PREVELANCE OF *HELICOBACTER PYLORI* AMONG STUDENTS OF COLLEGE OF MEDICAL SCIENCES AND FACULTY OF AGRICULTURE

BY

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CERTIFICATION

We the undersigned hereby certify that OSAWARU INNOCENT(BMS1802414) carried out this research in the Department of Medical Biochemistry, University of Benin, Benin city and thereby approve same as adequate in scope and quality for the award of Bachelor of Science Degree (B.Sc) in Medical Biochemistry.

Signed

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DEDICATION

With humility and love I would like to dedicate this project work to God Almighty, who is the source of all knowledge, Wisdom and Understanding. Furthermore, I dedicate this work to my mother (Late. Mrs. Rita Ngozi Osawaru) for the parental care, love and motivation.

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This project work is a product of much research, extensive discussion and analysis. I want to use this medium to acknowledge the input of various persons at the different stages of its development.

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ABSTRACT

Helicobacter Pylori infection has been identified as an important risk factor for the development of peptic ulcer disease (PUD) and gastric cancers. The purpose of this study aimed to determine the prevalence of H. pylori infection and associated risk factors in undergraduate students of college of medical science and faculty of Agriculture in University of Benin. Whole blood samples were randomly collected from 93 participants both male and female and analyzed using antibodies against H. pylori. Participants' demographic and clinical information were obtained using a structured questionnaire. The study results showed that out of 93 selected participants, 59 (63.9%) showed positive for Helicobacter Pylori antibodies, while 34(36.6.%) were negative for Helicobacter antibodies. Popularity of Helicobacter Pylori infection was found to be slightly higher in female participants compared with male participants. Based on age, the prevalence of Helicobacter Pylori infection was significantly higher ($P<0.05$) in participants from 16 to 25 years old compared to other age groups. Symptomatic participants primarily complained of heartburn, followed by loss of appetite, abdominal pain, nausea, bloating and eventually vomiting. However, none of them complained about vomiting blood. Identifiable risk factors associated with infection include misuse of pain killers drugs, infected drinking water, living with someone having the bacteria among others .The aim of this work was to generate information focusing on the prevalence of Helicobacter pylori infection in study participants. The information generated will help provide the public health intervention needed to examine these issues and provide evidence-based prevention to students in the College of Medical Sciences and the Faculty of Agriculture as well as the general population of the University of Benin

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CHAPTER ONE

INTRODUCTION

1.0 BACKGROUND OF THE STUDY

Helicobacter Pylori (*H. pylori),* formerly known as Campylobacter pylori, is Gram-negative, flagellated, spiral aerobic bacterium found in the stomach with abundant urease production, which is associated with to some acute upper gastrointestinal diseases manifesting as indigestion. It is the first officially recognized bacterial carcinogen and one of the most effective human pathogens, causally linked to gastritis and gastritis-related diseases, gastric ulcer disease, gastric adenocarcinoma and primary gastric lymphoma. Previous research has shown that *H. Pylor*i is present in patients with chronic gastritis and duodenal ulcers, conditions not previously thought to have a bacterial cause . *H. pylori* has been identified as an important risk factor for the development of peptic ulcer disease (PUD) and is perhaps the most important cause of recurrence in people who have previously been treated for ulcer disease. duodenal stomach. According to (Nwodo *et al*.,2009) *H. pylori* infection can lead to acute gastritis (abdominal pain, nausea, and vomiting) within two weeks of infection. Many patients infected with this organism have recurrent abdominal symptoms (non-ulcer dyspepsia) without ulceration. Duodenal inflammation (inflammation of the duodenum) also commonly occurs, as does peptic ulcer disease(Tijjani and Umar, 2008)

These are ulcers that develop in the lining of the stomach, lower esophagus, or duodenum:gastric ulcer, esophageal ulcer and duodenal ulcer. Common

risk factors for peptic ulcer disease include*:Helicobacter Pylor*i infection and nonsteroidal anti-inflammatory drugs (NSAIDs). Less common risk factors include alcohol, smoking, cocaine, serious illnesses, autoimmune problems, radiation therapy, and Crohn's disease, among others. In addition, peptic ulcer disease is one of the most common human diseases, affecting approximately 50% of the world's population (Higuera, 2015)

The most common symptom of peptic ulcer disease is severe abdominal pain that extends from the navel to the chest and can range from mild to severe. Other symptoms include: changes in appetite, nausea, bloody or dark stools (melaena), indigestion, vomiting and weight loss. Complications of peptic ulcer disease include bleeding, perforation, gastric tube blockage, and stomach cancer. Older adults are at higher risk of developing peptic ulcer disease due to taking high-risk medications, including antiplatelet drugs, warfarin, selective serotonin, proton pump inhibitors, and bisphosphonates (Bastos *et al*., 2013)

The prevalence of *H. pylori* infection varies between and within countries depending on the age, race, ethnicity, and geographic region of the population. The infection is commonly acquired in childhood in most countries. Infection rates in children are higher in developing countries than in industrialized countries, possibly due to poor sanitation, possibly combined with less use of antibiotics for unrelated illnesses. *H. pylori* infection is common worldwide, with prevalence rates ranging from 30 to 40% in the United States, 80 to 90% in South America, and 70 to 90% in Africa. It is more common in developing countries and its prevalence

increases with age from 20% in adolescents to 50 to 60% of subjects in the 6th and 7th decades of life. In highly endemic areas such as Nigeria, it is best to determine the true prevalence of Helicobacter Pylori infection using biopsy-based methods, but for the purposes of epidemiological investigations, screening can be performed. Quickly and easily screen *H. pylori* infection using this method. serological tests, although their ability to distinguish between past and present infections is low.

According to (Fashner and Gitu, 2015) urea breath testing and stool antigen testing are most accurate in identifying *Helicobacter Pylori* infection and can be used to confirm recovery. Additionally, the patient's serum may also be tested for the presence of anti-*H. pylori* antibodies.

More than 50% of the world's population, especially children and adolescents, harbor H. pylori in the upper digestive tract. . Infection is more widespread in developing countries. However, the level of awareness about this bacteria, the causative agent of gastric ulcer disease, appears to be very low among the student population.

Early detection of *H.pylori* infection can prevent peptic ulcer disease and its complications. However, the prevalence of *H. pylori* infection among students of college of medical science and faculty of Agriculture remains unknown. Furthermore, it is necessary to identify factors that may make youth in this setting susceptible to *H. pylor*i infection.

1.1 AIM OF THE STUDY

This study aims to assess the prevalence, determinants and knowledge of *Helicobacter pylori* infection among the students of college of Basic medical science and faculty of Agriculture of the University of Benin.

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CHAPTER TWO

LITERATURE REVIEW

2.0 Helicobacter pylori

Helicobacter pylori (*H.Pylori*) belongs to the Helicobacteraceae family. It is a Pathogenic, gram-negative, transmissible spiral-shaped bacteria are considered Food contaminants are digested instead of being actual invaders of the stomachmucosa (Samina et al.,2020).

H. pylori is one of the most common pathogens in the world. It invades about 60% of the area world population, causes gastritis and peptic ulcers and is closely related togastric adenocarcinoma and lymphoma. However, most individuals never developclinical disease. Thirteen years after Marshall and colleagues cultured *H. pylori* Warren, we still don't know its primary mode of transmission. Childhood symbolizes Infection occurs mainly in the third world, but infection is rare in children in the developed world. Possible routes of infection include the oral-oral or fecal-oral route,Iatrogenic spread due to inadvertent use of non-sterile pH probes and endoscopes, and vector spread by flies. Evidence supporting each route of transmission is provided, but there is no main routes. The only significant source of infection appears to be humans themselves. This organism has been found in some domestic and domestic cats primates are not humans, but opportunities for humans to interact with them are rare,making infection from this source unlikely. This organization doestends to become coccoid. This may represent a form of continuity in it *H. pylori* can survive in the environment, but it remains to be proven that it can survive return to copy form. (David *et a*t. 2019)

2.1 HISTORY OF *H.PYLORI*

In 1979, Robin Warren, a pathologist in Perth, Western Australia, began to discover that stomach biopsies sent for histological analysis often contained curved bacteria. These microorganisms are found in the mucus layer covering the tissues, but not in the gastric mucosa (Marshall *et al.*, 1989). According to Warren, similar species were described by European pathologists in the late 19th century but were eventually forgotten because they could not be isolated.Barry Marshall, a young internist, was fascinated by Warren's observations and the two worked together to try to separate the organisms from the biopsy tissues. The researchers used techniques to isolate Campylobacter species, which required inoculating biopsy samples into selective media and culturing under microaerobic conditions. Bacteria have a curved gram-negative rod shape. Plates with no apparent growth were discarded after three days because most Campylobacter grows in this medium within 48 hours. The first cultures from about 30 patients all tested negative, but coincidentally, a culture was grown over five days over the Easter holiday and colonies were detected. Subsequently, 11 patients had their respective bacteria isolated, described, and identified as Campylobacter pyloridis (later known as

Helicobacter pylori). Researchers around the world quickly confirmed the presence of these organisms in the stomach lining after this article was published. By 1984, it became clear that *H. pylor*i infection was closely linked to the development of inflammation of the gastric mucosa (chronic superficial gastritis), especially in cases of cellular infiltration. polymorphonuclear cells (chronic active gastritis). A growing body of research shows that once infected, *H. pylori* persists, often for life, unless treated with antibiotics.In 1994, a consensus meeting organized by the National Institutes of Health concluded that *H. pylori* contributes significantly to peptic ulcer disease and recommended treating people infected with ulcers to eliminate the bacteria. this object. Gastric adenocarcinoma, the most common type of stomach cancer in the world, is associated with chronic gastritis, but the cause of chronic gastritis was not recognized.. The World Health Organization's International Agency for Research on Cancer reviewed evidence that *H. pylori* causes cancer in humans in 1994.

2.2 EPIDEMIOLOGY OF *H. PYLORI*

H. pylori is found in the human stomach (and is often isolated from non-human primates) in all regions of the world. In developing countries, 70 to 90% of the population carries the *H. pylori* bacteria. Almost all get sick before the age of ten. In developed countries, infection rates are low, ranging from 25 to 50%. Data from developed countries also show that most infections occur during childhood. Consistent with this model, there is ample evidence that changes associated with industrial development have reduced the incidence of *H. pylori* infection. Thus, in the era of "emerging" bacteria, *H.pylori* has moved from a distribution that was thought to be nearly ubiquitous hundreds of years ago to a current situation where it is present in less than 10% of children. in developing countries are infected. I went into hiding." Most studies show that men and women are infected about equally, but at least one study shows that male gender is a major risk factor for infection rates , despite economic progress. However, high infection rates persist among some ethnic minorities.

