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# REVIEW ARTICLE ABOUT HELICOBACTER PYLORI IN IRAQ

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**Abstract:** Helicobacter pylori live in the stomach Infection with H. pylori is associated with a number of diseases including stomach and duodenal ulcers and cancer. experiences noticeable symptoms, Helicobacter pylori a cause of peptic ulcer disease, gastric adenocarcinoma, and low-grade gastric mucosa-associated lymphoid tissue. There are many prevalence and risk factors of H. pylori infection during lifetime as a function of age. H.pylori can be diagnosed by invasive and noninvasive methods. Due to h.pylori consistently increasing antibiotic resistance, there are many strategy done on developing viable vaccine

Studies such treatment, when effectively eradicating h.pylori from the stomach

Reduce inflammation and some of the histopathological abnormalities associated with the infestation. Usually, antibiotics is the first line of treatment, but 20 percent of patient failed with first line therapy, but second line therapy is a quadruple therapy. For third line treatment, it is proposed that patients who fail first- and second-line therapy. also, we have non antibiotic treatment like naturally by plant or herbal medication.

## 1. Introduction

*Helicobacter pylori* is a helical, or spiral-shaped, that lives in the stomach. Infection with H. pylori is associated with a number of diseases including stomach and duodenal ulcers, as well as certain cancers. However, only a small fraction of infected people experiences noticeable symptoms, and for that reason diagnosis of the infection is quite low when compared to the number of people who are infected. Helicobacter pylori a cause of peptic ulcer disease, gastric adenocarcinoma, and low-grade gastric mucosa-associated lymphoid tissue (MALT) lymphoma (Egan *et al*., 2007), has been falling due to improved sanitation and better living conditions over the decades in most countries (Parsonnet *et al*.,1995; Roosendaal *et al*.,1997). The changing epidemiology of the bacterium has been associated with a decline in the peptic ulcer disease (PUD) and gastric cancer (Fuccio *et al*.,2010) but it could increase gastroesophageal reflux disease and asthma which are related to acid or immunity. (Sonnenberg *et al*.,2013).

There are many studies regarding the prevalence and risk factors of *H. pylori* infection, and older age was commonly *considered* as the main risk factor (Lim et al.,2013). Adults have a continuous risk of *H. pylori* infection, resulting in the increased seroprevalence during lifetime as a function of age (Veldhuyzen van Zanten *et al*.,1994) However, this does not mean that young people have a higher seroprevalence when they get older, as cross-sectional presentation does not necessary give an accurate picture of lifetime trends. To compensate this there have been precious studies on lifetime trends for H. pylori seroprevalence (Lim *et al*.,2013*;* Banatvala et al.,1993; Kosunen *et al*.,1997).

**Aim of study**

a) *Helicobacter pylori (H. pylori)* is a common bacterial infection that is associated with significant morbidity and mortality worldwide.

b) This bacterium causes a chronic infection that is causally related to illnesses ranging from gastritis, peptic ulcer disease to gastric cancer.

## 2. Literature Review

### 2.1. Definition of H. pylori

The *Helicobacter* genus includes more than 35 species. *H. pylori*, the most important human pathogen, is Gram-negative, spiral shaped, acid-resistant, and microaerophilic (Tomb *et al*.,1997). Thus, H. pylori can be described as a cross between a commensal and a pathogenic bacterium. Typically, all Helicobacter species express urease enzymes, an enzyme that is essential for microbial survival. (Figura *et al*.,1999). Experimental deletion of the urease gene renders *H. pylori* unable to colonise gastric mucosa (McGee et al.,1999). The function of urease is to increase pH in the microenvironment by the generation of ammonia (NH3) from urea and to secure the nitrogen supply for bacterial protein production (Benno *et al*.,2011).

Urease is an intracellular enzyme that is bound to the outer membrane of other bacteria upon bacterial lysis. These non-covalently bound proteins may in turn impair the function of secretory IgA directed against H. pylori by antigen shedding. However, all clinical isolates produce urease and thus it cannot explain the occurrence of clinical disease. Several pathogenic bacterial factors have been identified which may have impact on the clinical presentation, but the only factors with consistent impact in laboratory and clinical studies were cytotoxin-associated gene A (CagA) and vacuolating cytotoxin gene (VacA). Both factors can be detected in strains from asymptomatic carriers. However, their 11 contributions to H. pylori-related pathology are academically interesting, but the overall impact is small. Alternatively, the main determinant may be differences between immune phenotypes of the infected individuals. Today the most thoroughly documented pathogenic factor of H. pylori is CagA (Glupczynski *et al.*,1997). The functions of the CagA protein are not fully understood, but several lines of evidence indicate that the protein is a phosphatase capable of altering activation states of proteins and transcription factors like NFκB in the target cell (Figura *et al*.,1999). All H. pylori strains contain the VacA gene, but five different genotypes exist rendering approximately 50% of the strains VacA protein negative. The VacA has been shown to induce vacuolization of epithelial gastric cell lines (Figura *et al*.,1999).

