CHAPTER III

LITERATURE REVIEW

1. General characteristic

1.1 History of *H.pylori*

Helicobacter pylori was first isolated in 1982 By Marshall and Warren in western Australia from gastric mucus of patients with chronic gastritis and duodenal ulcers. These investigators named the microogranism as Campylobacter-like bacteria (1). Skirrow, a microbiologist in the Campylobacter fieid, suggested that if the organisms were proved to be a member of the genus *Campylobacter*, the name *Campylobacter pyloridis* would be apt because of their specific location and association (47) . Successful culture of the bacterium resulted in the acceptance of this name, which was later corrected to *Campylobacter pylori* (48, 49).

Yet almost from its initial cultivation it was suspected that perhaps *Campylobacter pylori* was not a true *Campylobacter*. Early electron micrographs showed multiple sheathed flagella at one pole of the bacterium, in contrast to the single bipolar unsheathed flagellum typical of *Campylobacter* species (50). Further studies revealed that the organism differed sufficiently from true Campylobacters to justify the formation of a new genus *Helicobacter* (51). It soon becomes apparent that similar organisum coloized the stomach of a wide variety of animals other than humans, and that certain spiral bacteria colonizing the intestines of rodents and other animals also belonged to *Helicobacter* (51). It soon becomes apparent that similar organisms colonized the stomach of a wide variety of animals other than humans, and that certain spiral bacteria colonizing the intestines of rodents and other animals also belonged to *Helicobacter*. Ongoing study of this organism, particularly at the genetic level, resulted in the formation of a new genus, *Helicobacter* (helico = curved, bacter = staff), with *Helicobacter pylori* as the type species, the first member of the new genus *Helicobacter* (52) .

The analysis the sequence of the 16S rRNA gene led to the differentiation of *Helicobacter pylori* from *Campylobacter* species and this molecular tool also has been instrumental in the classification of the other members of this genus. Other important features that differentiate *Helicobacter* species from *Campylobacter* species included the possession of sheathed flagella, unique fatty acid profile, lack of respiratory quinones, active urease enzyme, and a distinct protein profile.

2. General characteristics of *Helicobacter pylori*

2.1 Morphology

Helicobacter pylori is a spiral to curved, rod-shaped bacterium approximately 0.5 μm in diameter and 3 to 5 μm long. This organism possesses the characteristic ultrastructure of a gram-negative bacterium. In older cultures, cells are seen to ball up, form U-shaped structures and lose their cytoplasmic cylinders and membrane integrity, resulting in the formation of coccoid cells (53). It has been proposed that this coccoid form is a viable but nonculturable form of the organism, which allows it to survive in hostile environments outside the gastric mucus (54)

Helicobacter pylori has 4 to 7 polar sheathed flagella which enable the bacterium to move freely in viscous environments as gastric mucus (55). Several studies have reviewed that this motility is essential for the bacterial colonization of its host (56). The flagella sheath is a membrance containing proteins and lipopolysaccharides which probably protects the flagella filaments from the gastric acidity (57). The flagella filament contains two different flagellin proteins, Fla A and Fla B, both of which have been shown to be necessary for the motility of the organism.

3. Classification of *H. pylori*

3.1 General characteristics of the genus Helicobacter

3.1.1 Cellular morphology and ultrastructure

Helicobacter are non-spore-forming gram-negative bacteria. The cellular morphology may be curved, spiral, or fusiform, typically 0.2 to 1.2 μm in diameter and 1.5 to 10.0 μm long and have rounded ends. The spiral wavelength may vary with the age, the growth conditions and the species identity of the cells. In old cultures or those exposed to air, cells may become coccoid .

Periplasmic fibers or an electron-dense glycocalyx or capsule-like layer has been observed on the cellular surface of several species (58-61). Electron-dense granular bodies have been observed in *H. pylori* (61) and *H. rodentium* (60). In *H. pylori* these bodies are known to be aggregates of polyphosphate and may serve as a reserved energy source.

Helicobacter cells are motile with a rapid cork-screw-like or slower wave-like motion due to flagella activity. Strains of most species have bundles of multiple sheathed flagella with a polar or bipolar distribution. The genus *Helicobacter* at present consists of 18 offical genera with another 10 potentially novel species (62). Of the offical genera, 8 are of gastric origin and the remaining 10 are found in the intestinal tract of a wide variety of animal species in *H. pylori* and *H. heilmanii* are the only 2 species which have been associated with human gastric disease. *H. cinaedi* and *H. fennelliae* are causes of enteritis and proctolitis. Distinguished features of 4 important member of the *Helicobacter* genus are compared in Table1.

Table1. Characteristics of member in genus *Helicobacter*

Helicobacter species	Host	Features	Disease associations
H. pylori	Human	multiple(4-6)sheathed flagella at one end	Causes gastritis in human aslo found sometimes in domesticated or caged animals e.g. pigs cats
H. heilmannnii	Human,cat,dog	Corkscrew appearance with between a and 20 turns, at least 12 sheathed flagella at one end, no axal periplasmic fibers noted	about 1% of human gastritis cases are caused by this bacterium, presumably acquire from cats and dogs
H. mustelae	Ferret	Several randomly placed flagella	Gastritis and ulcerations commonly develop in ferrets, useful modle for studying pathogenic mechanisms.
H. felis	cat, dog	Differs only form *H. heilmannnii* by the presence of axial periplasmic fibers noted by electron microscopy	Isolated from cats, can be propagated in mice, useful in screening trials for anti *H. pylori* chemotherapy agent

3.1.2 Growth characteristics

In laboratory conditions, strains typically grow under strictly microaerobic condition at 37°C. No growth is observed in aerobic conditions, *Helicobacter* will grow at 37°C on a variety of rich agar bases supplemented with 5% whole blood or serum. Many species require fresh media with moist agar surfaces for optimal growth conditions, though this is not usually the case for *H. pylori*.

3.1.3 Biochemical characteristics

Helicobacters are chemoorganotrophs and show a respiratory type of metabolism. They are asaccharolytic when sugar catabolism is examined by standard methods (neither oxidation nor fermentation is observed). Recent studies have, however, indicated that glucose oxidation occurs in at least *H. pylori* (63, 64).

Gelatin, starch, casein, and tyrosine are not hydrolyzed. Helicobacters are methyl red and Voges-Proskauer negative. Oxidase activity is present in all species. Strains of most species produce catalase. Many species produce urease, alkaline phosphatase, or both. There is no production of pigment (65) .

4. Culture and Identification

4.1 The atmosphere for culture of *Helicobacter pylori*

In general, primary cultures of *H. pylori* have less oxygen tolerance than most *Campylobacter* species with a growth maximum at 3 to 7% of O_2. *H. pylori* is usually grown in jars with gas-generation kits (1, 66) or a standard microaerobic atmosphere in CO_2 incubation or anaerobic chambers with a microaerobic atmosphere. Most studies with standardized atmosphere for culture of *H. pylori* have used 2 to 5% O_2, 5 to 10% (optimal closer to 10%) CO_2, and 0 to 10% H_2; high humidity are required for growth (67-70).

Optimal growth is obtained at 37°C after 4 to 5 days for primary culture or 2 days for subsequent subculture.

Plates must be incubated in a microaerobic environment (5 to 7% O_2) incorporating increased CO_2 (5 to 10%) and a high relative humidity. This environment may be created by using the campy Pak system (BBL Microbiology System, Cockeysville, Md.) in an anaerobic jar with a moistened towel in the bottom. A single evacuation of an anaerobic jar to 220 mm Hg (1 mm Hg = 133.322 Pa) and replacement with an anaerobic gas mixture (10% CO_2, 10% H_2, and 80% N_2 (71). The Campy Pouch (BBL), which holds one or two plates, has also been effective for subculture. Because an acceptable atmosphere is generated in the pouch, primary isolation is predicted even though no formal evaluation has been published. All biochemical and susceptibility tests for these organisms require microaerobic conditions (72).

4.2 Nonselective and selective media for growing *H. pylori*

H. pylori can grow on different solid media containing blood or blood products (blood or lysed blood agar plates). Most studies have Brucella agar or Columbia agar as the agar base. An amout of 7 to 10% blood improves the growth of *H. pylori* as compared with 5% blood. Horse blood may also improve the growth of *H. pylori* as compared to sheep blood (73). Suppplement of agar with cyclodextrin B can be used for blood-free culture media for *H. pylori* but with large difference between different batches of cyclodextrin (74).