Interestingly, a recent study by (Malaty *et al.,* 1999) has shown that children of matched socioeconomic classes but of different racial backgrounds acquire and lose infections consistently. different ways. In his 12-year follow-up serological study (Malaty *et al*., 1999), he found that the infection rate was four times higher in African American children than in white children. The 12-year rate of loss due to infection was significantly higher in white children (50%) than in African Americans (4%), the latter group remaining infected or reinfected. Additional indirect evidence for spontaneous clearance of infection in children comes from a recent seroprevalence study of 365 primary school children aged 4 to 7 years from a low-income area on the border of the United States and Mexico. It is The study showed a gradual decline in *H. pylori i*nfection rates from 36% in 4-year-olds to 24% in 5-year-olds, 20% in 6-year-olds and 14% in 7-year-olds. The study authors concluded that the downward trend in prevalence seen in these children suggests that transient infections may be common among young children.

2.3 BASIC MORPHOLOGY OF *H. PYLORI*

Helicobacter pylori is a gram-negative, highly motile, microaerobic, spiral-shaped bacterium belonging to the class Proteobacteria epsilon. The natural habitat of this pathogen is the human gastric mucosa, and infection in humans results in persistent gastritis, which can progress to peptic ulcer disease and adenocarcinoma. Therefore, many factors determine the type and severity of the disease, e.g., the state of the host's immune system, the pathogenicity of *H. pylori* strains, and the presence of environmental factors (diet, stress, hygiene level or presence of co-infections). Among them, the spiral shape of the cells is an important feature necessary for cells to effectively reach and colonize gastric mucus. Mutants exhibiting a rod phenotype have impaired gastric migration and motility. In *H. pylori,* maintenance of cell shape is apparently controlled by at least two independent mechanisms operating at two levels: peptidases influence cell shape by inducing peptidoglycan relaxation, and four so-called

coiled-coil-rich proteins (Ccrps) represent elements of the cytoskeleton that most likely influence cell shape by how to create an intracellular scaffold. Unlike many other bacteria, the cell morphology of *H. pylori* is not affected by the actin-like protein, MreB. Additionally, a bactofilin homologue has been identified, which is thought to be important for maintaining the characteristic spiral cell shape. Infection in mice demonstrated that bactofilin-deficient mutants of *H. pylori* were strongly defeated by wild-type bacteria, identifying *H. pylori* bactofilin as an important pathogenic factor. However, like all bactofilins studied to date, *H. pylori* bactofilin does not appear to be essential for bacterial survival, suggesting that the cell has other proteins that have redundant functions or that these proteins are involved in nonessential processes.

However, it remains unclear whether the unique bactofilin of *H. pylori* actually exhibits the biochemical properties of bactofilin and forms a cytoskeleton in this bacterium, and if so, what the putative cytoskeleton is. How is this related to spiral cell shape? We

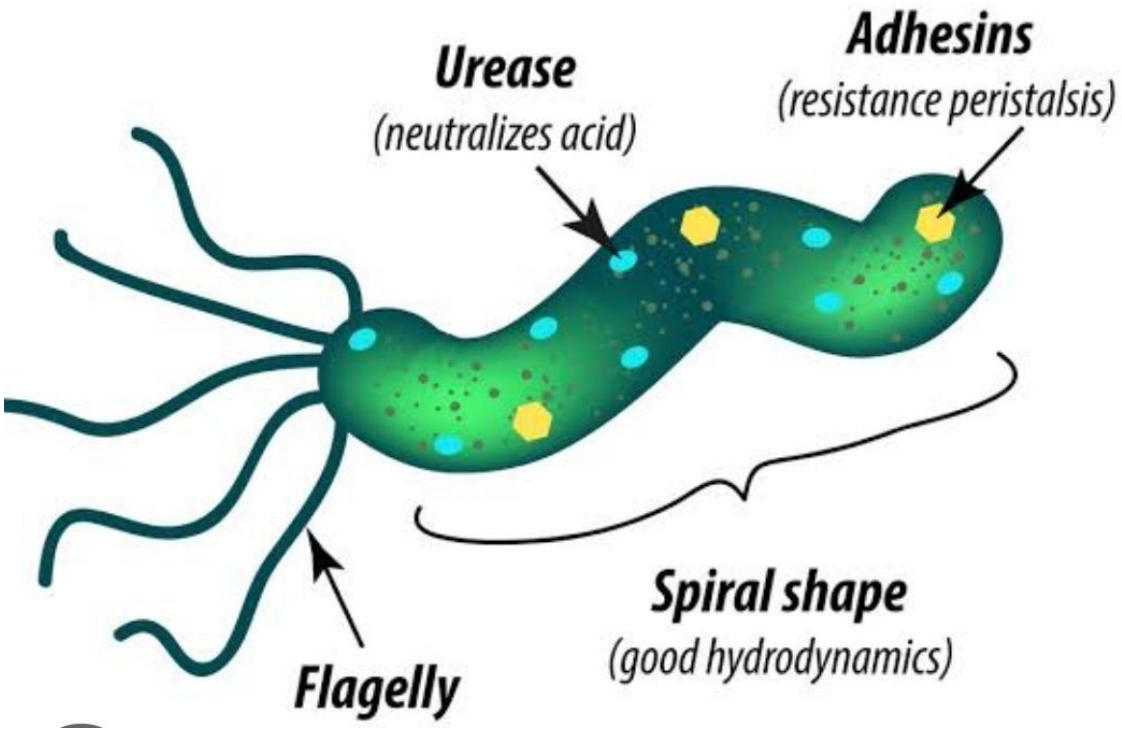


Fig 2.3 Diagrammatic expression of Helicobacter pylori

(Cheng *et al* 2015)

therefore sought to characterize the biochemical properties of the unique bactofilin HP1542 in vitro and study its localization in vivo. Our results thus provide the first insights into the assembly and localization properties of HP1542 and may thus serve as a starting point for the study of the cell physiology of bactofilin in organisms. causing this disease as well as designing targeting molecules. (hultrup., 2019) With the exception of the human stomach, there appears to be no significant reservoir of *H. pylori.* Although other animals harbor organisms similar to *Helicobacter pylori*, no animal species, with the exception of non-human primates and possibly cats under certain circumstances, harbor *H. pylori* . So the main problem with the infection lies in how *H. pylori* is transmitted from one person's stomach to another. Although there are no specific risks associated with handling this bacterium, laboratory personnel should exercise general precautions when handling clinical samples, remembering that *H. pylori* is a human pathogen. Perhaps most important is fecal-oral transmission. Although *H. pylori* has been isolated from the stool of infants infected with the pathogen, isolation in stool is rare. This may indicate that hair loss is sporadic. Water contaminated with feces is a potential source of infection, but no microorganisms have yet been isolated from the water. Transmission through food cannot be demonstrated. Finally, mouth-to-mouth transmission has been documented in African women who fed their infants pre-chewed foods. No link has been established between the infection and sexually transmitted diseases. So it's unusual for this to happen. *H. pylori infection* fection due to inhalation of vomit is also possible but has not been reported.

2 3.1 COCCOID FORM

The transformation of H.pylori from a culturable spiral to a non-culturable spherical form occurs when exposed to adverse conditions such as lack of nutrients (Enroth *et a*l., 1996), incubation time prolonged in vitro, presence of low or high temperatures (Nilsso *et al.*, 2002), incubation at alkaline pH, nutrients Significant change in culture medium

from rich medium to pure water, incubation in the presence or absence of CO2, or in contact with antibacterial compounds (e.g., antibacterial compounds). Antibiotics, proton pump inhibitors (PPIs) and compounds secreted by bacteria of the genera Lactobacillus and Streptococcus (Khosravi *et al.*, 2014). Spherical morphologies are commonly observed in laboratory studies of *H. pylori,* but the presence of these morphologies has been reported in many cases in *vivo* (Balakrishna *et al*. 2013). Although the spherical form is less pathogenic than the spiral form, it can produce urease, adhere to epithelial cells and cause gastritis. Studies on mice have shown that coccoids can spread in the body and cause full-blown disease. But most often near the stomach, a sphere appears next to the whorl. It is also reported to colonize only spherically in the gastric mucosa. Patients with gastric adenocarcinoma have more spherical forms of *H. pylori* than those with gastric ulcers, suggesting that these forms may be involved in carcinogenesis/progression. This is consistent with the observations of (Loke *et al*., 2019), who showed a high proportion of proteins involved in carcinogenesis in spherical Helicobacter in vitro. pylori occurs. In addition to the stomach, the oral cavity is also a potential place for this bacteria. *H.pylori* exists mainly in spherical form. There are also reports on the joint viability of individual and spherical helices. The morphology of oral *Helicobacter* is said to be*;H. pylo*ri corresponds to the conditions prevailing in this environment and the presence of physiological flora colonizing this area in the gastric mucosa.

2.3.2 ROD FORM

An alternative phenotype to spirals and spheres is the rod morphology (Fernandes *et al.* 2017). Little is known about the environmental conditions that stimulate the transition to the rod form, although it has been observed that in *H. pylori*: About 10-15% of cells become rod-shaped cells. During high-speed in vitro culture, the number of rods increased, with an inverse correlation observed in the number of spirals. The drastic

change in culture medium from nutrient-rich medium to pure water/brine medium contributes to the transition to spherical form of *H. pylori* (Sherin *et al.* 2003). A contrasting observation was made by (Fernandes et al., 2017) who, through prolonged acclimation of *H. pylori* to incubation in purified water, increased the isolation time of *H. pylori* with can be cultured. On microscopic examination, it was found that the adapted shape was mainly rod-shaped cells and the presence of individual cells with a very elongated shape (filamentous cells). These results are consistent with the hypothesis of a potential role of water as a source of *Helicobacter pylori* transmission (Bury-Mone *et al.* 2006).