### 2.2 History

By the late 19th and early 20th centuries, several investigators had reported the presence of spiral microorganisms in the stomachs of animals (Musa *et al*.,2013). Soon afterward, similar spiral bacteria were observed in humans (Krienitz *et al*., 1906*;* Luger et al., 1917*;* Pel *et al*.,1899) some of whom had peptic ulcer disease or gastric cancer. The etiological role of these bacteria in the development of peptic ulcer disease and gastric cancer was considered at the time, and patients were sometimes even treated with high doses of the antimicrobial compound bismuth (Pel *et al*.,1899). This possibility was later discarded as irrelevant, probably because of the high prevalence of these spiral bacteria in the stomachs of persons

without any clinical signs. The bacteria observed in human stomachs were thus considered to be bacterial overgrowth or food contaminants until the early 1980s. At this time, Warren and Marshall performed their groundbreaking experiments, leading to the identification of a bacterium in 58 of 100 consecutive patients, with successful culture and later demonstration of eradication of the infection with bismuth and either amoxicillin or tinidazole (Luger *et al*.,1917*;* Marshall *et al*.,1984*;* Warren *et al*.,1983).

The organism was initially named "*Campylobacter*-like organism," *"gastric Campylobacter*-like organism," *"Campylobacter pyloridis,"* and "*Campylobacter pylori*" but is now named *Helicobacter pylori* in recognition of the fact that this organism is distinct from members of the genus *Campylobacter* (Goodwin *et al*.,1989). It soon became clear that this bacterium causes chronic active gastritis, which in a subset of subjects may progress to other conditions peptic ulcer disease, distal gastric adenocarcinomas, and gastric lymphomas (Ernst *et al*., 2000).

### 2.3 Morphology

The simple morphology of *H. Pylori* have unique S-shape characteristics with Polar, sheathed flagellum which differ according to their spirals/turns form and number. (Pa *et al.* n.d.). Many aspects of the ultra-structural nature of Helicobacter have been involved, e.g., in their ability to function in hostile conditions such as acidic gastric mucosa and capability disease-causing, e.g., sheathed flagella and surface urease. Helicobacter pylori in vivo is an S-shaped pathogen with 1 to 3 turns, 0.5 x 5 µm in diameter, with a tuft of 5 to 7 polar sheathed flagella. (Ashwini *et al*. 2015b).

### 2.4 Epidemiology

The prevalence of *H*. *pylori* shows large geographical variations. In various developing countries, more than 80% of the population is H. pylori positive, even at young ages (Leja *et al*.,2016). The prevalence of H. pylori in industrialized countries generally remains under 40% and is considerably lower in children and adolescents than in adults and elderly people (Pounder et al.,1995). Within geographical areas, the prevalence of H. pylori inversely correlates with socioeconomic status, in relation to living conditions during childhood (Malaty *et al*.,1994). In Western countries, the prevalence of this bacterium is often considerably higher among first- and second-generation immigrants from the developing world (Perez *et al*.,2005; Tsai *et al*.,2005).

While the prevalence of *H. pylori* infection in developing countries remains relatively constant, it is rapidly declining in the industrialized world (Genta *et al*.,2002). The latter is thought to be caused by the reduced chances of childhood infection due to improved hygiene and sanitation and the active elimination of carriership via antimicrobial treatment. In developing countries, H. pylori infection rates rise rapidly in the first 5 years of life and remain constantly high thereafter, indicating that H. pylori is acquired early in childhood (Fiedorek *et al*.,1991). However, in industrialized countries the prevalence of H. pylori infection is low early in childhood and slowly rises with increasing age. This increase results only to a small extent from H. pylori acquisition at later age. The incidence of new H. pylori infections among adults in the Western world is less than 0.5% per year; the higher prevalence of infection among the elderly thus reflects a birth cohort effect with higher infection rates in the past (Kuipers et al.,1997*;* Granström *et al*.,1997). The active elimination of H. pylori from the population and improved hygiene and housing conditions have resulted in a lower infection rate in children, which is reflected in the age distribution of this lifelong-colonizing bacterium (Kosunen *et al*.,1997*;* Kosunen *et al*.,1997; Roosendaal *et al*.,1997). Overall, new infection more commonly occurs in childhood and lasts for life unless specifically treated.

