COMPARATIVE STUDY OF AGE AND SEX AS RISK FACTORS OF *HELICOBACTER PYLORI* AMONG STUDENTS OF NASARAWA STATE UNIVERSITY, KEFFI

BY

SALISU SULEIMAN

NSU/PGD/MCB/0050/18/19

PGD MICROBIOLOGY

DECEMBER, 2019

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A PROJECT SUBMMITTED TO THE SCHOOLL OF POST GRADUATE STUDIES, NASARAWA STATE UNIVERSITY KEFFI, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF POST GRADUATE DIPLOMA IN MICROBIOLOGY

DEPARTMENT OF MICROBIOLOGY

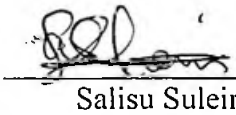
FACULTY OF NATURAL AND APPLIED SCIENCES

NASARAWA STATE UNIVERSITY, KEFFI - NIGERIA

DECEMBER, 2019

DECLARATION

I here declare that this project has been written by me and it is a report of my research work. It has not been presented in any previous application for degree. All quotations are indicated and sources of information specifically acknowledged by means of references.



Salisu Suleiman
NSU/PGD/MCB/0050/18/19

01-03-21
Date

CERTIFICATION

This is to certify that this project titled "Comparative Stu... ...nd Sex as Risk Factors of *Helicobacter pylori* among Students of Nasarawa State University, Keffi" meets the regulations governing the award of Postgraduate Diploma in Microbiology, of the School of Postgraduate Studies, Nasarawa State University, Keffi and is approved for its contribution to knowledge.

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DEDICATION

I dedicate this research project to my master, the key of all openings who emerged as a unique treasure of the truth and for that emerged as a divine distributor and the source of all things. Prophet Muhammad may the peace and blessings of Allah be upon him, his household and sublime companions.

ACKNOWLEDGEMENT

All thanks, glory and total submission are due to Allah who in his infinite mercy has seen me through my studies and has made this project a success. May his peace and mercy be upon his noble Prophet Muhammad (PBUH).

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And to my partner and friend Mr. Angba Amos, Muktar whom we work tirelessly to see that this project work becomes a success.

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ABSTRACT

A study to ascertain the age and gender determinants of *Helicobater Pylori* infection amongst students of Nasarawa State University Keffi was conducted. A total of eighty four (84) students were selected at random and blood samples were collected using the vene puncture technique and screened for the presence of *Helicobater Pylori* using Rapid Diagnostic test strip of the 84 samples examined 46 were positive which gives an overall prevalence of 54.8%. In relation to age He highest prevalence was regarded amongst those aged 21-40 years, while the lowest prevalence was recorded within the age bracket 41 years and above (40.0%). However, there is no statistically significant association with age at P=0.2522. In relation to gender determinant the males had the highest sero-positivity of the infection (59.4%) than the females with a point prevalence of 51.1%. Although there exist a statistically significant association for this parameter at P=0.0146. Consequently there is the need for the students to continuously monitor their status in order to effectively manage the disease, mean while, individuals with cases of Peptic Ulcer should desist from consuming spicy and sour foods as they have been shown to aggravate the condition.

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Helicobacter pylori infection is a global public health problem, affecting over 50% of the population worldwide including Africa and Asia; the two continents contributing two-third of the world's disease burden (Eshraghian, 2014).

Helicobacter pylori is motile, Gram-negative, microaerophilic bacteria. Barry Marshall and Robin Warren who isolated and identified *Helicobacter pylori* from the human stomach also drew attention to the association of *Helicobacter pylori*with gastritis; and much evidence showed that *Helicobacter pylori*causes peptic ulcers, and is also involved in the development of gastric mucosa-associated lymphoid tissue lymphoma (Morris, *et al*, Forman, *et al.*, 1990).

Infections are thought to occur early in life (during childhood) and the infection implicates several medical conditions including chronic gastritis, gastric cancer, gastric adenocarcinoma, mucosa-associated lymphoid tissue (MALT), lymphoma, and peptic ulcer disease (Plummer, *et al*, 2015). Infected individuals present with gastric reflux, abdominal pain, intestinal bleeding, occasional fevers, and loss of weight which if not treated can result in gastric ulceration and perforation (Bordin, *et al*, 2018). The incidence and prevalence rates of childhood infection with *Helicobacter pylori* vary greatly (Mohammed *et al*, 1996).

Infection with this bacterium has also been established as the etiologic factor in the development of gastric cancer, thus, *H. pylori* was designated a Class 1 carcinogen by the WHO (Alvarado-Esquivel, 2013).

Studies from many African countries reported similar prevalence rates of 91.7% in Egypt (El Dine, *et al,* 2008) 97% in Gambia (Seckae*t al.*, 2011) and 75.4% in Ghana (Baako and Darko ,1996)Similarly, in Asia, prevalence rates of 92% have been reported in Bangladesh (Ahmad *et al*, 1997) and 62% prevalence was found in Chinese (Shi *et al.,* 2008).

Studies from Nigeria by Bashir and Ali (Bashir and Ali, 2009) in Kano reported an *Helicobacter pylori* prevalence of 81%, Malu*et al.*(2000) in Jos, found a prevalence of 87%, while Aboderin*et al.,* (2007) reported 73% in South-West.

Screening, treatment, and prevention of *H. pylori* colonization can reduce the incidence of gastric cancer (Ferlay, *et al.,* 2008). Other interventions that may yield a similar effect, although of smaller magnitude, include promotion of a healthy lifestyle including dietary measures, nonsmoking, low alcohol intake, and sufficient physical activity. Furthermore, increasing evidence suggests that host factors, including genetic makeup, are also important determinants for carcinogenesis in *H. pylori* infection (Malaty, *et al.,* 1994).

Studies have shown that low socio-economy is a major risk factor in H. pylori acquisition (Tsai *et al.*, 2005). Allaker *et al.* (2002) noted that transmission of *H. pylori* is largely by the oral–oral or fecal–oral routes. This may have been encouraged by poor environmental sanitation, crowding, and fecal contamination of water source used for irrigation of vegetables in farms or domestic use. Jemikajah and Okogun (2014) and Etukudo *et al.* (2012) in separate studies showed that sourcing water from well and borehole confers a higher risk of H. pylori infection than pipe-borne water. Studies also by Bateson (1993) in Glasgow and Ogihara *et al.* (2000) in Jiangsu showed that there is increased prevalence of H. pylori in subjects who smoked cigarettes than in those who never smoked.

1.2 Statement of the Problem

Emotional stress and psychological factors are frequently identified as important contributors to PUD.(Peters M. N, 1983). Since the mid-20th century, stress has been considered as the main cause of PUD.(Alp M. H, et'al 1970).Several studies suggested that psychological stress may play a role in the onset and course of PUD.(Jones M. P. 2006). Studies have also shown that subjects with anxiety disorders (Rogers M. P, eta'al 1994) personality disorders.(Schuster eta'al, 2010). and panic disorders(Godwin R. D, et'al,2006). are more likely to have had PUD. Individuals with neuroticism.(Cox B. J, et'al, 2006) or with a history of childhood abuse.(Fuller-Thomson E, et'al 2011). are also more likely to have PUD. The detrimental effect of stress on PUD was shown in a Japanese study that demonstrated a rise in bleeding gastric ulcers in elderly people after a catastrophic earthquake in Japan.(Aoyama N, et'al 1998).

1.4 Justification

Studies have shown that low socio-economy is a major risk factor in H. pylori acquisition (Tsai *et al.*, 2005). Allaker*et al.* (2002) noted that transmission of *H. pylori* is largely by the oral–oral or fecal–oral routes. This may have been encouraged by poor environmental sanitation, crowding, and fecal contamination of water source used for irrigation of vegetables in farms or domestic use. Jemikajah and Okogun (2014) and Etukudo*et al.* (2012) in separate studies showed that sourcing water from well and borehole confers a higher risk of H. pylori infection than pipe-borne water. Studies also by Bateson (1993) in Glasgow and Ogihara*et al.* (2000) in Jiangsu showed that there is increased prevalence of H. pylori in subjects who smoked cigarettes than in those who never smoked.