2.3.3 FILAMENTOUS FORM

Another morphological pattern shown by *Helicobacter pylori* is the filamentous and elongated phenotype (Chaput *et al*. 2016). Normally, filamentation contributes to increased adhesion to mucosal surfaces, promoting slow, ligand-dependent absorption of filamentous bacteria within eukaryotic (invasive) cells and by multiplies cell length, this process also helps avoid phagocytosis (Yang *et al*. 2016). It has been suggested that the filament may play a protective role against the effects of genotoxic antibiotics. Multinucleated filaments increase the chances of repairing damaged genetic material, and during mutation accumulation, the promote recombination and selection for beneficial traits. In the case transmission of pylori, filamentation is triggered, among other things, by exposure to azithromycin or by incubation in a hyperosmotic medium (Takeuchi *et al.* 2006). The presence of individual filamentous cells was also observed during incubation in a CO2-free aerobic atmosphere and in adapted *Helicobacter pylor*i strains incubated in purified water. There was also a case where filamentous *Helicobacter pylori* bacteria were isolated from a stomach biopsy. The function of *Helicobacter pylori* elongating cells remains unknown, while it appears that unlike other bacteria, the filament does not participate in host colonization. The motility

and invasion capacity of C57/BL6J mice has been shown to be reduced in filamentous *Helicobacter pylor*i. However, they are believed to play a role in antibiotic resistance because the presence of these forms leads to a strong increase in the MBC value of amoxicillin, i.e. 0.06 compared to 32 μg/ml. Therefore, filamentation may be considered a protective mechanism against the antibacterial effect of this antibiotic against *Helicobacter pylori.* Based on some of these reports (filament induction by aerobic and hyperosmotic conditions (Gancz *et al*., 2008), it appears that filamentous forms of *Helicobacter pylori* may be more important in survival/ transmission of these bacteria to the external environment.human body.

2.4 TRANSMISSION OF *H.PYLORI*

With the exception of the human stomach, there appears to be no significant reservoir of H. pylori. Although other animals harbor organisms similar to Helicobacter pylori, no animal species, with the exception of non-human primates and possibly cats in certain cases, harbor *H.pylori* . So the main problem with the infection lies in how *H. pylori* is transmitted from one person's stomach to another. Some main routes have been described.

2.4.1 Oral-Oral transmission

No major route of *H.pylori* infection has been identified. Possibilities include oral-oral, fecal-oral, physician-borne, and vector-borne transmission. The advantages of each side depend on local factors. The data supporting word of mouth comes from a variety of observations*. H. pylori* is rarely cultured from the mouth. Transient invasion of the mouth can easily occur in people with free reflux or in people who have just had an endoscope inserted. Claims regarding the oral reservoir of *H.pylori* based on the rapid urease test should be viewed with caution because of the possibility that other urease-containing organisms are present in the oral cavity. Many researchers have detected *Helicobacter pylori* by polymerase chain reaction (PCR), with results ranging from high

rates of bacterial infection to no rates of infection. The PCR method used in one study was only able to detect *H. pylori* DNA on the dentures of one patient in one group, while *H. pylori* was detectable in the stomach in 54% of the patients treated. study.
Although there may be differences due to local factors, methodological differences probably explain most of the differences. Primer selection and careful optimization of conditions are clearly necessary to maximize PCR sensitivity and specificity.
Unfortunately, confirmation of specificity is rarely reported, but when it is, the data are more likely to be reliable. It is recommended to use *H. pylori*-specific 16S ribosomal RNA sequences or to bind specific virulence factors to unique DNA sequences. The use of urease-based probes can lead to problems of low specificity because the gene or its homologs are quite common in different bacteria. Only one of the studies claiming successful identification confirmed the observations by Southern blot analysis using a carefully validated chromosomal DNA probe. The same group was able to isolate *H. pylori* from the saliva of 1 of 9 subjects. The use of PCR techniques to detect *H. pylori* in the oral cavity has given rise to many assertions and refutations.
Indirect evidence supporting oral transmission comes from studies showing that gastroenterologists have higher rates of *H.pylori* infection than age-matched controls: 52% versus 21%, respectively. The difference was more evident in older gastroenterologists who did not wear gloves during their early years of practice. Endoscopy nurses and general practitioners were comparable to blood donor controls. In another study, nurses also had increased incidence at each time period examined. Overall, 39% of nurses tested positive, compared with 26% of the blood donor control group. In the youngest group, 20 to 34 years old, 25% of nurses tested positive compared with 13% in the control group; In the older group, >50 years old, 71% of nurses and 40% of controls tested positive. Presumably, they are more likely to be exposed to vomit, feces, and nasogastric tubes than dentists. In contrast, dental

workers known to have a high incidence of oral aerosol infections did not have an increased incidence. It has been suggested that vomiting mucus as a child contributes to the spread of *H. pylori;* however, the acquisition phase is mainly during childhood, which is rarely the case in developed countries. This can be important in daycare, but it is probably rare for adults to become infected in this way.

2.4.2 fecal-oral route

The possibility of fecal-oral transmission remains of great concern, but there is little data to support this concept (Thomas *et al.,*2019), reported isolation of *H. pylori* from 9 of 27 children aged 3 to 27 months in The Gambia. They used similar techniques to detect fecal shedding of *Helicobacter pylori* in adults with dyspepsia in the UK. In 12 of 25 patients with *Helicobacter pylori* gastric infection proven by endoscopy or I4C breath testing, they were able to isolate *H. pylori* colonies from the stool. They were then phenotypically identified as Helicobacter pylori and genotyped by PCR. Interestingly, the investigators found that they could still find *Helicobacter pylori* in stool, even after eliminating the stomach bacteria. This raises the question: If the current hypothesis is correct, where else is *H. pylori* found, other than in a stomach-like mucosa? Additionally, (Thomas *et al.*, 2017). It is possible that another type of Helicobacter with colon tissue tropism similar to Helicobacter cinaedi may have been found. Few other studies have been published attempting to demonstrate fecal shedding o*f H. pylori,* and none have been successful. So either this area was carefully avoided or it was technically very difficult. The first issue may actually be one that is supported by observations (Fox *et a*l., 2016). in mink. This group may show increased excretion of Helicobacter mustelae in the feces at times when ferrets are achlorhydric, in response to Helicobacter mustelae itself, or when gastric pH is neutralized by omeprazole. This raises an interesting question about the tendency of some Helicobacter species to reduce acid

secretion. Indeed, achlorhydria caused by *H. pylori* in humans occurs after the onset of infection and is too late to be considered a colonizing factor. However, this achlorhydria may be a factor that promotes *H. pylori* excretion. Some Gambian children who are cultured for *H.pylori* may have gastric acid deficiency for a number of reasons, including malnutrition and/or *Helicobacter pylori*-associated gastric acid deficiency. However, this possibility has not been tested. Adults with indigestion in the UK are unlikely to be acid deficient. Another factor that may reduce the ability to detect *H. pylori* in stool is the presence of bile salts. They have been shown to inhibit the growth o*f H. pylori* in culture, and after partial gastrectomy, the incidence of *H. pylori* decreased. This is thought to be secondary to reflux of bile into the remaining portion of the stomach, which is capable of destroying the Helicobacter pylori bacteria.

The indirect detection of *H. pylori* in stool by different researchers has been disturbing due to the presence of Taq polymerase inhibitors in the stool. Recent studies by (Monteiro *et al*., 2019). suggests that there are acidic polysaccharides in the stool that inhibit the reaction. (Li et al., 2018). developed a PCR method that produced a large number of positive *H. pylori* DNA results in saliva, which correlated well with gastric biopsy data. However, their success with stool is very limited. Special attention to DNA preparation appears to be essential. The question not yet answered by PCR concerns the form of Helicobacter pylori DNA found: is it DNA from replicative form, from dead bacteria, or from coccoid form? *H. pylori* exists in at least two forms. When initially isolated, it is present in a spirally replicating form, but if left for several days or deprived of nitrogen and carbon, it returns to its coccoid form. This form can also be caused in vivo by the use of antibiotics or omeprazole, or it may occur spontaneously in cultures that have expired over time. It is possible that the coccoid form of *H. pylori* is also a viable form. Evidence for this hypothesis has accumulated to the extent that it may have flagella; its evolution toward the coccoid form has been linked to the appearance of new

proteins and the loss of others. This demonstrates that it is a programmed response and not simply regenerative. This reaction is comparable to that observed in spore-forming bacteria. Coccoid *H.pylori* contains chromosomal DNA and polyphosphates, and its oxidative enzymes remain active for long periods of time. It induces similar cytoskeletal changes in 3D helical-associated AGS cells, but its binding to cells and cell matrices is less efficient than that of the replicative form .

The main question is whether the *H. pylori* coccoid can return to its spiral shape. Initial data in animals are controversial (Eaton *et al.,* 2013). Reversal could not be shown in gnotobiotic pigs when cultured at 16 days of age, but there is evidence that *H. pylori* can recur when inoculated to mice. Coccoid forms administered to BALB/c mice paralleled the replicate forms. Mice received 2 to 5 An intermediate situation has been reported in which coccoid forms can invade the mucosa and cause inflammation, but the replicative form cannot be recovered. The ability of *H. pylori* to exist in the air is very limited. (Gtube*l et al*., 2016). could not overcome the laboratory-adapted strain after 12 h of exposure to air. It has been reported that the replicative form can be recovered in river water after 10 days at 4°C. 35 However, because reversibility cannot be easily demonstrated, the notion that the coccoid form is environmentally persistent remains a tantalizing possibility. (Hopkins *et al.*, 2003). in a large-scale epidemiological study conducted in Chile, it was possible to show a statistical association between an increased risk of *H. pylori* infection and the consumption of raw vegetables. However, the sample size of those avoiding raw vegetables was small, and if this group avoids eating raw vegetables regularly, this raises questions about other concomitant hygiene practices that may reduce the incidence of *Helicobacter pylori* infection. The use of untreated wastewater as fertilizer for vegetables, especially if the coccoid form of *Helicobacter pylori* is reversible, is potentially dangerous. Several researchers have attempted to detect H. pylori in water sources, directly and indirectly. An

epidemiological study of water supplies in different municipalities of Lima, Petu, was carried out to analyze the risk associated with *H. pylori* infection. Clearly, there is a higher risk of infection in people who use external faucets rather than internal plumbing, regardless of their economic status. Another small group with high incomes and access to community well water through internal plumbing systems had very low disease rates. Subsequent evaluation of Lima water samples by PCR showed that half of the samples contained *H. pylori* DNA. No data are provided on whether the DNA is found in intact organisms or in the form of cocci or bacilli. Samples from community wells were negative. Indirect comparisons of *Helicobacter pylori* infection with hepatitis A have produced conflicting data.

A study conducted in China shows the difference between the two infections. *H.pylori* is common before age 10, while hepatitis A is rare because children only drink boiled water. This is powerful implying an alternative source of *H. pylori* for this population's water supply. Data reported by (Drum *et al*., 2017). showed that parents and siblings of first-born children infected with *H. pylori*, as measured by the presence of antibodies, had much higher rates of infection than those whose first-born children were negative. This is often interpreted to mean there is spread within the family. However, no isolates were identified and classified; thus there is an alternative explanation of the common source in the local environment. Transmission within the family has been examined using molecular techniques, where an identical isolate has been shown to spread both vertically and horizontally.4D However, within the same family, unrelated isolates were also identified. Thus, there is evidence of person-to-person transmission, but the sharp decline in the incidence of *Helicobacter pylori* infection since World War II is best explained by changes in health status. For example, outdoor (private) toilets were largely replaced by ceramic bowls in the 1950s in the United States. In contrast, interpersonal activities do not appear to change significantly over time, making oral

transmission less likely.