### 2.5 Pathogenesis

The gastric mucosa is well protected against bacterial infections. *H. pylori* is highly adapted to this ecologic niche, with a unique array of features that permit entry into the mucus, swimming and spatial orientation in

the mucus, attachment to epithelial cells, evasion of the immune response, and, as a result, persistent colonization, and transmission. The H. pylori genome (1.65 million bp) codes for about 1500 proteins. (Tomb *et al*.,1997) Among the most remarkable findings of two *H. pylori* genome-sequencing projects were the discovery of a large family of 32 related outer-membrane proteins (Hop proteins) that includes most known *H. pylori* adhesins and the discovery of many genes that can be switched on and off by slipped-strand mispairing-mediated mutagenesis. Proteins encoded by such phase-variable genes include enzymes that modify the antigenic structure of surface molecules, control the entry of foreign DNA into the bacteria, and influence bacterial motility. The genome of *H. pylori* changes continuously during chronic colonization of an individual host by importing small pieces of foreign DNA from other *H. pylori* strains during persistent or transient mixed infections. (Falush *et al*.,2001*;* Suerbaum *et al*.,1998). After being ingested, the bacteria have to evade the bactericidal activity of the gastric luminal contents and enter the mucous layer. Urease production and motility are essential for this first step of infection. Urease hydrolyzes urea into carbon dioxide and ammonia, thereby permitting *H. pylori* to survive in an acidic milieu. (Suerbaum *et al*.,2002) The enzyme activity is regulated by a unique pH-gated urea channel, ureic, that is open at low pH and shuts down the influx of urea under neutral conditions. (Weeks *et al*.,2000) Motility is essential for colonization, and *H. pylori* flagella have adapted to the gastric niche (Josenhans *et al*.,2001) *H. pylori* can bind tightly to epithelial cells by multiple bacterial-surface components. (Gerhard *et al*.,2001) The best-characterized adhesin, BabA, is a 78-kD outer-membrane protein that binds to the fucosylated Lewis B blood-group antigen. (Ilver *et al*.,1998) Several other members of the Hop protein family also mediate adhesion to epithelial cells. Accumulating evidence in animal models suggests that adhesion, particularly by BabA, is relevant in *H. pylori*-associated disease (Guruge et al.,1998) and may influence disease severity, although the results of several studies are contradictory. Most *H. pylori* strains express the 95-kD vacuolating cytotoxin VacA, a secreted exotoxin. (Montecucco *et al*.,2001) The toxin inserts itself into the epithelial-cell membrane and forms a hexameric anion-selective, voltage-dependent channel24 through which bicarbonate and organic anions can be released, (Szabò *et al*.,1999) possibly providing the bacterium with nutrients. VacA is also targeted to the mitochondrial membrane, where it causes release of cytochrome c and induces apoptosis. (Galmiche *et al*.,2000) The pathogenic role of the toxin is still debated. VacA-negative mutants can colonize in animal models, and strains with inactive vacA genes have been isolated from patients, indicating that VacA is not essential for colonization. However, VacA-negative mutants were outcompeted by wild-type bacteria in a mouse model, indicating that VacA increases bacterial fitness in this model. (Salama *et al*.,2001) The analysis of the role of VacA in disease is complicated by extensive variability in vacA. In Western countries, certain vacA gene variants are associated with more severe disease. (Atherton *et al*.,1997) However, similar associations have not been found in Asia, and the functional basis underlying these associations is unknown.

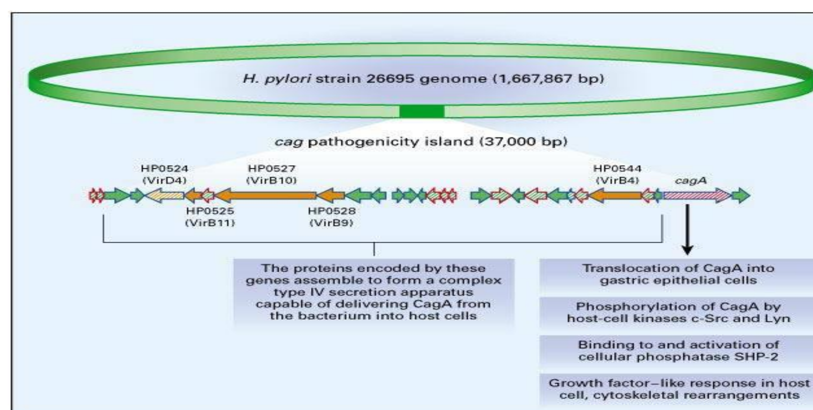


**Figure (1):** showing the cag Pathogenicity Island. (Censini *et al.,*1996)

## 2.6. Virulence factors of H pylori

### 2.6.1 Cag A

The primary determinant of *H. Pylori* virulence is the Cag Island of Pathogenicity. The island-positive strains were strongly associated with peptic ulcer and gastric ulcer pathogenicity. (Schreiber *et al.*, 2004). A type IV secretion mechanism via effector protein encoded by genes in the island of CagA pathogenesis is translocated to the cytoplasm of the host cell. (Basso *et al.,* 2008).

