Isolation and characterization of *Helicobacter pylori* in Gastroduodenal disease at a tertiary care hospital

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CERTIFICATE

This is to certify that the dissertation work entitled **"Isolation and characterization of *Helicobacter pylori* in Gastro duodenal disease at a tertiary care hospital"** submitted by **Dr. Lavanya J**, was done by her during the period of study in this department, from May 2014 to August 2015. This work was done under the direct guidance of Dr. J.Jayalakshmi, Professor, Department of Microbiology, PSG IMS&R.

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Introduction:

Helicobacter pylori is a less studied bacteria in our country. Association of virulence factors like *cagA* and *vacA* subtypes to the disease manifestations and resistance profile to the commonly used first line drugs varies widely among the geographical regions. For effective treatment of patients, we need to know these epidemiological profiles of *H.pylori* for every individual region. Also, among various methods to detect *H.pylori* infection, Rapid urease test (RUT) has remained the most widely used one. The efficacy of the various commercial kits and in-house preparations must be evaluated before using them as a routine diagnostic tool.

Aim:

To know the prevalence, recognize the specific gene variants and their association with the virulence state and identify the drug resistance among *H.pylori* in our population.

Material and Methods:

Gastric biopsy samples obtained from 165 patients with gastroduodenal diseases were included in the study. RUT and culture was compared against PCR for glmM gene. *CagA* and *vacA s and m* subtype status analysis of the infected patients was performed by PCR and their association with endoscopic findings was studied. Clarithromycin and Amoxicillin susceptibility testing were performed on the isolates using E-strips.

Result:

Prevalence of *H pylori* was found to be 37 percent. The RUT had a sensitivity and specificity of 93.4% and 90.4% respectively. There was a statistically significant association between presence of *cagA* & *vacA s1* subtype and peptic ulcer disease rather than the non ulcer dyspepsia ($p<0.05$). In this study the resistance to Clarithromycin and Amoxicillin was 7.62% and 15.38% respectively.

Conclusion:

RUT used in this study would serve as a better screening test. *CagA* and *vacA s1* positive strains are the virulent ones in this locale. Usage of Clarithromycin and Amoxicillin can be continued as first line drugs in *H.pylori* eradication therapy in our population.

Keywords: *H.pylori*, *cagA* gene, *vacA* gene, RUT

INTRODUCTION

Helicobacter pylori is the causative agent for variety of gastrointestinal disease-Chronic antral gastritis, Gastric ulcer, Duodenal ulcer, Gastric adenocarcinoma and Non Hodgkin's Lymphoma. Most of the infected patients remain asymptomatic. Disease manifestation is known to be associated with pathogenicity factors like proteins coded by *cagA* and *vacA* gene. However, there is a wide variation not only in the prevalence of these pathogenicity islands (PAI) in different ethnic groups, but also their association with clinical manifestations [(1)]. Studies from different parts of the world show different strains to be prevalent in their regions. In Iran, strains with vacAs1m2 genotype and cagA positivity are more prevalent and associated with Peptic Ulcer disease. [(2,3)] In Turkey, *s1am2* and *s1am1* both occur in equal rates, but *cagA* positivity is more with *s1m1* type.[(4)] *cagA* positivity is found to be 70% in India and Bangladesh, 56% in Pakistan, 70-76% in middle eastern countries and 90% in Japan.[(5,6)] In Jordan, predominant genotypes are *s1m1* and *s2m2*. In northeastern part of Mexico , *cagA+vacs1m1* strain are found mostly and are associated with intestinal metaplasia.[(7)] The study done by Chattopadhyay et al at National Institute of Cholera and Enteric Diseases found that in India *cagA+ s1m1* strains are predominant. Though the pattern is similar, these strains are associated with peptic ulcer disease in India.[(8)] Another study by Udhayakumar et al from south India found *cagA* positivity of 96% and *vac m2* to be the predominant one in Chennai. [(9)]

Study by Xue FB et al and Eslick GD et al found that *H pylori* is a definitive risk factor for gastric carcinoma.[10,11] Complete remission of low grade gastric MALT lymphoma was achieved in 60%–80% of patients following eradication of *H.pylori* [12,13]. Studies support the role of *H. pylori* in the development of gastric carcinoma and indicate that anti-*Helicobacter pylori* therapy may be effective in preventing gastric cancer.

Helicobacter pylori is also developing drug resistance and the prevalence of resistant strains are increasing at an alarming rate due to widespread use of antibiotics like Macrolides, Quinolones and Metronidazole. Systemic review of studies done from 2006 - 2009, by De Francesco V et al concluded that the overall antibiotic resistance rates were high for metronidazole (26.7%) followed by Clarithromycin (17.2%) and levofloxacillin (16.2%). Resistance to amoxicillin was 11.2 percent.[14] One of the multicentric study conducted in India recorded a high resistance rate to three most commonly used drugs- metronidazole (77.9%), clarithromycin (44.7%) and amoxycillin (32.8%). Multiple resistances were seen in 43.2% isolates. Resistance to the drug metronidazole was high in Lucknow, Chennai and Hyderabad and moderate in Delhi and Chandigarh. [15] Study by Ahmed et al in Hyderabad shows that 96% - 97% of the clinical isolates were sensitive to Tetracycline and ciprofloxacin, resistance to Amoxycillin, Clarithromycin and metronidazole was 80%, 76% and 100% respectively

[16].These are the first line drugs used routinely at our hospital. Though there is a need to know the sensitivity of our strains for effective management of these patients, difficulty in cultivating this fastidious organism makes it challenging.

Since *H pylori* genotypes vary from population to population, specific study is required to find the most prevalent genotypes and the commonest diseases associated with them to decide on the effective management. Also, resistance rates of the commonly used antibiotics have to be studied in order to choose the right treatment regime for the locale.

AIM & OBJECTIVES

AIM:

To study the prevalence of virulence factors of *H pylori* and their association with disease pattern among patients in a tertiary care hospital.

OBJECTIVES:

1. To isolate and characterize *H pylori* from gastric biopsy samples of patients attending the gastroenterology outpatient clinic at PSG Hospitals.
2. To compare the detection methods such as rapid urease test and culture against PCR in the diagnosis of *H pylori infections*.
3. To identify the *cagA and vac*A gene variants and their association with the virulence state.
4. To identify the prevalence of drug resistance among *H pylori* isolates using E test.

REVIEW OF LITERATURE

HISTORICAL PERSPECTIVES:

Over 100 years ago a Polish Clinical Researcher Prof W Jawroski described the presence of spiral shaped micro organisms in the human stomach. In 1893, G. Bizzazero described similar organism in animals which turned out to be *Helicobacter felis, H. canis* and *H. heilmannii.* [17] But their presence was not really taken seriously until the late 1970s when Robin Warren a Pathologist noticed the appearance of these bacteria in the gastric mucosa of inflamed tissues. This discovery changed the wide spread notion that chronic inflammation of stomach caused by immune reactions may well be caused by an infection**.**

In 1940, Freedberg- Anatomists and Baron-pathologists noticed spiral organisms in the carcinomatous human gastric mucosa now and then. They found spiral shaped organism in about 40% of resected specimens. But, they could not establish the association because, these bacteria disappeared with rigorous chemotherapy in these patients. [17]

Later in 1950s, Susumu Ito of Harvard Medical School made the first detailed description of the appearance of gastric mucosa under the electron microscope. Ito's photographs of the gastric corpus showed "spirilli" in some of his material. In 1967, he published a photograph showing enlarged *H. pylori* within a parietal cell gland. For the first time the sheathed flagella and typical spiral morphology was clearly demonstrated in these pictures [18].

In the mid-1970s, Steer and Colin-Jones made several failed attempts to culture *H.pylori*. Finally Robin Warren with Barry J Marshall, succeeded in cultivating this bacterium in an artificial medium for the first time in 1983 and named it *Campylobacter pyloridis* because it resembled the then known pathogenic bacteria of the *Campylobacter* genus in the intestinal tract. [17, 19] Later a new genus called *Helicobacter* was created since it did not fit into the genus *Campylobacter*. In 1983, McNulty and Skirrow succeeded in culturing it from a gastric ulcer patient in England. [20]

In 1983, at the Centre for Digestive Diseases, Borody came up with the first functioning Triple Therapy for eradication of *H. pylori* and cure of Duodenal Ulcer. This combination comprised of Bismuth sulfate, Tetracycline and Metronidazole. [21] Studies by Rauws and Tytgat, Coghlan et al and Graham et al proved that *H. pylori* eradication therapy cured peptic ulcer. [22-24]

Of the 100 patients studied by Marshall and Warren, patients who were found to be infected were more than 65% and almost all of them had gastritis. Almost 80- 100% of the patients who had duodenal ulcer and gastric ulcer were infected. Other patients who had ulcer without *H. pylori* were on nonsteroidal anti-inflammatory drugs. This finding motivated several researches all around the world and similar results were found in several countries within few years. [19] In the ensuing 5 years, several double-blind trials were conducted to study the infection eradication rates of the available therapies like bismuth with any one

anti-*H.pylori* antibiotic. Results were poor, with only one-fourth of the patients achieving eradication. [22-24]

MILESTONES IN *H.PYLORI* STORY

1893- Bizzozero reported existence of gastric spiral organisms in healthy dogs.

1906 -Krienitz first observed the spiral shaped organisms in human stomach

1938 -Doenges observed spiral bacteria in the gastric mucosa of about 50% of human stomach examined in autopsy

1940- Freedberg and Barron reported similar organisms in 37% of gastrectomy specimens from patients with peptic ulcer or carcinoma.

1975- Steer and Colin Jones came close to identification of *Helicobacter pylori* and its association with gastritis, but they misinterpreted the culture results and concluded that the organism was pseudomonas.

1982- Warren and Marshall were the first to culture, identify and establish the association between presence of the organism in gastric mucosa and occurrence of histological gastritis.

1984- Organism was named as *Campylobacter pyloridis* by Marshall et al.

1987- Eradication of *H.pylori* proved to be to a long term cure of duodenal ulceration.

1989- The Genus '*Helicobacter*' was created

1994- The International agency for research on Cancer working group WHO defined *Helicobacter pylori* as a grade –I or definite human carcinogen.

PHYLOGENY AND NOMENCLATURE:

Vandamme and associates placed *Helicobacter* under the phylogenetic group- rRNA superfamily VI along with *Campylobacter, Arcobacter* and *Wolinella* (based on DNA-rRNA hybridization, 16S rRNA sequence analysis and immunotyping analysis). Among this superfamily *Helicobacter* falls under RNA cluster III. [25]

Helicobacter pylori comes under the epsilon subdivision of Proteobacteria, and order *Campylobacter*ales. Skirrow proposed to name the new gastric bacteria as *Campylobacter pyloridis* and the culture from the Royal Perth Hospital 13487(NCTC 11637) was designated as type strain. [26]

A new classification was proposed and *H. pylori wa*s placed under the family *Helicobacteraceae* and genus *Helicobacter*. [27] The genus *Helicobacter* comprises of 23 species. Some of the medically important species are *H.pylori, H.cinaedi, H.fennelliae, CLO-3, Helicobacter Sp. Strain flexispira and H.heilmannii* (*Gastrospirillum hominis*). Among these only *H.pylori and H.heilmannii* are isolated from the gastric mucosa. Other species are recovered either from the rectal swab or feaces .Other nonhuman *Helicobacter*s seen in

rodents and other mammals are *H.canis , H.bilis, H.bizzozeronii, H.felis, H.hepaticus, H.muridarum, H.mustelae, H.nemestrinae, H.pametensis and H.pullorum* .They cause gastritis in their host animals.

Helicobacter pylori was initially called *Campylobacter pyloridis* and then *Campylobacter pylori*. On 16S rRNA study it was found to be different from *Campylobacter*. Henceforth the new Genus *Helicobacter* with *H.pylori* being the first species was created. Later other species were identified. [25,28]

Helicobacter pylori can be differentiated from *Campylobacter* by its multiple sheathes flagella, its strong urea hydrolysing property and its fatty acid profile (high percentage of 14:0 acid, low 16:0 acid and the presence of 3-OH-18:0 acid) [18]

MORPHOLOGY:

H. pylori is a Gram-negative bacteria. It appears as an s-shaped or curved rod with 1 to 3 turns in gastric biopsy specimen and fresh isolates which is described as Seagull appearance by some authors. It measures 0.5-0.9 µm wide and 2-4 µm long. Cells appear as singly curved rods owing to the less obvious spiral forms. Other rare forms reported in culture includes spherical, U form, V form (ox-bow) and straightened forms.

It is a non spore forming bacteria. *H. pylori* are motile with six polar sheathed flagella with a covering that is continuous with the outer membrane of

the cell wall. Though they are actively motile, cultures may appear to be non motile in hanging drop preparations. The normal configuration of flagella is found to be seven by freeze-fracture ultra structure studies. Each flagellum is 30 nm in diameter with a filament of 12-15 nm. Some flagella have distinctive terminal bulbs with no special function. Electron microscopy shows that external to the cell wall there is a 40 nm thick glycocalyx (capsule-like polysaccharide rich layer), which is thicker in vivo than in cultured bacteria.