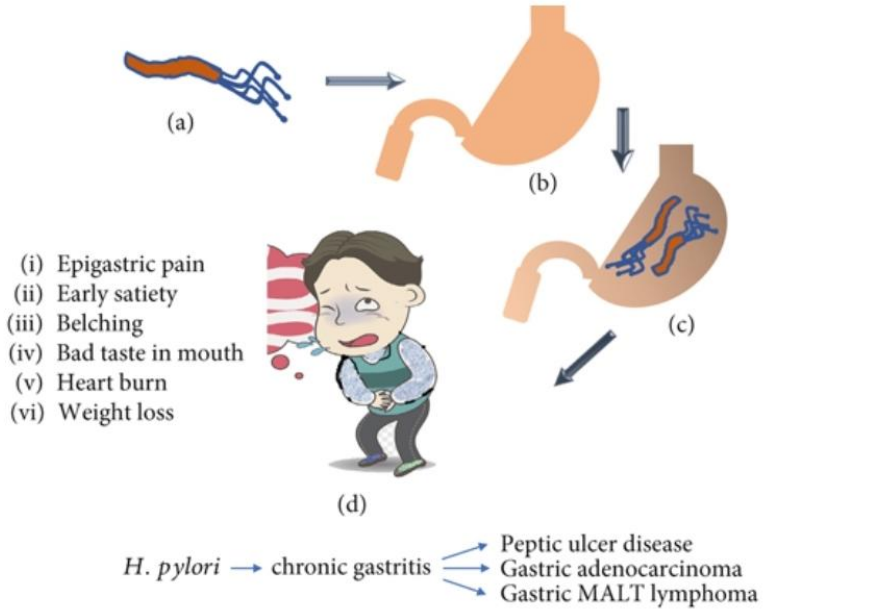
2.4.3 Iatrogenic Spread

Various procedures have been clearly associated with *H. pylori* transmission. A significant outbreak, in retrospect, of *Helicobacter pylori* infection, was reported by (Ramsey *et al*., 1999). right before *H. pylori* has been described. In this study of gastric secretions, a pH meter was used consecutively in volunteers, 17 of 34 of whom became chlorine deficient and developed acute gastritis. (Graham *et al*., 2003). reported details of another volunteer's endoscopic infection and again showed gastric acid deficiency and gastritis, the former of which resolved spontaneously. As mentioned earlier, 3 of the people who were treated for *H. pylori* was later found to have been reinfected during endoscopy. One can only guess at the organism's dose and the form in which it was transmitted, but the dose is probably very small. Data show that the risk of endoscopic infection is very low in instruments that are mechanically cleaned and then laundered.

2.4.4 Vectors

Recent data shows that domestic cats can carry the bacteria *Helicobacter pylori*. This study was based on the unexpected discovery that a commercial supplier was supplying cats to shelters with *H. pylori.* Domestic cats have been shown to carry Gastospirillum-like organisms, some of which are Helicobacter felis and others probably Helicobacter heilmannii. There are currently no large-scale epidemiological data on cats in community settings and on the existence or absence of significant colonization of cat populations. For now, the risk appears low. Non-human primates have been shown to be colonized by *Helicobacter pylori*-like organisms. However, colonizing the stomach of nonhuman primates with human-derived *H. pylori* can be difficult, revealing subtle differences. However, the limited interaction between humans and non-human primates

makes them a reservoir for *Helicobacter pylori* in humans. It has recently been shown that house flies are capable of mechanically transmitting the bacterium *Helicobacter pylori*. The ability of flies to transmit intestinal diseases has been known for decades. A recent study in Myanmar illustrated surprising rates of fly contamination, with over 70 fecal coliform species, 50 Salmonella species, and lower carrier rates of V cholerae and Shigella. (Grubel *et al.*34 fed newly hatched flies on plates of actively growing *H. pylor*i for 6 hours. *H. pylori* plates were removed and replaced with new, sterile plates every 6 hours and flies were sampled to test for the presence of *H. pylori*. The organism can be recovered from the external surface of the fly for up to 12 hours, but recovered from the gut and feces for up to 12 hours.



(a) *H. pylori* entry, adherence to gastric mucosa (b) exploitation of human immune system (c) multiplication, tissue damage and transmission to susceptible host (d) sign, symptoms and complication of *H. pylori* infection.

Fig 2. 3 Diagram showing process of *H. Pylori* transmission process (Smith *et al.,* 2019)

2.5 GASTRIC ULCER AND GASTRIC CANCERS

H. pylori infection is one of the most common infections in humans that can progress to stomach cancer. This bacterium causes gastric cancer through its specific virulence factors such as genes related to cytotoxin A, vacuolar toxin A and outer membrane proteins.

Cytotoxin-associated factor (CagA) and vacuolar toxin-associated factor (VacA) are H. pylori strain-specific, predominantly virulence factors associated with an increased risk of developing carcinoma stomach. The CagA protein is a 120–140 kDa protein that is translocated into host cells by the cag type IV secretion system, following H fixation. pylori and thus alter cell signaling mechanisms in gastric cells. The VacA protein is a cytotoxin produced by bacteria and causes vacuolization of epithelial cells. This gene is present in all strains but shows different variations in vacuolization activity. This aspect is characteristic of variations in the structure of the vacA gene in three regions: signal chain region (region s) (s1 or s2), middle region (region m) (m1 or m2) and middle region (region i) (i1, i2 or i3). These two virulence factors and their polymorphisms have been taken into account in many studies. Research (Matos et al., 2013) summarized the possible association between these genotypes and the risk of developing different gastric phenotypes. Scientists reviewed 44 studies, all of which had a case-control (n=13) or cross-sectional (n=31) design, including a total of 17,374 patients: 107 in the dysplasia group, 4994 in the gastric ulcer group, 5564 in the gastritis group and 6709 in the gastric cancer group. They confirmed an increased risk of gastric cancer in patients infected with H. pylori CagA+ strains and infected with VacA s1 and m1 strains. (Pandey et al., 2014) examined the comparative study of *H. pylori* and the possible influence of oncogenic CagA+ strains in the progression of gastric cancer. But not all *H. pylori* strains cause disease. Virulence is mainly determined by the cagA gene

and these strains are strongly associated with gastric cancer. They discuss the link between stomach cancer and stimulants such as tobacco and alcohol. According to their research, the number of *H. pylori* is found to be higher in precancerous and cancerous gastric lesions and is associated with smoking. This is because the infection is transmitted orally.

After entering gastric epithelial cells, unphosphorylated CagA interacts with E-cadherin (epithelial cadherin), leading to dissociation of the E-cadherin and β-catenin complex and accumulation of β-catenin in the cytoplasm and nucleus. Cadherins are proteins involved in selective adhesion between tissue cells. β-Catenin binding to α-catenin connects cadherins to actin filaments at the adhesion junction. The β-catenin/Tcf (T-cell factor) complex activates the expression of genes encoding cyclin D1 and c-Myc leading to abnormal cell proliferation. Unphosphorylated CagA also interacts with SOS (Son of Sevenless) binding to Grb-2 (growth factor receptor-associated protein 2), a guanine nucleotide exchange factor, and activates the Ras/MEK pathway /ERK leads to cell proliferation.

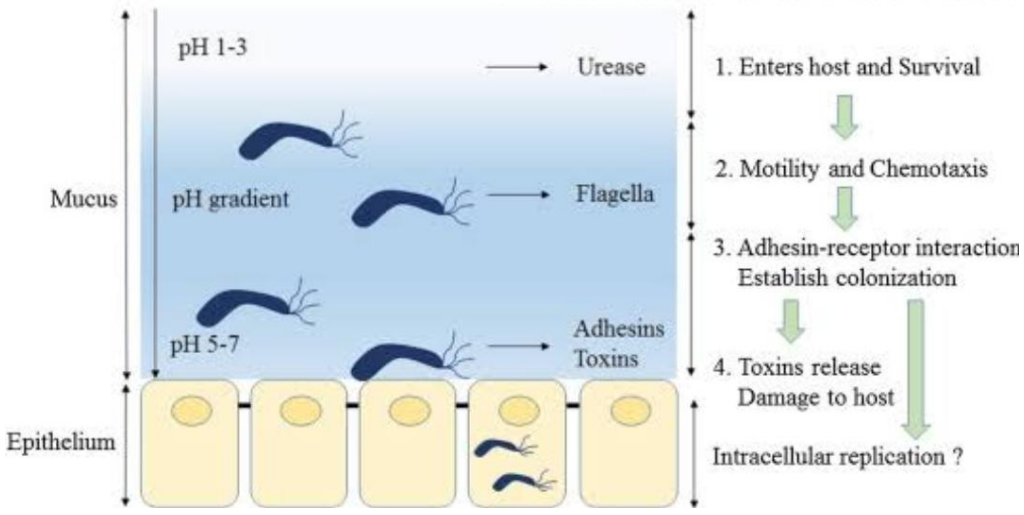


Fig 2.5 Mechnism of *H. Pylori* pathogenesis

(Cheng *et al* 2015)

