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COLLEGE OF GRADUATE STUDIES

Microbiological Investigation of *Helicobacter pylori* in Faeces by Culture and Polymerase Chain Reaction among Patients with Gastrointestinal Symptoms in Omdurman locality

التحقيق الميكروبيولوجي عن بكتريا الملوية البابية قي البراز بواسطة التزريع و تفاعل البلمرة المتسلسل بين المرضى الذين يعانون من أعراض الجهاز الهضمي في محلية أم درمان

A dissertation submitted in partial Fulfillment for the requirements of M.Sc. Medical Laboratory Science (Microbiology)

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الآيــة

قال الله تعالى: { وَعَسَى أَنْ تَكْرَهُوا شَيْئاً وَهُوَ خَيْرٌ لَكُمْ وَعَسَى أَنْ تُحِبُّوا شَيْئاً وَهُوَ شَرٌّ لَكُمْ وَاللَّهُ يَعْلَمُ وَأَنْتُمْ لا تَعْلَمُونَ}

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Dedication

I would like to dedicate this work firstly to my beloved Parents, brother, sisters and all teachers who suffered with me and also to my colleagues and dear friends.

Acknowledgement

First of all, Thank to AL MIGHTY ALLAH for helping me to complete this work. Thanks to my supervisor **Prof.Yousif Fadlalla** for his guidance, and understanding throughout this study. My thanks and appreciation is extended to my teachers specially Dr. Hisham Nour el-daeim El-tayib Mohammed and Ust. Maram Mustafa Mohammed Ali and special thanks to the staff of the Research Lab for their efforts and support that help me in complete this successful research. Thank extend to medical center workers and patients for their help.

Abstract

The aim of this study was to detect of *Helicobacter pylori* (*H. pylori*) in faeces by culture and Polymerase Chain Reaction among patients with gastrointestinal symptoms in relation to selected risk factors in Omdurman locality.

This was cross sectional case control study. The study was conducted fifty symptomatic patients (n=50)19 were males and 31 were female. The age of patients ranging from 10 –85 years. Sociodemographic data were collected from patient by using structured questionnaire.

All stool samples were cultured on selective Columbia blood agar media for isolation. DNA extraction by i-genomic Stool DNA Extraction Mini Kit and multiplex PCR were applied to these samples to detect *H.pylori Urea A* gene.

The results showed that out of 50 samples, no growth was seen on culture media. The prevalence of *H. pylori* according to Polymerase chain reaction is 58% . 20% were males and 38% were females which is statstically insignificant ($p = 0.547$). Statistical analysis showed that there was insignificant correlation between *H.pylori* infection and gender , age , social status , gastrointestinal symptoms , educational level , family history of infection with *H. pylori* , smoking behavior , source of water ($P > 0.05$).

المستخلص

كان الهدف من هذه الدراسة هو الكشف عن البكتريا الملوية البابية في البراز عن طريق التزريع و تفاعل البلمرة المتسلسل بين المرضى الذين يعانون من أعراض الجهاز الهضمي فيما يتعلق بعوامل الخطر المحددة في محلية أم درمان .

وكان هذا دراسة مقطعية مراقبة الحالة. وقد أجريت الدراسة خمسين مريضا يعانون من الأعراض (ن = 50) 19 كانوا من الذكور و 31 من الإناث. سن المرضى تتراوح بين 10-85 سنة. تم جمع البيانات الاجتماعية والديموغرافية من المريض باستخدام استبيان منظم .

تم تزريع جميع عينات البراز على وسائط الدم كولومبيا الانتقائية للعزل. تم استخلاص الحمض النووي بواسطة العدة المصغرة لاستخراج الحمض النووي للبراز الجينومي و تفاعل البلمرة المتسلسل طبق على هذه العينات لتحديد حمض اليوريا أ للبكتريا الملوية البابية.

أظهرت النتائج أنه من بين 50 عينة ، لم يُلاحظ أي نمو على وسائط التزريع . أنتشار الملوية البابية وفقا لتفاعل البلمرة المتسلسل هو 58 ٪. 20 ٪ من الذكور و 38 ٪ من الإناث وهو أحصائيا ضئيل (ب=0.547).

أظهر التحليل الإحصائي أن هناك علاقة غير ذات دلالة إحصائية بين الإصابة بالبكتيريا الملوية البابية والجنس والعمر والحالة الاجتماعية وأعراض الجهاز الهضمي والمستوى التعليمي والتاريخ العائلي للإصابة بالبكتيريا الملوية البابية وسلوك التدخين ومصدر المياه .

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List of abbreviation

Abbreviate	Full term
°C	Celsius
C^{13}	Carbon – 13
CO_2	Carbon dioxide
D.N.A	Di nucleic acid
D.W	Distilled water
EDTA	Ethylene diamine tetra acetic acid
IL	Interlukin
Kda	Kilodalton
min	Minute
ml	Milliliter
mm	Millimeter
N	Number of sample
PH	Potential hydrogen
qPCR	Quantitative Polymerase chain reaction
rpm	Round per minute
rRNA	Ribosomal Ribonucleic acid
RT	Room Temperature
sec	Second
µl	Microliter
TBE	Tris , Boric acid , EDTA
Primer (F)	Primer Forward
Primer (R)	Primer Reverse

Chapter one

Introduction and objectives

1.1 Introduction:

Helicobacter pylori (H.pylori) has been recognized as an important gastroduodenal pathogen. *H. pylori* infection is one of the most frequent bacterial infections in the world, and this organism has been acknowledged to be a crucial factor in several different diseases ranging from gastritis to gastric malignancies (Piccolomini *et al*., 1997). Approximately two third of the world population is infected with *H. pylori*, any age can get infection and women are affected just as often as men (Yamoka , 2010).

In February 1994, the National Institute of Health Consensus development conference concluded that *H. pylori* infection is major cause of peptic ulcer, and all patients with confirmed peptic ulcer associated with *H. pylori* infection should receive treatment with antimicrobial agent (Yamada *et al*., 2006). High prevalence of the pathogen was reported in developing countries as compared to low estimates in developed world. Several risk factors including; gender, age and existing lifestyle such as smoking habit have a role in varying this prevalence(Baik *et al.,* 2012) .To control such widespread infection, a recent quadrable treatment regimen combining proton pump inhibitor, clarithromycin, amoxicillin and nitroimidazole has been effectively used . *H. pylori* is present on the gastric mucosa of less than 20% of persons under age 30 but increases in prevalence to 40-60% of persons age 60, including persons who are asymptomatic (Gisbert and Calvet, 2012).

In developing countries, the prevalence of infection may be 80% or higher in adults. Person to person transmission of *H. pylori* is likely because intrafamilial clustering of infection occurs (Brook *et al.,* 2010).

In Sudan, information about the prevalence of *H. pylori* infection is very patchy. and there is only one study which showed high prevalence (80%) of *H. pylori* infection among patients with symptoms of gastritis, 56% with duodenal ulcer, while 60% with duodenitis and 16% apparently look normal (Elbagir and Ahmed, 2001). Early and prompt accurate diagnosis of *H. pylori* infection is crucially needed to identify any gastric diseases, limit further pathological complications and give rapid treatment to manage infected cases. The most frequent used diagnostic laboratory techniques are culture, stained smears, urease test, PCR assay, Western immunoblotting and histopathological examination of biopsy specimens (Rocha *et al*., 2003; Deankanob *et al.,* 2006).However, these methods necessitate obtained gastric biopsy materials by an endoscopic invasive procedure. (Hanvivatvong *et al*., 2004).

Proper and rapid laboratory assays are needed to diagnose infections in order to treat and manage the patient. Several techniques including culturing and molecular methods are present to identify the organism.

1.2 Rationale:

Prevalence of the pathogen was reported in developing countries as compared to low estimates in developed world. Several risk factors including; gender, age and existing lifestyle such as smoking, have a role in varying this prevalence (Baik *et al.*, 2012). In Sudan, information about the prevalence of *H. pylori* infection is very patchy and there is study which showed high prevalence (80%) of *H. pylori* infection among patients with symptoms of gastritis, 56% with duodenal ulcer, while 60% with duodenitis and 16% apparently look normal (Elbagir and Ahmed, 2001). A number of different diagnostic tests methods both invasive and non invasive are available. Invasive methods have high specificity and sensitivity in the detection of *H. pylori* infection, however they are expensive and need special gastroenterologist to be performed. In contrast, non-invasive methods such as antibody (Ab) detection is cheap, easy to performe but may give false positive result (Quartero *et al.*, 2000). We need a new non-invasive diagnostic tool like stool culture for detect the resistance of bacteria and detect the gene of *H. pylori* by PCR for specific result.

This study aimed to investigate about *H. pylori* by using stool culture and PCR and their risk factors among patients with gastrointestinal symptoms in Omdurman locality.

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1.3 Objectives of the study

1.3.1 General objective:

To investigate of *Helicobacter pylori* in faeces by culture and Polymerase Chain Reaction among patient with gastrointestinal symptoms in Omdurman locality.

1.3.2 Specific objectives:

1. To isolate of *H. pylori* from stool sample.
2. To detect the Urea A gene in *H. pylori* by polymerase chain reaction.
3. To determine selected risk factors associated with *H. pylori* infection.

Chapter Two

Literature review

2.1 Historical background of *H. pylori*:

In April 1982 in the Microbiology Department of Royal Perth Hospital, Perth, Western Australia, culture of endoscopic biopsy specimens of human gastric mucosa yielded a spiral organism with some features of the genus *Campylobacter,* which was named *Campylobacter pyloridis* **(**Marshall *et al.*, 1984**)** in September 1983 The organism resembled *Campylobacter* in several respects, including curved morphology, growth on rich media under microaerophilic conditions, failure to ferment glucose, sensitivity to metronidazole, and a G + C content of 34%. It was therefore first referred to as "pyloric *Campylobacter*" (pylorus, Greek, gatekeeper, or one who looks both ways) and validated as *Campylobacter pyloridis* (Pearson, 1983). In 1985 by anonymous validation the specific epithet was revised to *Campylobacter pylori* . In 1987 to conform to the correct Latin genitive of the noun pylorus(Marshall and Goodwin, 1987). Yet almost from its initial cultivation it was suspected that perhaps *C. pylori* was not a true *Campylobacter*. Early electron micrographs showed multiple sheathed flagella at one pole of the bacterium, in contrast to the single bipolar unsheathed flagellum typical of *Campylobacter* spp. (Goodwin *et al.*, 1985). Subsequent 16S rRNA sequence analysis showed that the distance between *C. pylori* and the true campylobacters was sufficient to exclude it from the *Campylobacter* genus (Romaniuk *et al.*, 1987), and it was renamed *Helicobacter pylori*, the first member of the new genus *Helicobacter* (Goodwin *et al*., 1989).

2.2 Classification of *H. pylori*:

The genus *Helicobacter* belongs to the subdivision of the *Proteobacteria*, order *Campylobacterales*, family *Helicobacteraceae*. This family also includes the genera *Wolinella*, *Flexispira*, *Sulfurimonas*, *Thiomicrospira*, and *Thiovulum*. To date, the genus *Helicobacter* consists of over 20 recognized species, with many species awaiting formal recognition (Fox, 2002). *Helicobacter* species can be subdivided into two major lineages, the gastric *Helicobacter* species and the enterohepatic (nongastric) *Helicobacter* species. Both groups demonstrate a high level of organ specificity, such that gastric helicobacters in general are unable to colonize the intestine or liver, and vice versa. An extensive review of non-pylori *Helicobacter* species is available (Solnick and Schauer, 2001).

2.3 General characteristic of *H. pylori*:

H. pylori, a Gram-negative bacterium with a curved, spiral, or gull-wing shape, is 2.5 to 3.5 mm long and 0.5 to 1.0 mm in diameter and has a periodicity of 1 to 2 mm. It has smooth surfaces, and one to six polar-sheathed flagellae emerge from one of its rounded ends. Since it is morphologically similar to *C. jejuni*, it was initially named "pyloric *Campy-lobacter*" and subsequently *C. pyloridis* and *C. pylori* before finally being named *H. pylori*. This organism colonizes only the non-acid-secreting mucosa of the stomach and is not found where parietal cells are numerous. Thus, it may be observed in the gastric antrum and the cardia, but also in the corpus, when atrophic gastritis is present, and attached to the gastric epithelial cells found in the duodenum, when gastric metaplasia is present(Solnick *et al.*, 1993).

2.4 Epidemiology of *H. pylori*:

The seroepidemiology of *H. pylori* has been extensively studied in the United States and in other countries (Taylor and Blaser, 1991). The high frequency of seropositivity(up to 100% in some age groups in Albania) and acquisition of the infection during infancy are characteristic of disadvantaged socioeconomic groups living in crowded or poor hygienic conditions and appears to be independent of gender and ethnic origin. In adults of higher socioeconomic groups, the rate of seroconversion is estimated at 0.5% per year, although the frequency of seropositivity increases with age and may be as high as 40% A longitudinal study has indicated that the high frequency of seropositivity in older adults might be due to a higher rate of *H. pylori* infection in Western countries in the years between the two world wars than during recent years (cohort effect) (Cullen *et al.*, 1993).Alternatively, the increase in frequency of infection in older adults might be due to years of low but cumulative risk for infection. Although the route of transmission for this infection is not known, the contamination of drinking water may play a role in certain developing countries (Klein *et al.*, 1991a). In the United States and in other regions, direct contact and/or consumption of food or water contaminated by saliva (Ferguson *et al.*, 1993), gastric contents, or feces (Thomas *et al.*, 1992) may be major factors. The recent observation that *H. pylori* can be isolated from cats (Handt *et al.*, 1994) suggests that transmission from pets to humans (or humans to pets) is also possible.

