Risk Factors Associated with *Helicobacter pylori* Infection in Gaza, Palestine

By

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Declaration

“I herby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another nor material which to a substantial extent has been accepted for the award of any other higher degree or diploma of the university or other institute of higher learning, except where due acknowledgement is made in the text”

Author

Rana Mohammed Abu-M Hughesieb

Signature: Rana Date: May- 2007

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Abstract

**Background:** *Helicobacter pylori* (*H. pylori*) infection is usually acquired in early childhood. *H. pylori* infection is associated with several upper gastrointestinal disorders. Local data on the epidemiology of the infection are scarce in Palestine. The purpose of this study is to measure the rate and to explore the associated factors among the population living in Gaza strip.

**Method:** This study included eighty nine randomly selected participants from non-hospitalized patients. Age, sex, socioeconomic status and other potential risk factors were assessed using a structured interview. Ultra Rapid Urease Test was performed on biopsy specimens followed by histology examined with Methylene blue stain, HpSAg test to detect antigen in stool specimen and *Hp* IgM antibody was measured in blood using ELISA technique.

**Results:** The study subjects comprised of 89 participants. Age ranged between 13-77 years, with mean age 37.03, (37.1%) were females and (62.9%) were males. The rate of *H. pylori* infection was (48.3%). There were variations between the different tests. URUT was easily performed, reliable and non-expensive test; HpSAg test was non invasive, simple and could be used for the diagnosis of *H. pylori* infection, histology by using methylene blue stain and serology by detection IgM antibody in blood. In crude analysis, the rate was associated with type of drinking water during childhood with $P$ value =0.018. *H. pylori* infection showed no significant correlation with age, sex, weight, marital status, smoking, education level, coffee drinking, oral hygiene, socioeconomic status including number of persons living in the accommodation, number of persons in each room, income, type of accommodation, contact with animals, travelling abroad, consumption of drugs and antibiotics. Tea drinking proved to be a protective factor against *H. pylori* infection.

**Conclusion:** The results of this work supported the hypothesis that *H. pylori* acquisition occurs early in childhood and persist throughout life. In addition, *H. pylori* infection appears to be multifactorial. Tea proved to have a protective effect against *H. pylori* infection.

**Keywords:** *H. pylori*, URUT, HpSAg, ELISA, Biopsy specimen, socioeconomic status, Gaza.
بحث

تمهيد: العدوى ببكتيريا (Helicobacter pylori) عادة ما تكتسب في الطفولة المبكرة، هذه العدوى يصاحبها مجموعة من الأمراض في الجزء العلوي من الجهاز الهضمي. لا يوجد معلومات وثبت أن هذه البكتيريا والأمراض التي قد تصاحبها في فلسطين. الغرض من هذه الدراسة تجديد عوامل الخطر التي قد تزيد من الإصابة بهذه البكتيريا.

طريقة البحث: في هذه الدراسة تم اختيار 89 مريضًا من عدة مستشفيات في قطاع غزة. كل مريض خضع لاستaneous تنقل بالعمر، نظام حياة، تاريخه المرضي بالإضافة لعدة أسئلة تشمل الوضع الاقتصادي. تم أخذ 3 عينات من كل مريض عينة دم لفحص IgM وعينة خزعة من المعدة أخذت بواسطة Antigen وفحص نسيجي باستخدام صبغة Methylene Blue.

النتائج: في هذه الدراسة التي شملت 89 مريضًا كانت أعمار المرضى تتراوح ما بين 13-77 سنة ومعدل العمر 37 سنة، نسبة النساء كانت 37.1% بينما كانت نسبة الرجال 62.9%. نسبة انتشار H. pylori (48.3%)، هناك اختلاف ما بين الفحوصات الأربع. من أهم النتائج في هذه الدراسة وجود علاقة مهمة بين الإصابة بهذه البكتيريا ومصادر شرب الماء في مرحلة الطفولة. لم يكن هناك أي علاقة مهمة ما بين الإصابة بالبكتيريا والعمر، نوع الجنس، الوضع الاقتصادي للمريض، عدد أفراد عائلته، أنواع الأدوية التي يستخدمها، التدخين، وشرب القهوة. من أهم النتائج في هذه الدراسة وجد أن شرب الشاي يعمل كعامل حماية من البكتيريا.

الاستنتاج: النتائج من هذه الدراسة أثبتت أن الإصابة ببكتيريا (H. pylori) تكون في مرحلة الطفولة مرتبطية بعوامل خطر مثل مصادر شرب الماء. كما استنتج من هذه الدراسة فائدة الشاي كمضاد للإصابة بهذه البكتيريا.
This thesis is dedicated to my father Dr. Mohammed, my Mother and my brothers, Ahmed, Sameh and my sister Sawsan who supported me all the way since the beginning of my study and always encouraged me to pursue my ambitions and my goals.

Finally, this thesis is dedicated to all those who believe in the richness of learning.
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<td>ALeb</td>
<td>Blood group A derivative of Lewis b.</td>
</tr>
<tr>
<td>BabA</td>
<td>Blood group antigen binding A adhesion.</td>
</tr>
<tr>
<td>BabB</td>
<td>Blood group antigen binding B adhesion</td>
</tr>
<tr>
<td>BE</td>
<td>Barrett’s Esophagus</td>
</tr>
<tr>
<td>BIC</td>
<td>Bovine Immune Colostrum</td>
</tr>
<tr>
<td>BLemb</td>
<td>Blood group B derivative of Lewis b.</td>
</tr>
<tr>
<td>Cag A</td>
<td>Cytotoxin-Associated Gene</td>
</tr>
<tr>
<td>Cag A</td>
<td>Cytotoxin-Associated Protein</td>
</tr>
<tr>
<td>cag-PAI</td>
<td>cag-Pathogenicity Island.</td>
</tr>
<tr>
<td>CFU</td>
<td>Colony Forming Unit</td>
</tr>
<tr>
<td>CGA</td>
<td>Cultured gastric adenocarcinoma</td>
</tr>
<tr>
<td>DU</td>
<td>Duodenal Ulcer</td>
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<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>ESPCG</td>
<td>European Society of Primary Care Gastroenterology</td>
</tr>
<tr>
<td>G-C ratio</td>
<td>Guanine-Cytosine ratio</td>
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<tr>
<td>GERD</td>
<td>Gastroesophageal Reflux Disease</td>
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<td>GI</td>
<td>Gastrointestinal tract</td>
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<td>HpSAg</td>
<td><em>Helicobacter pylori</em> Stool Antigen detection kit</td>
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<td>H. pylori</td>
<td><em>Helicobacter pylori</em></td>
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<td>HLA-DQA1</td>
<td>Human Leukocyte Antigen Locus</td>
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<td>HP-NAP</td>
<td><em>H. pylori</em> Neutrophil-Activating Protein</td>
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<td>IARC</td>
<td>International Agency for Research on Cancer</td>
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<td>IgM, A, G</td>
<td>Immunoglobulin M, A, G</td>
</tr>
<tr>
<td>ICAM</td>
<td>Intercellular Adhesion Molecule</td>
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<tr>
<td>IL</td>
<td>Interleukin</td>
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<tr>
<td>Le b, x, y</td>
<td>Lewis blood group antigen b, x and y.</td>
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<tr>
<td>LPS</td>
<td>Lipopolysaccharide</td>
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<tr>
<td>LT</td>
<td>Labile Toxin</td>
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<tr>
<td>MALT</td>
<td>Mucosa Associated Lymphoid Tissue</td>
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<td>MB</td>
<td>Methylene Blue</td>
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<tr>
<td>NADPH</td>
<td>Nicotinamide Adenine Dinucleotide Phosphate</td>
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<td>NASPGN</td>
<td>North American Society for Pediatric Gastroenterology</td>
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<tr>
<td>NF_κB</td>
<td>Nuclear Factor_κB.</td>
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<td>NIH</td>
<td>National Institute of Health</td>
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<td>NIS</td>
<td>New Israel Shekel</td>
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<td>NSAIDS</td>
<td>Non Steroidal Anti Inflammatory Drugs</td>
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<td>OMP</td>
<td>Outer Membrane Proteins</td>
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<td>PAI</td>
<td>Pathogenicity Island</td>
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<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
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<td>PMNs</td>
<td>Polymorphic Nucleated Cells</td>
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<tr>
<td>PPI</td>
<td>Proton Pump Inhibitor</td>
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ROS  Reactive Oxygen Species.
SabA  Sialic Acid Binding Adhesin.
SES  Socioeconomic Status
Sib-Sib  Sibling-Sibling
sLea  Sialyl-Lewis a Antigen
sLex  Sialyl-Lewis x Antigen.
Th  T helper cell
TMB  Tetra Methyl Benzidine
TLR  Toll-like receptors
UBT  Urea Breath Test
Urel  Urea Channels
URUT  Ultra Rapid Urease Test
UTIs  Urinary Tract Infections
Vac A  Vacuolating Cytotoxin Gene
Vac A  Vacuolating Cytotoxin
Chapter I
Introduction
Chapter 1
Introduction

1.1 Overview
Barry Marshall and Robin Warren of Perth, Western Australia discovered \textit{H. pylori} in 1983. Originally, the organism was named \textit{Campylobacter pylori} because it was structurally similar to other \textit{Campylobacter} species, such as \textit{C. jejuni} \cite{1}. Signs of \textit{H. pylori} infection such as gram-negative gastric bacilli, gastric urease and epidemics of hypochlorhydria have been described since the late nineteenth century \cite{2}. These observations could be better explained after Warren and Marshall in the early 1980’s managed to culture a bacterium that was to be designated \textit{Campylobacter pyloridis} \cite{3}. In 1989, the genus Helicobacter was created and the bacterium received the name \textit{H. pylori}\cite{4}.

\textit{H. pylori} is a small (0.5-1.0 μm in width and 2.5 to 5.0 μm in length), spiral shaped, highly motile, gram negative rod with 4-6 unipolar sheathed flagella \cite{5}. The microorganism grows slowly in vitro and requires special media and a microaerophilic (5% O$_2$, 10% CO$_2$, and 85% N$_2$) environment \cite{6}. The most striking biochemical characteristic is the production of large quantities of urease. This enzyme digests urea to produce carbon dioxide and ammonia. In the presence of water this leads to the formation of ammonium hydroxide \cite{7}. In this way, \textit{H. pylori} is able to neutralize the acid in its direct environment.

\textit{H. pylori}, colonizes the human stomach, can cause gastritis, is strongly associated with gastric and duodenal ulceration (DU) and has been implicated in the causation of gastric carcinoma and mucosa-associated lymphoid tissue (MALT) lymphomas. It has been reported that there is relationship between \textit{H. pylori} infection and children’s gastroenterological diseases \cite{8}. However, only 10-20% of infected individuals manifests severe
complications and this selectivity in disease progression is inadequately understood (9, 10). Epidemiological studies have shown that a weakly positive correlation exists between chronic gastric infection with *H. pylori* and coronary heart disease (11).

Urease, vacuolating cytotoxin Vac A, and the pathogenicity island (cag PAI) gene products, are the main factors of virulence of this organism. *H. pylori* LPS may have an important role in autoimmune-mediated damage in the gastric mucosa (12).

Half of the world’s population is estimated to be infected with *H. pylori*, which makes it one of the most common bacterial pathogens in humans (13). The prevalence of *H. pylori* infection worldwide is approximately 50%, it reaches as high as 80%–90% in developing countries, and about 35%–40% in the United States (14). Approximately 20% of persons infected with *H. pylori* develop related gastroduodenal disorders during their lifetime (14). The annual incidence of *H. pylori* infection is about 4%–15% in developing countries, compared with approximately 0.5% in industrialized countries (15). Documented risk factors include low socioeconomic status, overcrowding, poor sanitation or hygiene, and living in a developing country (16).

In "Israel", the prevalence of *H. pylori* infection is about 60%, and the annual incidence of gastric cancer is about 15 per 100,000 populations (17). The prevalence of *H. pylori* infection was 10% among children in Egypt (18). In crude analyses, prevalence was associated with increasing age, non-white skin color, lower current family income, lower education level, higher size of the family, low socio-economic conditions in childhood, higher number of siblings and attendance to day-care centers in childhood, and presence of dyspeptic symptoms. Socioeconomic conditions in childhood besides ethnicity and presence of dyspeptic symptoms were the factors significantly associated with the infection (19).
There are several methods of diagnosing *H. pylori* infection including invasive procedures using mucosa biopsies taken during endoscopy (mainly culture, histology and the rapid urease test) and noninvasive procedures (20).

Non-invasive testing methods for detection of *H. pylori* or confirmation of eradication include: 1) antibody tests (in serum, saliva or blood); 2) antigen tests (in stools, saliva or urine); and 3) radioactive or non-radioactive urea breath tests. The most interesting of the non-invasive tests is the detection of antigens in stool samples by enzyme immunoassay technique. While this test has good performance at a reasonable cost, doubts exist regarding patient and clinician compliance and actual performance, particularly with regard to inter laboratory variability. The urea breath test is based on analysis of samples of exhaled air before and after ingestion of urea containing specially labelled carbon (21).

PCR is a powerful technique for the detection of target DNA in various clinical specimens, but its application to fecal specimens has been limited due to the presence of substances inhibiting the reaction (22).

Eradication therapy of symptomatic *H. pylori* infection substantially reduces the recurrence of associated gastroduodenal diseases. Therapy entails complicated regimens of several antimicrobial agents for at least 2 weeks. In general, triple therapy regimens usually entail two of the following antimicrobial agents: metronidazole, amoxicillin, tetracycline, or clarithromycin in combination with a proton pump inhibitor or bismuth salts (23). The most common causes of treatment failure are patient noncompliance and antimicrobial resistance of the infecting *H. pylori* strain (24). Quadruple regimens are used as a salvage therapy when triple therapy regimens have failed (23). However, the success of treatment is usually dependent on early detection. Moreover, prevention of *H. pylori* infection seems to be a wise strategy. Prevention strategies require deep understanding of the transmission risks. *H. pylori* infection can be prevented
by interrupting the transmission of the infection by improving environment, socioeconomic status and personal hygiene. It has been shown convincingly that with the improvement of socioeconomic status in developed countries, *H. pylori* infection has gone down by 50% over the last 3 decades (25).

Recently in a mouse model it has been demonstrated that oral immunization with a crude lysate of *H. felis* induced protection against gastric infection by *H. pylori* (26). Further studies have shown that superficial gastric ulcers in a mouse model infected with *H. pylori* can be prevented by the administration of a recombinant oral vaccine with *E. coli* heat labile toxin (LT) given as adjuvant (27).

Despite the fact that Gaza is overcrowded, with poor sanitary conditions, there are no previous studies or data concerning the prevalence of *H. pylori* or the associated risk factors. Another vital issue regarding *H. pylori* diagnosis, is the absence of many of the simple accurate tests in Gaza.

### 1.2 Statement of the Problem

*H. pylori* affects around 50% of the population in their lifetime, it is the causative agent of serious disease and *H. pylori* classified as a class I carcinogen. There is a need to examine the extent of the disease in Gaza city and to study the risk factors associated with *H. pylori* infection and to evaluate various diagnostic approaches.

### 1.3 Objectives

The general aim of this study is to evaluate risk factors associated with *H. pylori* infection in Gaza, Palestine.

The following specific objectives were achieved:
1. The rate of *H. pylori* infection among the target population was estimated.
2. The performance of *H. pylori* detection techniques was evaluated.
1.4 Significance

The rate among middle-aged adults is over 80 percent in many developing countries, as compared with 20 to 50 percent in industrialized countries (28). *H. pylori* infection plays a role in the development of chronic gastritis, peptic ulcer and gastric cancer (29).

The local epidemiological data on *H. pylori* infection are scarce and there is no research done in Gaza on *H. pylori*. It is expected that data generated from this work would provide an insight on the risk factors associated with *H. pylori* infection. This may contribute to reducing the incidence of such infections.
Chapter II
Literature Review
Chapter II
Literature Review

2.1 The Microorganism

2.1.1 History of H. pylori microorganism

The species Helicobacter was probably first observed by Bizzozero, an Italian physiologist at the end of the 19th century. Bizzozero observed gram negative, spiral shaped bacteria in the stomach of dogs. At around the same time other medical professionals also reported presence of a spiral shaped bacteria in upper gastrointestinal disorders. However these discoveries went largely unnoticed. In 1938 an American pathologist discovered a spiral shaped bacteria in 43 % of 242 subjects in an autopsy study, further it was known that peptic ulcer disease responded well to treatment with bismuth salts. Any further research into the findings was hindered by the lack of fresh specimens of human gastric tissue as well as the fact that the newly discovered bacteria could not be cultured. Thus, the findings soon fell into oblivion (30).

In 1983, almost a century after it had first been discovered, two Australians, Barry Marshal and Robin Warren noticed a gram negative, flagellated, spiral shaped bacteria growing on agar plates containing human gastric biopsies, that were accidentally left in an incubator over the Easter holidays. The close physical resemblance of this new bacterium to that of the Campylobacter species and the fact that peptic ulcers frequently occur in the pyloric gland region of the stomach, led them to name it Campylobacter pyloridis. The bacterium has undergone two name changes since, first to C. pylori and then in 1989 the name was changed to the currently used Helicobacter pylori. Early observations established a link between H. pylori and gastroduodenal
disease, such as peptic ulceration and gastritis. But it was not until Dr. Marshall took it upon himself to fulfil Koch’s postulate by ingesting a liquid culture of the bacteria that the link was proven. After ingesting the culture, Marshall developed mild illness for 14 days; by day ten, gastritis had developed which lasted for four days. The discovery of *H. pylori* and the realization that it caused gastric ulcers earned the two Australians the 2005 Nobel Prize in physiology and medicine (30).

Helicobacter species belong to the epsilon sub-group of proteo-, or purple bacteria. The group contains over thirty members of the Helicobacter species colonizing a wide range of hosts (31). The species is diverse in morphology but some features are shared among almost all members of the genus Helicobacter, such as low (35-44 mol %) chromosomal guanine/ cytosine (G/C) content, strong urease activity and the presence of sheathed flagella (32). The fact that there is such a wide degree of morphological diversity and host specificity suggest that the Helicobacter spp. is an old bacterium and has co-evolved with its hosts for a long period of time (33).

### 2.1.2 General aspects of *H. pylori* microbiology and infection

#### 2.1.2.1 *H. pylori* characteristic

*H. pylori* is a microaerophilic, gram negative, spiral shaped rod, between 2.5 and 4 μm in size, and under certain circumstances it can be U-shaped or coccoid (34). *H. pylori* is actively motile using 4-6 unipolar, sheathed flagella (32). It resides naturally in the gastrointestinal tract of humans and non-human primates. There is also evidence that it can infect pigs, cats, sheep and pups (33, 35). In the stomach, the majority of *H. pylori* can be found in the gastric mucosa; however a few are found adhered to the gastric mucosal epithelium. The bacterium is highly adapted to survive in the hostile environment of the stomach where few other organisms can survive. Although *H. pylori* is considered to be a extra cellular bacteria, there is
evidence suggesting that the bacteria has a mechanism for intracellular invasion (30).