2.6 RISK FACTORS OF *H.PYLORI*

The exact mechanism o*f H. pylori* infection is currently unknown; however, socioeconomic and environmental factors are known to play an important role. H. pylori infection occurs in childhood and occurs silently over many years; Only 30% of people develop signs and symptoms of gastritis. Transmission occurs by oral-oral, fecal-oral and gastro-oral routes. For example, mother-to-child transmission occurs if the mother's saliva is contaminated or due to poor hand hygiene. Pathogens are transmitted through direct routes such as kissing or sharing utensils or by indirect routes such as drinking water, air, animals, flies and food. The presence of *H. pylori* infection promotes the transmission of contaminants in the stool in a young population hospitalized during a gastroenteritis epidemic. Similarly, in frozen foods, pathogens can survive for a short time and be a source of infection. In addition, it is also related to eating habits, including milk, meat and vegetables. An Indian study found that the disease is more common in lower socioeconomic groups. Consuming unfiltered water, tobacco, and meat are risk factors for developing *H. pylori.* Additionally, drinking unboiled water in restaurants in urban areas increases the spread of pathogens. Pathogen colonization and transmission increase in crowded living conditions, with the use of non-steroidal anti-inflammatory drugs, in blood type O, with obesity and a family history of stomach disease. Consumption of fried foods is positively correlated with infection. Antibiotic resistance is another factor that contributes to *H. pylori*, because antibiotic resistance is the main cause of failure to completely eliminate *H. pylori*. In some cases, patients will undergo 14 days of treatment with four drugs and then have the infection relapse or develop complications from *Helicobacter pylori* infection. The main cause of antibiotic resistance is the overuse of antibiotics; Metronidazole has the highest resistance rate and is widely used in most countries to treat gastrointestinal diseases. The global emergence of antibiotic-resistant bacteria, which threatens the effectiveness of antibiotics, is a major cause of increasing rates of bacterial infections, including *H.*

pylori infections. who have transformed medicine and saved countless lives. The main cause of antibiotic resistance is the overuse or misuse of antibiotics because many people mistakenly believe that antibiotics can cure all diseases. This causes people to view antibiotics as a panacea and regularly consult their doctors, even for viral diseases. This combined with improper use of antibiotics leads to drug resistance. In addition, improper antibiotic prescribing also contributes to antibiotic resistance. Research shows that in 30 to 50% of cases, the indication for treatment, antibiotic choice, and duration of antibiotic treatment are incorrect. In many countries, antibiotics are available without a prescription; This ease of obtaining antibiotics also contributes to the problem. Lack of regulation and easy availability of many cheap antibiotics in the market as well as online lead to overuse and misuse of antibiotics. Finally, the pharmaceutical industry lacks new drug development due to reduced financial incentives and onerous regulatory requirements. contributing to the antibiotic resistance crisis. New research policies and coordinated efforts to control antibiotic resistance are urgently needed. This will help kill disease-causing bacteria. The lifetime risk of developing ulcer disease is 10-20%, the risk of distal gastric cancer is 1-2%, and the risk of mucosa-associated lymphoid tissue (MALT) lymphoma is <0 .1% in patients positive for Helicobacter pylori. Gastric and duodenal ulcers are closely associated with this pathogen. During the first decade after the discovery of *Helicobacter pylori*, it was discovered that 95% of duodenal ulcers and 85% of gastric cancers were caused by *Helicobacter pylori.* The development of peptic ulcer disease (PUD) is often caused by the use of *H. pylori* and nonsteroidal anti-inflammatory drugs (NSAIDs); however, any individual using NSAIDs and infected with Helicobacter pylori did not develop PUD. Patients with duodenal ulcers often complain of abdominal pain two to three hours after eating on an empty stomach or stomach pain at night, while patients with gastric ulcers complain of nausea, vomiting, weight loss, and abdominal pain. after meal. Complications of PUD are bleeding, perforation, penetration, gastric

tube obstruction, and gastric malignancy (adenocarcinoma and MALT). Bleeding is the most common complication of PUD, and nearly 40% to 60% of upper gastrointestinal bleeding is caused by PUD. Regarding stomach cancer, As the fifth most common cancer and third leading cause of death worldwide, *H. pylori* is a major risk factor for its development. Precancerous lesions are often caused by *H.pylori* infection with transformation of normal mucosa to non-atrophic gastritis, which can lead to atrophic gastritis and intestinal metaplasia.

All these complications of *H. pylori* is thought to be due to the severity of gastritis, which is determined by a number of host and bacterial factors. The cytotoxin A pathogenicity island-associated gene (cag PAI) is a determinant of *H. pylori*-specific virulence. It is a 42 kb insertion element consisting of 32 genes encoding the type 4 bacterial secretion system. The cag A pathogenicity island is present in 60–70% of H. pylori and almost 100% of East Asian *H. pylori* strains. The presence of cag PAI is associated with more severe gastritis, peptic ulcer disease, atrophic gastritis, and gastric cancer. Among host factors, evidence emphasizes acid production induced by *H. pylori.* Increased acid production limits *H.pylori* gastritis in the antrum, increasing the risk of duodenal ulcers, while reducing acid secretion promotes more proximal inflammation, thereby promoting the risk of atrophic gastritis and gastric ulcers. stomach and stomach cancer.

Although the global incidence of peptic ulcer disease (PUD) is decreasing, PUD remains one of the most common upper gastrointestinal diseases worldwide due to *H. pylori* infection and increased use of antibacterial drugs. nonsteroidal inflammation. In Korea, the rate of *H. pylori* infection is also decreasing but is still the leading cause of PUD. The consequences of *H.pylori* infection are due to an imbalance between bacterial virulence factors, host factors and environmental influences.

2.7 SYMPTOMS OF *H.PYLORI*

Most people with chronic gastritis or duodenitis caused by *H. pylori* have no symptoms. However, about 5 to 10 percent of people develop more serious problems, such as stomach and duodenal ulcers, and rarely stomach tumors.

Ulcers may cause a variety of symptoms or no symptoms at all. The most common symptoms of ulcers are:

Pain and discomfort (mainly in the upper abdomen)

Swelling

Feeling full with a small amount of food

Anorexia

Nausea or vomiting

Bloating

Unintentional weight loss

Dark or tarry stools

Indigestion

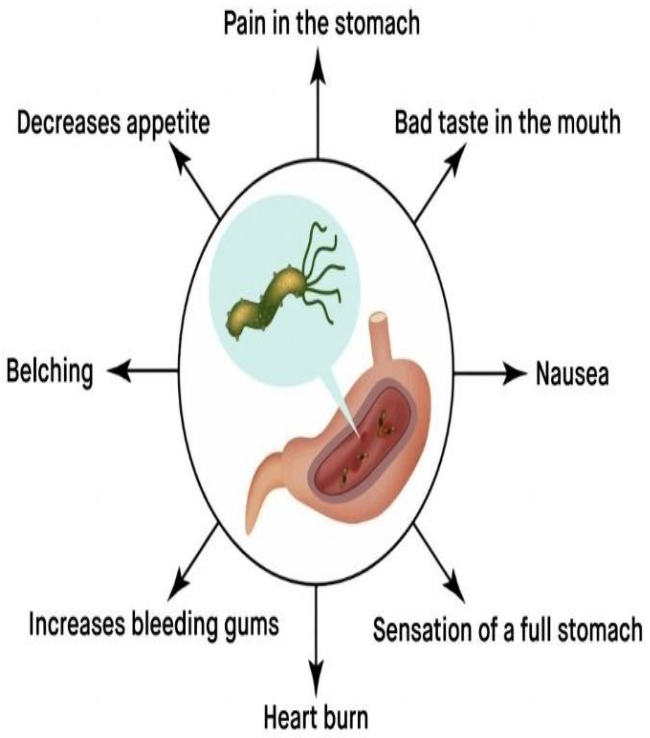


Fig 2.6 Diagram showing symptoms o*f H.pylori* infection

(Smith *et al* 2019)

2.8 DIAGNOSIS OF *H. PYLORI*

Accurate diagnosis of *H. pylori* infection is essential for effective treatment of various gastroduodenal diseases. A number of tests have been developed over time to detect *H. pylori* infection. Each test has advantages and limitations. In the clinical setting, the use

of a single test is often sufficient for diagnosis. Usually, tests are divided into two types, which are invasive and non-invasive tests.

Invasive testing relies on endoscopic biopsies performed in the duodenum for histology, culture, rapid urease testing, and molecular methods.

Noninvasive tests rely on peripheral samples, such as blood, breath, and stool samples, to detect antibodies, bacterial antigens, and urease activity. Fecal antigen testing and urea breath testing are the most widely used noninvasive tests in clinical settings for the detection of *Helicobacter* pylori and during follow-up after antibiotic treatment, while blood samples are used for screening and epidemiological studies.

2.8.1 BLOOD TEST

Test for antibodies (infection-fighting cells) against *Helicobacter pylori* Inspection procedures:

A health care professional will take a blood sample from a vein in your arm using a small needle.

After the needle is inserted, a small amount of blood is collected in a test tube or vial.

This serological test occur whereby the pouch is brought to room temperature before opening and the test device is removed from the sealed pouch which is been used as soon as possible And the device is been placed on a clean and level surface before venipuncture. ; When venipuncture occur the dropper is hold vertically and 2 drops of the whole blood (approximately 50µL) is transferred to the specimen well (S) of the test device, and also 1 drop of buffer which was actually timed. The last part was to wait for the colored line(s) to appear. Results at 10 minutes and this was adhere to

2.8.2 Stool EXAMINATION

Fecal antigen testing. This is the most common stool test to detect *H. pylori*. The test looks for proteins (antigens) associated with *H. pylori* infection in the stool.

2.8.3 SENSORY PCR TEST

A laboratory test called a stool polymerase chain reaction (PCR) test can detect *H. pylori* infection in the stool. The test can also identify mutations that may be resistant to antibiotics used to treat *H. pylori.* However, this test is more expensive than the stool antigen test and may not be available at all medical centers.

2.8.4 BREATH TEST

During the breath test - called a urea breath test - you swallow a pill, liquid or pudding that contains labeled carbon molecules. If you are infected with *Helicobacter pylori,* carbon is released when the solution comes into contact with *H. pylori* in your stomach.

2.8.5 SCOPE TEST

The health care provider may perform a scope test, called an upper endoscopy test. Your doctor may perform this test to investigate symptoms that may be caused by conditions such as peptic ulcer disease or gastritis that may be caused by *H. pylori*. For this test, you will be given medicine to help you relax. During the procedure, your doctor will pass a long, flexible tube with a small camera (endoscope) through your throat and esophagus, then through your stomach and the first part of your intestines (duodenum). This tool allows your doctor to visualize any problems in your upper digestive tract. Your provider may also take a tissue sample (biopsy). These samples are tested for *H. pylori* infection. Because this test is more invasive than a breath or stool test, it is often performed to diagnose other digestive problems as well as *H. pylori* infection. Healthcare providers may use this test to perform additional tests and check for other digestive disorders. They can also use this test to determine exactly which antibiotic is best to treat an *H. pylori* infection, especially if the first antibiotic tried does not clear the infection.

This test may be repeated after treatment, depending on what was discovered during the initial endoscopy or if symptoms persist after treatment for *H. pylori* infection. However, complications of *H. pylori* infection are not diagnosed with noninvasive tests. People infected with *H. pylori* often develop certain antibodies in their circulation, such as IgA, IgG, and IgM. These antibodies are determined by specific serological tests, which are noninvasive, rapid, inexpensive, and useful for diagnosing *H. pylori* infection or when There are equivocal test results with other methods due to complications, such as bleeding ulcers, or when the patient is taking antibiotics or antisecretory therapy. Detection of serological responses to cytotoxin A-associated gene (cag A) provides useful information in cases of serious gastroduodenal infections, such as gastric malignancy. thick and MALT.