2.5 Pathogenesis of *H. pylori*:

Helicobacter pylori is considered a pathogen because its presence is always associated with chronic active gastritis, and eradication of the bacterium is always followed by resolution of gastritis. In addition, nearly all patients with duodenal ulcer disease have *H. pylori* gastritis, and ulcer relapse is exceptional after *H. pylori* eradication. Thus, the presence of *H.pylori* seems necessary for the production of duodenal ulcers, with the exception of ulcers attributed to the use of nonsteroidal anti- inflammatory agents or to the Zollinger-Ellison syndrome . The association with gastric ulcers is not as strong, although *H. pylori* infection is present in 80% of patients with gastric ulcers who do not consume nonsteroidal anti-inflammatory agents .However, most *H. pylori* infected persons do not report any clinical symptoms. This may be because these persons are colonized by less virulent strains or because other host or bacterial cofactors are required for overt disease. In addition, three prospective cohort studies have demonstrated that *H. pylori*-infected persons have an increased risk of developing intestinal-type, but not undifferentiated, gastric adenocarcinoma .(NIH Consensus Conference , 1994).

2.6 *H. pylori* Virulence Factors:

2.6.1 Urease :

Urease is an important virulence factor for *H. pylori* and is critical for bacterial colonization of the human gastric mucosa. *H. pylori* urease metabolizes urea producing ammonia to neutralise the microenvironment in which the bacterium resides (Bode *et al.,* 1993). The ammonia production can damage the gastric mucosa through the disruption of tight junctions and the alteration of permeability of gastric epithelium. Moreover, urease stimulates activation of

mononuclear phagocytes and production of inflammatory cytokines (Harris *et al.*,1996). The native *H. pylori* urease consists of a nickel-containing hexameric molecule with a molecular mass of approximately 540 kDa made up of two subunits: UreA [30 kDa] and UreB [62 kDa]. The urease gene cluster contains nine genes, including *ureA* and *ureB* structural genes (Mobley *et al.*,1995).

2.6.2 Phospholipases :

Helicobacter pylori phospholipases induce generation of products such as lysolecithin which disrupt the protective phospholipid-rich layer on the apical membrane of mucus cells (Mauch *et al.,* 1993).

2.6.3 Flagella:

The presence of flagella is an essential factor of colonization in *H. pylori*. A flagellate strains are not able to colonize gnotobiotic piglets (Eaton *et al.*, 1996). *H. pylori* possesses two to six polar flagella characterized by two types of flagellin proteins coded by *flaA* and *flaB* genes that are required for full motility and persistent infection of the gastric mucosa (Schmitz *et al.*,1997). A recent study demonstrated that flagellar biosynthesis and urease activity may be linked (McGee *et al.,* 1999).

2.6.4 Neutrophil activating protein:

Helicobacter pylori is able to activate neutrophils and to increase neutrophil adherence to endothelial cells through the expression of a 150 kDa activating protein (Hp-Nap), made up of 10 identical subunits, coded by the *napA* gene (Evans *et al.,* 1995).

2.6.5 Adhesins :

It is widely accepted that *H. pylori* adheres to receptors in the gastric epithelium by means of adhesins. Several specific receptors are involved in these mechanisms including lipids, gangliosides and sulfated carbohydrates, and different types of adhesins have been characterized (Simon *et al.,* 1997).

An interesting study revealed the presence of a protein (coded by *babA* and *babB* genes) able to bind the human blood group antigen Lewis b (Leb) to human gastric epithelial cells (Ilver *et al.,* 1998). Furthermore, the chemical structure of LPS of some strains of *H. pylori* has been found to mimic Lewis *x* and Lewis *y* blood group antigens expressed in the gastric mucosa; this may serve to down regulate the immune response in patients with acute and chronic infections (Appelmelk *et al.,*1997).

2.6.6 IceA

The gene encoding IceA has been identified in isolates from patients with peptic ulcer, independently of the *vacA* and *cagA* genotype (Yamaoka *et al.,* 1999) . The expression of *iceA* is induced by adherence of *H. pylori* to gastric ephitelium (Peek *et al.,* 1998). DNA sequencing has revealed the presence of two families: *iceA1* and *iceA2*. Strains with the *iceA1* gene are most frequently associated with peptic ulceration and increase the production of IL-8 (Peek *et al.,* 1995).

2.6.7 Vacuolating Cytotoxin (VacA)

One of the primary virulence factors described for *H. pylori* is VacA . *VacA* is an oligomeric toxin composed of 87 kDa active subunits obtained by treatment at low pH. An antiserum produced against these purified proteins neutralizes the cytotoxic activity (Cover and Blaser, 1992). able to induce acidic vacuoles in the cytoplasm of eukaryotic cells (Cover *et al.,* 1992). VacA induces an inactivation of energy metabolism followed by mitochondrial damage, leading to impairment of the cell cycle in gastric epithelial cells (Kimura *et al.,* 1999). VacA is immunolocalised in the periplasm and outer membrane of whole bacteria and also in vesicles and outer membrane blebs (Fiocca *et al .,* 1999).

vacA genotypes are important *in vivo* because of the diversity- pathogenicity relationship among *H. pylori* strains (Zhongming and Taylor, 1998). The *s1a*

strains produce higher levels of cytotoxin with more severe gastric inflammation and duodenal ulceration than the other two allelic s types *(s1b* and *s2*) (Atherton *et al.,* 1995). The *m1* middle region allele is more frequently associated with a higher level of gastric damage as compared with the *m2* form and it is toxic for Hela cells (Atherton *et al.,* 1997).

2.6.8 Cag Pathogenicity Island:

Helicobacter pylori strains isolated from gastric epithelium can be classified in at least two groups, named type I and type II, on the basis of genotypic and phenotypic differences. Infections by type I strains are associated with the more severe forms of disease with respect to the less virulent type II strains (Censini *et al* ., 1996) (Covacci *et al.,* 1999) . Two type I *H. pylori* strains, 26695 (Tomb *et al.,* 1997) and J99 (Alm *et al.,* 1999), have been entirely sequenced and they differ from type II strains by the presence of a 40 Kb locus, containing 31 genes, inserted into the chromosomal glutamate-racemase gene, named *Cag* pathogenicity island (abbreviated *Cag*PAI or *Cag* region) (Censini *et al.,* 1996) .This secretory system is involved in the induction of increased gastric mucosal levels of members of the C-X-C chemokine family, which includes the neutrophil chemoattractant IL-8 (Baggiolini *et al.,*1994, 1997), and promotes neutrophilic infiltration into the gastric epithelium. It has recently been demonstrated that multiple genes in the left half of the CagPAI are required for transcriptionof the IL-8 gene in gastric epithelial cells and that this is related to the activation of protein tyrosine kinase (Li *et al.,* 1999). Several CagPAI genes are homologous to genes of other pathogens that encode for subunits of the specialized type IV secretory system that deliver bacterial virulence factors. across the bacterial membrane to the surface or into host cells (Covacci *et al.,* 1999). *H. pylori* containing *Cag*PAI is associated with the development of chronic active gastritis (Peek *et al.,* 1995), peptic ulceration

(Walker and Crabtree, 1998) and atrophic gastritis with an increased risk of gastric cancer (Webb *et al.,* 1999). Before the characterization of *Cag*PAI, the development of clinical disease related to *H. pylori* was associated with the expression of *cagA* gene (Covacci *et al.,* 1993). CagA is described as an immunodominant antigen with a molecular mass of 120 kDa able to express the cytotoxin encoded by *vacA.* This gene is at one end of the *Cag*PAI and is considered the marker of its presence (Censini *et al.,* 1996). Recently, the presence of CagA positive *H. pylori* infection has been related to food allergy (Figura *et al.,* 1999). In fact, the enhanced mucosal and inflammatory lesions could increase the epithelial permeability with a non selective passage of allergens which, in atopic subjects, could stimulate the IgE response. The incidence of *Cag*PAI positive strains is 60–70% all over the world, except in Korea and Japan where it is nearly 100% (Covacci *et al.,* 1997; Maeda *et al.,* 1999). Recent studies (Rothenbacher *et al.,* 1999) have demonstrated the presence of both *Cag* positive and *Cag* negative strains in the same patient, suggesting a dynamic equilibrium among strains in which the prevalence of one type over the other modulates the expression of the disease (Covacci *et al.,* 1997).

2.7 Transmission of *H. pylori*:

The mode of transmission of H. pylori remains poorly understood; no single pathway has been clearly identified. Grubel *et al.* (1997) demonstrated that the housefly has the potential to transmit *H. pylori* mechanically, and thus fly excreta might theoretically contaminate food. This hypothesis may be of the most significance in areas of the world with poor sanitation. Person-to-person contact is considered the most likely transmission route. (Dunn *et al.,* 1997). The second possible route is faecal–oral. Failure to recover the bacterium from

faeces might be due to the toxic effect of faeces (Sahay and Axon, 1996) or the methods used may not have been suitable (Me´graud, 1995). Faeces-contaminated water may be a source of infection; an association between *H. pylori* and the absence of hot running water was found in some studies (Neale and Logan, 1995). In addition, an increased risk of infection was observed in children who swam in rivers, streams, or swimming pools in the southern Colombian Andes (Goodman *et al.*, 1996). However, the organism has not been isolated from water (Neale and Logan, 1995). Three epidemiological studies South America have suggested that transmission occurred through food or water. In Chile, consumption of uncooked vegetables that had been irrigated with water contaminated with untreated sewage was associated with *H. pylori* seropositivity. (Hopkins *et al.*, 1993). The third possible route of transmission is oral–oral. Few reliable studies have cultured *H. pylori* from the oral cavity; only sporadic isolates from dental plaqueand saliva have been recorded (Namavar *et al*., 1995).

Helicobacter pylori infection is uncommon among dental professionals (Lin *et al.*, 1998). In addition, studies using the polymerase chain reaction have given contradictory findings (Dunn *et al.*, 1997). Via the last two routes, but possible transmission via intimate oral–oral contact has been suggested indirectly by the fact that spouses and children of individuals infected with *H. pylori* were more often seropositive than spouses and children of noninfected individuals (Me´ graud, 1995). Without specifying the exact mode of transmission, evidence for oral–oral exposure has been suggested by a population-basedstudy in Victoria, Australia, in 1994–95: a significant association was found between positive test results for *H. pylori* and increased number of tooth surfaces with plaque (Peach *et al.*, 1997). Intrafamilial clustering of infections and the higher prevalence found in institutionalized populations may indicate that person-to-person

contact is a route of transmission, but this could also indicate that there had been a common source of transmission, such as contaminated drinking water or food. The use of molecular typing on bacterial strains isolated from infected members of a family might indicate whether there had been a common source.

2.8 Symptoms and complication of *H. pylori* infection:

Helicobacter pylori is possibly the most common human infection. While most individuals are asymptomatic, infection acts as a co-factor to produce gastrointestinal disease in a small but significant minority. It associated with over 90% of duodenal ulcers and 70%_ 80% of gastric ulcers. *H.pylori* does not appear to play a role in gastroesophageal reflux disease or non-ulcer dyspepsia, most commonly presenting with persistent or recurrent pain in the upper abdomen in the absence of structural evidence of disease. (Richard *et al.*, 2013). It has been strongly linked aetiologically to chronic gastritis and duodenal ulcer disease but its role in non-ulcer dyspepsia, gastric ulcer disease and gastric carcinoma has been less well established (Hopkins and Morris, 1994).

2.9 Diagnosis of *H.pylori* infection

2.9.1 Invasive Tests:

2.9.1.1 Histopathology:

The presence of typical spiral motile bacteria accompanied by inflammatory reaction in the histopathological sections of stomach was the first described method used for the diagnosis of the *H. pylori* (Lee and Kim, 2015).

A routine hematoxylin and eosin (H&E) stain detects *H. pylori* and inflammation (gastritis type) (Rajindrajith *et al.,* 2009). When this stain has produced inconclusive results, special stains, such as Giemsa, Warthin-Starry, acridine orange, toluidine blue, Dieterle, Genta, Romanouski and McMullen stains, or immunochemical methods can be utilized. The Genta stain, which combines silver, H&E, and Alcian blue stains, is used to observe both inflammatory cells and *H. pylori*, but it is expensive and technically complex However, the Giemsa stain is much easier to perform, highly sensitive, inexpensive, and the preferred method for clinical practice (Garza-González *et al.,* 2014). Histologic testing has a few disadvantages: The use of some medications, such as antibiotics, bismuth, and proton pump inhibitors (PPIs) decreases the sensitivity and specificity of the test. The cost of the special stains is quite high, and skilled personnel are needed to examine the slides (Kanna *et al.,* 2013).

2.9.1.2 Culture:

Culture that requires an endoscopy is the gold standard and the most specific method for diagnosing *H. pylori.* It is used for determining antibiotic susceptibility of *H. pylori* in clinical practice. (Garza-González *et al.,* 2014)

Because of the demanding character of this bacterium, this method remains challenging. This technique, although highly specific, is not as sensitive as other tests like histology and the rapid urease test. As well as for purposes of scientific research, cultured live *H. pylori* is used for diagnostic approaches and for the detection of antibiotic resistance if treatment failure is suspected (Taj *et al.,* 2003). *H. pylori* requires a microaerophilic atmosphere (5% to 10% oxygen, 5% to 12% carbon dioxide and 80%–90% nitrogen with humidity) and a complex culture media. The most commonly used media contains *Brucella* , Columbia Wilkins-Chalgren, brain-heart infusion or trypticase agar bases, supplemented with sheep or horse blood (Ndip *et al.,* 2003) due to the presence and growth of numerous other bacteria and especially microorganisms phenotypically similar to *H. pylori*, colonies have to be further characterized by other methods. Moreover, it is possible that the bacterium goes into a viable form that cannot be cultured (coccoid form) which leads to false negative results (Azevedo *et al.,* 2007). However, the results vary according to the microbiologist's experience, transport media, and specimen quality used.

2.9.1.3 Rapid Urease Test:

The Rapid Urease Test (RUT) is a popular invasive diagnostic *H. pylori* test that is relatively quick, cheap and simple to perform. It detects the presence of urease in or on the gastric mucosa. Best results for RUT are obtained if biopsies are taken from both the antrum and corpus. The biopsy used for RUT can also be used for other tests such as for molecular-based tests of microbial susceptibility or for host factors (Uotani and Graham, 2015).