*H. pylori* is the best known member of the Helicobacter genus, which includes dozens of species that primarily colonize the gastrointestinal tract of a variety of animals (33). *H. pylori* are a curved gram-negative bacillus with a bundle of unipolar flagella (figure 2.1). Biochemical identification of *H. pylori* relies on the activities of the urease, catalase and oxidase enzymes. The bacterium is slow-growing and requires a rich medium and a microaerophilic atmosphere for in vitro culture. After starvation through prolonged culturing, a coccoid form can be found in the cultures and it has been debated whether this form represents dormant or degenerated, non-viable bacteria (36).

![Figure 2.1: H. pylori](image1.png)  ![Figure 2.1: H. pylori](image2.png)

**Figure (2.1):** *H. pylori*. The curved bacillus with unipolar flagella is visualized by a scanning electron microscope (left) and depicted in a schematic drawing (right) (37).

### 2.1.2.2 Bacterial infection

The human stomach is an inhospitable milieu and a fasting stomach is normally devoid of bacterial species other than *H. pylori* and some Lactobacilli. *H. pylori* is well adapted to its gastric niche and has developed a broad spectrum of functions that enable colonization. For example, bacterial
urease hydrolyzes urea with the formation of carbon dioxide and ammonia, providing protection against the highly acidic gastric environment. The capability of *H. pylori* to maintain a chronic infection is of particular interest and can be facilitated by i) protected localization and adherence, ii) evasion and regulation of the immune response and iii) adaptation to changing conditions (37).

The infection can be patchy and is primarily localized to the distal parts of the stomach, but can spread proximally, especially in persons with low gastric acid secretion (38). The majority of the bacteria are regarded to be free-living in the gastric mucus layer, which can provide some protection against the harsh environment (38). Part of the bacterial population adheres to the gastric epithelial cells, which may benefit interactions with the host and maintenance of the colonization. A significant role of adherence is indicated by the array of products that contribute to this purpose in *H. pylori* (39, 40). The bacterium is generally extracellular, but may invade host cells, although the significance of internalization is uncertain (38).

Long-term persistence of the infection is likely to require evasion or modulation of the immune response. However, the inflammation may benefit the bacteria to some extent by disrupting the tissue integrity, thereby making nutrients available. Thus, bacterial interactions with the immune system may need to somewhat balance the risk of eradication against a more favorable environment (41).

### 2.1.2.3 Structures

**A. Flagella**

The unipolar flagella of *H. pylori* enable motility, which is an important bacterial feature (42). Two different flagellin proteins constitute the flagellar filament, but about 40 additional genes are involved in the secretion and
assembly of the whole flagellar apparatus (37). Chemotactic systems offer means for spatial orientation, for example towards the mucosal cell lining where the pH is higher, nutrients can be more abundant and closer interactions with host cells are possible (42).

B. Outer membrane proteins

A relatively large proportion (4%) of the coding capacity of the *H. pylori* genome is devoted to outer membrane proteins (OMP) (37). Several of these proteins have been suggested to possess adhesive properties. The receptors include glycoconjugates expressed on host cells, such as the Lewis carbohydrate blood group antigens, extra cellular matrix components are unknowns (38). Two OMPs have received particular attention for their ability to bind to host receptors. First, the Blood group antigen binding A (BabA) adhesin binds to Lewis b that is expressed by gastric epithelial cells (37) and the presence of this adhesin has been suggested to be associated with more severe disease (43). Second, the Sialic acid binding Adhesion (SabA) mediates a weaker and more intimate adherence by binding to sialyl-Lewis x, which is upregulated by the inflammation (39). Accordingly, a model was proposed where initial binding is mediated by Lewis b and BabA, which results in inflammation, induction of sialyl-Lewis x and binding through SabA. Altered adhesive properties may provide mechanisms for *H. pylori* to regulate its interactions with the host if, for example, the immune response would necessitate less tight adherence (39) or when the availability of receptors differ between human populations (37). Such modulation of the binding properties may occur by changed expression or evolution of functional variants through frame-shift mutation or recombination between homologous loci (39).
C. Lipopolysaccharide

Lipopolysaccharide (LPS) covers the surface of *H. pylori* and other gram-negative bacteria and sustains membrane integrity and can mediate interaction with the host. An unusual feature of *H. pylori* LPS is the expression of Lewis antigens on the polymeric carbohydrate O-antigen constituent, resembling blood group antigens expressed on various host tissues. About 80-90% of *H. pylori* strains express Lewis antigens, where of Lewis x and Lewis y are most common (44). Fucosyl transferases involved in the synthesis of Lewis antigens can undergo slipped strand miss-pairing, generating variability of Lewis antigen expression, which may aid adaptive evolution (45). The molecular mimicry of *H. pylori* and human Lewis antigen expression has been suggested to facilitate bacterial immune evasion and give rise to autoimmunity (37). *H. pylori* Lewis antigens could further interact with dendritic cells to balance the T helper cell (Th1/Th2) responses and have been described to mediate adhesion (37). However, much of the evidence regarding the biological roles of *H. pylori* Lewis antigen expression is inconclusive and awaits confirmatory data (39, 44).

D. The genome

*H. pylori* has a small genome (1,667,876 base pair- that is, 1.7 Million base), as compared with those of bacteria that can live in a wide range of habitats such as *Escherichia coli* (4.6 million base). *H. pylori* has many fewer regulatory genes of the type environment. Thus, this finding support epidemiologic evidence that *H. pylori* lives only in the human stomach and that the enzymatic pathway it needs for survival in this harsh milieu are the continuous switched on (46). *H. pylori* is the first bacterial species for which two genomes, those of strains 26695 and J99, were completely sequenced. A third sequenced genome, AG7:8, is currently in the final stages of annotation and analysis (37). The *H. pylori* genome is small and compact and the 1.65 million base pairs accommodate about 1,500 genes. The limited metabolic potential and the low number of regulatory networks have been
interpreted to reflect a restricted gastric niche of the bacterium (37). After a revision of the annotation, 77% of the genes have been assigned a functional category (47). Comparison of the two sequenced genomes revealed some larger genomic rearrangements, but the gene order and metabolic potential were relatively conserved (37). Strain-specific genes, 67% of the genes, were concentrated in two genomic regions that hence were designated “plasticity zones”. These zones have thereafter also been discernible by microarray-based comparative genomics of other strains (37). Some gene functional classes have been found to be especially variable, including genes related to DNA metabolism and the cell envelope (45, 47).

2.1.2.4 Biodiversity

Here are three general kinds of biodiversity discussed: habitat diversity, genetic diversity, and species diversity. The survival of each is linked to the safety of the other two, and together they comprise the resources of bacterial ecosystems.

A-Habitat diversity

Here, habitat diversity refers to the variety of organs and/or pathological alterations caused by bacterial infection; i.e. Helicobacter species exist in the stomach for H. pylori, the intestinal tract for H. canadensis, liver for H. hepaticus, and the gall bladder for H. bilis. The natural habitat of H. pylori appears to be the gastric mucus and the mucus producing epithelium. In the duodenum of H. pylori infected patients, the bacterium is always found closely associated with gastric metaplastic cells, which is a precancerous condition and relatively common in the upper Gastrointestinal tract (GI) tract (39). The formation of gastric-type epithelium in the duodenum seems to be related to increase gastric acid output. This new habitat could be essential for colonization of H. pylori when gastric changes such as chronic active atrophic gastritis or cancer induced by bacteria take place in the natural habitat (39).
B-Genetic diversity and species diversity

The genetic diversity within a species is mainly the variety of populations that comprise it. The more variation within an *H. pylori* population in an infected individual, the better the chance that some of the phenotypes will have an allelic variant that is suited to the changing environment in the stomach, and that it will produce offspring with variants that will in turn reproduce and continue the population into subsequent generations (39). Compared to most other organisms in the human biosphere, *H. pylori* is highly diverse. The identification of genetically divergent sub-clones within individual hosts indicates that *H. pylori* diversification continues during its decades-long colonization of the host. This genetic diversity is generated through multiple mechanisms, including spontaneous point mutations, recombination with other bacterial cells, and intragenomic rearrangements involving mobile genetic elements or repetitive DNA sequences (39). The *babA* and *babB* genes code for a family of *H. pylori* OMPs, which have appreciable N and C-terminal identity. Recently, Pride et al., (2002) analyzed the nucleotide sequences of *babA* and *babB* and showed that geographic origin was the major determinant of phylogenetic relationships. Simultaneous colonization of the human stomach with more than one strain of *H. pylori* can be detected in about 5-10% of patients in the United States, and this may occur more commonly in other populations. Such mixed infections, even if transient, provide an opportunity for genetic exchange between strains. Kersulyte et al. (1999) could show different genetic exchanges between single cell clones from a patient who was naturally infected with two different *H. pylori* strains (39). One exchange resulted in deletion of the *cag*-PAI, while other recombination involved a region encoding outer membrane proteins that could be involved in adherence activities. Genetic exchange may play an important role in the biology of *H. pylori* by generating new genotypes much more rapidly than is possible by mutation alone, thereby allowing genera of pathogenic bacteria to adapt to other organs (48).
2.1.2.5 Virulence factors

A virulence factor contributes some function that renders the microorganism more pathogenic, that is, increases the likelihood for disease development. *H. pylori* infection is usually lifelong and asymptomatic and disease may be attributed to the host response towards the colonization. Thus, some of the factors commonly designated as virulence factors in *H. pylori*, for instance the flagella, may rather be regarded as “colonization factors” (38). These factors primarily facilitate establishment and persistence of the infection, which however, naturally also increases the risk for disease, blurring a distinction from virulence factors. Studies of the contributions of individual bacterial factors to infectivity and pathogenicity have to be cautiously interpreted. Associations between different factors may give rise to confounding (43) and the influences of unrecognized subtle mutations may result in spurious findings (40).

A-The cag PAI and VacA

The cag- pathogenicity Islands (cag PAI) is one of the most studied loci in the *H. pylori* genome and is present in the majority of strains worldwide (49). The locus is associated with a more vigorous host response characterized by IL-8 induction (49) and an increased risk for ulceration and cancer (43, 49). The cag PAI is an almost 40 kb stretch of DNA that encodes nearly 40 genes, many of which are homologous to type IV secretion system components. Type IV secretion systems assemble into a syringe-like structure that mediates secretion of molecules extracellularly or into the cytosol of host cells. The secretion system of *H. pylori* delivers the cag PAI-encoded and immunodominant Cytotoxin Associated protein (CagA) protein into the gastric epithelial cells. CagA gene codes for a relatively long (1186 amino acid) protein, upon translocation, CagA is phosphorylated and initiates signal transduction that results in cytoskeletal rearrangements and an inflammatory response (37). The secretion system may also mediate transfer of *H. pylori*
peptidoglycan into the epithelial cells where intracellular pathogen-recognition molecule can initiate an immune response. PAIs are typically prone to horizontal genetic transfer. The *cag PAI* exhibits signs of such mobility by the differing GC content compared to the rest of the genome and the presence of flanking direct repeats and insertion sequences (37). Accordingly, excision and insertion of the *cag PAI* can result in mixed infections with regard to *cag PAI* status. Intermediate strains that lack some of the *cag PAI* genes have also been described (49).

Early on, *H. pylori* was found to possess a cytotoxic ability involving formation of vacuoles in epithelial cells, which could be attributed to the bacterial exotoxin Vaculating Cytotoxin (VacA) (37). The *vacA* gene appears to be universally present, but there are alleles with different signal (*s1/s2*) and mid regions (*m1/m2*). The *s1/m1* variant is most cytotoxic and the *s1* and *m1* genotypes have been proposed to be correlated with the pathogenic potential of the infection (43). VacA can induce apoptosis of gastric cells, which may provide the bacteria with nutrients or reduce the acid output through the killing of parietal cells. Furthermore, VacA mediated inhibition of antigen presentation and activation of T lymphocytes could play a role in immune evasion (37).

The cytotoxic variant of Vaculating Cytotoxin gene (*vacA*) is in linkage disequilibrium with the *cag PAI*, hence the gene name cytotoxin-associated gene A (*cagA*). The *vacA* and *cag PAI* loci are situated at distant sites on the chromosome and their linkage is inadequately understood. Nevertheless, the loci form the basis for a classification of virulence of *H. pylori* strains. The more virulent type I strain express CagA and a cytotoxic variant of VacA, while the less virulent type II strains do not express CagA and harbor a non-toxic form of VacA. The serological response against CagA has been used as a marker of more virulent strains, but serological methods have been questioned due to limited sensitivity (0.71–0.90) and specificity (0.80–0.90) (37).
B- Adhesions

A wide variety of molecules present on adherent structures of bacteria can function as adhesions. In bacterial-eukaryotic interfaces, there are apparently general systems for recognition of certain microbial cell surface carbohydrate characteristics (39). Like other microorganisms, *H. pylori* require adhesive molecules for colonization and persistence. *H. pylori* has a wide range of adhesion properties and has been suggested to bind to many different carbohydrates, mediated by various bacterial components (50). The Leb and sLex antigens binding adhesins, BabA and SabA, respectively, are the best described (51). Hemagglutination of RBCs by bacteria has been used to study bacterial binding specificities and to identify the cognate bacterial adhesions. The *H. pylori* sialic acid dependent hemagglutination was a subject for researchers for more than a decade but recently, this sialic acid-dependent binding activity has been shown to be mediated by SabA, since the corresponding *sabA* deletion mutant lacks all hemagglutination properties (39).

B-1. Consequences of bacterial adhesion

It is becoming increasingly obvious that the act of adhering to the host cells induces intense changes in the adherent organism, regardless of the origin of the host cells. It is also known that adhesion of an organism to a host receptor greatly affects its rate of growth, carbohydrate utilization, protein synthesis and energy generation, as well as its cell wall composition and production of adhesive molecules. The host cell and its adherent bacterium exchange signals (*i.e.* they engage in molecular “crosstalk”), which brings about changes in both cell types (figure2.2) (52).
Figure (2.2): Adhesion of *H. pylori* to gastric epithelial cells induces a multitude of changes to the host cell, the most common of which are shown in the diagram (39).

**B-2. Effect on the bacterial cells**

There are a number of reasons why the bacterial cell has to adjust its phenotype once it has adhered to a cell surface. Prior to any interaction with its host, *H. pylori* is probably exposed to different environmental conditions (*e.g.* different pH, high turnover, shear force, osmolality, nutrient concentration) (39). Then, adherence is required for microbial survival in this hostile environment. Attachment of *H. pylori* to the gastric mucosa (*e.g.*
mediated by Leb) activates the type IV secretion system which results in the translocation of CagA protein into the host cells (53) and triggers inflammation. Having reached an inflamed epithelial surface with the new carbohydrate structure as a ligand (which may not necessarily be its ultimate destination), H. pylori will then have to adapt to this new environment and this will require the up regulation or suppression of various genes such as SabA. Such changes will certainly be induced in response to alterations in a number of environmental factors, but some are recognized to be triggered by the adhesion process itself. Recently, changes in H. pylori gene expression induced by bacterial adhesion to CGA (cultured gastric adenocarcinoma) cells were reported. Several genes and those for two outer membrane proteins in particular, were upregulated (54). Attachment to the host cell may be only the first step in a sequence of events that could involve invasion of the cell. Adhesion of H. pylori mediated by SabA may be used as a signal for the microbe to begin synthesis of those molecules essential to initiate invasion of the host cell. There have been many reports suggesting that bacteria do respond in this way to contact with the host cell. As an example of this, similar crosstalk has been described for Porphyromonas gingivalis adherence to epithelial cells, which induces the secretion of a multitude of proteins and downregulation of the production of extracellular proteases (55).

**B-3. Effect on the host cells**

A number of bacterial species capable of inducing pathology in humans also adhere to epithelial cells without actually inducing any apparent changes in these cells. However, although the adhesion process itself may not affect the structure or function of the host cell, microorganisms also produce toxins – enzymes that may ultimately damage host cells. H. pylori induces the morphological alterations in gastric epithelial cells (56). H. pylori-related chronic inflammation in gastric tissue has also been reported to modulate the glycosylation patterns of epithelial cells. In contrast, normal human gastric epithelial cells are essentially devoid of such sialylated glycoconjugates (57).
In addition, recent studies have indicated that adherence of *H. pylori* induces cell proliferation and apoptosis during the early phase of chronic inflammation of the gastric mucosa (58). A characteristic feature of any bacterial infection is the migration of PMNs towards the site colonized by the infecting organism. Binding of *H. pylori* to the gastric epithelial cells induces the expression and secretion of IL-8 (39). In response to a chemotactic gradient from the site of infection, PMNs first adhere to, and then traverse, the vascular endothelium (59). This process involves the interaction between E-selectin, intercellular adhesion molecule (ICAM) 1 and 2 on the surface of the endothelium cell, and sLex and 2-integrins on the PMN surface (60). PMNs are rich in various sialylated glycoconjugates, which *H. pylori* can bind to. An important consequence would be the bacterial interaction with, and activation of PMNs (39). Nutrients released by the inflamed and damaged cells might be used by *H. pylori* as an energy source (61).

**B-4. Invasion of host cells**

A characteristic feature of *H. pylori*-induced inflammation is massive attraction of phagocytes (particularly PMNs) to the site of infection. This can be achieved by the production of *H. pylori* neutrophil-activating protein (HP-NAP). The protein was found to promote the adhesion of PMNs to endothelial cells by upregulating adhesion receptors of the 2-integrin family (62). Satin and colleagues showed that HP-NAP stimulates (NADPH) oxidase assembly and production of reactive oxygen species (ROS). Also, as PMNs consistently outnumber macrophages in *H. pylori* infected stomach; it induces a state of chronic acute inflammation. Previous reports (63) have suggested that *H. pylori* is capable of invading epithelial cells in the gastric mucosa; however, the role of invasion in *H. pylori* pathogenesis remains unclear. The following mechanisms have been postulated to explain how *H. pylori* evades phagocytosis: 1- Strong binding between *H. pylori* and phagocytes correlates with a rise in the level of urease on the surface of *H. pylori*, thus retarding phagocytosis and strong respiratory burst in the phagocytes (64). 2-
Catalase, alkyl hydroperoxide reductase, and factors (*cag* pathogenicity island type IV secretion apparatus) that are unique to type I strains allow bacteria to resist phagocytic killing (65). 3- Delayed phagocytosis is linked to intracellular survival, since type I *H. pylori* persists inside macrophages within a novel vacuole called the megasome (66).