1.5 Aims of the Study

Comparative study of age and sex as risk factors of *Helicobacter Pylori* among students of Nasarawa State University, Keffi

1.6 Objectives

The objectives of this study include:

i. To determine the presence of fecal occult blood in stool of peptic ulcer patient.

ii. To provide recommendation on the management of peptic ulcer patient thereby providing health advocacy

1.7 RESEARCH HYPOTHESIS

H_1: There is a strong association between the risk factors to be studied and increased prevalence of H. pylori infection

H_0: There is no strong association between the risk factors to be studied and increased prevalence of H. pylori infection

H_1: Decision rule: Accept H_1 if $P<0.05$ statistical level of significance otherwise reject.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Epidemiology of *Helicobacter pylori*

2.1.1 Prevalence and Geographical Distribution

The prevalence of H. pylori shows large geographical variations. In various developing countries, more than 80% of the population is *H. pylori* positive, even at young ages (Perez-Perez *et al.*, 2004). The prevalence of *H. pylori* in industrialized countries generally remains under 40% and is considerably lower in children and adolescents than in adults and elderly people (Pounder *et al.*, 1995). Within geographical areas, the prevalence of *H. pylori* inversely correlates with socioeconomic status, in particular in relation to living conditions during childhood (Malaty and Graham 1994.). In Western countries, the prevalence of this bacterium is often considerably higher among first- and second-generation immigrants from the developing world (Perez-Perez *et al.*, 2005) (Tsai *et al.*, 2005). While the prevalence of *H. pylori* infection in developing countries remains relatively constant, it is rapidly declining in the industrialized world (Genta, 2002). The latter is thought to be caused by the reduced chances of childhood infection due to improved hygiene and sanitation and the active elimination of carriership via antimicrobial treatment. In developing countries, *H. pylori* infection rates rise rapidly in the first years of life and remain constantly high thereafter, indicating that *H. pylori* is acquired early in childhood (Fiedorek *et al.*, 1991).

In Europe, the prevalence of *H. pylori* seems to be lower in Northern countries than in Southern and Eastern countries. In the Netherlands, a randomly selected sample of 1550 blood donors from four different regions was tested for the presence of antibodies against *H. pylori* and the

CagA antigen (van Blankenstein *et al.*, 2013). In Eusebi et al., (2014) study, only native Dutch subjects were evaluated excluding non-European immigrants. Eusebi et al., (2014) reported a 32% prevalence of *H. pylori* infection, with 28% of *H. pylori* positive subjects carrying a CagA-positive strain. The seroprevalence of H. pylori declined from 48% in subjects born between 1935 and 1946 to 16% in those born between 1977 and 1987, as a likely consequence of a birth cohort effect. Also the proportion of CagApositive subjects decreased from 38% to 14% in the same age cohorts. These data would suggest that a further reduction of *H. pylori* prevalence in the Netherlands over the coming decades could be expected. Additionally, from the Netherlands, a population based prospective study of a cohort of more than 6500 pregnant women was published (den Hollander *et al.*, 2013;).

Study found that the prevalence of *H. pylori* in Dutch women was 24%. The most important finding was that the prevalence of *H. pylori* was much higher in non-Dutch women with 64% of them being *H. pylori* seropositive. Moreover, in the latter group, infected subjects born abroad (first-generation immigrants) had a higher risk of *H. pylori* infection than second-generation immigrants. Thus, ethnicity was a strong predictor for *H. pylori* in this study. In contrast with northern European countries, a higher prevalence of *H. pylori* was reported in Portugal, where the prevalence of infection was 84.2%, with 61.7% of strains also positive for CagA (Bastos, *et al.*, 2013). A second evaluation, based on a proportion of included subjects, defined an incidence rate of infection of 3.6/100 person-years, confirming that Portuguese rates of *H. pylori* infection remain among the highest in Europe. Similar high values were reported in Eastern Europe. In Turkey, in a population-based cross-sectional survey, more than 4600 subjects were tested across the country, resulting in a weighted overall prevalence of infection of 82.5% (Ozaydin *et al.*, 2013). Interestingly, the prevalence was lowest among individuals living in the southern part of

the country who usually have a citrus fruit rich diet, as this is the major citrus fruit-growing area. Indeed, vitamin C is effective in the prevention of most infections; thus, the authors suggested that it might also play a role in *H. pylori* infection. In North America, the prevalence of *H. pylori* seems to be similar to northern Europe. Further evidence was provided by a Canadian study where the presence of *H. pylori* infection was evaluated in 203 aboriginal patients with dyspepsia referred for gastroscopy. *H. pylori* infection was reported by histology in 37.9% of patients (Sethi *et al.*, 2013). To the contrary, a study from Mexico (Alvarado-Esquivel, 2013) confirmed the previously reported (Porras, *et al.* 2013) high prevalence of *H. pylori* infection in Latin America. A seroprevalence of 52.2% was reported among 343 pregnant women living in rural areas in Mexico. In Asia, the studies published over the last year showed high prevalence rates of *H. pylori* infection ranging from 54% to 76% (Lim , *et al.*, 2013; Zhu *et al.*, 2014; Sodhi, *et al.*, 2013; Adlekha *et al.*, 2013; Benberin *et al.*, 2013;Vilaichone *et al.*, 2013;Dorji *et al.*, 2014) Only one study carried out on healthy individuals in Saudi Arabia showed a low prevalence of infection of about 28% (Hanafi and Mohamed 2013).

In Korea, in a large cross-sectional nationwide multicenter study, more than 10,000 asymptomatic subjects without a history of *H. pylori* eradication were enrolled (Lim, *et al.*, 2013). The seroprevalence of infection was 54.4%. However, this estimate was lower than that reported in the same country by two similar surveys performed in 1998 (Kim *et al.* 2001) and 2005 (Yim, *et al.*, 2007), where the prevalence of *H. pylori* was 66.9% and 59.6%, respectively. This decrease was significant across all age groups and in most areas of the country. In China, a survey of *H. pylori* infection was carried out on a sample of the general population from areas with high incidence of gastric cancer (Zhu *et al.*, 2014). A total of 5417 healthy individuals aged between 30 and 69 years were tested with the 13C-urea breath test. The prevalence of *H. pylori*

underwent gastroscopy with gastric biopsies, were evaluated for the presence of *H. pylori* infection (Yu *et al.*, 2014). The histologic examination of gastric biopsies showed a 32.1% prevalence of *H. pylori* infection. A higher rate of infection in children was reported in Iran, where Ghasemi-Kebria *et al.* found a seroprevalence of 50.5%, with 61.7% of children also positive for CagA (Ghasemi-Kebria *et al.*, 2013).

2.1.2 Risk Factors for *Helicobacter pylori* Infection

Several studies investigated putative risk factors for *H. pylori* infection. Gender and age do not seem to be associated with an increased risk of infection. Indeed, most studies reported no significant difference of *H. pylori* infection between men and women, both in adults (van Blankenstein *et al.*, 2013; Adlekha *et al.*, 2013; Vilaichone *et al.*, 2013;Hanafi and Mohamed 2013; Benajah *et al.*, 2013;Mathewos *et al.*, 2013) and in children (Mana *et al.*, 2013;Bastos *et al.*, 2013;Ghasemi-Kebria *et al.*, 2013). No significant association was found between infection and age in the adult population (den Hollander *et al.*, 2013;Alvarado-Esquivel 2013;Adlekha *et al.*, 2013; Benberin *et al.*, 2013;Dorji *et al.*, 2014;Hanafi and Mohamed 2013;Mana et al., 2013). The age-specific gradient in *H. pylori* prevalence reported by some studies seems to be related to a birth cohort effect (van Blankenstein *et al.*, 2013, Lim, *et al.*, 2013; Benajah *et al.*, 2013, Mathewos *et al.*, 2013; Yu *et al.*, 2014).

Several socioeconomic factors have been associated with *H. pylori* infection. In particular, subjects with a low socioeconomic status (den Hollander *et al.*, 2013;Hanafi and Mohamed 2013), measured also as a low family income (Lim *et al.*, 2013; Zhu *et al.*, 2014), had a higher likelihood of carrying *H. pylori* infection. Furthermore, an inverse association between educational level and *H. pylori* infection was found in the majority of the studies (den Hollander *et al.*, 2013;Ozaydin *et al.*, 2013;Benajah *et al.*, 2013); indeed, except for two cases (Alvarado-

Esquivel, 2013; Zhu *et al.*, 2014;), individuals with lower educational levels had a higher risk than those with a higher education. The same association concerning the parents' education was also found in studies on children (Mana *et al.*, 2013; Bastos *et al.*, 2013).