Some members of the microbiota in the oropharynx make urease; however, this weaker enzyme is destroyed rapidly when it reaches the stomach due to the

high acidity of the gastric juice. In addition, a recent intake of bismuth compounds, antibiotics, PPIs and patients with achlorhydria can result in false-negative urease test.(Garza-González *et al.,* 2014) Therefore in these cases a negative RUT result should be complemented by a second method .

2.9.1.4 Molecular Methods:

PCR based detection of *H. pylori* could be categorized under invasive as well as non-invasive methods (Yang and Rothman, 2004). The PCR can be performed rapidly and cost-effectively, used to detect different bacterial genotypes, and employed in pathogenic and epidemiological studies (Garza-González *et al.,* 2014). The PCR can be carried out on tissue and stool specimens and helps identify genes related to antibiotic resistance and virulence (Guarner *et al.,* 2010). Kalach *et al*. described a quantitative real-time PCR (qPCR) used to detect *H. pylori* in gastric biopsy samples of French children. They reported that qPCR is a more sensitive test than histology, culture, or RUT alone, and allows detecting low bacterial loads.(Kalach *et al.* , 2015). Fluorescent *in situ* hybridization (FISH) is a recently developed technique, which is used to detect the resistance of *H. pylori* to clarithromycin. (Garza-González *et al.,* 2014).

However, conventional methods for antibiotic susceptibility testing, such as E-test and agar dilution method are dependent on bacterial growth.(Caristo *et al.* , 2008). In addition, the test is expensive, labor intensive, and not widely used in clinical studies (Garza-González *et al.,* 2014). Omics-based methods are increasingly being used for diagnosis of *H. pylori* infection.(Mentis *et al.,* 2015) Authors detected *H. pylori* by pyrosequencing method of the 16S ribosomal ribonucleic acid gene in all samples that were detected to be *H.*

pylori positive by conventional methods and in 60% of the *H. pylori*-negative samples (Kim *et al.* ,2015).

2.9.2 Noninvasive Tests:

2.9.2.1 Urea Breath Test (UBT):

Urea breath test (UBT) has been used for almost 30 years and is still the most popular and accurate noninvasive test for diagnosis of *H. pylori* infection. By the urease activity of *H. pylori*, the ^{13}C- or ^{14}C-labeled urea ingested by the patient is hydrolyzed to labeled CO_2 in stomach, then labeled CO_2 is absorbed in the blood and exhaled by breathing in which labeled CO_2 can be measured. Although several factors including patient, bacteria and the test itself influence the results of UBT, the UBT is a highly accurate and reproducible test with near 95% sensitivity and specificity under standardized procedures (Ferwana *et al.* , 2015). The [^{13}C] UBT is the best detection test in children aged 5 years and older and may be accepted as a "gold standard," especially if endoscopy is not routinely performed (Czinn, 2005). Due to radioactivity risks in children, a few reports are available on the use of ^{14}C-labeled UBT in children. (Walker *et al.*, 2004) False-negative results can occur in patients who have recently received bismuth compounds, antibiotic agents, or gastric acid antisecretory agents (Czinn , 2005).

2.9.2.2 Stool Antigen Test:

Stool antigen test (SAT) is the other noninvasive method with good sensitivity and specificity, 94% and 97% respectively in global meta-analysis, in the diagnosis of *H. pylori* infection . This method detects the presence of *H. pylori* antigen in stool samples. There are two types of SATs used for H. pylori

detection, enzyme immunoassay (EIA) and immunochromatography assay (ICA) based methods, using either polyclonal antibodies or monoclonal antibodies. Many SATs are available now for the diagnosis of *H. pylori* infection and different diagnostic accuracy are showed from different studies with different SATs and different study design. In general, monoclonal antibody-based tests are more accurate than polyclonal antibody-based tests (Gisbert *et al.*, 2006). The current guideline evaluates the use of the stool antigen test as equivalent to the UBT if a validated laboratory-based monoclonal antibody is used (Malfertheiner *et al.*, 2012). In a recent study, the Tesmate pylori antigen (TPAg) EIA utilizing a monoclonal antibody to check native *H. pylori* catalase showed 92.4% sensitivity and 100% specificity in adult when compared with RT-PCR and the accuracy of this test was 94.9% (Okuda *et al.*, 2014). Furthermore, the available stool antigen tests have been shown tobe able to distinguish infected from treated patients (Gisbert *et al.*, 2002).

2.9.2.3 Serological test:

Serological tests are qualitative, commonly used to detect immunoglobulin (IgG, IgA, or IgM antibodies to *H. pylori* infection, and are accepted as first-line non-invasive diagnostic methods among adults with suspected *H. pylori* infection in Europe(Czinn, 2005). Serology is the only test which is not affected by those local changes in the stomach that could lead to a low bacterial load and to false negative results (Malfertheiner *et al.*, 2012) also frequently been used in screening for epidemiological studies because of their inexpensive, rapid and acceptability to patients(Ueda *et al.*, 2014) . However, serology does not indicate whether or not the infection is active or past (Czinn , 2005). In general, no serological assays can be utilized on their own in

adolescents and children for diagnosing *H. pylori* infection. They cannot be used to observe the success of eradication therapy since the sensitivity and specificity for determination of antibodies (IgG or IgA) to *H. pylori* in children differ commonly. A positive IgG test can result several months or even years after the infection, and is not reliable for diagnosis or treatment outcomes.(Guarner, 2010).

2.10 Treatment of *H. pylori*:

Helicobacter pylori is sensitive to many antimicrobial drugs in vitro, it is difficult to eradicate from the stomach. This may be ascribed to antibiotic breakdown by gastric acid, clearance by gastric emptying, and the difficult-to-penetrate mucous layer in which the bacterium resides. Resistance of *H. pylori* to specific antibiotics, especially metronidazole, is also frequent. Therefore, it is generally accepted that a combination of at least two, and possibly three, antimicrobial agents should be given for a minimum of 1 week. The regimen found to be most effective is the administration of amoxicillin (or tetracycline) plus metronidazole and bismuth subsalicylate 2 to 4 times a day for 2 to 3 weeks (Chiba *et al.*, 1992). Recently, it was shown that at least a 7-day course of any of these regimens is required to obtain a high (90%) cure rate, but that continuing treatment for more than 10 days does not significantly improve its efficacy. Finally, topical therapy for 1 h was recently tried with excellent results, albeit in only one center at this time (Kimura *et al.*, 1995). This treatment involves a 2-day administration of a mucolytic agent to dissolve the mucous layer and of a proton pump inhibitor. On the third day, a balloon is introduced into the second portion of the duodenum under fluoroscopic control,

and a solution of pronase, amoxicillin, metronidazole, and bismuth subsalicylate is injected into the stomach, where it is left for 1 hour. The presence of the duodenal balloon appears to prevent emptying of the antibiotics and the mucolytic agent, thus ensuring maximum efficacy of the therapy.

Chapter three

Materials and Methods

3.1 Study design:

This is a cross sectional hospital based study.

3.2 Study area:

The research was conducted in Omdurman locality.

3.3 Study populations:

Patients with gastrointestinal symptoms.

3.4 Data collection:

Interviewed each patient and complete a detailed questionnaire about risk factors.

3.5 Ethical consideration:

The study was approved by College Graduate Studies, and verbal consents from patients was taken.

3.6 Sample size:

Fifty individuals were included in this study, they were investigated for the presence of gastric *H. pylori* infection.

3.7 Inclusion criteria:

Patients complain of gasterointestinal symptoms.

3.8 Exclusion criteria:

Patients did not complain of gastrointestinal symptoms.

3.9 Sample collection:

The stool samples were collected into sterile plastic spoon-cover and outer labeled stool container for culture and extraction of DNA.

3.10 Sample processing:

3.10.1 Stool Culture:

A fresh fecal sample was obtained from each of the patients. The samples were delivered within 2 to 6 hours in plastic container kept on ice. The samples were emulsified in phosphate buffered saline (PBS was in room temperature) and pretreated with 1g cholestyramine(Pharma Science, Canada) to remove the bile that might inhibit *H. pylori* growth and then homogenized for 2 -5 minutes. This suspension was then cultured onto selective media Oxoid;Dent-supplemented Columbia agar base and(0.11 mg / ml) nalidic acid, (0.25µl / ml) gentamycin and 7% wt/vol horse blood was added. The plates were incubated at 37°C in a gas jar that contained a gas bag (Manufactured by Thermo Fisher Scientific, United State) create microaerophilic environment (contain 5%O_2, 10%CO_2 and 85%N_2) for 7 days. The agar plates were checked for growth from day 3 to day7. Isolates were identified as *H. pylori* on the basis of colonial morphology. The colonies were small, round and grayish. Colonies were diagnostic and the presence of characteristic curved Gram negative short rods on Gram stain. Positive catalase, oxidase and urease reaction.

3.10.1.1 Gram's stain:

Smear was done from small, round and grayish colonies that grow then, by sterile loop take small portion from colony to drop of normal saline on clean dry slide then mix and spread in circular manner. Then slide was left to dry and

fixation was done by gentle heat. Crystal violate was added to smear for 1minute,and then washed by tap water ,logul's iodine was added for 1minute.then washed by tap water ,aceton alcohol added for seconds and washed by tap water .finally ,the smear covered by saffranin for 2 minutes , and washed by tap water, the smear was left to dry by air ,a drop of oil was added and examined under light microscope(carl zeiss) by oil lens x100 (Cheesbrough, 2006).

3.10.1.2 Catalase test:

By using wooden stick several colonies of tested organism were immersed in tube with 2-3 ml of 3%H_2O_2, presence of air bubbles was indicated of catalase positive and no presence of air bubbles was indicated of catalase negative.

3.10.1.3 Oxidase test:

By using wooden stick small portion of the colony of tested organism was placed in oxidase disk, presence of purple color was indicated of oxidase positive and no change in color was indicated of oxidase negative (Cheesbrough, 2000).

3.10.51.4 Urease test:

By using sterile straight loop the organism under test was inoculated in urea ager. The tubes incubated over night at 37˚c. Presence of pink color was indicated of ureas positive and no change in color was indicated of urease negative (Cheesbrough, 2006).

3.11 Data analysis:

The data were analyzed by Chi-square test using Statistical package for the social science (SPSS) version 16.

3.12 DNA extraction:

DNA was extracted from stool samples by using i-genomic Stool DNA Extraction Mini Kit. Amount 180-220 mg stool was transferred in a 1.5 ml micro-centrifuge eppendrof tube, when stool was fluid, 200 µl was pipetted into the microcentrifuge tube and then 200 µl of Buffer SPL(pre-lysis buffer) was added to each stool sample . The sample was vortexed continuously for 1 min until the stool sample is thoroughly homogenized and then heated for 5 min at 70 °C in incubater . Then, the i-genomic Stool IR Spin Column (inhibitor absorption column) was placed in to a new 1.5 ml tube and transferred the supernatant promptly into the i-genomic Stool IR Spin column and centrifuged for 1min at 13,000 rpm (RT), and then removed the IR Spin Column from the 1.5 ml tube. Then 200 µl of Buffer SL (lysis buffer), 10 µl of proteinase K and 5 µl of RNase were added to mixture in 1.5 ml tube and then mixed by vortexing and incubated the lysate for 30 min at 65 °C (mix for 5 or 6 times during incubation by inverting tube) and then after lysis completely, 200 µl of Buffer SB (binding buffer) was added to the lysate , and mixed by pipetting or gently inverting 5 to 6 times (not vortexed) . After mixing, spinned down to remove drops from inside the lid. Then 250 µl of 80 % ethanol was added to the lysate , and mixed by pipetting or gentely inverting 5 to 6 times without vortexing . After mixing, spinned down to remove drops from inside lid and then pipetted 750 µl of the mixture in to the spin column inserted in a 2.0 ml collection tube. Centrifuged at 13,000 rpm at RT for 1 min, and discard flow-through and collection tube altogether and placed the spin column into a new 2.0 ml collection tube. 700 µl of Buffer SWA (washing buffer A) was added to the spin column and centrifuged at 13,000 rpm for 1 min. discarded the flow-through and reuse the collection tube. Then added 700 µl of Buffer SWB(washing buffer B) to the spin column , and centrifuge at 13,000 rpm for

1min to dry membrane , discarded the flow-through and collection tube altogether. Then the spin column was placed into a new 1.5 ml tube, 50 µl Buffer SE (elution buffer) directly onto the membrane. Incubated for 1 min at room temperature and then centrifuge at 13,000 rpm for 1 min to elute (shown in appendix).

3.13 DNA storage:

The DNA from extraction was stored at -20°C, the DNA purity was determined by 1.5% agarose gel electrophoresis.

3.14 PCR Master Mix:

3.14.1 The preparation of pre – Mix of PCR:

The primer was firstly prepared according to manufacture instruction (Macrogen, Korea). Stock solution was prepared by adding 300 µl of sterile D.W in vial of primer, and for making working solution 5 µl was taken from stock solution and 95 µl from sterile D.W. Then in 0.2 eppendrof tube add 7 µl of master mix (manufacture by Dongsheng BioTech) is a premixed reagent ready to use and 0.5 of (F) primer and 0.5 µl of (R) primer were used from working primers solution. Then added 10 µl of D.W and 2 µl from the sample (the extracted DNA) were added, and then vortex. The total volume was 20 µl in each appendrof tube.

3.14.2 The PCR Protocol:

PCR (manufacture by Biometra TAdvanced , Germany) assay was performed to detect Urea A gene by using specific primer in table 3.3 .

PCR amplification involved initial denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 94°C C for 30 sec, annealing at 53°C for 30 sec

and extension at 72 °C for 45 sec, and a final extension at 72 °C for 5 min (Tamer *et al*., 2013).showen in table 3.1

3.15 Electrophoresis:

The agarose gel prepared by weight 1.5 gm agarose powder that was dissolved in 100 ml 1XTBE Buffer in small flak covered by tin (10 ml of 10X TBE Buffer added to 90 ml of distilled water), to prepare 1.5% agarose gel . Agarose was dissolved in microwave for 90 sec, 2 µl of 1% ethidium bromide was added to the molten agarose before it cooled down . mixed well and poured in a casting tray that had been taped up appropriately and equipped with spacers and suitable comb to form wells and left to solidified, after solidification the comb and the spacers were gently removed. Then, poured enough 1XTBE buffer into the buffer chamber to barely cover the top surface of gel , then ladder 5 µl and the PCR prduct 4 µl were added into the wells in the gel , 100 bp DNA ladder was used and the electric current was 124 volts for 30 min . then bands were visualized under U.V transilluminator(UVitec – UK) to detect the specific amplified products by comparing with 100 base pairs standard ladders (Tables 3.2 ,3.3 and 3.4) .

