 2.1 (range: 16- 73). The frequency of *H. pylori* organism growth in selective media was 56% of all population**.**

Cross tabulation between gastritis patient's gender and *H. pylori* organism growth was shown in Table 1.

Within gender concept, 54.2% of the males were negative to *H. pylori* growth and 45.8 were positive. Where, 34.6% of females were negative to *H. pylori* growth and 65.4 were positive**.** Within organism growth concept, 59.1% of negative growth was males and

40.9% were females. While, 39.3% of positive growth were males and 60.7% were females**.**

Along the fifty patients participating in this study, 28 (56%) were showing positive growth for *H. pylori* organism. Within these 28 patients, 22 were males and 17 were females**.**

For the positive growth patients we examine different diagnostic investigations, they were, Gram Stain (GS) and Urease Test (UT) against the golden standard test Stool Antigen Analysis (SAA). In the gold standard test Stool Antigen Analysis 4 patients were showing negative result and 24 were showing positive result. Table 2 was representing the mean age in respect to different diagnostic tool. In all diagnostic investigations the mean male age was more than the mean female age.

## Sensitivity and specificity of different testes used in *H. pylori* detection

The sensitivity and specificity of gram stain and Urease test against gold standard stool antigen analysis used in *H. pylori* detection was shown in (Table3)**.** Both gram stain and urease test were show the same figure against the gold standard stool antigen analysis where both were have the sensitivity of 87.5% and specificity of 75%**.**

When testing the sensitivity of all tests used in *H. pylori* detection against positive biopsy the sensitivity of both gram stain and urease test were 78.6% and for stool antigen analysis it was 85 7% (Table 4).

**Table.1** Cross tabulation between gastritis patient's gender and *H. pylori* organism growth with risk estimate between males and females

| | | | *H. pylori* Growth | | P |
|---|---|---|---|---|---|
| | | | Negative | Positive | |
| Gender | Male | Count | 13 | 11 | 0.134 |
| | | % within Gender | 54.2% | 45.8% | |
| | | % within Growth | 59.1% | 39.3% | |
| | Female | Count | 9 | 17 | |
| | | % within Gender | 34.6% | 65.4% | |
| | | % within Growth | 40.9% | 60.7% | |

Risk Estimate

| | | 95% Confidence Interval | |
|---|---|---|---|
| | Value | Lower | Upper |
| Odds Ratio for Gender (M / F) | 2.23 | 0.71 | 6.97 |

**Table.2** Mean age and standards error of the mean for participants in respect to gender and organism growth as well as different diagnostic tools

| Gender | N | Mean | Std. Error Mean | Significance |
|---|---|---|---|---|
| Male | 11 | 38.27 | 5.54 | |
| Female | 17 | 44.05 | 3.39 | 0.35 |
| G Stain | | | | |
| Positive | 22 | 44.31 | 3.47 | |
| Negative | 6 | 32.50 | 4.15 | 0.04 |
| Urease Test | | | | |
| Positive | 22 | 43.95 | 3.54 | |
| Negative | 6 | 33.83 | 3.94 | 0.07 |
| Stool Ag | | | | |
| Positive | 24 | 42.95 | 3.30 | |
| Negative | 4 | 34.75 | 6.30 | 0.30 |

**Table.3** The sensitivity and specificity of gram stain and Urease test against gold standard stool antigen analysis used in *H. pylori* detection

| **Item** | **Gram Stain** | **Urease Test** |
|---|---|---|
| **True Positive** | 21 | 21 |
| **False Negative** | 3 | 3 |
| **True Negative** | 3 | 3 |
| **False Positive** | 1 | 1 |
| **Sensitivity** | 78.5 | 78.5 |
| **Specificity** | 75 | 75 |
| **PPV** | 95.9 | 95.9 |
| **NPV** | 50 | 50 |
| **Accuracy** | 85.7 | 85.7 |

**Table.4** The sensitivity of all tests used in *H. pylori* detection against positive biopsy

| **Investigation** | True Positive | False Negative | Sensitivity |
|---|---|---|---|
| **Gram Stain** | 22 | 6 | 78.6 |
| **Urease Test** | 22 | 6 | 78.6 |
| **Stool Antigen** | 24 | 4 | 85.7 |

