بسم الله الرحمن الرحيم

# *Diagnosis of Helicobacter pylori Infection :Detection of iceA & glmM Genes By Polymerase Chain Reaction (PCR)*

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In

**Applied Medical Chemistry**

By

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B.Sc. Special Biochemistry, Faculty of Science,
Alexandria University 2003

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# تشخيص الإصابة بالميكروب الحلزوني: تحديد وجود الجينات أي سي أي أ ، جلم إم بطريقة تفاعل البلمرة المتسلسل

رسالة علمية

مقدمة الى معهد البحوث الطبية

جامعة الأسكندرية

استيفاء للدراسات المقررة للحصول على درجة

## الماجستير

فى

## الكيمياء الطبية التطبيقية

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٢٠٠٩

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# LIST OF ABBREVIATION

| | |
|---|---|
| AG | Atrophic gastritis |
| bp | Base pairs |
| CAG | Chronic active gastritis |
| *cagA* | Cytotoxic-associated gene A |
| *vacA* | Vacuolating cytotoxin A |
| *IceA* | Induced contact epithelium |
| *ureC(glmM)* | Urease C gene |
| Hsp | Heat Shock Protein |
| CLO test | *Campylobacter*-like organism-test |
| DGAC | Diffuse gastric adenocarcinoma |
| DNA | Deoxyribonucleic acid |
| RNA | Ribonucleic acid |
| dNTPs | Deoxyribonucleoside triphosphates |
| GERD | Gastroesophogeal reflux diseas |
| GI | Gastrointestinal |
| H & E | Haematoxylin-eosin |
| IGAC | Intestinal gastric adenocarcinoma |
| IgG | Immunoglobulin G |
| IgA | Immunoglobulin A |
| IM | Intestinal metaplasia |
| KCl | Potassium Chloride |
| Kd | Kilo dalton |
| MALT | Mucosa-associated lymphoid tissue |
| $Mg^{++}$ | Magnesium ion |
| $MgCl_2$ | Magnesium chloride |
| NaCl | Sodium chloride |
| NaOH | Sodium hydroxide |
| $NH4^{+}$ | Ammonium ions |
| PCR | Polymerase Chain Reaction |
| ROS | Reactive Oxygen Species |
| rpm | Round per minute |
| Taq polymerase | Thymus aquatics polymerase |
| TBE | Tris Boric EDTA |
| TE | Tris EDTA |
| Tm | Melting temperature |
| UV | Ultra Violt |
| WHO | World Health Organization |

# INTRODUCTION

*Helicobacter pylori* (*H. pylori)* is a helical shaped, gram-negative bacterium that is strongly associated with gastroduodenal disease, including chronic active gastritis, peptic and duodenal ulcer disease, and gastric cancer. [1-3]

*Helicobacter* species are the only known microorganisms that can survive in the highly acidic environment of the stomach, its helical shape (from which the genus name is derived) is thought to have involved to penetrate and favor its motility in the mucus gel layer.[4]

## History

In 1875, German scientists found helical shaped bacteria in the lining of the human stomach. The bacteria could not be grown in culture and the results were eventually forgotten.[5,6] In 1893, the Italian researcher Giulio Bizzozero described helical shaped bacteria living in the acidic environment of the stomach of dogs.[7]

In 1899, Professor Walery Jaworski of Jagiellonian University in Krakow investigated that the sediments of gastric washings obtained from humans. contain some rod-like bacteria, he also found bacteria with a characteristic helical shape, which called Vibrio rugula. He was the first to suggest a possible role of this organism in the pathogenicity of gastric diseases. [8]

In 1979, the bacterium was rediscovered by Australian pathologist Robin Warren, who did further research on it with Barry Marshall beginning in 1981; they isolated the organisms from mucosal specimens from human stomachs and were the first to successfully culture them.[9] Barry Marshall and Robin Warren described the successful isolation and culture of a spiral bacterial species, known as *H. pylori*. [10,11] Warren and Marshall, 2005 were awarded the Nobel Prize in Medicine for their work on *H. pylori.*[11,12]

The organism was initially named "*Campylobacter*-like organism," "gastric *Campylobacter*-like organism," "*Campylobacter pyloridis*," and "*Campylobacter pylori*" but it is now named *H. pylori* in recognition of the fact that this organism is distinct from members of the genus *Campylobacter.* [13]

The genus *Helicobacter* belongs to the subdivision of the *Proteobacteria*, order *Campylobacterales*, family *Helicobacteraceae*, *Helicobacter* consists of over 20 recognized species ,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. [14]

*Helicobacter* species can be subdivided into two major lineages, the gastric *Helicobacter* species and the enterohepatic (nongastric) *Helicobacter* species. Both groups demonstrate a high level of organ specificity, such that gastric *Helicobacter*s in general are unable to colonize the intestine or liver, and vice versa. [15]

## Structure and Morphology

Thin section of *H. pylori* reveal the typical cell wall detail of a gram-negative bacterium that consists of outer and inner, or plasma, membranes separated by the periplasm of approximately 30 nm thickness. The dense cytoplasm contains nucleoid material and ribosomes, [16] it is a microaerophilic bacterium that colonizes gastric mucosa

and damages epithelial cells by association and cytotoxin release, it is the principal cause of non-autoimmune gastritis , peptic ulcer, and an aetiological factor in gastric carcinoma. [17-20]