In older cultures, *H. pylori* undergo morphological change from bacillary to coccoid form with an associated loss in cultivability. These forms are viable but more resistant and dormant. This is a temporary adaptation to a hostile environment - stress caused by nutrient deprivation, antibiotic exposure or extended incubation. It is hypothesized, but not yet proved, that these coccoid forms can revert to an infectious bacillary form under appropriate conditions.

GENETICS:

H.pylori has a single circular DNA with a mean size of 1.71 Mb ranging from 1.40-1.73 Mb. Its base composition is in the range of 35-37 mol% G+C. Despite evidence showing re-arrangements in gene order, DNA-DNA hybridizations shows a high level (1>65%) of sequence homology between strains. The complete sequences of the genomic DNA from several strains have been determined. Extra chromosomal plasmid DNA is found in half of the strains,

although the type strain (NCTC 11637) is plasmid free. Plasmid varies in size from 1.8 to 63 kbps.

*Helicobacter*s have a typical cell wall of Gram negative bacteria made of Lipopolysaccharide (LPS) attached to the outer membrane, a peptidoglycan layer, and an inner cell membrane. The major cellular fatty acids are tetradecanoic acid and octadecanoic acid, with smaller amounts of hexadecanoic acid and 3-hydroxydecanoic acid. The main respiratory quinone is menaquinone-6 but thermoplasmoquinone-6 is lacking. These characters exclude this bacterium from *Campylobacter*. [20]

LPS of about 80% of strains express Lewis x and y blood group antigens which are not found in other Gram-negative bacteria. Structural analysis of the O-polysaccharide chains resemble fucosylated Lewis x and y antigens. Example: the O-chain of the type strain (NCTC 11637) mimics Lewis x antigen. This LPS helps in attachment of bacteria to human gastric epithelial cell. [20]

HABITAT:

H.pylori has been isolated from gastric and duodenal biopsies. It colonizes the mucus layer of surface epithelia in a patchy fashion. [29] They are located predominantly in the antrum followed by corpus and bulb of the stomach. They are densely distributed around the lumen of gastric pits, but when subjected to acidic stress, they extend into the lumen of gastric glands. [30]

CULTURE OF *HELICOBACTER PYLORI*:

Transport media:

Transport medium is used mainly to prevent exposure to atmospheric oxygen and also to avoid drying of biopsy specimen. Survival is better when transported at 4^{o}C and plated immediately. Some of the media are Brucella broth, Thioglycolate broth, Stuart's medium, Carry and Blair medium. A biphasic medium has also been described to give better result. [20]

H. pylori is a microaerophilic organism, growing best in the presence of 5% oxygen along with 5-10% CO2 on blood containing non selective media eg: Oxoid brain heart infusion agar (BHI) and 5% horse blood agar enriched with 1% IsoVitaleX, which is a well defined supplement having vitamin B,2, L-glutamine, L-cysteine, and various other growth promoting compounds. These media can be made selective by adding antibiotic supplements. It has a respiratory type of metabolism. The optimum temperature for growth is 37°C. [18,20]All strains can grow within a narrow temperature range of 33- 40°C, whereas growth is poor at temperatures both below and above the range and none grew at 25°C. *H. pylori* grows over a wide pH range (5.5-8.5), but optimum growth occurs between pH 6.9 and 8.0. *H. pylori* do not tolerate low pH in vitro. It takes a minimum of 3-5 days for an observable growth in a well defined media.

List of media that can be used for culture:

Marshall et al, during their first isolation used BHI chocolate agar supplemented with 7% horse blood. [19] Several nutrient-rich media such as tryptic soy broth, brucella broth/agar, brain heart infusion broth/agar, Columbia blood agar and Mueller-Hinton broth/agar (MHB) has been shown to support the growth of *H.pylori*.

Fetal calf serum (FCS) is one of the most commonly used supplements. Solid media are usually supplemented with blood and serum. Other typical supplements added to growth media include lysed erythrocytes or hemin, yeast extract(YE), peptone, IsoVitaleX, starch, ferrous sulfate plus sodium pyruvate (FP), cyclodextrins, mucin, cyanobacterial extract.[31] Egg yolk emulsion has also been found to be a good supplement. Although some of these enhance the growth of *H. pylori*, the serum or blood in the culture media can't be replaced.

Listed below are some of the preparations used for culturing *H. pylori* [32- 34]

1. The formula of Goodwin et al. (GDW agar) contained brain heart infusion agar, 7% whole defibrinated horse blood, and 1% IsoVitaleX.
2. The medium of Glupczynski et al. (GLU agar) contained brain heart infusion agar, 10% horse serum, 0.2% charcoal, 1% yeast extract, and 40 mg of triphenyl tetrazolium chloride per liter.

3. Columbia blood agar as described by Dent and McNulty (D&M agar) consisted of Columbia agar and 7% lysed horse blood.
4. EYE (Egg Yolk Emulsion) agar consisted of Columbia agar, 10% EYE, 1% IsoVitaleX, and 40 mg of triphenyl tetrazolium chloride per liter.
5. Brain heart infusion egg (BHIE) agar was made from brain heart infusion agar with 10% EYE and 1% IsoVitaleX
6. BBA with 7% sheep blood.
7. HPBSA
8. Modified Thayer Martin agar.

The following antibiotics supplements are used in different combination to avoid growth of contaminants.

Vancomycin 3-6 mg/l

Amphotericin B 2-6 mg/l

Trimethoprim 5-20 mg/l

Colistin 25,000IU/l

Cefsulodin 5 mg/l

Skirrow's antibiotic supplement is the most commonly used one in day to day practice. It contains a combination of vancomycin, Polymyxin B and Trimethoprim.

Several liquid media has also been used for mass production of bacteria for storage, antigen preparation and other purposes. [35, 36]

BIOCHEMICALS:

H. pylori are inactive in most of the conventional biochemical tests. Carbohydrates are neither fermented nor oxidized. *H. pylori* tests positive for catalase and cytochrome oxidase but the most notable features are its high level of urease and alkaline phosphatase activity. *Helicobacter pylori* are a homogeneous species in its enzyme profile, with few exceptions of some minor strain differences in amino peptidase and other preformed enzyme activities. Very rare reports of catalase and urease negative strains have been quoted but isolation of such strains from a biopsy material is uncommon. An important strain to strain variability is their difference in vacuolating cytotoxin.

Both *H.pylori* and *Helicobacter* sp. strain flexispira are strong urease producers. Main differentiating feature between them is their susceptibility to cephalothin. *H.pylori* is inhibited by cephalothin whereas Flexispira strain is susceptible. *H.pylori* is resistant to nalidixic acid too. [18, 37]

Reactions shown by Helicobacter pylori

Catalase	Positive
Oxidase	Positive
Urease	Positive
Alkaline phosphatase test	Positive
Acid phosphatase test	Positive
Naphthol-AS-Bl-phosphohydrolase test	Positive
Leucine arylamidase test	Positive
Gamma glutamyl transpeptidase test	Positive
Hippurate hydrolysis	Negative
Nitrate reduction	Negative
Indole	Negative
Arylsulphatase test	Negative
Growth with 1 % and 3.5% NaCl	Negative
Indoxylacetate hydrolysis test	Negative
Growth at $42^{o}C$	Negative

VIRULENCE & PATHOGENICITY:

FACTORS ASSOCIATED WITH COLONIZATION OF GASTRIC MUCOSA:

- Neutralization of acid
- Motility and pH taxis
- Adherence to mucin and gastric epithelium

UREASE

Enzyme urease plays a major role in acid neutralization. It constitutes up to 10% of the total cell protein. This is an important enzyme in bacterial physiology as it is needed for survival in an acidic environment. This enzyme is composed of two subunits, UreA and UreB, which are encoded by the *ureAB* genes.

It's a constitutive enzyme found both in cytoplasm as well as the surface of the bacteria. Surface urease brings down the environmental acidity. Cytoplasmic urease decreases the acidity in periplasmic space. [38]

In the *ureIEFGH* operon, *ureI* gene is the first gene. It codes for UreI channel transporter. Urea moves into cytoplasm through this protein. The other genes of the operon code for accessory proteins involved in urease activity. Through the UreI channel, urea is transported into the periplasmic space, making it available to the cytoplasmic urease. This results in buffering of the periplasm and making the survival of this pathogen in an acidic environment easier. [39]

OTHER MECHANISMS INVOLED IN pH balance:

FUNCTION	**GENE**
Production of ammonia	Ami E, Ami F and Rocf (aliphatic amidase, Formamidase, Arginase)
Regulation of ammonia synthesis	Nix A, Nik R and ars RS

H pylori has a H+/K+ ATPase proton pump of P type, similar to the one normally found in the parietal epithelial cells. This bails out any proton that gets into the cell, thus helping to maintain a proton gradient across the bacterial cell membrane.

FLAGELLA:

Flagella are made up of flagellin subunits which are encoded by genes - flaA and flaB. The bacteria move through the mucus to reach the epithelial surface as the pH there, is approaching neutral. The flagella act like screws in propelling the bacteria through mucus. When the non-motile mutants were tested for their ability to infect gnotobiotic piglets, they were shown to be deficient in colonization of the stomach. This proved the significance of motility in colonization and persistence of infection in the host. [(40)]

Bacterial flagella are generally potent antigens, activating the innate immune system. Owing to the modifications in the flagellin structure, Toll like Receptor-5 does not recognize *H. pylori* flagellin efficiently. Hence it is a poor activator of innate immunity, yet another feature contributing to the persistence of infection in the host. [41]

THIOREDOXIN REDUCTASE:

This enzyme breaks down the mucin and helps penetration of the bacilli from the surface of mucus into deeper layers. This is encoded by hp825 coding sequence.

pH TAXIS:

H. pylori sense the pH gradient, so that it can move away from the acidic surface of mucus, toward the epithelial surface. This sense of a chemical gradient in coordination with the flagellar determines the direction of the bacterial movement. When the Genes involved in pH sensing i.e. *cheY* [42] and *tlpB* [43] are mutated these strains are defective in the colonizing the stomach.

ADHESINS:

Adhesion of Helicobacter to the gastric epithelium is mediated by numerous outer membrane proteins of the bacteria. Their expression is regulated in response to environmental cues.[44,45] Examples of some adhesins are the Adherence-

associated lipoproteins (AlpAB), Sialic acid-binding adhesin (SabA), Blood group antigen-binding adhesin A (BabA) and OipA

PROTEIN	**FUNCTION**
alpAB	Adherence, colonization and Initiation of proinflammatory signaling cascades
sabA/sabB	Binds to sialyl-Lewis x and a antigens on gastric epithelial cell
babA	Binds to Lewis b (Leb) blood group antigen on the human gastric epithelial cell
oipA	For bacterial adhesion. Associated to *cag* status and development of peptic ulcer
Trx	Colonization of gastric mucosa through disruption of structure of mucin
napA	Bacterial adhesion and modulation of the immunological response

FACTORS HELPING IN COPPING WITH IMMUNE RESPONSE:

1. Bacterial location within the lumen away from the reach of IgG & IgM.
2. Shedding of large quantities of antigens which mop-up the IgA antibodies in the lumen
3. Presence of feeble endotoxins which does not stimulate immunity effectively.
4. Having the enzyme catalase it neutralizes any hydrogen peroxide released.
5. Heat shock proteins produced by *H.pylori* under stress conditions, act like chaperons, protecting the protein molecules and helping the bacteria survive in harsh environment.