**Table.5** Cross tabulation of positive gastritis patients to *H. pylori* organism between clarithromycin, metronidazole, tetracycline, amoxicillin and furazolidoneantibiotic sensitivity between them

| **Item** | Sensitive | Resistance |
|---|---|---|
| Clarithromycin | | |
| Count | 25 | 3 |
| **%** within Clarithromycin | 89.2% | 10.7 |
| Furazolidone | | |
| Count | 24 | 4 |
| **%** within Furazolidone | 85.7% | 14.2% |
| Tetracycline | | |
| Count | 24 | 4 |
| **%** within Tetracycline | 85.7% | 14.2% |
| Amoxicillin | | |
| Count | 21 | 7 |
| **%** within Amoxicillin | 75.0% | 25.0% |
| Metronidazole | | |
| Count | 25 | 3 |
| **%** within Metronidazole | 89.2 | 10.7 |

## Antibiotic sensitivity to *H. pylori* growth

Different antibiotic were used to test *H. pylori* response, they were Clarithromycin, Metronidazole, Tetracycline, Amoxicillin and Furazolidone. The sensitivity of each antibiotic was shown in table 5.

## General population

The present study paid attention to evaluate the incidence of *H. pylori* infection among gastritis with peptic ulcers patients. The patients' selection was confirmed by biopsy specimens. Since the first description of *H. pylori* by Warren and Marshall (1983), bacterium has been thought to be one of the most common human infections worldwide.

It was believed that it affected approximately half of the world's population with geographic prevalence variations (O'Connor *et al.,* 2017).

The prevalence of *H. pylori* in the general population may be related to prevalence in symptomatic patients particularly those in whom no disease was found at endoscopy. The present work frequency of *H. pylori* organism growth in selective media was 56% of all population, this in concomitant withAdu-Aryee*et al.,* 2016 where theyield of *H. pylori* infection was 55.3 %.

Along different studies the prevalence of *H. pylori* was varied depending on different factors mainly the country and the site of infection where Darko *et al.,* (2015) found a decreased among Ghanian patients over the period, 69.7% in 1999 to 45.2% in 2012. While Obayo *et al.,* (2015) *H. pylori* was diagnosed in 75.6% among the biopsied patients. Oling *et al.,* 2015 found the prevalence of *H. pylori* gastritis was 36%. In China the mean prevalence of *H. pylori* infection in the endoscopy-referral patient population was 31.97 %, and it mirrored a marked significant linear decline trend from

42.40 % in 2003 to 23.82 % in 2012 as reported by Jiang*et al.,* 2016.Ansari *et al.,* 2016 found the overall prevalence of colonization of *H. pylori* was found as less as to be 16%.

The present work was recruited fifty gastritis peptic ulcers patients, (48%) of them were males and (52%) were females. The mean age and standard error were 39.7 ± 2.1 (range: 16-73). Within gender concept, 45.8 % of the males were positive to *H. pylori* growth while, 65.4 % of the females were positive to *H. pylori* growth. Within organism growth concept, 39.3% of positive growth were males and 60.7% were females (OR= 2.23, IC 95% = 0.71- 6.97; P>0.134).

The present study result was in contrary with Darko *et al.,* (2015) where their sex differences in *H. pylori* infection was identified (higher among males) and young adults (21-40 years).While in Obayo *et al.,* (2015) study, thepatient's age were ranged from 18 to 90 years, with a mean of 53.5 years old presenting with *H. pylori* growth.

## Positive population for *H. pylori* growth in media

Along the fifty patients participating in this study, 28 (56%) were showing positive growth for *H. pylori* organism. Within these 28 patients, 22 (61%) were males with mean age of 38.27 years and 17 (39%) were females with mean age of 44.05 years. In a study concerning different age ranges (Ansari *et al.,* 2016) found that overall prevalence of colonization of *H. pylori* was found to be 16 % (male-13.3 % and female- 20.0 %). Highest number (36.0 %) of the patients was under the age of 10 years followed by 28.0 % from 11 to 20 years and 8.0 % above 60 years. Out of 16 positive cases, 75.0 % were below 10 years and remaining 25.0 % in the age of 21–40 years**.**

In the positive growth patients, *H. pylori* organism was identified with different diagnostic investigations, they were, Gram Stain (GS) and Urease Test (UT) against the golden standard test Stool Antigen Analysis (SAA). Where 6 (21%) patients were showing negative GS with main age of 32.50 and 22 (79%) were showing positive GS with main age of 44.31. Where 6 (21%) patients were showing negative UT with main age of 33.83and 22 (79%) were showing positive UT with main age of 43.95. In the gold standard test Stool Antigen Analysis 4 (14%) patients were showing negative result with main age of 34.75 and 24 (86%) were showing positive result with main age of 42.95. In all diagnostic investigations the mean male age was more than the mean female age. The female's prevalence of positive for *H. pylori* organism is more than male.

