The use of different laboratory methods in diagnosis of *Helicobacter pylori* infection; a comparative study

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Abstract: Background: *Helicobacter pylori* are well recognized as a major cause of gastrointestinal illnesses and gastric cancers. Therefore, the current study aimed to assess different methods for detection of *H. pylori* in the oral cavity (saliva and dental plaque) and in gastric biopsy among patients with gastric affection, as well as, detection of *H. pylori* antigen in stool, moreover, to evaluate the antibiotic susceptibility testing of the isolated strains.
Methods: Specimens were obtained from Endoscopy Unit, Internal Medicine Department, Faculty of Medicine, Beni Suef University Hospital, Egypt. Thirty patients were subjected to detailed history and different sampling; gastric biopsy, oral and stool samples. The oral and gastric samples were processed and cultured. Thereafter, microscopic examination and rapid urease tests (RUTs) were conducted. *H. pylori* antigen detection was carried out in the stool samples, as well as, susceptibility testing to several antibiotics for all isolates identified.
Results: The selected patients had a mean age of 36.23 ± 6.317 years. They included 17 males (56.7%) and 13 females (43.3%). 90% of the cases were found positive by culture of the gastric biopsies, while, 96.7% were positive in oral cultures. 92.5% of the gastric samples showed positive results by microscopic examination, however, RUTs were positive in 63.3% of the gastric samples and in 73.3% of the oral samples, meanwhile, 66.7% of patients were found positive by testing their stool for *H. pylori* antigens. The prevalence of resistance among gastric and oral isolates to Amoxycillin, Amoxycillin/Clavulinic acid, Ampicillin/Sulbactam, Clarithromycin , Tetracycillin and Metronidazole were; (3.7 and 17.2 %), (11.1 and 24.1 %), (11.1 and 20.7 %), (11.1 and 24.1 %), (25.9 and 37.9 %) and (96.3 and 100 %) respectively.
Conclusion: There is an evidenced association between gastric affection and oral *H. pylori* recognition that, even exceeds stool detection of *H. pylori* antigen. Moreover, continuous evaluation of antibiotic susceptibility should be carried out and clinicians should be aware about it to select the appropriate empiric regimen for *H. pylori* eradication.
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1. Introduction

Helicobacter pylori is a gram-negative bacterium that colonizes the gastric mucosa, and is associated with chronic gastritis, peptic ulcer disease, mucosa-associated lymphoid tissue lymphoma and gastric adenocarcinoma. Investigators found that chronic *H. pylori* infection is in approximately one-half of the world's population and is etiologically linked to 63% of all stomach cancer or approximately 5.5% of the global cancer burden, and approximately 25% of cancers associated with infectious etiology. They also suggested it would nearly double between the years 2005 and 2050 if no sufficient measures were taken to eradicate such infection ***(Megraud et al., 1993, NIH Consensus Development Panel, 1994, Mbulaiteye et al., 2009 and Ford and Axon, 2010).***

Around the world, the prevalence of *H. pylori* infection ranges from 20% to over 90% in adult populations. Infection rates average at about 30% in Western populations ***(Breckan et al., 2009 and Sykora et al., 2009),*** while, infection rates in Asian countries and in developing countries are higher and range from 60% to 90% ***(Prinz et al., 2005).*** Moreover, ***Mohammad et al. 2008*** found that, the overall prevalence of *H. pylori* infection was 72.38% among Egyptian school children.

Although *H. pylori* was first isolated nearly 30 years ago ***(Marshall and Warren, 1983)***, the process of infection, reinfection or human transmission remains unclear and there are few reports of its prevalence in the oral cavity of individuals with periodontal disease and gastric diseases ***(Riggio and Lennon, 1999).*** Many researchers have assumed that the primary extragastric reservoir for *H. pylori* is the oral cavity and may be the source of infection and transmission. The first documentation of the presence of *H. pylori* in the oral cavity was reported in 1989, when the bacterium was cultured from the dental plaque of one of 29 patients with *H. pylori* associated gastric disease ***(Krajden et al., 1989).*** Since then, some

reports indicated that *Helicobacter* may be present in oral cavity which can serve as a reservoir for bacteria and a source of gastric reinfection. *H. pylori* have also been detected by culture and PCR in both dental plaques and saliva ***(Veerman et al., 1997, Alm et al., 2000, Fritscher et al., 2004 and Sayed et al., 2011).***

Several diagnostic strategies for *H. pylori* are available. They can be divided into two groups: invasive and noninvasive tests. All invasive test methods are based on endoscopic examination during which biopsy specimens are obtained for direct (histological analysis, isolation) or indirect (urease test) diagnosis of *H. pylori* infection. Noninvasive methods reveal the presence of *H. pylori* by measuring the activity of urease (urea breath test), then by confirming the presence of antibodies in the serum. Meanwhile, other noninvasive tests were also evaluated in several studies including, detection of *H. pylori* antigens in stool and presence of *H. pylori* in the saliva ***(Ozdemir et al., 2001, Viara et al., 2002 [a] and Viara et al., 2002 [b]).***

However, culture and identifying *H. pylori* in gastric biopsy, still, the most definite method for identification. Nevertheless, it requires experience, accuracy and dexterity, as identification and culturing may sometimes be difficult and tedious process. In addition, the erratic distribution of *H. pylori* could also lead to flawed results. Microscopy and RUT 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***).

On the other hand, noninvasive tests for *H. pylori* are important in primary care, both for initial diagnosis of *H. pylori* infection and for confirmation of eradication. Current guidelines recommend noninvasive testing for diagnosis and treatment follow up of young dyspeptic patients without alarming symptoms as a primary care setting by using low-cost noninvasive tests ***(Vaira et al., 2002 [a])***, including oral cavity bacterial isolation and identification. Therefore, determining the type of strain prevalent in the oral cavity or saliva, it could be easy to diagnose the strain colonizing the gastric mucosa as reported by ***Li et al., 1996*** who demonstrated that same strain was present in both niches. Consequently, saliva specimens may potentially be reliable, and could serve as an effective and valuable noninvasive specimen to diagnose and monitor the efficacy of eradication therapy ***(Tiwari et al., 2005 and Sayed et al., 2011).***

Resistant strains of *H. pylori* are now widely prevalent in the United States and Europe, and eradication therapy with current regimens fails in 10% to 20% of patients ***(Vakil et al., 1998 and Megraud et al., 2012)***. *In vitro* antibiotic sensitivity for *H. pylori* should be done to guide the selection of antibiotic and to predict the clinical response to treatment aiming at successful eradication ***(Hunt et al., 2000)***.