FACTORS ASSOCIATED WITH DISEASE:

Cag PAI:

The *cag*PAI is a genomic region found in the glutamate racemase gene (*glr*) of *H. pylori*. It is approximately 40 kb in size. It is involved in expression of the type IV secretion system and translocation of bacterial products to the host cell. The *cagA* gene is a marker for the presence of *cag*PAI. It encodes for *CagA*, the first proteins considered to be a virulence factor of *H. pylori*. [46, 47]

CagA has a unique structure compared to other known proteins. *CagA* positivity has been widely accepted as a predictor of severe gastritis and an increased risk of peptic ulcer disease, atrophic gastritis and gastric cancer, particularly in western populations. [48] About 60-70 per cent of *H. pylori* strains in the West were found to be *cagA*+ and these strains were associated with Duodenal ulcer and Gastric carcinoma. [49] However, more than 90 per cent of *H. pylori* strains in Asia are *cagA*+ irrespective of their disease. [9, 50] *cagA* positivity rate in Indian population is found to be generally more than 50%. [51]

The Type IV secretion apparatus translocates cagA protein into the cytoplasm of the gastric epithelial cells. *CagA* proteins are phosphorylated by host kinases in the cell and *CagA* interacts with the host signaling molecules such as the Src kinase, tyrosine phosphatase and Crk adaptor protein. This brings about changes in the host epithelial cell.[52]

Cag A brings about cell elongation processes called as the hummingbird phenotype, Cytoskeleton rearrangement, loss of epithelial barrier function by disrupting tight junction and loss of cell polarity. *CagA* helps in the activation of NF-κB, the proinflammatory transcription factor. [53]

The *cagE* gene is a more accurate marker of *cag*PAI than *cagA*. *Cag E* induces IL-8 production by host cells. It's known to be associated with peptic

ulcer disease. The *cagT* gene codes for an extracellular lipoprotein situated at the base of the Type IV Secretion System. [54]

In a given strain, the *cag*PAI can undergo partial or complete deletions. Similarly *Cag* PAI positive and negative strains can co exist in same patient. The balance between these strains is a significant contributing factor for colonization and persistence of *Helicobacter pylori* in the host. [55]

Vacuolating Cytotoxin:

The *vacA* is universally present in *H. pylori* and it is encoded by *VacA* gene. The 140 kDa precursor protein of *vacA* is cleaved to yield a mature toxin and secreted in to the host environment. Mature protein has two parts, free and bound part. The free molecule is released into the environment and its function is not known. The bound part remains in contact with the bacterial cell surface and is more toxigenic. Consequent to the contact with the gastric epithelium, this bound part is transferred to host cells. [56] This toxin creates pores in host-cell membranes through which anions and small molecules like urea leaks out. [57-59] Formation of large acid vacuoles is induced by this toxin. It disrupts intercellular tight junction in gastric epithelial calls. It induces apoptosis of gastric epithelial cells by damaging the mitochondrial inner membrane and causing efflux of cytochrome C. [61] It blocks various steps in the pathway of immune response like antigen

presentation to T cells, activation and maturation of phagosomes in macrophages. [61, 62]

The *vacA* is a polymorphic gene, showing variability in the nucleotide sequence in the three following regions: signal sequence (s), intermediate (i) and middle regions (m). There are two types of each sequence- signal sequence (s1 and s2), middle sequence (m1 and m2) and intermediate sequence (i1 and i2). Hence the *vacA* gene of a given strain can have any one combination of s and m region sequence types. Literature cites that the s2/m1 combination is rare. [63]

Specific *vacA* genotypes are associated with level of toxin production and clinical diseases like Peptic ulcer disease and Gastric Carcinoma in different part of the world. [50] However, study by Udhayakumar et al showed significant association between the *cagA* and *m2 of vacA* among gastric ulcer patients also suggesting *Helicobacter pylori* strains with the *vacA* m2 region were predominant in Chennai, South India. This study also showed that studying the *cagA* gene from biopsy specimens through PCR has a potential value. [9] Both s1m1 and s1m2 variants of *vacA* are known to be associated with gastric diseases like ulcers and cancer especially in western countries. [64, 65] The degree of illness and the pattern of association vary with ethnicity. [10]

The biological activity of *VacA* is blocked by a short N-terminal extension in s2 forms. [11] Hence *H. pylori* strains that harbour s2 genotype encode proteins with low toxicity and are not frequently associated in gastric diseases. [66, 67]

The *vacA* shows polymorphism related to the 'i' region. This region codes for restriction endonuclease. *VacA* s1m1 and s2m2 types were invariably typed as i1 and i2, respectively. Only the vacuolating activity of s1m2 and s2m1 strains were found to depend on the 'i' type. Recent studies show that *vacA* i1 has significant association with adenocarcinoma and peptic ulcer. [68, 69]

The *cag*PAI and *vacA* are located separately in the genome of *Helicobacter pylori*. Yet the presence of *cagA* and active s1 form of *vacA* positive *H. pylori* strains in a patient seems to be more associated with gastritis, peptic ulcers and gastric cancer. [67, 70]

DISEASES CAUSED BY *H pylori*:

Infection with *H.pylori* produces a highly variable picture which is influenced by variety of factors like dietary habits, lifestyle, host immunity, virulence of the infecting strain. Colonization of gastric mucosa with *H.pylori* invariably results in inflammatory response. Only in few patients this leads to severe diseases like peptic ulcer, MALT lymphoma, gastric adenocarcinoma, etc. Rest others have mild asymptomatic gastritis which goes unnoticed.

Chronic Gastritis:

Gastritis is of two major form Type A and B. Type A is an autoimmune disease where antibodies are produced against acid secreting parietal cells.

Inflammation is confined to corpus of stomach predominantly. Type B gastritis affects the gastric antrum involving the mucus secreting cells. Association of *H.pylori* and gastritis is found to be 70-90%. [71]

Peptic Ulcer:

Majority of patients with duodenal and gastric ulcer are infected with *H.pylori.* Majority of the ulcers are in the first part of duodenum and the lesser curvature of stomach. [72] Studies done in different geographic locations point towards the same. Eradication of infection with combination therapy has proved to heal the ulcers. [73] Peptic ulcers occur more commonly in men than in women with the average ratio of 18:1 in developing countries .This indicates the influence of environmental factors on disease manifestations. [74]

Gastric Carcinoma:

This is the second common cause of fatal malignancy in the world accounting for more than a half million deaths annually. *H.pylori* infection leads to a chronically inflamed state of the gastric milieu. This is found to be a significant risk factor contributing to the development of adenocarcinoma. [75, 76]

In the year 1994, the International agency for research on Cancer working group WHO defined *H.pylori* as a grade–I or definite human carcinogen. [77]

Study by Xue FB et al states that "*H. pylori* infection pre-exists in gastric carcinoma and precancerous lesions, the results of Meta analysis present a strong

evidence to support the conclusion that *H. pylori* infection is a risk factor for gastric carcinoma". [(10)] Eslick GD et al found that there is a 2 fold increase in risk of gastric carcinoma among *H. pylori* infected patients. [(11)]

Gastric Malt Lymphoma:

Gastric lymphoma is the proliferation of mucosa associated monoclonal B lymphocytes. There is a strong correlation between prevalence of gastric MALT-oma and *H.pylori* colonization. In-vitro studies on the proliferation of cells derived from these lymphomas show that, this change is dependent on *H.pylori* specific T cells and their products and not due to the bacteria themselves. [(78)] Eradication of *H.pylori* results in regression of this tumor. Gastric MALT lymphoma is the only known malignancy which can be prevented by elimination of *H.pylori* infection. [(79, 80)]

Though majority of MALT lymphoma patients are positive for *H. pylori* infection, only less than 1% of those who tested positive for *H. pylori* developed MALT lymphoma [(81)]. The eradication of the bacteria in MALT patients can lead to complete remission in 60%–80% of patients with stage 1 low-grade gastric MALT lymphoma. [(82,83)] In Uemura et al study gastric cancer was seen in approximately 3% of infected patients, compared to none of the uninfected patients. This study also supported the fact that eradication of infection would decrease the risk of gastric cancer in infected individuals without premalignant lesions. [(64)] Taken together, these studies support an unequivocal role

for *Helicobacter pylori* in the development of gastric carcinoma and that the anti-*H. pylori* therapy will be an effective means of prevention.

DIAGNOSIS:

Various tests are available to diagnose *H.pylori* infection. They are broadly categorized into

1. Invasive tests (Direct)
2. Non invasive tests (Indirect)

The invasive tests are based on Gastric Biopsy. Direct microscopy with appropriate staining, Rapid urease tests, PCR, Immuno histochemistry are some of the methods used in diagnosing the infection in tissue sample.

Non invasive tests obviate the need for biopsy. It comprises of serology, urea breath test and stool antigen test. Due to the patchy distribution of this bacteria in the stomach, biopsy based tests have a slight disadvantage of missing the diagnosis of infection as opposed to non invasive methods. But the endoscopy allows the assessment of disease state and the need for treatment. [20]

INVASIVE TESTS:

Endoscopy must be performed at least 4 weeks after the last treatment with antibiotics and proton pump inhibitor. Biopsy must be taken from the antrum and

body of stomach. The sample must be processed immediately or can be stored at $4^{o}C$ for one hour.

1. Rapid urease test:

H.pylori has a very high urease activity that can be utilized for diagnosis of infection. This test was first described by Mc Nulty and Wise by using Christensen's urea broth incubated up to 24 hours. [84] Later several modifications were made to make the test rapid. Currently several commercial preparations like CLO test, Stuart's urease test broth, Pylori-tek are available which are designed to give results in few minutes. Positive predictive value of these tests is high. At least 10^4 organisms are required to give a positive result. Sensitivity of the test is 85-95% and specificity is 90-95%. [85]

2. Microscopy:

Several staining methods are available to stain the tissue sections for demonstrating *H.pylori*. Advantage of H & E stain is that it allows studying the nature of the disease in the gastric tissues as well. This could demonstrate the various diseases associate with *H.pylori*.

Some of them are Modified Giemsa stain, Hematoxylin and eosin stain, Gimenez, Warthin –starry stain, Leifson, Acridine orange. Methylene blue staining can also be used. Among these modified Giemsa stain is simple, cheap

and less labor intensive. [18] Immuno-histochemistry and fluorescence in situ hybridization are few other sensitive methods to demonstrate *H.pylori* in tissue. [86]

3. Culture:

Culture is the most specific method (100%) to establish diagnosis.[87] But its sensitivity depends on the effective transport of samples, skill of the laboratory personel and the sophistications available in lab. Culture is done on biopsy sample from antrum and corpus of stomach, but antral lesser curvature sample yields the best result.

Specimens must be plated on both selective and non selective media. The sensitivity of culture varies from 80-95%. Samples are incubated under microaerophilic condition at 37^{o}C under humid conditions. Colonies are visible after 3-5 days of incubation. They are small, translucent, dome shaped more than 3mm. On Gram stain- Gram negative curved rods, that tests positive for urease, catalase and oxidase is considered to be *H.pylori*. [19]

Advantage of culture is that it allows for performance of antibiotic sensitivity profile on the isolated bacteria. But the major disadvantage is its time consumption and requirement of expertise to culture making this mostly a research methodology rather than a regular diagnostic procedure. Delay in transport and inefficient handling of sample can have an adverse impact on recovery of this organism by culture.

4. Molecular methods:

Molecular tests are performed on samples like gastric biopsy and gastric mucosal brushing. [88] Several molecular methods based on hybridization and amplification (PCR, LAMP) have been developed. They are useful in diagnosis and post treatment evaluation. Sensitivity and specificity of PCR are 95% and 100% respectively.[89] Apart from diagnosis, PCR also allows for detection of diversity, virulence and resistance pattern. Primers for detection of housekeeping genes involved in urease production are used for identification (*Ure A, B, C gene*). *glmM* gene (*ureC*) is considered most specific for *H.pylori* detection. [90] RFLP analysis can be used to separate isolates based on the restriction fragment sizes.

NON INVASIVE TESTS:

1. **Urea Breath test:**

Urease activity can be detected by radioactive carbon ^{13}C and ^{14}C urea breath test. The sensitivity and specificity ranges from 90-100%. [91] However this test is not well approved for routine diagnosis because of risk of radiation exposure and requirement of expensive equipments.

2. **Stool antigen assay:**

It is a valuable, non invasive alternative to UBT. *H.pylori* is constantly shed in stool along with the gastro-intestinal epithelial cells. Detection of shed antigen using anti-*H.pylori* antibody based on ELISA, Immuno-

chromatography, Lateral flow assay, etc is available. [92] Usage of monoclonal antibodies is found to be better than polyclonal antibody based detection. ICT is cheap and field based method. It can be used in smaller laboratories that do not have equipment for performing EIA. Immuno Card STAT HpSA has been reported to have sensitivity of 96% and specificity of 90%. [93]

3. **Antibody detection (serology):**

This method is simple, cost effective and has better reproducibility. Yet the method is not specific enough to be used as a screening procedure in the endemic population. IgM develops soon after patient gets infected followed by IgG and IgA. Various methods for detection of these antibodies are complement fixation, agglutination, immunoblot and ELISA. The antigens for ELISA are whole cell lysate, acid glycine extract and high molecular weight associated recombinant proteins of *UreB, VacA and CagA.*

In Immunoblot assay the above said proteins are immobilized on a nitrocellulose membrane and used for detection of IgG in patient's serum. The sensitivity and specificity of the test is 90% compared to that of ELISA. [94]

4. String test:

Patients are asked to swallow an absorbent string on an empty stomach and it is allowed to stand in stomach for 15-30 minutes. It is then withdrawn and the distal end is used for testing urease, culture and PCR. This is a non invasive

method for collection of sample for more specific tests. The sensitivity of this method of collection is good for PCR compared to culture. [95, 96]

EPIDEMIOLOGY:

Although *Helicobacter pylori* infection is found in more than 50% of the world population, there is an increased prevalence in developing countries rather than the developed ones. [97] Maximum prevalence of *H.pylori* infection of more than 90% is seen in the African and few of the Asian countries. Prevalence in India is estimated to be 88% in adults by World Gastroenterology Organization in 2010. [98] Its high in South India compared to Northern states.

There is a significant variation in prevalence between countries and also in different regions within the same country. *H.pylori* sero-positivity rate increases progressively with age, reflecting a cohort phenomenon. Most of the infections occur during childhood and there is a significant reduction in incidence with improving hygienic practices. Studies show that the incidence of *H.pylori* infection is 2.7-36% in children as opposed to 0.4% in adults. [90]

Person to person transmission is the most likely mode. This occurs either by the oral-oral route or the fecal-oral route. This would explain the clustering of *H. pylori* infection within families and higher incidence of infection among institutionalized children and adults. Fecal contamination of the drinking water is

another possible mode of spread of infection. The only proven mode of iatrogenic transmission of *H. pylori* is following endoscopy. [97]

Adequate nutritional status like consumption of fruits rich in vitamin C and vegetables appears to protect against infection with *Helicobacter pylori*. Risk factors of infection include inadequate sanitation practices, crowded or high-density living conditions, low social class and consumption of food prepared under poor hygiene.