Darko *et al.,* (2015) demonstrate that, the relatively more females referred for upper gastrointestinal tract endoscopy compared to males. As they measure the *H. pylori* prevalence in two different time periods, there were significantly more males in the first time period (1999) compared to females, whereas females were in the majority in the second time period (2012), that the prevalence of *H. pylori* was highest among those aged between 21 and 50 years,

In the present study the diagnosis of *H. pylori* organism was confirmed with different diagnostic tests they were, Gram Stain (GS) and Urease Test (UT) against the golden standard test Stool Antigen. Detection of *H. pylori* with both gram stain and urease test were show the same figure against the gold standard stool antigen analysis where both were have the sensitivity of 87.5% and specificity of 75%. When testing the sensitivity of all tests used in *H. pylori* detection against positive biopsy the sensitivity of both gram stain and urease test

were 78.6% and for stool antigen analysis it was 85 7%.

By screening different studies concerning the prevalence of *H. pylori* infection only one test was applied per the study beside the golden standard test of endoscopy investigation as urease test was used in many studies, (Obayo *et al.,* 2015 &Adu-Aryee*et al.,* 2016). Darko *et al.,* (2015)use also urease test as its results are read between one minute and 24 hours (the sensitivity and specificity of these methods are in the region of 95%. Jiang*et al.,* (2016) useRUT (Rapid urease test kit) for *Helicobacter pylori* infection status determination with a sensitivity of 99 % and specificity of 100 % as a single test.

*H. pylori* antigen present in stool specimen (HpSAg) was detected by immuno-chromatographic method using cassette (Ansari *et al.,* 2016)withover 95 % sensitivity, and 94 % specificity. HpSAg is used for non-invasive and costs a fraction of what is usually charged for endoscopy. Oling *et al.,* (2015) diagnosis of *H. pylori* infection in biopsy specimen using the Modified Giemsa stain which is easy to interpret, inexpensive, and takes about 5 min to perform, and rarely requires repeat stains. It has a sensitivity of 98% and specificity of 90%.

## Antibiotic sensitivity to *H. pylori* growth

In the present study different antibiotic were used to test *H. pylori* response, they were Clarithromycin, Metronidazole, Tetracycline, Amoxicillin and Furazolidone. The sensitivity of each antibiotic was calculated and statically analyzed.

*H. pylori* response to both Clarithromycin and Metronidazole antibiotic sensitivity was giving the same figures. In respect to gender, 90.9 % of males and 88.2 % of females were sensitive to both antibiotics. Tetracycline antibiotic sensitivity was showing that 81.8 % of the males and 88.2 % of the females were sensitive to Tetracycline antibiotics. Amoxicillin antibiotic sensitivity was showing that, 81.8 % of the males and 70.6 % of the females were sensitive to Amoxicillin antibiotics. Finally Furazolidone antibiotic sensitivity was showing that, 100 % of the males and 76.5 % of the females were sensitive to Furazolidone antibiotics.

Darko *et al.,* (2015)given the importance of eradicating *H. pylori* infection in patients with peptic ulcer disease, it is vital that infection is treated optimally with a combination regime that has an acceptable high eradication rate in the region of 90%. The incidence of *H. pylori* infection in Ghana may be lower than previously reported due to changing socioeconomic factors, increased PPI and antibiotic useAdu-Aryee*et al.,* 2016). The burden of *H. pylori* infection among dyspeptic patients was high which decrease after antibiotic empirical treatment (Oling *et al.,* 2015).

In conclusion, from this study we found that, females were more susceptible to *H. pylori* infection than male. Stool antigen analysis was more sensitive than either gram stain or urease test but still not reaching 100 percent as biopsy culture do. *H. pylori* are more sensitive to Clarithromycin and Metronidazole while, Amoxicillin show less sensitivity in response to *H. pylori* infection. From the study we may recommend to detect *H. pylori* infection using molecular biology technique for detection of *H. pylori* gene to increase the sensitivity to *H. pylori* infection diagnosis.

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