Therefore, the current study aimed to assess different methods of detection of *H. pylori* in saliva and dental plaque of the oral cavity and in gastric biopsy among Egyptian patients with gastric affection, as well as, detection of *H. pylori* antigen in stool. Moreover, the investigation also evaluated the antibiotic susceptibility testing of the isolated strains in the present study.

2. Subjects and Methods

In the present study, specimens were obtained from Endoscopy Unit, Internal Medicine Department, Faculty of Medicine, Beni Suef University Hospital, Egypt, during the period from December 2008 to April 2009. Detailed personal history (including age, sex, occupation, smoking, etc.), patients complaints, family history, past history (especially any previous gastric complaint) as well as blood group type were recorded for each patient.

1- Selection criteria

The selected group of this study consisted of 30 patients referred for gastroscopy with upper GIT symptoms at the endoscopy unit. All subjects were more than 21 years of age and had received no previous treatment for ulcer or gastritis. Individuals who had received antibiotic treatment in the last 2 months, with history of alcohol abuse, having chronic debilitating disease, pregnant and lactating females were excluded from the study. The individuals who were included in the study showed good oral hygiene, although small amounts of supra-gingival plaque could be visually detected at the time of sampling, and proven to be with gastric affection in the form of ulcer, erosion or gastritis shown by gastroscopy. All individuals signed an informed consent in order to be included in the study group. Scientific Research Ethical Committee of Beni Suef Hospital approved the study. Control group of 20 (13 males and 7 females) individuals were included in the present study. They were free from any upper GIT symptoms, did not take any treatment for gastritis or ulcer before and had no antibiotics for the previous 2 months.

2-Sampling:

a- Oral specimens (saliva and dental plaque) were collected from each volunteer before undergoing an upper gastric endoscopy. The samples were collected from the patients and the control group, in sterile containers for performing culture for *H. pylori*. The dental plaque sampling was conducted using cotton swab and placed in a tube containing the saliva collected and treated as a single specimen. Unstimulated salivary flow was collected in amount of 1-2 ml in long tubes that can accommodate the dental plaque swabs ***(Tiwari et al., 2005).*** After centrifugation

(14,000 x g) for 10 min, the supernatant was transferred into a fresh microtube and stored at 70ºC for future analysis ***(Kignel et al., 2005).***

b- Gastric biopsy: two biopsies of the gastric antrum were collected, during upper gastric endoscopy, under complete aseptic condition in 2 sterile cups sized 2x2 cm^3 containing 1 ml of sterile saline ***(Bayerdorffer et al., 1989).*** One specimen was used for doing rapid urease test (RUT) and the other was used for direct Gram staining and culture of *H. pylori*. During endoscopic examination, patients were evaluated for the presence of gastritis, gastric ulcers or erosion to undergo the current investigation.

c- Stool samples were collected for performing stool antigen of *H. pylori* from the selected group as well as the control group.

3- Microbiological examinations:

The microbiological part of this work was performed at the Medical Microbiology & Immunology Department, Faculty of Medicine, Beni Suef University.

a- Rapid urease test (RUT):

This test was performed directly on the unprocessed gastric biopsies and oral samples, using urea soft agar tube, by dipping the sample into the soft agar. The agar was inspected at 30 minutes, 1, 2 and 24 hours for a colour change from yellow to pink indicating a positive result ***(Logan and Walker, 2001)***.

b- Stool antigen detection:

This was done for stool samples using "The One Step *H. pylori* Antigen Test Device (feces)", which is a qualitative test using immunoassay for detection of *H. pylori* antigens in human feces specimens providing results in 10 minutes.

c- Gastric biopsy processing:

Processing was done for biopsy specimens used for culture of *H. pylori*. A sterile automated mechanical homogenizer (house developed) with a speed of about 60-100 run/minute was used for gastric biopsy specimens processing for 2-3 minutes to mince the biopsy into very small pieces. This was followed by centrifugation at 6000 x g for 20 minutes ***(Logan and Walker, 2001)***.

i. Supernatants from the homogenized tissues: were used for direct Gram stain

ii. Deposits from the homogenized tissues: were used for culture of *H. pylori*, freshly prepared blood agar plates (containing citrated sheep blood and made selective by addition of Dent supplement ‹Oxoid; Basingstoke, Hampshire, England›) were used to perform the culture of *H. pylori* and were incubated at 37ºC for 3-7 days under microaerophilic conditions. Colonies were identified as *H. pylori* by its colonial morphology, typical appearance on Gram stain as well as catalase, oxidase and urease tests.

iii- Cultures were performed on saliva and dental plaque specimens: Culture and identification of *H. pylori* were performed as mentioned above.

iv- Antimicrobial susceptibility testing for *H. pylori:* Antibiotic sensitivity of the isolates was determined using the Kirby-Bauer antibiotic testing (KB testing or disk diffusion method) as recommended by the Clinical and Laboratory Standards Institute (CLSI, previously called NCCLS) (2006 guidelines). In this method, a microbial suspension equal to 4 McFarland turbidity (12×10^8CFU/ml) was prepared and cultivated on Muller-Hinton agar (Merck, Germany) supplemented with 10% sheep blood ***(Mishara et al., 2006)*** , using the following antibiotics; Amoxycillin (AML), Amoxycillin/Clavulinic acid (AMC), Unasyn (Ampicillin/Sulbactam) (SAM), Clarithromycin (CLR), Tetracycillin (TE) and Metronidazole (MTZ).

4- Statistical analysis:

Data were collected and analyzed statistically using Statistical Package for Social Sciences program (SPSS v16). The following tests were used in this study: mean, standard deviation, T test for independent samples, ANOVA test (analysis of variance). Significance levels: $P>0.05$ insignificant, $P<0.05$ significant and $P<0.001$ highly significant.

3. Results

The current investigation included 30 patients their ages ranged between 20 and 60 years, with mean age of 36.23 $\pm$ 6.317 years. The highest prevalence of *H. pylori* infection was noted among 31-40 Yrs age group as shown in table 1. The tested cases included 17 males (56.7%) and 13 females (43.3%). Moreover, 20 volunteers were enrolled as the control group, their ages ranged between 21 and 68 with mean age of 41.1$\pm$13.1Yrs, including 13 (65%) males and 7 (35%) females.

Table (1): Comparison between different age groups among the studied cases:

Age group (Yrs)	**No**	**Percent**
21-30	7	23.3
31-40	16	53.3
41-50	6	20
51-60	1	3.3
Total	30	100

Risk factors were assessed in the current study among the 30 patients with gastric affection including blood groups, smoking habit and occupational stress. All patients had occupational stresses; however, 50% of them were smokers. Regarding blood groups, 50% of the tested group was from the O group type, followed by A group

(26.7%) while, the B group patients represented 23.3% and none were from AB group blood type (Figure 1).