### 2.6.2 Vac A

Vac A, the vacuolating cytotoxin encoding with the Vac A gene, It is a powerful precursor of *H*. Virulence *Pylori,* That induces vacuolization of epithelial cells, Endo somal/lysosomal pathway disruption, cell signaling disturbance, T-cell proliferation, and inhibition. (Basso *et al.*, 2008). Vac A is mature with 87-KDa monomers in various, strains of H pylori, and And it has been observed that only 50 percent of *H pylori* strains have cytotoxic activity. (Basso *et al.,* 2008) An elevated incidence of carcinoma in the stomach occurring in combination polymorphism of s1m1 was seen in patients classified as Vac A seropositive. (Berg *et al.*, 1997).

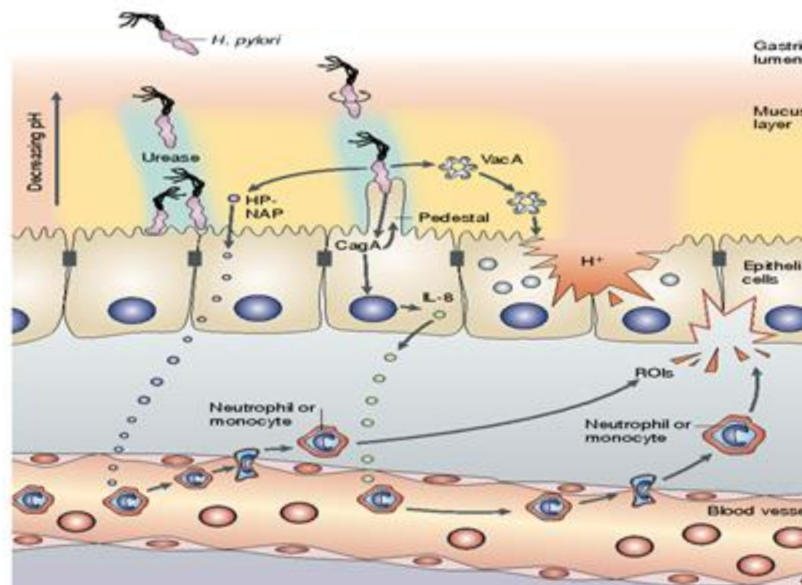


**Figure 2**: *colonization of the gastric mucosa by helicobacter pylori* (Montecucco *et al*., 2001)

## 2.7 Transmission of *H.pylori*

Now it has been established that *H. pylori* is mainly transmitted through person-to-person, especially in the childhood and intrafamilial transmission (Raymond *et al*.,2004; Drumm *et al*.,1990; Fujimoto *et al*.,2007) However, it is not clear how the bacteria are transmitted person-to-person and why the colonization does not occur in some persons, but it persists forever in others. The main route of transmission is regarded through oral-oral, fecal-oral, or gastric-oral. As the route of transmission could be different depending on developing or developed countries because the exposed age, race, and socioeconomic status are variable in these countries (Suerbaum *et al*.,2002).

### 2.7.1Transmission of H. pylori in the Developed Countries

In the developed countries, parental transmission has been frequently reported (Okuda *et al*.,2015) but the transmission route is not so simple because of the immigrants from countries with a high prevalence of H. pylori. For instance, the seroprevalence of German children in Germany was 13.1%, but that of Turkish children in Germany was 30.4% and Turkish in Turkey was 44.5%. suggesting that seroprevalence could be

different depending on race and socioeconomic status. Similarly, among 7-9-years-old Germans, H. pylori prevalence was 9.4% by UBT, but a subgroup analysis showed that prevalence in German children was 7.1% and that of the immigrants' children was 28.2% (Porsch *et al*.,2003) In this study, the number of family members was found to be a risk fac tor, and the prevalence rate was high when a family member had gastrointestinal symptoms (Porsch *et al*.,2003) The intrafamilial transmission became strong from many evidence in the developed countries. One study which performed finger printing showed that among 35 families, the strain of 29 families (81%) was the same In addition, the concordance between mother and children was 56% (10 among 18 families), but none between father and children, and 22% between husband and wife, suggesting that a close relationship increases the possibility of transmission (Kivi *et al*.,2003).