Often *H. pylori* grows poorly or not at all on selective media containing antibiotics. Skirrows and Dents selective media seen to be the best available commercial selective media and have been used in several studies (75-77). There seem to be greater difference between horse and sheep blood agar, in with an without antibiotics (73). By comparing agar plates containing 5% horse blood, 10% horse blood, 7% lysed horse blood, 7% lysed horse blood with trimetroprim and selective *campylobacter* plates, revealed that *H. pylori* with more plates than on the other media, but the numbers of *H. pylori*-positive patients were almost equal with all media.

Like *Campylobacter*, *H. pylori* is stictly microaerophilic and CO_2 (5 to 10%) and high humidity are required for growth. *H. pylori* requires media containing supplements similar to those used for *Campylobacter* ; blood, serum, haemin, starch, or charcoal. However,*H. pylori* is inhibited by the bisulphate in the FBP *Campylobacter* "aerotolerance supplement". Growth is best on media such as moist freshly prepared heated (chocolate) blood agar, and nutrient-rich media such as brain heart infusion agar or brucella agar supplement with 5 to 7% horse blood are adequate as nonselective media (75, 76). An egg yolk emulsion agar with Columbia agar base has also been shown to yield excellent nonselective growth of *H. pylori* when compared to other culture media for *H. pylori* as shown in Table 2. (78).

Usually *H. pylori* grows slowly in liquid media with formation of a high numbers of coccoid forms (79, 80). Contaminating microorganisms (Staphylococci, yeasts, etc.) usually grow much faster than *H. pylori* and make liquid media useless for primary culture of biopsies. Becauses of the risk of contaminated samples, a selective medium is usually recommended in addition to the nonselective media for routine culture.

4.3 Transportation and handling of biopsies for *H. pylori*

Gastric biopsy specimens are the only ones likely to be used for the primary isolation of *H. pylori* they should be transported in a moist state and cultured within 2 hours of collection. Storage beyond this time should be at 4°C or at -20°C if the period is more than 2 days, whereas a higher temperature (about 20°C) decreases the number of positive cultures significantly (81, 82). Thus, a decrease in culture rate of about 15% was found when biopsies were transported or stored overnight. A long transportation time decreases the number of *H. pylori* especially after antibiotics therapy, and if the number of bacteria is low, culture may be become false negative.

Various transport media have been described for transporting biopsy samples, including cysteine brucella broth, normal saline, glucose, milk, stuart's medium, semi-solid agar, brain-heart infusion broth and Clary-Blair medium (66).

Table 2. Comparison of culture media and atmospheric conditions for growth of *H. pylori* (78)

Agar Media	Conditions of Incubation				
	Anaerobe Jar (no Catalyst)	Campy GasPak Jar	Anaerobe Jar (no Catalyst)	Poly Bag	Atmosphere Air With 5% CO_2
Brucella with 5%sheep blood	Best growth β-hemolysis	Very good growth β-hemolysis	No growth	No growth	No growth
Tryptic soy With 5% sheep blood	Very good growth	Good growth	No growth	No growth	No growth
Brain heart Infusion with 5% horse blood	Very good growth	Good growth	No growth	No growth	No growth
Chocolate (BD)	No growth	No growth	No growth	No growth	No growth
Chocolate (GIBCO)	Small colonies	Small colonies	No growth	No growth	No growth
Chocolate (freshly)	Very small colonies	Very small colonies	No growth	No growth	No growth
Campy-BAP	No growth	No growth	No growth	No growth	No growth

4.4 Specimens for culture of *H. pylori*

H. pylori is the microorganism most frequently found in the human gastric mucosa in assaciation with gastric epithelial cells, but other curved bacteria have also been found in the gastric mucosa.

4.4.1 Gastric specimens

H. pylori is most regularly found in the antral part of human gastric mucosa of untreated persons. In persons treated with acid-suppressive drugs (proton pump inhibitiors and H_2 antagonists), *H. pylori* may be present in higher numbers in the body of the stomach. *H. pylori* is more frequently found in gastric antrum than in duodenal biopsies even in persons with duodenitis and duodenal ulcer. *H. pylori* can only be cultured from gastric juice in about 15% of persons with *H. pylori* cultured from gastric antrum and from less than 50% of esophageal biopsies from untreated persons with esophagitis, even though *H. pylori* can be cultured from gastric antrum (83, 84). The number of biopsies necessary to diagnose *H. pylori* by culture has been estimated in a study where more than 95% of *H. pylori* was cultured from one antral biopsies (68). It is generally accepted, according to the modified Sydney classification of gastritis (85) that at least one biopsy from antrum and two biopsies from corpus should be taken for culture to ensure a sufficient diagnosis.

4.4.2 Extragastric specimens

H. pylori has occasionally been cultured from ectopic gastric mucosa in Meckel's diverticulum, esophagus, rectum, urinary bladder, dental plaque, and feces (54, 86-89). Detection of extragastric *H. pylori* from plaque, fecal samples, atherosclerotic plaque and liver was mainly achieved by genome methods and serology (90-92) Recently, *H. pylori* has also been detected by PCR in specimens from gallbladder and liver (93). No systematic studies have been carried out to recommend optimal sample sites for extragastric *H. pylori* infections.Culture-confirmed microbiological identificaion is preferable to ensure the

bacteriological diagnosis of isolates from these sites, a least until molecular biological methods have been better evaluated than they are today.

5. Identification of *H. pylori*

The optimal temperature of incubation in 35 to 37°C. Colonies from primary isolation are generally observed by 3 to 4 days of incubation. Colonies of *H. pylori* are small (0.5 to 2 mm), translucent to yellowish colonies on 7% lysed horse blood agar and with translucent to pale grayish colonies of 0.5 to 1 mm in size on blood agar. In very young cultures, *H. pylori* may appear as almost straight rods on microscopy. After 3 to 5 days of incubation the bacteria look pleomorphic, with irregular curved rods, several being U shaped. In old cultures, *H. pylori* appears as degenerative coccoid forms that Gram stain poorly. Because of their small size, *H. pylori* colonies may be difficult to identify and isolate when there are few colonies and additional contaminating oral microbiota is present. Some contaminating microorganisms may grow as small colonies but usually differ from *H. pylori* in color.

H. pylori is biochemically closely related to *Campylobacter*, *Arcobacter*, and *Wollinella* species but also resembles *Bacteroides*, *Thiovulum*, and *Selenomonas* species. They are all characterized as being gram-negative rods that are able to grow microaerobically or anaerobically. The rods may be more or less curved, depending on the growth conditions. The urease reaction is a key reaction in identifying *Helicobacter* species, but some *Campylobacter lari* stains are urease positive and at least one urease-negative *H. pylori* strain has been isolated from a patient. Several *Helicobacter* species are gram negative, motile curved rods that are oxidase, catalase, and urease positive, and it may, therefore, be necessary to undertake protein profiles or genomic analysis to ensure the correct identification.

6. Diagnostic Testing

Currently, there are several popular methods for detecting the presence of *H. pylori* infection, each having its own advantages, disadvantages, and limitations. Basically, the tests available for diagnosis can be separated according to whether or not endoscopic biopsy is necessary. Histologic evaluation, culture, polymerase chain reaction (PCR), and rapid urease test are typically performed on tissue obtained at endoscopy. Alternatively, simple breath tests, serology, and stool assays are sometimes used, and trials investigating PCR amplification of saliva, feces, and dental plaque to detect the presence of *H. pylori* are ongoing (94) as seen in Table 3

6.1 Histology

Histologic evaluation has traditionally been the gold-standard method for diagnosing *H. pylori* infection. The disadvantage of this technique is the need for endoscopy to obtain tissue. Limitations also arise at times because of an inadequate number of biopsy specimens obtained or failure to obtain specimens from different areas of the stomach (95). In some cases, different staining techniques may be necessary, which can involve longer processing times and higher costs. However, histologic sampling does allow for definitive diagnosis of infection, as well as of the defree of inflammation or metaplasia and the presence/absence of MALT lymphoma or othe gastric cancers in high-risk patients.