Table 3.1: The PCR Protocol:

PCR cycle	Temperature	Time	Cycle
Initial Denaturation	94°C	3 min	1
Denaturation	94 °C	30 sec	35
Annealing	53 °C	30 sec	35
Extension	72 °C	45 sec	35
Final Extension	72 °C	5 min	1

Table 3.2: Preparation of 10XTBE:

Substance	**Amount**
Tris HCL	48.45 mg
Boric acid	5.5 mg
EDTA	7.44 mg
D.W.	1000 ml

Table 3. 3: Primers sequences: (Kelly *et al.*, 1994)

Primers	**Primer sequences**	**Product length**
(F) primer	5´AACCGGATGATGTGATGGAT 3´	217 bp
(R) primer	5´GGTCTGTCGCCAACATTTTT 3´	

Table 3.4: Preparation of PCR pre-mix:

PCR reaction mixture	**Amount**
Master mix (Dongsheng BioTech)	7µl
Primer (F)	0.5 µl
Primer (R)	0.5 µl
D.N.A	2 µl
D.W	10 µl
Total	20 µl

Chapter four

Results

The frequency according to gender was 19 males and 31 females (n=50), The average age was 33.14 years old, the youngest was 10 years old and the oldest was 85 years old. Of the population, 38% were men, whose average age was 26.52 years, and 62% were women, whose average age was 37.19 years. (Table 4.1, 4.2).

The different clinical symptoms include most of patients (56%) suffering from nausea , (42%) vomiting , (other such as fever , fatigue , palpitation, diarrhea , loin pain ,dizzy , loss of appetite , belching) (Table 4.3).

Table 4.4 shows smoking behavior and most patient 94% were non smoker.

Table 4.5 shows the frequency according to educational level. The results show that the primary level was more than the other level.

Table 4.6 shows the frequency according to family history, which showed that the patients without family history were 54% more than patient with family history (46%).

Table 4.7 shows frequency according to social status .Which shows the number of married were 56 % more than the unmarried and widows.

Table 4.1 Frequency of patients' according to gender (N= 50)

Gender	**N (%)**
Male	19 (38%)
Female	31(62%)
Total	50 (100%)

Table 4.2 Frequency of patients according to age

Age (years)	**N (%)**
10 – 19	9 (18%)
20 – 29	18 (36%)
30 – 39	9 (18%)
40 – 49	6 (12%)
50 – 59	3 (6%)
60 – 69	1 (2%)
≥ 70	4(8%)

Table 4.3 Frequency of symptoms among study population

Symptoms	Yes N (%)	No N (%)
Nausea	28(56%)	22(44%)
Vomiting	21 (42 %)	29(58%)
Weight loss	10(20%)	40(80 %)
Abdominal pain	17(34%)	33(66%)
Heartburn	6(12%)	44(88%)
Headache	18(36%)	32(64%)
Other	30(60%)	20(40%)

Table 4.4 Frequency of patients' according to smoking behavior

Smoking behavior	N (%)
Never	47(94%)
Former	1(2%)
Current	2(4%)

Table 4.5 Frequency of patients' according to educational level

Educational level	N (%)
Uneducated	11(22%)
Primary	15(30%)
Secondary	13(26%)
Higher	11(22%)

Table 4.6 Frequency of patients' according to family history of infection with *H. pylori*:

Family history	N (%)
Yes	23(46%)
No	27(54%)

Table 4.7 Frequency of patients' according to social status:

Social status	N (%)
Married	28 (56%)
Unmarried	21 (42%)
Widow	1(2%)

Prevalence of *H.pylori* infection by stool culture and PCR:

Table 4.8 demonstrated the result of PCR were 58% positive and 42% negative and stool culture show all negative.

Table 4.8 Frequency of *H.pylori* infection according to stool culture and PCR

Method	**Number positive for *H.pylori* (%)**	**Number negative for *H.pylori***	**Total n=50**
Culture (stool)	0(0%)	50 (100%)	50(100%)
PCR	29(58%)	21(42%)	50(100%)

Figure 4.1 show result of PCR

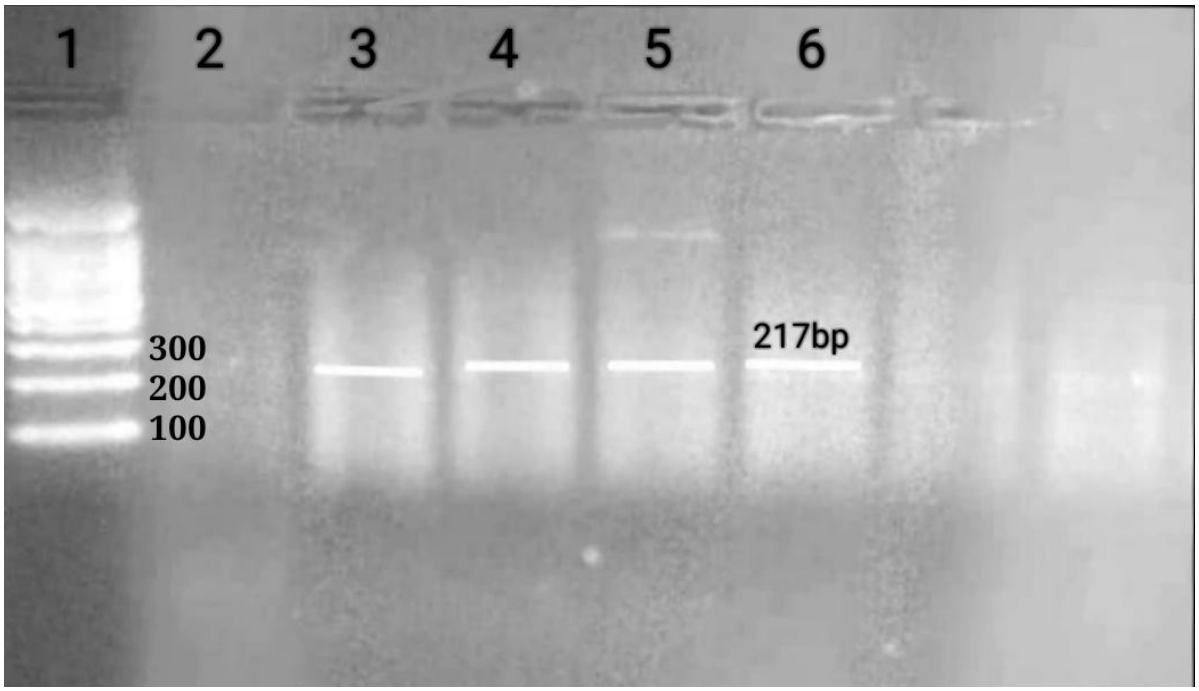


Figure 4.1 Multiplex PCR for amplification of *H. pylori ureA* gene on 1.5% agarose gel electrophoresis. Marker (Ladder): 100bp fragments. Lane 1: Marker, Lane 2: negative sample, Lane 3: positive control (from hospital) Lane 4 - 6: positive samples (Amplicons 217 bp represent)

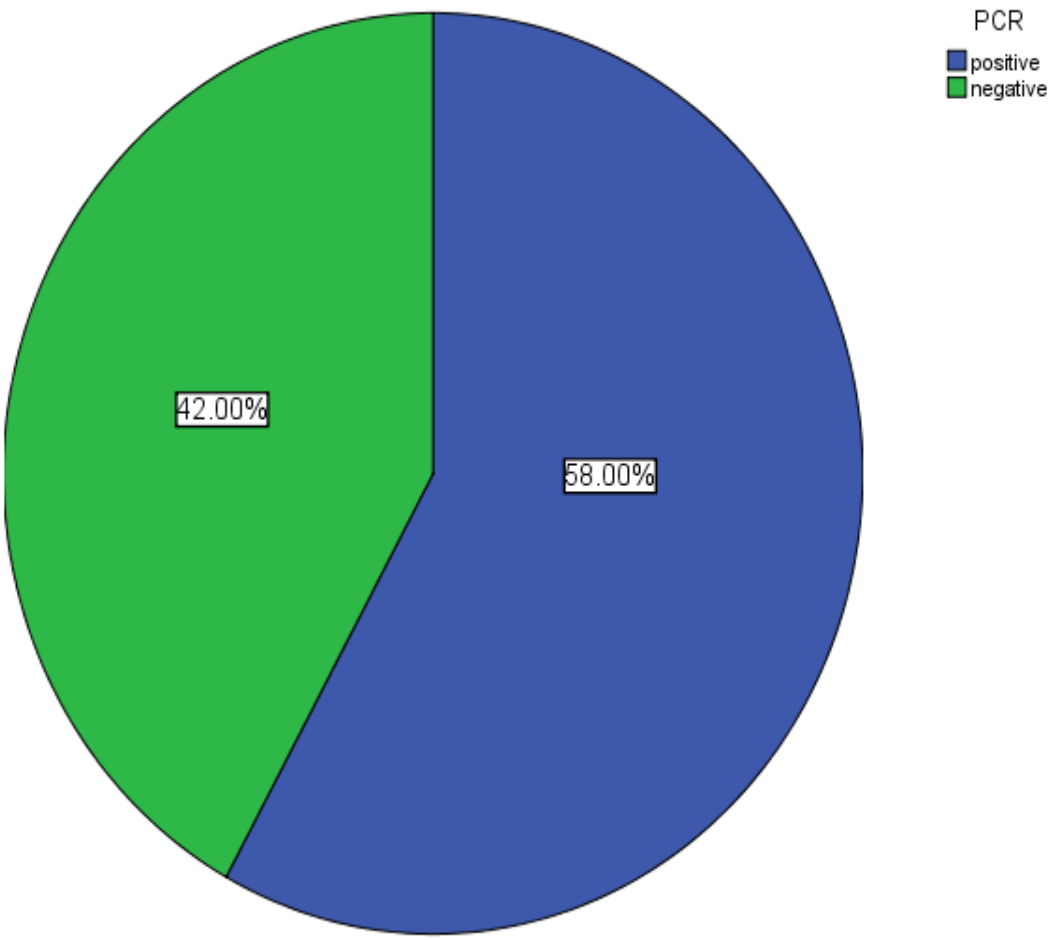


Figure 4. 2 The prevalence of *H. pylori* infection among study samples using PCR

The relationship of *H.pylori* infection diagnosed tested by PCR with participant characteristic:

According to table 4.9 overall prevalence of *H. pylori* was 58%. The prevalence of *H. pylori* on males and females was 20% and 38%, respectively.

Table 4.10 shown the frequency of *H. pylori* using PCR among different ages, showed insignificant association between age group and PCR results.

Table 4.11 show that no association between social status and PCR results (P.value >0.05).

Table 4.12 show whether specific symptoms were correlated with *H. pylori* prevalence, and found that there was no association but in vomiting and abdominal pain show slightly correlation p = .06 and .07 respectively.

Table 4.13 the findings show no association between *H. pylori* infection and the educational status.

Table 4.14 the findings show no association between *H. pylori* infection and the family history.

Table 4.15 the findings show no association between *H. pylori* infection and the smoking behavior.

Table 4.16 the findings show no association between *H. pylori* infection and the drinking water.

Relationship between *H. pylori positivity* and risk factors for infection:

Table 4.9 Relationship between *H.pylori* positivity by PCR and gender

Gender	Positive N (%)	NegativeN (%)	Total	P-value
Male	10(20%)	9(18%)	19(38%)	0.547
Female	19(38%)	12(24%)	31(62%)	0.547

Table 4.10 Relationship between *H. pylori* positivity by PCR and age

Age	Positive N (%)	Negative N (%)	Total	P-value
10-19	6(12%)	3(6%)	9(18%)	0.561
20-29	11(22%)	7(14%)	18(36%)	0.738
30-39	6(12%)	3(6%)	9(18%)	0.561
40-49	2(4%)	4(8%)	6(12%)	0.192
50-59	2(4%)	1(2%)	3(6%)	0.754
60-69	0(0%)	1(2%)	1(2%)	0.235
≥ 70	2(4%)	2(4%)	4(8%)	0.735

Table 4.11 Relationship between *H.pylori* positivity by PCR and social status

Social status	Positive N (%)	NegativeN (%)	Total	P-value
Married	16(32%)	12(24%)	28(56%)	0.890
Unmarried	12(24%)	9(18%)	21(42%)	0.917
Widow	1(2%)	0(0%)	1(2%)	0.390

Table 4.12 Relationship between *H.pylori* positivity by PCR and symptoms

Symptoms	PositiveN (%)	NegativeN(%)	Total	P-value
Nausea	16(32%)	12(24%)	28(56%)	0.890
Vomiting	9(18%)	12(24%)	21(42%)	0.065
Weight loss	4(8%)	6(12%)	10(20%)	0.197
Abdominal pain	13(%)	4(%)	17(%)	0.073
Heartburn	3(6%)	3(6%)	6(12%)	0.672
Headach	10(20%)	8(16%)	18(36%)	0.793
Other	14(28%)	14(28%)	28(56%)	0.196

Table 4.13 Relationship between *H.pylori* positivity by PCR and educational level

Educational level	PositiveN(%)	NegativeN(%)	Total	P-value
Uneducated	7(14%)	4(4%)	11(22%)	0.668
Primary	7(14%)	8(16%)	15(30%)	0.288
Secondary	8(16%)	5(5%)	13(26%)	0.764
Higher	7(14%)	4(8%)	11(22%)	0.668

Table 4.14 Relationship between *H.pylori* positivity by PCR and family history

Family history	PositiveN(%)	NegativeN(%)	Total	P-value
Yes	13(26%)	10(20%)	23(46%)	0.847
No	16(32%)	11(22%)	27(54%)	0.847

Table 4.15 Relationship between *H.pylori* positivity by PCR and smoking behavior

Smoking behavior	PositiveN(%)	NegativeN(%)	Total	P-value
Never	26(52%)	21(42%)	47(94%)	0.128
Former	1(2%)	0(0%)	1(2%)	0.390
Current	2(4%)	0(0%)	2(4%)	0.219

Table 4.16 Relationship between *H.pylori* positivity by PCR and drinking water

Drinking water	PositiveN(%)	Negative N (%)	Total	P-value
Tape water	29(58%)	20(40%)	49(98%)	0.235
Filtered water	0(0%)	1(2%)	1(2%)	
Other	0(0%)	0(0%)	0(0%)	

Chapter five

5.1 Discusion

H. pylori infection is a cofactor in the development of three important upper gastrointestinal diseases, duodenal or gastric ulcers, gastric cancer and gastric mucosa-associated lymphoid tissue (MALT) lymphoma (Kenneth and McColl , 2010). The risk of *H. pylori* infection in developing countries with low socioeconomic status is much higher (> 80%) than that in developed countries (< 40%)(Pounder and Ng , 1995 ; Brown *et al.*,2002).