Osaki et al. performed a multilocus sequence typing DNA analysis using the stools of parents belonging to three families with a child positive for H. pylori infection (Osaki *et al*.,2013) The study showed an intrafamilial transmission in all selected families, with a mother-to-child transmission in at least two families. Similarly, Urita et al. investigated the intrafamilial transmission of H. pylori infection by testing 838 children and their family members from a small town in Japan. The H. pylori prevalence in children was 12.1%, and most risk factors were the siblings, mother, and grandmother, but the father and grandfather were not a risk factor (Urita *et al*.,2003). Indeed, it seems that mothers transmit the infection through mouth secretions, using common spoons or tasting the child's food (Eusebi *et al*.,2013). Grandmothers might take care of their grandchildren when mothers are at work increasing the risk of transmission. Similar report has come out from Ireland that children could be infected when the mother or siblings had H. pylori infection. Taken together, H. pylori infection in the developed countries could have characteristics of developing countries due to the immigrants from countries with a high prevalence of *H. pylori*. However, the oral-oral especially mother-to-child transmission is the main route. (Rowland et al.,2006)

## 2.8. MICROBIOLOGY

### 2.8.1 Genus Description and Phylogeny

The genus Helicobacter belongs to the ε subdivision of the Proteobacteria, order Campylobacterales, family Helicobacteraceae. This family also includes the genera Wolinella, Flexispira, Sulfurimonas, Thiomicrospira, and Thiovulum. To date, the genus Helicobacter consists of over 20 recognized species, with many species awaiting formal recognition (Fox *et al*.,2002). Members of the genus Helicobacter are all microaerophilic organisms and in most cases are catalase and oxidase positive, and many but not all species are also urease positive.

Helicobacter species can be subdivided into two major lineages, the gastric Helicobacter species and the enterohepatic (non-gastric) Helicobacter species. Both groups demonstrate a high level of organ specificity, such that gastric helicobacters in general are unable to colonize the intestine or liver, and vice versa. An extensive review of non-pylori Helicobacter species is available (Solnick *et al*.,2001) and here we briefly discuss those Helicobacter species that are either associated with human disease or have relevance for animal models of human Helicobacter infections (Table1) (Labigne *et al*.,1996).

**TABLE 1**. Characteristics of selected *Helicoactebr* species. (Labigne *et al*.,1996)

| Species | Primary mammalian host | Pathology | Animal model |
|---|---|---|---|
| Gastric *Helicobacter* spp. | | | |
| *H. pylori* | Human, primate | Gastritis, peptic ulcer disease, gastric adenocarcinoma, MALT lymphoma | Mouse, Mongolian gerbil, guinea pig, gnotobiotic piglet |
| *H. felis* | Cat, dog, mouse | Gastritis in natural host; | Mouse |

| | | | |
|---|---|---|---|
| | | may cause peptic ulcers or gastric adenocarcinoma in mouse | |
| *H. mustelae* | Ferret | Gastritis, peptic ulcer disease, gastric adenocarcinoma, MALT lymphoma | None |
| *H. acinonychis* | Cheetah, tiger, other big cats | Gastritis, peptic ulcer disease | Mouse |
| *H. heilmannii* | Human, dog, cat, monkey, cheetah, rat | Gastritis, dyspeptic symptoms, MALT lymphoma | Mouse |
| *Enterohepatic Helicobacter spp.* | | | |
| *H. hepaticus* | Mouse, other rodents | Proliferative typhlocolitis, hepatitis, hepatocellular carcinoma | None |

### 2.9 Diagnostic methods of *H. pylori*

Currently, there are various diagnostic methods used for *H. pylori* infection in different subjects (children and adults), but the only methods with both high sensitivity and high specificity remain useful and recommendable. In other words, precisive detection of this bacterium in different clinical specimens (e.g., urine, stool, saliva, biopsy, and gastric juice) attributed with successful therapeutic practice will be listed in hot topic research interest globally (Marshall *et al.,*1984; Fock *et al*,.2013). According to a traditional classification, *H. pylori* infection can be diagnosed by noninvasive tests such as *H. pylori* antigen in stool specimen, UBT (Urea Breath Test), serology, and invasive tests such as PCR (polymerase chain reaction), culture, and histology which require endoscopic surgery and biopsy specimens (McMahon *et al*.,2016) (Kim *et al*.,2014). Invasive tests (e.g., Histological examination, culture, and polymerase chain reaction) require endoscopy and noninvasive techniques (e.g., serology and urea breath) are independent of endoscopic surgery. Nonetheless, for having the best management of *H. pylori*-related diseases, we need to specific and accurate diagnosis, especially for treatment courses (pretreatment and posttreatment of *H. pylori* infection). In fact, the selection of choice method is highly dependent on the availability and feasibility of many circumstances (Mégraud *et al*.,2007). To now, many tests had been invented for diagnosis of *H. pylori*; however, each one has certain advantages and disadvantages (Mégraud *et al*,.2007; Granstrom *et al*.,2008)