6.2 Culture

Because *H. pylori* is difficult to grow on culture media, the role of culture in diagnosis of the infection is limited mostly to research and epidemiologic considerations. Although costly, time-consuming, and labor intensive, culture does have a role in antibiotic susceptibility studies and studies of growth factors and metabolism. However, in the United States, culture should not be considered a routine, first-line means of diagnosis (96).

6.3 Polymerase Chain Reaction

With the advent of PCR, many exciting possibilities emerged for diagnosing and classifying *H. pylori* infection. PCR allows identification of the organism in small samples with few bacteria present and entails no special requirements in processing and transport. Moreover, PCR can be performed rapidly and cost-effectively, and it can be used to identify different strains of bacteria for pathogenic and epidemiologic studies. As suggested earlier, PCR also is being evaluated for its utility in identifying *H. pylori* in samples of dental plaque, saliva, and other easily sampled tissues (94).

The major limitation of PCR is that relatively few laboratories currently have the capability to run the assay. In addition, because PCR can detect segments of *H. pylori* DNA in the gastric mucosa of previously treated patients, flase-positive results can occur, and errors in human interpretation of bands on electrophoretic gels can likewise lead to false-negative results.

6.4 Rapid Urease Testing

Rapid urease testing takes advantage of the fact that *H. pylori* is a urease-producing organism (97). Samples obtained on endoscopy are placed in urea-containing medium; if urease is present, the urea will be broken down to carbon dioxide and ammonia, with a resultant increase in the pH of the medium and a subsequent color change in the pH-dependent indicator. This test has the advantages of being inexpensive, fast, and widely available. It is limited, however, by the possibility of false positive results; decreased urease activity, caused either by recent ingestion of antibiotic agents, bismuth compounds, proton pump inhibitors, or sucralfate or by bile reflux, can contribute to these false-positive results (98).

6.5 Urea Breath Test

A urea breath test similarly relies on the urease activity of *H. pylori* to detect the presence of active infection. In this test, a patient with suspected infection ingests either ^{14}C-labeled or ^{13}C-labeled urea; ^{13}C-labeled urea has the advantage of being nonradioactive and thus safer (theoretically) for children and women of chidbearing age. Urease, if present, splits the urea into ammonia and isotope-labeled carbon dioxide; the carbon dioxide is absorbed and eventually expired in the breath, where it is detected.

Besides being excellent for documenting active infection, this test is also valuable for establishing absence of infection after treatment, an important consideration in patients with a history of complicated ulcer disease with bleeding or perforation (99). In addition, a urea breath test is relatively inexpensive (whichever isotope is used), is easy to perform, and does not require endoscopy. However, if the patient has recently ingested proton pump inhibitors, antibiotic agents, or bismuth compounds, a urea breath test can be of limited value. Therefore, at least 1 week should separated the discontinuing of antisecretory medications and testing for active infection (100), and 4 weeks should separate treatment of *H. pylori* infection and testing for eradication of the organism. Moreover, except for major medical centers or tertiary referral centers where results are usually available in fewer than 24 hours, a urea breath test may be further limited by a turnaround time of several days or longer required for transport of samples and analysis by specialized laboratories not present in many community settings.

6.6 Serologic Tests

In response to *H. pylori* infection, the immune system typically mounts a response through production of immunoglobulins to organism-specific antigens. These antibodies can be detected in serum or whole-blood samples easily obtained in a physician's office. The presenct of IgG antibodies to *H. pylori* can be detected by use of a biochemical assay, and many different ones are available.

Serologic tests offer a fast, easy and relatively inexpensive means of identifying patients who have been infected with the organism. However, this method is not a useful means of confirming eradication of *H. pylori*; several different samples and changes in titers of specified amount over time would be needed (101). In addition, few patients become truly seronegative, even after eradication of the organism (102).

In low-prevalence populations, serologic tests should be a second-line methodology because of low positive predictive value and a tendency toward false-positive results. Serologic tests may be useful in identifying certain strains of more virulent *H. pylori* by detecting antibodies to virulence factors associated with more severe disease and complicated ulcers, gastric cancer, and lymphoma.

6.7 Stool Antigen Testing

Stool antigen testing is a relatively new methodology that uses and enzyme immunoassay to detect the presence of *H. pylori* antigen in stool specimens. A cost-effective and reliable means of diagnosing active infection and confirming cure, such testing has a sensitivity and specificity comparable to those of other noninvasive tests (103). Questions remain regarding possibel crossreactivity with other *Helicobacter* species present in the intestines, but definitive studies are lacking.

Table 3. Summary of tests for detection of *H. pylori*

Test	Sensitivity (%)	Specificity (%)	Cost*	Endoscopy required	Comments
Culture	77-94	100	+++	Yes	"gold standard"
Histology	93-99	95-99	+++	Yes	Demonstrate host response and integrity of mucosa
Rapid urease Test (CLO test)	86-97	86-98	+	Yes	Can be performed rapidly
^{13}C breath test (no radioactive)	96-100	80-99	++	No	Preferred for pregnant Women and children; not Widely available;excellent For early posttreatment Tracking
^{14}C breath test	90-100	92-100	++	No	Small amount of radiation Exposure; excellent for Posttreatment tracking
Serology	83-98	56-100	+	No	Readily available; useful Seroepidemiological tool; Limited value in short-term Tracking of therapy

* + = least expensive, ++ = moderately expensive, +++ = most expensive

7. Epidemiology and Transmission

7.1 Prevalence

Helicobacter pylori infects more than half the people in the world. The prevalence of the infection varies among countries and among differention groups within the same country. The highest rates of infection are associated with low socioeconomic status, crowding, poor sanitation, and unclean water supplies (104). *H. pylori* is the most common chronic bacterials infection in humans. Infection is strongly correlated with socioeconomic conditions, with a prevalence of over 80% in developing world , compared with 20-50% in highly industrialized countries (2). *H. pylori* is highly adapted for the human stomach, where it has lived for many thousands of years, as determined by geographical associations of *H. pylori.* It is predominantly found in modern populations that derive their gene pools from ancestral populations that arose in africa, central Asia and east Asia, with subsequent spread attributable to human migratory fluxes (105).

H. pylori infection is typically acquired in childhood. The prevalence of the infection at age 20 provides a reasonable estimate of the frequency of infection in that birth cohort throughout the remainder of their lives. In adults, the frequency of acquiring the infection is about 0.5% a year. In developed countries, the rate of acquisition of the infection is now less than the rate of loss of the infection, probably as a consequence of the use of antibiotics for a different medical problem (eg, respiratory or urinary tract infections). Thus, overall, the prevalence of *H. pylori* infection is decreasing among all birth cohorts. Nonetheless, the indigent and socially disadvantaged populations in the United States still have a relatively high rate of acquisition and, along with immigrants, remain as a reservoir in the population.

Age is the most important variable related to the prevalence of infection. In general, prevalence increases with age (106) This rising infection prevalence with age is largely apparent, rather than real, reflecting a continuing overall decline in the prevalcnce of *H. pylori* infection. Because the infection is typically acquired in childhood and is lifelong the

high proportion of older individuals who are infected is the long-term result of infection in childhood when standards of living were lower. The prevalence will decrease as current 40-year-olds with their lower rate of infection reach age 60, a birth cohort phenomenon.

Children in developing nations between the ages of 2 years and 8 years acquire the infection at a rate of about 10% a year. This significant difference in the rate of chilhood acquisition is responsible for the differences in epidemiology between developed and developing countries. The annual incidence reported in 3 adult studies in developed countries was between 0.3% and 0.5% per year (107, 108).

Socioeconomic differences are the most importand predictor of the prevalence of infection in any group. Higher standards of living, higher levels of education, and better sanitation correlate with lower prevalence of infection. The rate of infection in those with several generations of high socioeconomic status is between 8% and 15% (109). This is probably the lowest the prevalence will spontaneously fall until eradication or vaccination programs are instituted.