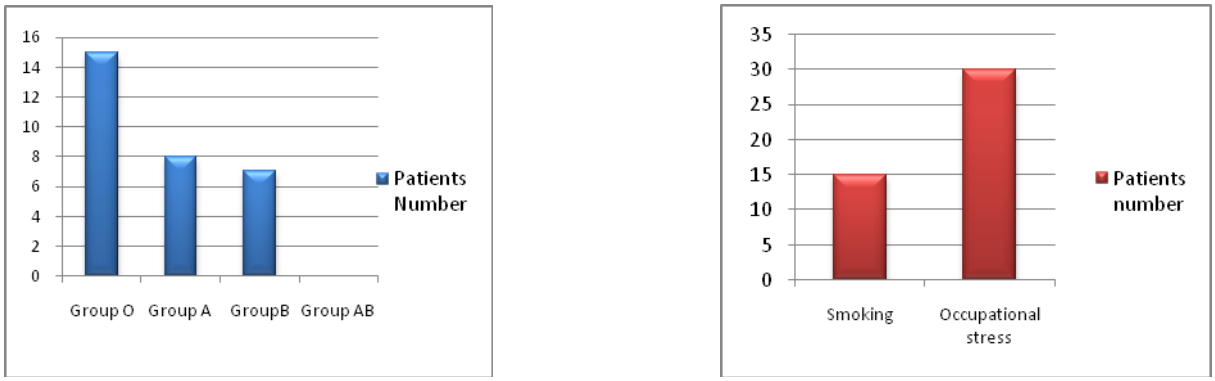

Figure 1: The distribution of risk factors among the tested group.

Out of the 30 patients examined, 27 (90%) were found positive by culture of the gastric biopsy. On the other hand, oral cultures were positive in 29 cases representing 96.7%. The correlation between the culture results is shown in table 2.

On comparing the culture results of the oral samples with the control group, it showed high significance ($p \leq 0.001$); as the cultures of the oral samples of the control group were positive in only 40% of the specimens (Figure 4). Specificity and sensitivity of the oral culture results compared with gastric cultures were 50% and 96.4% respectively.

Table (2): Results of culture of *H. pylori* in different specimens:

Oral specimens' culture	Gastric biopsies' culture		Total
	Positive	Negative	
Positive	26 (96.3%)	3 (100%)	29**(96.7%)**
Negative	1 (3.7%)	0 (0%)	1 (3.3%)
Total	27 **(90%)**	3 (10%)	30 (100%)

Direct microscopic examination was performed on the gastric biopsies showing positive results in 25/ 27 culture positive specimens representing 92.5%. Specificity and sensitivity of the results compared with gastric culture were 100% and 93.1% respectively.

Rapid urease test was performed on the gastric biopsy samples as well as oral specimens; it was found positive in 63.3% of the gastric samples and in 73.3% of the oral samples, meanwhile, represented 70.3% out of the 27 positive gastric cultures, and 75.8% out the positive 29 dental and saliva samples (table 3, Figure 2). The control group showed positive results of RUT in their saliva and dental samples in only 40% of the volunteers, which, when compared with the cases showed evident statistical significance representing $p \leq 0.01$ (Figure 4). Specificity and sensitivity of the RUT of gastric biopsies results compared with gastric culture were 100% and 77.1% respectively, nevertheless, RUT of oral samples when compared with oral culture, they showed specificity and sensitivity of 100% and 80.5% respectively.

Table (3): Results of Rapid Urease Test (RUT):

RUT of oral Specimens	Gastric biopsy RUT		Total
	Positive	Negative	
Positive	15 (78.9%)	7 (63.6%)	22**(73.3%)**
Negative	4 (21.1%)	4 (36.4%)	8 (26.7%)
Total	19 **(63.3%)**	11(36.7)	30 (100%)

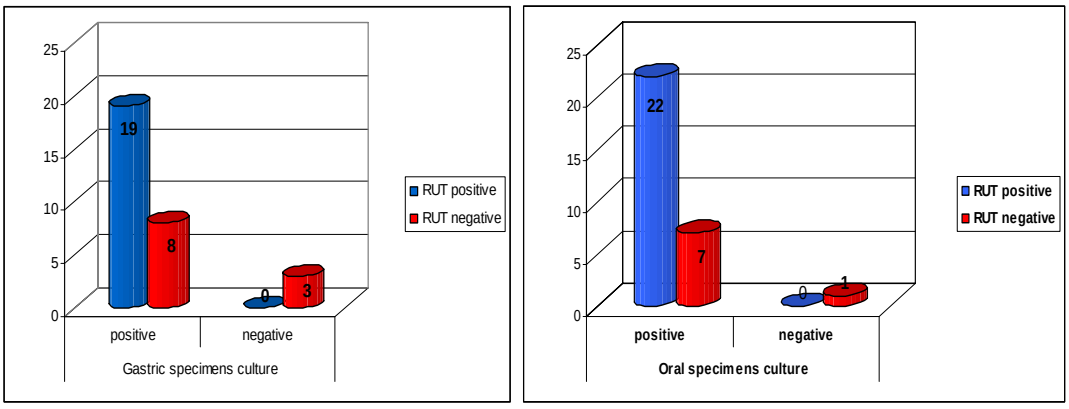

Figure 2: The results of RUTs *versus* culture results of gastric and oral samples.

The *H .pylori* isolates from gastric and oral samples were subjected to oxidase, urease and catalase tests. Among the isolates from gastric biopsies oxidase test was found positive in 25/27 (92.6%) gastric isolates, while, 28/ 29 (96.7%) oral yields were found positive by the test.

Urease test showed positive results in all gastric yields; however, it only gave positive results in 26 /29 (89.7%) isolates taken from oral samples. In addition, catalase test was found reactive in 23/27 (85.2%) gastric isolates, similarly in 25/29 (86.2%) oral *H .pylori* isolates (Figure 3).