Moreover, several factors related to residence have been found to be associated with the infection. Indeed, living in a rural area (Lim *et al.*, 2013;Vilaichone *et al.*, 2013;Hanafi and Mohamed, 2013), in crowded homes (Dorji *et al.*, 2014;Hanafi and Mohamed, 2013; Bastos *et al.*, 2013), and having contaminated sources of drinking water (Ozaydin *et al.*, 2013)were risk factors for *H. pylori* infection. Among the main lifestyle habits, smoking and alcohol consumption showed discordant results: Although in most studies, there was no significant association with *H. pylori* infection (den Hollander *et al.*, 2013; Zhu *et al.*, 2014;Benajah *et al.*, 2013), some authors reported that regular smokers (Ozaydin *et al.*, 2013; Hanafi and Mohamed, 2013) and drinkers (Hanafi and Mohamed, 2013) were at higher risk. In contrast, in one study, regular alcohol drinking was a protective factor for *H. pylori* infection (Ozaydin *et al.*, 2013).

Table 1: Prevalence of *Helicobacter pylori* infection in adults reported by studies published in 2013

Country	Setting	Number	Diagnostic method	Prevalence of *Helicobacter pylori* (%)
Western Europe				
The Netherlands	Blood donors	1550	Serology	31.7
The Netherlands	Pregnant women	6837	Serology	46
Portugal	General population	2067	Serology	84.2
Eastern Europe				
Cyprus	Patients with dyspepsia	103	PCR	39.8
Turkey	General population	4622	UBT	82.5
America				
Canada	Aboriginal population	203	Histology	37.9
Mexico	Pregnant women	343	Serology	52.2
Asia				
Saudi Arabia	Healthy individuals	456	Serology	28.3
Korea	Routine health check-up	10796	Serology	54.4
India	Patients with dyspepsia	2000	Histology RUT	58
India	Patients with dyspepsia	530	Histology Urease test	62
China	Healthy individuals	5417	UBT	63.4
Bhutan	Volunteers	372	Histology RUT Culture Serology	73.4
Bhutan	Patients with dyspepsia	241	Serology	86
Kazakhstan	Asymptomatic and patients with dyspepsia	835	Serology	76.5
Africa				
Ethiopia	Selected population	1388	Serology	65.7
Morocco	Patients with dyspepsia	429	Histology RUT Culture	75.5
Nigeria	Patients with dyspepsia	125	Serology Histology	93.6 80

UBT, urea breath test; RUT, rapid urease test.

Table 2 Prevalence of *Helicobacter pylori* infection in children reported by studies published in 2013

Country	Age of included subjects (years)	Number	Diagnostic method	Prevalence of *Helicobacter pylori* (%)
Western Europe				
Belgium	12–25	516	UBT	11
Portugal	13	1312	Serology	66.2
America				
Brazil	2–19	129	Histology RUT Culture	41.1
Asia				
China	1–18	1634	Histology RUT	32.1
Iran	1–15	194	Serology	50.5

UBT, urea breath test; RUT, rapid urease test.

Source : Special Issue: The Year in Helicobacter 2014, Volume19, Issues1.

Epidemiology of *Helicobacter pylori* Infection

2.1.3 Transmission

There appears to be no substantial reservoir of *H. pylori* aside from the human stomach. Other animals harbor organisms that resemble *H. pylori*, but with the exception of nonhuman primates (Dubois *et al.*,1994) and, under particular circumstances, perhaps cats (Fox *et al.*, 1995), none harbor *H. pylori*. Thus, the major question of transmission is how *H. pylori* travel from the stomach of one person to that of another. Three routes have been described. The first and least common is iatrogenic, in which tubes, endoscopes, or specimens in contact with the gastric mucosa from one person are introduced to another person (Akamatsu *et al.,* 1996). Improved disinfection of endoscopes has reduced the incidence of transmission (Katoh *et al.*, 1993) (Tytgat, 1995). Interestingly, endoscopists, especially those who did not wear gloves during procedures, were at increased risk of becoming infected (Mitchell *et al.*, 1989) Occupationally acquired infections also have been reported (Sobala *et al.*, 1991); although there does not appear to be any special risk associated with handling this organism, laboratory workers should use universal precautions when handling clinical specimens and should remember that *H. pylori* strains are human pathogens. Fecal-oral transmission is perhaps most important. Although *H. pylori* has been isolated from the feces of young children infected with the organism (Thomas *et al.*, 1992), fecal isolation is not common; this could indicate that shedding is intermittent. Fecally contaminated water may be a source of infection (Klien *et al.*, 1991), but the organism has not been isolated from water. Food-borne transmission has not been substantiated.

2.2 MICROBIOLOGICAL CHARACTERISTICS

2.2.1 Morphology

Helicobacter pylori is a helix-shaped (classified as a curved rod, not spirochaete) Gram-negative bacterium about 3 μm long with a diameter of about 0.5μm. H. pylori can be demonstrated in tissue by Gram stain, Giemsa stain, haematoxylin–eosin stain, Warthin–Starry silver stain, acridine orange stain, and phase-contrast microscopy. It is capable of forming biofilms (Stark *et al.*, 1999) and can convert from spiral to a possibly viable but nonculturable coccoid form. (Chan *et al.*, 1994). Helicobacter pylori has four to six flagella at the same location; all gastric and enterohepatic Helicobacter species are highly motile owing to flagella. (Josenhans *et al.*, 2000) The characteristic sheathed flagellar filaments of Helicobacter are composed of two copolymerized flagellins, FlaA and FlaB (Rust *et al.*, 2008).

2.2.2 Genome, plasmids, and strain diversity.

The size of the two sequenced *H. pylori* genomes is approximately 1.7 Mbp, with a GC content of 35 to 40%. The *H. pylori* strain 26695 genome includes 1,587 genes, whereas the genome of strain J99 includes only 1,491 genes (Boneca *et al.*, 2003). Both genomes contain two copies of the 16S, 23S, and 5S rRNA genes. Many strains carry one or more cryptic plasmids, which do not seem to carry antibiotic resistance genes or virulence genes (Heuermann and Haas, 1995). The existence of *H. pylori*-infecting bacteriophages has been reported, but detailed characterization is lacking (Schmid *et al.*, 1990). In contrast to other bacterial pathogens that are highly clonal (such as Shigella dysenteriae and Mycobacterium tuberculosis), H. pylori is genetically heterogeneous, suggesting a lack of clonality. This results in every *H. pylori*-positive subject carrying a distinct strain (Kansau *et al.*, 1996), although differences within relatives may

be small. The genetic heterogeneity is possibly an adaptation of *H. pylori* to the gastric conditions of its host, as well as to the distinct patterns of the host-mediated immune response to H. pylori infection (Kuipers, *et al.*, 2000). Genetic heterogeneity is thought to occur via several methods of DNA rearrangement and the introduction and deletion of foreign sequences (Achtman and Suerbaum 2000; Falush *et al.*, 2003; Suerbaum and Achtman 2004). The latter usually have an aberrant GC content and often carry genes involved in virulence. A striking example of this in *H. pylori* is the cag PAI, but other plasticity regions have also been suggested to play a role in the pathogenesis of *H. pylori* infection (Jonge *et al.*, 2004; Lehours, *et al.*, 2004; Occhialini *et al.*, 2000; Santos *et al.*, 2003). Diversity is also seen at the nucleotide level via several mechanisms, including transcriptional and translational phase variation and mutation (Achtman and Suerbaum 2000; Falush *et al.*, 2001). Phase variation often occurs via reversible slipped-strand mispairing in homopolymeric G or C tracts, which causes a shift in translation of the affected gene, thus resulting in phase variation via a single mutation. This leads to a reversible phenotypic diversity with only minor genetic variation. Several virulence genes, such as the sabA, sabB, hopZ, and oipA outer membrane protein-encoding genes, display such phenotypic variation, as do lipopolysaccharide (LPS) biosynthetic enzymes (Mahdavi *et al.*, 2002).

2.3 Virulence Factor of *Helicobacter pylori*

Although infection with *H. pylori* almost always results in chronic active gastritis, most infected patients develop no other complications and are free of any obvious clinical symptoms of this infection (Blaser and Atherton , 2004.). This led to the notion that some strains may be more virulent than others. Early investigations of the differential pathogenic properties of H. pylori strains indicated that this increased pathogenicity correlated with the ability of these more

virulent strains to induce morphological changes, vacuolization, and successive degeneration of in vitro-cultured cells (Leunk, *et al.*, 1988). This activity was then linked to the presence of a protein with a molecular mass of approximately 140 kDa that was named CagA (for "cytotoxin associated gene A").