The present study included 50 patients with different age groups and both genders (19 males and 31 females). The results of PCR for *H. pylori* detection in patients stool was 29 (58%). According to elbagir and Ahmed (2001) the disease prevalence was 80%. This variation may be because of using different test and in this case the PCR is more accurate than Immunochromatography test (ICT).

In stool culture; it was negative for *H.pylori* and it agree with Julie *et al.* (1999). Despite 58% of patients were PCR positive but non- of the stool culture was positive that because its fastidious organism with special requirement and there is inhibitor in stool which interrupt the growth of the bacteria but it need more research for suitable condition to growth .

In this study, as shown in Table 1, the prevalence of *H. pylori* infection according to PCR result is higher in females (38%) than males(20%). which observed that the females were more affected than males, but it correlation was statistically insignificant (P.value 0.547). Which appear there is no association between *H.pylori* infection and gender, this result agree with Ruihua *et al.*(2008) and Rasheed *et al.* (2012) but disagree with Bello *et al.* (2018) and

with Malcolm *et al.* (2004) who reported that prevalence of *H. pylori* is increased in males and statistically significant when compared with females . As for age, the result is agreed with Ruihua *et al.* (2008) and disagreed with Rasheed *et al.*, (2012), Klein *et al.* (1991b) and *Malcolm et al.* (2004).There was no association to marriage status which agrees with Ruihua *et al.* (2008) and disagreed with Malcolm *et al.* (2004) .

H. pylori infection was negatively correlated with the gastrointestinal symptoms which agreed with Ruihua *et al.* (2008) and Rasheed *et al.* (2012). Although no significant associations were seen for the subjects' level of education which agreed with Ruihua *et al.* (2008) .The *H. pylori* infection was negatively correlated to smoking which agreed with Ruihua *et al.* (2008) and disagreed with Bello *et al.* (2018) in our study, no association was found between *H. pylori* infection and the source of drinking water. Which agree with Ruihua *et al.* (2008) and Rasheed *et al.* (2012). This is because most people used the same source of drinking water from tape water that come from the river making it difficult to differentiate between sources which agree with Ruihua *et al.* (2008) and Rasheed *et al.* (2012).

The results that show in the risk factors may be due to different in geographical area, environmental condition and individual behavior.

5.2 Conclusion

Prevalence of *H.pylori* was high (58%) but there was insignificant correlation between gender , age , social status , gastrointestinal symptoms , educational level , family history of infection with *H. pylori* , smoking behavior , source of water . (P >0.05).

5.3 Recommendation

1. Increase sample size to verity these result.
2. Polymerase chain reaction is the most reliable tests for the diagnosis of *H. pylori* infection, but it is expensive and need technical demand.
3. More studies about the risk factors and mode of transmission of *H. pylori* are needed to cut the circle of transmission.

References

Alm RA., Ling LS., Moir DT., King BL., Brown ED., Doig PC., Smith DR., Noonan B., Guild BC., deJonge BL., Carmel G., Tummino PJ., Caruso A., Uria-Nickelsen M., Mills DM., Ives C., Gisbson R., Merberg D., Mills SD., Jiang Q., Taylor DE., Vovis GF., Trust TJ (1999). Genomic-sequence comparison of two unrelated isolates of the human gastric pathogen *Helicobacter pylori*. *Nature* **397**: 176–80.

Appelmelk BJ., Negrini R., Moran AP., Kuipers EJ (1997). Molecular mimicry between *Helicobacter pylori* and the host. *Trends Microbiol* **5** : 70–3.

Atherton JC., Cao P., Peek RM Jr., Tummuru MK ., Blaser MJ., Cover TL (1995).Mosaicism in vacuolating cytotoxin alleles of *Helicobacter pylori*. Association of speci.c VacA types with cytotoxin production and peptic ulceration. *J Biol Chem.* **270**: 17771–7.

Atherton JC., Peek RM., Tham KT., Cover TL., Blaser MJ (1997). Clinical and pathological importance of heterogeneity in *VacA*, encoding the vacuolating cytotoxin of *Helicobacter pylori*. *Gastroenterology***112**: 92–9.

Azevedo NF., Almeida C., Cerqueira L., Dias S., Keevil CW., Vieira MJ (2007). Coccoid form of *Helicobacter pylori* as a morphological manifestation of cell adaptation to the environment. *Appl. Environ. Microbiol* **73** : 3423–3427.

Baggiolini M., Dewald B., Moser B (1994). Interleukin-8 and related chemotactic cytokines-CXC and chemokines. *Adv Immunol* **55**: 97–179.

Baggiolini M., Dewald B., Moser B (1997). Human chemokines: an update. *Annu Rev Immunol* **15**: 675–705.

Baik SJ., Yi SY., Park HS., Park BH (2012).Seroprevalence of *Helicobacter pylori* in female Vietnamese immigrants to Korea. *World J Gastroenterol* **18**(6): 517-521.

Bello AK., Umar AB., Borodo MM (2018). Prevalence and risk factors for *Helicobacter pylori* infection in gastroduodenal diseases in Kano, Nigeria. *Afr J Med Health Sci* **17**:41.

Bode G., Malfertheiner P., Lehnhardt G., Nilius M., Ditschuneit H (1993). Ultrastructural localization of urease of *Helicobacter pylori*. *Med Microbiol Immunol* **182**: 233–42.

Brooks GF., Carroll KC., Butel JS., Morse SA., Mietzner TA (2010). *Medical Microbiology*. Jawetz, Melnick and Adelbergs, 25th Edition, McGraw-Hill Companies, 235- 240.

Brown LM., Thomas TL., Ma JL., Chang YS., You WC., Liu WD., Zhang L., Pee D., Gail MH (2002). *Helicobacter pylori* infection in rural China: demographic, lifestyle and environmental factors. *Int J Epidemiol* **31**:638–45.

Caristo E., Parola A., Rapa A., Vivenza D., Raselli B., Dondi E., Boldorini R., Orderda G (2008). Clarithromycin resistance of *Helico-bacter pylori* strains isolated from children' gastric antrum and fundus as assessed by fluorescent in-situ hybridization and culture on four-sector agar plates. *Helicobacter*. Dec;**13**(6):557–563.

Censini S., Lange C., Xiang Z., Crabtree JE., Ghiara P., Borodovsky M., Rappuoli R., Covacci A (1996). Cag, a pathogenicity island of *Helicobacter pylori*, encodes type I-speci.c and disease-associated virulence factors. Proc Natl Acad Sci USA **93**: 14648–53.

Cheesbrough, M, (2006). Discrit laboratory practice in tropical countries. 2nd ed.part 2. UK:Cambridge university press.UK.

Cheesbrough. M., (2000). Distruct labrotary practice in tropical countries, part 2. UK:Cambridge university press.

Chiba N., Rao BV., Rademaker JW., Hunt RH (1992). Metaanalysis of the efficacy of antibiotic therapy in eradicating *Helicobacter pylori. Am J Gastroenterol* **87**:1716-27.

Covacci A., Censini S., Bugnoli M., Petracca R., Burroni D.,Macchia G., Massone A., Papini E., Xiang Z., Figura N., Rappuoli R (1993). Molecular characterization of the 128-kDa immunodominant antigen of *Helicobacter pylori* associated with cytotoxicity and duodenal ulcer. *Proc Natl Acad Sci USA* **90**: 5791–5.

Covacci A., Falkow S., Berg DE., Rappuoli R (1997). Did the inheritance of a pathogenicity island modify the virulence of *Helicobacter pylori*? *Trends Microbiol* **5**: 205–8.

Covacci A., Telford JL., Del Giudice G., Parsonnet J., Rappuoli R (1999). *Helicobacter pylori* virulence and genetic geography. *Science* **284**: 1328–33.

Cover TL., Blaser MJ (1992).Purification and characterization of the vacuolating toxin from *Helicobacter pylori. J Biol Chem* **267**: 10570–675.

Cover TL., Halter SA., Blaser MJ (1992). Characterization of Hela cell vacuoles induce by *Helicobacter pylori* broth culture supernatant. *Human Pathol* **23**: 1004–10.

Cullen DJ., Collins BJ., Christiansen KJ., Epis J., Warren JR., Surveyor I., Cullen KJ (1993). When is *Helicobacter pylori* infection acquired? *Gut* **34**:1681-2.

Czinn SJ. (2005). *Helicobacter pylori* infection: detection, investigation, and management. *J Pediatr*. Mar;**146**(Suppl 3):S21–S26.

Deankanob W., Chomvarin C., Hahnvajanawong C., Intapan PM., Wongwajana S., Mairiang P., Kularbkaew C., Sangchan A (2006). Enzyme

linked immunosorbent assay for Serodiagnosis of *Helicobacter pylori* in dyspeptic patients and volunteer blood donors. *Southeast Asian J Trop Med Public Health* **37**(5): 958-965.

Dunn BE., Cohen H., Blaser MJ (1997). *Helicobacter pylori*. *Clinical Microbiology Reviews* **10** : 720–741.

Eaton KA., Suerbaum S., Josenhans C., Krakowka S (1996).Colonization of gnotobiotic piglets by *Helicobacter pylori* deficient in two flagellin genes. *Infect Immun* **64** : 2445–8.

Elbagir M and Ahmed K (2001) Duodenal ulcer in Sudan .*Sudan Medical Journal* **24** :1-39.

Evans DJ Jr., Evans DG., Takemura T., Nakano H., Lampert HC., Graham DY., Granger DN., Kvietys PR (1995) Characterization of *Helicobacter pylori* neutrophil activating protein. *Infect Immun*; **63** : 2213–20.

Ferguson DA., Li C., Patel NR., Mayberry WR., Chi DS., Thomas E (1993) .Isolation of *Helicobacter pylori* from saliva. *J Clin Microbiol* **31**:2802-4.

Ferwana M, Abdulmajeed I, Alhajiahmed A, Madani W, Firwana B, Hasan R, Altayar O, Limburg PJ, Murad MH, Knawy B. (2015).Accuracy of urea breath test in *Helicobacter pylori* infection: meta-analysis. *World J Gastroenterol*.**21**:1305–1314.

Figura N., Perrone A., Gennari C., Orlandini G., Bianciardi L., Giannace R., Vaira D., Vagliasinti M., Rottoli P (1999). Food allergy and *Helicobacter pylori* infection. *Ital J Gastroenterol Hepatol* **31**: 186–91.

Fiocca R., Necchi V., Sommi P, Ricci V., Telford J., Cover TL., Socia E (1999). Release of *Helicobacter pylori* vacuolating cytotoxin by both a speci.c secretion pathway and budding of outer membrane vesicles. Uptake of released toxin and vesicles by gastric epithelium. J Pathol **188**: 220–6.

Fox, J. G. (2002). The non-*H. pylori* helicobacters: their expanding role in gastrointestinal and systemic diseases. *Gut* **50** :273–283.

Garza-González E., Perez-Perez GI., Maldonado-Garza HJ., Bosques-Padilla FJ., Javier F (2014). A review of *Helicobacter pylori* diagnosis, treatment, and methods to detect eradication. *World J Gastroenterol*. Feb;**20**(6):1438–1449.

Gisbert JP and Calvetx (2012) update on non – bismuth quardryple therapy for eradication of *Helicobacter pylori*. *Clin Exp Gastroentterol* **5**:23-34 .

Gisbert JP., de la Morena F., Abraira V (2006). Accuracy of monoclonal stool antigen test for the diagnosis of *Helicobacter pylori* infection: a systematic review and meta-analysis. *Am J Gastroenterol* **101**:1921–1930.

Gisbert, JP., Cabrera M.M.M, Pajares J.M (2002). Stool antigen test for initial *Helicobacter pylori* diagnosis and for confirmation of eradication after therapy. *Med. Clin. (Barc).*, **118 :** 401–404.

Goodman KJ., Correa P., Tengana Aux HJ., Ramirez H., Delany JP., Guerrero Pepinosa O., Lopez Quinones M., Collazos Parra T (1996). *Helicobacter pylori* infection in the Colombian Andes: a population-based study of transmission paths. *American Journal of Epidemiology* **144**: 290–299.

Goodwin C. S., McConnell W., McCulloch R. K., McCullough C., Hill R., Bronsdon M. A., Kasper G (1989) **.**Cellular fatty acid composition of Campylobacter pylori from primates and ferrets compared with those of other campylobacters. *Journal of clinical microbiology*, ***27***(5), 938-43.

Goodwin C. S., McCulloch R. K., Armstrong J. A., Wee S. H (1985) .Unusual cellular fatty acids and distinctive ultrastructure in a new spiral bacterium (*Campylobacter pyloridis*) from the human gastric mucosa. *J. Med. Microbiol.* **19**:257–267.

Grubel P., Hoffman JS., Chong FK., Burstein NA., Mepani C., Cave DR (1997). Vector potential of houseflies (Musca domestica) for Helicobacter pylori. *Journal of Clinical Microbiology* **35** : 1300–1303.

