REVIEW ARTICLE

RECORDS OF PHARMACEUTICAL AND BIOMEDICAL SCIENCES

Helicobacter pylori infection in Egypt: A review on the epidemiology, mode of transmission, diagnosis, and management of the infection

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Abstract

Helicobacter pylori, previously known as *Campylobacter pylori*, is the most famous causative agent of stomach ulcer, duodenal ulcer, gastritis, and gastric cancer and it has a direct relation with other gastric malignancies. It is a ubiquitous organism that is present in a percent of 30-100% of the global population. This percentage varies according to different geographical locations. Clinical manifestation, transmission, method of diagnosis, management of the infection, and even the treatment differs from region to region. In this review, we are concentrating on the above-mentioned factors that affect *H. pylori* infection epidemiology and we are directing the spotlight towards the epidemiology of this bacterium in Egypt. We have summarized different tools of diagnosis and the most famous modes of transmission of this infection in Egypt and mentioned our recommendations for future research investigation in these aspects. For example, using pulsed-field gel electrophoresis and next-generation sequencing for the full genomic characterization of *H. pylori* samples from this geographical area.

Keywords: *Helicobacter pylori*; Epidemiology; Diagnosis; Urease test; Management; Transmission.

1. Introduction

Helicobacter pylori (Garibyan and Avashia 2013) was first known as separate bacterial species in 1983 by Barry Marshall and Robin Warren. Before that, it was considered a species of *Campylobacter*. They were the first to establish Koch postulates to assure the fact that this bacterium causes peptic ulcer. **Figure 1** (J. Robin Warren 1983).

H. pylori is a spiral, negative gram, microaerophilic and pathogenic bacterial species that inhabit mainly the stomach (Allahverdiyev, et al. 2014). It is a motile, lophotrichous bacteria as it has six flagella at one or both poles (Diane, et al. 1992).
H. pylori can also transform from a spiral bacillary form into coccoid form in unfavorable conditions (A. Al-Sulami, et al. 2012).

This bacterium survives gastric acidity by secreting urease enzyme. It can colonize gastric epithelium depending on flagellar motility, adhesins, and cellular receptors. Then, secretion of virulence factors occurs (Sallas, et al. 2017).

H. pylori is the most famous causative agent of stomach ulcer, duodenal ulcer, gastritis, and gastric cancer (Enzo, et al. 2015). *H. pylori* infection has a direct relation with other gastric malignancies for example gastric adenocarcinoma and mucosa-associated lymphoid tissue carcinoma (MALT) (Roesler, et al. 2014). In 1994, the International Agency for Research on Cancer announced that *H. pylori* is classified as a group I carcinogen (Cancer 1994).

The development of gastric carcinoma has been due to the interaction between *H. pylori* virulence factors and genetic information of host gastric epithelial cells. This interaction induces genetic and epigenetic alternation in host genetic information (Oluwasola 2014).

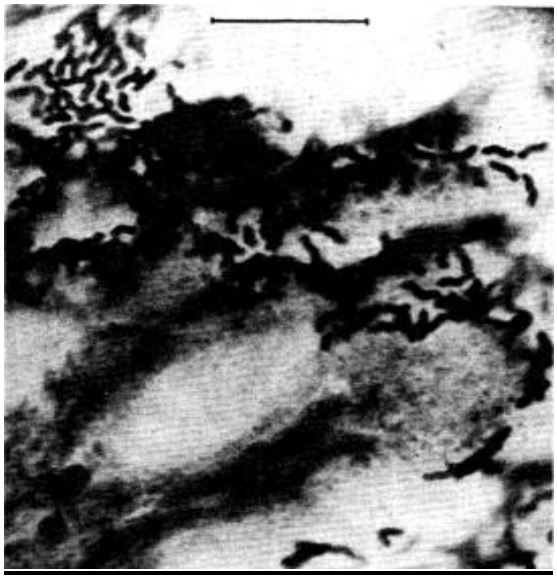

Figure 1 Curved bacilli on gastric epithelium.