7.2 Prevalence of *H. pylori* in different ethnic groups

The prevalence of *H. pylori* infection varies among nations with developed, Western nations exhibiting lower rates of infection than developing countries. Even within Western nations, the rate of infection varies with ethnicity and race. In the United States, whites have a lower prevalence of *H. pylori* infection than do blacks or Hispanics. For example, in the metropolitan Houston ares, the prevalence of *H. pylori* infection in blacks and Hispanics is twice that of the age-adjusted white population (110). The difference in prevalence remained when differences is the following were controlled: income, educational level, current socioeconomic status, housing type, and use of tobacco and alcohol. Race was shown not to be the determining factor because, despite different rates of infection for whites and blacks, Hispanics (an ethnic group independent of race) had a rate of infection equal to that of blacks. When difference in socioeconomic status during childhood were included in the model, the

differences in prevalence among the various groups disappeared, confirming that childhood is the critical period for acquisition of this typically lifelong infection (111). Thus, the prevalence of *H. pylori* in any group of adults reflects the rate of acquisition in childhood.

7.3 Genetic factors

Although genetic factors were not an important explanation for the differences among racial or ethnic groups, genetic factors are important in determining the bacteriumhost interaction and outcome of the infection (112, 113). Genetic influences are best demonstrated in studies comparing outcomes in twins raise together and separated at or near birth. This allows for comparison of the outcome in monozygotic twins, who share the same genes, with that in dizygotic twins, who are genetically different. The importance of environmental factors is seen in the difference in prevalence between those reared apart compared with those reared together. Such studies have shown that monozygotic twins reared apart have concordance rates for *H. pylori* infection that are much higher than dizygotic twins reared apart. The correlation coefficient (a measure of the influence of heritability on the prevalence of infection) was high (0.66). The possible range of this coefficient is between 0 and 1 (112, 113). The major conclusions are that genetics are significant in acquisition of the infection (114), but environmental factors are also important.

7.4 Family studies

Transmissiblity of the infection is emphasized by several studies showing that any activity that brings together infected and uninfected individuals in stuations where sanitation may be compromised is associated with a high prevalence of *H. pylori* infection. For example living in crowded circumstances in childhood has repeatedly been shown to be an epidemiologic link to risk of acquistion of the infection. Multiple studies have demonstrated an association between the number of children in the household and the risk of acquistion of the infection (115-117). Previous studies have demonstrated an association between the number of children in the household and the risk that adult family members will acquire the

infection (114, 118). Studies of asymptomatic families showed that if the index case had *H. pylori* infection, the other members of the family were likely to be infected. If the index parent was not infected, other members of the household were also unlikely to have *H. pylori* infection. The primary source of infection within a family probably varies depending on which parent has the most contact with the children. The fact that the prevalence of *H. pylori* infection in the spouse, who is genetically unrelated to the other spouse, and in the children could be predicted by the *H. pylori* status of the index case is consistent with the notion that the environment is the most important factor in acquisition of *H. pylori* infection. The results in families with children contrast markedly to studies of infertile couples, in which the data show little tendency for transmission from one spouse to the other (119).

8. Transmission

South Korea has undergone a huge change in its economic fortunes in the last several decades, altering itself from an underdeveloped nation to a fully industrialized country. This economic transformation makes Korea a particularly apt location to study the effects of socioeconomic differences on the acquisition of *H. pylori* infection. Korean adults have a very high rate of infection, equivalent to rates in developing nations, relecting the living conditions during their chidhood. However, Korean children from high socioeconomic-status families had much lower prevalence rates (22%) than did children in developing nations or children of Koreans with lower socioeconomic status (120). As in other countries, social class was inversely related to prevalence of *H. pylori*. Most Korean adults are infected, yet children from higher socioeconomic-status parents are becoming infected at a markedly lower rate than other socioeconomic classes, suggesting that it is possible to break the pattern of transmission. The behavior modification or mechanism resulting in the reduced rate of transmission remains unclear and in one of the important questions of further research

Previous studies suggest a fecal-oral mode of transmission (121), although some investigators support an oral-oral mode of transmission (122-124). *H. pylori* can reach the mouth via reflux of gastric contents. While *H. pylori* can be found in dental plaque, dental workers are not at increased risk to develop the infection. This would be categorized as

gastric-oral and would be to the increased risk seen in gastroenterologists and endoscopy nurses who work with gastric secretions. Nurses in one study had a 39% rate of seropositivity compared with 26% in age-matched controls (125). The source of *H. pylori* infection in nurses could be fecal-oral or gastric-oral, associated with handling of nasogastric tubes or endoscopes.

9. Pathogenicity of *H. pylori*

Once acquired, *H. pylori* infection persists in its host indefinitely, apparently for life. Its persistence can be attributed to features that allow it to colonize the stomach . *H. pylori* is motile by polar flagella, allowing it to access susceptible areas. In addition, it is able to withstand the local pH. Which leads to the formation of ammonia on the gastric mucosa, thereby increasing the pH of its environment. The organism also releases cytotoxin, toxic proteins, platelet activating factor, and lipopolysaccharide. The latter is produced in its outer membrane. Colonization of the stomach by *H. pylori* leads to inflammatory reaction with neutrophilic gastritis that ultimately results in the clinical manifestations of the infection. This process is mediated by host factor, including interleukin1, 2, 6, 8, and 12; interferon gamma; tumor necrosis factor-α; T and B lymphocytes; and phagocytic cells that is mediated by several of the bacterium's virulence determinants, which ultimately cause injury to the stomach tissues. These factors mediate injury through release of reactive oxygen species and inflammatory cytokines cause injury of epithelial cell (126). *H. pylori* additionally appears to increase the rate of mucosal programmed cell death also known as apoptosis (127).

10. Clinical Manifestations

The clinical outcomes of *H. pylori* infection vary from asymptomatic chronic gastritis, chronic *H. pylori* associated dyspepsia, peptic ulcer disease, gastric adenocarcinoma and mucosal associated lymphoid tissue lymphoma

10.1 Non-ulcer dyspepsia

Non-ulcer dyspepsia (NUD) is a common heterogenous condition with multifactorial causes. In the past the presence rate of *H. pylori* associated in NUD has been found to be 25-30% (128). The role of *H. pylori* causing dyspeptic symptoms remains unclear and controversial. Recent meta-analysis from McColl (129) and Jaakkimainen (130) have indicated that dyspepsia is improved if *H. pylori* is eradicated. Of note, many dyspeptic patients do continue anti acid drug therapy after *H. pylori* cure. It is not possible currently to predict which patient would respond to eradicate therapy and treatment can not guarantee improvement in all cases of *H. pylori* related NUD. Multiple designed randomized controlled trials comparing the eradication of *H. pylori* with placebo or alternative pharmacological therapics have provided conflicting, but mainly negative, results. However, previous studies concluded that treating *H. pylori* has a small but statistically significant beneficial effect in treating non-ulcer dyspeptic patients (131).

10.2 Chronic gastritis

The majority of patients infected with *H. pylori* are asymptomatic despite the presence of gastric mucosal inflammation. The activity of the inflammation is correlated with the presence of virulence factors (*cagA* PAI and *vacA*) in the organism. On pathology basis, long standing chronic inflammation represents loss of glands which are named atrophy and ultimately intestinal metaplasic change. This occure mostly in the antrum, less in the corpus and cardia. Inflammation and development of atrophy worsen particularly in the

corpus upon acid suppressant therapy (132). *H. pylori* gastritis and particularly, subsequently atrophic gastritis increase the risk for gastric carcinoma on a multifactorial basis.

10.3 Peptic ulcer

With circumstantial evidence and with Koch's postulates arguably fulfilled for a direct link between *H. pylori* and peptic ulcer disease (PUD), the combination weight of all evidence leads to the unequivocal conclusion that PUD is an infectious disease. That PUD also occurs as a result of other factors and in the absence of *H. pylori* infection should not be misinterpreted as casting doubt on the causative line between *H. pylori* and most cases of PUD (133). Long term follow-up studies have confirmed that permanent cure of the infection and healing of the gastric mucosa has been realized (133).

The two major causes of peptic ulcers are chronic *H. pylori* infection and the use of non-steroidal anti-inflammatory drugs (NSAIDs). In patients not taking NSAIDs, the eradication of *H. pylori* leads to long-term remission from chronic peptic ulcer disease. Data have been conflicting regarding interactions between *H. pylori* and NSAIDs, but in a meta-analysis, *H. pylori* infection and NSAIDs have a synergistic effect on increasing the risk of peptic ulceration and ulcer bleeding (134). Furthermore, Chan et al (135), reported from Hong Kong that for patients with dyspepsia or a past history of peptic ulcer who require long-term NSAID treatment, screening and treatment for *H. pylori* infection significantly reduced the risks of an ulcer developing. Such evidence formed the basis of a recommendation in the Masstricht 2-2000 Consensus Report of a panel of mainly European experts that *H. pylori* should be eradicated before commencing NSAID therapy (131).