TREATMENT:

Indications for treatment of *Helicobacter pylori* infection are [99]

1. Past or present duodenal or gastric ulcer patients, with or without complication.
2. Post-resection of gastric cancer
3. Gastric (MALT) lymphoma
4. Atrophic gastritis
5. Dyspepsia
6. Patients who have first-degree relatives with gastric cancer

Multidrug regimens are commonly used. Triple therapy or quadruple combinations are the most successful ones. Triple-therapy with PPI + two antibiotics is most accepted worldwide. Here the patients are treated for 7-14 days.

Antibiotics normally chosen are amoxicillin and Clarithromycin or metronidazole and Clarithromycin. Failure to eradicate the infection, with this method, is surfacing in recent years due to Clarithromycin resistance.

In Quadruple therapy bismuth salicylate is added to PPI with amoxicillin + Clarithromycin or metronidazole + tetracycline and administered for 10-14 days. It is cheaper than triple therapy.

Proton pump inhibitors are added to increase the pH in stomach, for the better action of the other drugs in the regimen. [100]It also alleviates the symptoms of acidity.

All the drugs used in these therapies have a high incidence of adverse effects like nausea, metallic taste leading to discontinuation of treatment adding to the failure rate.

Eradication is defined as negative test for *H.pylori* by urea breath test or stool antigen test or check endoscopy, at least 28 days after the end of antimicrobial therapy.

Apart from the above mentioned, other First-line therapies [101] are

1. Concomitant therapy- PPI + clarithromycin + amoxicillin and metronidazole for 7–10 days

2. Hybrid therapy - 7-day dual therapy with a PPI + amoxicillin followed by a 7-day quadruple therapy with a PPI + amoxicillin + Clarithromycin + metronidazole
3. Sequential therapy - 5day dual therapy with a PPI and amoxicillin followed by a 5-day triple therapy with a PPI+ clarithromycin and metronidazole

DRUG RESISTANCE:

Like any other bacteria, *H pylori* is also developing drug resistance and the prevalence of resistant strains is increasing at an alarming rate due to the widespread use of antibiotics like Macrolides , Quinolones and Metronidazole. Systemic review of studies done from 2006 - 2009, by De Francesco V et al concluded that the antibiotic resistance rates of *Helicobacter pylori* were 26.7% for metronidazole, 17.2% for clarithromycin, 16.2% for levofloxacin, 11.2% for amoxycillin, 5.9% for tetracycline, 1.4% for rifabutin and 9.6% for multiple antibiotics. Prevalence of resistance to clarithromycin, metronidazole, and levofloxacin has increased in Asia, America and Africa compared to Europe. Tetracycline resistance is less than <3% in all countries except Africa (43.9%). [(14)]

Study conducted by Thyagarajan et al showed that the *H. pylori* resistance rate was 77.9% to metronidazole, 32.8% to amoxycillin and 44.7% to

clarithromycin. [15] Multiple resistances were seen in 43.2% isolates. Patterns with two, three and four drug resistance were seen in 13.2, 32 and 2.56% samples respectively to the following drugs- metronidazole, Clarithromycin and amoxycillin. Metronidazole resistance was high in Lucknow (68%), Chennai (88.2%) and Hyderabad (100%) and moderate in Chandigarh (38.2%) and Delhi (37.5%). Ciprofloxacin and tetracycline had the least resistance (1.0 to 4%) [48]. *H.pylori* shows a marked allelic diversity due to their higher mutation rate compared to many other bacteria due to an incomplete DNA mismatch repair system (mutS1/MutL/mutH) and lack of enzymes involved in base excision repair. This may be one of the reasons for its high-level resistance to commonly used antibiotics such as clarithromycin. [102]

The rate of Clarithromycin resistance, globally, varies widely from 49% in Spain to 1% in The Netherlands. In places where Clarithromycin resistance is <10%, a standard triple therapy can be employed to achieve an eradication rate of >90%. On the other hand this standard therapy should be avoided in areas with clarithromycin resistance ≥20% (ie, Turkey, Spain, China, Alaska and Japan). Performance of culture and Antibiotic susceptibility testing guided choice of therapy is a better method to treat patients who failed with first line eradication methods.

In drug resistant patients, second line therapies with Fluoroquinolones are used in place of Clarithromycin. Levofloxacin and moxifloxacin are the commonly chosen ones. [101]

PREVENTION AND CONTROL:

Spread of infection within the family members can be controlled by educating the people about the hygienic practices and need for diagnosis and treatment of infected patients. Practices of good hand hygiene, eating properly cooked food and consuming safe drinking water can reduce the infection rate to significant extent. Strict implementation of sanitary measures helps to avoid infection in community. At present there is no vaccine available to prevent *H.pylori* infection.

MATERIALS & METHODS

This prospective observational study was conducted in the Department of Microbiology, PSGIMS&R after obtaining institutional Human ethical committee's approval.

STUDY PERIOD: May 2014- June 2015.

SAMPLE SIZE:

Gastric biopsy samples were collected from **165** patients with gastro duodenal diseases undergoing Upper GI Endoscopy in Gastroenterology department in PSG Hospitals, Coimbatore.

SAMPLE SIZE JUSTIFICATION:

Formula used: $n= t2 \times p (1-p)/m^2$

Where;

n=required sample size

t=confidence level at 95% (standard value of 1.96)

p=estimated prevalence of *H pylori*

m=margin of error at 5% (standard value of 0.05)

Estimated prevalence from hospital statistics (p)=0.88

n=1.96X1.96X 0.88(1-0.88)/0.05X0.05

n=165 **(**162)

INCLUSION CRITERIA:

Patients with symptoms suggestive of following conditions were included in the study

1. Non Ulcer Dyspepsia (NUD)
2. Peptic Ulcer Disease (PUD)
3. Gastric carcinomas
4. MALT lymphoma

EXCLUSION CRITERIA:

Patients who had received antibiotics within the past 1 month period and anti secretory drugs within the past 2 weeks prior to endoscopy were excluded

CONSENT:

Written Informed consent to be enrolled in the study was obtained from patients before performing Endoscopy.

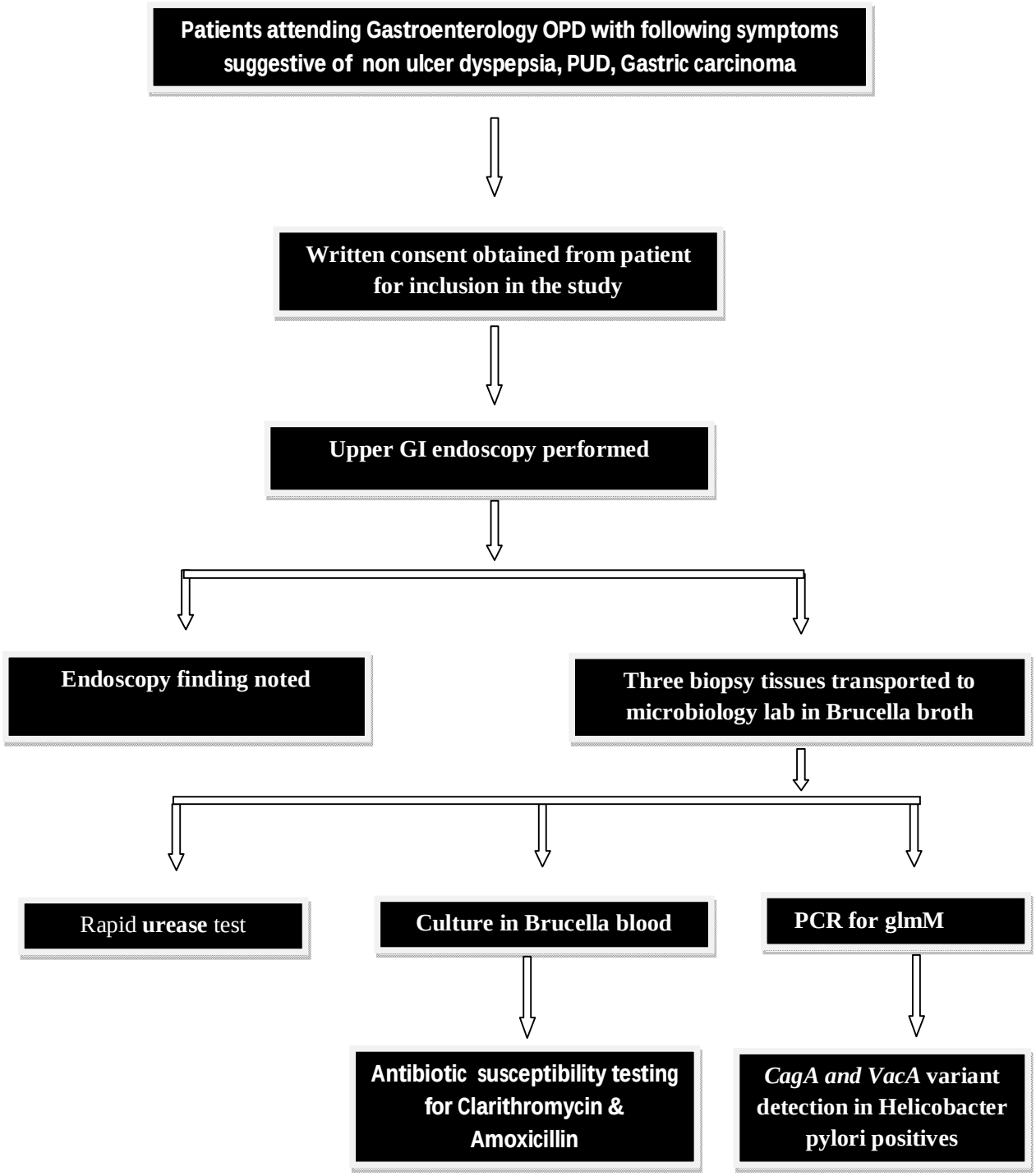
ETHICAL CLEARANCE:

This prospective study was approved by the Ethical Committee of PSG IMS&R

STATISTICAL ANALYSIS:

Statistical analysis was performed using SPSS software (Statistical Product and Services Solutions, version 17, SPSS Inc, Chicago, Il, USA) to analyze data. Association between gastric lesions and genotypes was tested independently using Pearson's chi^2 test and Fischers exact test. All Chi^2 test and Fischers exact test results with P-values less than 0.05 were considered statistically significant

WORK FLOW DIAGRAM



ENDOSCOPY PROCEDURE:

Patients were instructed to fast for a minimum of 12 hours before endoscopy. The Fibreoptic gastroduodenoscope was rinsed thoroughly with water and disinfected by placing in a solution of 2% gluteraldehyde for 20 minutes. The endoscope was rinsed in sterile physiological saline just before the procedure. Patient's throat was sprayed with lignocaine spray to reduce gagging and they were made to lie down in left lateral position comfortably. The end of the endoscope was lubricated with lignocaine jelly before inserting into the mouth. The patient was asked to swallow until the scope reached the esophagus. Internal structure of stomach and duodenum were studied for ulcers, erosion, inflammation, discoloration and abnormal growth. Findings were noted and 3 biopsy samples were taken preferably from the site of lesion and gastric antrum using biopsy forceps.

Three biopsy bits were transported in Brucella Broth to microbiology laboratory within an hour of collection. One bit was used for performing each of the below mentioned tests.

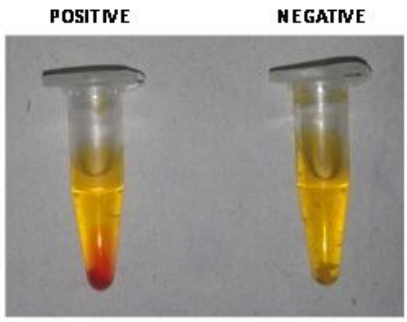
1. Rapid urease test
2. Culture
3. Polymerase Chain Reaction

RAPID UREASE TEST:

First bit was used for Rapid urease testing. Freshly prepared rapid urease broth was used. To a 100 ml of sterile distilled water, 20 grams of urea was weighed and added with decreased chances of contamination. pH of this broth was adjusted to 5 with 1N HCl and confirmed with litmus paper. Phenol red 0.002gm (indicator) was added and filtered in membrane filter and dispensed as 1ml broths, in autoclaved sterile eppendorf tubes.[103] The final color of the medium was yellowish brown. The biopsy bit was added to this broth as soon as possible and the tubes were incubated at 37^{o}C. An uninoculated tube was kept as negative control. Positives were recorded when the tissue sample turned pink within 20 minutes. The entire 1 ml broth turned pink in next few hours. Few samples gave weak positive result which appeared only after incubation for 4 hours.

H pylori produces large amount of urease enzyme which splits urea into ammonia and carbon dioxide. This test detects the increase in pH of the medium due to ammonia using an indicator (phenol red). Change in color from yellow to reddish pink indicates positive.