2. Epidemiology of *H.pylori*:

Epidemiology is the study of how often diseases occur in different groups of people, and why? It is also used to correlate between the prevalence of certain diseases and other factors like social conditions, other illnesses or -in this study - genetic characters. Epidemiology can be used to formulate strategies for managing an established illness, as well as for preventing further cases (Stewart 2016).

H. pylori affects more than 90 % of the world population but not all infected patients show clinical symptoms. Many patients may have the infection without even knowing. *H. pylori* infection is more common in developing countries due to lack of well sanitary disposal of sewage matters (Graham 1997 Nevoa, et al. 2017 Rafeey, et al. 2013).

Direct epidemiologic comparisons of peptic ulcer disease (PUD) between developing and developed countries are complex due to differences in symptoms, availability, and accessibility of the tests required for diagnosis vary widely (World Gastroenterology 2011).

Most of the statistical reports in last years indicated that the risk of *H. pylori* infection is higher in children and declines in the elder population (Salih 2009) and infection of one of the family members, especially the mother, is the main cause of pediatric infection (Osaki, et al. 2015 Salih 2009).

A study on Egyptian schoolchildren has stated that the prevalence of *H. pylori* infection among children has reached about 72.38 % (70.34 in girls & 73.8 among boys) (Mohammad, et al. 2008). This study also stated that school children living in Suhag have a higher infection rate than those in Giza and Cairo (96.7% - 61.9 % respectively). This indicates the effect of geographical location and socio-economic status on the prevalence of infection. Similar results were reported in 2005 by (Enany, et al. 2005) on dyspeptic patients.

The rate of *H. pylori* infection reaches 82.9% in gastritis patients and 71.7% in dyspeptic patients. This indicates a strong association between *H. pylori* infection and gastritis (Khedmat, et al. 2013). Besides, *H. pylori* is considered to be the main risk factor for gastric cancer due to prolonged chronic atrophic gastritis (Kishikawa, et al. 2020). Moreover, Previous studies have stated that symptoms may vary geographically (Yamaoka 2010). For example, the percent of gastric cancer incidence increases in East Asia, conversely it decreases in Africa and South Asia (Kao, et al. 2016).

3. Mode of Transmission:

The main mode of transmission is human to human through oral-oral, gastro-oral, fecal-oral by respiratory droplets, saliva, and vomits but the isolation of bacterium from environmental samples failed (Alexander, et al. 2010). As stated in (Salih 2009), mothers have a role in the transmission of *H. pylori* infection to their children. This has been confirmed by the fact that the genetic fingerprint of strains in infected children and parents are always identical (Roesler, et al. 2014). According to many studies, contaminated water was suspected to be one of the causes of transmission of *H. pylori* (Aziz, et al. 2015 Bahrami, et al. 2013).

In Egypt, (El-Sharouny, et al. 2015) detected *H. pylori* in water samples from Abu El Matamir-Beheira, and Sidi Bishr-Alexandria after in both PCR and culture tests.

Other ways such as inappropriate disinfected gastric devices and endoscopes may also have a role in *H. pylori* transmission between patients. Gastroenterologists and nurses have an increased risk for infection due to their occupational exposure to infected patients. (Sualeh, et al. 2012).

In Egypt, a study by (Galal, et al. 2019) stated that there are many sociodemographic variables that are related to *H. pylori* infection residence in Egypt like; illiteracy of mothers, absence of pure water supply, and eating from street vendors.

In general, improper hygiene practices, overcrowded living conditions, and low socioeconomic status may be the main reason for the prevalence of *H.pylori* infection (Cheng, et al. 2009 Salih 2009).

4. Pathogenesis:

H. pylori can survive gastric acidity because it is embedded in gastric mucosa. It can also assure survival by secreting urease enzyme which has an important role in pathogenicity and colonization in gastric mucosa. Urease enzyme secreted in large quantities by the bacteria hydrolyzes urea into carbon dioxide and ammonia which neutralize the gastric acidity to a higher sustainable pH for *H. pylori* (Diane, et al. 1992).