**C- *H. pylori* enzymes**

*H. pylori* produce catalase, oxidase and urease enzymes. Urease is an abundantly produced enzyme. *H. pylori* has developed a unique mechanism to control urease-dependent pH buffering. The urea channels (UreI) present in the inner membrane are opened at pH below 6.5, to allow for delivery of urea to intracellular urease. The NH₃ produced can then buffer the periplasm (67). Urease activity is therefore essential for maintenance of cytosolic pH levels compatible with efficient metabolism and survival of *H. pylori* in the acidic environment of the stomach (39).

**2.1.3 Gastric helicobacter species**

To date nine Helicobacter species have been cultured from the stomach of humans and land animals (table2.1), all are capable of hydrolyzing urea (68). These can be further classified into Lockard types 1, 2 and 3: type 1 has a fusiform to slightly spiral morphology with tapered ends; type 2 is spiral and has sparsely distributed periplasmic fibers and can appear singly or in groups of two to four; and type 3 is more tightly coiled and lacks periplasmic fibers. In general the morphology of gastric Helicobacter species isolated from animals other than cats and dogs can sometimes be distinctive and sometimes resemble *H. pylori*. Phylogenetic analysis of current gastric, enteric and hepatobiliary Helicobacter species, based on 16s rRNA similarity has been performed (68).
<table>
<thead>
<tr>
<th>TAXONOMY</th>
<th>NATURAL HOST</th>
</tr>
</thead>
<tbody>
<tr>
<td>H. acinonychis</td>
<td>Cheetah</td>
</tr>
<tr>
<td>H. bizzeroni</td>
<td>Dog</td>
</tr>
<tr>
<td>Candidatus Helicobacter bovis</td>
<td>Cattle</td>
</tr>
<tr>
<td>H. felis</td>
<td>Cat</td>
</tr>
<tr>
<td>H. heilmannii</td>
<td>Human, non human primate, pigs</td>
</tr>
<tr>
<td>Candidatus Helicobacter suis</td>
<td>Pig</td>
</tr>
<tr>
<td>H. mustelae</td>
<td>Ferret</td>
</tr>
<tr>
<td>H. nemestrinae</td>
<td>Macaque</td>
</tr>
<tr>
<td>H. pylori</td>
<td>Human, monkey, sheep</td>
</tr>
<tr>
<td>H. salomonis</td>
<td>Dog</td>
</tr>
<tr>
<td>H. suncus</td>
<td>Shrew</td>
</tr>
</tbody>
</table>

Table (2.1): Human and land animal gastric Helicobacter (68).

2.1.4 Taxonomy of H. pylori

*H. pylori* is helix shaped (hence the name helicobacter) and can literally screw it self into the stomach lining to colonize it. The scientific classification of the bacteria is:

**Kingdom:** Bacteria

**Phylum:** Proteobacteria

**Class:** Epsilon Proteobacteria

**Order:** Campylobacterales

**Family:** Helicobacteraceae

**Genus:** Helicobacter

**Species:** *H. pylori* (3)
2.2 Epidemiology of *H. pylori* Infection

2.2.1 Prevalence of *H. pylori* infection

This gram-negative bacterium infects more than half the world’s population and its prevalence has been shown to correlate with poor socio-economic conditions. In many underdeveloped nations, more than 80% of the population is infected with this pathogen (10).

*H. pylori* infection is one of the commonest infections worldwide, occurring in all regions and infecting at least half of the world’s population (69). The prevalence of *H. pylori* infection worldwide is approximately 50% (14), as high as 80%–90% in developing countries, and ≈35%–40% in the United States (14). While within countries, the prevalence is higher among groups with lower socioeconomic status (70, 71). *H. pylori* prevalence is generally found to increase with age, reaching 20–50% in adult populations in Europe and North America (72). *H. pylori*-positivity in adults is more closely associated with living conditions and with the parents’ socioeconomic status in childhood than with current living conditions and socioeconomic status (73).

The infection is also associated with low Socio Economic Status (SES) within countries (74). In the United States, for instance, a significantly lower prevalence was found in Caucasians (26%) compared to Hispanics (65%) and Afro-Americans (66%) (74). This dissimilarity was interpreted to reflect the different socioeconomic backgrounds of the groups. In a follow-up study, it was found that the difference in prevalence between Afro-Americans and Caucasians resulted from different seroconversion rates, although the rate of seroreversion could also have played a role (75). *H. pylori* infection is usually acquired before the age of five (75).
This pattern has been interpreted to partly reflect a birth-cohort phenomenon caused by a higher incidence in the past due to poorer living conditions and sanitation (37). Indeed, mathematical modeling has suggested that the infection will eventually disappear in high-income countries even without intervention (76).

2.2.2 Incidence of *H. pylori* infection

The annual incidence of *H. pylori* infection is ≈4%–15% in developing countries, compared with approximately 0.5% in industrialized countries (14). Documented risk factors include low socioeconomic status, overcrowding, poor sanitation or hygiene, and living in a developing country (15).

In adults, the incidence rates are mostly derived from retrospective longitudinal sero surveys, and these studies are mostly conducted in industrialized countries. It appears that acquisition of the infection during adulthood is a rare event: seroconversion (i.e. change of the serostatus from seronegative to seropositive) occurs generally at a rate less than 1% per year of follow-up. Seroreversion appears to occur approximately at the same or even higher rate than seroconversion (77). Data about the incidence of *H. pylori* infection among adults in developing countries are scarce. In Brazil, an annual seroconversion rate of 1.1% and a seroreversion rate of 0.2% were found during 56 months of follow-up (78). Annual incidence rates over 20% have been reported in early childhood in low-income countries (37).

2.2.3 Risk factors for infection

An obvious necessary cause for acquisition of *H. pylori* infection is exposure to the bacterium. The probability of exposure depends on the characteristics of the infective source and contact, but factors of the recipient host and the bacterium may also influence the probabilities of acquisition and persistence.
The bacterium has to overcome numerous barriers to successfully establish an infection in a new individual (37).

1. Exit from an infected individual.
2. Transient survival outside the gastric niche.
3. Introduction into a new host.
4. Colonization of the new gastric mucosa.
5. Maintenance of the colonization.

A predominantly person-to-person transmission has been postulated. This notion is based on the clustering of the infection in families (79) and in institutionalized individuals while consistent and verified environmental reservoirs are absent (80).

2.2.3.1 The family

The family stands out as the most important framework for transmission and a child’s risk of being infected is associated with having infected family members (79). Family size and residential crowding (persons per room or m²) are frequently described as risk factors for \textit{H. pylori} infection and may be regarded as proxies for the number of infected family members (81). Similarly, having familial connections to high-prevalence regions is associated with infection in children living in low-prevalence areas and this effect decreases in successive generations (82). The living conditions during childhood can be predictive of infection in adulthood being in accordance with \textit{H. pylori} acquisition in childhood from household members (83).

Having an infected mother has been found to be a more prominent risk factor for childhood infection than having an infected father, supporting primarily mother-child transmission. Transmission among siblings has also been indicated by clustering of the infection in sib ships (79).
Even though the infection is usually initiated in early childhood, some epidemiological data point to the possibility of acquisition in adulthood from infected family members. Having an infected spouse has been described as a risk factor for infection (84). Furthermore, having more children has been identified as a risk factor for infection in adults, possibly indicating that children may serve as mediators of transmission within families (85).

2.2.3.2 Environmental and behavioral factors

Reasons for the association between *H. pylori* infection and low SES have been sought among environmental and behavioral factors. A shared environmental source of the infection could theoretically contribute to the observed intrafamilial clustering. Possibly contaminated water has been suggested as an infection source since using particular water sources, such as wells, has been correlated to the infection (86). However, other studies have not found the water source to be associated with infection (87). More indirect environmental transmission has also been proposed following the identification of the consumption of raw vegetables as a risk factor for infection (88). Furthermore, *H. pylori* has been proposed to possess zoonotic potential and suggested reservoirs include cats, houseflies and sheep, but these theories are controversial (37).

Behavioral factors may influence the risk of *H. pylori* acquisition and persistence. Residence in a high-prevalence country can facilitate acquisition, as frequent close contact with infected individuals and poor sanitary practices may enhance bacterial exposure (86). Intimate contact has likewise been suggested to explain other observed risk factors, such as bed sharing and breastfeeding. Breastfeeding has also been speculated to possibly provide protection against early infection by passive immunization. However, such protection, if any, should be of limited relevance after weaning, as supported by negative findings (87). Moreover, *H. pylori* infection has been negatively correlated with antibiotic consumption (37). In another
study, however, a similar negative association disappeared when the country of origin was taken into account, which could be explained by the higher antibiotic consumption in low-prevalence countries (81). Some behavioral factors have been assessed as determinants for *H. pylori* infection specifically in adults. Examples include a possible negative association with alcohol intake (89) and perhaps a positive relationship with smoking, but data are discrepant (83).

### 2.2.3.3 Host and bacterial factors

*H. pylori* strains differ in their ability to establish and maintain an infection in a given host, which can be attributed to host and bacterial factors and their compatibility (45). The transient infections in childhood may reflect instances where the bacterium is not optimally suited for the new host and adaptation is not feasible or rapid enough, leading to the host succeeding in clearing the infection (75).

Host genetics have been indicated to be involved in susceptibility to *H. pylori* infection, based on a higher concordance of infection in monozygotic (81%) than in dizygotic (63%) twin pairs. The specific genetic components of this suggested predisposition are unknown, but some host factors that may contribute susceptibility to the infection have been proposed. Expression of blood group antigens that mediate bacterial adherence to the gastric mucosa has been suggested to be important for susceptibility to *H. pylori* infection (37). Some research indicates that *H. pylori* strains have adapted their binding affinities in accordance with the blood group antigen expression of different human populations (90). Furthermore, individuals that excrete receptors in body fluids, offering removable binding sites that can compete with the tissue-bound receptors, have been reported to have a lower risk of being infected (91). However, some studies have shed doubt on the theory that blood group antigen-mediated adhesion contributes to susceptibility to infection (40).
The immune system may also be involved in determining predisposition to \textit{H. pylori} infection. This notion is supported by studies that describe alleles within the human leukocyte antigen locus HLA-DQA1 to be correlated with the infection (\textsuperscript{92}). An interleukin (IL)-1 receptor polymorphism of unknown functional consequence has also been correlated with the infection (\textsuperscript{93}). Furthermore, the spread of the bacterium may be promoted by low gastric acid secretion, which may be especially relevant in young children and during infective gastroenteritis (\textsuperscript{94}). Some studies have found a slightly higher prevalence of \textit{H. pylori} infection in males (\textsuperscript{83}). The reasons for this tendency are unclear and other studies have not been able to confirm this correlation (\textsuperscript{75}).

\textit{H. pylori} has developed a repertoire of functions for survival in the harsh gastric niche, including acid tolerance, motility, adherence, immune evasion and mechanisms for adaptive evolution. These features are all involved in the interplay between the host and the bacterium and may influence acquisition and persistence of infection, as is typically studied in animal models. Bacterial acid tolerance and motility were among the first factors found to play a role in colonization (\textsuperscript{37}). Global mutagenesis approaches have verified these findings and have expanded the collection of putative essential genes (\textsuperscript{95}). However, the relevance of these observations in human populations is largely unknown. In a Finnish population, strains with a bacterial virulence factor, the \textit{cag} pathogenicity island (PAI), have been indicated to disappear more rapidly than strains without the \textit{cag} PAI (\textsuperscript{96}). This could speculatively be explained by reduced transmissibility or persistence of \textit{cag} PAI strains in this population. However, \textit{cag} PAI+ infections constitute the majority of \textit{H. pylori} infections worldwide (\textsuperscript{49}).
2.2.4 Transmission of *H. pylori* infection

The exact routes of transmission are not definitely known due to the inability to clinically detect acute *H. pylori* infection as well as due to technical difficulties in isolating the microorganism from sources other than the gastric mucosa (77).

Transmission of the infection probably occurs in multiple pathways, which may differ in different societies and age groups. As childhood is a period of high risk for *H. pylori* acquisition, a good understanding of the mode(s) of transmission in children is required to identify how to break the chain of transmission of the infection (77).

The minimum infectious dose of *H. pylori* for humans is not yet established. In human volunteers, ingestion of $10^4 - 10^{10}$ of *H. pylori* after administration of famotidine resulted in infection in 18 out of 20 subjects (77). For non-human primates, the established minimum infectious dose of *H. pylori* is $10^4$ cfu (68).

The most important reservoir of *H. pylori* is the human stomach; and potentially, *H. pylori* may pass from the stomach into the external environment by feces, vomitus or gastric regurgitation (77).

2.2.4.1 Fecal-oral and gastro-oral routes

For fecal-oral transmission, *H. pylori* must be excreted by feces, viable and at sufficiently high concentrations. Culturing of *H. pylori* from normal feces has been rarely successful, although higher isolation rates have obtained recently by using modified culturing conditions (77). *H. pylori* can be more easily isolated from diarrheal stool (97) indicating that *H. pylori* may preserve its viability better if the transit time through the GI tract is shorter. The number of bacteria isolated in diarrheal stool, though, is relatively small, 5–2125 cfu/ml. The shedding of a large number of viable *H. pylori*, up to 30,000 cfu/ml has
found in artificially induced vomitus, and *H. pylori* has been isolated from the air sampled in the vicinity of the vomitus. *H. pylori* has been successfully cultured also from naturally secreting vomitus in a child with acute gastroenteritis. This proves that the organism is potentially transmissible during episodes of gastrointestinal tract illness, particularly with vomiting (97). History of vomiting in siblings was found to be an independent risk factor for *H. pylori* infection in children followed prospectively neurologically handicapped children living in an institution, and found a chronological link between outbreaks of gastroenteritis and new cases of *H. pylori* infection. Therefore, the decreased incidence of diarrheal diseases, parallel with socioeconomic development, is one of the possible explanations for the decreased incidence of *H. pylori* infection (77).

The iatrogenic transmission of *H. pylori* from stomach to stomach via contaminated endoscopic devices is possible but rare event. Proper disinfection of endoscopes prevents iatrogenic spread. Hands may serve as a potential vector of the infection. In rural Guatemala, carriage of *H. pylori* under fingernail was detected in 58% of studied persons using PCR method (77).

### 2.2.4.2 Oral-oral route

Human oral cavity has been implicated as a possible source of *H. pylori* infection, although there exists controversy as to whether the oral cavity can act as a temporary reservoir of *H. pylori*, or whether the microorganism can be detected only occasionally in saliva or on the oral mucosal surfaces as a result of gastroesophageal reflux or vomiting. The fastidious nature of *H. pylori* and the complexity of the oral microflora make the isolation of the microorganism from the oral cavity complicated. Most reports about presence of *H. pylori* in the oral cavity are based on detection of a specific DNA, which has been found in the dental plaque (98), in the periodontal pockets, and in saliva. The detection rate, though, has shown a great deal of variation,
from less than 10% among the subjects harboring the organism in the stomach to 100% among the subjects under study, irrespective to their gastric *H. pylori*-status (98).

A major weakness of PCR is its inability to distinguish between viable or dead microorganisms, and therefore, detection of the DNA of the microorganism in the oral cavity is not sufficient evidence for considering it a reservoir of the infection (77). Although successful culture of *H. pylori* from the oral cavity has also been reported, the success rate is low (97). The number of organisms in the oral cavity, if present, is rather small: using a competitive PCR assay the median number of *H. pylori* in dental plaque of adults with gastric *H. pylori* infection was found to be 25 cells/mg; in the postemesis saliva collected half an hour after vomiting *H. pylori* cultures had counts ranging from 50–500 cfu/ml (97). Some epidemiological results also favor spread of *H. pylori* by an oral source. Pre-mastication of food and plate sharing were found to be independent risk factors for *H. pylori* infection of children (77).

### 2.2.4.3 Environmental sources: water and food

The analysis of the genome has shown that it is very unlikely that *H. pylori* can multiply in environment. *H. pylori* can survive in water, milk and in various foods under refrigerated storage for several days, suggesting that the water or food contaminated with *H. pylori* could be potentially infectious to humans (77). In water, *H. pylori* remained culturable for up to 24 hours at 20–23 °C and for 2–3 days at 16 °C (99). *H. pylori* has not been cultured from natural freshwater, but was isolated from wastewater in Mexico (77). Using PCR method, *H. pylori* DNA has been detected in drinking water. *H. pylori* DNA has been detected also in biofilms within water storage pots or water distribution systems. Few quantitative data are available. Krumbiegel et al (2004) found that the *H. pylori* DNA was present in approximately one tenth of the private wells in rural counties in Germany, and that estimated average
infestation was approximately one *H. pylori* cell per ml. However, a positive PCR result does not prove that the organism is viable and transmissible (100). Under unfavourable conditions, *H. pylori* may transform from an actively dividing spiral-shaped form into a non-cultur able coc coid form, which may represent an alternative survival system (99) or a morphologic manifestation of cell death (77). These coccoid forms may persist for extended periods in water (99). The question whether the coccoid form of *H. pylori* is able to establish infection in humans is still unclear. In laboratory conditions, coccoid *H. pylori* organisms given at high dosage to mice were able to colonize their gastric mucosa and cause inflammation (101).

In epidemiological studies, generally no association has been found between *H. pylori* infection and a water source in industrialized countries, possibly because of high quality water treatment. Water-borne transmission may occur in regions of the world where the quality of drinking water is low (77). Stored household water, contaminated in home, may also serve as a vehicle of the infection (87).

### 2.2.4.4 Animal reservoirs

Animals are unlikely to be an important reservoir of *H. pylori* infection, although in specific settings, zoonotic transmission may occur. Sheep and Monkey have been reported to harbor *H. pylori* in their stomachs. *H. pylori* was isolated from sheep’s milk, suggesting that it might be a transmission vehicle of *H. pylori*. Regular professional contact with sheep results in almost 100% *H. pylori* prevalence: 98% of studied shepherds in Italy and in Poland were seropositive (77). The role of insects as a potential vector has been studied as well. Houseflies and cockroaches, when fed pure cultures of *H. pylori*, were able to harbor the microorganisms in their gut and excreta for more than 24 hours after initial exposure. However, *H. pylori* was not recovered from any of the houseflies fed human feces either naturally.
infected or artificially infected with *H. pylori*, thereby not confirming that houseflies are vectors for transmission (77).

### 2.2.4.5 Interfamilial transmission

*H. pylori* infection clusters within families: children living with infected parents have higher prevalence of the infection than those living with uninfected parent. Molecular studies have shown that family members often share the same strain of *H. pylori* (77). Intrafamilial clustering of the infection suggests either a person-to-person transmission within family or exposure to a common environmental source of the infection. Close intrapersonal contact appears to facilitate spread of the infection: domestic overcrowding in childhood has been consistently found to be a significant risk factor for *H. pylori* infection for children as well for adults, which supports the theory of person-to-person transmission (102).