FIGURE 1: IN-HOUSE RAPID UREASE TEST



POLYMERASE CHAIN REACTION:

Second bit was stored at -80^{o}C in 70% ethanol under sterile condition for Extraction and PCR. Extraction was done using QIAmp DNA MINI extraction kit (QIAGEN). Procedure given by the manufacturer was followed for extraction.

Principle of PCR:

PCR is based on the principle of exponential amplification of a desired fragment of DNA, based on DNA replicative mechanism. It involves denaturation of double stranded DNA followed by annealing of primers for the desired amplicon and extension of primer.

Instruments and materials:

Vortex mixer

Refrigerated micro centrifuge –Legend Micro 21R

Thermo cycler

Dry bath - Thermocon DB900

Micropipettes 1000 µl, 100 µl and 200 µl

Barrier tips 1000 µl and 200 µl

Components of QIAamp DNA MINI kit:

Tissue lysis buffer (ATL)

Lysis buffer (AL)

Proteinase K

Wash buffer I & II

Elution buffer

Spin columns

2 ml collection tubes

The kit can be stored at room temperature (15-25^{o}C)

Extraction Procedure:

Pre-extraction steps

25ml of absolute ethanol was added to wash buffer 1(AW1)

30ml of absolute ethanol was added to wash buffer 2(AW2)

Dry baths were set at 56^{o}C and 70^{o}C

Extraction steps

- In a sterile 1.5 ml centrifuge tube, gastric biopsy tissue was taken and 180µl of tissue lysis buffer and 80 µl of phosphate buffer saline was added and the tissue was ground to make a homogenous mixer.
- To this 100 µl of ATL and 20 µl of proteinase K was added.
- Mixer was vortexed and incubated at 56^{o}C in dry bath for 2-3 hours with intermittent vortex until the pellet was completely lysed.
- Tube was removed from dry bath and 200 µl of AL was added.
- The mixture was vortexed well and incubated at 70^{o}C for 10 minutes in dry bath.

- The tube was removed from the dry bath. To this 200 µl of absolute ethanol was added and vortexed.
- The lysate was transferred to the upper reservoir of a labeled Spin column tube without wetting the rim. The tube was centrifuged at 8000 rpm for 1 minute in cold centrifuge.
- Collection tube with contents was discarded. Spin column was transferred to a new 2ml collection tube. To this 500 µl of AW1 was added and centrifuged at 8000 rpm for 1 minute.
- The above step was repeated with 500 µl of AW2 and centrifuged at 14000 rpm for 3 minutes.
- Spin column tube is transferred to a new 2ml collection tube and centrifuged at full speed 14800 rpm for 1 minute for final filtration.
- Spin column tube was placed in a new sterile 1.5ml eppendorf tube for elution of DNA. To this 200 µl of elution buffer was added and held at room temperature for 5 minutes. Centrifuged at 8000 rpm for 1 minute and eluted material is store at 4^{o}C for PCR.

Cells are lysed during the incubation period with proteinase K in the presence of chaotropic salts and detergents in the lysis buffers. Once the digest is centrifuged, the DNA in it binds to the silica in the spin column tubes. On subsequent washing steps the impurities like proteins and polysaccharides are

removed. Finally a low salt elutes like Tris is added to elute the DNA bound to the silica.

PCR FOR THE DETECTION Of *glmM* GENES:

PCR was performed for 165 tissue samples for detection of glmM gene which is one of the house keeping genes of *Helicobacter pylori*. The primers used were obtained from Sigma Aldrich, Mumbai, which were based on reference article from a previous study. The primers used were as follows.

Gene	Primer Sequence(5′-3′)	Amplicon size (bp)	Reference
glmM	F- AAGCTTTTAGGGGTGTTAGGGGTTT R- CGCAATGCTTCAATTCTAAATCTTG	136	104

Reaction mixture:

Each single reaction mixture (25µl) contained 4 µl of DNA suspension, 12.5 µL of Master Mix (10mM dNTPs, 1 U *Taq* DNA polymerase, 25mM $MgCl_2$ and 2.5 µl of **10** x *Taq* buffer) and 1µM of each primer (Sigma- Aldrich, Mumbai). The remaining volume was adjusted with PCR grade water.

Positive and negative controls were setup with each batch of tests run.

Polymerase chain reaction:

The PCR was performed by conventional method using a Eppendorf thermal-cycler, under the following conditions.

Initial denaturation at 95℃ for 10 minutes, 35 cycles of 95°C for 30 seconds, annealing temperature 60^{o}C for 45 seconds and extension at 72°C for 1 minute and a final elongation at 72°C for 5 minutes

The products after amplification are stored at **-20°C** until they were subjected to agarose gel electrophoresis.

Agarose gel electrophoresis:

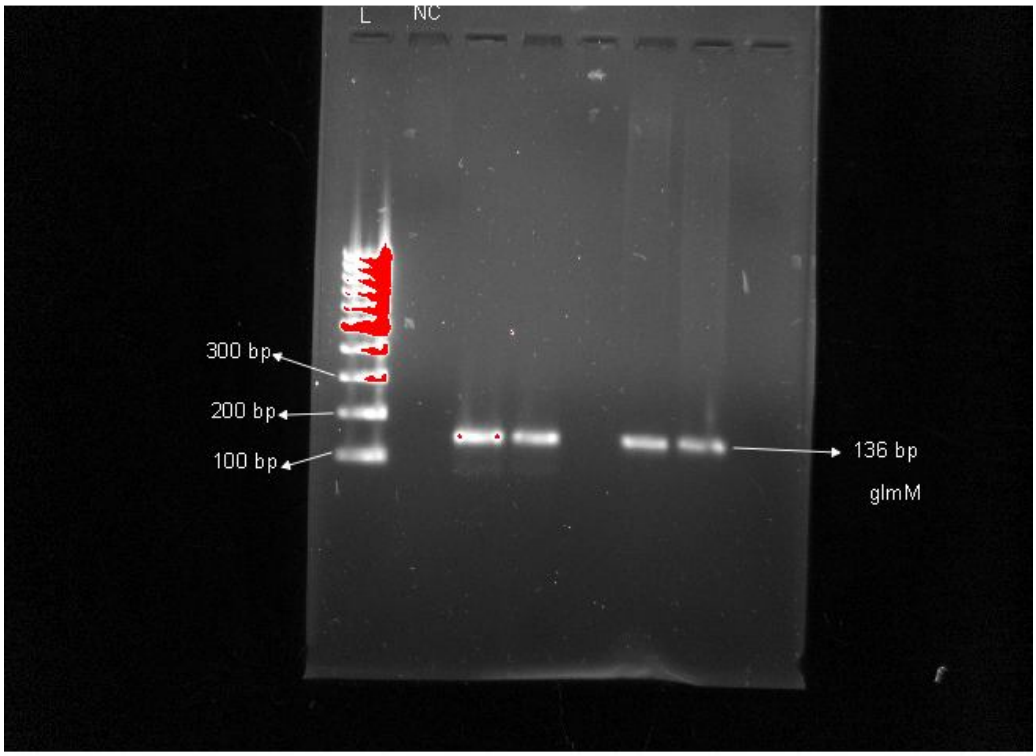
The amplified product is visualized by use of agarose gel electrophoresis. The agarose gel was prepared to a ratio of 1% by adding agarose gel powder with Tris Borate EDTA (TBE) buffer. This mixture was heated in microwave till it becomes a clear solution and followed by the addition of *Ethidium bromide was added* to visualize the amplified DNA under UV light. The mixture was allowed to set in an electrophoresis tank with comb in place.

A 100 base pair ladder was used as the molecular marker to measure the size of the amplified product from the PCR in the first well and the rest of the wells had 5µl of the amplified product along with 3 µl of the loading dye from Sigma Aldrich was used to visually see the movement of the product. The gel electrophoresis was performed by placing the gel in an electrophoresis tank containing Tris Borate EDTA (TBE) buffer at 100 volts for 40 minutes.

Interpretation:

The ethidium bromide was used to stain the amplified DNA for visualization under UV illumination. Then images of the gel were captured by Gel doc. Sixty one samples were found to be positive for glmM gene having 136bp, confirmed by the 100bp ladder that was used for this purpose.

Figure 2: Gel Electrophoresis picture showing glmM gene



PCR FOR *cagA* And VARIANTS OF *vacA* GENES:

PCR for analysis of *VacA* s1/s2 and *VacA* m1/m2 mosaic structure analysis and the presence of *cagA* gene was performed for the 61 isolates which were positive for *glmM* gene. The primers used were obtained from Sigma Aldrich, Mumbai, which were based on reference article from a previous study. The primers used were as follows

Genes	Primer name and Sequence(5′-3′)	Amplicon size (bp)	Reference
VacA s1/s2	VAI-F ATGGAAATACAACAAACACAC VAI-R CTGCTTGAATGCGCCAAA	259/286	8, 64
VacA m1/m2	VAG-F CAATCTGTCCAATCAAGCGAG VAG-R GCGTCAAAATAATTCCAAGG	567/642	28, 105
cagA	cag5c-F GTTGATAACGCTGTCGCTTC cag3c-R GGGTTGTATGATATTTTCCAT AA	350	8

Each single reaction mixture (25µl) contained 4 µl of DNA suspension, 12.5 µL of Master Mix (10mM dNTPs, 1 U *Taq* DNA polymerase, 25mM $MgCl_2$ and 2.5 µl of **10** x *Taq* buffer) and 25pmol of each primer for VacA genes and 10pmol of each primer for *cagA* gene (Sigma- Aldrich, Mumbai). The remaining

volume was adjusted with PCR grade water. Positive and negative controls were setup with each batch of tests run.

Polymerase chain reaction:

The PCR was performed by conventional method using a thermo-cycler, under the following conditions, which were same for all the four genes.

Initial denaturation at 94°C for 3 minutes, 35 cycles of 94°C for 1 minute, annealing temperature of 55^{o}C for 1 minute and extension at 72°C for 1 minute, and a final elongation at 72°C for 10 minutes

The amplified products were stored at **-20°C** until they were subjected to agarose gel electrophoresis as described above

Interpretation:

All glmM gene positivity indicates that the patients harbor Helicobacter pylori in their gastric tissue. Strain was determined to be positive or negative for cagA gene based on the presence or absence of specified 136bp amplicon respectively.

In VacA s region analysis, amplicon size of 259 and 286 indicates vacAs1 and s2 respectively. Similarly amplicon size of 567 and 642 base pairs indicates VacAm1 and m2 respectively. A single strain harbors any one of the variant in each gene eg. VacA s1/m1, VacA s1/m2, etc.