H. pylori move toward host gastric epithelium by flagella-mediated motility. Then, bacterial adhesins interact with receptors on gastric host epithelium. This prevents peristalsis and gastric emptying from the displacement of bacteria from the stomach. Toxins like cag pathogenicity protein (*cagA*) and vacuolating cytotoxic protein (*vacA*) are then released and cause damage to host tissue. **Figure 2** (Kao, et al. 2016)

H. pylori directly activates neutrophils by inducing neutrophil oxidative burst. A protein named HP-NAP (*H. pylori* neutrophil-activating protein) has been identified and it may be responsible for this function. After neutrophils activation, they produce oxygen free radicals and adhere to endothelial cells (Atherton 1998 Polenghi, et al. 2007).

Polymorphism in cytokine gene affects the cytokine release and so affects clinical outcome and symptoms. Polymorphism in toll-like receptors (TLR4) also affects the pathogenesis of *H. pylori*. Hypermethylation of tumor suppressor genes promotor in host epithelial cells is one of the epigenetic changes due to bacterium–host interaction. Some studies stated that there are combined effects of certain IL-1 genotypes and (*cagA*, *vacA*) virulence factors that decide higher risk for developing gastric carcinoma (Amieva and El–Omar 2008).

The pathogenicity of *H. pylori* infection is highly related to its genetic content. There are many strain-specific factors that affect *H. pylori*-related diseases like peptic ulcers, duodenal ulcers, and gastric cancer. These factors are not independent, and they are closely related. These factors are *vacA*, *iceA*, outer membrane protein (*babA2*), duodenal ulcer associated protein (*dupA*), and *oipA* genes (Yamaoka, et al. 2002).

Due to its role in increased IL8 production and nuclear factor-kB activation, *cagA* gene is mostly used as a measure for *H. pylori* virulence activity and is reported to be responsible for mucosal inflammation and gastric cancer development (Abu-Taleb, et al. 2018). Another virulence factor is the sialic acid-binding adhesion *(sabA)* that has been proved before to be associated with gastric cancer in Egyptian patients (Enany 2015).

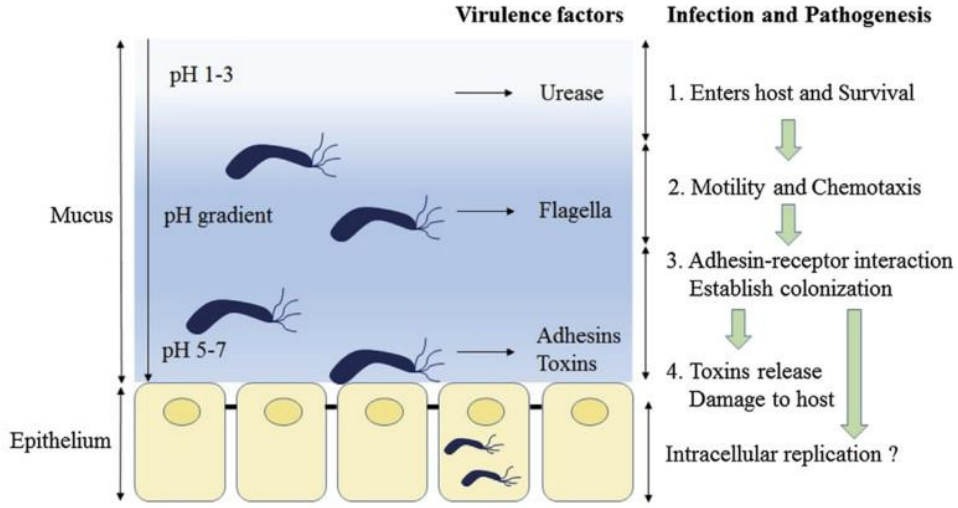

Figure 2 Schematic diagram of steps of *H. pylori* pathogenesis

5. Diseases and clinical manifestation:

Dyspepsia, epigastric pain, and vomiting are the main symptoms associated with *H. pylori* infection (Magid, et al. 2014). It has been stated that it is the main cause of recurrent abdominal pain in 10%-20% of normal children and that it can be related to other health problems like β-thalassemia and hemolytic anemia (Yousab Feiby, et al. 2015). However, not all infected persons develop the same clinical symptoms. Some infected persons show no symptoms at all.