10.4 Gastroesophageal reflux disease

Chronic *H. pylori* infection involving the corpus with *cagA* positive appears to decrease the risk of reflux disease because of its interference on gastric acid secretion. Decrease of acid secretion due to corpusitis and ammonia related neutralization can be

considered as the equivalent of chronic intake off a mild acid suppressant (132). Removal of this acid inhibition may unmask reflux disease. Some investigators propose not to treat *H. pylori* infection in order not to aggravate the tendency of reflux disease and esophageal columnar metaplasia. However, the risk of gastric adenocarcinoma related *H. pylori* infection is higher than the risk of esophageal malignacy in noninfected hosts. In routine practice, it is inconclusive at the present time to eradicate *H. pylori* in patients with gastro-esophageal reflux disease.

Considerable controversy exists regarding the relationship between *H. pylori* and esophagus. *H. pylori* is relatively infrequent in gastroesophageal reflux disease (GERD), and the presence of *H. pylori* improves the efficacy of proton pump inhibitors in suppressing gastric acid secretion. Because some studies suggest that the eradication of *H. pylori* may cause GERD, whereas others found *H. pylori* to be less prevalent in patients with GERD-associated distal esophageal adenocarinoma, *H. pylori* may conceivably 'protect' against GERD and its deleterious consequences (136). However, subsequent studies have not generally supported this hypothesis (137), and the benefits of eradicating *H. pylori* lower risk of peptic ulcer discase and distal or noncardia gastric cancer probably outweigh and potentially harmful esophageal consequences.

10.5 Gastric adenocarcinoma

Atropic gastritis and intestinal metaplasia are well accepted precancerous conditions for gastric cancer. Gastric adenocarcinoma are related to *H. pylori* gastritis, most of this gastritis is atrophic in the microscopic phenotype and exhibits intestinal metaplasia as an underlying mucosal lesion, in addition to the loss of the mucosal gland(138). The relationship between gastric cancer and atrophic gastritis of the automimmune type suggests that the presence of *H. pylori* is not necessary for the development of gastric malignancy in atrophic gastritis, but *H. pylori* is the key phenomenon in the triggering of the gastritis related process and the subsequent carcinogenic events.

Recently, 119 gastric cancer cases were reported from Thailand (139). The most common age group was 22-91 years and the most common site of the cancer was the antrum followed by lessor curvature and greater curvature. The histological section revealed that 68 percent of cases had adenocarcinoma mention the incidence of *H. pylori* infection.

Up to 50% of all *H. pylori*-infected individuals will progress some way down the pre-neoplastic sequence of histological change towards gastric cancer (140), although fewer than 2% will develop a frank malignacy. Several *H. pylori* virulence-associated genes have been found in Western populations to be associated with an increased risk of gastric cancer and pre-cancerous lesions. However, the extent of the relationship varies considerably across populations, and explains only a fraction of the association of *H. pylori* with specific diseases. A major advance in our understanding of the pathogenesis of *H. pylori* was the demonstration that the gastric immune and epithelial response to *H. pylori* are partly dependent on cytokine gene polymorphisms (141).

Subsequent studies from Japan have confirmed that IL-1β polymorphisms do contribute to the gastric acid secretory response to *H. pylori* infection and subsequently to clinical sequelae. These outcomes range from, at one end of the spectrum, hypochlorhydria and atrophic gastritis with an increased risk of cancer to, at the other end, high acid secretion and duodenal ulcer disease (142, 143).

In an important extension of this work, Figueriedo et al.(144) genotyped a large population with chronic gastritis and gastric cancer for polymorphisms of the genes for both IL-1β and its receptor, and for the *vacA* and *cagA* genotype of the infecting *H. pylori* strain. Combinatorial analysis of both bacterial and host genotypes demonstrated an enormous (up to 90-fold) difference in the risk of gastric cancer, depending on particular mixtures of *H. pylori* virulence and host genetic factors, thus demonstrating the importance of considering both *H. pylori* and host genetics in gastric cancer risk assessment.

The cellular and molecular mechanisms linking *H. pylori* to inflammation and gastric cancer. In *vitro*, *H. pylori* interferes with the host cellular machinery that normally limits DNA damage through mismatch repair mechanisms (145). Chronic *H. pylori* infection is also associated with increased gastric cell turnover, probably of importance in malignant transformation. Increased gastric cell proliferation (but not increased apoptosis) may be related to the *babA2* adhesin of *H. pylori* (146). In related work, strains of *H. pylori* carrying the cag pathogenicity island (PAI) were found to be antiapoptotic, through a pathway involving nuclear factor kappa B (147), indicating that by modulating gastric cell cycling *H. pylori* may cause the aberrant growth of neoplastic and preneoplastic gastric epithelial cells.

Treatment of *H. pylori* infection in gastric cancer can decrease the inflammation of gastric mucosa, apoptosis and intestinal metaplasia (148). At the present time in order to prevent gastric cancer, there is not sufficient evidence support eradication of *H. pylori* infection in normal hosts. Other important factors including race and family history of gastric or other gastrointestinal malignancy should be considered before eradication of this organism.

10.6 Mucosa-associated lymphoid tissue lymphoma

The association between MALT lymphomas and *H. pylori* was postulated for the first time in 1988 with the recognition that chronic infection of *H. pylori* is the cause of acquired gastric MALT lymphomas. The role of *H. pylori* in gastric MALT lymphomas is consolidating. A mutation of fld-A-gene appears to be involved in the pathogenesis . Antibody to such a mutated antigen could become useful as a serologic detector of this disease. Early lesion limited to the wall of the stomach in the absence of metastasis, gastric MALT lymphomas should be eligible for initial attempt at *H. pylori* cure(149).

The regression of gastric low-grade MALT lymphomas after *H. pylori* therapy occurs in most cases. A small case study of seven such patients who refused further management (surgery, radiation or chemotherapy) showed that they came to no harm with a watch-and-

wait strategy over approximately 3 years' follow-up (150). Further verification by following a larger group of patients over a longer period with suitable control groups would be important for clinicians managing such patients.

In a murine model of *H. pylori*-induced MALT lymphoma that faithfully reproduces almost all of the histological features of the human malignancy, Mueller et al (151). elucidated the gene expression profiles characteristic and predictive of the various histopathological stages of the disease. The study should pave the way for an improved understanding of the molecular mechanisms of MALT lymphomagenesis in humans.

11. Virulence factors of *H. pylori*

Several possible disease-specific virulence factors have been postulated associated with *H. pylori* infection. The true virulence factor must pass the tests of biological feasibility and data regarding the disease associated factors must be experimentally and epidemiologically consistent. Covacci et al.(152) defined the possible virulence factors into colonization factors and disease associated factors as summarized in Table 4.

Table 4. *H. pylori* virulence factor in gastroduodenal pathogenesis.

Colonization factor	Disease-associated factor
Urease	*cag* PAI
Flagelins	*cagA*
Motility	*vacA*
Catalase	*babA*
Adhesins	*iceA*
Internalization	

Colonization factors such as urease, motility, and adhesins are particularly important because they are potential targets for anti-*H. pylori* therapy and also help us understand the bacterial interaction with the host and gastroduodenal mucosa.

11.1 Urease

H. pylori urease is important in the regulation of the microenvironment immediately surrounding it and for optimal survival of the micro-organism in the gastric mucosa. The role of urease may not be the sole factor in protecting the organism from acid. Urease converts urea, of which there is an abundant supply in stomach (from saliva and gastric juices), into bicarbonate and ammonia which are strong bases. This creates a could of acid neutralizing chemicals around the *H. pylori*. Recent data, however, have shown that clinical urease-negative *H. pylori* isolates can colonize and infect animal hosts (153), emphasizing other factors except urease, may be essential for initial and chronic infection.

11.2 *cagA* gene

The cytotoxin-associated gene A (*cagA* gene) located on the right end of *cag* PAI serves as the marker for the pathogenic island (Figure 1). The *cag* PAI is an approximately 40 kb genomic region that contains 25 to 30 genes important to increase inflammation and for secretion of virulence-associated gene products. The express *cagA* gene product, CagA, is highly immunogenic.