2.3.1 *Cag*PAI

*Cag*PAI is a 40kb region of chromosomal DNA encoding approximately 31 genes that forms a type IV secretion system and can be divided into two regions, cag I and cag II, according to a novel insertion sequence (Censini *et al.*, 1996). This secretion system forms a pilus that delivers *Cag*A, an oncoprotein, into the cytosol of gastric epithelial cells through a rigid needle structure covered by CagY, a VirB10-homologous protein, and CagT, a VirB7-homologous protein, at the base (Covacci *et al.*, 2000; Rohde *et al.*, 2003; Backert and Selbach, 2008).

Upon delivery into host cells by the cag secretion system, the product of the terminal gene in the island, CagA, undergoes Src-dependent tyrosine phosphorylation and activates an eukaryotic phosphatase (SHP-2), leading to dephosphorylation of host cell proteins and cellular morphological changes (Odenbreit *et al.*, 2000; Higashi *et al.*, 2002). CagA has also been shown to dysregulate b-catenin signaling (Franco *et al.*, 2005; Murata-Kamiya *et al.*, 2007) and apical–junctional complexes, (Amieva, 2003) events that have been linked to increased cell motility and oncogenic transformation in a variety of models (Suzuki *et al.*, 2005; Franco *et al.*, 2008).In addition, some studies have reported that *cag*PAI appears to be involved in the induction of gastric interleukin-8 (IL-8) production, a potent neutrophil-activating chemokine (Brandt *et al.*, 2005).

Consequently, the presence of *cag*A gene has been associated with higher grades of inflammation, which may lead to the development of the most severe gastrointestinal diseases, such as peptic ulcer disease (Figueiredo *et al.*, 2001) and GC (Wang, *et al.* 2007; Roesler et *al.*, 2011).

In Western countries, it has been reported that individuals infected with *cag*A-positive strains of *H. pylori* are at a higher risk of peptic ulcer disease or GC than those infected with *cag*A-negative strains (Yamaoka 2008; van Doorn , *et al.*, 1998).

However, in East Asia, most strains of *H. pylori* have the *cag*A gene irrespective of the disease (Yamaoka *et al.*, 1999).

Furthermore, cagA is a polymorphic gene that presents different numbers of repeated sequences located in its 3' region. Each repeated region of CagA protein contains Glu-Pro-IleTyr-Ala (EPIYA) motifs, including a tyrosine phosphorylation site (Hatakeyama, 2004).

According to the sequences flanking the EPIYA motifs, four distinct EPIYA segments, EPIYA-A, EPIYA-B, EPIYA-C, and EPIYA-D, each of which contains a single EPIYA motif, have been identified in the EPIYA-repeat region. The EPIYA-repeat region of *Cag*A from Western *H. pylori* isolates is in arrangement of EPIYA-A, EPIYA-B, and EPIYA-C segments (A–B–C-type *Cag*A). The EPIYA-C segment variably multiplies (mostly one to three times) in tandem among different Western *Cag*A species. *Cag*A from East Asian *H. pylori* isolates also possesses EPIYA-A and EPIYA-B segments, but not the repeatable EPIYA-C segment. Instead, it has a distinct EPIYA-containing segment (it is the EPIYA-D segment), which is unique to East Asian *Cag*A. Accordingly, the EPIYA-repeat region of East Asian *Cag*A is in an arrangement of EPIYA-A, EPIYA-B, and EPIYA-D segments (A–B–D-type *Cag*A) (Higashi, *et al.*, 2002;

Hatakeyama, 2009).Analysis using a series of EPIYA mutants of CagA revealed that SHP-2 specifically binds to the tyrosinephosphorylated EPIYA-C or EPIYA-D segment. The sequence flanking the tyrosine phosphorylation site of EPIYA-D segment perfectly matches the consensus high affinity binding sequence for the SH2 domains of SHP-2, whereas that flanking the tyrosine phosphorylation site of the EPIYA-C segment differs from the consensus sequence by a single amino acid at the pY+5 position. As a result, East Asian CagA, which contains the EPIYA-D segment, exhibits stronger SHP-2 binding than does Western CagA, which contains the EPIYA-C segment. Within Western CagA species, those having a greater number of EPIYA-C segments exhibit stronger activity to interact with SHP-2 and are more closely associated with precancerous lesions and GC (Higashi, *et al* 2002; Hatakeyama, 2009).

As regard to the function of the repeated regions, initial demonstrations suggest that *H. pylori* strains that have a larger number of EPIYA segments in their regions are less resistant to gastric acid (Yamaoka *et al.*, 1999). This finding seems to indicate that *H. pylori* strains containing many EPIYA segments can survive only in the presence of advanced atrophic gastritis, in which gastric acid secretion is low (Yamaoka, 2011).

2.4 Pathophysiology of *Helicobacter pylori*

In order to avoid the acidic environment of the interior of the stomach (lumen), *H. pylori* uses its flagella to burrow into the mucus lining of the stomach to reach the epithelial cells underneath, where it is less acidic. (Amieva and El-Omar, 2008). *H. pylori* is able to sense the pH gradient in the mucus and move towards the less acidic region (chemotaxis). This also keeps the bacteria from being swept away into the lumen with the bacteria's mucus environment, which is constantly moving from its site of creation at the epithelium to its dissolution at the lumen interface. (Schreiber *et al.*, 2004). *H. pylori* is found in the mucus, on the inner surface of the

epithelium, and occasionally inside the epithelial cells themselves (Petersen and Krogfelt 2003). It adheres to the epithelial cells by producing adhesins, which bind to lipids and carbohydrates in the epithelial cell membrane. One such adhesin, BabA, binds to the Lewis b antigen displayed on the surface of stomach epithelial cells. (Ilver *et al.*, 1998) H. pylori adherence via BabA is acid sensitive and can be fully reversed by increased pH. It has been proposed that BabA's acid responsiveness enables adherence while also allowing an effective escape from unfavorable environment at pH that is harmful to the organism. (Bugaytsova *et al.*, 2017). Another such adhesin, SabA, binds to increased levels of sialylLewis x antigen expressed on gastric mucosa (Mahdavi *et al.*, 2002). "In addition to using chemotaxis to avoid areas of low pH, *H. pylori* also neutralizes the acid in its environment by producing large amounts of urease, which breaks down the urea present in the stomach to carbon dioxide and ammonia. These react with the strong acids in the environment to produce a neutralized area around *H. pylori* (Mobley, *et al.*, 2001). Urease knockout mutants are incapable of colonization. In fact, urease expression is not only required for establishing initial colonization but also for maintaining chronic infection(Debowski *et al.*, 2017).

2.5 Clinical Aspects of *H. pylori*-Associated Diseases

2.5.1 Association with Particular Diseases

All *H. pylori*-infected patients develop chronic gastric inflammation (Blaser, 1990), but this condition usually is asymptomatic. This gastritis is not a disease or illness per se. Peptic ulcer disease had been considered to be idiopathic or to be due to agents such as aspirin or nonsteroidal anti-inflammatory drugs or, rarely, to Zollinger-Ellison syndrome, Crohn's disease, and several other inflammatory disorders (Peterson, 1991). The idiopathic form of peptic ulcer disease represents 60 to 95% of all cases (depending on the extent of nonsteroidal anti-

inflammatory drug use in the population); we now know that *H. pylori* is the cause of nearly all of these cases in adults (NIH Consensus Conference, 1994) and that treatment that eradicates *H. pylori* leads to cure of the ulcers. Thus, in any population, *H. pylori* causes the majority of all cases of both gastric and duodenal ulcers. Carriage of *H. pylori* also is strongly associated with the risk of development of atrophic gastritis (Blaser, 1990), which is a precursor lesion to gastric cancer. Thus, not surprisingly, *H. pylori* carriage also is associated with adenocarcinoma of the distal but not the proximal (cardia) stomach (Talley *et al.*, 1991).Infection is associated with both the intestinal and diffuse histologic types of tumors. This association is extremely important since, in total, gastric cancer is the second leading cause of cancer death in the world (Neugut, 1996).