In developing countries, where there are large families with many siblings, sibling-sibling transmission might be more important (87). In developed countries, where the number of children in families is generally small, mothers may play the key role (103). Studies have generally failed to identify *H. pylori*-positive father as a risk factor of the infection in offspring (79). Child-to-adult transmission seems to be an unlikely event. During a nine-year follow-up of 46 families in Japan no case was identified where the infection spread from an infected child to an uninfected parent (104). In Sweden, no increased risk for *H. pylori* infection was seen among children who had attended day-care centers in comparison with those who had been exclusively looked after at home (77).
2.3 Clinical Manifestations of \textit{H. pylori} Infection

2.3.1 Natural course of \textit{H. pylori} infection

The ability of \textit{H. pylori} to maintain persistent colonization is of key importance for disease development \cite{37}. Acute \textit{H. pylori} infection in adults is accompanied by mild to moderate dyspeptic symptoms and occasional vomiting, which appear few days after challenge, peak during the second week and then resolve. The clinical course of chronic \textit{H. pylori} infection is highly variable and influenced by microbial, host and environmental factors. In virtually all infected individuals \textit{H. pylori} causes chronic inflammation in the gastric mucosa \cite{77}. Gastritis develops rapidly after acquisition of \textit{H. pylori} infection and persists. Along with its persistence, through several years of the infection, chronic gastritis may gradually progress to atrophic gastritis. The annual incidence of atrophic gastritis among \textit{H. pylori}-positive adults is approximately 1–3\% \cite{105}. Most people with \textit{H. pylori} infection are asymptomatic, but a proportion of infected individuals develop severe gastroduodenal disease, including Duodenal Ulcer (DU), gastric ulcer and rarely gastric adenocarcinoma and gastric Mucosa Associated Lymphoid Tissue (MALT) lymphoma \cite{77}. \textit{H. pylori} infected persons have approximately a 4–6-fold increased risk of developing peptic ulcer disease and approximately a 6-fold increased risk of non-cardia gastric adenocarcinoma and gastric MALT lymphoma compared with uninfected persons. In \textit{H. pylori}-infected subjects, the estimated lifetime risk of peptic ulcer is 6–20\% ; the estimated lifetime risk of gastric cancer is 1–2\% in Western societies and 11–12\% or even higher in Japan. It is hypothesized that early life acquisition of \textit{H. pylori} may increase the risk of subsequent development of gastric cancer. In an animal experiment with Mongolian gerbils, early acquisition of \textit{H. pylori} significantly increased their susceptibility to gastric chemical carcinogenesis as compared with the case of later infection \cite{77}.
2.3.2 Gastritis

Acute *H. pylori* infection causes gastritis and hypochlorhydria and symptoms such as vomiting and dyspepsia have been associated with acquisition (2). Persistent infection causes chronic gastritis in virtually all infected individuals. The gastric inflammation involves infiltration of immune cells, such as neutrophils, lymphocytes, plasma cells and macrophages, and secretion of a multitude of cytokines, of which IL-8 seems to have a central role (10). The chronic gastritis is usually asymptomatic, but eradication of *H. pylori* in non-ulcer dyspeptic patients alleviates symptoms in a fraction of the patients (37).

2.3.3 Peptic ulcer disease

The discovery of the role of *H. pylori* in the development of peptic ulcer disease has lead to a paradigm shift in the treatment of ulcer patients (106). The lifetime risk for peptic ulcer in infected individuals ranges from 3% in the United States to 25% in Japan (10). It has been estimated that 95% of DU and 70% of gastric ulcers can be attributed to *H. pylori* (107). Duodenal ulcers are associated with *H. pylori*-induced antrum-predominant gastritis, decreased somatostatin levels and augmented gastrin and acid secretion (10). Development of gastric metaplasia in the duodenum can allow further bacterial colonization, leading to duodenitis and epithelial damage. Gastric ulcers are associated with corpus gastritis, which is believed to damage the epithelium (9).

2.3.4 Gastric cancer

Gastric cancer ranks as the second most frequent cause of cancer deaths worldwide despite a decreasing incidence in high-income countries (108). The World Health Organization classified *H. pylori* as a class I carcinogen in 1994 due to its definite carcinogenic potential in humans (37). Subsequent studies have corroborated the association to gastric cancer and about 70% of
non-cardia adenocarcinomas have been attributed to *H. pylori* infection \((109)\). However, only a few percent of infected individuals develop gastric cancer \((9)\).

Persons with low gastric acid secretion and corpus-predominant gastritis, leading to atrophic gastritis, loss of parietal cells and further hypochlorhydria, are at increased risk for gastric cancer \((10)\). Postulated carcinogenic mechanisms include the increased epithelial turnover caused by the inflammation. Furthermore, the development of gastric atrophy and hypochlorhydria can result in impaired antioxidant absorption, infection with other carcinogenic microorganisms and formation of carcinogenic compounds \((9)\). *H. pylori* infection up regulates the proinflammatory cytokine IL-1, which is also a potent inhibitor of acid secretion. Polymorphisms considered to increase the activity of IL-1 have been proposed to be associated with hypochlorhydria and cancer, supporting the central role of gastric acidity in cancer development \((110)\).

### 2.3.5 Other *H. pylori*-associated conditions

*H. pylori* has been assessed for its involvement in various additional conditions. An association between the infection and gastric MALT lymphoma belongs among the generally accepted findings and antibiotic treatment alone leads to regression of the cancer in many cases \((111)\). Moreover, it has been debated whether there is a causal relationship between the parallel decline of *H. pylori* prevalence and the increase of gastroesophageal reflux disease (GERD) and esophageal adenocarcinoma in high-income countries. There is accumulating evidence in favor of a protective role of *H. pylori* infection against these conditions, but there are a number of additional contributing factors \((112)\). The high *H. pylori* prevalence in many parts of the world accentuates the public health importance of any associations between *H. pylori* and disease. This may be particularly noteworthy for associations that are of modest strength or involve relatively
benign conditions, as these associations may otherwise tend to be neglected (112).

2.3.6 Extragastric manifestations of *H. pylori* infection in children

2.3.6.1 Iron-deficiency anemia

*H. pylori*-infected subjects have lower mean serum ferritin levels compared with the non-infected ones irrespective to their iron intake (113). *H. pylori* infection is more prevalent among subjects with low serum ferritin level than among subjects with normal serum ferritin level, both of adults and children (113). A number of case reports and case series have described resolution of refractory iron deficiency anemia only after eradication of *H. pylori* infection, thereby supporting the hypothesis that *H. pylori* infection causes iron deficiency and not vice versa (114). The mechanisms through which *H. pylori* infection can cause iron deficiency and further lead to anemia have not been fully elucidated. It has been suggested that *H. pylori* infection may increase the iron demand as *H. pylori* itself uses iron for its growth and may capture ingested iron and iron from human lactoferrin (115).

2.3.6.2 Atopic diseases

The composition of the microflora of the gastrointestinal tract may play a role in development of and protection from allergy (116). The increase in the occurrence of atopic diseases appears to have coincided with the decrease in the prevalence of *H. pylori* infection (77).

In some studies, which have addressed predominantly respiratory allergy, decreased prevalence of atopic diseases and/or allergen-specific IgE antibodies has been found among *H. pylori*-positive adults. whereas some pediatric case-control studies point out that *H. pylori* infection, especially the
infection with a CagA-positive strain may actually increase the risk of food allergy (77).

Accordant with these results, the animal experiments and in vitro studies have demonstrated that H. pylori infection increases the transcellular passage of macromolecules and inhibits development of oral tolerance to food antigens, thereby suggesting that H. pylori infection may predispose to food allergy (117).

2.3.6.3 Acute intestinal infections

Some studies have suggested that H. pylori infection may predispose to various gastrointestinal infections as cholera and shigellosis. Newly acquired H. pylori infection in children was followed by increased occurrence of diarrhea (118). Transient hypochlorhydria has thought to be the mechanism underlying this phenomenon. Passaro et al. (2001) postulated that H. pylori-promoted gastroenteritis some months after acute infection which is a biologically plausible mechanism for successful spread of the H. pylori infection (118). In contrast, in other studies, H. pylori infected children and adults had significantly less acute diarrheal illnesses than non-infected subjects (119). It cannot be excluded that some confounding factors may also explain the conflicting results of these observational studies (77).

2.3.6.4 Diminished growth

Several population-based cross-sectional studies, conducted both in developing as well as developed countries, have found an association between diminished growth and H. pylori infection in children, whereas others have not found such association (77).
2.3.7 Barrett’s esophagus

Barrett’s esophagus (BE) is the condition in which columnar epithelium replaces the squamous epithelium. The condition develops when gastroesophageal reflux disease damages the squamous esophageal mucosa, through a metaplastic process in which columnar cells replace squamous epithelium. The abnormal columnar epithelium that characterizes BE is an incomplete form of intestinal metaplasia (called specialized intestinal metaplasia) that predisposes patients to adenocarcinoma (120). Inflammation of gastric cardiac mucosa decreases in prevalence from controls to patients with GERD, and *H. pylori* and *cagA*-positive strains have been suggested to protect against the development of BE (121). It is important to distinguish BE from intestinal metaplasia related to carditis, because these conditions have a different natural history, risk of malignancy, and treatment. BE is the most important risk factor for the development of esophageal adenocarcinoma. The risk of developing esophageal adenocarcinoma from BE is estimated to be 0.5% per patient per year (39).

2.3.8 Gastroesophageal reflux (GERD)

Even though GERD is primarily a motility disorder, other pathophysiological disturbances seem to play a role in its pathogenesis. Whether *H. pylori* may be one of the players remains controversial and conflicting results have been reported. Indications that the curing of *H. pylori* infection in DU patients may provoke reflux esophagitis have been reported (122). Although not finding any significant difference in the prevalence of *H. pylori* carriage in patients with GERD and its sequel compared with the controls, Vicari found that patients carrying *CagA*-positive strains might be at a decreased risk of complications of GERD (123).
2.3.9 Idiopathic thrombocytopenic purpura

*H. pylori* causes an inflammatory response and provokes an immunologic reaction. It has been proposed that other chronic immune disorder may be caused by an immunologic reaction to *H. pylori* antigen in anti-bodies that cross-react with human tissues. Uncontrolled studies have suggested a role for *H. pylori* in chronic idiopathic thrombocytopenia (124).

2.4 Diagnostic Tests for Detection of *H. pylori* Infection

The diagnostic tests for *H. pylori* infection can be roughly divided into two categories: biopsy-based tests which are invasive tests because as they require gastroscopy, and non-invasive or minimally invasive tests where no gastroscopy is required and the serum, whole blood, feces, expired air, saliva or urine are used for testing. Selection of the test depends on the purpose of testing, sensitivity and specificity of the test, cost-effectiveness of testing strategy and availability of the test (77).

2.4.1 Biopsy-based tests

Biopsy-based tests include histological examination, culture, rapid urease test and tests based on molecular methods. Histological examination: *H. pylori* can be directly visualized by histological examination on the gastric mucosa as characteristic spiral or curve-shaped bacteria. This allows estimation of *H. pylori* colonization density as well as provides permanent record (77). For optimal assessment and for minimizing sampling error, at least two biopsy samples from both the gastric antrum and corpus mucosa should be taken (125). The Giemsa stain appears to be the preferred stain for evaluation of *H. pylori* infection on the basis of its good sensitivity, excellent specificity, and lack of technical difficulty in preparation (126). Besides diagnosing *H. pylori* infection, histology has the advantage of allowing the excellent visualization of morphological changes in the gastric mucosa; for the latter purpose, hematoxylin and eosin stain are generally
used (77). The results of histological examination are somewhat dependent upon experience and on the accuracy of the pathologist, but generally, histological examination has high sensitivity and specificity for detecting *H. pylori* infection, with high inter observer agreement (127).

Culture is a very specific method for diagnosing *H. pylori* infection, however, culturing is technically demanding, time-consuming, and its sensitivity varies among laboratories (128). In experienced laboratories, sensitivity higher than 90% is achieved. The benefit of culture is the possibility to determine the susceptibility of the strain to antimicrobial agents (77).

Rapid urease test is based on the capacity of *H. pylori* to secrete the enzyme urease. When *H. pylori* infected biopsy specimen is placed in a urea-containing medium, the bacterial urease splits urea into ammonia and CO₂, and pH of the medium rises. This is detectable by the color change of the pH indicator dye (77). It is a quick and simple test with a sensitivity about 88–90% and a specificity close to 100% (129). The sensitivity of the test is lower in patients with active or recent upper gastrointestinal bleeding. The test indicates only the presence or absence of the infection and gives no additional information (77).

Tests based on molecular methods, mainly the PCR are used in order to detect *H. pylori* from biopsy specimens with high sensitivity and specificity (130), to detect point mutations encoding *H. pylori* resistance to antibiotics, especially to clarithromycin and to detect virulence factors (131).

### 2.4.2 Tests based on detection of specific anti-*H. pylori* antibodies

*H. pylori* infection induces a humoral immune response in the host, resulting in an early increase of specific IgM antibodies, and later a persistent increase of specific IgG and IgA antibodies (132). Specific antibodies can be detected in serum, whole blood, urine and saliva, both from fresh and freeze- stored
samples (133). The limitation of the tests based on specific antibody detection is that acute infection can be missed due to the delay in antibody production as IgG sero-conversion occurs approximately 7–14 weeks after infection (134). On the other hand, false-positive results may occur, as antibody titers may remain positive even for years after resolution of the infection (135). However, the titer of specific antibodies has tendency to fall: a 25–50% drop in the IgG antibody titer in paired sera 6 months after treatment confirms eradication in most cases (136).

Enzyme-linked immunosorbent assay (ELISA) is commonly used for detection of specific antibodies in the serum, as it is easy to perform, inexpensive, widely available, and suitable also for large-scale screening (137). The meta-analysis of 21 studies with commercially available ELISA kits reported overall sensitivity of 85% and specificity of 79%. The performance of different assays may vary between patient populations from different geographic regions and ethnic backgrounds due to the differences between antigenic properties of bacterial strains colonizing patients (138). Therefore, the test should be locally validated. In young children, the sensitivity of ELISA is low when the cut-off values validated for adults are used (133).

Immunoblot is more costly and time-consuming than ELISA, but permits a detailed analysis of antibody reactivity to the different H. pylori proteins. It has been a useful tool that complement serology in children, particularly in cases with doubtful ELISA results (139).

Several near-patient or office-based tests on the whole blood have been developed, they are quick and easy to perform, however, the diagnostic accuracy of these tests is inadequate for recommending them for widespread usage (140). Tests on the saliva and urine are attractive because samples can be easily obtained. Salivary tests have shown inconsistent results with
less than optimum sensitivity and specificity, urine antibody tests have demonstrated accuracy comparable to that of serum-based ELISA (141).

2.4.3 Urea breath test

Urea breath test (UBT) is based on the capacity of *H. pylori* to secrete the enzyme urease, which hydrolyses urea to ammonia and carbon dioxide. The test substance used is urea, labelled with a carbon isotope, either with 14C (a radioactive isotope) or 13C (a stable isotope). The 13C-UBT is most frequently employed for its safety; however, the cost of 13C-UBT is higher than of 14C- UBT. In non-infected individuals, ingested urea leaves the stomach unchanged, while in infected subjects, urea is split into ammonia and CO₂, which can be detected in the expired air. Labeled urea is usually given to the patient with a test meal to delay gastric emptying and to increase the time of contact with the mucosa (77). The UBT detects current *H. pylori* infection with a sensitivity and specificity of >95% (140). The test is indicated for initial diagnosis of the infection and for follow-up of eradication therapy; in the latter case, the UBT should not be performed before an interval of four weeks has elapsed (142). False-negative results may occur during treatment with proton pump inhibitors (143).

2.4.4 Tests based on detection of bacterial antigens or bacterial DNA in feces

Identification of bacterial antigens in stool using enzyme immunoassays has emerged in recent years, and tests based on polyclonal antibodies (144) and monoclonal antibodies (145) have been developed. A systematic review of 89 studies including 10,585 patients concluded that stool antigen test is an accurate method for diagnosing *H. pylori* infection: the mean sensitivity and specificity were 91% and 93%, respectively (146). Overall results revealed that tests based on monoclonal antibodies are slightly more sensitive and specific and have greater reproducibility than tests based on polyclonal
antibodies (146). PCR-based methods of detecting *H. pylori* DNA in stool samples have also been developed (147). They allow genotyping of strains, detection of virulence factors and testing of antibiotic susceptibility (148).

2.5 Treatment of *H. pylori* Infection

The goal of treatment of *H. pylori* infection is complete eradication of the organism. Eradication is currently defined as absence of *H. pylori* for a minimum of 4 weeks after treatment, as confirmed by biopsy-based test, UBT or stool antigen test (142). Clinically relevant eradication regimens must have cure rates of at least 80%, according to intention-to-treat analysis, without major side effects and with minimal induction of bacterial resistance (10).

2.5.1 Indications for treatment

Eradication of *H. pylori* infection is an effective way to cure *H. pylori*-associated peptic ulcer disease, as it facilitates ulcer healing, prevents recurrences in vast majority of patients (108), and significantly reduces the risk of complications. Eradication of *H. pylori* infection leads to resolving of gastric inflammation both in adults and in children, and it may lead to reversion of existing atrophy in the gastric mucosa (149). Eradication of *H. pylori* induces remission of gastric MALT lymphoma (150) and would likely prevent development of gastric cancers if intervention comes early enough in the sequence of carcinogenesis (151).

Whether the eradication of *H. pylori* results in improvement of symptoms in patients with non-ulcer dyspepsia or not is still debatable. Meta-analyses of randomized controlled trials have yielded equivocal results: one showed no improvement of symptoms with *H. pylori* eradication, while another revealed a statistically significant but small benefit (77). At the best, one case of non-ulcer dyspepsia is being cured for every 10 persons whom the infection was
eradicated; at the worst, eradication induces exacerbation of gastro-esophageal reflux disease (152).

A number of guidelines on management of *H. pylori* infection have been compiled, based on published clinical evidence and expert opinion, as the Maastricht 2–2000 Consensus Report (142) and guidelines from European Society of Primary Care Gastroenterology (ESPCG) (153).

All guidelines and consensus statements recommend testing for *H. pylori* infection only when subsequent treatment of *H. pylori* infection is planned, and recommend eradication of *H. pylori* in patients with peptic ulcer disease. Other recommendations vary somewhat between different guidelines according to the population and setting applied (77).