Most *H. pylori* strains contain the *cag* PAI, a region with a different GC content to the rest of the *H. pylori* genome, which suggests that it was originally acquired by horizontal transmission from another species. Carriage of the *cag* PAI is associated with the induction of a more severe inflammatory response and, in Western populations, with disease states (peptic ulcer disease and gastric cancer). The relationship between *cag* carriage and disease is much less clear-cut in the devloping world, where almost all strains are *cag*-positive(154).

The *cag* PAI comprises approximately 30 open reading frames, including several that form a type IV secretory apparatus capable of transferring bacterial products directly into gastric epithelial cells. Subsequently, the modulation of epithelial cell signal transduction pathways results in the activation of inflammatory cascades and other events of potential importance in pathogenesis. Many studies have shown that the CagA protein is associated with more severe clinical diseases. Infection with *H. pylori* strains possessing the high density of *cagA* gene could cause increased gastric inflammation by inducing interleukin 8 formation (132) and may be related to the greater risk of duodenal ulcer and gastric cancer (155-157).

11.2.1 Biological activity of CagA

One bacterial protein transferred to epithelial cells is the immunodominant CagA protein, which is then tyrosine phosphorylated by intracellular Src homology 2-containing tyrosine phosphatase (SHP2). This anchors SHP2 to the plasma membrance and activates downstream pathways (158). The tyrosine phosphorylation of CagA by Src family tyrosine kinases is itself downregulated by phosphorylated CagA (159), leading through cortactin dephosphorylation to action rearrangement and crosslinking, resulting in a 'spreading' morphology in the AGS gastric epithelial cell line. This change is believed to be important for neoplastic transformation. The transfection of tyrosine phosphorylated CagA inhibits Src activation (159), but the pathway may be indirect, through stimulating C-terminal Src kinase activity and inactivating Src family protein tyrosine kinases (160). Furthermore, the ectopic over-expression of CagA induces apoptosis through the activation of SHP2 (160). CagA may therefore stimulate both oncogenic and apoptotic pathways simultaneously. CagA may also activate signal transduction without being tyrosine phosphorylated. CagA derivatives resistant to tyrosine phosphorylation can also increase cell proliferation by interacting with Grb2 and the Ras/MEK/ERK MAP kinase pathway (161) involved in the induction of abnormal proliferation and movement of gastric epithelial cell, a cellular condition eventually leading to gastritis and gastric carcinoma.

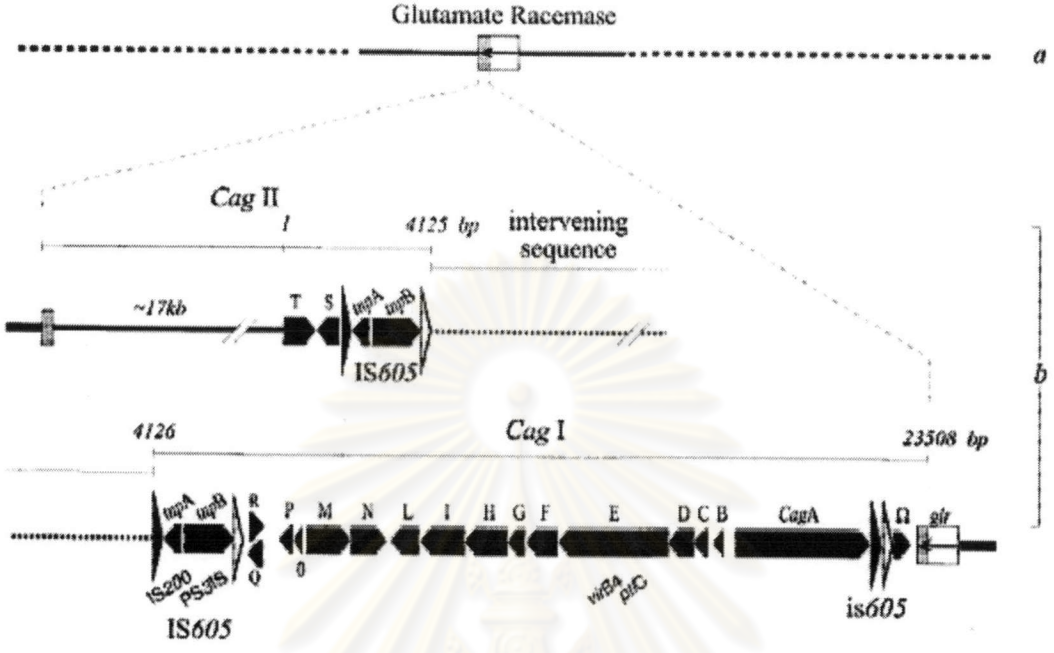

Figure 1. Schematic representation of the *cag* region (25). The *cag* integration site within the glutamate racemase gene is shaded (a). *cag* structure : the putative ORFs are represented by arrow (b).

11.3 *vacA* gene

All *H.pylori* strains contain a copy of the toxin gene, *vacA*. The *vacA* gene of *H. pylori* encodes a multimeric exotoxin producing endosomal vacuoles in epithelial cells. *H. pylori* strains expressing the high activity forms of VacA are associated with an increased risk of ulcer disease. This had been thought to be due to the ability of VacA to bind to gastric epithelial cell receptors and, after internalization, form endocytic vacuoles and cell death. However, mice deficient in protein tyrosine phosphartase receptor type Z incorporate VacA and demonstrate vacuolization but incorporate VacA and demonstrate vacuolization but do not develop ulcers (162). VacA may therefore act as a ligand for protein tyrosine phosphatase receptor type Z, although other groups have proposed other proteins and structures including lipid rafts (163) to be the elusive VacA receptor.

11.3.1 Biological activity of VacA

VacA is predicted to encode a protoxin with a mass of about 140 kDa, but the mature secreted VacA toxin migrates as a band of approximately 90 kDa (42). A comparison of the amino-terminal sequence of the mature secreted toxin with that predicted for the protoxin indicates that a 33-amino-acid amino-terminal signal sequence is cleaved during the process of VacA secretion (Figure 2). VacA causes epithelial cell vacuolation *in vitro*, for primary human gastric epithelial cells exposed to high does of toxin that occur cell death (164). VacA is a secreted protein toxin that is responsible for the gastric epithelial erosion observed in infected hosts. It causes vacuolar degeneration of target cells by interfering with intracellular membrance fusion. The vacuoles appear to be a hybrid between lysosomal and late endosome compartment, and the generation requires the vacuolar adenosine triphospate-dependent proton pump and the small guanosine triphosphate-binding protein Rab7(165). There are two alleles (m1 and m2) of a 300 - amino acid region containing the cells binding domain of VacA, which have different target cells specificities. Only the m1 form is toxic on Hela cells in the standard assay of vacuolization (42, 166). However, both of them are active on primary gastric cells. It is not clear why this functional polymorphism has evolved, but it may reflect

human genetic polymorphism, because the m1 form is predominant in western, Korean and Japanese isolates, whereas the m2 form is found in 75 % of Chinese isolates (35, 167, 168).