6. Diagnosis of *H. pylori* infection:

There are many factors affecting the choice of the most suitable diagnostic test such as clinical situation, population, cost, test performance, the use of Proton-pump inhibitors (PPIs) or antibiotics as treatment, and other factors that may affect test results (World Gastroenterology 2011). Method of diagnosis may be endoscopic or non-endoscopic as indicated in **Table 1**.

6.1. Endoscopic diagnosis methods:

6.1.1. Rabid urease test:

It is a rabid and cheap test but it has low sensitivity after treatment (World Gastroenterology 2011). It can be in the form of a freshly prepared buffered solution of urea with an acid-base indicator or in the form of test strips which are inoculated with the biopsy sample (Buharideen, et al. 2015)

6.1.2. Histology:

Using special stains such as: Warhin-starry silver stain or Giemsa stain protocol (World Gastroenterology 2011).

6.1.3 Culture and isolation:

Culture may not be applied in all countries as the main diagnostic method due to the experience required, cost, and poor sensitivity if adequate transport media are not available. However, it is very important for studying resistance patterns and it is considered an important tool for selecting the most suitable treatment protocol (World Gastroenterology 2011). *H. pylori* grows perfectly on enriched media in microaerophilic conditions. it should be cultured for 5 days at 37° C, iron supplement is very important for the growth of *H. pylori* (Merrell, et al. 2003). The viability of *H. pylori* for culture is affected by many factors like the transportation media, smoking, alcohol, nonsteroidal anti-inflammatory drugs, or PPIs use (Nordenstedt, et al. 2013).

6.1.4. Fluorescence in situ hybridization (FISH):

In this method, a fluorescence-labeled oligonucleotide probe specifically targets and hybridizes special sequences of ribosomal RNA (rRNA), so that the whole bacteria in the specimens can be visualized directly by a fluorescence microscope. Indeed, FISH is a molecular technique that permits us to detect the morphology of the bacteria. The advantage of FISH over PCR is that the extraction of DNA from the bacteria is omitted in FISH. Furthermore, there is no need for prior culturing of the samples (Samarbaf-Zadeh, et al. 2006).

6.1.5. Polymerase Chain Reaction (PCR):

It is a sensitive and specific test, but it is not standardized. It depends on DNA extraction from biopsy samples then test for *ureA* gene or 16SRNA gene (World Gastroenterology 2011).

6.2. Non-endoscopic diagnosis methods

6.2.1. Stool antigen test (HPSAT):

It has high sensitivity and specificity before and after treatment (World Gastroenterology 2011). It has low validity in predicting *H. pylori*-associated diseases (Segamwenge, et al. 2014).

Table 1: Tests for *H. pylori* infection (World Gastroenterology 2011)

Endoscopic diagnosis methods	Non-endoscopic diagnosis methods
-Rabid urease test (RUT)	-Stool antigen test (SAT)
-Histology	-Finger-stick serology test
-Culture	-Whole blood serology
-Fluorescence in situ hybridization (FISH)	-13C urea breath test
-Molecular approach: polymerase chain reaction (PCR)	-14C urea breath test

Stool antigen test (HPSAT) is done by using polyclonal antibody against alkyl hydroperoxide reductase (*AhpC*) of *H. pylori*. Another monoclonal antibody-based immunoassay has been used too (David Y. Graham 2009).

6.2.2. Whole blood serology test:

Serological tests were the first non-invasive technique based on the detection of a specific anti-*H. pylori* IgG antibody in patients' serum. Nevertheless, they have present limitations. The most important is that with this test we can detect exposure of the host to the bacterium, but we cannot know exactly if there is an ongoing infection. Persistent antibodies will lead to false-positive results (Vaira, et al. 2002).
In high-prevalence areas, the definition of the serological cut-off value distinguishing between active infection and background infection may be problematic (Hunt, et al. 2010).

6.2.3. Finger-stick serology test:

It is a next-generation in-office, whole-blood antibody test that can achieve a sensitivity and specificity similar to or better than those of widely used quantitative laboratory serological tests and may be used as the initial screening tests of choice for *H. pylori* (Laine, et al. 1999). However, its results are very poor and cannot be equated with ELISA serology (Hunt, et al. 2010).