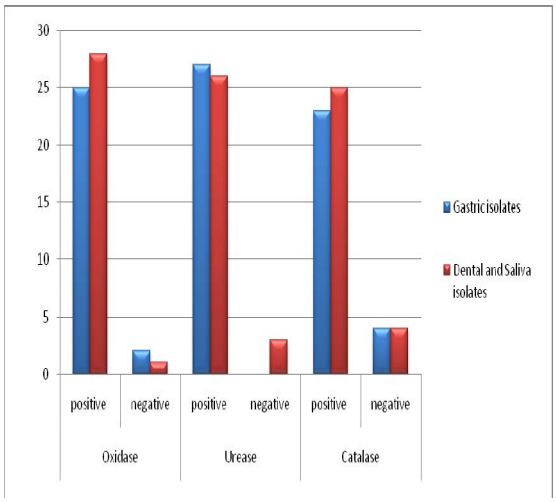

Figure 3: The results of biochemical reactions among the tested isolates.

Twenty patients were found positive by testing their stool for *H. pylori* antigens representing 66.7%. On comparing the cases with the control group it showed high significance ($p \leq 0.001$) (Figure 4). The correlation between the stool antigen results and gastric culture is shown in Table 4. Specificity and sensitivity of the stool antigen compared with gastric culture were 60% and 71% respectively.

Table (4): Results of stool antigen detection compared with gastric biopsy culture:

Gastric biopsy results	Results of stool antigen		Total
	positive	Negative	
Positive	18 (90%)	9 (90%)	27 (90%)
Negative	2 (10%)	1(10%)	3 (10%)
Total	20 **(66.7)**	10 (33.3)	30 (100%)

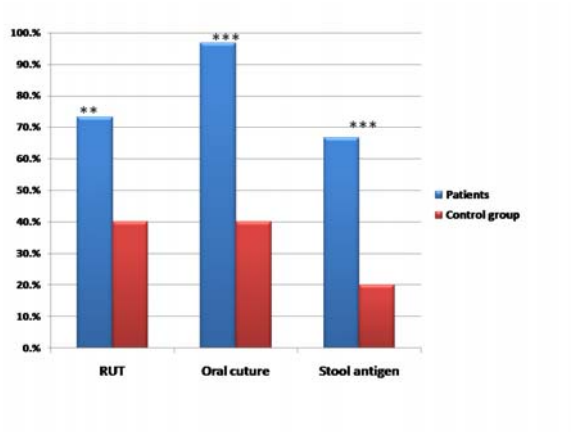

Figure 4: The correlation between the positive results of patients' specimens and the control group samples. The values are expressed in % of positive results. ***P < 0.001 **P < 0.01.

All the isolates from gastric biopsy and oral specimens were subjected to antibiotic sensitivity testing on Muller Hinton sheep blood agar to the following antibiotics: amoxycillin, amoxycillin clavulinic, ampicillin salbactam, clarithromycin, tetracycline and metronidazole.

The isolated strains of gastric biopsy samples showed high sensitivity to amoxicillin followed by clatithromycin, while, showed least sensitivity to metronidazole (3.7%).

Meanwhile, the oral isolates showed the highest sensitivity to amoxicillin, amoxicillin clavulinic and clatithromycin. The strains were completely resistant to metronidazole (Table 5, Figure 5). Antimicrobial resistance was not statistically significantly associated with sex or age (p >0.05).

Table (5): Antibiotic susceptibility patterns of gastric and oral isolates:

	Gastric strains (Isolates number=27)						**Oral strains (Isolates number=29)**					
	Sensitive		**Resistant**		**Intermediate**		**Sensitive**		**Resistant**		**Intermediate**	
	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%
AML	26	96.3	1	3.7	0	0	24	82.8	5	17.2	0	0
AMC	15	55.6	3	11.1	9	33.3	15	51.7	7	24.1	7	24.2
SAM	14	51.9	3	11.1	10	37	11	37.9	6	20.7	12	41.4
CLR	16	59.3	3	11.1	8	29.6	13	44.8	7	24.1	9	31.1
TE	13	48.2	7	25.9	7	25.9	11	37.9	11	37.9	7	24.2
MET	1	3.7	26	96.3	0	0	0	0	29	100	0	0

AML: Amoxycillin, AMC: Amoxycillin clavulinic, SAM: Ampicillin salbactam, CLR: Clarithromycin, TE: Tetracycline, MET: Metronidazole.

A. B.

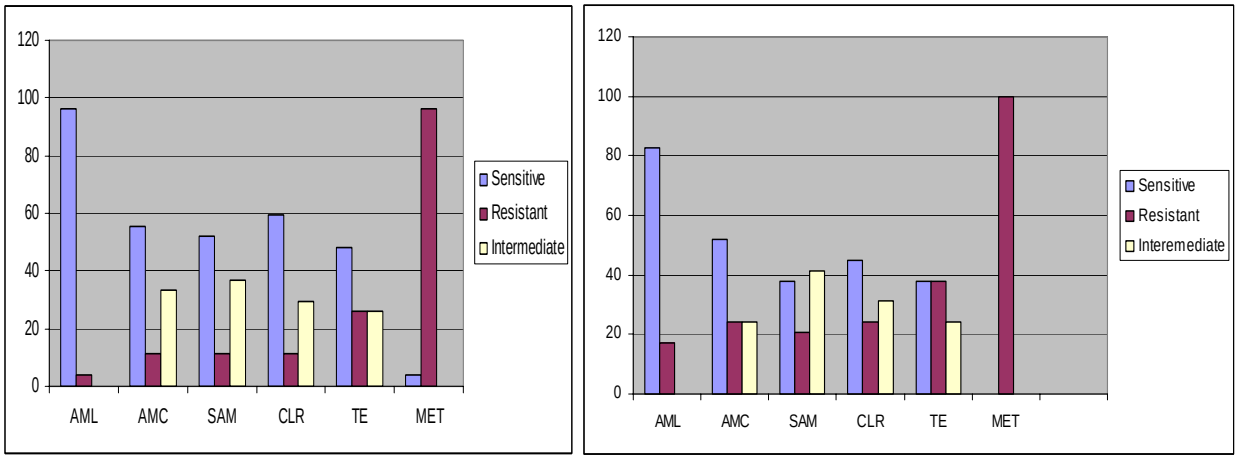

Figure (5): A. Antibiotic sensitivity pattern of the gastric isolates, **B.** antibiotic sensitivity pattern of the oral strains

On comparing the oral and gastric isolates, 17 (65.3%) showed good matching regarding their colony morphology, biochemical reactions and antibiogram. However, 5 (19.2%) yields showed minor differences in 2 or 3 of the tested antibiotics, meanwhile, 4 (15.3%) of them were completely different in 4 or more of the tested reactions, antibiogram and/ or colony morphological characters.

4. Discussion

H. pylori have been designated as key organisms in the etiology of chronic gastritis, peptic ulcers ***(Megraud, 1993)*** and gastric cancer ***(NIH Consensus Development Panel, 1994 and Mbulaiteye et al., 2009)*** and their suppression and elimination has been considered the gold standard therapy for infectious gastric diseases.