Vacuolating cytotoxin gene, *vacA*

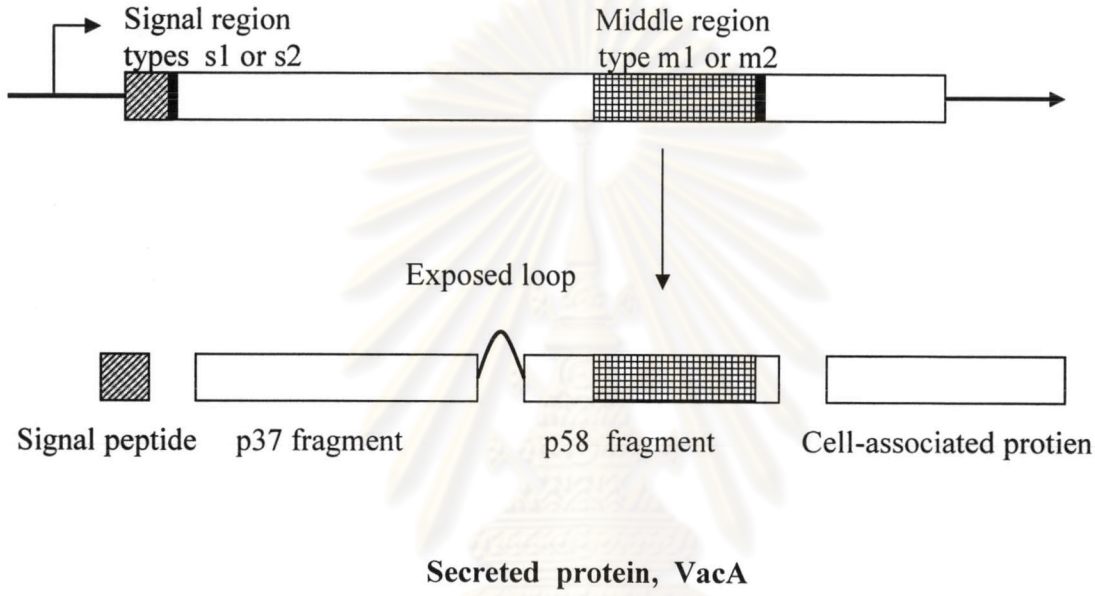

Figure 2. Schematic representation of the vacuolating cytotoxin gene, *vacA* and protein, VacA (42).

11.4 *iceA* gene

iceA is a gene that is induced by contact with the epithelium. The *iceA* gene encodes a protein of 178 amino acids with a MW of 20.6 kDa. To investigate *iceA* diversity, the primers for *iceA* were used to amplify genomic DNA form the gastritis strain J178. The longest possible ORF in this region of J178 strain predicted a protein of 59 amino acids, and the sequence in the J178 strain was designated *iceA2*. The published genome sequence of *H. pylori* strain 26695 was shown to contain an *iceA1*, but not *iceA2* (64). Recently, a novel *H. pylori* gene *iceA* was identified following transcriptional upregulation on contact with gastric epithelial cells (45). *iceA* exists as two distinct genotypes, *iceA1* and *iceA2* (Figure 3), and only *iceA1* RNA is induced following adherence *in vitro* (45). *H. pylori iceA1* demonstrated strong homology to a restriction endonuclease *nla*IIIR in *Neisseria lactamica* (169), and *in vivo* carriage of *H. pylori iceA1* strains has been reported to be associated with peptic ulceration and enhanced acute neutrophilic in filtration (170). However, linkage between the *iceA* genoype and ulcer disease is not universal (35), and thus maybe population dependent. In contrast, with *iceA1* and *iceA2* has no significant homology to known and its structure reveals patterns of repeated protein cassettes. Recently, the genetic organization and sequence heterogeneity of *iceA2* has been studied (169) revealing five distinct *iceA2* subtype. While *iceA*2 strains are more prevalent among patients with gastritis and non-ulcer dyspepsia (171). Interestingly, *iceA1* positive strains were significantly associated with peptic ulceration and increased mucosal concentrations of IL-8. Similary, van Doorn et al.(44) reported that the *iceA* allelic type was independent of the *cagA* and *vacA* status, and there was significant association between the presence of the *iceA1* allele and peptic ulcer disease. In contrast,Yamaoka et al. (35), demonstrated that no significant association between *iceA1* and clinical outcome including gastritis, peptic ulcer diseases and gastric cancer. The hypothesis that *iceA* is associated with gastroduodenal disease has been subsequently tested in many studies.

***iceA* gene structure**

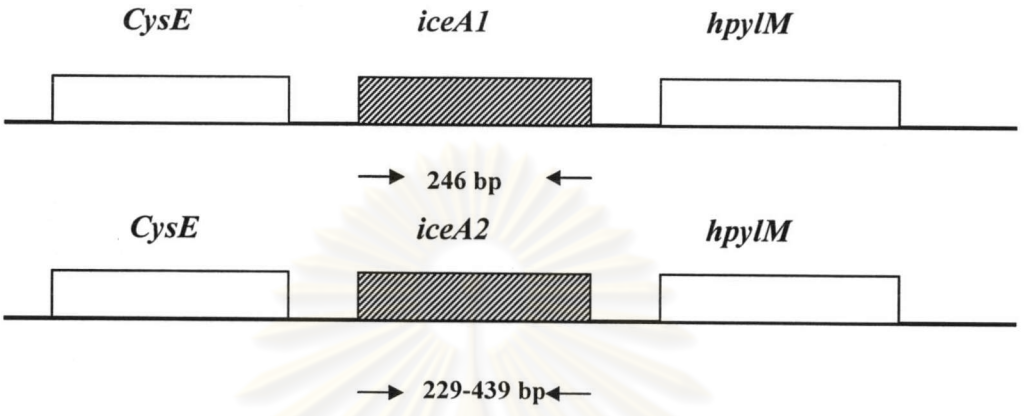

Figure 3. Schematic representation of *iceA* genes (46)

12. The Association between genes *cagA*, *vacA* and *iceA* in *H. pylori* and clinical outcome

During the past decade, cumulative evidence supports virulence genes associated with clinical outcome. However, the results are still controversial and varied between each ethnic group.

12.1 *cagA* gene

The *cagA* gene located at the right end of cag pathogenicity island that contains approximately 30 genes, encodes the CagA protein which varies in molecular mass between 120 and 140 kDa (22, 23). The variation of *cagA* gene was attributed to the pressence of a variable number of repeated sequences in the 3' region of the gene (22, 23, 172, 173). Previous reported that the sequence of the 3' region of the *cagA* gene form isolate in Western countries demonstrated *cagA* type 2 a and in East Asian countries demonstrated *cagA* type 1 a (23, 174).

The presence of gene *cagA* have been reported to be associated with clinical outcome mainly in Western countries. In Netherlands, van Doorn et al. demonstrated that *cagA* was strongly associated with peptic ulcer disease. The *cagA* gene was present in 63(67 %) of 94 cases ($P = 0.0019$) (44). In UK, Warburton JV et al. demonstrated the prevalence of *cagA* positive in duodenal ulcer patients (94 %) was highly significantly greater than in those with non-ulcer dyspepsia (56 %) (175). As in other reports, Stephens JC et al. the presence of the *cagA* gene was significantly associated with peptic ulcer diseases ($P = 0.006$) (176). Moreover some experiment demonstrated the importance of gene *cagA* positive are associated with DU prevalence (177). On the other hand, reports from other geographical regions like Asian and some Latin American countries have found no association between the presence of *cagA* and clinical outcome of *H. pylori* infection (26, 35, 154, 178, 179).

Rencenly reported in Turkish population suggested that there is a significantly positive association between *cagA* gene and peptic ulcer disease (29).

12.2 *vacA* gene

In 1995s to 2004 many authors reported the *vacA* gene. The *vacA* gene which encodes the vacuolating cytotoxin is the major toxin secreted by *H. pylori* that induces vacuolation in the human epithelial cell in *vitro* (38, 39, 180). Previous studies permitted a comprehensive description of the *vacA* signal (s) and middle (m) regions, which exist as s1 or s2 and m1 or m2 respectively (38). In Western countries, the presence of *vacA*s1 reported to be significantly associated with peptic ulcer disease (43, 181, 182), whereas such association has not been reported in Asian countries (35, 168).

The *vacA* s1 region was subtyped into s1a, s1b and s1c; the m1 region was subtyped into m1a, m1b; m2 region was subtyped into m2a and m2b (43, 168, 183, 184). It has been show that *vacA* type s1/m1 strain produce more cytotoxins than type s1/m2 and that type s2/m2 strains do not produce active cytotoxins (180). Many studies have confirmed these finding (22, 176, 185). The prevalence of the different *vacA* alleles has been reported in *H. pylori* isolates from different regions. A high prevalence of *vacA* s1 allele and its uniform distribution among the various diseases has been observed in Asian countries (167).