#### 2.9.1 Endoscopy: A Pivotal Approach in Diagnosis of *H. pylori*

Although various methods had been attempted to accurately detect the *H. pylori* infection, noninvasive methods were preferred by gastroenterologists for many reasons (Abadi *et al*.,2o14; Megraud *et al*.,2007). The whole advantages and disadvantages of invasive and noninvasive methods are listed in detail (see Table 2). In a short sentence, the main rationale for choosing the noninvasive methods is to avoid endoscopy. Relatively high numbers of guidelines were recommending the noninvasive tests as first choice (Malfertheiner *et al*.,2012)

(Abadi *et al*.,2016). What should not be forgotten is that the endoscopy surgery is an unpleasant and uncomfortable approach for investigating the *H. pylori* in dyspeptic patients (Kiesslich *et al*.,2005; Dinis-Ribeiro *et al*.,2012). Additionally, there are other drawbacks which limit using the invasive methods such as endoscopy:

(i) patients need for 1–3 days off for this surgery.

(ii) high cost for disposable forceps and other stuffs, and

(iii) high risk of contamination by some viruses such as human immunodeficiency virus (HIV) and hepatitis C virus (HCV) (Kovaleva *et al*.,2017; Sakai *et al*.,2017). Of course, valid evidence indicating transmission of HIV and HCV among the subjects for endoscopy is not well-documented, but many patients declined this surgery just for this unpleasant probability released in media over the last years (Kovaleva *et al*.,2013; Fischer *et al*.,2010) Disparate distribution (patchy) of *H. pylori* in stomach is causing the bias in sampling (false negative) (Kusters *et al*.,2006). Indeed, taking a biopsy specimen (maximally 3-4 $mm^2$) cannot guarantee the existence of *H. pylori*-colonized in stomach environment (500–1000 $mm^2$ in different persons). A solution would be to increase the number of taken gastric biopsies, but for ethical limitations, gastroenterologists are highly prohibited to take 6 or more biopsies from a patient. Lastly, endoscopy is an impossible procedure for subjects such as pregnant women, children, and elderly patients (Mégraud *et al*.,2007; Ricci *et al*.,2007). There are two major approaches to noninvasive tests to diagnosis the *H. pylori* infection: UBT and serological examinations (Mégraud *et al*.,2007; Bessède *et al*.,2017). The main superiority of these methods is their easy applications in epidemiological studies. Furthermore, their easy application is affecting their high popularity in studies investigating the eradication rate following the antibiotic therapy (Ricci *et al*.,2007; Cutler *et al*.,1995; Monteiro *et al*.,2001).

**Table 2**: Overview of *H. pylori* diagnosis with invasive and noninvasive methods.(Talebi Bezmin Abadi *et al*.,2018)

| Name | Type | Reference method | Characteristics | Advantages | Disadvantages |
|---|---|---|---|---|---|
| UBT* | Noninvasive | No | Sensitivity: >95% Specificity: >95% | (i) High specificity and sensitivity (ii) Useful to confirm H. P eradication (iii) Useful to detect gastroduodenal bleeding (iv) Relatively cheap, simple and safe (v) A gold standard only for asymptomatic patients (vi) No sampling errors, good for epidemiological studies (vii) practically useful for children ~100% sensitivity | (i) Rarely false positive results refer to urease positive organisms (ii) Radiation in the case of application of $^{14}C$-UBT (iii) No data about antibiotic resistance |
| Serology | Noninvasive | No | Sensitivity: >96% Specificity: 60–90% | (i) Has no false negative result (ii) Cheap, simple and safe (iii) Highly recommended for initial *H. pylori* screening (iv) Not affected by gastric bleeding (v) No false negative result in the case of PPI** consumption (a unique character) | (i) No data about antibiotic resistance (ii) Failure in distinguish between active and past infection (iii) No application in clinical practice and hospitals |
| SAT*** | Noninvasive | No | Sensitivity: >95% Specificity: >95% | (i) High specificity and sensitivity (ii) Good popularity among patients (iii) Relatively fast and simple (iv) Easy modification to produce better results (v) No need to skilled staffs | (i) No data about antibiotic resistance (ii) The false positive result in the case of PPI and antibiotics (iii) Variation in specificity and sensitivity over the |