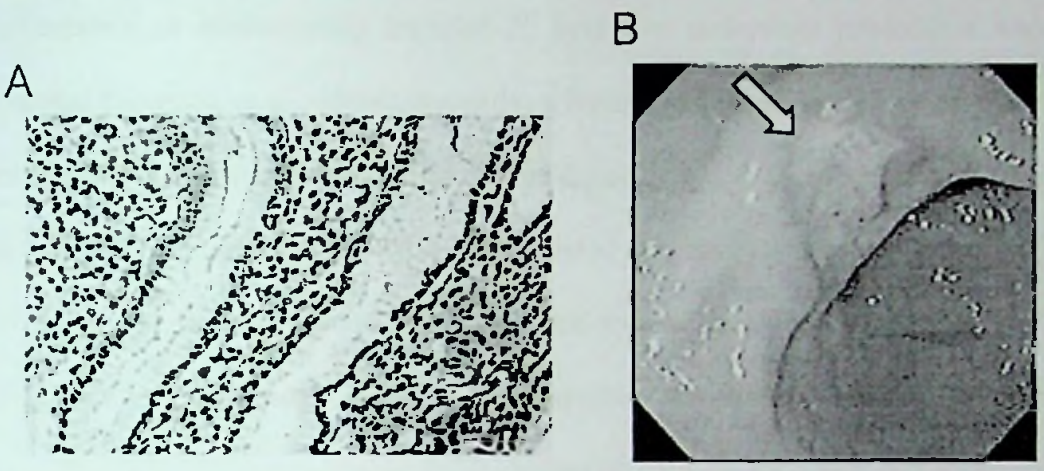
2.5.2 Disease Types

Although gastric colonization with *H. pylori* induces histologic gastritis in all infected individuals, only a minority develop any apparent clinical signs of this colonization. It is estimated that *H. pylori*-positive patients have a 10 to 20% lifetime risk of developing ulcer disease and a 1 to 2% risk of developing distal gastric cancer (Kuipers, 1998). The risk of development of these disorders in the presence of *H. pylori* infection depends on a variety of bacterial, host, and environmental factors that mostly relate to the pattern and severity of gastritis (*Fig. 2*).

2.5.3 Acute and chronic gastritis

Colonization with *H. pylori* virtually always leads to infiltration of the gastric mucosa in both antrum and corpus with neutrophilic and mononuclear cells (*Fig. 1A*). This chronic active

gastritis is the primary condition related to *H. pylori* colonization, and other H. pylori-associated disorders in particular result from this chronic inflammatory process.



(1A) Gastric glands abundantly colonized with Helicobacter pylori, shown as dark, curved bacilli closely aligning with the mucosal surface. (1B) Endoscopic view of a gastric ulcer, with a clean base at the angulus.

Source: Clinical Microbiology Reviews July, 2006 American Society for Microbiology

2.5.4 Acute Gastritis

Data on the acute phase of infection are scarce and largely come from reports of subjects who deliberately or inadvertently ingested *H. pylori* or underwent procedures with contaminated material (Solnick, *et al.*, 1999). Recently, a human challenge model for *H. pylori* infection was introduced; it allowed controlled studies of the acute phase of infection with deliberate infection of healthy volunteers with a well-characterized laboratory strain of *H. pylori* (Graham *et al.*, 2004). Together, these reports showed that the acute phase of colonization with *H. pylori* may be associated with transient nonspecific dyspeptic symptoms, such as fullness, nausea, and vomiting, and with considerable inflammation of both the proximal and distal stomach mucosa, and pangastritis. This phase is often associated with hypochlorhydria, which can last for months. It is unclear whether this initial colonization can be followed by spontaneous clearance and resolution of gastritis and, if so, how often this occurs. Follow-up studies of young children with serology or breath tests suggested that infection may spontaneously disappear in some patients in this age group (Granstrom, *et al.*, 1997; Malaty, *et al.*, 1999. Perez-Perez, *et al.*, 2003); this has not been observed in adults other than under specific circumstances, such as development of atrophic gastritis. However, studies of homozygotic twins showed a concordance in their *H. pylori* status irrespective of whether they had grown up together or apart (Malaty, *et al.*, 1994). Such a concordance was not observed among heterozygotic twins. This suggests that some individuals are prone to *H. pylori* colonization while others may be able to prevent colonization or clear an established infection. This hypothesis is also supported by the observation that in many developing countries the level of exposure to *H. pylori* is very high (i.e., 90%) at young ages and yet some individuals never develop persistent *H. pylori* infection.

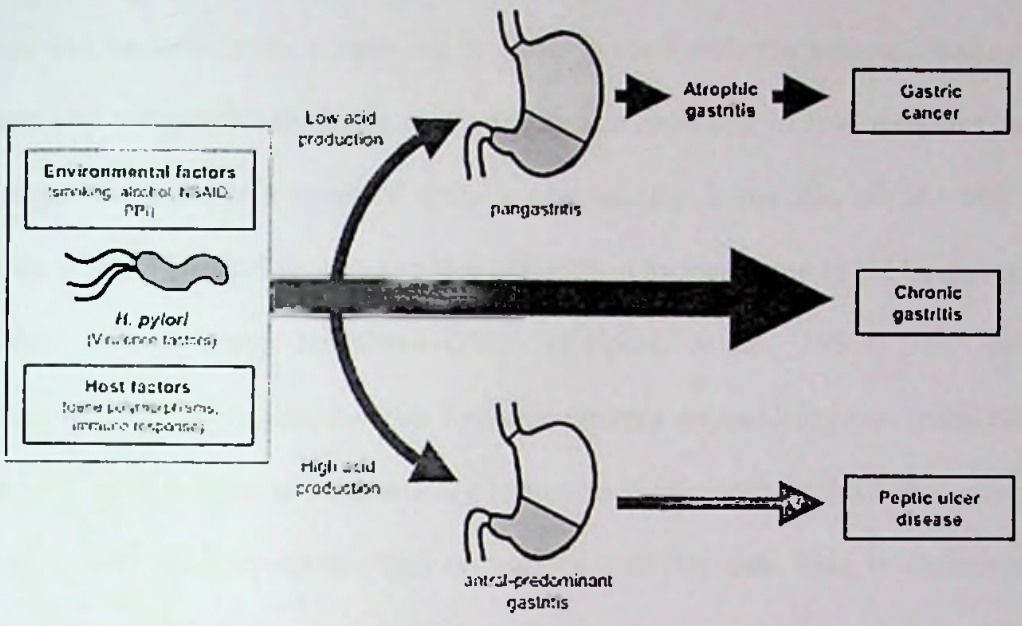


FIG. 2. Schematic representation of the factors contributing to gastric pathology and disease outcome in H. pylori infection.

Source: Clinical Microbiology Reviews July, 2006 American Society for Microbiology

2.5.5 Chronic gastritis

When colonization does become persistent, a close correlation exists between the level of acid secretion and the distribution of gastritis (*Fig. 3*). This correlation results from the counteractive effects of acid on bacterial growth versus those of bacterial growth and associated mucosal inflammation on acid secretion and regulation. This interaction is crucial in the determination of outcomes of *H. pylori* infection. In subjects with intact acid secretion, *H. pylori* in particular colonizes the gastric antrum, where few acid-secretory parietal cells are present. This colonization pattern is associated with an antrum-predominant gastritis. Histological evaluation of gastric corpus specimens in these cases reveals limited chronic inactive inflammation and low numbers of superficially colonizing *H. pylori* bacteria. Subjects in whom acid secretion is

impaired, due to whatever mechanism, have a more even distribution of bacteria in antrum and corpus, and bacteria in the corpus are in closer contact with the mucosa, leading to a corpus-predominant pangastritis (Kuipers, *et al.*, 1995). The reduction in acid secretion can be due to a loss of parietal cells as a result of atrophic gastritis, but it can also occur when acidsecretory capacity is intact but parietal cell function is inhibited by vagotomy or acid-suppressive drugs, in particular, proton pump inhibitors (PPIs) (Kuipers, *et al.*, 1995). The resulting active inflammation of the corpus mucosa further augments hypochlorhydria, paralleling the acute phase of infection, as local inflammatory factors such as cytokines, including interleukin-1 (IL-1), have a strong suppressive effect on parietal cell function. This is illustrated by various observations. Firstly, *H. pylori* corpus gastritis is often associated with hypochlorhydria, and eradication therapy leads to increased acid secretion in these subjects (El-Omar, *et al.*, 1997; Ruiz, *et al.*, 1996). Secondly, *H. pylori* corpus gastritis augments the acid-suppressive effects of PPIs (Verdu', *et al.*, 1995). As a result, *H. pylori* positive patients with gastroesophageal reflux disease (GERD) may respond somewhat faster to PPI treatment both with respect to symptom resolution and with healing of esophagitis (Holtmann, *et al.*, 1999), but this effect is minimal and largely irrelevant in daily clinical practice. This means that there is no general need to take H. pylori status into account when decisions on the dose of PPI treatment for GERD must be made. A third observation in support of the acid-suppressive effects of active corpus gastritis comes from more recent, important research showing that subjects with proinflammatory genotypes have a higher risk of corpus-predominant pangastritis, predisposing them to atrophic gastritis, intestinal metaplasia, and gastric cancer (El Omar, *et al.*, 2000). Although colonization with *H. pylori* is almost invariably associated with the presence of gastritis, and gastritis is mostly due to *H. pylori* colonization, other causes of gastritis include infections such as cytomegalovirus,

chronic idiopathic inflammatory and autoimmune disorders such as Crohn's disease and pernicious anemia, and chemical damage due to alcohol abuse or nonsteroidal anti-inflammatory drug (NSAID) use.