It is microaerophilic bacterium, ie: it requires oxygen but at lower levels than those contained in the atmosphere. It contains a hydrogenase which can be used to obtain energy by oxidizing molecular hydrogen ($H_2$) that is produced by other intestinal bacteria. [21]

*H. pylori* in vivo and under optimum conditions in vitro is an S-shaped bacterium, with 1 to 3 turns. [22, 23]It measures 2 to 4 µm in length and 0.5 to 1 µm in width.it is usually spiral-shaped, and can appear as a rod, while coccoid shapes appear after prolonged in vitro culture or antibiotic treatment, [24] coccoid forms may represent a viable, nonculturable state. [25]

The organism has 2 to 6 unipolar, sheathed flagella of approximately 3 µm in length, [11,26] and is 30 nm in diameter, consisting of an internal filament ~12 nm in diameter surrounded by a sheath, the outer membrane of which is continuous with the outer membrane of the cell [22,23], which often carry a distinctive bulb at the end for fixation, these flagella confer motility and allow rapid movement in viscous solutions such as the mucus layer overlying the gastric epithelial cells. [26]

The following figure shows electron micrographs of negatively stained preparations of *H. pylori* in lag and exponential phases of growth.

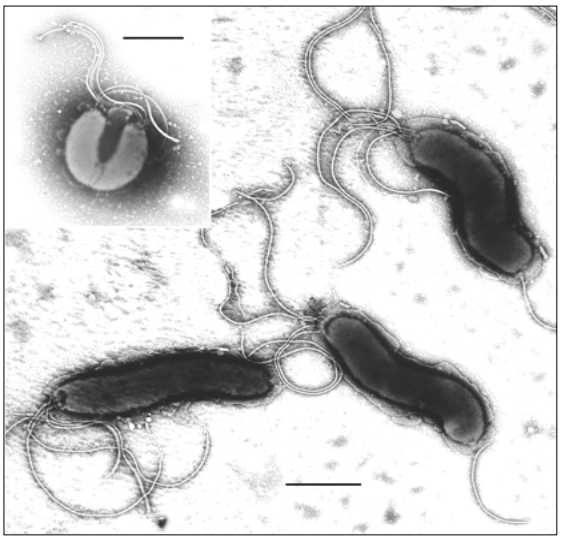

**Figure (1): Illustrating non-dividing bacteria with helical forms and multiple polar flagella** [17]

It has been observed that *H. pylori* cells can transform in vitro from a culturable spiral-shaped form to an as-yet nonculturable coccoid form. This change in morphology is accompanied by physiological changes. [27-29] The following figure show the coccoid forms of *H. pylori*.

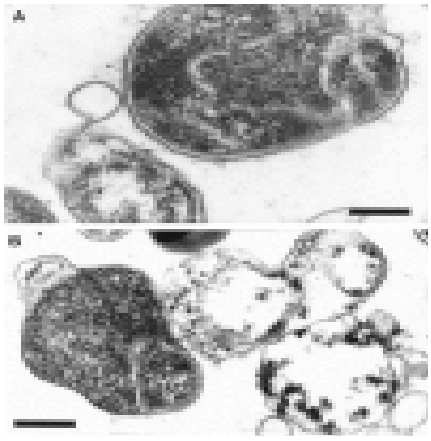

**Figure (2): Coccoid forms of *H. pylori*** [24]

## Growth requirements

A key feature of *H. pylori* is its microaerophilicity, with optimal growth at $O_2$ levels of 2 to 5% and the additional need of 5 to 10% $CO_2$ and high humidity. Although its natural habitat is the acidic gastric mucosa, *H. pylori* is considered to be a neutralophile. The bacterium will survive brief exposure to pHs of <4, but growth occurs only at the relatively narrow pH range of 5.5 to 8.0, with optimal growth at neutral pH. [30, 31]

## Colonization

*Helicobacter pylori* senses the pH gradient within the mucus layer and swims away from the acidic contents of the lumen,[32] where it produces large amounts of urease enzymes which are localized inside and outside of the bacterium. Urease metabolizes urea (which is normally secreted into the stomach) to carbon dioxide and ammonia which neutralizes gastric acid. The survival of *H. pylori* in the acidic stomach is dependant on urease and it would eventually die without it. The ammonia that is produced is toxic to the epithelial cells, and with other products of *H. pylori*, including protease, catalase, and phospholipases, causes damage to those cells. Some strains of the bacteria have a particular mechanism for "injecting" the inflammatory inducing agent peptidoglycan from their own cell wall into epithelial stomach cell. [33] Colonization of the stomach by *H. pylori* results in chronic gastritis, an inflammation of the stomach lining. [34] Duodenal and stomach ulcers result when the consequences of inflammation allow the acid and pepsin in the stomach lumen to overwhelm the mechanisms that protect the stomach and duodenal mucosa from these caustic substances. The type of ulcer that develops depends on the location of chronic gastritis, which occurs at the site of *H. pylori* colonization. [35]

# *Pathogenesis*

Gastric colonization with *H. pylori* can lead to variety of upper gastrointestinal disorders, such as chronic gastritis, peptic ulcer disease, gastric mucosa-associated lymphoid tissue (MALT) lymphoma, and gastric cancer, the persistence of a pathogen in