The current study included 30 patients with upper GIT symptoms proven to be with gastric affection in the form of ulcer, erosion or gastritis shown by gastroscopy, subjected to Outpatient Clinic of Internal Medicine and Endoscopy Unit, Faculty of Medicine, Beni Suef University.

Ages of the study participants ranged between 20 and 60 years, with mean age of 36.23 $\pm$ 6.317 years. There was association between 31-40 Yrs age group and infection density, though it did not reach statistical significance (Table 1). These results were comparable to European studies which reported correlation between age and gastric affection ***(Breckan et al., 2009 and Jackson et al., 2009)***; however, these studies found increased prevalence of gastritis and *H. pylori* colonization with increasing age.

A higher level of gastric affection and *H. pylori* isolation was observed among male patients (56.7%) rather than females (43.3%). This may be due to the poor oral hygienic conditions and tobacco smoking. This was in agreement with ***Lindella et al., 1991*** who stated that there is an epidemiological evidence of an association between cigarette smoking and gastritis. Our results also agreed with those reported by ***Khulusi et al. 1995*** who assessed the same risk factors (sex and smoking), nevertheless, many studies contradicted these findings and found no such relation ***(Roma et al., 2009 and Zhang et al., 2009).***

In the present study, 50% of the tested group was from the O blood group, followed by A group (26.7%) while, the B group patients represented 23.3%. None of the patients were from the AB group (Figure 1). Though the highest prevalence was among the O group patients, it did not reach statistical significance among the patients or in relation to *H. pylori* infection. The same finding was reported by ***Demir et al., 2009 and Petrovic et al., 2011*** who stated that different ABO blood groups may show insignificant rates in the colonization numbers of the bacteria (p value > 0.05). However, this contradicted with ***Bhuiyan et al., 2009*** who stated that children with blood group "A" were more susceptible to *H. pylori* infection than those with other ABO blood groups. In the current study, the cause of higher incidence among the O group patients were not clear and the etiology of gastric affection is unknown, nevertheless, some studies have justified these findings by similarities between blood cell surface proteins and surface proteins on bacteria that live in the intestines of normal humans. Patients with blood type O have antibodies against A and B antigens, so, they have antibodies against these surface proteins. *Helicobacter* and other bacteria in the gut have type A and B surface proteins. Therefore, antibodies that react strongly with *Helicobacter* cause more swelling and inflammation, more stomach pain, more belching and more ulcers ***(Araya et al., 2000 and Alkout et al., 2000).***

It was observed in our study that psychological, occupational stress and smoking were considered as a risk factor of peptic diseases caused by *H. pylori* infection. This was consistent with the findings of ***Levenstein, 1998*** who reported that psychological

stress and smoking probably functions most often as a cofactor with *H. pylori*. In addition, four studies conducted in European populations among adults in the United Kingdom revealed that, male gender, increasing age, tobacco use and lower socioeconomic status were all significantly associated with positive *H. pylori* colonization (***Jackson et al., 2009).*** Therefore, *H. pylori* may be inadequate as a sole explanation for peptic ulcers.

Different diagnostic measures were performed in the current investigation and compared with the mostly used method for diagnosis; culture of gastric antral biopsy on selective media. They included RUT, microscopic examination, and culture of oral (dental plaque, saliva) samples, biochemical reactions for the positive isolates as well as stool antigen detection.

The present findings showed high positivity rate of *H. pylori* colonization in both gastric and oral samples in the examined group, in a percentage of 90% and 96.7% respectively (Table 2). On comparing the culture results of the oral samples with the control group, it showed high significance ($p \leq 0.001$) (Figure 4). Specificity and sensitivity of the oral results compared with gastric culture were 50% and 96.4% respectively. These results may justify the question raised by many studies, whether the oral cavity is a reservoir for gastric *H. pylori* infection or not ***(Loste et al., 2006 and Bürgers et al., 2008).*** Our results were comparable to those of another study done in Egypt as well; it revealed 100% prevalence in patients with gastric affection and showed specificity and sensitivity of 50% and 100% respectively ***(Sayed et al., 2011).*** This same study found also high significance when compared patients with gastric pathology with another group with no gastric affection ($p \leq 0.0017$). However, many other studies showed much lower rates of *H. pylori* colonization among dyspeptic patients, patients with gastric pathology and even among the control group with no gastric complaints ***(Souto and Colombo, 2008 and Silva et al., 2009).***

These contradictions in the current findings from other investigations may be explained by local environmental factors which influence the establishment and composition of the plaque needed for microbial adhesion to tooth surfaces. Environmental conditions are not uniform and the microbial composition at the site depends on the outcome of a variety of host-microbial and microbial-microbial interactions ***(Scheie, 1994)***.

The higher prevalence of oral *H. pylori* in rural community, such as presented in the current investigation, may be explained by the characteristics of the patients investigated, little access to standard medical and dental care, close contact, overcrowding, poor sanitation, low level of education and low level of socio-economic status ***(Al Asqah et al., 2009)*** or may be due to iatrogenic transmission during the poor dental care service ***(Madinier et al., 1997).***

For patients undergoing upper gastrointestinal endoscopy, RUT is considered to be a cheap and a reliable test that allows screening of *H .pylori* infection ***(Perri, 2003).*** In the present study, 19 isolates (70.3%) out of the 27 positive gastric yields were found reactive by RUT, meanwhile, it was positive in 22 (75.8%) out the positive 29 dental and saliva samples (Table 3, Figure 2). On comparing the culture results with the control group, it was statistically significant ($p \leq 0.01$) (Figure 4). Specificity and sensitivity of the RUT of gastric biopsies results compared with gastric culture were 100% and 77.1% respectively, nevertheless, RUT of oral samples when compared with the oral culture, they showed specificity and sensitivity of 100% and 80.5% respectively. These results were comparable to those of ***Pajares et al.(1998)*** who found that the sensitivity and specificity of the rapid urease test ranged between 80 and 90%. Negative RUTs in our study may be explained that patients were in acute phase of ulcer bleeding ***(Schilling et al., 2003)***, or patients were on acid reducing drugs since recent use of the latter can cause false negative results. RUTs should be carried out 4 weeks after the completion of therapy ***(Yakoob et al., 2008).***

In the present study, *H. pylori* yields from gastric biopsies as well as oral samples had undergone several biochemical reactions; urease, oxidase and catalase tests. The results of oxidase test were positive in 92.6% of the gastric biopsy samples and in 96.7% of oral samples. While, urease test were 100% positive in gastric biopsy samples and 89.7% in oral samples. However, catalase test was 85.2% positive in gastric biopsy samples and 86.2% in the oral samples (Figure 3).