2.9 TREATMENT OF *H.PYLORI*

People with a history of peptic ulcer disease, progressive gastric ulcer disease, or progressive duodenal ulcer disease associated with *H. pylori* infection should receive treatment. Successful treatment of Ms. *H. pylori* promotes ulcer healing, prevents ulcer recurrence, and reduces the risk of ulcer complications (such as bleeding). U.S. and international guidelines recommend eradication of *H. pylori* and, if infected, treatment to eradicate Helicobacter pylori infection. There are no medications that can cure *H pylori* infection. Most treatment plans involve taking multiple medications over 14 days. Most treatments include drugs called proton pump inhibitors. This medication reduces the production of stomach acid, allowing tissue damaged by infection to heal. Examples of proton pump inhibitors include lansoprazole (Prevcid), omeprazole (Prilosec), pantoprazole (Protonix), rabeprazole (AcipHex), dexlansoprazole (Dexilant), and esomeprazole (Nexium). Usually, two antibiotics are also recommended. This reduces the risk of treatment

failure and antibiotic resistance. The number of patients infected with *H.pylori* is increasing. Therefore, it is important to take all prescribed medications for the entire course of treatment (usually 10 to 14 days) and then get tested to make sure that the infection is gone. Up to 50% of patients experience side effects when treated with *H. pylori*. Side effects are usually mild and less than 10% of patients discontinue treatment due to side effects. People who are susceptible to side effects may find relief by adjusting the dosage and duration of treatment. Some treatment plans use drugs called metronidazole (Flagyl) or clarithromycin (Biaxin). These medications can cause a metallic taste in the mouth and nausea.

Avoid alcoholic beverages (beer, wine, etc.) while taking metronidazole. This combination may cause skin flushing, headache, nausea, vomiting, sweating and increased heart rate.

Bismuth found in some formulations can cause black stools and constipation. Many treatments cause diarrhea and abdominal pain. Treatment failure – Up to 20% of patients infected with *H. pylori* do not recover after completing the first treatment. In this case, a second treatment regimen is often recommended. Retreatment typically requires the patient to take a proton pump inhibitor plus two antibiotics and bismuth subsalicylate ("quadruple therapy") for 14 days. At least one of the antibiotics is different from the antibiotic used in the initial treatment. After completing treatment for *H. pylori,* testing should be repeated to ensure the infection has been eliminated. This is usually done through a breath test or stool test. Blood tests are not recommended for initial diagnosis or follow-up testing; Antibodies detected by a blood test often remain in the blood for four months or more after treatment, even after the infection has cleared.

CHAPTER THREE

METHODOLOGY

3.0 MATERIALS AND METHODS

3.1 Study setting and design

The sample collection and analysis was carried in the Faculty of College of Medical Sciences and and faculty of Agriculture of the university of Benin, Nigeria. The study involved 93 both male and female undergraduate students systematically recruited from across the university. The study will adopt a cross-sectional design.

3.1.1 Ethical Consideration

Approval for the study protocol was obtained from the Ethics and Research Committee of the Faculty of Pharmacy, University of Benin (Appendix I). Also, informed consent was obtained from all the study participants (Appendix III)

3.1.2 Inclusion Criteria

Considering that *H. pylori* infection can be asymptomatic in 80% of the infected, and the main symptoms and complications of *H. pylori* infection are gastroduodenal diseases, the target population for this study were students with symptoms of gastroduodenal disorders. The inclusion criteria were;

1. Willingness to sign an informed consent form for the study
2. Aged 18 years or older
3. A registered student across some faculties

3.1.3 Exclusion Criteria

a) Students who are taking PPIs, bismuth-containing drugs and antibiotics

b) Students who will not consent to the study.

c) Students who are below 18 years of age.

3.2 Data and sample collection

Firstly, following the expression of willingness to participate in the study, students were administered questionnaire containing sociodemographic characteristics (e.g., age, sex, faculty, accommodation type, among others) and clinical information (e.g., awareness of having any form of ulcer, experience of any signs and symptoms of illness, family member with ulcer, among others). The students were asked questions on their knowledge of *H. pylori* infection and its clinical significance. The blood samples were collected from the vein of each study

participant by an experienced phlebotomists from University of Benin Teaching Hospital (UBTH) and kept in a plain sample container for analysis.

3.3 Detection of Helicobacter pylori antibody in the blood

3.3.1 Principles of the Rapid Test Kit

The *H. pylori* Rapid Test Device (Whole Blood/Serum/Plasma) is a simple test that utilizes the combination of *H. pylori* antigen coated particles and anti-human IgG to qualitatively and selectively detect *H. pylori* antibodies in Whole Blood, serum, or plasma in just minutes.

The *H. pylori* Rapid Test Device (Whole Blood/Serum/Plasma) is a qualitative membrane based immunoassay for the detection of *H. pylori* antibodies in the whole blood, serum or plasma. In this test procedure, anti-human IgG is immobilized in the test line region of the test. After specimen is added to the specimen well of the device, it reacts with *H. pylori* antigen coated particles in the test. This mixture migrates chromatographically along the length of the test and interacts with the immobilized anti human IgG. If the specimen contains *H. pylori* antibodies, a colored will appear in the test line region indicating a positive result. If the specimen does not contain *H. pylori* antibodies, a colored line will not appear in this region indicating a negative result. To serve as a procedural control, a colored line will always appear in the control line region, indicating that proper volume of specimen has been added and membrane wicking has occurred.

3.3.2 Reagents

The test contains *H. pylori* antigen coated particles and anti-human IgG coated on the membrane

3.4 Materials

Diagnostic Test kit

Package insert

Droppers

Buffers

Specimen collection containers

Syringes(2ml)

Centrifuge

Heparinized capillary tubes and dispensing bulb (for fingerstick whole blood only)

Timer

Torniquette

Disposable nylons and Nose masks

Hazards bars

Hand gloves

Cotton wool

Methylation spirit

Weighing scale

Hand sanitizer

3.5 Method

The test, specimen, buffer and/or controls were allowed reach room temperature (15-30°C) prior to

1.The pouch is brought to room temperature before opening and the test device was removed from the sealed pouch which was used as soon as possible

2. And the device was placed on a clean and level surface before venipuncture

Venipuncture Whole Blood specimens; The venipuncture whole blood specimen was adopted in this study which the dropper is hold vertically and 2 drops of the whole blood (approximately 50μL) is transferred to the specimen well (S) of the test device, and also 1 drop of buffer which was actually timed.

3. The last part was to wait for the colored line(s) to appear. Results at 10 minutes and this was adhere to

3.5.1 Interpretation of Results

POSITIVE; Two lines appeared. One colored line wad on the control line region (C) and another apparent colored line was on the test line region (T).

NOTE; The intensity of the color in the test line region (T) will vary depending on the concentration of *H. pylori* antibodies in the specimen. Therefore, any shade of color in the test line region (T) was considered positive.

NEGATIVE; One colored line appeared in the control line region (C). No line appeared in the test line region (T).

INVALID; Control line failed to appear. Insufficient specimen volume or incorrect procedural techniques were mostly the reasons for control line failure. The procedure was reviewed and repeated with a new test test kit.

3.5.2 Quality Control ; An internal procedural control was included in the test. A colored line appearing in the control line region (C) is an internal procedural control. It confirms sufficient specimen volume, adequate membrane wicking and correct procedural techniques.

Control standards were not supplied with this kit; however, it is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

3.6 Data Analysis

All analysis was done in Stata version 16, indicating statistical significance as p-value of less than 0.05. Descriptive analysis will be used to describe the study population, using frequency and percentages for binary/categorical variables, mean and standard deviations for normally distributed continuous variables, and median and interquartile range for non-normally distributed continuous variables. The prevalence of H. pylori carriage among the student population will be presented in terms of proportions with 95% confidence intervals (CIs), while the associated factors with *H. pylori* carriage will be presented in terms of adjusted odds ratio with 95% CIs

CHAPTER FOUR

RESULTS AND DISCUSSION

Table 4.0 shows the Rapid test outcome of H.pylori among students college of Basic Medical Sciences, and Agriculture of the University of Benin

The Rapid test outcome shows that total number of participants of 93, which 59 students came out with a negative test result (approx. 63.4%). While 34 students (approx. 36.6%)came out Positive.

4.0 Rapid diagnostic test outcome for *H. pylori* test

RDT *H. pylori* test	**Frequency (%)**
Negative	59 (63.4)

Positive	34 (36.6)
Total	93 (100.0)

Table 4.1 shows the prevalence of H.pylori among students in College of Basic Medical Sciences, and faculty of Agriculture and its associated risk factors

4.1.1 AGE CATEGORY

In the course of this study, it was observed from the data collected that the prevalence of *Helicobacter pylori* among the above various faculties majorly affected those between the ages of 21 and 25 with a percentage distribution of; 59 Negative cases and 36 positive cases with a

mean value of 21.4 Negative and 21.1 positive cases. The age category for the negative cases include age ranges from 16 to 20 is 29(49.2%), age range from 21 to 25 is 23(38.0%) while age range from 26 to 59 Is 7(11.9%) respectively. For the positive cases age range from 16 to 20 is 16(47.1%), age range from 21 to 25 is 16(47.1%) while from 26 to 59 is 2(5.8%) as represented in Table 4.0

4.1.2 SEX

For the course of this study, a total of 93 student participants were taken from the faculty of Medicine, Dentistry, Nursing, Basic Medical Sciences and Agriculture. The essence of this data collection was to determine the percentage of genders most likely prone to the bacteria infection and from the table above which shows total of male cases 16(47.1%) and female 18(52.9%). From this study there is a probability of females been proned to H.pylori infection.

4.1.3 LEVEL OF STUDY

During this study, the level of study by student was also considered which could also be a risk factor, from the Table 4.1 it shows that students in 100 level 14 cases(41.2%)is at higher risk of this bacteria infection.

4.1.4 PREVIOUS DIAGNOSIS OF ULCER

During the process of data collection, participants were asked for a possible diagnosis of ulcer from previous medical tests carried out. From the faculty of Medicine, Dentistry, Nursing, Basic Medical Sciences and Agriculture, it was observed that out of 84 students who said “NO” about

52 came out Negative, while 32 came out with a positive result. And 9 students who responded "YES" to a previous diagnosis 7 came out Positive while 2 came out Positive. The essence of this question was to provide an equal opportunity for all participants to confirm and re-confirm (in the case of those with previous diagnosis) the presence or absence of the bacterium antigen.