| | | | | | |
|---|---|---|---|---|---|
| | | | | | different clinical circumstances |
| Culture | Invasive | Yes | Sensitivity: 50–95% Specificity: >95% | (i) Existing the data about antibiotic resistance (ii) High specificity but low sensitivity (the most specific method existing) (iii) The possibility of having the pure bacterium and chance of preservation for a long time | (i) Need optimal incubation conditions and highly skilled operators (ii) Fast processes after endoscopy in necessary to avoid bacterial death (iii) Risk of the false negative result in the case of PPI and antibiotic consumption (iv) Need strict condition in transport before culturing (cool temperature) (v) Time-consuming and also the most expensive method |
| Histology | Invasive | Yes | Sensitivity: 60–90% Specificity: >95% | (i) The gold standard for direct *H. pylori* detection (ii) Almost cheap method for using in the universal scale (iii) Simple method | (i) Contradictory results following the PPI consumption (ii) Need extra biopsy sample and facing with ethical limitations (iii) Fluorescent microscope required method (limiting wide-spread usage) (iv) The relatively high rate of false negative reports |
| RUT**** | Invasive | No | Sensitivity: 95% Specificity: 80–90% | (i) Rapid, simple and cheap method (ii) High specificity (~99%), but low sensitivity (~80%) (iii) The most handful test in a clinical setting | (i) No data about antibiotic resistance (ii) Decreased sensitivity in patients with gastric bleeding (iii) Increased false negative results in the case of antibiotics & PPI consumption and achlorhydria (iv) Not useful for screening the eradication in epidemiologic studies |
| PCR | Invasive | No | Sensitivity: 80% Specificity: 100% | (i) Existing data about antibiotic resistance (ii) High specificity and sensitivity (iii) Tracking the mutations involved in antibiotic resistance (iv) The possibility of virulence typing (v) Useful to detect the bacterium in environmental samples (vi) Rapid and accurate results | (i) High cost (ii) Risk of contamination (iii) Time consuming and requirement to skilled staff (low feasibility in all laboratories) (iv) Lack of data about phenotypic antibiotic susceptibility profile |

Urea breath test; **proton pump inhibitor; ***stool antigen test; ****rapid urease test.

### 2.9.2. Urea Breath Test

The first report of the application of UBT was about 60 years ago by Kornberg et al. investigating the intravenous injection of 14C urea into the cat and determining the amount of decreased 14C-CO2 in animal breath (Kornberg *et al*.,1954; Kornberg *et al*.,1954). Their finding was due to the colonization of Helicobacter felis in the cat. When ingesting 13C- or 14C-labeled exposed to the bacterial urease in H. pylori positive patient, hydrolyzation results in the production of CO2 in the stomach. Thereafter, labeled CO2 enters the bloodstream and is exhaled in the lung. Consequently, the amount of trapped labeled CO2 will be measurable in the exhalation (Atherton *et al*.,1994). As mentioned above, the principle of this test is based on the intrinsic ability of *H. pylori* to break down absorbed 13C or 14C-labeled urea into CO2 in acidic gastric condition. If one is colonized actively with *H. pylori,* the urea is metabolized to the ammonia and labeled bicarbonate [14C-CO2]. Thereafter, labeled bicarbonate is transferred to the lung and produces labeled carbon dioxide. This product is detectable by the machine to confirm the existence of the infection. Because of high sensitivity and specificity, UBT is a very attractive method to measure the H. pylori active infection by microbiologists and clinicians. At least for asymptomatic subjects, the UBT is a gold standard method (Sardarian *et al*.,2013; Metanat *et a*l.,2015). Another preference of UBT is that the method is free of sampling errors (lack of endoscopic surgery). This superiority made it very popular for clinicians to confirm bacterial eradication, especially in asymptomatic, elderly, and pediatric subjects. Clinicians need to wait 1-2 months for performing UBT to confirm successful bacterial eradication. As noted, the false-positive result is a minor problem with UBT, and clinicians need to take care of other urease-producing organisms which may be able to change the result. Overall, the specificity and sensitivity of the UBT are mostly more than 95%. Although these high rates for both sensitivity and specificity are an advantage for this test, lack of data on antibiotic resistance and further analysis is the main limiting feature of this popular method to detect active *H. pylori* infection (Pantoflickova *et al*.,2003; De Francesco *et al*.,2010). A major consideration for this test is about its radiation. So far, decreased dosage of radiation made it a bit convenient for children but is still prohibited for pregnant women. In recent years, stool antigen test (SAT) and UBT became more acceptable diagnostic tests to detect active *H. Pylori* Infection. Lacking a universal protocol to perform UBT is an unsolved problem. Till now, only the manufacturer's experiences guided current standards to perform this method. Given high sensitivity (>95%) in posttreatment procedures (Moshkowitz *et al*.,1995), one of the disadvantages with UBT is the chance of colonization by urease-forming pathogens than *H. pylori* (Osaki *et al*.,2008). This probability is existing by the relatively low rate of current reports which increased our hopes to generalize application of UBT in routine and posttherapy *H. pylori* detection.