The patient should be investigated for *H. pylori* infection only when they present with symptoms and signs suggestive of organic disease, such as peptic ulcer, and that optimal approach to investigation for *H. pylori* infection is upper gastrointestinal endoscopy with multiple biopsies. “Test-and-treat” strategy, i.e. verifying presence of *H. pylori* infection by UBT or stool antigen test and prescribing anti-*H. pylori* treatment (77). There is some disagreement regarding whether *H. pylori*-positive children with abdominal complaints but without peptic ulcer should receive eradication treatment. The North America society for Pediatric Gastroenterology (NASPGN) guidelines state that there is no compelling evidence to recommend eradication treatment (154), whereas the proceedings of the European and Canadian consensus conferences state that if symptoms are severe enough to justify endoscopic evaluation, and if *H. pylori* infection is found, then the treatment is indicated (155).
2.5.2 Combined antibacterial regimens against *H. pylori* infection

*H. pylori* is susceptible to many antibiotics *in vitro*, e.g. penicillin, ampicillin, cefotaxime, erythromycin and tetracycline. *In vivo*, however, the effectiveness of a treatment with a single agent is very low, less than 15% (154). For a successful treatment, combined anti-bacterial therapy is needed, and because low acidity influences the effectiveness of some antimicrobial agents, combinations with proton-pump inhibitors (PPI) or ranitidine are often used (77).

A large number of different medication and their combinations have been evaluated to find optimal therapeutic regimen(s), and optimizing of treatment is an ongoing challenge. Laheij et al. (1999) reviewed studies performed up to 1998 and identified 132 different medication combinations used. Oderda et al. (2000) reviewed studies done on pediatric population until 1999, and found that a total of 22 different treatment combinations were used. The most effective treatment combinations in adults comprise with three medicaments: a so-called proton pump inhibitor based triple therapy which consists of a proton pump inhibitor together with two antibiotics, usually clarithromycin with either amoxicillin or metronidazole, or so-called classic triple therapy consisting of a bismuth compound together with metronidazole and tetracycline or amoxicillin. The cure rates of different triple regimens, analyzed on intention-to-treat basis, are roughly 75–85% (156).

The recommended first-line therapy for adults according to the Maastricht 2–2000 Consensus Report is a proton pump inhibitor (alternatively ranitidine bismuth citrate) combined with clarithromycin and amoxicillin or metronidazole. The recommended duration of the treatment ranges from 7 to 14 days (142).

In children, few randomized double-blinded trials have been performed on eradication of *H. pylori* infection (157), and the best treatment strategies have
to be identified. Existing data indicate that dual therapies might be as effective as triple therapies (158). whereas the European Pediatric Task Force on *H. pylori* has not given any specific recommendations regarding optimal treatment due to the insufficient data in relation to this issue (155).

### 2.5.3 Factors influencing treatment results

None of the regimens cure *H. pylori* infection in 100% of the patients; a number of factors, as bacterial resistance to antibiotics, patient compliance, high bacterial load, low gastric pH and others may affect outcome of eradication therapy (159). The most important reason for treatment failure appears to be drug resistance, especially clarithromycin-resistance, which leads to about 70% decrease in efficacy of the clarithromycin-containing regimen, whereas the impact of metronidazole resistance on clinical outcome is relatively modest, leading to about 25% decrease in efficacy (160). The controversy can be attributed to the methodological problems in determination of metronidazole resistance of *H. pylori* (161). The pattern of resistance to clarithromycin and metronidazole varies within wide limits in different regions (162). The resistance of *H. pylori* to amoxicillin and tetracycline is low, less than 1% of isolates worldwide (161). The primary or acquired resistance of *H. pylori* to bismuth salts has never been reported (163).

The other important determinant of successful eradication therapy is patient’s compliance with the prescribed regimen (164). The common reason of non-compliance is occurrence of side effects (164), while convenience also plays a role: patients receiving a 2–3-times-a-day regimen were significantly more compliant than those receiving a 4-times-a-day regimen (165). Eradication treatment appears to be more effective in patients with peptic ulcer disease than without it, irrespective of patient compliance (24).
2.5.4 Alternatives for treatment of *H. pylori* infection

The suboptimal success rate of current antibacterial regimens and emerging antibiotic resistance has lead to the search of new modalities to treat *H. pylori* infection (77). Investigation of the possible role of probiotics in the adjunctive treatment and prophylaxis of *H. pylori* infection has yielded encouraging results (166). The effects of medicinal plants on *H. pylori* have been studied as well (167). Bovine lactoferrin in combination with antibiotic therapy has improved the *H. pylori* eradication rate (168).

One of alternative approaches is passive immunization with orally administered antibodies (77). One of the best accessible sources of immunoglobulins is bovine colostrum, the milk secreted by cows during the first four days after parturition. The bovine colostrum contains approximately 60 g immunoglobulins per liter (range 30–200 g/l); majority of it, over 75% comprises IgG1 (169). The concentration of specific antibodies against pathogens in the colostrum can be increased by immunizing cows with these pathogens or their antigens (170). Such colostrum is referred to as immune colostrum or hyperimmune colostrum. Orally administered bovine immune colostrum (BIC) has been shown to provide effective protection against various gastrointestinal infections such as rotaviral infection, shigellosis, and infection with enterotoxigenic *E. coli*. Successful therapeutic interventions have been reported in childhood rotaviral infections and cryptosporidial infections in immunocompromized patients (171). *In vitro*, BIC exhibits high bactericidal activity against *H. pylori* (172) and inhibits *H. pylori* adherence to the gastric mucosa in a dose-dependent manner (170). Promising results have been obtained in animal experiments: mice given monoclonal IgA anti-*H. felis* antibodies at the time of initial challenge were protected from infection (26). Marnila et al. (2003) demonstrated that the BIC derived from cows immunized with *H. felis* but not control colostrum protected some mice against infection: after an experimental challenge, 10 out of 17 mice in immune preparation group remained *H. felis* negative versus none out of 18
mice in control group \( p<0.005 \) \( (172) \). Casswall et al. (2002) reported eradication of \( H. pylori \) infection in mice receiving BIC \( (170) \).

2.5.5 Recurrence of \( H. pylori \) infection after eradication

Recurrence refers to a situation where tests for \( H. pylori \) infection, which were negative at four weeks after cessation of treatment, become positive at a later stage \( (173) \). Recurrence is the result of either reinfection or recrudescence. Recrudescence is a situation where the pre-treatment strain of \( H. pylori \), which was suppressed by treatment and was undetectable one month after treatment, becomes detectable at a later stage, usually within first three months after treatment \( (77) \).

Reinfection is acquisition of a new infection, either a new strain of \( H. pylori \) or a genetically identical strain, after the original strain of \( H. pylori \) has been completely eradicated \( (173) \). Due to the high grade of genetic diversity of \( H. pylori \) strains among unrelated patients, distinguishing between recrudescence and true reinfection by comparing pre- and post-treatment isolates by genotyping methods is suggested \( (173) \). However, the results of genetic analysis of the strains should be interpreted with caution, since an individual patient may be colonized with multiple strains \( (174) \), also there may occur reinfection by a strain identical to the one present prior to treatment \( (77) \).

The recurrence rate during the first 6–12 months is inversely associated with the efficacy of the original course of eradication therapy \( (175) \). This suggests that in cases of low effective treatment, most recurrences that occur shortly after treatment are actually recrudescence \( (77) \). The risk of reinfection appears to be different in different regions of the world. In industrialized countries, reinfection is a rare event: the reported recurrence rates in adults are generally lower than 2% per patient per year. The lowest recurrence rate,
virtually no recurrences during 2 years after treatment has been reported in a
study conducted in Germany (176).

In developing countries, the reported recurrence rates are variable, being
generally higher than 5% per patient-year. The highest reinfection rates,
>15% per patient-year, have been reported from Peru and Bangladesh (177). A
recurrence rate as low as 1.1% per patient-year was found in a study
conducted in China (178).

Prevalence of the infection among family members seems not to predict
recurrence rate. In Germany, there were no recurrences detected in 96
successfully treated adult patients during a 2–year follow-up in spite of the
fact that 56% of the spouses were infected (176). The reinfection was an
unlikely event also among Irish children (reinfection rate 2.0% per patient-
year), even though 66% of the siblings, 78% of the mothers and 65% of the
fathers in this study were infected, and having infected parents and siblings
was not an independent risk factor for recurrences (179). Farrell et al. (2004)
conducted a prospective randomized study to investigate whether eradication
of H. pylori in the entire family reduces the risk of childhood reinfection, and
found no significant effect. These data suggest that H. pylori eradication
therapy should not be prescribed for infected spouses or siblings with the aim
to prevent reinfection of the patient (180).

### 2.5.6 Prevention of H. pylori-associated disease

Prevention of H. pylori-associated disease benefits from predictions of who
will become clinically ill (37). Accordingly, current treatment guidelines advise
prophylactic H. pylori eradication for some individuals at higher risk for
disease, for example patients with atrophic gastritis or taking Non Steroidal
Anti Inflammatory Drugs NSAIDs (10). Some studies have also targeted high-
risk population groups to study the effect of H. pylori eradication (37). Anti-H.
pylori treatment has been reported to increase regression of cancer
precursor lesions (181) and, despite low power and a lack of studies, there is some evidence that *H. pylori* eradication may protect against gastric cancer (151). Future prevention approaches may possibly benefit from a deeper knowledge of the pathogenic mechanisms by allowing more precise identification of individuals at high risk for disease (37).

Indiscriminate treatment of *H. pylori* infections has been proposed as an approach to limit the burden of *H. pylori*-associated disease (37). The appropriateness of such a large-scale and crude intervention has been questioned due to the uncertain full spectrum of possible harmful consequences, for example the development of antibiotic resistance (10). Testing and treating large numbers of persons would also imply significant costs and would therefore be unrealistic in many parts of the world (37).

An alternative approach could be to target the acquisition or persistence of the infection, while limiting the use of antibiotics (37). The role of an *H. pylori* vaccine is uncertain given the common failure of the immune system to clear the infection and the apparently inadequate protective immunity against reinfection (177). A protective vaccine would also have to be administered at an early age before the infection is acquired. At this age, an immature immune system may not respond sufficiently to immunization. Another approach could perhaps be a therapeutic vaccine that would circumvent problems with antibiotic resistance (37). There have been considerable efforts to develop vaccines against *H. pylori*, but despite some encouraging results further work is needed to bring about effective and safe candidates for humans (182). Moreover, probiotics have been suggested to be capable of contributing to control of *H. pylori* infection, but this area of research is in its infancy (166). Any future prevention of *H. pylori*-associated disease should likely be primarily aimed at high-risk populations and target both the infection and other known risk factors. However, understanding and interfering with the acquisition or persistence of the infection by other means may become useful supplemental strategies. This is likely to be especially true in some
(low-income) populations, where effective antibiotic regimens may be impaired by high cost, poor compliance, antibiotic resistance and high reinfection rates (37).
Chapter III
Materials and Methods
CHAPTER III

Materials and Methods

The optimal diagnostic approach in patients with dyspepsia is still controversial. Upper endoscopy is frequently performed as the primary diagnostic test, but it is costly and in most patients no underlying disease can be identified (183). It has been suggested that a strategy based on non-invasive testing for H. pylori could be more cost-effective. Such a strategy could either imply the referral of only H. pylori-positive patients for endoscopy ("test and scope" strategy) (184) or subjecting H. pylori-positive patients to anti-H. pylori treatment ("test and treat" strategy) (185). Whatever approach is chosen, its efficacy is highly dependent on the accuracy of the test used to diagnose H. pylori infection. This study aims at comparing between two invasive tests and two non-invasive tests. In addition, risk factors associated with H. pylori infection were evaluated.

3.1 Materials and Reagents
- Sterile syringes and needles.
- Alcohol.
- Red top tubes for serum.
- Plasters.
- Eppendorf tubes.
- Glass slides.
- Sterile containers for stool collection.
- Ice box for keeping the sample through transportation.
- Deep freezer for specimen storage.
- ELISA Kit for IgM antibodies detection in serum (DRG-Germany).
- HpSAg detection Kit to detect antigen in stool (DRG-Germany).
- Loeffler’s Methylene blue for staining.
- Methyl Alcohol (absolute) for staining.
- Ultra Rapid Urease Test (prepared in house) for biopsy specimens.
3.2 Permissions and Ethical Consideration
The appropriate permissions were taken from the concerned authorities for sample collection, and permissions were taken from the patients to make the interview and the sample collection. Patients were informed about the purpose of this research and a verbal agreement was given by the patient before any sample collection or interview.

3.3 Patients
Eligible participants with possible *H. pylori* infection were defined as those patients independently assessed by their attending physician based on clinical symptoms (186). There was no specific age limitation for participant.

3.4 Sample Size
One hundred twenty randomly selected eligible patients were subjected to endoscopy for exploration and gastric or duodenal biopsy collection by there attending physicians. In addition, blood and stool samples were collected. All patients were interviewed. However, only 89 participant submitted the three types of samples.

3.5 Sample Collection

3.5.1 Gastric biopsies.
Gastric biopsies sampling was performed by a specialized physicians were the patient underwent upper gastrointestinal endoscopy and a biopsy was collected, placed in a sterile phosphate buffer for ultra rapid urease testing (URUT) and for imprint smears (187). Biopsy tissue was collected by using standard endoscopy biopsy forcipes, gastroscopies which were used are(Olympus GIF Type Q40 – 2300903), Olympus Videotrolley tv-z CLE-10.
Figure (3.1): Olympus Videotrolley tv-z CLE-10 machine

Figure (3.2): A patient undergoing gastric biopsy collection.
3.5.2 Blood sample for serological evaluation
About 5 ml of venous blood were collected from patients in a plain tube with no anticoagulant. The tube was labeled with the patient name, number and date of collection. The tubes were placed in a water bath at 37 °C for 30 minutes or until clotted. The clotted blood was centrifuged and serum aspirated into several (200 µl) Eppendorf tubes and stored at -70 °C until tested.

3.5.3 Stool sample for antigen detection
About 20 grams of stool were collected into a sterile container. All stool samples were frozen at -70 °C until tested for *H. pylori* antigen.

3.6 Sample processing

3.6.1 Blood sample
Serum was brought to room temperature for IgM detection by IgM enzyme-linked immunosorbent assay (ELISA kit). Cat # EIA-2111, Tests: 96 wells, DRG Instruments GmbH, Germany (188).

3.6.1.1 Principle of the ELISA test
Purified *H. pylori* antigen is coated on the surface of microwells. Diluted patients serum is added to the wells and the *H. pylori* IgM- specific antibody, if present, binds to the antigen. All unbound materials are washed away. Enzyme conjugate is added, which binds to the antibody-antigen complex. Excess enzyme conjugate is washed off and a solution of TMB Reagent is added. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the color generated is proportional to the amount of IgM-specific antibody in the sample. The results are read by ELISA reader compared in a parallel manner with calibrator and control.
3.6.1.2 Assay procedure
1) The desired number of the coated wells was secured in the holder.
2) The followings were prepared; 1:40 dilution for test samples, negative control, positive control and calibrator by adding 5 µl of the sample to 200 µl of sample diluents and mixed well.
3) One hundred µl of diluted sera, calibrator and controls were dispensed into the appropriate wells. For the reagent blank, 100 µl sample diluents was dispense in A1 well position. The holder was Tapped to remove air bubbles from the liquid and mixed well.
4) The plate was incubated at room temperature for 30 min.
5) At the end of the incubation period, liquid from all wells was removed. The microtiter plate was rinsed and flicked 4 times with diluted wash buffer (1x) and then one time with distilled water.
6) Enzyme conjugate (100 µl) was dispensed to each well and mixed gently for 10 seconds.
7) Incubated at room temperature for 30 min.
8) Enzyme conjugate was removed from all wells. Microtiter plate was rinsed and flicked 4 times with diluted wash buffer (1x) and then one time with distilled water.
9) TMB reagent (100 µl) was added to each well and mixed gently for 10 seconds.
10) Microtiter plate was incubated at room temperature for 20 min.
11) One hundred µl of stop solution was added to each well including the 2 blanks.
12) The plate was mixed gently for 30 seconds. All the blue color changed to yellow color.
13) The optical density was read at 450nm within 15 min with ELISA reader.

3.6.1.3 Calculation of result:
1) The mean of duplicate calibrator value was calculated.
2) The mean of duplicate positive control, negative controls and patient's samples were calculated.
3) Then the *H. pylori* IgM EIA index of each determination was calculated by dividing the mean values of each sample by calibrator mean value.

### 3.6.2 Biopsy specimen

#### 3.6.2.1 Ultra rapid urease test (URUT)

This test is based on the high concentration of pre-formed urease enzyme in *H. pylori* infected gastric biopsy samples. This will bring about a pH change when placed in a urea containing medium. The sensitivity of the test depends on the number of bacteria present in the sample, which may have consequences for its use when evaluating treatment failures etc. The specificity is very good when the test is read within 1 minute, but declines with the length of the incubation.

Biopsy tissue was collected by (Olympus GIF Type Q40 – 2300903), Olympus Videotrolley tv-z CLE-10. Each biopsy tissue was placed immediately into capped Eppendorf tube containing 0.5 ml of freshly prepared solution of 10% urea in deionized water, to which had been added two drops of 1% phenol red as pH indicator. Positive result was indicated by change in the color of the solution from orange to pink within the first minute (189).

#### 3.6.2.2 Microscopy

After reading the result of the ultra rapid urease test (URUT) the biopsy was removed from the urea solution and imprint smears were made by lightly rolling it on a clean glass slide, using a hypodermic needle. The imprint smear was air dried and fixed in absolute methanol. Imprint smears were stained by loeffler’s methylene blue stain. Slides were read using the oil immersion objective to search for the curved bacilli (187).
3.6.3 Stool sample

3.6.3.1 Kit components

*H. pylori* Ag (stool) ELISA, Cat #: EIA-4354 Kit from DRG, Germany Instruments GmbH. Tests: 48 wells. Two kits were used. Antibody coated microwells, positive control, negative control, sample diluent, wash buffer, enzyme conjugate, substrate, stop solution, transfer pipettes, strip holder, strip sealer and wooden stick applicators.

3.6.3.2 Pre assay controls and operations

1) Stool sample was prepared as described in figure 3.3, which illustrate the instructions for the proper use of *H. pylori* Ag extraction kit.

2) The liquid components were checked by the naked-eye for visible particles or aggregates (to eliminate the possibility of contamination). The chromogen/substrate was checked for color (colorless or pale blue) by aspirating a small volume of it with a sterile transparent plastic pipette. All other components were checked according to the manufacturer recommendations.

3) The content of the 20x concentrated wash solution was diluted with the proper diluent.

4) The calibrator set were dissolved.

5) All the other components were allowed to reach room temperature (about 1 hr) and then mixed by vortex.

6) The ELISA incubator was set at 37 °C.

7) The ELISA reader was turned on at least 20 minutes before reading.
Instruction for use

Open the collection device and introduce the extraction brush deeply into the specimen. Rotate the brush 3-4 times in order to collect the right amount of sample (about 0.2 g).

Transfer the brush into a test tube. Add 1ml Extraction Buffer and then mix on vortex for 1 min in order to dissolve the sample in solution.