4.1.5 THINK YOU HAVE AN ULCER

Participants were asked if they think they currently have an ulce and what they know about the infection. The overall results obtained from the students in the College of Basic Medical Sciences, and faculty of Agriculture shows that high majority of students think they dont have the bacteria infection but know about it.

4.1.6 MAJOR WATER SOURCE

From data collected during the study, it was concluded that most students' water source is the borehole.

4.1.7 AVAILABILITY OF SOAP IN THE TOILET

Helicobacter pylori infections can also be associated with poor hygiene. During this study, students were asked if there was always an available soap for use in the toilet. The majority answered "NO" which 76.5% came out Positive while others negative according to Table 4.1

4.1.8 LIVE WITH SOMEONE WHO HAS ULCER (SCHOOL AND FAMILY)

In previous studies, it was observed that people who stay in close contact with someone who has a positive result for an ulcer can be prone to having the presence of *Helicobacter pylori* antigen in them. Therefore, during this study, we asked each study participant if they live with someone who has an ulcer, either in school or a family member. The Majority responded "NO" however there was a higher percentage of positive results which 85.3% came out Positive.

4.1.9 PRIMARY FOOD SOURCE PER WEEK

Taking into consideration hygiene as a risk factor, student participants were asked what their individual primary source of food was per week. The results obtained from this study indicate the majority of students eat home-cooked meals followed by mixed meals. Taking into consideration only those who eat home-cooked meals, a total of 41.2.% came out Positive and also for participants who eat mixed meal with also 41.2%.

4.1 Sociodemographic characteristics of the study participants in relation to *H. pylori* positivity and risk factors

Characteristic	**Negative (n=59)**	**Positive (n=34)**
Mean (SD) age, year	21.4 (4.3)	21.1 (2.7)
Age category, year		
16-20	29 (49.2)	16 (47.1)
21-25	23 (39.0)	16 (47.1)
26-59	7 (11.9)	2 (5.8)
Missing	0 (0.0)	0 (0.0)
Sex		
Female	30 (50.9)	18 (52.9)
Male	29 (49.1)	16 (47.1)
Level of study		
100 level	27 (45.8)	14 (41.2)

200 level	7 (11.9)	4 (11.8)
300 level	5 (8.5)	4 (11.8)
400 level	12 (20.3)	8 (23.5)
500-600 level	8 (13.6)	4 (11.8)
Missing	0 (0.0)	0 (0.0)
Previous diagnosis of ulcer		
No	52 (88.1)	32 (94.1)
Yes	7 (11.9)	2 (5.9)
Think you have an ulcer		
No	45 (76.3)	28 (82.4)
Yes	10 (17.0)	5 (14.7)
Missing	4 (6.8)	1 (2.9)
Reliable water source		
No	53 (89.8)	28 (82.4)
Yes	6 (10.2)	6 (17.7)
Major water source		
Borehole	52 (88.1)	29 (85.3)
Reservoir/well	3 (5.1)	3 (8.8)
Mixed	4 (6.8)	2 (5.9)
Availability of soap in the toilet		
Yes	6 (10.2)	8 (23.5)
No	53 (89.8)	26 (76.5)
Live with someone with an ulcer (school)		
No	49 (83.1)	29 (85.3)
Yes	10 (16.9)	5 (14.7)
Family member with ulcer		
No	34 (57.6)	23 (67.7)
Yes	25 (42.4)	11 (32.4)

Primary food source per week		
Home/Personal	28 (47.5)	14 (41.2)
Restaurant (Buka)	4 (6.8)	6 (17.7)
Mixed	27 (45.8)	14 (41.2)
Share toilet		
No	25 (42.4)	10 (29.4)
Yes	34 (57.6)	24 (70.6)

CHAPTER FIVE
DISCUSSION AND CONCLUSION

5.0 DISCUSSION

Helicobacter pylori infection is estimated to affect 50% of the global population and is a public health problem (Mladenova, 2021). This present study was conducted to evaluate the prevalence of *Helicobacter pylori* among students in College of Medical science and faculty of Agriculture health sciences and faculties of agriculture. A total of 93 (100%)participants were systematically recruited for the study, of whom 59 (63.4%) tested positive and 34 (36.6%) tested negative. It was observed that females had a higher number of positive cases 18 (52.9%) than male 16 (47.1%) but not with a high margin.This is consistent with reports by (Oti *et al.,* 2021) among students of Nasarawa state university and (Ombugagu *et al*., 2018) among dyspepsia patients in Jos. Part of the study assessed their knowledge about *H. Pylori* and ulcers, which was impressive. This is similar to the results of students' *H. Pylori* knowledge reported in

Saudi Arabia (Hafiz et al., 2021). This may be because medical students on campus have taken medical microbiology courses and, therefore, may have been exposed to some information about *H. Pylori* Infection.This study highlights the necessity for educating and enlightening students at various universities about *Helicobacter pylori,* its potential sources, risk factors, mode of transmission, and potential control measures. Ultimately, this research contributes to a deeper understanding of *Helicobacter pylori* and its implications for this specific demographic. It also underscores the importance of proactive healthcare measures in promoting healthcare awareness among University students. The research highlights varying prevalence rates of *Helicobacter pylori* among university students. Such prevalence data offer valuable insights into the overall health of this age group. High prevalence rates may indicate a need for increased awareness and intervention measures. One of the notable aspects of the study is its exploration of risk factors associated with *Helicobacter pylori* infection. Lifestyle choices, hygiene practices, and dietary habits play a crucial role. Understanding these factors can help in formulating preventive strategies for students.The high prevalence in this study may be due to the fact that many of these students live in homes and out-of-school settings where good hygiene is not observed, constituting a risk factor for H. Pylori transmission.

5.1 CONCLUSION

During this study about *Helicobacter pylori* and its prevalence among students in College of Basic Medical Sciences and faculty of Agriculture of the University of Benin , the result revealed that 3 out of 10 students are at risk of this bacteria infection and the need for good hygiene, environmental sensitization should be thoroughly looked upon.

REFERENCE

Balakrishna, N ., Krishnaveni, D., Bhayal, A. C., Porika, S. K., Malladi, U. D. Macherla, R. A. Jyothy, N. and Pratibha, A. V.(3013).; Association of endothelial nitric oxide synthase gene T-786C promoter polymorphism with gastric cancer:World Journal of Gastrointestinal Oncology 7 (7), 87-99

Bastos, J., Peleteiro, B., and Barros, R. (2013) Sociodemographic Determinants of Prevalence and Incidence of Helicobacter pylori Infection in Portugese adults. Helicobacter 18(6): 413-422.

Bury-Moné, S. N., Kaakoush, C., Asencio, F., Mégraud, M. T., Hilde, D. R. and , George, L.(2006).:Is Helicobacter pylori a True Microaerophile?;Helicobacter 11 (4), 296-303

Chaput, C. C., Ecobichon, N., Pouradier, J. C. Rousselle, A., and Namane, I. G. (2016).:Role of the N-Acetylmuramoyl-l-Alanyl Amidase, AmiA, of Helicobacter pylori in Peptidoglycan Metabolism, Daughter Cell Separation, and Virulence;Microbial Drug Resistance 22 (6), 477-486

Cheng, Y. K., Bo S. S., and Jiunn, J. W.(2015. An overview of bacteria virulence factor and pathogenesis. Biomedical Journal.39(3)14-23

David, Y., Graham, Y. L., Ming–Shiang, W.(2019).Rational Helicobacter pylori therapy: evidence-based medicine rather than medicine-based evidence;Clinical Gastroenterology and Hepatology 12 (2), 177-186

Drum, L. M., Elizabeth, J. W., and Graham, S.(2010).Role of Helicobacter pylori in gastric cancer. Journal of clinical pathology. 8(5)345-377

Eaton, K. A., Brooks, D. R. and Morgan, S. K.(1991):Essential role of urease in pathogenesis of gastritis induced by Helicobacter pylori in gnotobiotic piglets;Infection and immunity 59 (7), 2470-2475

Enroth, H., Kristina, H., Sae-Won, H., Peter, D. K., Arkansas, R. O, Robert, H. G., Dolores, G. Evans, L. E. David, Y., and Graham, F. A. (1996). Helicobacter pylori in drinking water ;GASTROENTEROLOGY-BALTIMORE THEN PHILADELPHIA- 110, 1031-1035,

Fashner, J., and Gitu, C. A. (2015) Diagnosis and Treatment of Peptic Ulcer Disease and H. pylori Infection, American Family Physician 91(4): 236-242.

Fernandes, E. E., De Koster, M., Buset, M., and Deltenre, W.(2017):Helicobacter pylori: the link with gastric cancer European journal of cancer prevention 3 (3), 247-258

Fox, G. J. and Alexander, S.(2016):The role of the gastrointestinal microbiome in Helicobacter pylori pathogenesis;Gut microbes 4 (6), 505-531

Gancz, H., Kathleen, R. M., and Jones, D. S.(2008):Sodium Chloride Affects Helicobacter pylori Growth and Gene Expression;Journal of bacteriology 190 (11), 4100-4105

Graham, D. T., Nguyen, D., Ramsey, Y., Shaib, S., Shiota, M. Velez, R. C., Bhupinderjit, A. M. and Vela, H. B.(1999).The Prevalence of Helicobacter pylori Remains High in African American and Hispanic Veterans;Helicobacter 20 (4), 305-315

Grubel, K. O., and David R. C.(2016):How is Helicobacter pylori transmitted?;Gastroenterology 113 (6), S9-S14

Hafiz, T. A., D'Sa, J. L., Zamzam, S., Dionaldo, M. L., Mubaraki, M. A., and Tumala, R. B.(2021).Helicobacter pylori infection:Comparison of Knowledge between Health science and Non-health science students. International journal of Environmental Research and Public Health. 18(15),8173

Higuera, V. (2005) Peptic Ulcer, Healthline 1(1): 3-5.

Hopkins, J. R., Luigi, S., and Girardi, E. A(2003) :Special reports and reviews;Gastroenterology 110 , 1244-1252

Khosravi, Y., Yakhya D., Bee Hoon Poh, C. N., Mun Fai Loke, K. G.,and Jamuna, V. (2014);Culturable Bacterial Microbiota of the Stomach of Helicobacter pylori Positive and Negative Gastric Disease Patients;The Scientific World Journal.23(5)34-55

Li, Y., Qing, L.X., Meng, C. Z., Guo-Yi, T., Sha, L., and Ren-You, G. H. (2018):Natural Products for the Prevention and Management of Helicobacter pylori Infection;Comprehensive reviews in food science and food safety 17 (4), 937-952

Loke, M., Anthony, P. M., Andrew, C. J., Houghton, N. T., James, G., Fox, T. C. and Wang, E. A.(2019):Intact Gram-Negative Helicobacter pylori, Helicobacter felis, and Helicobacter hepaticus Bacteria Activate Innate Immunity via Toll-Like Receptor 2 but Not Toll Infection and immunity 72 (11), 6446-6454.