The results of "The One Step *H.pylori* Antigen Test Device (Feces)" revealed that 20 out of the 30 patients were found positive by testing their stool for *H. pylori* antigens, representing 66.7%. On comparing the cases with the control group it showed high significance ($p \leq 0.001$) (Figure 4). Specificity and sensitivity of the stool antigen compared with gastric culture were 60% and 71% respectively. This was different from the findings of ***Forné et al.(2000***), ***Manes et al.(2001)and Krausse et al.(2008)*** who stated that *H. pylori* stool antigen represents a sensitive test and suitable for detecting *H. pylori* infection with sensitivity similar to that obtained with other standard tests. Also these results were different from those of ***Vaira et al., 2002 [b]*** who reported that the stool antigen detection of *H. pylori* had a sensitivity of 94% and a specificity of 97% before and after treatment. However, ***Gulcan et al., 2005*** found similar results with a percentage of 61% of the selected group; nevertheless, the specificity and sensitivity were higher than the current findings. These discrepancies in the results may be due to the variability in the tests used and/or

following certain antibiotic treatments that the patients did not reveal, which may cause the level of *H. pylori* antigens to decrease to the concentration below the minimum detection level of the test ***(Soll. 1990, Anand et al.1996 and Cutler. 1996).***

Treatment of *H. pylori* infection is becoming a very relevant problem especially in the developing countries. Although different therapeutic regimens are currently available, treatment failure remains a growing problem in daily medical practice. Several factors could play a role in the eradication failure, but the most relevant are antibiotic resistance and patient's compliance**.** In our study, it was found that *H. pylori* strains were almost completely resistant to metronidazole in both gastric biopsy and oral isolates. This came in accordance with ***Torres et al. (2001)*** who reported that resistance to mertonidazole is even more prevalent in the developing countries, where up to 95% of isolates may be resistant. Resistance to metronidazole may be explained by the frequent use of nitonidazole derivatives (metronidazole, ornidazole) in treatment of amebiasis and giardia infections which renders the bacteria resistant against the drugs ***(Zwet et al 1994).*** Regardless of the reason, it is now clear that metronidazole should not be included in treatment regimens for *H. pylori* in Egypt ***(Sherif et al., 2004).***

In the present study, the resistant rate was the least among isolates treated with amoxicillin among gastric yields (3.7%), while, it was higher among oral strains (17.2%). Whereas ***Kato (2002)*** reported 0% resistance to amoxicillin, higher resistance rates were reported in a study done in Iran (11.6 %) ***(Milani et al., 2012).*** On the other hand, resistance to clarithromycin was found to be 11.1% in gastric biopsy samples and 24.1% in oral samples, these findings agreed with the result reported by ***Fijen (2003)*** who stated 9.1% resistance rate among subjects of African descent. Resistance to clarithromycin is becoming more prevalent in some European countries, where the prevalence may be as high as 17% ***(Romano and Cuomo, 2004 and Megraud et al., 2012)***.

Moreover, it was found that *H. pylori* were less susceptible to tetracycline, about 48.2% sensitivity rate in gastric biopsy samples and 44.8% in oral samples. This susceptibility to tetracycline was in a disagreement with ***Kim et al (2003)*** findings. They reported that *H. pylori* resistance to tetracycline was only 6.8% of the isolated strains. However, higher resistance rates have been reported in other studies from Iran ***(Milani et al., 2012).*** Interestingly, in the work of ***Van der Wouden et al., 1999*** isolates exhibited cross-resistance to metronidazole, and the cross-resistance could be transferred to tetracycline-susceptible *H. pylori* strains, which may be comparable with our findings**.**

Amoxicillin clavulinic and ampicillin salbactam showed moderate sensitivity rates among the isolates with better susceptibility among the gastric isolates rather than oral yields in the following order; 55.6, 51.9% in gastric strains and 51.7, 37.9% in oral isolates. Antimicrobial resistance showed no statistical significance in association with certain sex or age groups, which agreed with the work of ***Milani et al., 2012*** who concluded the same results in their study.

Many characteristics were different between the gastric and oral isolates regarding colony morphology, biochemical reaction and it was even clearer in their antibiotic susceptibility patterns. This may be explained by the environmental inconsistency between the oral and gastric habitats and colonization and biofilm formation. This variability may affect the genetic presentation of the bacteria or their gene expression as suggested by many studies ***(Cole et al., 2004, Pattiyathanee et al., 2009 and Andersen and Rasmussen, 2009).***

5. Conclusion and Recommendations

In conclusion, there is no one non invasive test enough to diagnose *H. pylori* infection and subsequently follow up of treatment outcomes, nevertheless, saliva and dental plaque culture propose a good prediction tool associated with gastric affection caused by *H. pylori* infection, with fairly good specificity and sensitivity, that was found better than that of stool antigen detection in the current study. Moreover, appropriate eradication of *H. pylori* is necessary as many gastric cancers could be prevented by elimination of *H. pylori.* Antibiotic susceptibility patterns vary widely in different time and place, therefore, continuous evaluation should be carried out and clinicians should be aware about it to select the appropriate empiric regimen for *H. pylori* therapy.

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References

Al Asqah M, Al Hamoudi N, Anil S, Al Jebreen A, *et al*. (2009). Is the presence of *H. pylori* in dental plaque of patients with chronic periodontitis a risk factor for gastric infection? Can J Gastroenterol; 23(3):177-9.

Alkout AM, Blackwell CC, Weir DM *et al*. (2000). Increased inflammatory responses of persons of blood group O to Helicobacter pylori. Journal of Infectious Diseases, 181(4): 1364-1369

Alm RA, Bina J, Andrews BM, Doig P, *et al*. (2000). Comparative genomics of *Helicobacter pylori*: analysis of the outer membrane protein families. Infect Immun; 68:4155–68.

Anand BS, Raed AK, Malaty HM, *et al*. (2000). Prevelance of *H. pylori* in normal individual with *H.pylori* infection. Am J Gasteroenterol, 91:1112-1115.

Andersen LP and Rasmussen L (2009). *Helicobacter pylori*-coccoid forms and biofilm formation. FEMS Immunol Med. Microbiol; 56(2):112-5.

Araya JC, Villaseca MA, Roa I, *et al*. (2000). Helicobacter pylori and chronic gastritis: Relationship between infection and inflammatory activity in a high risk population for gastric cancer. Revista Medica de Chile; 128(3):259-265.