Pattern of gastritis	*Gastric histology*	*Duodenal histology*	*Acid secretion*	*Clinical condition*
 Pan-gastritis	• Chronic inflammation • Atrophy • Intestinal metaplasia	• Normal	• Reduced	• Gastric ulcer • Gastric cancer
 Antral-predominant	• Chronic inflammation • Polymorph activity	• Gastric metaplasia • Active chronic inflammation	• Increased	• Duodenal ulcer

FIG. 3. Acid secretion and the associated pattern of gastritis play an important role in disease outcome in H. pylori infection. The figure displays the correlations between the pattern of H. pylori colonization, inflammation, acid secretion, gastric and duodenal histology, and clinical outcome.

Source: Clinical Microbiology Reviews July, 2006 American Society for Microbiology

2.5.6 Peptic Ulcer Disease.

Gastric or duodenal ulcers (commonly referred to as peptic ulcers) are defined as mucosal defects with a diameter of at least 0.5 cm penetrating through the muscular is mucosa (Fig. 1B). Gastric ulcers mostly occur along the lesser curvature of the stomach, in particular, at the transition from corpus to antrum mucosa (Veldhuyzen *et al.*, 1999). Duodenal ulcers usually occur in the duodenal bulb, which is the area most exposed to gastric acid. In Western countries, duodenal ulcers are approximately fourfold more common than gastric ulcers; elsewhere, gastric ulcers are more common. Duodenal ulcers in particular occur between 20 and 50 years of age, while gastric ulcers predominantly arise in subjects over 40 years old.

2.5.7 Association with *H. pylori*

Both gastric and duodenal ulcer diseases are strongly related to *H. pylori*. In initial reports from all over the world in the first decade after the discovery of *H. pylori*, approximately 95% of duodenal ulcers and 85% of gastric ulcers occurred in the presence of *H. pylori* infection (Kuipers, *et al.*, 1995). Several cohort studies estimated that the lifetime risk for ulcer disease in *H. pylori*-positive subjects is 3 to 10 times higher than in *H. pylori*-negative subjects (Nomura, *et al.*, 1994) and that 10 to 15% of *H. pylori*-positive subjects developed ulcer disease during long-term follow-up (Cullen, *et al.*, 1993). These data came from studies in developed areas of the world. It is unknown whether H. pylori-positive subjects in developing countries have similar disease risks. Introduction of *H. pylori* eradication regimens completed the evidence for a causal relation between *H. pylori* and ulcer disease by showing that eradication of this bacterium strongly reduced the risk of recurrent ulcer disease (Rauws, *et al.*, 1990).This has had a major impact on the treatment and course of peptic ulcer disease in daily clinical practice. In earlier days, this disease was a chronic, recurrent disorder with high morbidity, frequently requiring

acid-suppressive maintenance therapy or surgery. Approximately 50% of patients with *H. pylori*-associated peptic ulcer disease suffered ulcer recurrence within 1 year (Rauws, *et al.*, 1990). Eradication of H. pylori dramatically changes the natural course of ulcer disease and almost completely prevents ulcer recurrence (268, Rauws, *et al.*, 1990; van der Hulst, *et al.*, 1997). Ulcer recurrences after H. pylori eradication therapy can be due to persistent or renewed H. pylori infection, use of NSAIDs, or idiopathic ulcer disease. Ulcer development in the presence of H. pylori is influenced by a variety of host and bacterial factors. Ulcers mostly occur at sites where mucosal inflammation is most severe (Veldhuyzen , *et al.*, 1999) (*Fig. 3*). In subjects with decreased acid output, this usually is the gastric transitional zone between corpus and antrum, giving rise to gastric ulcer disease. If acid production is normal to high, the most severe inflammation usually is found in the distal stomach and proximal duodenum, giving rise to juxtapyloric and duodenal ulcer disease.

2.6 DIAGNOSIS

Various tests have been developed for the detection of *H. pylori*, each with their specific advantages and disadvantages. A variety of tests are now available to diagnose *H. pylori* infection. Histological examination of gastric tissue, bacterial culture, rapid urease testing, use of DNA probes, and PCR analysis, when used to test gastric tissue, all require endoscopy; therefore they incur expense and a risk, albeit slight, of complication due to the procedure. In contrast, breath tests, serology, gastric juice PCR, and urinary excretion of [^{15}N] ammonia are noninvasive tests that do not require endoscopy. The choice of test used for diagnosis of *H. pylori* infection will depend, in most cases, on the clinical information sought and the local availability and cost of individual tests.

2.6.1 Methods Requiring Endoscopy

At endoscopy, many adults with *H. pylori*-associated gastritis have normal-appearing gastric mucosa. The distribution of *H. pylori* and the associated inflammation is often patchy. The patchy nature of infection can lead to endoscopic sampling error, resulting in false-negative biopsy, culture, and rapid urease test results. At a minimum, two biopsy specimens taken from within 5 cm of the pylorus should be obtained at endoscopy, with multiple sections being examined histologically (Sobala *et al.*, 1991). Genta and Graham (1994) however, reported a sensitivity of 100% with biopsy specimens taken from the angularis of the stomach. Two conditions observable directly by the endoscopist, antral nodularity and uncomplicated duodenal ulcer disease, are almost always associated with *H. pylori* infection (Laine *et al.*, 1995); hence, testing for *H. pylori* in these settings may not be necessary.

2.6.2 Culture

Culture of *H. pylori* has two major advantages. First, it allows antimicrobial susceptibility testing; second, isolates obtained by culture can be characterized in detail. Although the sensitivity of culture in experienced laboratories is greater than 95%, other methods for the diagnosis of *H. pylori* infection are simpler, prone to less variability, and more timely. Culture of gastric biopsy specimens typically provides the greatest yield of *H. pylori*. However, culture of gastric juice occasionally has been successful, and there are reports of successful culture from feces (Kelly Pitcher *et al.*, 1994; Thomas, *et al.*, 1992). Only the principles of culture techniques for gastric biopsies are reviewed below. Although preimmersion of biopsy forceps in formaldehyde does not appear to adversely influence the recoverability of organisms in culture (Yousfi, *et al.*, 1996), we recommend that gastric mucosal samples for culture be obtained initially (before sampling for histology or other tests). Saline is a simple acceptable short-term

(,6 h) transport medium (Han, *et al.*, 1995; Veenendaal *et al.*, 1993). If the culture is to be delayed, more complex media such as Stuart's medium or supplemented brain heart infusion broth should be used (Han, *et al.*, 1995). Media containing glycerol are suitable for long-term storage of biopsy specimens at 270°C (Han, *et al.*, 1995), or the specimens can be immediately frozen at 270°C without a fluid medium. A variety of selective and nonselective media are available commercially for culture of *H. pylori* (Hachem *et al.*, 1995). The use of multiple media may increase sensitivity. *H. pylori* requires a microaerobic environment, high humidity, and incubation at 35 to 37°C for a maximum of 7 to 10 days. Positive cultures are usually detected after 3 to 5 days of incubation. *H. pylori* is identified on the basis of colony morphology (translucent colonies varying in size from barely detectable with the naked eye to approximately 3 mm); colonies consist of gram-negative, curved (not usually helical) rods that are urease, catalase, and oxidase positive. The addition of tetrazolium salts aids in the identification of *H. pylori* colonies cultured on agar media (Queiroz, *et al.*, 1987).