Malaty, M. H., David Y G., Wendy, A. W., Sathanur, R., Srinivasan, Michael, O., and Gerald S. B.,(1999):Natural history of Helicobacter pylori infection in childhood Clinical Infectious Diseases. The Lancet. 28 (2), 279-282,

Marshall, B. J., Warren, J. R, and Levi, O. K.(1989). Role of Helicobacter pylori in duodenal ulcer. Journal of clinical pathology ; 39(4)356-3

Matos, I. J. Henrique, A. C., Ricardo, M. P., and Mario, D. R.(2013).Helicobacter pylori CagA and VacA genotypes and gastric phenotype: a meta-analysis;European journal of gastroenterology & hepatology 25 (12), 1431-1441

Mladenova, I. (2021).Clinical relevance of Helicobacter pylori infection. Journal of clinical medicine 10(3)452-455

Monteiro, L. M., Oleastro, P., and Lehours, F.M.(2009):Diagnosis of Helicobacter pylori Infection;Helicobacter 14, 8-14

Nilsson, H. O., Jens, B,. Waleed, A., Al-Soud, A. L , Leif, P. A., and Torkel, W.(2002).Effects of cold starvation, acid stress, nutrient on metabolic activity of Helicobacter pylori ;Applied and Environmental Microbiology 68 (1), 11-19

Nwodo, E. N. Yakubu, S. E., Jatau, E.D., and Yabaya, A. (2009) Seroprevalence of Helicobacter pylori Infection in Patients with Gastritis and Peptic Ulcer Disease in Kaduna, African Journal of Basic and Applied Sciences 1(5-6): 123-128.

Ombugadu, D. O. , Oladele, O. V., Onuoha, S. C., Omisope, O., and Ani, A.,(2018).Prevalence of Helicobacter pylori igG and stool antigen detection from dyspeptic patients in Jos, Nigeria. African Journal of Cliinical and Experimental Microbiology. 19(3),177-185

Oti, B. V., Pennap, R. G., Dennis, O., Ajegena, S. A., and Adoga, M. P.(2017).Prevalence and predictor of Helicobacter pylori among patient attending a health care facility in North Central Nigeria. Asian pacific journal of Tropical disease 7(6),352-355

Samina, K. B., and Muhammad, Khan.(2020) - Overview of Helicobacter pylori;Advanced Gut & Microbiome Research.2(2)35-66

Sherin, H., John, T., Pinto, L. U., Liu, M., Merzianu, E. M., and Sordillo, S. F., (2003):Helicobacter pylori decreases gastric mucosal glutathione;Cancer letters 164 (2), 127-133

Smith, S., Fowora, M., and Pellicano, R.(2019).Infection with Helicobacter pylori and challenges encountered in Africa. World Journal of Gastroenterology. 25(25)334-355

Takeuch, H. I., Nishimori, T., Minakuchi, K., Morimoto, S., Sano, S., Onishi, D., Vullo, A. and Scozzafava, C. T.(2006):Carbonic Anhydrase Inhibitors: DNA Cloning and Inhibition Studies of the α-Carbonic Anhydrase from Helicobacter pylori, A New Target for Developing;Journal of medicinal chemistry 49 (6), 2117-2126

Tijjani, B.,and Umar, A. (2008) Peptic Ulcer Disease and Helicobacter Infection at Kano, Nigeria. The Internet Journal of Gastroenterology 8(1): 1-4.

Thomas, G. J., and Steven, L. P.(2019),:Transmission of Helicobacter pylori and the role of water and biofilms;Journal of water and health 7 (3), 469-477

Yang, H. W., and Jianfu, J.(2016),:Using Probiotics as Supplementation for Helicobacter pylori Antibiotic Therapy;International journal of molecular sciences 21 (3), 1136

APPENDIX I

ETHICS COMMITTEE
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10th August, 2023

Our Ref: EC/FP/023/10

Omorede Ikponmwonsa-Eweka
Department of Medical Biochemistry
Faculty of Basic Medical Sciences
University of Benin
Benin City

Dear Mrs Ikponmwonsa-Eweka,

RE: ETHICAL APPROVAL

Your request for ethical approval to carry out your research titled: Prevalence of *Helicobacter pylori* and its determining factors among University of Benin students: a cross sectional study has been reviewed by the Ethics Committee and you are hereby granted approval for the study.

Note that you are to adhere strictly to the methods described in your proposal. You are also to limit your research to the Questionnaire and to the sample size specified in the proposal. If there is any need in the course of the study for protocol variation, it should be re-submitted to the Committee for consideration.

Yours faithfully,

Prof (Mrs) O.J Owolabi
Chairperson, Ethics Committee

A study on the prevalence of *H. pylori* and its associated factors among university students in Benin City, Nigeria

Participant ID:

Sociodemographic and clinical characteristics

Faculty of study:

Department of study:

Year of study:

Mobile number:

Age, in year

Sex: Male ☐ Female ☐

State of residence while on holiday:

Height (cm):

Weight (kg):

Waist circumference:

What is your blood group:

what is your genotype:

Have you ever been diagnosed with an ulcer? No ☐ Yes ☐

- If yes, when was the diagnosis made (try to remember the month and year):
- If not, do you think that you might have an ulcer? No ☐ Yes ☐

What's your tribe?

Setting of residence outside school: Urban ☐ Rural ☐ Semi-urban ☐

Signs and symptoms

I have experienced this/these symptoms **in the last 7 days** (Tick all that apply):

Sign/symptom	**No**	**Yes**
I experience an ache or burning pain in the stomach (abdomen)		
I experience stomach pain that may be worse when hungry (e.g., not eaten)		
I feel nauseous		
I lose appetite		
I experience frequent burping or belching		
I experience bloating		
I have experienced unintentional weight loss		

APPENDIX II

Potential risk factors for *H. pylori*

What is your accommodation type: Self-contain ☐ Shared accommodation ☐

Accommodation location: School hostel ☐ Off-campus (student hostel) ☐ Off-campus (family) ☐

How many hours do you sleep at night?

How many people sleep in your room?

Do you have a reliable water source within your compound/room? No ☐ Yes ☐

- If yes, what is your major water source?

Do you ALWAYS have soap available for use after using the toilet? No ☐ Yes ☐

Do you live with someone known to have an ulcer at school? No ☐ Yes ☐

Highest level of education of mother/female guardian: Primary ☐ Secondary ☐ Post-secondary ☐

Highest level of education of father/male guardian: Primary ☐ Secondary ☐ Post-secondary ☐

Occupation of mother/female guardian:

Occupation of father/male guardian:

Are you a vegetarian? Yes ☐ No ☐

what is your smoking status? Never ☐ Ex-smoker ☐ Current smoker ☐

If a current smoker, what is your smoking frequency in a week: Once ☐ 2-3 times ☐ >3 times ☐

What is your predominant source of food per week? Home/personal cooking ☐ Restaurant (Buka) ☐
Mixed (both home and restaurant) ☐

How many times do you take fruits in a week? None ☐ Once ☐ Twice ☐ ≥Thrice ☐

Do you drink non-filtered or non-boiled water (i.e., directly from a tap or well)? Yes ☐ No ☐

Does anyone in your family have an ulcer? No ☐ Yes ☐

Would you consider yourself a chilli pepper lover? No ☐ Yes ☐

Estimated **monthly** allowance: <10,000 ☐ 10,000-19,999 ☐ 20,000-30,000 ☐ >30,000

Do you currently take non-steroidal anti-inflammatory drugs (e.g., ibuprofen)? No ☐ Yes ☐

Do you enjoy taking soft drinks regularly, say three times per week? No ☐ Sometimes ☐ Always ☐

What is your preferred tooth brushing/cleaning method: Chewing stick ☐ Toothpaste ☐ Mixed ☐

- If toothpaste: how many times do you use it **in a day**? Once ☐ Twice ☐ Thrice ☐

Do you share the toilet with anyone while in school? No ☐ Yes ☐

- If yes, with how many people?

APPENDIX III

Informed Consent Form

A study on the prevalence of *H. pylori* and its associated factors among university students in Benin City, Nigeria

Hello! My name is_____. I would like to invite you to take part in this study about *Helicobacter pylori* among university students in Benin City. *Helicobacter pylori* is a bacterium that has the potential to cause ulcers (it is not the only cause of ulcers, and its presence does not mean that one has an ulcer). This is part of our undergraduate final-year project. You are invited to take part in this study but taking part in this study is voluntary.

Study Procedures

If you take part in this study, we will ask you a few questions concerning yourself, including questions related to your faculty and department of study. In addition, we will take blood, about 2 ml, to do a rapid diagnostic test for the bacterium. You will get the result on the spot. The interview and test will take about 30-40 minutes.

Confidentiality

The information will be collected on paper. The information is stored securely and can only be accessed by approved study staff. The interview will take place in private. Everything you tell us is strictly confidential. Your identifying information will never be used in any reports. All information reported from this study will not be able to be linked to you.

Potential Risks

None is expected, other than the time you will spend in participating in the collection of data for the study.

Potential Benefits

You may or may not benefit by taking part in this study. If you take part in this study, you may help other students in the future as we would have identified the students most at risk of developing ulcer, and will improve our understanding of how to implement interventions targeting the health condition overall. However, as s token of appreciation for your time, we shall provide light refreshments.

Costs to Participate

It will not cost you anything to take part in this study other than your time.

Confidentiality and Access to your Health Information

Access to the information you provide will be limited to persons involved with this study and will be protected in a secure place. Efforts will be made to protect your information and your answers to the interview questions. A unique number will be used instead of your name to identify your personal information and the answers you give. Only study staff can use this number to link your responses to you. Any answers included in the final report will not have your name or personal information on it.

Consent Statement

I have read this form and/or someone has read it to me. I was encouraged to ask questions and given time to ask questions. Any questions that I had have been answered satisfactorily. I agree to voluntarily take part in the study. I know that after choosing to take part in the study, I may withdraw at any time. I have been offered a copy of this consent form.

1. Do you agree to participate in the study? 'YES' means that you agree to do the interview. 'NO' means that you will NOT do the interview.
   _____Yes _____No

Participant signature _____ Date: ___/___/___

DD/MM/YYYY

Printed name of participant _____

Participant ID number _____

Signature of person obtaining consent_____ _____ Date: ___/___/___

DD/MM/YYYY