FIGURE 3: GEL ELECTROPHORESIS PICTURE SHOWING *CagA* GENE

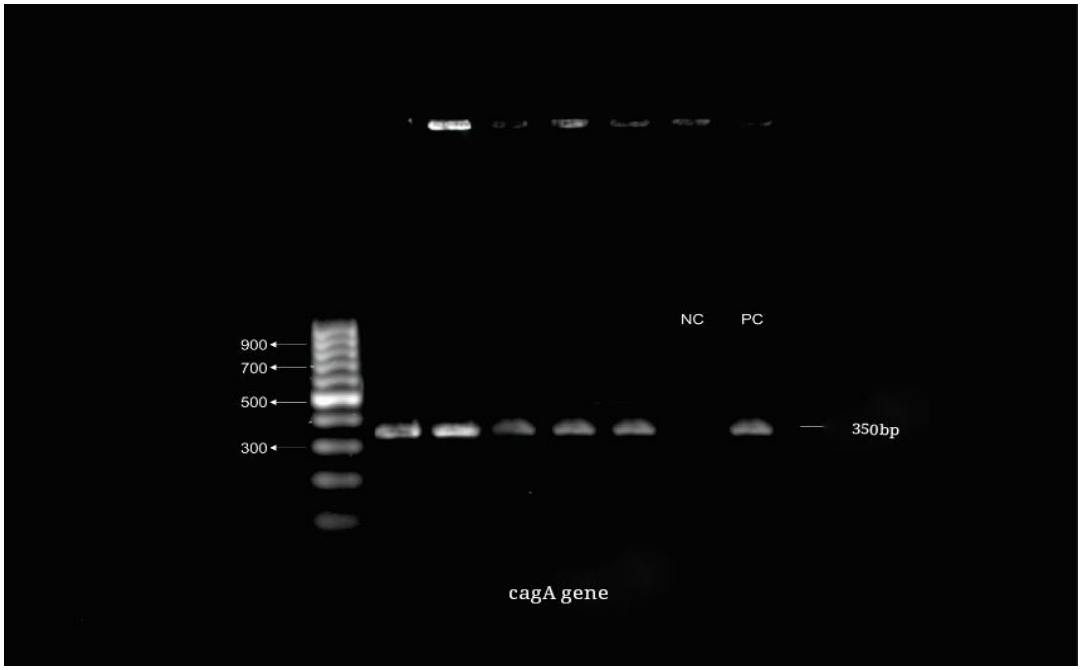


FIGURE 4: GEL ELECTROPHORESIS PICTURE SHOWING *VacA* s1/s2 GENE

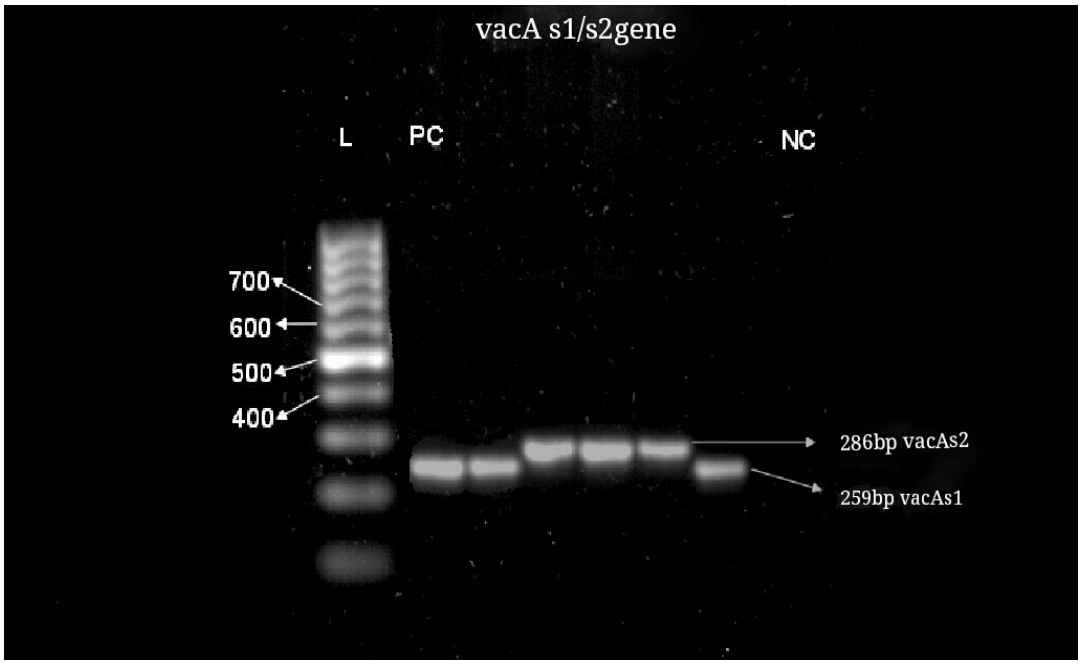


FIGURE 5: GEL ELECTROPHORESIS PICTURE SHOWING *VacA* m1/m2 GENE

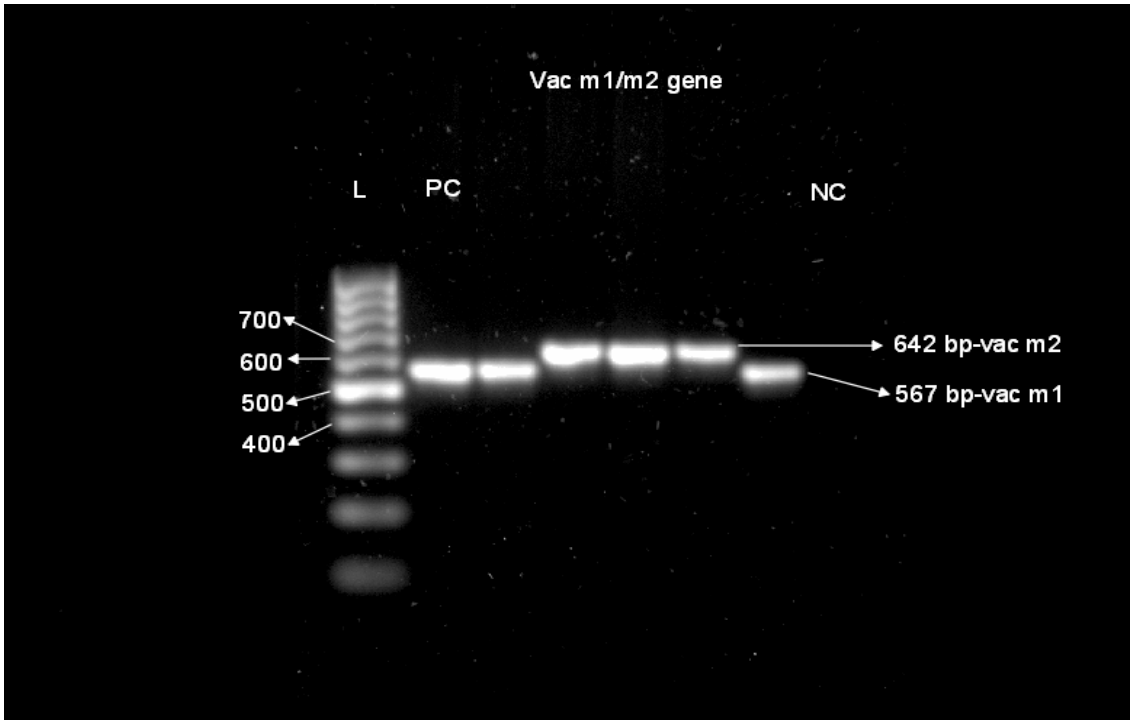


FIGURE 6: MATERIALS REQUIRED FOR MOLECULAR STUDY

CULTURE:

The biopsy specimen collected in Brucella broth was processed within 2-3 hours from the time of collection. The biopsy tissue was minced to pieces using a sterile no.22 scalpel blade on a sterile glass slide. The minced tissue was inoculated to a freshly prepared selective medium, Brucella chocolate agar [106, 107] supplemented with vancomycin, Polymyxin B and amphotericin B. This antibiotic combination was chosen based on the common contaminants encountered during processing. Prepared plates were stored in sterile box at $4^{o}C$ for not more than a week. Culture plates were incubated under microaerophilic atmosphere 10% CO_2, 5% O_2, 85% N_2 at $37^{o}C$ using Anoxomat system. The incubation jar was opened on $3^{rd,}$ $5^{th,}$ 7^{th} and 10^{th} day and checked for growth. Plates which had no growth were discarded after 10^{th} day.

Helicobacter pylori grew on plate as tiny (0.5-1 mm), moist, convex and watery colonies (translucent). Identification was confirmed by Gram stain, catalase, oxidase and urease tests. (Fig 7)

Single colony was emulsified in a drop of saline on a clean glass slide. The smear was air dried and fixed with a few drops of methanol. On Gram stain's' shaped or seagull shaped Gram negative spirals were seen. (Fig 8)

A colony was picked with sterile glass rod and dipped into 3% hydrogen peroxide solution in a test tube. Appearance of prompt bubbling indicates a positive reaction. *H.pylori* is strongly catalase positive. (Fig 9)

A suspected colony was smeared on to a dry filter paper impregnated with Tetramethyl para-phenylene diamine dihydrochloride. *H.pylori* shows change of colour from white to purple within 10 seconds indicating oxidase positivity. (Fig 9)

When a few colonies were streaked on the slant of Christensen's urease agar, a rapid color change from yellow to pink occurred, demonstrating the presence of urease and indicating the presence of *H pylori*. (Fig 9)

The growth was stored in broth made of equal parts of BHIB with 15% glycerol and skimmed milk at -80^{o}C.

FIGURE 7: GROWTH OF *H.pylori* IN CHOCOLATED BRUCELLA AGAR

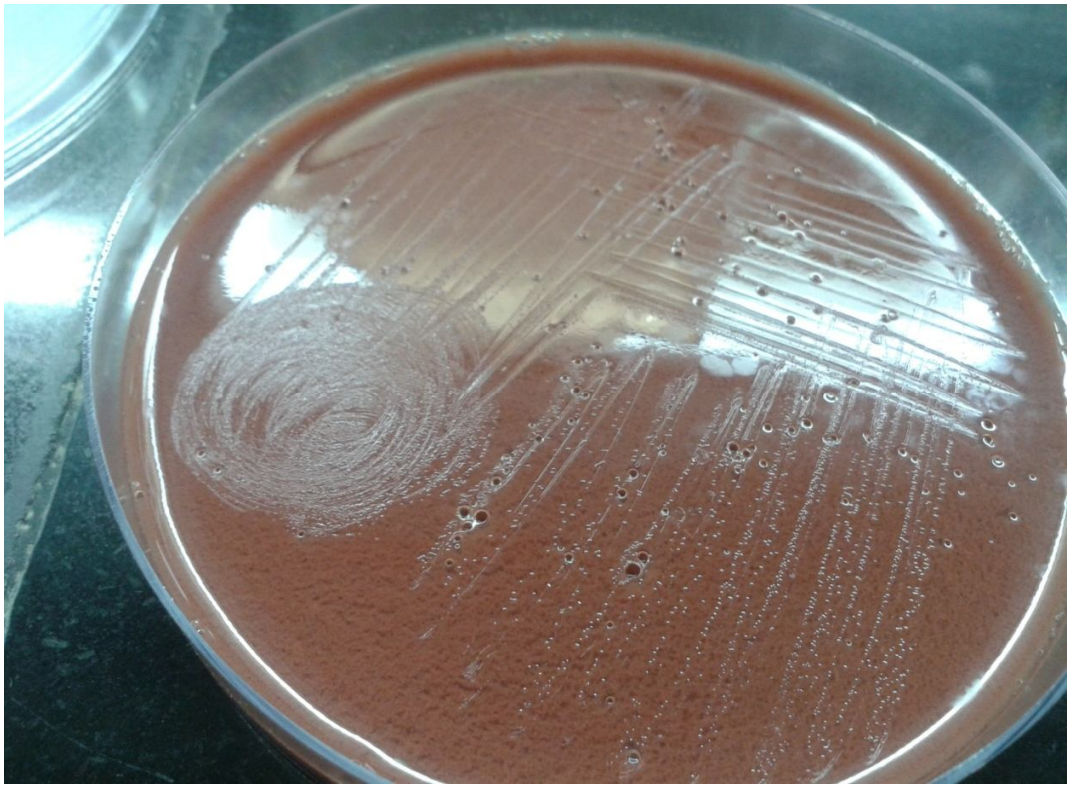


FIGURE 8: GRAM STAIN IMAGE OF *H.pylori*

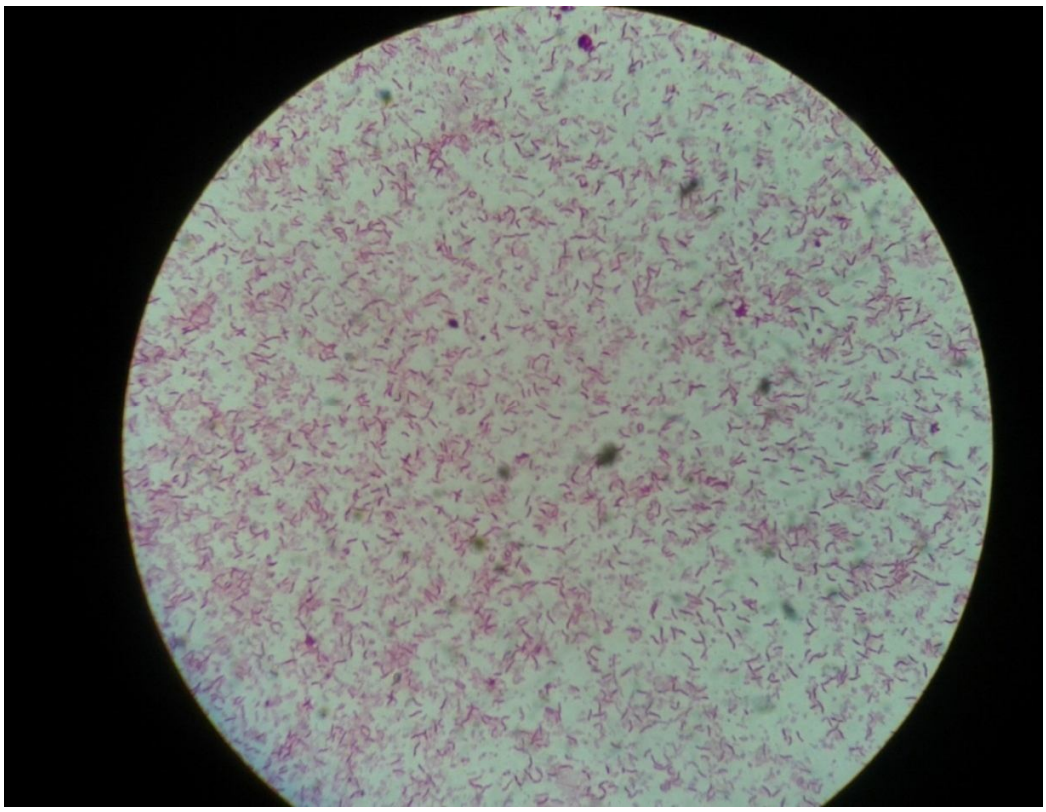
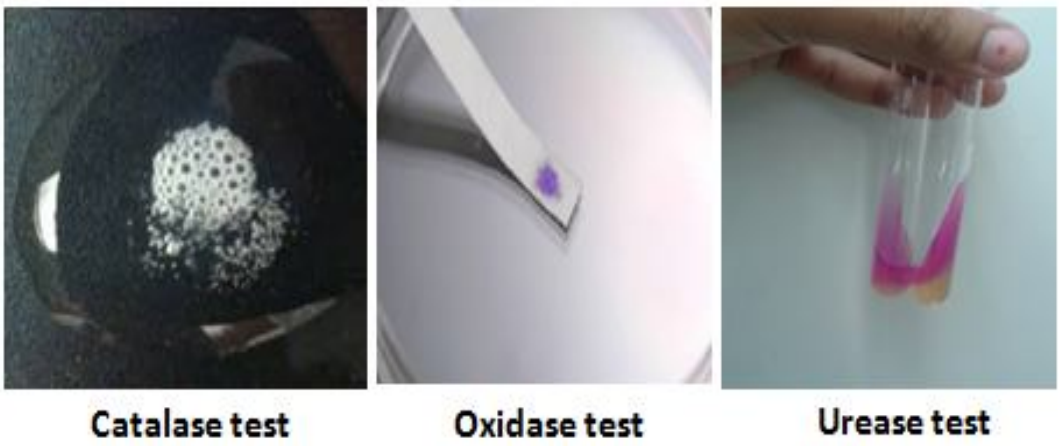