6.2.4. Urea breath test using C13 & C14:

It is a noninvasive test that is recommended before treatment and a preferred for confirming eradication of the infection. Proton pump inhibitors and antibiotic therapy affect the results of this test (World Gastroenterology 2011). In patients with atrophic gastritis, there are urease-positive bacteria in the oral cavity. This may give a false-positive result for *H. pylori*. In patients younger than 6 years old the endogenous CO_2 production may also give a false-positive result (David Y. Graham 2009).
Endoscopy followed by rapid urease testing is considered as the gold standard for *H. pylori* infection but one of the most important precautions before any diagnostic test that the patient should be free from medication with PPIs or h2 receptor antagonists for 2 weeks at least and antibiotics for 4 weeks at least (World Gastroenterology 2011).

7. Biochemical tests:

Many enzymes are secreted by *H. pylori* to enhance the survival of the bacterium. For example, urease and catalase. Using specific reagents for these enzymes can help us in detecting the presence of these enzymes and so the presence of any bacteria.

7.1. Urease test:

This test is used to identify bacterial ability to produce urease enzyme. This enzyme hydrolyzes urea into weak base and ammonia. This test is performed by preparing urea as a substrate with an acid-base indicator (phenol red) system. The presence of urease enzyme will hydrolyze urea into a weak base and ammonia which will change the color of media into pink color (Abdalla, et al. 1989).

7.2. Catalase test:

This test is used to identify bacterial ability to produce catalase enzyme. This enzyme hydrolyzes hydrogen peroxide into water and oxygen which produce strong effervescence for a positive result (Hazell, et al. 1991).

7.3. Oxidase test:

This test is used to identify bacterial ability to produce cytochrome oxidase by the introduction of oxidase reagent (tetra-methyl- p-phenylenediamine dihydrochloride solution) artificial electron donors and acceptors. When an electron donor is oxidized by cytochrome oxidase, it turns dark purple (Shields, et al. 2010).

8. Management of H. pylori infection:

In general, following good hygiene measures like washing hands, drinking water from clean sources, and eating well-cooked food is very important for avoiding infection in the first place (World Gastroenterology 2011).

As mentioned before *H. pylori* infection complications vary from gastric ulcer to gastric cancer and lymphoma. The only golden key for preventing such complications is ensuring complete eradication of this infection. Multidrug regimens have been approved as the standard treatment for *H. pylori* infection as illustrated in **Table 2** (World Gastroenterology 2011)

Adverse drug reactions, presence of other medical conditions, allergy to penicillin’s and Antibiotic resistance should be taken into consideration while choosing antibiotics for these regimens. Antibiotic sensitivity testing may be a must if treatment fails (World Gastroenterology 2011).

In Egypt, many physicians are using quadrable therapy for treating *H. pylori* (Afifi, et al. 2020). However, resistance against first-line antibiotics, especially Clarithromycin, Tetracycline, and metronidazole is starting to emerge (Pohl, Keller et al. 2019).

Table 2 Multidrug regimens for treatment and management of *H. pylori* infection. World Gastroenterology, 2011

Triple drug therapy	Quadruple drug therapy
-PPI + TWO antibiotics Amoxicillin & Clarithromycin Or Metronidazole & Clarithromycin Or Amoxicillin & Levofloxacin From 7 days to 14 days	-PPI + bismuth + TWO antibiotics Amoxicillin & Clarithromycin Or Metronidazole & Tetracycline Or Amoxicillin & Levofloxacin -use three antibiotics in case of unavailability of bismuth - For 10 days

9. Future recommendations:

H. pylori has been extensively studied in different parts of the world due to its determinant role in developing many gastric malignancies. However, little is known about *H. pylori* strains in Egypt. For that reason, we recommend the application of more advanced molecular techniques such as pulsed-field gel electrophoresis (PFGE) and Next-generation sequencing techniques for the full characterization of *H. pylori* infection in this important geographical region.

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