Bayerdorffer E, Oertel H, Lehn N, *et al*. (1989). Topographic association between active gastritis and *Campylobacter pylori* colonisation. J. Clin. Pathol. 42:834–839.

Bhuiyan TR, Qadri F, Saha A, *et al*. (2009). Infection by *H. pylori* in Bangladeshi children from birth to two years: relation to blood group, nutritional status, and seasonality. Pediatr Infect Dis J; 28(2):79-85.

Breckan RK, Paulssen EJ, Asfeldt AM, *et al*. (2009). The impact of body mass index and Helicobacter pylori infection on gastro-oesophageal reflux symptoms: a population-based study in Northern Norway. Scand J Gastroenterol; 44:1060–6.

Burgers R, Schneider-Brachert W, Reischl U, *et al*. (2008). *Helicobacter pylori* in human oral cavity and stomach. Eur J Oral Sci; 116:297–304.

Clinical and Laboratory Standards Institute (2006). Performance standards for antimicrobial susceptibility testing, 16th informational supplement, M100-S16. Wayne, PA: CLSI, 2006.

Cole SP, Harwood J, Lee R, *et al*. (2004). Characterization of Monospecies Biofilm Formation by *Helicobacter pylori*. J Bacteriol; 186(10): 3124–3132.

Cutler AF. (1996). Testing for *H. pylori* in clinical practice. Amj. Med; 100:35S-41S.

Demir T, Uslu H, Orbak R, *et al*. (2009). Effects of different blood groups on the reproduction of periodontal pocket bacteria. - Int Dent J; 59(2):83-6.

Fijen CA. (2003). Antibiotic resistance of *H. pylori*: across-sectional study in consecutive patients, and relation to ethinity. Clin Microbiol Infect; 9:600-604.

Ford AC and Axon ATR. (2010). Epidemiology of *Helicobacter pylori* infection and Public Health Implications. *Helicobacter* 15 (Suppl. 1): 1–6

Forné M, Domínguez J, Fernández-Bañares F, *et al*. (2000). Accuracy of an enzyme immunoassay for the detection of *H. pylori* in stool specimens in the diagnosis of infection and post-treatment check-up. : Am J Gastroenterol; 95(9):2200.

Fritscher AM, Cherubini K, Chies J, *et al*. (2004). Association between *Helicobacter pylori* and recurrent aphthous stomatitis in children and adolescents. J Oral Pathol Med; 33:129–132.

Gulcan EM, Varol A, Kutlu T, *et al*. (2005). *Helicobacter pylori* Stool Antigen Test. Indian Journal of Pediatrics, 72:675-678.

Hunt RH, Smaill FM, Fallone CA. *et al*. (2000). Implications of antibiotic resistance in management of *H. pylori* infection. Can. J. Gastroenterol; 14 (19): 862-868.

Jackson L, Britton J, Lewis SA, *et al*. (2009). A population-based epidemiologic study of *Helicobacter pylori* infection and its association with systemic inflammation. *Helicobacter*; 14:460–5.

Kato S, Fujimura S, Udagawa H.*et al*. (2002). Antibiotic resistance of *H. pylori* strains in Japanese children. Journal of clinical Microbiology; 40:649-653.

Kignel S, de Almeida Pina F, Andre´ EA, *et al*. (2005). Occurrence of *Helicobacter pylori* in dental plaque and saliva of dyspeptic patients. Oral Dis; 11(1):17–21.

Kim JJ, Kim GJ and Kuwon DH. (2003). Mixed-Infection of Antibiotic Susceptible and Resistant *Helicobacter pylori* Isolates in a Single Patient and Underestimation of Antimicrobial Susceptibility Testing. *Helicobacter*; 8(3): 202-206.

Khulusi S, Mendel MA, Patel A .*et al*. (1995). *H. pylori* infection density and gastric inflammation in duodenal ulcer and non ulcer subjects. Gut; 37:319-324.

Krajden S, Fuksa M, Anderson J, *et al*. (1989). Examination of human stomach biopsies, saliva, and dental plaque for Campylobacter pylori. J Clin Microbiol; 27:1397–1398.

Krausse R, Muller G and Doniec M. (2008). Evaluation of a Rapid New Stool Antigen Test for Diagnosis of *Helicobacter pylori* Infection in Adult Patients._JOURNAL OF CLINICAL MICROBIOLOGY; 46(6):2062–2065.

Levenstein S. (1998). Stress and peptic ulcer: life beyond *Helicobacter*. BMJ; 316(7130):538-541.

Lindella G, Hesselvikb M, Schalénc C, *et al*. (1991). *H. pylori*, Smoking and Gastroduodenitis; 49(4):192-197.

Li C, Ha T, Ferguson DA, *et al*. (1996): A newly developed PCR assay of *H. pylori* in gastric biopsy, saliva and feces. Evidence of high prevalence of *H. pylori* in saliva supports oral transmission. Dig Dis Sci; 41:2142-2149.

Logan RP and Walker MM. (2001). ABC of the upper gastrointestinal tract: Epidemiology and diagnosis of *Helicobacter pylori* infection. BMJ; 323(7318):920–2.

Loster B, Majewski S, Czensikiewicz-Guzik M, *et al*. (2006). The relationship between the presence of *Helicobacter pylori* in the oral cavity and

gastritis in the stomach. J Physiol Pharmacol; 57:91–100.

Madinier IM, Fosse TM and Monteil RA. (1997). Oral carriage of *H. pylori*: a review. J Periodontol; 68(1):2-6.

Manes G, Balzano A, Iaquinto G, *et al*. (2001). Accuracy of the stool antigen test in the diagnosis of *H. pylori* infection before treatment and in patients on omeprazole therapy. Aliment Pharmacol Ther; 15(1):73-9.

Marshall BJ and Warren JR. (1983). Undentified curved bacilli on gastric epthelium in active chronic gastritis. Lancet; 1:1273–5.

Mbulaiteye SM, Hisada M, El-Omar EM. *et al*. (2009) *Helicobacter pylori* associated global gastric cancer burden. Front Biosci; 14:1490–504.

Megraud F. (1993). Epidemiology of *Helicobacter pylori* infection. Gastroenterol Clin North Am; 22:73–88.