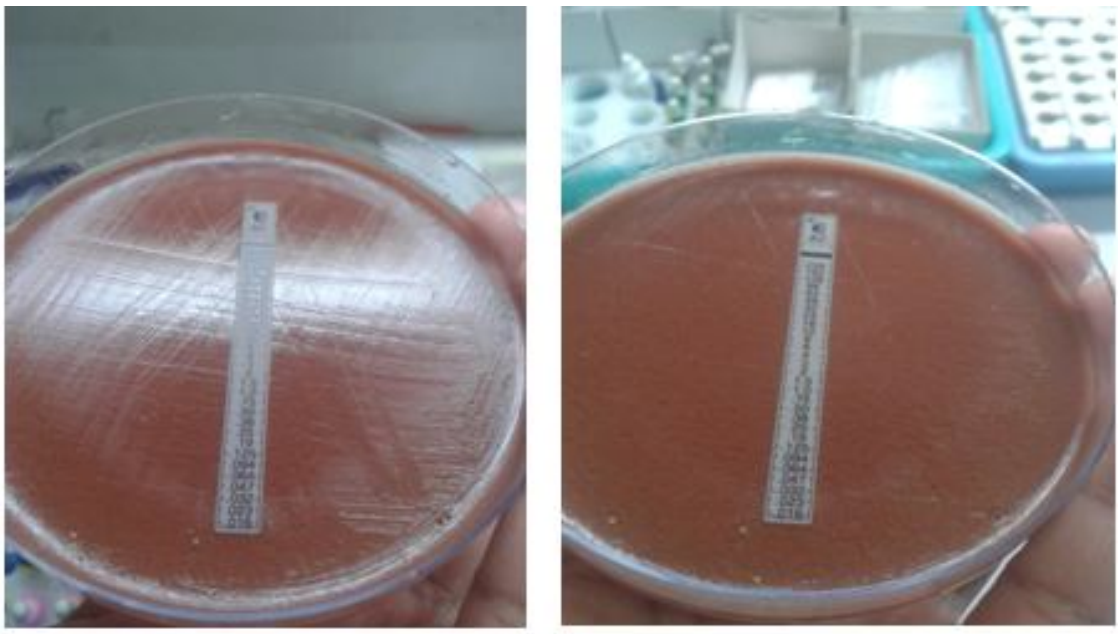
FIGURE 9: BIOCHEMICAL TEST FOR IDENTIFICATION OF *H.pylori*



ANTIBIOTIC SUSCEPTIBILITY TESTING:

Isolates were tested for Clarithromycin and Amoxicillin. Isolated organism was sub cultured on antibiotic free Brucella chocolate agar. On an average, organism took 3 days to grow on sub culture. The growth was emulsified in Brucella broth and matched to Mc Farland standard of 4. Lawn culture was made in a Brucella chocolate agar with sterile cotton swab. E-strip (Biomerieux) was placed at the center with a sterile applicator and the plates were incubated in micro-aerophilic atmosphere at 37^{o}C for 72 hours. [108] Reading on the strip at the point of intersection of the growth was taken as MIC of that isolate. (Fig 10)

FIGURE 10: E-TEST METHOD OF ANTIBIOTIC SUSCEPTIBILITY TESTING



MIC CUT OFF FOR DETERMINATION OF ANTIMICROBIAL SUSCEPTIBILITY IS AS FOLLOWS

ANTIBIOTICS	SUSCEPTIBLE	INTERMEDIATE	RESISTANT	REFERENCE
CLARITHROMYCIN	≤0.25	0.5	≥1.0	109
AMOXICILLIN	≤0.25	0.5	≥1.0	110

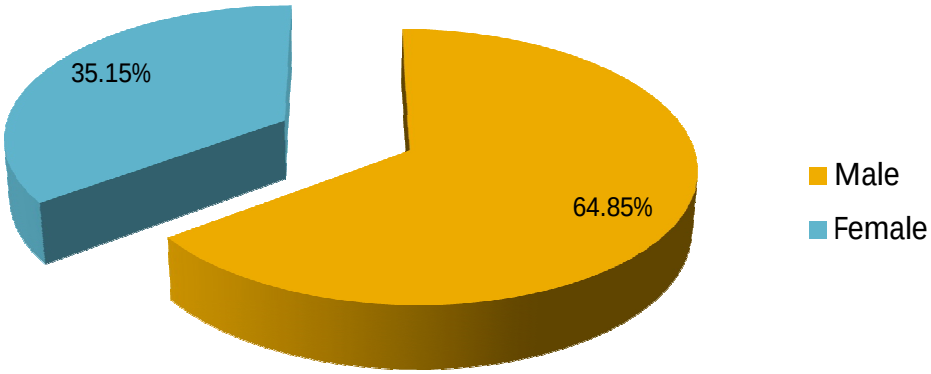
ENDOSCOPY FINDINGS:

Upper GI endoscopy findings were noted down in endoscopy unit following the procedure as normal, Gastritis, Gastro duodenitis, Gastric ulcer, Duodenal Ulcer, CA Stomach and others.

RESULTS & ANALYSIS

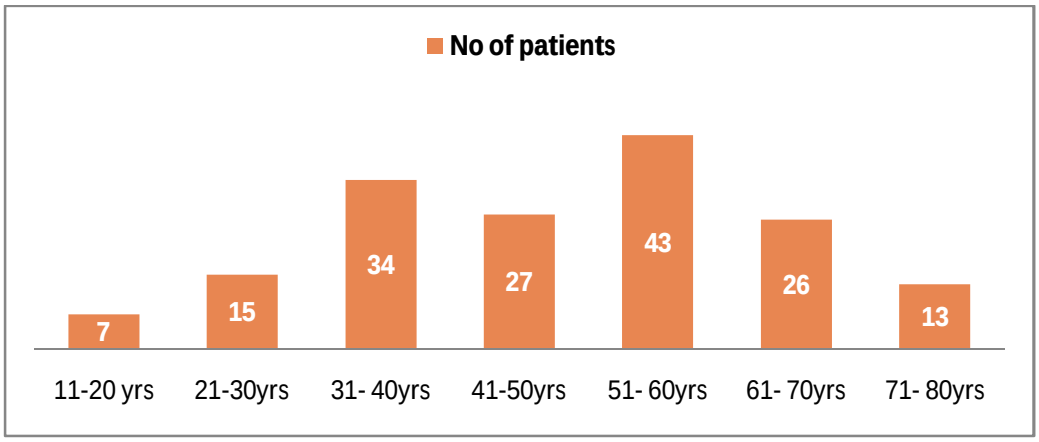
Out of the 165, 107 (64.85%) were male and 58 (35.15%) were female. This shows the increased prevalence of gastro duodenal disease among male compared to female.

Figure 11: Sexwise distribution of study group



The selected 165 patients were analyzed based on their age. Of the 165, maximum number of cases were under the age group of 51-60 years, 43 (26%) followed by 34 (20.6%) falling under 31-40 years. The study population falling under the age groups of 41-50 and 61-70 years were 16% each. The rest were below 30 years and above 70 years. The mean age of the cases was 49 years.(Fig - 12)

FIGURE 12: GRAPH OF AGE WISE DISTRIBUTION OF STUDY GROUP



The above graph shows that majority of the patients undergoing Upper GI endoscopy for gastro duodenal diseases fall under the age group of 30-70 years.

TABLE 1: DISTRIBUTION OF CASES AMONG THE STUDY GROUP

ENDOSCOPIC FINDINGS	**NO. OF CASES (%)**
Gastritis	101(61.2)
Gastric ulcer	14(8.4)
Duodenal ulcer	3(1.8)
Gastroduodenitis	11(6.7)
Carcinomatous growth	3(1.8)
Normal study	23(14)
Others	10(6.1)
TOTAL	**165(100%)**

The Table 1 shows that among the 165 dyspeptic patients, endoscopic finding suggestive of gastritis (antral, fundal, diffuse or erosive gastritis) was seen in 101 (61.2%) patients, gastric ulcer and duodenal ulcer were seen in 14 (8.4%) and 3 (1.8%) patients respectively and Gastroduodenitis was seen in 11 (6.7%) cases. Three patients had growth in stomach which was later identified as gastric carcinoma. Fourteen percent of patients had normal study. Six percent of patients had other conditions like polyps, Barrett's esophagus, diverticula, Tropical sprue, etc

FIGURE 13: CHART SHOWING DISTRIBUTION OF STUDY POPULATION WITH RESPECT ENDOSCOPIC FINDINGS

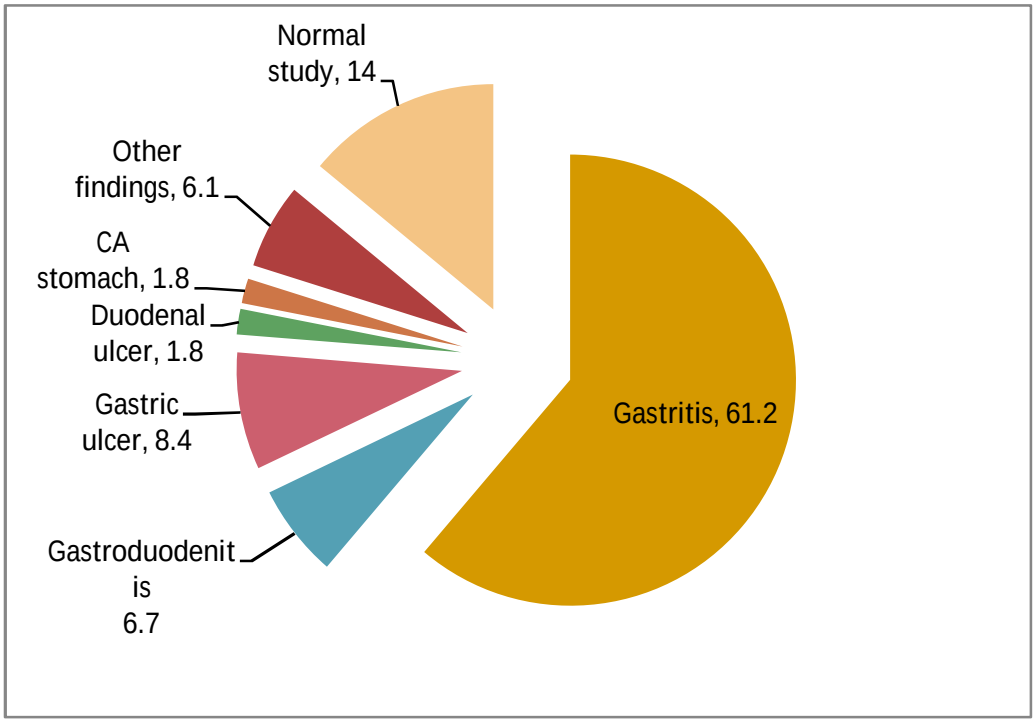
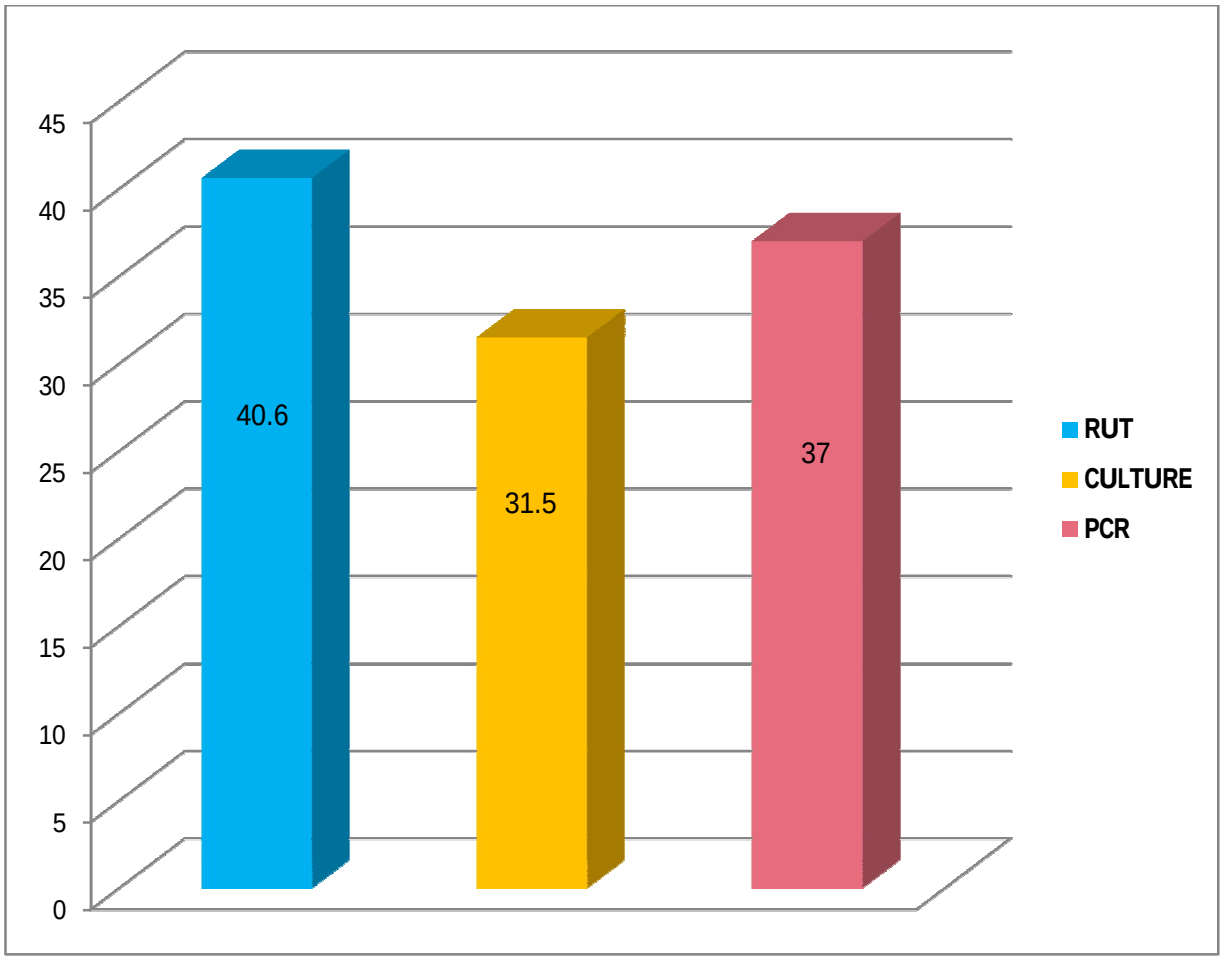