Discard the brush and insert the filter piston into the tube. Push the piston down to the bottom in order to release a soluble filtered material with the Pasteur pipette.

Figure (3.3): Schematic presentation of the HpSAg detection procedure
3.6.3.3 Assay procedure (Quantitative Assay)

1) The required number of strips was placed in the plastic holder and the wells for calibrator and samples were carefully labelled. A1 + B1 wells were left empty for blanking purposes.

2) One hundred µL Calibrators was pipetted in duplicate into the calibrator wells.

3) With the Pasteur pipette supplied, 3 drops of the extracted stool sample were aspirated and dispensed into the sample well.

4) Then 100 µl enzymatic conjugate were dispensed in all wells, except for A1 + B1, used for blanking operations.

5) Following the addition of the conjugate, the color of the samples has turned from brown to pale reddish and the microplate was incubated for 120 min at 37 °C.

6) When the first incubation is over, the microplate was washed 5 times.

7) Two hundreds µl chromogen/substrate were added into all wells including A1+B1. The microplate was incubated protected from light at room temperature (18-24 °C) for 20 min.

8) Sulphuric acid (100 µl) were pipetted into all the wells to stop the enzymatic reaction, using the same pipetting sequence as in step 7.

9) The color intensity of the solution was measured in each well, using 450 nm filters and at 620-630nm filters.

3.6.3.4 Calculation of result

For qualitative reading
The test results were calculated by means of cut-off value determined from the OD450nm value of the (CAL0) and the OD450nm of the CAL0.1 µg/ml with the following formula: \( \text{Cut-off} = \frac{(\text{CAL0} + \text{CAL0.1})}{2} \)

3.7 Questionnaire

An interview questionnaire was used to collect data from eligible patients. The independent variables included in the questionnaire were; gender,
weight, height, age, civil state, tea and coffee drinking, smoking, type of food ingested (meat, fish, vegetables, others), type of water drinking during childhood and adulthood (filtered, municipality or well water), garbage collection in the neighbourhood, indicators of socioeconomic status: educational level, family income, type of accommodation (house, flat, villa), water supply (well, municipality), sewage system, number of rooms and number of persons residing in each dwelling, contact with animal, travelling abroad, consuming antibiotics, consuming drugs and dental complain (See annex for the Arabic questionnaire).

3.8 Analysis of Data
Data generated from the study was tabulated as Microsoft Excel sheets and uploaded to Statistical Package for Social Sciences (SPSS version 11). Cross tabulation of variables were generated. Chi square was used to detect statistically significant correlation among variables.
Chapter IV
Results
Chapter IV
Results

4.1 Study Sample Description

This study was conducted during the period from September 2006 to March 2007. During the study period, 122 patients undergoing upper gastrointestinal endoscopy were interviewed and they answered several questions regarding personal information and their life style. A serum, gastric biopsy and stool specimens were collected. Among them only 89 patients provided the three specimen types.

Those patients are non-hospitalized patients from different hospitals across Gaza strip. Approximately 10% from patients were from Al-shifa hospital, 45% from Balsam-hospital, 30% from the European-hospital and 15% from the Al-karamah-hospital. The following table is a concise description of the study sample.

Table (4.1): Age and sex distribution of the study sample

<table>
<thead>
<tr>
<th>Age group</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
</tr>
<tr>
<td>13-20</td>
<td>1</td>
<td>16.7</td>
<td>5</td>
</tr>
<tr>
<td>21-35</td>
<td>37</td>
<td>78.7</td>
<td>10</td>
</tr>
<tr>
<td>36-50</td>
<td>11</td>
<td>50</td>
<td>11</td>
</tr>
<tr>
<td>Over 51</td>
<td>8</td>
<td>57.1</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>56</td>
<td>62.9</td>
<td>33</td>
</tr>
</tbody>
</table>

The study population age ranged between 13-77 years, with mean age 37.03. (37.1%) are females and (62.9%) are males. Males constituted about two third of the age group 21-35 years.
4.2 Ultra Rapid Urease Test
The test which was performed on biopsy collected from patients during upper gastroscopy proved to be rapid and simple. The following table (4.2) shows the results of rapid urease test which indicate 32.6% positivity. Figure (4.1) shows positive (pink to red) and a negative (yellow to orange) URUT.

![Ultra rapid urease test](image)

**Figure (4.1):** Ultra rapid urease test (left positive; right negative).

4.3 Gastric biopsies Stained with Methylene Blue
Although simple to perform and does not require special equipment, the reading and interpreting of results was somewhat tedious and requires time. From the 86 biopsies stained with Methylene blue, 40 showed *H. pylori* constituting 46.5% (table 4.2). Three samples were lost during the course of this work. Figure 4.2 illustrates a positive Methylene blue stained smear for *H. pylori*.
Figure (4.2): Methylene blue stained gastric biopsies showing the helical bacilli

4.4 *H. pylori* Serum IgM

The result of *H. pylori* Serum IgM performed using ELISA Kit is listed in table (4.2). According to the manufacturer recommendations of interpreting absorbances of serum samples, 40 (44.9%) of the samples were considered positive.

4.5 *H. pylori* antigen Detection From Stool

*H. pylori* antigen detection from stool is receiving attention from service laboratories as well as from researchers. According to the manufacturer recommendation of interpreting absorbances of the extracted stool samples, 32 (36%) were considered positive (table 4.2).
Table (4.2): Distribution of positive and negative results in each of the four tests used

<table>
<thead>
<tr>
<th>H. pylori</th>
<th>¹URUT</th>
<th>²MB stain</th>
<th>³HpSAg</th>
<th>⁴IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Negative</td>
<td>60</td>
<td>67.4</td>
<td>46</td>
<td>51.7</td>
</tr>
<tr>
<td>Positive</td>
<td>26</td>
<td>32.6</td>
<td>40</td>
<td>46.5</td>
</tr>
<tr>
<td>Total</td>
<td>89</td>
<td>100</td>
<td>86</td>
<td>96.6</td>
</tr>
</tbody>
</table>

¹URUT; Ultra Rapid Urease Test, ²MB; Methelyene Blue stain, ³HpSAg; H. pylori Stool antigen and ⁴IgM; H. pylori Immunoglobuline M

From the table one can notice the variation between the four tests, the highest positivity for H. pylori was in the IgM test and methylene blue 44.9% followed by HpSAg test 36.0% and the lowest positivity was in urease test 32.6%.

4.6 True Positive For H. pylori Infection.

As indicated in table (4.2), there are variations in the percentage of positive results for the four employed tests. A true positive was assumed if URUT and/or Methylene blue tests were positive (table 4.3) (189). All the subsequent correlations between possible risk factors and H. pylori infection were done with the true positive.

Table (4.3): True H. pylori positive

<table>
<thead>
<tr>
<th>H. pylori</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>46</td>
<td>51.7</td>
</tr>
<tr>
<td>Positive</td>
<td>43</td>
<td>48.3</td>
</tr>
<tr>
<td>Total</td>
<td>89</td>
<td>100.0</td>
</tr>
</tbody>
</table>
4.7 Statistical Analysis of *H. pylori* Tests Results (Chi square)

In table (4.4), (4.5) and (4.6) there were statistically significant differences between MB and URUT, IgM and HpSAg test with *P* value < 0.01. As shown in table (4.4) the agreement in positive result between MB and URUT test was 92.9%. In table (4.5) the difference between IgM test and MB were significant with *P* value < 0.01. The agreement were 69.2% in positive result. In table (4.6) the agreement between MB and HpSAg test were 96.8% in positive result and in table (4.7) the agreement between URUT and HpSAg test were 89.7% in positive result, URUT and HpSAg gave statistical significant differences with *P* = 0.01. In table (4.8) the difference between IgM and URUT was not significant (*P* = 0.415), but the difference between HpSAg and IgM that showed in table (4.9) was significant with *P* = 0.034 and the agreement between the positive result 47.5%.

**Table (4.4):** Chi square test for statistical differences between the results of Methylene blue stain and URUT

<table>
<thead>
<tr>
<th>Ultra rapid urease test</th>
<th>MB test</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Negative</td>
<td>44</td>
<td>75.9</td>
<td>14</td>
<td>24.1</td>
</tr>
<tr>
<td>positive</td>
<td>2</td>
<td>7.1</td>
<td>26</td>
<td>92.9</td>
</tr>
<tr>
<td>Total</td>
<td>46.0</td>
<td>53.5</td>
<td>40</td>
<td>46.5</td>
</tr>
</tbody>
</table>

**Table (4.5):** Chi square test for statistical differences between the results of Methylene blue stain and IgM in serum

<table>
<thead>
<tr>
<th>IgM in serum</th>
<th>MB test</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Negative</td>
<td>34</td>
<td>72.3</td>
<td>13</td>
<td>27.7</td>
</tr>
<tr>
<td>positive</td>
<td>12</td>
<td>30.8</td>
<td>27</td>
<td>69.2</td>
</tr>
<tr>
<td>Total</td>
<td>46</td>
<td>53.5</td>
<td>40</td>
<td>46.5</td>
</tr>
</tbody>
</table>
Table (4.6): Chi square test for statistical differences between the results of Methylene blue stain and HpSAg

<table>
<thead>
<tr>
<th>HpSAg</th>
<th>MB test</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>No</td>
<td>%</td>
<td>Positive</td>
<td>No</td>
<td>%</td>
<td>P value</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>negative</td>
<td>45.0</td>
<td>81.8</td>
<td>10.0</td>
<td>18.2</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>positive</td>
<td>1.0</td>
<td>3.2</td>
<td>30.0</td>
<td>96.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>46.0</td>
<td>53.5</td>
<td>40.0</td>
<td>46.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table (4.7): Chi square test for statistical differences between the results of URUT and HpSAg

<table>
<thead>
<tr>
<th>URUT</th>
<th>HpsAg</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>No</td>
<td>%</td>
<td>Positive</td>
<td>No</td>
<td>%</td>
<td>P value</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>negative</td>
<td>54.0</td>
<td>90.0</td>
<td>6.0</td>
<td>10.0</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>positive</td>
<td>3.0</td>
<td>10.3</td>
<td>26.0</td>
<td>89.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>57.0</td>
<td>64.0</td>
<td>32.0</td>
<td>36.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table (4.8): Chi square test for statistical differences between the results of IgM and URUT

<table>
<thead>
<tr>
<th>IgM in serum test</th>
<th>Ultra rapid urease test</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>No</td>
<td>%</td>
<td>Positive</td>
<td>No</td>
<td>%</td>
<td>P value</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>negative</td>
<td>34</td>
<td>69.4</td>
<td>15</td>
<td>30.6</td>
<td>0.415</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>positive</td>
<td>26</td>
<td>65.0</td>
<td>14</td>
<td>35.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>67.4</td>
<td>29</td>
<td>32.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table (4.9): Chi square test for statistical differences between the results of IgM and HpSAg

<table>
<thead>
<tr>
<th>IgM in serum test</th>
<th>Negative</th>
<th>Positive</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Negative</td>
<td>36</td>
<td>73.5</td>
<td>13</td>
</tr>
<tr>
<td>positive</td>
<td>21</td>
<td>52.5</td>
<td>19</td>
</tr>
<tr>
<td>Total</td>
<td>57</td>
<td>64.0</td>
<td>36</td>
</tr>
</tbody>
</table>

Table (4.10): Pearson correlation between the different *H. pylori* tests.

<table>
<thead>
<tr>
<th>Test</th>
<th>MB</th>
<th>URUT</th>
<th>IgM</th>
<th>HpSAg</th>
<th>True positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>MB</td>
<td>Correlation 0.646(<strong>) 0.415(</strong>) 0.757(<strong>) 0.954(</strong>)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sig. (2-tailed) 0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>URUT</td>
<td>Correlation 0.646(**)</td>
<td>0.047</td>
<td>0.778(<strong>) 0.719(</strong>)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sig. (2-tailed) 0.0</td>
<td>0.7</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>IgM</td>
<td>Correlation 0.415(**) 0.047</td>
<td>0.217(*) 0.347(**)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sig. (2-tailed) 0.0</td>
<td>0.7</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>HpSAg</td>
<td>Correlation 0.757(<strong>) 0.778(</strong>) 0.217(*)</td>
<td>0.728(**)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sig. (2-tailed) 0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>True positive</td>
<td>Correlation 0.954(<strong>) 0.719(</strong>) 0.347(<strong>) 0.728(</strong>)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sig. (2-tailed) 0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

Table (4.10) shows that there were correlation between the four tests, MB shows a significant results with *P* value <0.01 with the other three test and there is a high compatibility of negative and positive results with URUT, IgM, HpSAg test and increased to 0.954 with the true positive infection.
4.8 *H. pylori* Infection among the Study Sample

As indicated earlier, only those with URUT and or Methylene blue positive subjects were considered as having active *H. pylori* infection. According to this criterion only 48.3% were considered positive. Table (4.11) illustrates the distribution of positive *H. pylori* subjected according to age groups. The table shows that the highest positive result was among the age group 21-35 years (51.1%).

Table (4.11): Age distribution of *H. pylori* positive subjects

<table>
<thead>
<tr>
<th>Age</th>
<th><em>H. pylori</em> infection</th>
<th>Total</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>13-20</td>
<td>4 66.7</td>
<td>2 33.3</td>
<td>6   100.0</td>
</tr>
<tr>
<td>21-35</td>
<td>23 48.9</td>
<td>24 51.1</td>
<td>47   100.0</td>
</tr>
<tr>
<td>36-50</td>
<td>11 50.0</td>
<td>11 50.0</td>
<td>22   100.0</td>
</tr>
<tr>
<td>above 51</td>
<td>8 57.1</td>
<td>6 42.9</td>
<td>14   100.0</td>
</tr>
<tr>
<td>Total</td>
<td>46 51.7</td>
<td>43 48.3</td>
<td>89   100.0</td>
</tr>
</tbody>
</table>

4.9 Risk factors For *H. pylori* Infection.

In the literature, there were many implicated risk factors for the acquisition of *H. pylori*. There were also variations and conflicting reports about almost each of the implicated factors. These variations were attributed by most of the reviewed literature to differences either in methodologies of data collection or geographical variations. This study attempted to determine risk factors for the acquisition of *H. pylori* by the study population.
4.9.1 Personal variables

Among the 89 subjects who completed data, the highest positive result was found in the age group 21-35yr (51.1%) while the highest negative result was in age group 13-20yr (66.7%). The highest positive result was in female and it constituted 53.1%, while in male it was 45.6%. There were no significant statistical results related to age or sex to be considered as a risk factor.

The highest positivity result was found in weight group 35-55kg it was 87.5% but the lowest results were in weight group 98-118kg. With regard to marital status, there were no significant differences. Both married and single subjects were approximately equally effected (table 4.12).

Table (4.12): Personal factors

<table>
<thead>
<tr>
<th>Variable</th>
<th>Negative</th>
<th>Positive</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13-20y</td>
<td>4</td>
<td>66.7</td>
<td>2</td>
</tr>
<tr>
<td>21-35y</td>
<td>23</td>
<td>48.9</td>
<td>24</td>
</tr>
<tr>
<td>36-50y</td>
<td>11</td>
<td>50.0</td>
<td>11</td>
</tr>
<tr>
<td>&gt;51y</td>
<td>8</td>
<td>57.1</td>
<td>6</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>31</td>
<td>54.4</td>
<td>26</td>
</tr>
<tr>
<td>Female</td>
<td>15</td>
<td>46.9</td>
<td>17</td>
</tr>
<tr>
<td>Weight</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>35-55</td>
<td>1</td>
<td>12.5</td>
<td>7</td>
</tr>
<tr>
<td>56-76</td>
<td>23</td>
<td>57.5</td>
<td>17</td>
</tr>
<tr>
<td>77-97</td>
<td>20</td>
<td>52.6</td>
<td>18</td>
</tr>
<tr>
<td>98-118</td>
<td>2</td>
<td>66.7</td>
<td>1</td>
</tr>
<tr>
<td>Martial status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>38</td>
<td>50.7</td>
<td>37</td>
</tr>
<tr>
<td>Single</td>
<td>8</td>
<td>57.1</td>
<td>6</td>
</tr>
</tbody>
</table>
4.9.2 Life style variables

Life style varies from region to region and depends on many factors such as socioeconomic factors, geographical distribution and religion. Such variations are used in justifying conflicting results obtained by different investigators.

As shown in table (4.13), there is no significant difference between smokers and non smokers with regard to \textit{H. pylori} infection. The percentage of positive results for smokers and non smokers were 46.9\% and 49.1\% respectively. Even the number of cigarettes seems not to affect or increase the possibility of infection. There is a statistically significant differences among subjects who drinks tea and those who do not with \textit{P} value = 0.045.

Subjects who drinks coffee are less likely to develop \textit{H. pylori} infection (41.0\%) as compared to those who don’t drink coffee (54.0\%). However, the difference is not statistically significant. With regard to the number of cups per day, lower percentage of \textit{H. pylori} infection was observed among those who consume more than 5 cups per day.

From statistical analysis of data, the type of water drunk during childhood could be considered as a risk factor with \textit{P} value=0.018. As shown in table (4.13), the positive results were high in subjects who drunk municipality or well water during childhood 53.2\% while subjects who drunk filtered water during childhood have 16.7\% positive results. However, the type of drinking water during adulthood did not influence the outcome of \textit{H. pylori} infection.

Oral hygiene status of the subjects was assessed by asking the patient if he/she had a dental complains. \textit{H. pylori} infection was high in subjects with dental complains 64.3\%, but this result was not statistically significant.

Contact with animals was evaluated as a possible risk factor. The subjects were classified as either those who handle animals or those who do not. It
was found that 50% was positive among those who have contact with animals and 47.8% for those who don’t.

The subjects were asked if they traveled abroad or not and the positive result in patients who traveled was 41.2% while the positive result in patients who did not travel was 50% and there was no statistical significance difference observed.