Megraud F, Coenen S, Versporten A, *et al*.; on behalf of the Study Group participants. (2012). *Helicobacter pylori* resistance to antibiotics in Europe and its relationship to antibiotic consumption. Gut. 2012 May 12. [Epub ahead of print]

Milani M, Ghotaslou R, Akhi MT, *et al*. (2012) The status of antimicrobial resistance of *Helicobacter pylori* in Eastern Azerbaijan, Iran: comparative study according to demographics. J Infect Chemother. 2012 May 12. [Epub ahead of print]

Mishara K, Srivastava S, Garg A and Ayyagari A. (2006). Antibiotic susceptibility of *Helicobacter Pylori* clinical isolats: Comparative evalution of disk-diffusion and E-test methods. Current Microbiol.; 53: 329-34.

Mohammad M, Hussein L, Coward A and Jackson S. (2008). Prevalence of *Helicobacter pylori* infection among Egyptian children: impact of social background and effect on growth. Public Health Nutr;11(3):230–6.

NIH Consensus Development Panel. (1994). *Helicobacter pylori* in peptic ulcer disease. J Am Med Assoc; 272:65–9.

Ozdemir A, Mas MR and Sahin S. (2001). Detection of *Helicobacter pylori* colonization in dental plaques and tongue scrapings of patients with chronic gastritis. Quintessence Int;32:131–4.

Pajares-García JM. (1998). Diagnosis of *H. pylori*: invasive methods. Ital J Gastroenterol Hepatol; 30 Suppl 3:S320-3.

Pattiyathanee P, Vilaichone R and Chaichanawongsaroj N. (2009). Effect of curcumin on *Helicobacter pylori* biofilm formation African Journal of Biotechnology; 8(19):5106-5115.

Perri F. (2003). *Helicobacter pylori* infection: the diagnostic dilemma is still going on! Dig Liver Dis; 35(2):71-2.

Petrovic M, Artiko V, Novosel S., *et al*. (2011). Relationship between *Helicobacter pylori* infection estimated by 14C-urea breath test and gender, blood groups and Rhesus factor. Hell J Nucl Med; 14(1): 21-24

Prinz C, Schwendy S and Voland P. (2005). *H pylori* and gastric cancer: shifting the global burden. World J Gastroenterol; 12:5458–64.

Riggio MP and Lennon A. (1999). Identification by PCR of *Helicobacter pylori* in subgingival plaque of adult periodontitis patients. J Med Microbiol; 48(March (3)):317–22.

Roma E, Panayiotou J, Pachoula J, *et al*. (2009). Intrafamilial spread of *Helicobacter pylori* infection in Greece. J Clin Gastroenterol; 43:711–5.

Romano M and Cuomo A. (2004). Eradication of *H. pylori*: A Clinical Update. MedGenMed.; 6(1): 19.

Sayed MM, Ibrahim WA, Abdel-bary SA, *et al*. (2011). Salivary PCR detection of *Helicobacter pylori* DNA in Egyptian patients with dyspepsia. The Egyptian Journal of Medical Human Genetics 12, 211–216.

Scheie AA. (1994). Mechanisms of dental plaque formation. Adv Dent Res; 8(2):246-253.

Schilling D, Demel A, Adamek HE, *et al*. (2003). Negative RUT is unreliable for exclusion of *H. pylori* infection during acute phase of ulcer bleeding. Dig. Liver Dis, 35(4):215-216.

Sherif M, Mohran Z, Fathy H, *et al*. (2004). Universal high-level primary metronidazole resistance in *H. pylori* isolated from children in Egypt. J Clin Microbiol; 42(10):4832-4.

Silva DG, Stevens RH, Macedo JM, *et al*. (2009). Detection of cytotoxin genotypes of *Helicobacter pylori* in stomach, saliva and dental plaque. Arch Oral Biol; 54:684–8.

Soll AH. (1990). Pathogenesis of peptic ulcer and implication for therapy. New England J.Med; 322:909-16.

Souto R and Colombo A. (2008). Detection of *Helicobacter pylori* by polymerase chain reaction in the subgingival biofilm and saliva of non-dyspeptic periodontal patients. J Periodontol; 79:97–103.

Sykora J, Siala K, Varvarovska J, *et al*. (2009). Epidemiology of *Helicobacter pylori* infection in asymptomatic children: a prospective population-based study from the Czech Republic. Application of a monoclonal-based antigen-in stool enzyme immunoassay. *Helicobacter*;14:286–97.

Tiwari S, Khan A, Ahmed K, *et al*. (2005). Rapid diagnosis of *Helicobacter pylori* infection in dyspeptic patients using salivary secretion: a

non-invasive approach. Singapore Med J; 46(5):224–8.

Torres J, Camorlinga-Ponce M,Perez-prerz G. *et al*. (2001). Increasing multidrug resistance in *H. pylori* strains isolated from children and adults in Mexico.J.Clin.Microbiol; 39(7):2677-2680.

[a]Vaira D, Gatta L, Ricci C and Miglioli M. (2002). Review article: diagnosis of *Helicobacter pylori* infection. Aliment Pharmacol Ther; 16(1):16–23.

[b]Vaira D, Vakil N, Menegatti M, *et al*. (2002). The Stool Antigen Test for Detection of *H. pylori* after Eradication Therapy. Ann Intern Med; 19, 136(4):280-287.

Vakil N, Hahn B and McSorley D. (1998). Clarithromycin-resistant *H. pylori* in patients with duodenal ulcer in the United States. Am J Gastroenterol; 93: 1432-1435.

Van der Wouden EJ, Thijs JC, Sluiter WJ, and Kleibeuker JH. (1999).The influence of in vitro nitroimidazole resistance on the efficacy of nitroimidazole-containing anti-*H. pylori* regimens: a meta-analysis. Am. J. Gastroenterol; 94: 1751–1759.

Veerman EC, Bank CM, Namavar F, *et al*. (1997). Sulfated glycans on oral mucin as receptors for *Helicobacter pylori*. Glycobiology; 7:737–43.

Yakoob J, Jafri W, Abbas Z, *et al*. (2008). The Diagnostic Yield of Various Tests for *H. pylori* Infection in Patients on Acid-Reducing Drugs, ,Volume 53, Number 1; 95-100.

Yoshida H, Hirota K, Shiratori Y, *et al*. (1998): Use of a gastric juice-based PCR assay to detect *H. pylori* infection in culture-negative patients. J Clin Microbiol; 36:317-20.

Zhang D-H, Zhou L-Y, Lin S-R, *et al*. (2009). Recent changes in the prevalence of *Helicobacter pylori* infection among children and adults in high- or low-incidence regions of gastric cancer in China. Chin Med J; 122:1759–1763.

Zwet AA, Thıjs JC and Vrıes WS. (1994). In vitro studies on stability and development of metronidazole resistance in *H. pylori*. Antimicrob Agents Cheomother; 38: 360-2.

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