Guarner J., Kalach N., Elitsur Y., Koletzko S (2010). *Helicobacter pylori* diagnostic tests in children: review of the literature from 1999 to 2009. *Eur J Pediatr*. Jan;**169**(1):15–25.

Handt LK., Fox JO., Dewhirst FE., et al. (1994).*Helicobacter pylori* isolated from the domestic cat: public health implications. *Infect Immun* **62** :2367-74.

Hanvivatvong O., Atinop P., Niramol T., Pinit K (2004). Evaluation of commercial Immunoassays for detection of antibody against *Helicobacter pylori* in Thai Dyspeptic Patients. *Clin Diagn Lab Immunol* **11**(3): 618-620.

Harris PR., Mobley HL., Perez-Perez GI., Blaser MJ., Smith PD (1996). *Helicobacter pylori* urease is a potent stimulus of mononuclear phagocyte activation and in• ammatory cytockine production. *Gastroenterology* **111**: 419–25.

Hopkins RJ and Morris JG.(1994)*Helicobacter pylori:* The missing link inperspective. *Am Med* **97**:265-77.

Hopkins RJ.,Vial PA., Ferreccio C., Ovalle J., Prado P., Sotomayor V., Russell RG.,Wasserman SS., Morris JG Jr (1993). Seroprevalence of *Helicobacter pylori* in Chile: vegetables may serve as one route of transmission. *Journal of Infectious Diseases* **168**: 222–226.

Ilver D., Arnqvist A., Ogren J., Frick IM., Kersulyte D., Incecik ET., Berg DE., Covacci A., Engstrand L., Borén T (1998). *Helicobacter pylori* adhesin binding fucosylated histo-blood group antigens revealed by retagging. *Science* **279**: 373–7.

Julie parsonnet MD. , Haim shmely ,MD Thomas Haggerly ,BS (1999). fecal and oral shedding of *Helicobacter pylori* from healthy infected adults .*JAMA*[online] **282** (12) .p. 2240-2245.

Kalach N., Gosset P., Dehecq E., Decoster A., Spyckerelle C., Papadopolos S., Dupont C., Raymond J (2015). Usefulness of gastric biopsy-based real time-PCR for the diagnosis of *Helicobacter pylori* Infection in children. *J Pediatr Gastroenterol Nutr*. Sep;**61**(3):307–312.

Kanna MS., Romereo CM., Fas R(2013). Diagnostic tests for *Heli-cobacter pylori. . Gastroenterol Endosc News* **11**(Special Edition):51–58.

Kelly SM., Pitcher MC., Farmery SM., Gibson GR (1994).Isolation of *Helicobacter pylori* from feces of patient with dyspepsia in United Kingdom.*Gastroenterology***107**(6):1671-4.

Kenneth EL and McColl MD (2010). *Helicobacter pylori* Infection. *N Engl J Med* **362**: 1597-1604.

Kim J., Kim N., Jo HJ., Park JH Nam RH., Seok YJ., Kim YR., Kim JS., Kim JM., Kim JM., Lee DH., Jung HC (2015). An appropriate cutoff value for determining the colonization of *Helicobacter pylori* by the pyrosequencing method: comparison with conventional methods. *Helicobacter*. Oct;**20**(5):370–380.

Kimura K., Ido K., Saifuku K., Taniguchi Y., Kihira K., Satoh K., Takimoto T., YoshidaY (1995). A 1-h topical therapy for the treatment of *Helicobacter pylori* infection. *Am J Gastroenterol*;90:60-3.

Kimura M., Goto S., Wada A., Yahiro K., Niidome T., Hatakeyama T., Aoyagi H., Hirayama T., Kondo T(1999). Vacuolating cytotoxin causes mitochondrial damage in human gastric cells. *Microb Pathogenesis* **26** : 45–52.

Klein PD., Graham DY., Gaillour A., Opekun AR., Smith EO (1991a). Gastrointestinal Physiology Working Group. Water source as risk factor for *Helicobacter pylori* infection in Peruvian children. *Lancet* **337**:1503- 6.

Klein PD., Opekun AR, Smith EO., Graham DY., Gaillour A (1991b). Water source as risk factor for Helicobacter pylori infection in Peruvian children. *Lancet* **337**:1503-1506.

Lee, J.Y. and Kim, N.(2015). Diagnosis of *Helicobacter pylori* by invasive test: Histology. *Ann. Transl. Med.* **3 :**10.

Li SD., Kersulyte D., Lindley IJD., Neelam B., Berg DE., Crabtree JE (1999). Multiple genes in the left half of the Cag pathogenicity island of *Helicobacter pylori* are required for tyrosine kinase-dependent transcription of interleukin-8 in gastric epithelial cells. *Infect Immun* **67**: 3893–9.

Lin SK., Lambert JR., Schembri MA., Nicholson L., Johnson IH (1998). The prevalence of *Helicobacter pylori* in practicing dental staff and dental students. *Australian Dental Journal* **43**: 35–39.

Maeda SH., Yoshida T., Ikenoue K., Ogura K., Kanai F., Kato N., Shiratori Y., Omata M (1999).Structure of cag pathogenicity island in Japanese *Helicobacter pylori*. *Gut* **44**: 336–41.

Malcolm CA., MacKay WG., Shepherd A., Weaver LT(2004). *Helicobacter pylori* in childern is strongly associated with poverty .*Scottish medical journal.* **49** (4)136-138.

Malfertheiner P., Megraud F., O'Morain C.A., Atherton J., Axon A.T.R., Bazzoli F., Gensini G.F., Gisbert J.P., Graham DY., Rokkas T., El-Omar EM., Kuipers EJ., European Helicobacter Study Group(2012). Management of *Helicobacter pylori* infection-theMaastricht IV/Florence Consensus Report. *Gut* **61 :**646–664.

Marshall B. J., H. Royce, D. I. Annear, C. S. Goodwin, J. W. Pearman, J. R. Warren, and J. A. Armstrong (1984). Original isolation of *Campylobacter pyloridis* from human gastric mucosa. *Microbios Lett*. **25**:83-88**.**

Marshall B.J. and Goodwin C. S. (1987). Revised nomenclature of *C. pyloridis*. *Int. J. Syst. Bacteriol* **37**:68.

Mauch F., Bode G., Ditschuneit H., Malfertheiner P (1993). Demonstration of a phospholipid-rich zone in the human gastric ephitelium damaged by *Helicobacter pylori*. *Gastroenterology*; **105**: 1698–704.

McGeeDJ., May CA., Garner RM., Himpsl JM., Mobley HLT (1999) .Isolation of *Helicobacter pylori* genes that modulate urease activity. *J Bacteriol* **181**: 2477–84.

Me´ graud F. (1995). Transmission of *Helicobacter pylori*: faecal-oral versus oral-oral route. *Alimentary Pharmacology and Therapeutics*, **9** (Suppl. 2): 85–91.

Mentis A., Lehours P., Megraud F.(2015).Epidemiology and diagnosis of *Helicobacter pylori* infection. Helicobacter. Sep;**20** (Suppl 1):1–7.

Mobley H., Island MD., Hausinger RP (1995). Molecular biology of microbial ureases. *Microbiol Rev* **59**: 451–80.

Namavar F., Roosendaal R., Kuipers EJ., Groot P De., Van der Bijl MW., Pena AS., Graaff J De (1995). Presence of Helicobacter pylori in the oralcavity, oesophagus, stomach and faeces of patients with gastritis.*European Journal of Clinical Microbiology and Infectious Diseases* **14**: 234–237.

Ndip RN., MacKay WG., Farthing M.J.G., Weaver L.T. (2003). Culturing *Helicobacter pylori* from clinicalspecimens: Review of microbiologic methods. *J. Pediatr. Gastroenterol. Nutr* **36** : 616–622.

Neale KR. And Logan RPH (1995). The epidemiology and transmission of Helicobacter pylori infection in children. *Alimentary Pharmacology and Therapeutics* **9** (Suppl. 2): 77–84.

NIH Consensus Conference (1994). *Helicobacter pylori* in peptic ulcer disease. *JAMA* **272**:65-9.

Okuda M., Osaki T., Kikuchi S., Ueda J., Lin Y., Yonezawa H., Maekawa K., Hojo F., Kamiya S., Fukuda Y (2014). Evaluation of a stool antigen test using a mAb for native catalase for diagnosis of *Helicobacter pylori* infection in children and adults. *J Med Microbiol*.**63**:1621–1625.

Peach HG., Pearce DC., Farish SJ. (1997). *Helicobacter pylori* infection in an Australian regional city: prevalence and risk factors. *Medical Journal of Australia* **167**: 310–313.

Pearson AD (1983). and Great Britain Public Health Laboratory Service *Campylobacter II: Proceedings of the Second International Workshop on Campylobacter Infections*, Brussels, 6–9 September. Public Health Laboratory Service, London, United Kingdom.

Peek RM Jr., Miller GG., Tham KT., Perez-Perez GI., Zhao X., Atherton C., Blaser MJ (1995). Heightened in• ammatory response and cytokine expression *in vivo* to CagA» *Helicobacter pylori* strains. Lab Invest **73**: 760–70.

Peek RM Jr., Thompson SA., Donahue JP., Tham KT., Atherton JC., Blaser MJ., Miller GG(1998). Adherence to gastric epithelial cells induces expression of a *Helicobacter pylori* gene, *iceA*, that is associated with clinical outcome. *Proc Assoc Am Physicians* **110**: 531–44.

Piccolomini RD., Bonaventura G Festi, D.catamo G (1997). Optimal combination of media for primary isolation of Helicobacterpylori from gastric biopsy specimen . *Journal of Clinical Microbiology* **53**(6).p.1541-1544.

Pounder RE., Ng D.(1995). The prevalence of *Helicobacter pylori* infection in different countries. *Aliment Pharmacol Ther*; **9** :33–9.

Quartero A., Numans M,de Melker R., de Wit N.(2000).Impractice evaluation of whole-blood Helicobacter pylori test: its usefulness in detecting peptic ulcer disease . Br J Gen Pract. **50** (450):13.

Rajindrajith S., Devanarayana NM., de Silva HJ. (2009). *Helicobacter pylori* infection in children. *Saudi J Gastroenterol*. Apr;**15** (2) :86–94.

Rasheed F, Ahmad T, Bilal R. (2012). Prevalence and risk factors of Helicobacter pylori infection among Pakistani population. *Pak J Med Sci*; **28**(4):661-665.

Richard V. Goering, Hazel M. Dockrell ,Mark Zuckerman , Ivan M.Roitt , Peter L.Chiodini (2013).*Mims' Medical Microbiology* .5th Ed. China: Elsevier saunders.

Rocha GA., Rocha AM., Silva LD., Santos A., Bocewicz AC., Queiroz RM., Bethony J., Gazzinelli A., Correa-Oliveira R., Queizroz DM (2003). Transmission of *Helicobacter pylori* infection in families of preschool-aged children from Minas Gerais, Brazil. *Trop Med Int Health* **8**(11): 987-991.

Romaniuk P. J., Zoltowska B., Trust T. J., Lane D. J., Olsen G. J., Pace N. R., Stahl D. A. (1987). *Campylobacter pylori*, the spiral bacterium associated with human gastritis, is not a true *Campylobacter* sp. *J. Bacteriol.* **169**:2137–2141.

Rothenbacher D., Bode G., Berg G., Knayer U., Gonser T., Adler G., Brenner H (1999).*Helicobacter pylori* among preschool children and their parents: evidence of parent-child transmission. *J Infect Dis*; **179**: 398–402.

Ruihua Shi , Shunfu Xu , Hongjie Zhang , Yanbing Ding , Guiqian Sun ,Xiayue Huang , Xiaoxing Chen, Xueliang Li , Zhigang Yan, Guoxin Zhang

(2008). prevalence and risk factors for Helicobacter pylori infection in Chinese population .*Helicobacter* [online] **13** :157-165.

Sahay P., Axon ATR (1996). Reservoirs of Helicobacter pylori and modes of transmission. *Helicobacter* **1**: 175–182.

Schmitz A., Josenhans C., Suerbaum S (1997). Cloning and characterization of *Helicobacter pylori*• *flbA* gene, which codes for a membrane protein involved in coordinated expression of flagellar genes. *J Bacteriol* **179**: 987–97.

Simon PM., Good PL., Mobasseri A., Zopf D.(1997).Inhibition of *Helicobacter pylori* binding to gastrointestinal epithelial cells by sialic acid-containing oligosaccharides. *Infect Immun*; **65** : 750–7.

Solnick JV., O'Rourke J., Lee A., Tompkins LS. (1993). Molecular analysis of urease genes from a newly identified uncultured species of *Helicobacter*. *J Infect Dis***168**:379-83.

Solnick, J. V., and D. B. Schauer (2001). Emergence of diverse *Helicobacter* species in the pathogenesis of gastric and enterohepatic diseases. *Clin. Microbiol*. Rev. **14**:59–97.

Taj Y., Essa F., Kazmi SU., Abdullah, E. (2003). Sensitivity and specificity of various diagnostic tests in the detection of *Helicobacter pylori*. *J. Coll. Physicians Surg. Pak.*,**13** : 90–93.

TamerE.,Wail, H Israr, S, Walid, S, Mohammad, AF (2013). Determination of *Helicobacter pylori* virulence genes in gastric biopsies by PCR. *ISRN Gastroenterology*, article ID: 606258.

Taylor DN. and Blaser MJ.(1991).The epidemiology of *Helicobacter pylori* infection. *Epidemiol Rev***13**:42-59.

Thomas JE., Gibson CR., Darboe MK., Dale A., Weaver LT. (1992). Isolation of *H. pylori* from human faeces. *Lancet*; **340**:1194-5.

Tomb JF., White O., Kerlavage AR., Clayton RA., Sutton GG., Fleischmann RD., Ketchum KA., Klenk HP., Gill S., Dougherty BA., Nelson K., Quackenbush J., Zhou L., Kirkness EF(1997). The complete genome sequence of the gastric pathogen *Helicobacter pylori*. *Nature* **388**: 539–47.