Table (4.13): Life style variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>H. pylori infection</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Negative</td>
<td>Positive</td>
<td>P value</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td>No</td>
<td>29</td>
<td>50.9</td>
<td>28</td>
<td>49.1</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>17</td>
<td>53.1</td>
<td>15</td>
<td>46.9</td>
</tr>
<tr>
<td>If smoking</td>
<td>1-20</td>
<td>13</td>
<td>48.1</td>
<td>14</td>
<td>51.9</td>
</tr>
<tr>
<td>No. of cigarettes per day</td>
<td>&gt;20</td>
<td>4</td>
<td>80.0</td>
<td>1</td>
<td>20.0</td>
</tr>
<tr>
<td>Drink tea</td>
<td>No</td>
<td>1</td>
<td>14.3</td>
<td>6</td>
<td>85.7</td>
</tr>
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<td></td>
<td>Yes</td>
<td>45</td>
<td>54.9</td>
<td>37</td>
<td>45.1</td>
</tr>
<tr>
<td>How many cups per day</td>
<td>1-5</td>
<td>36</td>
<td>54.5</td>
<td>30</td>
<td>45.5</td>
</tr>
<tr>
<td></td>
<td>&gt;5</td>
<td>9</td>
<td>56.3</td>
<td>7</td>
<td>43.8</td>
</tr>
<tr>
<td>Drink of coffee</td>
<td>No</td>
<td>23</td>
<td>46.0</td>
<td>27</td>
<td>54.0</td>
</tr>
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<td></td>
<td>Yes</td>
<td>23</td>
<td>59.0</td>
<td>16</td>
<td>41.0</td>
</tr>
<tr>
<td>How many cups per day</td>
<td>1-5</td>
<td>20</td>
<td>55.6</td>
<td>16</td>
<td>44.4</td>
</tr>
<tr>
<td></td>
<td>&gt;5</td>
<td>3</td>
<td>100-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Type of drinking water during childhood</td>
<td>Municipality or well water</td>
<td>36</td>
<td>46.8</td>
<td>41</td>
<td>53.2</td>
</tr>
<tr>
<td></td>
<td>Filtered water</td>
<td>10</td>
<td>83.3</td>
<td>2</td>
<td>16.7</td>
</tr>
<tr>
<td>Type of drinking water during adulthood</td>
<td>Municipality or well water</td>
<td>8</td>
<td>50.0</td>
<td>8</td>
<td>50.0</td>
</tr>
<tr>
<td></td>
<td>Filtered water</td>
<td>37</td>
<td>50.7</td>
<td>34</td>
<td>46.5</td>
</tr>
<tr>
<td>Dental complains</td>
<td>Yes</td>
<td>5</td>
<td>35.7</td>
<td>9</td>
<td>64.3</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>41</td>
<td>54.7</td>
<td>34</td>
<td>45.3</td>
</tr>
<tr>
<td>Consumed drugs</td>
<td>No.</td>
<td>28</td>
<td>49.1</td>
<td>29</td>
<td>50.9</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>18</td>
<td>56.3</td>
<td>14</td>
<td>43.8</td>
</tr>
<tr>
<td>Consumed antibiotics in the last month</td>
<td>No</td>
<td>23</td>
<td>46.9</td>
<td>26</td>
<td>53.1</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>23</td>
<td>57.5</td>
<td>17</td>
<td>42.5</td>
</tr>
<tr>
<td>Contact with animals</td>
<td>No</td>
<td>36</td>
<td>52.2</td>
<td>33</td>
<td>47.8</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>10</td>
<td>50.0</td>
<td>10</td>
<td>50.0</td>
</tr>
<tr>
<td>Traveling abroad</td>
<td>No</td>
<td>36</td>
<td>50.0</td>
<td>36</td>
<td>50.0</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>10</td>
<td>58.8</td>
<td>7</td>
<td>41.2</td>
</tr>
</tbody>
</table>
4.9.3 Drugs history
As shown in table (4.14), the history of medication with aspirin, other anti-inflammatory drugs and gastric medication (PPI,H2 antagonist) was evaluated and the results showed no significant differences.

Table (4.14): Drug intake relation to \textit{H. pylori} infection

<table>
<thead>
<tr>
<th>Do you take drug</th>
<th>\textit{H. pylori} infection</th>
<th>\textit{P} value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>No</td>
<td>28</td>
<td>49.1</td>
</tr>
<tr>
<td>Yes</td>
<td>18</td>
<td>56.3</td>
</tr>
<tr>
<td>Total</td>
<td>46</td>
<td>51.7</td>
</tr>
</tbody>
</table>

4.9.4 Antibiotics intake during the last month
As shown in table (4.15) the history of antibiotics intake in the last month was evaluated and the results showed no significant results.

Table (4.15): Antibiotics intake relation to \textit{H. pylori} infection

<table>
<thead>
<tr>
<th>Do you take antibiotics</th>
<th>\textit{H. pylori} infection</th>
<th>\textit{P} value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>No</td>
<td>23</td>
<td>46.9</td>
</tr>
<tr>
<td>Yes</td>
<td>23</td>
<td>57.5</td>
</tr>
<tr>
<td>Total</td>
<td>46</td>
<td>51.7</td>
</tr>
</tbody>
</table>
4.9.5 Socioeconomic status

For all 89 subjects who had participated in this study, the level of education, monthly income, type of accommodation, number of rooms, number of persons lived in each room, type of water supply and the sewage system were recorded and correlated to *H. pylori* infection.

As shown in table (4.16), the level of education did not significantly influence *H. pylori* infection. The highest positive result was found in those with intermediate school level (66.7%) and the lowest positive result was in university level (36.8%).

The monthly income of subjects was classified to those with 1000 NIS, 1000-2000 NIS, 2000-3000 NIS and more than 3000 NIS. The lowest positive result was observed in subjects with more than 3000 per month (22.0%). However, this result was not statistically significant.

With regard to the type of accommodation, the number of persons lived in the accommodation, type of water and type of the sewage system, no significant differences were observed.
### Table (4.16): Effects of socioeconomic status

<table>
<thead>
<tr>
<th>Variable</th>
<th>H. pylori infection</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>%</td>
<td>Positive</td>
<td>%</td>
<td>P value</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No</td>
<td></td>
<td>No</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Education</td>
<td>University</td>
<td>12</td>
<td>63.2</td>
<td>7</td>
<td>36.8</td>
<td>0.311</td>
</tr>
<tr>
<td></td>
<td>High school</td>
<td>19</td>
<td>52.8</td>
<td>17</td>
<td>47.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Intermediate school</td>
<td>6</td>
<td>33.3</td>
<td>12</td>
<td>66.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Less</td>
<td>9</td>
<td>56.3</td>
<td>7</td>
<td>43.8</td>
<td></td>
</tr>
<tr>
<td>Monthly income</td>
<td>Below 1000</td>
<td>5</td>
<td>45.5</td>
<td>6</td>
<td>54.5</td>
<td>0.370</td>
</tr>
<tr>
<td></td>
<td>1000-2000</td>
<td>22</td>
<td>46.8</td>
<td>25</td>
<td>53.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2000-3000</td>
<td>12</td>
<td>54.5</td>
<td>10</td>
<td>45.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;3000</td>
<td>7</td>
<td>77.8</td>
<td>2</td>
<td>22.2</td>
<td></td>
</tr>
<tr>
<td>Garbage collecting system</td>
<td>No</td>
<td>8</td>
<td>44.4</td>
<td>10</td>
<td>55.6</td>
<td>0.336</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>38</td>
<td>53.5</td>
<td>33</td>
<td>46.5</td>
<td></td>
</tr>
<tr>
<td>Type of accommodation</td>
<td>Flat</td>
<td>23</td>
<td>51.1</td>
<td>22</td>
<td>48.9</td>
<td>0.134</td>
</tr>
<tr>
<td></td>
<td>House</td>
<td>19</td>
<td>47.5</td>
<td>21</td>
<td>52.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Villa</td>
<td>4</td>
<td>100.0</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Number of rooms in the</td>
<td>&gt;5</td>
<td>1</td>
<td>50.0</td>
<td>1</td>
<td>50.0</td>
<td>0.768</td>
</tr>
<tr>
<td>accommodation</td>
<td>3-5</td>
<td>39</td>
<td>53.4</td>
<td>34</td>
<td>46.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>6</td>
<td>42.9</td>
<td>8</td>
<td>57.1</td>
<td></td>
</tr>
<tr>
<td>Number of persons in the</td>
<td>&gt;10</td>
<td>13</td>
<td>76.5</td>
<td>4</td>
<td>23.5</td>
<td>0.073</td>
</tr>
<tr>
<td>accommodation</td>
<td>5-10</td>
<td>27</td>
<td>46.6</td>
<td>31</td>
<td>53.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1-4</td>
<td>6</td>
<td>42.9</td>
<td>8</td>
<td>57.1</td>
<td></td>
</tr>
<tr>
<td>Number of persons in each</td>
<td>&gt;5</td>
<td>3</td>
<td>100.0</td>
<td>0</td>
<td>-</td>
<td>0.227</td>
</tr>
<tr>
<td>room</td>
<td>2-4</td>
<td>43</td>
<td>50.7</td>
<td>33</td>
<td>49.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>9</td>
<td>47.4</td>
<td>10</td>
<td>52.6</td>
<td></td>
</tr>
<tr>
<td>Type of water supply</td>
<td>Municipality</td>
<td>41</td>
<td>51.9</td>
<td>38</td>
<td>48.1</td>
<td>0.586</td>
</tr>
<tr>
<td></td>
<td>Well</td>
<td>5</td>
<td>50.0</td>
<td>5</td>
<td>50.0</td>
<td></td>
</tr>
<tr>
<td>Sewage system</td>
<td>Poor</td>
<td>12</td>
<td>48.0</td>
<td>13</td>
<td>52.0</td>
<td>0.421</td>
</tr>
<tr>
<td></td>
<td>Good</td>
<td>34</td>
<td>53.1</td>
<td>30</td>
<td>46.9</td>
<td></td>
</tr>
</tbody>
</table>

*P value <0.05 significant*
Chapter V
Discussion
Chapter (V)
Discussion

*H. pylori* is a spiral-shaped microaerophilic Gram-negative bacterium that colonizes the gastric mucosa of human beings. The microorganism is the major agent of gastritis and plays an important role in the pathogenesis of peptic ulcer and gastric cancer (190). *H. pylori* is believed to infect the host by the fecal-oral route and home to the gastric mucosa. Although it is acid-sensitive, *H. pylori* can survive in the stomach for short periods by neutralizing the gastric acid. The current therapy for *H. pylori* infection is efficacious but the treatment regimen is complex and demanding on the patient and it does not provide resistance to future infections (191). Current data suggest that the overall prevalence of *H. pylori* infection is higher both in developing countries and in lower socioeconomic groups in the developed world. Probably these populations are exposed to conditions that favor the acquisition of the microorganism such as precarious hygiene, crowded household conditions and deficient sanitation (192).

The major risk factor for acquiring *H. pylori* infection is poor socioeconomic conditions during childhood. The rate of *H. pylori* infection in developed countries ranges from 10% in children to 60% in 60 year olds (193).

Various methods are available for detecting *H. pylori*, but all have limitations. *H. pylori* infection can be diagnosed by invasive test tests requiring endoscopy (rapid urease test, histology, culture) and by non-invasive tests (carbon-\(^{13}\) urea breath test, serology, stool tests) (194).

The aim of this study is to determine the rate of *H. pylori* infection in the population of Gaza strip, and to evaluate possible risk factors linked to acquisition of the microorganism.
In this study, 122 patients undergoing upper gastrointestinal endoscopy were interviewed and serum, biopsy and stool specimens were collected. Among them, only 89 patients provided the three specimen types. Data concerning age, sex, use of medication, water supply, sewage, number of rooms in the household, number of persons residing in each dwelling and monthly family income were obtained during interviews. Categorical variables were analyzed using the Chi-square test. The study population age ranged between 13-77 years, with mean age of 37.03, (37.1%) are females and (62.9%) are males.

### 5.1 *H. pylori* Tests

In our study, four tests were used to evaluate *H. pylori* infection.

1. Invasive test required endoscopy for (URUT, MB staining).
2. Non-invasive test including IgM detection in serum sample and HpSAg test in stool sample.

#### 5.1.1 Evaluation of *H. pylori* Tests

Unfortunately, at present, no single test can be relied upon to detect definitely *H. pylori* infection (195) and a combination of tests is recommended as gold standard (196). A patient was considered an *H. pylori*-positive if culture alone or histology plus rapid urease test (RUT) were positive (197).

This fact was also evident in our study. As shown in table (4.2), there are variations in the results between the four tests that we used in this study and the highest positive results were observed with the IgM test (44.9%) then MB test (46.5%), HpSAg test (36.0%) while the least positivity was observed in the URUT (32.6%).

These variations could be attributed to several factors; one factor would be the nature of the test itself; for instance, IgM test detects immunoglobulin M which is the early responder. IgM is elevated during the early stages of an exposure to *H. pylori*. There are two possible outcomes, either the immune system will eradicate the bacteria or the bacteria will begin to populate the
mucosal lining of the upper GI tract. If IgM is the only elevated antibody to *H. pylori*, it must be correlated with active symptoms to warrant treatment. If asymptomatic, it is quite possible that the patient’s immune system has won the battle with *H. pylori* on its own. If treatment is not chosen at this time, it would be prudent to retest in 2-3 months to make sure that the patient’s immune system has in fact eradicated the infection (198).

The decision by the physician to select the proper part to collect a biopsy during gastroscopy would greatly affect the outcome of the invasive tests such as methylene blue staining and Ultra rapid urease tests. Patients on or with a previous history of antibiotic therapy or proton pump inhibitors may show negative results with tests that depend on the activity of *H. pylori* rather than its mere presence. Another possible cause of such variations is the cross-reactivity with other bacteria (e.g. campylobacter) by some tests such as HpSAg. These differences between the four tests may in part be due to patchy distribution in gastric mucosa, fastidious nature, mucosal atrophy, intestinal metaplasia (in stomach), administration of antibiotics (due to some other infection or protozoal infestation) and proton pump inhibitors. If patients under medication or antibiotics against *H. pylori* infection, this may lead to the death or inhibition of bacteria and as a consequence will not produce urease enzyme and exhibit negative results in URUT and reduce the number in MB stained smears. While IgM test may not be affected as well as HpSAg (199).

In a study done by Silvio K Ogata (2002) in Brazil, the results showed that the highest agreement between two tests occurred in histology plus rapid urease test combination (91.7%). Similar to results were observed in other study (200).

In another study in Pakistan, (52/109) 48% of the study population used PPI before presentation to the outpatient clinics, Pronto Dry (a commercial urease test) was positive in 40% (44/109) and negative in 60% (65/109). Histopathology was positive for *H. pylori* in 57% (62/109) and negative in
43%. In this study, treatment with a PPI before endoscopy reduced the sensitivity of urease test from antral biopsies for *H. pylori* detection. Ideally PPI should be discontinued before the endoscopy (201). In patients on PPI, the biopsy specimen may contain low bacterial density of viable cells giving a negative urease test. This also leads to lack of *H. pylori* identification on histology. Of the various tests that are available for *H. pylori* detection, histological examination of gastric biopsy is considered the most accurate method of diagnosis (202). The migration of *H. pylori* from the antrum to the fundus was also associated with a corresponding decrease in the activity of antral gastritis and matched by a progressive fall in the excretion of $^{13}$C urea breath test (203). If more than one gastric biopsy tissue is used to inoculate the rapid urease test, a positive test might appear, thus improving the test sensitivity without compromising its specificity. The diagnostic yield is said to be increased by over 5% by taking more than a single biopsy (202).

Biopsy urease tests for *H. pylori* all have inherent inadequacies, notably in patients with bleeding peptic ulcers, in which a false-negative result might be found. Chan *et al.* found that in 480 *H. pylori*-associated ulcers, 49 *H. pylori* infections were missed by a biopsy urease test, which was performed at initial endoscopy during the bleeding episode. This result is probably caused by the buffering effects of blood and plasma, which can interfere with the pH indicator used in urease test (204). Gisbert *et al.* pointed out in his meta-analysis that the diagnostic sensitivities of biopsy urease tests and histology could be increased to 78% and 83%, respectively, if both antrum and corpus biopsies were obtained. The specificity of biopsy-based *H. pylori* detection per se is excellent (93%-100%) (205). Multiple *H. pylori* diagnostic tests, such as the biopsy urease test, should be performed in the diagnosis of a bleeding ulcer, and if negative, histology should be examined to increase the accuracy of diagnosing *H. pylori* infection (206).
5.1.2 The rate of *H. pylori* infection

In our study there were variations in the percentage of positive results for the four employed tests. A true positive was assumed if URUT and/or Methylene blue tests were positive (table 4.3) (189). The rate of *H. pylori* infection according the positive results was 48.3%. Our results do not agree with the prevalence of *H. pylori* infection among patients in other countries in the Middle East. These differences in the prevalence is might be due to the fact that the number of patients included in our study was limited, different tests used in other countries, different possible risk factor, source of drinking water, eating habits among the people..

A study in Yemen (1998) showed that the prevalence of *H. pylori* infection among 275 dyspeptic patients was 82.2%. In their study they depended on URUT for detect the infection (207).

Another study in Saudi Arabia (2005) showed that the prevalence among 120 volunteer students was low (35%) in comparison to other study conducted on a larger number of the Saudi population which showed prevalence of 80% (208).

5.2 Risk factors Associated with *H. pylori* infection

5.2.1 Age

This study demonstrated that the highest positive results were in the age group of 21-35 yr (51.1%). There were no statistically significant results and all age groups were equally exposed to infection. This finding does not agree with similar studies in developed countries which showed that the infection begin in younger age and increasing annually with age. This is may be due to the reason that the number of participant of the older age in our study was limited.

A study in Brazil, where the infection rate was of 84.7% in subjects 18 to 30 years of age, increasing to 92% in subjects 46-60 years old, while in subject
above 60 years old, the prevalence decreased slightly. As a whole, the prevalence of infection did not increase significantly ($P=0.147$) with age. There were no significant differences in the prevalence of $H.\, pylori$ infection, when patients were classified by age. This has been explained as being due to a reduction in the specific serological response among older individuals and/or to a decreased number of microorganisms as a consequence of gastric atrophy (209).

In another study conducted in south of Brazil (2005), the author showed totally different results. Among the 563 eligible individuals, the prevalence rate of $H.\, pylori$ infection was 63.4%. In crude analyses, prevalence was associated with increasing age (19).

5.2.2 Sex

As shown in table (4.12) there is no significant difference in the overall prevalence of $H.\, pylori$ infection between males and females. And both of them appear to be equally exposed. This result is in agreement with other studies. In Brazil, two hundred and four individuals participated in the study, 49 males and 155 females, with ages ranging from 18 to 80 years. 165 of 204 participants (80%) were $H.\, pylori$ positive, with no significant gender differences ($P= 0.49$) (209).

This finding is also supported by those reported from south-eastern and central south Brazil, and from Africa and India (210). As has also been found in other studies made in developing countries, there were no gender differences in the risk of acquisition of infection (211).

5.2.3 Weight

In table (4.12) the highest positive result was in weight group 35-55 Kg (87.5%) and this percentage did not increase with increasing weight. This could be explained by the fact that malnutrition could lower immunity and therefore increase the susceptibility of those patients to be infected with $H.$
pylori organism. A study in UK (2005) demonstrated a relation between H. pylori infection and weight lose. Ninety seven patients were H. pylori positive. Their results suggest that children with dyspepsia and H. pylori infection are shorter and lighter compared to children without the infection. It is possible that H. pylori infection may have some detrimental effect on growth, especially during the pubertal growth spurt (212).