Many studies demonstrated that the *vacA* s1 genotype was found predominant in patients with peptic ulcer or non-ulcer dyspepsia in Europe such as Portugal, UK, Netherland, Germany, France, Sweden(39, 44, 175, 186-188) and United States (43) and in Asia such as China, Japan, Taiwan(35, 168, 189). In addition, previous study of Atherton CJ et al, the results showed that specific *vacA* genotypes of *H. pylori* strains are associated with the level of in *vitro* cytotoxin activity as well as clinical consequences (38). In the Brazilian study from Minas Gerais reported in 1998, it was found a prevalence of 94% for the s1 *vac A* allele-harbouring *H. pylori* isolates in patients with DU much higher than the 54% found in patients with gastritis and thus an association of s1 allele with DU (190, 191). Recently,

previous studies in Brazil demonstrated that there was a strong association between the genotype *cagA*-positive *vacA*s1 and PUD. However, logistic regression anlysis showed that the *vacA*s1 the only predictive factor for PUD that support the hypothesis of virulent strains may protect against the development of gastroesophageal reflux disease(171).

In contrast, several reports revealed that there was no association between *vacA* status and clinical outcome. Studies in Chile suggested that the presence of allelic variant s1 *vacA* alone do not have a predictive value as a risk markers of severe gastric pathologies in the Chilean population (192), similar to previous studies in four different countries, Yamaoka et al. have found no relationship between the prevalence of *vacA* and clinical outcome (35). In addition, previous studies in Turkey demonstrated that the presence of *vacA* is not a predictive marker for peptic ulcer and NUD in our patients although *vacA* positivity in ulcer patients was higher than that in NUD group the difference was not statistically significant associated with PUD ($P>0.05$) (193). That supporting the results of previous studies in Korean patients that supports many reported the results showed that most *H. pylori* in Korea carry *vacA* gene, but *vacA* genes no correlate with peptic ulcer in the Korean patients (34).

12.2.1 *vacA* s region

Previous studies showed that the *vacA* s1a or s1b genotype were predominant in strains from Western countries, whereas *vacA* s1c is highly prevalence strains from East Asia (39, 182). A high prevalence of the s1a subtype observed in different regions in the world (38, 167), similar to previous studies in mainland China reported that subtype s1a exists in all strain (194). In contrast, previous studies in Central and South American countries demonstrated that a greater prevalence of subtype s1b (182, 191). The previous studies in patients living in Portugal and Netherlands demonstrated that the majority of Portuguese strain (72%) contained the s1b allele, whereas most of the Dutch strain (61%) contained type s1a. This highly significant difference is observed the NUD and the ulcer patients (186).

In spite of the efforts in the past decade to prove association of the infection by *vacA* genotype *H. pylori* strains and the development of a specific disease, the finding were not conclusive (27, 195).

12.2.2 *vacA* m region

Evidence for the presence of the *vacA* middle region may be still underestimated, especially in relation to the nucleotide sequence of *vacA* middle region. The data did not indicate any *vacA* m genotype as a significant virulence factor, as previously described (196). Interestingly, the previous studies demonstrated that the presence of *vacA* m1a and m2a genotype were predominant in strains from Western countries, m1c genotype was predominant in strain from South Asia and the m1b and m2b genotype were predominant in strains from East Asia (174, 182).

The relationship between *vacA* s1,m1 and peptic ulcer remain controversial even though this genotype has been correlated with clinical outcome (196). Recently, previous studies demonstrated that *vacA* s1, m1 genotype was more frequently in *H. pylori* from gastric cancer than from benign gastroduodenal disease and the frequently of *iceA1* and *cagA* did not differ among the group (197). In contrast, previous studies indicated that the *vacA* s1,m2 genotype was associated with duodenal ulcer, logistic regression analysis suggested that this genotype could be due to a higher prevalence of the *vacA* s1 allele among patients with duodenal ulcer (44).

Overall strains from Western countries predominantly possessed *cagA* type 2a; *vacA* s1a, s1b, or s2/m1a; or m2a genotype. Strain from South Asia predominantly possessed *cagA* type 2a and *vacA* s1a/m1c genotypes, whereas strains from East Asia predominantly possessed *cagA* type 1a, *vacA* s1c/m1b, or m2b genotype (174, 182).

12.5 *iceA* gene

The *iceA* contains two main allelic variants, *iceA1* and *iceA2* of which *iceA1* is associated with peptic ulcer disease (44, 45). Previous studies demonstrated that *iceA1* expression was found to be significantly related to the host mucosal response in United states and Holland (182, 198). However, the *iceA1* genotype was not associated with peptic ulceration in Japan, Korea, Columbia, the United States (Texas) and India (35, 184, 199). In addition, previous studies demonstrated that the results obtained with from Dutch, Japanese and Korean patients *iceA2* was the most frequently genotype detected in their population (35, 186, 199). Interestingly, previous studies in the United States (45) and the Netherlands (44) have demonstrated a strong association between *iceA* and peptic ulcer disease. Previous studies demonstrated that mixed *iceA* genotype had wide rage of prevalence 15% in Netherlands, 22% in Columbia and 40% in South Africa (35, 44, 46). It is possible that the high prevalence about 40% of mixed *iceA* strains in peptic ulcer disease patients may obscure any potential relationship between the allele and the disease. Furthermore, Kidd M et al (46) reported a strong relationship between the combination of *vacA* s region and *iceA* alleles.

According to previous reports, as shown in Table 5 represented the positive association between genes *cagA*, *vacA* and *iceA* and clinical outcome. However, some controversy results were also reported for example, reports from four different countries by Yamaoka et al.(35) show no significant association genes *cagA*, *vacA* and *iceA* between clinical outcomes.

Table 5. Positive association between genes *cagA*, *vacA* and *iceA* of *H. pylori* and clinical outcome

Author (Reference number)	Country	Specificity	*P* value
Atherton et al. (43)	USA	Significant association between *vacA* s1a and clinical outcome	$P<0.05$
van Doorn et al. (44)	Netherland	Significant association with genes *cagA,vacA* s1 and *iceA* that are more likely to lead to ulcer diseas	*cagA* , *vacA* s1 $P=10^{-5}$ *iceA* $P= 0.0042$
Pan et al. (168)	China	No association between any *vacA* allele and clinical outcome	$P>0.05$
Ito et al. (167)	Japan	No association between gene *vacA* and level of cytotoxin activity or clinical outcome	$P=1.0$
Wang et al. (189)	Taiwan	Significant association between gene *vacA* s1a/m1 and clinical outcome	$P<0.05$
Warburton et al. (175)	UK	Significant association between genes *cagA,vacA* s1 and clinical outcome	*cagA* $P<0.0001$ *vacA* s1 $P=0.049$
Celik et al.(187)	Sweden	No association between genes *vacA, cagA* and clinical outcome	$P>0.05$
Stephen et al.(176)	UK	Significant association between genes *cagA,vacA* s1 and clinical outcome	*cagA* $P=0.006$ *vacA* s1 $P=0.01$
Rudi et al. (39)	Germany	Significant association between genes *vacA s1* and peptic ulceration	$P<0.0001$
Han et al. (183)	Germany	Significant association between genes *cagA,vacA* s1,m1 and gastric cancer	*vacA* s1,m1 $P=0.005$ *cagA* $P=0.01$

Table 6. Positive association between genes *cagA*, *vacA* and *iceA* of *H. pylori* and clinical outcome

Author (Reference number)	Country	Specificity	*P* value
Yamaoka et al.(35)	four different	No association between genes *cagA*, *vacA* and *iceA* status and clinical outcome	*cagA*, *iceA* $P>0.6$ *vacA* $P>0.7$
Ashour et al. (199)	Brazil	No association between gene *iceA1* status and clinical outcome	$P> 0.05$
Miehlke et al.(197)	Germany	Only the *vacA* s1 ,m1 genotype is associated with gastric cancer	$P<0.05$
Ribeiro et al.(171)	Brazil	A strong association between the genotype *cagA* positive, *vacA* s1 and peptic ulcer disease	$P=0.001$
Bulent et al.(29)	Turkey	Association between gene *cagA* and peptic ulcer disease	$P<0.01$
Kidd et al. (46)	South Africa	Association with gene *iceA* and peptic ulcer disease. Combination analyses of *iceA* genotype and *vacA* alleles supported these associations	*iceA* $P<0.01$
Kim et al.(34)	Korea	No association between genes *cagA*, *vacA* and *iceA* status and peptic ulcer disease	$P>0.05$
Faundez et al.(192)	Chile	The combination *cagA* positive *vacA* s1,m1 associated with peptic ulcer disease	$P=0.035$
Saribasak et al.(193)	Turkey	The presence of the *cagA* gene was strongly associated with that of the *vacA* s1a genotype and clinical outcome	$P<0.0001$