Ueda J., Okuda M., Nishiyama T., Lin Y., Fukuda Y., Kikuchi S.(2014). Diagnostic accuracy of the E-plate serum antibody test kit in detecting *Helicobacter pylori* infection among Japanese children. *J Epidemiol*. **24**:47–51.

Uotani T., Graham D.Y. (2015). Diagnosis of *Helicobacter pylori* using the rapid urease test. *Ann. Transl. Med* **3** : 9.

Walker MM. and Crabtree JE. (1998). *Helicobacter pylori* infection and the pathogenesis of duodenal ulceration. *Ann NY Acad Sci* **859**: 96–111.

Walker WA., Goulet O., Kleinman RE., Sherman PM., Shneider BL., Sanderson IR. (2004). *Helicobacter pylori* and peptic ulcer disease. . In: Rowland M., Bourke B., Drumm B., editors; Pediatric gastrointestinal disease. Ontario: BC Decker;. p. 491–512.

Webb P., Crabtree JE., Forman D., and the Eurogast Study Group.(1999). Gastric cancer, cytotoxin associated gene A positive *Helicobacter pylori* and serum pepsinogens: an international study. *Gastroenterology* **116**: 269–76.

Yamada S., Mastsuhisa T., Makokawkeyoon l., Chaidatch S., Kato S., Matsukura N(2006). *Helicobacter pylori* infection in combination with the serum pepsinogen I / II ratio and Interleukin-1 beta -511 polymorphisms are independent risk factor for gastric cancer in thais.*JGastroenterol* **41**(12):1169-1177.

Yamaoka Y., Kodama T., Gutierrez O., Kim JG., Kashima K., Graham DY. (1999).Relationship between *Helicobacter pylori* iceA, cagA, and vacA status

and clinical outcome: studies in four different countries. *J Clin Microbiol* **37**: 2274–9.

Yamoka ,Y.(2010). Mechanisms of disease :*Helicobacter pylori* virulence factors . *Nat Rev Gastroenterol Hepatol* **7**(11):629-641.

Yang S. and Rothman, R.E. (2004). PCR-based diagnostics for infectious diseases: Uses, limitations, and future applications in acute-care settings. *Lancet. Infect. Dis*. 4:337–348.

Zhongming G. and Taylor DE. (1998).*Helicobacter pylori*-molecular genetics and diagnostic typing. *Br Med Bull* **54**: 31–8.

Appendix

Questionnaire

- Date:..... - Hospital:.....

- Participant name:.....

- ID number..... - Age:.....years

- Sex: Male ( ) Female ( )

-social status : married ( ) unmarried ( ) separate or die ( )

-individual education : uneducated ( ) primary ( )

secondary ( ) higher ( )

-smoking: Never( ) former( ) current( )

- Signs and symptoms:

Heart burn ( ) Unexplained weight loss ( )
Nausea ( ) vomiting ( )

Headach ( ) abdominal pain ( )

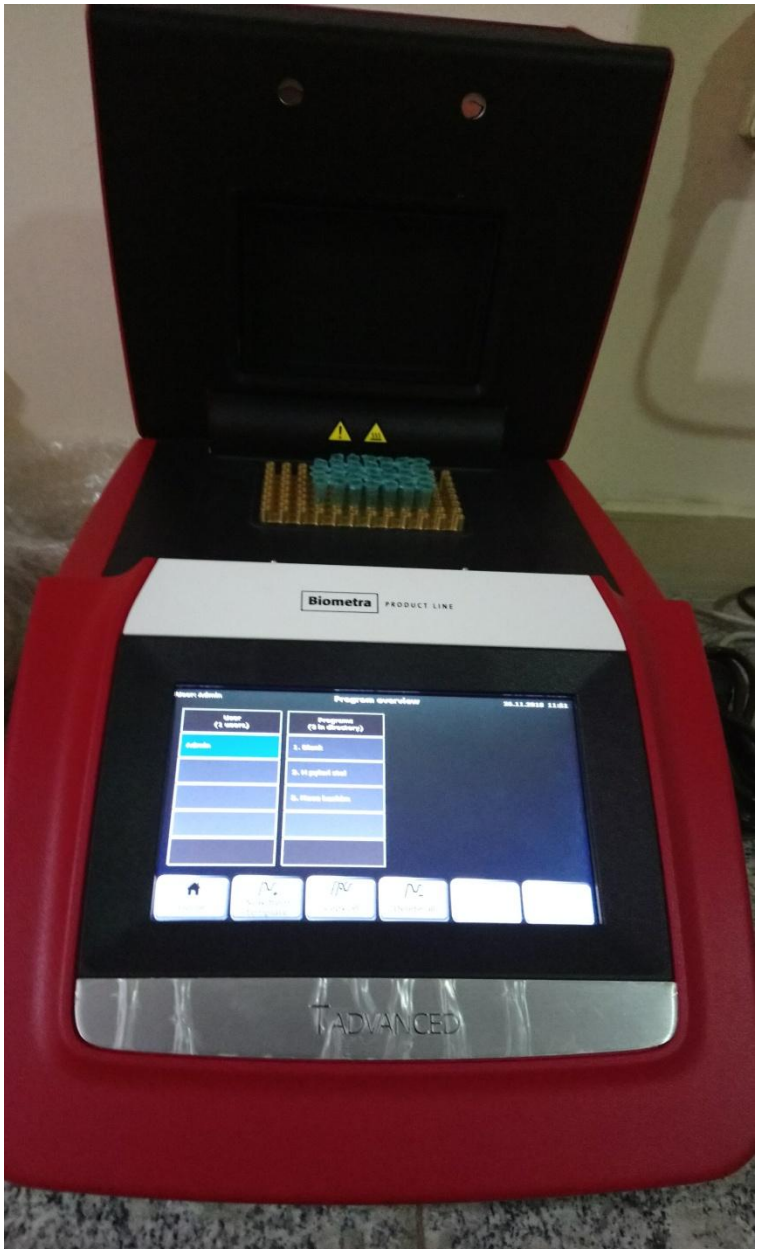
other ( )

- family history of infection with H.pylori : Yes ( ) No ( )

- source of water ( )

-result of stool culture : ( )

- result of PCR : ( )



PCR machine

i-genomic stool DNA Extraction mini kits

Microcentrifuge

RECOVERY OF PURIFIED DNA

Determination of concentration, yield, and purity DNA yield is determined from the concentration of DNA in the eluate, measured by absorbance at 260 nm. Absorbance readings at 260 nm should fall between 0.1 and 1.0 to be accurate. Sample dilution should be adjusted accordingly: for example, an eluate containing 25 ~ 50 ng DNA/ μl (A_{260} = 0.5 ~ 1.0) should not be diluted with more than 4 volumes of buffer. Use elution buffer or water (as appropriate) to dilute samples and to calibrate the spectrophotometer. Measure the absorbance at 260 and 280 nm, or scan absorbance from 220 ~ 320 nm (a scan will show if there are other factors affecting absorbance at 260 nm). Both DNA and RNA are measured with a spectrophotometer; to measure only DNA, a fluorimeter must be used. Purity is determined by calculating the ratio of absorbance at 260 nm to absorbance at 280 nm. Pure DNA has an A_{260}/A_{280} ratio of 1.7 ~ 2.0. DNA purified by the i-genomic Stool Kit procedure is free of protein and other contaminants that can inhibit PCR or other enzymatic reactions. The purified DNA can be used immediately or safely stored in Buffer SE at –20°C for later use.

EQUIPMENT AND REAGENTS TO BE SUPPLIED BY USER

i-genomic Stool DNA Mini Kit provides almost

- ✓ PBS (phosphate-buffered saline) may be required for some samples
- ✓ Ethanol (96 – 100%)
- ✓ Pipettes and pipette tips
- ✓ Micro-centrifuge (e. g., CENDORI™ S-12; iNtRON, Cat. No. 50100)
- ✓ Water bath or heating block, Vortex mixer
- ✓ Other general lab equipments

IMPORTANT POINTS BEFORE STARTING

▪ Buffer SWA / SWB

: Buffer SWA / SWB is supplied as concentrate. Before using for the first time, be sure to add 28 ml / 40 ml of absoulute ethanol (96 ~ 100%) to obtain a working solution.

▪ Lyophilized RNase A

Dissolve the RNase A in 0.3 ml of pure D.W. to each vial. The lyophilized RNase A can be stored at room temperature (15–25°C) until the expiration date without affecting performance. The lyophilized RNase A can only be dissolved in D.W.; dissolved RNase A should be immediately stored at -20℃. The RNase A solution is stable at -20°C for up to 24 months and 20 times frozen-thawing until the kit expiration date.

▪ Lyophilized Proteinase K

Dissolve the Proteinase K in 1.1 ml of pure D.W. to each vial. The lyophilized Proteinase K can be stored at room temperature (15–25°C) until the expiration date without affecting performance. The lyophilized Proteinase K can only be dissolved in D.W.; dissolved Proteinase K should be immediately stored at -20℃. The Proteinase K solution is stable at -20°C for up to 24 months and 20 times frozen-thawing until the kit expiration date.

▪ Preheat a water bath or heating Block

▪ Centrifugation

: All centrifugation steps are carried out at RT (15 ~ 25°C) in a micro-centrifuge.

COLUMN INFORMATION

▪ i-genomic series Spin Column, applied to CAPS

CAPS, Clean Automatic Packaging System

Column Membrane[1]	Silica-based membrane
Spin Column[1]	Individually, in inserted in a 2.0 mL Collection Tube[2].
Loading Volume	Maximum 800 μl
DNA Binding Capacity	Maximum 45 μg
Recovery	85 ~ 95% depending on the elution Volume
Elution Volume	Generally, eluted with 30 ~ 200 μl of Elution Buffer

[1] Do not store the Column packs under completely dried conditions. It may be affected to DNA binding capacity. The Spin Columns are stable for over 1 year under these conditions

[2] Additional Collection Tubes (100 ea) are also supplied for your convenient handling.

PROTOCOL

: *Refer to the "VISUAL PROTOCOL"*

1. **Weigh 180 ~ 220 mg in a 1.5 ml tube and place the tube on ice.**
   Note : Exceeding the recommended optimal amount of starting material will result in inefficient lysis, resulting in low DNA yield and purity. To prevent thaw the frozen sample during transfer it, previously chill the spatula and 1.5 ml tube in liquid nitrogen. The freezing-thawing repetition of frozen sample will result in the DNA degradation.
2. **Add 200 μl of Buffer SPL to each stool sample. Vortex continuously for 1 min and incubate 70°C for 5 min.**
   Note : If the Buffer SPL become solid, incubate in 80°C for 10 min.
3. **Place the i-genomic Stool IR Spin Column into a new 1.5 ml tube (not supplied), and transfer supernatant promptly into the i-genomic Stool IR Spin column.**
4. **Centrifuge for 1 min at 13,000 rpm (RT), and remove the IR Spin Column from the 1.5 ml tube.**
5. **Add 200 μl of Buffer SL, 20 μl of Proteinase K and 5 μl of RNase A solution in 1.5 ml tube and mix by vortexing.**
6. **Incubate the lysates for 15 min at 65°C.**
   Note : For complete lysis, mix 5 or 6 times during incubation by inverting tube. The incubation time can be prolonged for more yields of DNA. The complete lysis let you see clear lysate.
7. **After lysis completely, add 200 μl of Buffer SB to the lysate, and mix by pipetting or gently inverting 5 to 6 times. DO NOT vortex. After mixing, spin down to remove drops from inside the lid.**
8. **Add 250 μl of 80% ethanol to the lysate, and mix by pipetting or gently inverting 5 ~ 6 times. DO NOT vortex. After mixing, spin down to remove drops from inside the lid.**
   Note : It is essential that the sample and 80% ethanol are mixed thoroughly to yield a homogeneous solution. But do NOT vortex vigorously, because high speed of vortexing can give occasion to shearing of genomic DNA.
9. **Pipette 750 μl of the mixture from step 8 into the spin column inserted in a 2.0 ml collection tube. Centrifuge at 13,000 rpm at RT for 1 min, and discard flow-through and collection tube altogether.**
   Note : The maximum volume of the spin column reservoirs is 800 μl. In case of the large volume of binding mixture, divide the binding mixture into halves and load the halve of binding mixture.
10. **Place the spin column into a new 2.0 ml collection tube (additionally supplied), add 700 μl of Buffer SWA to the spin column, and centrifuge at 13,000 rpm for 1 min. Discard the flow-through and reuse the collection tube in step 10.**
    Note : Ensure that 28 ml of ethanol (EtOH) has been added to Buffer SWA.
11. **Add 700 μl of Buffer SWB to the spin column, and centrifuge at 13,000 rpm for 1 min to dry membrane. Discard the flow-through and collection tube altogether.**
    Note : Ensure that 40 ml of absolute ethanol (EtOH) has been added to Buffer SWB.
    Note : It is very important to dry the membrane of the spin column since residual ethanol may inhibit subsequent reactions. Following the centrifugation, remove carefully the spin column from the collection tube without contacting with the flow-through, since this will result in carryover of ethanol.
12. **Place the spin column into a new 1.5 ml tube (not supplied), and 50 μl Buffer SE directly onto the membrane. Incubate for 1 min temperature, and then centrifuge at 13,000 rpm for 1 min to elute.**
    Note : Elution with 30 μl increases the final DNA concentration, but reduces overall DNA yield conventionally. Alternatively, if you need larger amounts of DNA, eluting with 100 μl increases generally overall DNA yield.
    Note : A new 1.5 ml tube can be used for the second elution step to prevent dilution of the first elute. Alternatively, the tube can be reused for the second elution step to combine the elutes.

VISUAL PROTOCOL

I Preparation step

1

Prepare the sample


Weigh 180 ~ 220 mg of sample


Transfer into 1.5 ml tube

II Pre Lysis step

2

Add 200 ㎕ Buffer SPL

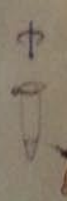
Mix by vortex vigorously


Incubate at 70℃ for 5 min

3

Place IR Spin Column into a new 1.5 ml tube


Transfer the prelysate into the IR Spin column.