5.2.4 Marital status
Marital status is not a risk factor as shown in table (4.12). Although a slightly higher percentage of H. pylori infection was observed among married than single subjects. But the increase was not statistically significant. This increase may be attributed to age and personal contact. A Libyan study showed a higher prevalence of H. pylori in married subjects (84%) in comparison to single subjects (68%) (213).

5.2.5 Smoking
Smoking is considered as a risk factor for many diseases and is implicated by several studies in the literature as a risk factor for H. pylori infection. However, the results of this study showed no statistical differences between smokers and non-smokers (15 (46.9%), 28 (49.1%) respectively) with regard to H. pylori infection (table 4.13). A striking observation is that smoking of more than 20 cigarettes reduced the incidence of H. pylori.

There are reasons why smoking might have little effect on, or even increase, the hostility of the gastric environment to H. pylori. The acid gastric pH prevents most organisms from thriving or even surviving in the stomach. H. pylori, however, has an electropositive internal milieu; twice the number of basic amino acids, arginine and lysine, as Haemophilus influenzae and Escherichia coli; and powerful urease activity, with the ability to produce both ammonia and factors that inhibit parietal cell acid production (214). All these attributes make survival of H. pylori in the stomach less influenced by the reduction in pH which may accompany with smoking consumption (215).
In another study which disagree with our result, El-Barrawy, demonstrated that infection prevailed mostly (70%) in smokers (113 out of 161). According to odds ratio, the risk of infection was 5.3 times higher for smokers than non-smokers, which was significant. They attributed their finding to the destructive effect of smoking on the immunity of gastric mucosa and lining layers and hence increasing its susceptibility to infection by *H. pylori*. Communal Shisha smoking might carry the risk of passing the infection from a diseased person to an uninfected one, as oral-to-oral infection (216).

**5.2.6 Coffee drinking**

There was no statistically significant difference between those who drinks or don’t drinks coffee. In fact a little bit lower percentage of positive *H. pylori* was found in those who drinks coffee (41.0%) than those who do not (54.0%). The number of cups consumed per day seems to affect the outcome of *H. pylori* infection. Among those who drinks more than 5 cups, none were positive.

A survey in Germany in 1997 on 447 patients with an overall prevalence of 21%, coffee consumption showed a positive dose-response relation with active infection. The positive relation between coffee consumption and *H. pylori* infection identified in that study is consistent with results from a cohort study among epidemiologists in which the risk of seroconversion (change from negative to positive results for antibodies to *H. pylori* in serum) was 4.6 times higher among those who drank more than 2 cups of caffeinated drinks a day than among the others. The mechanisms underlying this association require further research (217).

**5.2.7 Tea drinking**

A significant finding of this study is that (as shown in table 4.13) tea consumption is a protective factor. Only 45.1% of those who drinks tea were infected, a very much higher percentage was found among those who do not
drink tea (85.7%). This could be explained by the fact that tea has medicinal effects. This finding is supported by a Japanese study (1999) on the benefits of tea. Recent studies have presented data that show a variety of biological activities of tea catechins, compounds which constitute about 15% (dry weight) of tea. They investigated the antibacterial activity of catechins against *H. pylori* in vitro and in vivo. Effect of these compounds were investigated on the gastric mucosal injury induced by this organism in Mongolian gerbils. *H. pylori* was eradicated in about 10% of the gerbils in each of the catechin. Although the mechanism of this action is still obscure, structure-activity relationship studies indicated that the antibacterial activities of catechins were predominantly related to the gallic acid moiety and the number of hydroxyl groups. It has also been reported the catechins can damage the membrane lipid bilayer. Catechins probably damage the membrane of *H. pylori*. However, the exact mechanism is still unknown. Moreover, catechins inhibits the urease activity and motility of *H. pylori* (218).

5.2.8 Type of drinking water

Type of water drunk during childhood proved to be a detrimental factor with a statistically significant results (*P* value=0.018). *H. pylori* infection rate was high in subjects who consumed municipality or well water during childhood (53.2%) in comparison with subjects who consumed filtered water during childhood (16.7%).

The type of drinking water during adulthood is not considered as a risk factor for *H. pylori* infection. Both municipality or well water and filtered water consumers showed similar infection rates (50.0%) and (47.9%) respectively. This result is in agreement with other studies in developed and developing countries. They implicated the type of water during childhood as the main risk factor for *H. pylori* infection. The microorganism is transmitted by the fecal-oral routes in the infected water to the child and persist all the life and as the result showed that the type of water during adulthood does not affect the infection rates.
In a study conducted in Leipzig, Germany, which consisted of a self-administered or parent-completed questionnaire (age-dependent), eliciting information on lifestyle habits and their use/drinking the well water as well as the \textit{H. pylori} infection. A total of 91 subjects (44 users of \textit{H. pylori} positively and 47 negatively tested wells) were screened for their \textit{H. pylori} status. The group was comprised of 42 males and 49 females, i.e., 73 adults and 19 children under the age of 18 (mean age 39.5 years with a range between 3-80 years). Logistic regression analyses identified the drinking of well water as the significant risk factor for a positive colonization status ($P<0.001$). Water supplies have been identified as possible reservoirs to acquire the bacterium (219).

\textbf{5.2.9 Oral hygiene}

Higher percentage of \textit{H. pylori} infection was observed among those with dental complains (64.3\%) than those with out dental complains (45.3\%). Although, the difference is not statistically significant, the result is in conformity with finding of many investigators who claimed that the bacteria may persist in the oral cavity and in dental plaques of people with poor hygiene.

In a study in Australia by Hedley G. Peach on 217 adults randomly selected from the electoral roll, they found that the positive \textit{H. pylori} status was significantly associated with increasing number of tooth surfaces with a high plaque, they confirmed that living in an overcrowded household during childhood and visiting a dentist less than once a year are positively associated with \textit{H. pylori} infection in Australia. And they found the Negative \textit{H. pylori} status was significantly associated with increasing education, having ever lived on a farm. \textit{H. pylori} has been cultured from dental plaque, and identical \textit{H. pylori} ribotypes were found in the mouth and gastric antrum of ulcer patients. Moreover, \textit{H. pylori} has been detected by reverse transcription polymerase chain reaction in only moderate to heavy accumulations of
plaque. If *H. pylori* inhabits plaque, scaling may lead to ingestion of the organism and inoculation of the stomach (220).

### 5.2.10 Antibiotic consumption

Among the study subjects, 40 took antibiotics one day to one month prior sample collection, 57.5% of them were shown to be negative while among the 49 who did not consume antibiotics, only 46.9% of them were negative for *H. pylori*. Although not statistically significant, the results showed a reduction in positive cases of *H. pylori* among those who consumed antibiotics (table 4.15).

This reduction could be explained by the fact that both URUT and Methylene blue depends on the activity and the presence of *H. pylori* in the biopsy and antibiotic consumption may reduce the number and activity of the organism, therefore interfering with the result outcome producing false negative results.

### 5.2.11 Drug consumption

Among the study subjects, 32 took drugs, 56.3% of them were shown to be negative while among the 57 who did not consume drugs, only 49.1% of them were negative for *H. pylori*. Although not statistically significant, the results showed a reduction in positive cases of *H. pylori* among those who consumed drugs (table 4.14).

A rapid urease test is highly specific and simple, and can also be performed on biopsy samples; however, it may have false-negative results, particularly if the patient has recently taken a proton pump inhibitor (PPI) (221).

Proton pump inhibitors are known to decrease the activity of *H. pylori* organisms within the stomach and to shift their distribution proximally. This effect may reduce the sensitivity of histological examination and rapid urease testing for *H. pylori* on biopsies taken from recommended sites. It is of
particular relevance if a proton pump inhibitor has been taken before the patient has undergone diagnostic endoscopy (201).

Dickey and Kenny, investigated patients referred to open-access upper gastrointestinal endoscopy service who had either been on no medication (controls) or were already taking proton pump inhibitors. Biopsies taken from the gastric antrum and corpus were used for rapid urease testing and for histological examination. Sera taken from patients who had no evidence of *H. pylori* in biopsies, they were tested for IgG *H. pylori* antibodies as an alternative indicator of infection. *H. pylori* organisms were detected by histological examination in 27 of 40 controls (68%) and in 13 of 25 patients taking proton pump inhibitors (52%). Among patients with positive histology (organisms detected in either antral or corpus biopsies, or both), only the sensitivity of the antral urease test read at 1 h was significantly lower in patients taking proton pump inhibitors than in controls, with no significant difference in sensitivities of the antral urease test at 24 h, of the corpus urease test at 1 or 24 h, or of histology from the antrum or corpus. Of patients with negative histology, none of 13 controls compared with six of 12 patients taking proton pump inhibitors (50%) had positive serology (*P* = 0.005). Five (83%) of the six histology-negative, seropositive patients taking proton pump inhibitors had histological changes consistent with *H. pylori* gastritis even though no organisms were detected. Treatment with a proton pump inhibitor before endoscopy reduces the sensitivity of antral and corpus biopsies for *H. pylori* detection, both by urease testing and histological examination. If proton pump inhibitors already prescribed cannot be discontinued for an adequate period before endoscopy, patients should have biopsies taken from the corpus as well as from the antrum, and serum should be tested for *H. pylori* (201).

### 5.2.12 Education

Regardless of the educational level, the study population showed no significance variation (table 4.16). The highest positive result were in intermediate school level (66.7%) and the lowest positive result was in the
university level (36.8%). This finding is in agreement with a study conducted in Brazil (2005). The study showed that in 165 out of 204 participants (80%) were *H. pylori* positive; there were no significant differences in the prevalence of *H. pylori* infection, when patients were classified by educational level (209).

Another study (2005) in south of Brazil conducted by Ina and Jose, showed different results. The prevalence rate of *H. pylori* infection was 63.4%. In crude analyses, prevalence was associated with increasing lower education level. This variation in findings could be explained by the fact that *H. pylori* infection is multifactorial (19).

### 5.2.13 Income

The highest positive rate was observed in subjects with monthly income of less than 1000 NIS (54.5%) and the lowest positive result were in patients with income more than 3000 NIS per month (22.0%). However there is no statistically significant variation. This contradicts the finding of Ina and Jose (2005) who noted high prevalence rate in the low income group (19).

### 5.2.14 Number of persons in the accommodation

As shown in table (4.16), the number of persons living in the accommodation did not show significant differences. This finding is similar to that of Maria and Dulciene (2005), their study in poor urban community in Brazil, showed that in 165 of 204 participants (80%) were *H. pylori* positive. The number of persons per room, the number of children per household, the number of adults per household and household pets, were not risk factors suggesting that the infection, even in a poor population, may be acquired predominantly during childhood; the relatively high prevalence that was observed may be more due to a cohort effect than to acquisition of infection during adulthood (209). Factors linked to household conditions, such as present family size, were not relevant determinants of *H. pylori* infection status in this adult population, a finding also observed by others, both in developed and in
developing countries. In most of these studies, a clear association was seen between socioeconomic status and overcrowding in childhood, but not with present household conditions (222). In fact, residential crowding is known to facilitate transmission of infection within families (223).

Another study in south of Brazil (2005), showed different results. Of 563 eligible individuals, 363 agreed to perform the $^{13}$C-UBT (refusal rate of 35.5%). The prevalence rate of *H. pylori* infection was 63.4% (95%CI 59.3%–69.3%). In crude analyses, prevalence was associated with increasing higher size of the family, low socio-economic conditions in childhood, higher number of siblings and the presence of dyspeptic symptoms. In their study past socio-economic variables in childhood presented the strongest association with the occurrence of the infection in adult life (19). This finding is in agreement with the "birth cohort phenomenon", person-to-person contact appears to be the most likely mode of transmission, number of siblings; particularly the number of older siblings, domestic crowding and living in orphanages are important determinants of the prevalence of *H. pylori* infection (224).

5.2.15 Type of accommodation
Variations in housings and accommodations probably reflect variations in socioeconomic conditions, however, in this study; *H. pylori* appear to ignore such variations. As shown in table (4.16) the highest positive results were in patients who lived in house (52.5%). Therefore, type of accommodation may not be a risk factor. There was a similar finding by Fiedorek et al (1991) in a study on children. They found no significant differences in *H. pylori* infection related to gender, type of housing and location of housing (225). The type of water in the accommodation whether municipality or private well was not significant risk factor for *H. pylori* infection. Both groups exhibited similar positive rates.
Chapter VI
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Chapter VI

Conclusions and Recommendations

6.1 Conclusions

This study is the first in Gaza strip to detect *H. pylori* infection and attempted to determine possible risk factors associated with the acquisition of the microorganism. From the result of this work, the following conclusions were drawn:

1. The rate of *H. pylori* infection among 89 subjects undergoing upper endoscopy was 48.3%.

2. Ultra Rapid Urease test proved to be reliable, easy to prepare and to perform, not expensive and the patient can get quick result while still in the physician office. However, the exclusive use of the rapid urease test for the diagnosis of *H. pylori* cannot be recommended in patients with prior medication use.

3. Drugs and antibiotics consumption appears to interfere with tests that depend on either the presence or activity of *H. pylori*.

4. No single test among those used in this study could be used reliably to diagnose active *H. pylori* Infection.

5. HpSAg test, none invasive, simple and accurate test for detecting *H. pylori* in stool sample, however, its use for diagnosis should be evaluated.

6. Also it can be concluded that drinking and/or using *H. pylori* contaminated municipality or well water is a risk in the acquisition of *H. pylori*. Given the
public health impact of *H. pylori* infection, this should be taken into account when measures of prevention are considered.

7. Age, sex, weight, marital status, smoking, coffee consumption, oral hygiene status, socioeconomic status include, education level, income, type of accommodation, number of persons living in the accommodation, number of persons in each room, type of water, the sewage system, contact with animals, traveling abroad and drug consumption could not be considered as a risk factor of *H. pylori* infection as demonstrated by the results of this study.

8. The statistical analysis of data obtained from this work demonstrated a significance protective property of tea against *H. pylori* infection with a significant *P* value.

9. Our results support the hypothesis that *H. pylori* infections in developing countries is predominantly acquired during childhood.

**6.2 Recommendations**

1. As a result of our study we recommend that further investigation to detect the possible sources of *H. pylori* infection especially drinking water

2. We recommend using urease test at the physician office as a simple, one minute test.

3. More than one biopsy during gastroscopy is recommended to increase the sensitivity of tests depending on the activity of the bacterium (URUT and MB).

4. We recommend that tea leaves should be tested for their effect on *H. pylori* both in *vivo* and *vitro*. 
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Appendices
The aims of this questionnaire is to determine the risk factor associated with \textit{H.pylori} infection.

All personal information will be confidential

Thanks for your corporation ……

Sample number…………………… Patients name……………………
1-Age………………
2- Sex ………… Male □ Female □
3-Weight …………
4-Martial Status….. Married □ Single □ Divorced □
5-Do you smoke? Yes □ No □
6-How many cigarettes per day? ……………
7- Do you drink tea? Yes □ No □
8- How many cups of tea do you drink per day? ……………
9-Do you drink coffee? Yes □ No □
10- How many cups of coffee do you drink per day? ……..
11- What type of water did you drink during childhood?
   Municipality or well water □ Filtered □
12-What type of water did you drink during adulthood ?
   Municipality or well water □ Filtered □
13-Have you any dental complains?
   No □ Yes □
14-What is your education level?
University □  High school □  Intermediate school □  Less □
15-What is your family income?………………
16-What is the socio-economics level of your neighborhood?
Good □  Fair □  Poor □
17-Is there a garbage collecting system in your area?
Yes □  No □
18-What is the type of your accommodation?
Villa □  House □  Flat □
19-How many rooms are in your accommodation? ………
20-How many members are in your family? ……………
21-How many persons are living in each room?………..
22-What's the source of water in your house?
Municipality □  Wells □
23- Evaluation of sewage system in your house
Good □  Fair □  Poor □
24- Do you take drugs? Yes □  No □
25- Did you take any antibiotics in the last month? Yes □  No □
26- Have you any animals in your house? Yes □  No □
27- Did you ever travel abroad?
Yes □  No □

Medical Tests

1-(Ultra Rapid Urease Test)………………
2-(Methylene Blue stains)…………………..
3-(IgM in serum)………………
4-(HpSAg)…………………..
يهدف هذا الاستبيان إلى معرفة عوامل الخطر المرتبطة بيكتيريا H. pylori المسببة للقرحة المعدة:

كل البيانات ستبقى قيد السرية 침امة......

نرجو مساعدتك في إنجاز هذه الدراسة

شاكرين لكم حسن تعاؤكم

اسم المريض: ........................................

رقم العينة: ........................................

1- العمر ........................................

2- الجنس  ذكر □ أنثى □

3- الوزن □ مطلق او منفصل □ أعزب □ مزوج □ نعم □ لا

4- الحالة الاجتماعية □ هل تدخن السجائر او الشيشة؟ □ نعم □ لا

5- إذا كنت تدخن فكم مرة باليوم؟ □ نعم □ لا

6- هل تشرب الشاي؟ □ نعم □ لا

7- إذا كنت تشرب الشاي كم كوبا من الشاي تشرب في اليوم؟ □ نعم □ لا

8- هل تشرب القهوة؟ □ نعم □ لا

9- إذا كنت تشرب القهوة، كم فنجان في اليوم تشرب؟ □ نعم □ لا

10- ما هو نوع الماء الذي تشربك في الطفولة؟ □ مفلتة □ بلدية أو من الأبار □ المهاجرة □ نعم □ لا

11- ما هو نوع الماء الذي تشربك حاليًا؟ □ مفلتة □ بلدية أو من الأبار □ المهاجرة □ نعم □ لا

12- هل تعاني من مشاكل صحية في أسنانك؟ □ جامعية □ ثانوي □ متوسط □ دون ذلك

13- هل تعاني من مشاكل صحية في أسنانك؟ □ نعم □ لا □ متوسط □ دون ذلك

14- هو مستوى التعليم الذي حصلت عليه؟ □ جامعية □ ثانوي □ متوسط □ دون ذلك
15 ما هو الدخل المادي للعائلة؟

16 هل الحي الذي تسكنه يُصنف؟

17 هل يتم جمع القمامة باستمرار؟

18 ما هو نوع السكن؟

19 ما هو عدد الغرف في المنزل؟

20 كم عدد أفراد العائلة؟

21 كم عدد شقته؟

22 ما هو مصدر الماء في المنزل؟

23 كيف هو نظام الصرف الصحي لدينا؟

24 هل تتناول أي مسكن باستمرار؟

25 هل تناولت أي مضاد حيوي خلال الشهر الماضي؟

26 هل يوجد حيوانات بالمنزل أو بالجوار؟

27 هل سافرت إلى الخارج؟

الفحوصات التي تم إجراؤها:

Urea Rapid Urease Test -1
Methylene Blue stain -2
(IgM in serum) -3
(HpSAg) -4