2.6.2 Histologic assessment

H. pylori can be visualized at high magnification with conventional hematoxylin and eosin (H&E)- stained sections. Bacteria are located in the mucus adherent to the surface epithelium and are often found deep within the crypts. However,H&E staining may be unreliable when few bacteria are present. In addition, luminal debris on the surface of the epithelium can be mistaken for *H. pylori* in H &E stained sections. Histological identification of bacteria is facilitated by using special stains such as the Warthin-Starry and modified Giemsa stains (el-Zimaity, *et al.*, 1996; Genta and Graham, 1994). The distribution of *H. pylori* in the stomach is not uniform, nor are organisms usually found in areas of intestinal metaplasia (Genta, *et al.*, 1996). Histologic identification of bacteria with the characteristic morphology of *H. pylori* is, in part, observer

dependent. Factors that influence the ability to correctly identify *H. pylori* include bacterial density, type of stain used, and the enthusiasm and experience of the laboratorian (Laine, *et al.*, 1997; Molyneux and Harris, 1993). A sensitive staining technique consisting of a combination of H & E, Steiner silver stain, and alcian blue has been developed by Genta *et al.* (1994). This stain reportedly allows ready detection of *H. pylori* while simultaneously allowing evaluation of gastric histology, thus obviating the need for additional staining. However, the Genta stain procedure can be technically difficult, and its acceptance among gastrointestinal pathologists has not been universal (Laine, *et al.*, 1997). Selection of cases needing special staining (if not performed routinely) may be guided by the inflammatory infiltrate of the gastric biopsy specimens. In the absence of inflammation, the likelihood of *H. pylori* infection is remote, and special stains are not indicated. However, if an active inflammatory infiltrate is present and *H. pylori* is not detected, special stains may be appropriate. In fact, the presence of histologically active gastric inflammation in a patient not previously treated for *H. pylori* infection may be pathognomonic for *H. pylori* infection (Cutler, *et al.*, 1995.). In summary, sole reliance on H&E staining when few *H. pylori* organisms are identified or when *H. pylori* is not detected in the presence of significant inflammation may be imprudent. Immunohistochemical staining techniques also have been developed to detect *H. pylori* (Ashton-Key *et al.*, 1996).

CHAPTER THREE

METHODOLOGY

3.0 METHOOLOGY

3.1 Study Location

Keffi is a city found in Nasarawa State, Nigeria. It is located at 8.85^0 latitude and 7.87^0 longitudes and it is situated at elevation 321 meters above the sea level. Keffi has a population of about 85,911 making it the second biggest city in Nasarawa State. South Atlantic Petroleum Medical Center is located within the environs of Nasarawa State University, keffi where must of the University community members seek medical care.

3.2 Study Population

The study population cover the University community members who are peptic ulcer positive and seek their care in South Atlantic Petroleum Medical Center keffi, Nasarawa State. Ethical approval was obtained from research and Ethics committee of South Atlantic Petroleum Medical Center, Keffi, Nigeria

3.3 Data Collection

A comprehensive Secondary data of those who were screened for *H. pylori* infection from May to November, 2019 was obtained from South Atlantic Petroleum Medical Center, Keffi, Nasarawa State. Which include gender, age, occupation, current history, previous history, nature of Specimen, *H. pylori* status.

3.4 Statistical Analysis

The data generated were coded, entered, validated, and analyzed using SPSS. Associations between categorical variables were tested using the chi-squared test with reports of the corresponding p-values. In some instances where there were small numbers in a given cell (<5), Fischer's exact test was used and the corresponding p-value reported. The odds ratio and the corresponding 95% confidence intervals (95% CI) were used to summarize the strength of association between *H. pylori*sero positivity and risk factors in a multinomial logistic regression test. The level of statistical significance for the study was set at $p<0.05$. In all the tests, p-values less than 0.05 or near 0.05 were used as statistical association for risk factors of *H. pylori* infection.

3.5 Diagnostic tests

Diagnostic tests are indicated in patients:

1) With active peptic ulcer disease (duodenal or gastric),

2) With a history of peptic ulcer disease, who have not been previously treated,

3) With low-grade gastric MALT lymphoma,

4) Who have undergone endoscopic resection of early gastric cancer

5) With uninvestigated dyspepsia, younger than 55 years old (without alarm symptoms).

Available tests for the detection of Helicobacter pylori include:

- Antibody tests,
- Urea breath tests,

- Stool antigen tests,
- Endoscopic biopsies.

Blood tests detect the presence of antibodies of Helicobacter pylori. However, blood antibodies can persist years after the complete eradication of the bacteria. They may be useful in diagnosing the infection, but they are not good for a determined successful eradication.

The urea breath test (UBT) is a safe, easy, and accurate test for the detection of the presence of Helicobacter pylori in the stomach. Ten to twenty minutes after swallowing a capsule containing urea, a breath sample is collected and analyzed for labeled carbon dioxide breath. A positive test signifies that there is an active infection. The test becomes negative shortly after eradication. Individuals concerned with the minute amounts of radioactivity can be tested with urea labeled with heavy, nonradioactive carbon.

Endoscopy is an accurate test for diagnosing the infection as well as the inflammation and ulcers. Endoscopy also allows the determination of the severity of gastritis with biopsies as well as the presence of ulcers, MALT lymphoma and cancer. Biopsies may also be cultured in the bacteriology laboratory for the presence of Helicobacter pylori.

Stool sample: the test uses an antibody of Helicobacter pylori to determine if Helicobacter pylori antigen is present in the stool, which means that there is a Helicobacter pylori infection in the stomach. The stool test can be used to determine if the eradication has been effective after the treatment. In 2012, the FDA approved that the urea breath test was performed in children aged 3 to 17 year

CHAPTER FOUR

RESULT

4.1 Result

Chi-Square Analysis for Age and Sex Determinants of *Helicobacter pylori* Infection Status among Students of Nasarawa State University, Keffi

Hypothesis

H_1:- There is a statistically significant relationship between the prevalence of *Helicobacter pylori* and age/sex of the students.

H_0:- There is no statistically significant association between the prevalence and distribution of *Helicobacter pylori* with the studied parameters

Interpretation

Significance level = 0.05

Degree of freedom = n-1

Where,

n= No. of observations

Confidence Limits = 95%

Decision: if $P \leq 0.05$ accept H_1; reject H_0

If $P > 00.5$accept H_0; reject H_1

4.2 Data Analysis

Table 1: Seroprevalence of *Helicobacter pylori* Infection amongst Students of NSUK in Relation to Age

Age (Years)	No. Screened	No. Infected	% Prevalence
0-20	29	15	51.7
21-40	50	29	58.0
41 and above	5	2	40.0
Total	**84**	**46**	**54.8**

$X^2 = 2.7549$

$P = 0.2522$

(Result not statistically significant at $P>0.05$)

In Table 1: The highest percentage prevalence was recorded with the age bracket of (21-40) years with 58.0% while the lowest fall within the age bracket of (41and above) with 40.0%.

Table 2: Seroprevalence of *Helicobacter pylori* Infection amongst Students of NSUK in Relation to Gender

Gender	No. Screened	No. Infected	% Prevalence
Males	37	22	59.4
Females	47	24	51.1
Total	**84**	**46**	**54.8**

$X^2 = 5.9605$

$P_{value} = 0.0146$

(Result is statistically significant at $P<0.05$)

In Table 2: The males have the highest percentage prevalence of 59.4% though with the lowest number screened at 37 and 22 infected respectively while the females have the lowest percentage prevalence of 51.1% with the highest number screened at 47 and 24 infected respectively.

Basic Decision

Table 1: There is no statistically significant relationship between the age of the students and the distribution of *Helicobacter pylori* ($P>0.05$)

Table 2: There is a statistically significant association between the distributions of *Helicobacter pylori* ($P\leq0.05$)

Chi-Square Analysis for Occupation and History Determinants of *Helicobacter pylori* Infection Status among Students of Nasarawa State University, Keffi

Hypothesis

H_1:- There exist a statistically significant association between the prevalence of *Helicobacter pylori* infection and occupation and previous/current infection history of the students.

H_0:- There is no statistically significant association between the prevalence of *Helicobacter pylori* with occupation and history of the infection.

Basic Decision and Interpretation of Data

Confidence limit = 95%

Level of significance = 0.05

Degree of freedom = n-1; where:

n= Total number of observations

Decision: if $P \leq 0.05$ accept H_1; reject H_0

If $P > 00.5$accept H_0; reject H_1

Table 3: Seroprevalence of *Helicobacter pylori* Infection amongst Students of NSUK in Relation to Occupation

Occupation	No. Examined	No. Positive	% Prevalence
Students	81	45	55.6
Civil Servants	3	1	33.3
Total	**84**	**46**	**54.8**

$X^2 = 4.2189$

$P_{value} = 0.0399$

(The result is statistically significant at $P<0.05$)

In Table 3: The highest percentage in relation to occupation prevalence was recorded among students with 55.6% with 81 students examined and 45 tested positive respectively while the lowest percentage prevalence was recorded among civil servants with 3 examined and only one tested positive with 33.3%.