4

Centrifuge 13,000 rpm for 1 min

Discard

III Lysis step

5

Add 200 ㎕ of Buffer SL
20 ㎕ of Proteinase K
5 ㎕ of RNase A


Mix by vortexing vigorously (or mix by pipetting)

6

Incubate at 65℃ for 15 min (invert the tube every 2 ~ 3 min)

IV Binding step

7

Add 200 ㎕ of Buffer SB

Mix by pipetting or inverting
Do NOT vortex

8

Add 250 ㎕ of 80% EtOH

Mix by pipetting or inverting
Do NOT vortex

9

Place Spin Column into a new 2.0 ml Collection tube


Add 750 ㎕ of mixture (Lysate)

-> Centrifuge at 1min


Discard

V Washing step

10

Place Spin Column into a new 2.0 ml Collection Tube


Add 700 ㎕ of Buffer SWA
Note :
Ensure that 28 ml of EtOH has been added to buffer SWA


Centrifuge 13,000 rpm for 1 min

Discard the flow-through

11

Add 700 ㎕ of Buffer SWB
Note :
Ensure that 40 ml of EtOH has been added to buffer SWB


Centrifuge 13,000 rpm for 1 min

Discard the flow-through


Centrifuge 13,000 rpm for 1 min

Discard the flow-through

VI Elution step

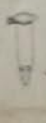
12

Transfer Spin Column to new 1.5 ml tube


Add 50 ㎕ of Buffer SE directly onto the membrane


Incubate at RT for 1 min


Centrifuge 13,000 rpm for 1 min


Recover the final eluate (gDNA)

Discard

ISO 9001/14001 Certified Company

i-genomic Stool DNA Extraction Mini Kit

Cat. No. 17451 50 Columns

DESCRIPTION

The i-genomic Stool DNA Extraction Mini Kit provides fast and easy purification of stool DNA from fresh or frozen stool samples. i-genomic Stool DNA Extraction Mini Kit purified DNA is of high quality and is ideal for reliable use in PCR and other downstream enzymatic reactions. The i-genomic Stool DNA Extraction Mini Kit is designed to extract genomic DNA in 50 minutes from a small amount of stool matter from any of a variety of animals, including human, rat, bird, cat, and cow. The simple i-genomic spin procedure yields pure DNA ready for direct use in less than standardization and ease of use. Purification requires no phenol-chloroform extraction or alcohol precipitation, and involves minimal handling. DNA is eluted in low-salt buffer and is free of protein, nuclease, and other impurities or inhibitors. The purified DNA is ready for use in PCR and other enzymatic reactions, or can be stored at -20℃ for later use.

Genomic DNA obtained from stool samples provides a window into animal pathophysiology. For example, changes in the methylation patterns in fecal DNA may be a promising marker for human colorectal cancer screening. In rural watersheds, *E. coli* typing can help track the animal sources of fecal water pollution. In addition, PCR can be used to detect intestinal protozoan infections in avian wildlife populations, in humans, or for human biome studies.

Stool samples typically contain many compounds that can degrade DNA and inhibit downstream enzymatic reactions. To ensure removal of these substances, the i-genomic Stool DNA Extraction Mini Kit contains i-genomic Stool IR Spin Columns, a special tool provided in a convenient spin column form. i-genomic Stool IR Spin Columns efficiently absorb these substances early in the purification process so that they can easily be removed by a quick centrifugation step from stool samples.

KIT CONTENTS AND STORAGE

Label	Description	Contain
Buffer SPL	Pre-lysis Buffer	12 ml
Buffer SL	Lysis Buffer	12 ml
Buffer SB	Binding Buffer	12 ml
Buffer SWA (concentrate) [1]	Washing Buffer A	12 ml
Buffer SWB (concentrate) [2]	Washing Buffer B	10 ml
Buffer SE	Elution Buffer	20 ml
IR Spin Columns	Inhibitor Absorption Column	50 columns
Spin Columns (Violet O-ring color)	Inserted into the collection tubes (2.0 ml tubes)	50 columns
Collection Tubes (2.0ml tubes)	Additionally supplied.	50 tubes X 2ea
RNase A (Lyophilized powder) [3]	Dissolve with Pure DW 0.3 ml	3 mg
Proteinase K (Lyophilized powder) [4]	Dissolve with Pure DW 1.1 ml	22 mg

[1] Buffer SWA is supplied as concentrates. Add 28 ml of ethanol (96~100%) according to the bottle label before use.
[2] Buffer SWB is supplied as concentrates. Add 40 ml of ethanol (96~100%) according to the bottle label before use.
[3] After dissolving, store at -20℃. The RNase A is completely free of DNase activity.
[4] After dissolving, store at -20℃. After thawing, freshly use. We recommend to aliquot to small volume of Proteinase K.

QUALITY CONTROL

As INtRON quality control program, the performance of INtRON's products are monitored routinely on a lot-to-lot basis. The genomic DNA yield of i-genomic series Genomic DNA Mini Kit is tested by preparing various sample and assaying the genomic DNA yield spectrophotometrically. The quality of isolated genomic DNA is checked by restriction digestion, PCR, agarose gel electrophoresis, and spectrophotometry. i-genomic Stool DNA Mini Kit is tested to ensure the absence of DNase contamination. All buffers are each incubated with 1 mg pUC18 DNA for 6 hours at 37℃. The DNA is then visualized by electrophoresis on an agarose gel and compared to a positive control to determine if any linear or nicked plasmid DNA is visible.

i-genomic series DNA Extraction Mini Kits

i-genomic series DNA Extraction Mini Kits provide nine kind of kits according to the type of samples as seen below table. These i-genomic series Kits provide a fast and easy way to purify DNA from various samples. The kits procedures provide pure genomic DNA for reliable PCR and Southern blotting in less than 1 ~ 2 hours. Purification requires no phenol or chloroform extraction or alcohol precipitation. Purified DNA extracted by i-genomic series Kits is eluted in low-salt buffer or water, ready for use in downstream applications, including PCR, RAPD analysis, AFLP analysis, RFLP analysis, Southern blotting, microsatellite analysis, SNP genotyping, and quantitative real-time PCR. Purified DNA has an A_{260}/A_{280} ratio of 1.7 ~ 2.0, indicating high purity of the DNA.

Nine Kinds of the i-genomic series DNA Extraction Mini Kits

Purpose	Product Name	Cat. No.	Samples
Specific Sample use (Big 5 Series)	i-genomic CSI DNA Extraction Mini Kit	17992	Micro-amount sample (Forensic Sample)
	i-genomic Urine DNA Extraction Mini Kit	17391	Urine / Urinal swab
	i-genomic Food DNA Extraction Mini Kit	17401	Vast kinds of Food
	i-genomic Stool DNA Extraction Mini Kit	17451	Stool / Stool swab
	i-genomic Soil DNA Extraction Mini Kit	17421	Soil / Soil bacteria
General Use (KIAGEN series)	i-genomic CTB DNA Extraction Mini Kit	17341	Cells Tissues Gram(-) Bacteria
	i-genomic Blood DNA Extraction Mini Kit	17351	Bloods
	i-genomic BYF DNA Extraction Mini Kit	17361	Gram(+) Bacteria Yeast Fungi
	i-genomic Plant DNA Extraction Kit	17371	Plant

PRODUCT USE LIMITATIONS

All i-genomic series Kits are developed, designed, and sold for research purpose only. They are not to be used for human or animal diagnosis of diseases. Do not use internally or externally in humans or animals. Be careful in the handling of the products.

PRECAUTIONS AND SAFETY INFORMATION

All chemicals should be considered as potentially hazardous. When working with chemicals, always wear a suitable lab coat and disposable glove. Some buffer contain the chaotrophic salt which may be an irritant and carcinogen, so appropriate safety apparel such as gloves and eye protection should be worn. If a spill of the buffers occurs, clean with a suitable laboratory detergent and water. If the liquid spill contains potentially infectious agents, clean the affected area first with laboratory detergent and water, then with a suitable laboratory disinfectant. Only persons trained in laboratory techniques and familiar with the principles of good laboratory practice should handle these products.

PRODUCT WARRANTY AND SATISFACTION GUARANTEE

All products undergo extensive quality control test and are warranted to perform as described when used correctly. Immediately any problems should be reported. Satisfaction guarantee is conditional upon the customer providing full details of the problem to INtRON within 60 days, and returning the product to INtRON for examination.

TECHNICAL INFORMATION

EXPERIMENTAL INFORMATION

• Genomic DNA Purification

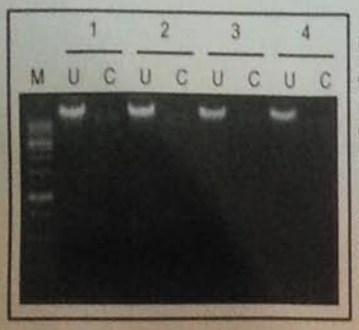


Fig. 1. Results of DNA purification
After eluting genomic DNA with 50 μl Buffer SE, each of 100 ng of DNA were used in DNA electrophoresis.
Panel 1, 2, i-genomic Stool DNA Extraction Mini Kit
Panel 3 , 4, Company A's product
Lane M, 1 kb Ladder DNA marker; **lane U,** Purified genomic DNA from Stool; **lane C,** Genomic DNA digested with *EcoR*I

Table 1. DNA purity by performing ratio absorbance measurements

	A_{260} (Average)	A_{280} (Average)	Purity (A_{260}/A_{280})	Yield (μg)
iNtRON	1.862	0.857	2.1	4.7
Company A	1.599	0.763	2.0	4.0

• Genomic DNA PCR

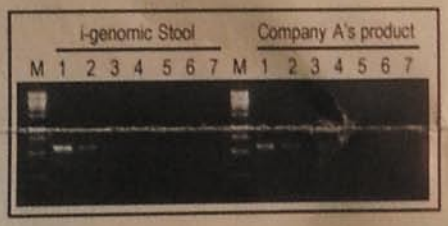


Fig. 2. GAPDH amplification
The housekeeping gene GAPDH (mammalian, 575 bp) was amplified with the purified DNA as template(5 μl) using *Maxime* PCR PreMix Kit (i-Taq) (Cat. No. 25025). The template DNA was serially diluted 10^0 ~ 10^{-6}.
Lane M, 1 kb Ladder DNA Marker, **lane 1,** 10^0 of template DNA (10 ng); **lane 2,** 10^{-1} of template DNA; **lane 3,** 10^{-2} of template DNA; **lane 4,** 10^{-3} of template DNA; **lane 5,** 10^{-4} of template DNA; **lane 6,** 10^{-5} of template DNA; **lane 7,** 10^{-6} of template DNA

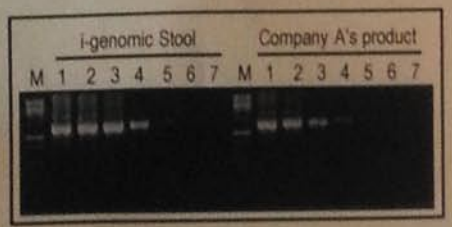


Fig. 3. 16S rRNA amplification
The 16s rRNA(bacteria, 1.6 kb) was amplified with the purified DNA as template(5 μl) using *Maxime* PCR PreMix Kit (i-Taq) (Cat. No. 25025). The template DNA was serially diluted 10^0 ~ 10^{-6}.
Lane M, 1kb Ladder DNA Marker; **lane 1,** 10^0 of template DNA (10 ng); **lane 2,** 10^{-1} of template DNA; **lane 3,** 10^{-2} of template DNA; **lane 4,** 10^{-3} of template DNA; **lane 5,** 10^{-4} of template DNA; **lane 6,** 10^{-5} of template DNA; **lane 7,** 10^{-6} of template DNA

• Pathogen genomic DNA PCR

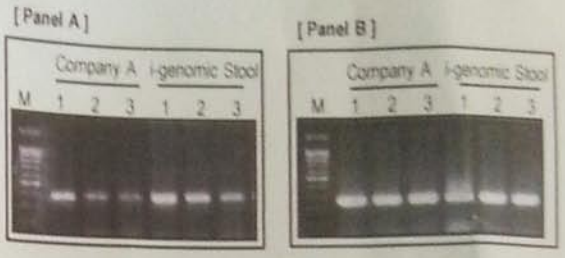


Figure 4. PCR results of *Clostridium perfringens* (CLOS) and *Canine parvovirus*(CPV)
CLOS(bacteria, 405 bp) and CPV(virus, 257 bp) were amplified with the purified DNA from infected stool sample as template (5 μl/each) using VeTeK™ CLOS Detection Kit (iNtRON, Cat. No.D10400) and VeTeK™ CPV Detection Kit (iNtRON, Cat. No.D10230).
Panel A , CLOS; **Panel B**, CPV
Lane M, 1 kb Ladder DNA Marker; **lane1 ~ lane 3,** Extracted genomic DNA of CLOS and CPV infected stool

TROUBLESHOOTING GUIDE

Problem	Possible Cause	Recommendation
Low DNA yield	Too much starting material	- Check the step 1 of protocol. Reduce the amount of starting material used.
	Reagents correctly were stored	- Check the "STOAGE". Buffer SPL tend to become solid at RT. If Buffer SPL become solid, incubate in 80°C.
	Incomplete lysis	- Lysis time and buffer volume not correct for sample size.
	DNA inefficiently eluted	- Elute product with 100 ~ 200 μl of the Buffer SE to obtain best result. - Depending on starting material size, decrease or increase volume of Buffer SE. - Add Elution buffer to the center of the spin column to ensure that the Buffer SE completely covers the membrane.
	Buffer SWA, Buffer SWB did not contain 100% EtOH	- 100% EtOH must be added to Buffer SWA, Buffer SWB before use.
Problems in the downstream application	Ethanol carryover	- Ensure that during the Buffer SWB, the spin column is spun at maximum speed 1min to dry spin column membrane.

RELATED PRODUCTS

Product Name	Cat. No.
Maxime™ PCR PreMix (*i*-Taq)	25025
Maxime™ PCR PreMix (*i*-StarTaq)	25165
SIZer™-1000 DNA Marker	24074
RedSafe™ Nucleic Acid Staining Solution	21141