### 2.9.3. Stool Antigen Test (SAT)

Historically, serology approach was the first suggestion in order to diagnose H. pylori infection. Although the SAT is an accurate and precise method this accuracy is influenced by several limiting factors: upper gastrointestinal bleeding, antibiotic consumption, bowel movement, and also proton pump inhibitors (PPIs) uptake (Shimoyama *et al*.,2013). This noninvasive and almost cheap test became recommended whenever UBT was not available (Table 1). The superiority of UBT versus SAT was also found by Perri et al., while they showed that the diagnosis with UBT is undertaken with higher accuracy (Perri *et al*.,2002). Sequentially, SAT was introduced following the UBT into the clinic. Polyclonal antibodies-EIA gave useful reports on the diagnostic practices, but occasional inconsistent findings (mostly false-positives) forced clinicians to start application of the monoclonal antibody-based approach. An actual improvement in this technique was the higher specificity which reduced the false-positive findings (Gisbert et al.,2006; Koletzko et al.,2003). As the constructive shift in this immunologic assay, using monoclonal antibodies provided improved sensitivity and specificity rates in comparison with UBT. There are two major preferences for SAT in comparison with UBT; less expensive chemicals and materials and not high-technology equipment are necessary. Another advantage of this method was that patients could have stored the samples at home

and send it to laboratories at a suitable time. The partial insufficient condition of preservation of the stool sample in-house (optimal temperature, shaking, and transport medium to the laboratory) beside that applied cut-off value in final measurement can determine any bias in the diagnosis of the infection. To detect H. pylori infection, there are two main types of SAT: based on enzyme immunoassay (EIA) and immunochromatography (ICA) (Konstantopoulos *et a*l.,2001). In clinical practices, the ICA-based test is more convenient to run in the small clinics and hospitals since so complex procedures are involved. In 1997, this test was suggested for the first time and the found acceptable sensitivity and specificity (88% and 94%, respectively) initiate other groups to apply it over the clinical practices (Makristathis *et al*.,1998). A very attractive advantage of this method is to measure successful eradication of the infection using a simple laboratory examination (Vaira *et al*.,1999). Of course, to target pediatrics, SAT using monoclonal antibodies can give better feasibility since it is of low cost and is easy to handle by regular laboratories personals. In recent years, a new generation of stool antigen tests was invented. The test mate pylori antigen EIA and Test mate rapid pylori antigen are the major examples (Shimoyama *et al*.,2011; Calvet *et al*.,2013).

### 2.9.4. Serological Tests

In general, detection of specific-antibody following the exposure to the various H. pylori antigens can be a useful method in clinical practices. As application and logic procedure was undertaken for many other pathogenic microorganisms, H. pylori discovery was not far from serological diagnosis (Jones *et al*.,1984) outer membrane proteins, LPS, heat shock protein (HSP), catalase, and cagA protein and many of the adhesions were applied to induce specific antibodies in the host for facilitating the serological assay (Konstantopoulos *et al*.,2001; Mitchell *et al*.,1996; Khanna *et al*.,1998). Broadly defined, human immune response to the H. pylori is very complicated since many surface antigens are contributed. Routine *H. pylori* serologic methods can only detect specific IgG antibodies. The clinical importance of this test emerges when antibiotics and PPIs consumption are reported. Indeed, false negative results observed for other methods can have different response using serologic analysis. In addition to those drug uptakes, gastric bleeding and gastritis atrophic condition were also caused by false negative results for other methods; again, the serologic assay can be helpful for clinicians (Mitchell *et al*.,1998; Oksanen *et al*.,1998).

The highlighted problem with the serologic approach is the weak distinguishing power of this test to discriminate active and inactive infection. Due to the different backgrounds in host genetics, it can be expected that various H. pylori strains induce different levels of antibodies and it may be a considerable item in explaining the reported findings (Vaira *et al*.,2001). Because of acceptable sensitivity and specificity rates observed in many commercial IgG-bases tests exist and have been validated in recent years (Lee *et al*.,2015; Blecker *et al*.,993). One of the interesting aspects of serology method is following the antibiotic therapy; the long-lasting antibodies are still existing and it may cause the false-positive result (Lahner *et al*.,2002; Cutler *et al*.,1993).

This point should be cautiously considered by epidemiologists and gastroenterologists in their examinations in populations. In total, serologic tests are inexpensive; thus the application of these antibodies-based tests in some geographical area such as developing countries is highly acknowledged. A major consideration for the regions with a low prevalence of H. pylori is that suboptimal specificity can increase the false-positive results. Moreover, IgA-based measurement was also suggested but noted that the test is less trustful and reliable than IgG-based assays (Pandya *et al*.,2014; Granberg *et al*.,1993).

In some interesting studies, examinations of H. pylori-specific antibodies in other sample sources than serum were investigated (Berloco *et al*.,2001; Christie *et al*.,1996). In brief, saliva and urine were checked but because of the lower titer of antibodies in these samples in comparison with serum, clinicians are not so eager to check this sort of samples for H. pylori. Taking together, the antibody-based examination cannot guarantee the accuracy of reported *H. pylori* status following the antibiotic treatment; thus further analysis is needed (Korean *et al*.,1998).

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