Table 4: Past and Current status of Peptic Ulcer due to *Helicobacter pylori* Infection amongst Students of NSUK

Variable	No. Identified	% Distribution
Formally infected & currently infected	25	55.6
Past infected, currently negative	15	33.3
Formally negative currently positive	21	25.0
Formally negative & currently negative	23	27.4
Total	**84**	**100.0**

Currently positive 46

Total prevalence 54.8%

$X^2 = 1.3692$

$P_{value} = 0.7127$

(Result not statistically significant at P>0.05).

Table 4: There is a statistically significant association between the prevalence of *Helicobacter pylori* infection and the occupation of the students at NSUK ($P \leq 0.05$)

Table 4: There is no statistically relationship between the prevalence of *Helicobacter pylori* infection and its distribution in terms of past and current status in the students of NSUK studied at (P>0.05).

4.3 Discussion

The prevalence of *Helicobacterpylori* infection is high worldwide and it is more common in developing countries than the developed ones (Magalhães and Luzza, 2006). It is a major risk factor for chronic gastritis, peptic ulcer and gastric cancer (Fock and Ang, 2010).*Helicobacter pylor*i can cause severe illnesses with high morbidity and mortalityrates, the complex interactions between this microbe and humans, particularly its transmission pathways to humans and reservoirs, are largely unknown (Seckaet *al.*, 2013). However, recent report by Mitchell and Katelaris (2016) had considered *H. pylori* to be a foodborne pathogen because of its microbiological and epidemiological characteristics. Even so, Aktepe and colleagues (2011) had suggested that the accurate detection of *H. pylori* is essential for the management of patients and for the eradication of the bacterium following treatment.

The prevalence of *H. pylori* infection worldwide varies among countries and population groups in the same country. This infection is more common in developing countries where the prevalence rate ranges between 70–90% as compare to developed countries(Olokobaet *al.*, 2013). Thus a study to ascertain the age and gender determinant of infection due to peptic ulcer amongst students of Nasarawa State University was conducted. In this present study, 84 students of varying socio-demographic characteristics were screened, of which 46 representing an overall prevalence of 54.8% were infected by *H. pylori.* The results recorded in this study are comparable to those reported elsewhere, such as those of Akbar and El-Tahary (2005), Agüloluet *al.* (2006), Daniellaet *al.* (2008),

Tijanni and Umar (2008),Shresthaet *al.* (2012), Campanatiet *al.* (2013) and Isa *et al.* (2015).

Age-related prevalence indicates the highest occurrence of 58.0% amongst those students aged between 21–40 years old, followed closely by those within the age bracket ≤20 years old with a point prevalence of 51.7%. Interestingly, students within the higher age bracket had the least prevalence of 40.0% with respect to age. The determinant in relation to age shows that the highest sero-positivity is skewed towards younger students. This is in consonance to an earlier report by Amberbiret *al.* (2011)who suggested that age influence the acquisition and colonization of the intestinal walls by the causative agent of peptic ulcerin their study of the effects of *Helicobacter pylori*and helminthes infection amongst Ethiopian children. Shi *et al.* (2008) and Kato and Fujimura (2009) also note that the risk for infection due to *H. pylori* is higher in people of younger age particularly in children.

This tends to disagree with an earlier report by Al-Molgedet *al.* (1990) that seroprevalence of *H. pylori* increases as the age subjects advances. Also, the low prevalence of *H. pylori* among subjects of higher age disagrees with other studies previously described (Langat *et al.*, 2006). Though, the high prevalence of *H. pylori* among children has been reported to be due to poor socio-economic condition (Langat *et al.*, 2006). Nevertheless, the seroprevalence of *H. pylori* was statistically insignificant irrespective of the ages of subject at $P>0.05$ and this implies that age may not be a factor for high prevalence of *H. pylori.* In another related report, infected mothers or older siblings, low standards of living, and crowded households have been shown to be major risk factors for contracting *H. pylori* (Hunt *et al.*, 2011). In fact, during the past decades,

several reports indicated a correlation between *H. pylori* infection and various extra gastric disorders (Banić*etal.*, 2012).

Meanwhile, Ikeda and colleagues (2013) found high prevalence of *Helicobacter pylori* infection in middle-aged Japanese children majority of whom are college students; this correspond directly to the findings of this present study since it was specifically undertaken in a university environment. On a similar note, Kang *et al.* (2011) in their study of the risk factors for peptic ulcer infection due to *H. pylori* implicated age, diet, presence of other disease, overcrowding and certain group of people to be predisposing factors that increase vulnerability to contracting the infection. This singular cohort research reinforced the findings of this present study. Thus, proper hygiene and sanitary behaviours should be encouraged among the general population, particularly among students living in crowded houses.

This study observed higher prevalence amongst the male students (59.4%) than the females with a relatively lower prevalence rate of 51.1%. This corroborates the works of Xu*et al.* (2000) and Ndip*et al.* (2009) who reported a significantly higher prevalence rate among the males. Ahmad *et al.* (1997) reported a much higher seroprevalence rate of 92% among male subjects in his pilot serological study of *H. pylori* in Bangladesh which concur with the finding of the present work. Although most studies as observed in the literatures suggest that gender don't necessarily influence the seroprevalence rate, studies such as those of Mégraud*et al.*(1990), Granström *et al.* (1997), Nafic*yet al.*(2000), Dore *et al.* (2002), Akbar and El-Tahary (2005), Shi *et al.* (2008) and Etukudo*et al.* (2012).Conversely, Tijjani and Umar (2008), Nwodo*et al.* (2009) and Rasmi*et al.* (2009) refuted the arguments of the researchers mentioned above by demonstrating from their respective studies that both males and females are infected almost at the same rate.

CHAPTER FIVE

SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.1 Summary

1. This study sought to identify age and gender determinants in the acquisition of peptic ulcer infection due to *Helicobacter pylori* amongst students of Nasarawa State University Keffi.
2. A total of 84 students were selected at random and screened using the rapid diagnostic test strip, of which 46(54.8%) were found to harbour the bacteria.
3. In terms of age, the infection is highest amongst students aged 21–40 years (58.0%), followed by those aged 0–20 years (51.7%) and lastly those aged ≥41 year (40.0%). There is no statistical significant relationship with age at $P>0.05$.
4. In relation to gender, the males (59.4%) were more infected than the females (51.1%); although gender is statistically significant to the prevalence of *H. pylori* at $P<0.05$.

5.2 Conclusion

Seroprevalence of *H. pylori* was high in this study, though it was less than other studies reported elsewhere and in view of this, it may have public health implication. The infection was highest amongst the younger students and in males than in the female students. However, agein this study does not influence the acquisition of peptic ulcer infection statistically ($P= 0.2522$); while a statistically significant association was observed for the gender of the students ($P= 0.0146$).

This study therefore revealed a higher seroprevalence of *H. pylori* among some selected students of Nasarawa State University Keffi. One of the major justification of this study is that it is conducted among a general population of the study area, not among people in a hospital settings (whom of course are there due to sicknesses), and can perhaps be a true representation of the prevalence of *H. pylori* among the general population within the university community in particular. Nonetheless, a community study is desirable as this will give a more true representation of the prevalence.

5.3 Recommendations

In view of the high seroprevalence of the infection recorded among the subjects in the study area, a more thorough epidemiological survey is warranted to determine the prevalence of *H. pylori* in Keffi town in particular and Nasarawa State in general. This in turn will open avenues for more studies about the risk factors and mode of transmission of *H. pylori*. Also, various diagnostic tests for *H. pylori* infection may give an ambiguous results and the use of multiple tests may help to provide a more accurate diagnosis of *H. pylori* infection.

Although, in spite of the introduction of the first, second and third line therapies for *H. pylori*treatment and possible eradication; their eradication rate has not reached 100%. Antibiotic resistance is still a problem in many countries including Nigeria. To face treatment failures, several third-line 'rescue' therapies have been tried. This third-line therapy has not been used in many countries. Therefore further trials are still needed to get a better *H. pylori* eradication rate through a cheap and effective treatment regimen. It is also suggested that some medicinal plants with anti-*Helicobacterpylori* activity should be considered as alternatives for ulcer therapy; the plants include extracts of *Allium sativum, Curcuma longa, Mallotusphillipinesis, Sapindusmukarossi*and *Rheum emodii*.

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