FIGURE 14: PERCENTAGE OF POSITIVITY FOR *H.pylori* BY VARIOUS METHODS



Out of 165 samples tested, 67 (40.6%) were positive by rapid urease test, 52 (31.5%) by culture and 61 (37%) by PCR.

TABLE 2: POSITIVITY OF VARIOUS TESTS AMONG VARIOUS GASTRODUODENAL DISEASES

ENDOSCOPIC DIAGNOSIS	NO. OF CASES	NO. OF POSITIVES BY VARIOUS METHODS		
		RUT N (%)	Culture N (%)	PCR N (%)
GASTRITIS	101	38 (37.6)	29 (28.7)	35 (34.7)
GASTRODUODENITIS	11	6 (54.5)	6 (54.5)	6 (54.5)
GASTRIC ULCER	14	12 (85.7)	10 (71.4)	11 (78.6)
DUODENAL ULCER	3	3 (100)	3 (100)	3 (100)
CARCINOMA STOMACH	3	1 (33.3)	0	0
NORMAL STUDY	23	6 (26.1)	4 (17.4)	5 (21.7)
OTHERS	10	1 (10)	0	1 (10)
TOTAL	**165**	**67 (40.6)**	**52 (31.5)**	**61 (37)**

TABLE 3: ASSOCIATION OF ABNORMAL ENDOSCOPIC FINDING AMONG POSITIVE AND NEGATIVE CASES

H.pylori* infection	**ENDOSCOPIC FINDINGS**							**P value**
	Gastritis N (%)	**Gastroduodenitis N (%)**	**Gastric Ulcer N (%)**	**Duodenal Ulcer N (%)**	**CA stomach N(%)**	**Others N (%)**	**Normal N (%)**	
Positive	35(34.7)	6(54.5)	11(78.6)	3(100)	0(0)	1(10)	5(21.7)	**<0.001**
Negative	66(65.3)	5(45.5)	3(21.4)	0(0)	3(100)	9(90)	18(18.3)	

*As per PCR for *glmM* gene

Table-3 shows that there was a significant statistical association ($p< 0.001$) between the abnormal endoscopic finding and *H.pylori* infection.

Results of the association between the individual endoscopic findings and *H.pylori* infection is shown in the next table

TABLE 4: STATISTICAL ANALYSIS OF THE ASSOCIATION OF *H.pylori* INFECTION WITH ENDOSCOPIC FINDING

ENDOSCOPIC FINDINGS	NO. OF CASES	*H.pylori* POSITIVE CASES*(%)	P Value
Gastritis	101	35(34.7)	0.232
Gastroduodenitis	11	6(54.5)	0.114
Gastric ulcer	14	11(78.6)	0.001
Duodenal ulcer	3	3(100)	0.022
Carcinomatous growth	3	0(0)	1.000
Others	10	1(10)	0.640
Normal study	23	5(21.7)	
TOTAL	**165**	**61(37)**	

**H.pylori* positive by PCR

Among the 101 gastritis patients 34.7% (35) were positive for *H.pylori* infection by PCR. Among gastric and duodenal ulcer patients 78.6% and 100% were found infected with *H.pylori*. 6 (54.5%) out of 11 Gastroduodenitis patients had *H.pylori* infection. None of the gastric cancer patients had *H.pylori* infection. Among the normal study cases 21.7% were infected with *H.pylori.*

There was a strong association between *H.pylori* infection and peptic ulcer disease compared to Non ulcer dyspepsia and CA stomach ($P < 0.001$ for gastric ulcer and $p< 0.05$ for duodenal ulcer)

TABLE 5: SEX-WISE DISTRIBUTION OF *H.pylori* POSITIVE CASES:

SEX	NO. OF CASES INCLUDED IN THE STUDY	*H.pylori* +ve (%)
MALE	107	44 (72)
FEMALE	58	17 (28)
TOTAL	**165**	**61**

Out of the 61 total positives, 44 (72%) were male patients and 17(28%) were female cases. This clearly shows that the prevalence of *H.pylori* infection was more common among male patients than female in Coimbatore region.

TABLE 6: AGE-WISE DISTRIBUTION OF *H.pylori* POSITIVE CASES:

AGE GROUP IN YEARS	NO. OF CASES INCLUDED IN THE STUDY	*H.pylori* +ve (%)
11-20	7	5 (8.2)
21-30	15	5 (8.2)
31-40	24	**9 (14.8)**
41-50	37	**14 (22.9)**
51-60	43	**13 (21.3)**
61-70	26	**10 (16.4)**
71-80	13	5 (8.2)
TOTAL	165	61 (100)

Out of the 61 PCR positive *H.pylori* cases, 75.4% were within the age group of 31-70 years. Maximum number of cases (14) was seen within age group of 41-50 years followed by 51-60 year group (13).

TABLE 7: COMPARISON OF RAPID UREASE TESTS AGAINST PCR

RUT	**PCR**		
	POSITIVE	**NEGATIVE**	
POSITIVE	57	10	67
NEGATIVE	4	94	98
TOTAL	61	104	**165**

Sensitivity of RUT = 57/61 X 100= 93.4%

Specificity of RUT = 94/104 X 100= 90.4%

Positive predictive value = 57/67 X 100= 85.1%

Negative predictive value =94/98 X 100= 96%

Accuracy =57+94/165 X 100 = 91.51%

Table 7, shows that among the 61 patients who had the infection (by PCR method), RUT was positive in 57 cases. False negative occurred in 4 cases. Sensitivity and specificity of rapid urease test when evaluated against the Polymerase Chain Reaction, was 93.4% and 90.4% respectively. Positive

predictive value was 85.1% and negative predictive value was 96%. Diagnostic accuracy of RUT was 91.51%.

TABLE 8: COMPARISON OF CULTURE AGAINST PCR

CULTURE	PCR		
	POSITIVE	**NEGATIVE**	
POSITIVE	52	0	52
NEGATIVE	9	104	113
TOTAL	61	104	**165**

Sensitivity of culture = 52/61 X 100= 85.2%

Specificity of culture = 104/104 X 100= 100%

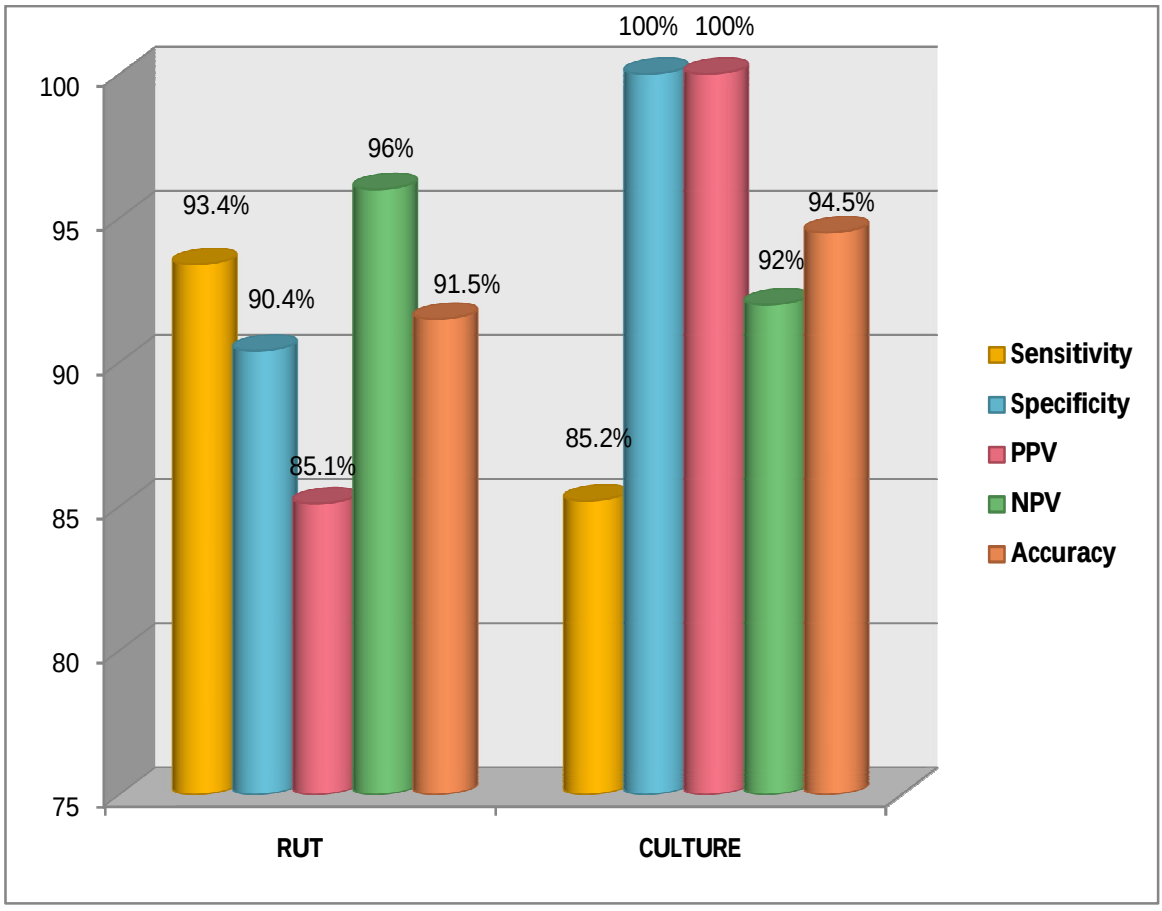
Positive predictive value = 52/52 X 100= 100%

Negative predictive value =104/113 X 100= 92%

Accuracy = 52+104/165 X 100= 94.54%

From the Table 8, sensitivity of culture was 85.2% when evaluated against the gold standard test- Polymerase Chain Reaction. Specificity of culture was 100%, positive predictive value was 100% and negative predictive value was 92% when evaluated against Polymerase Chain Reaction. Diagnostic accuracy of RUT was 94.54%.

FIGURE 15: PERFORMANCE CHARACTERISTICS OF VARIOUS DIAGNOSTIC TESTS



Rapid urease test had better sensitivity of 93.4% compared to culture, but culture had the highest specificity and a PPV of 100%. Culture had the highest positive predictive value while rapid urease test had a better negative predictive value.

TABLE 9: ASSOCIATION OF *cagA* with DISEASE STATUS

ENDOSCOPY FINDING	TOTAL CASES	PCR POSITIVE	*cagA* POSITIVE	P VALUE (Fischer's exact test)
NORMAL	23	5	2	0.642
DISEASED	142	56	33	
TOTAL	**165**	**61**	**35**	

Though percentage of *cagA* positivity was higher in patients with significant endoscopic finding (58.9%) compared to the normal cases (40%), this difference wasn't found to be statistically significant ($p > 0.05$). This may be due to the less number of normal cases in this study.

TABLE 10: PERCENTAGE OF *CagA* POSITIVITY AMONG INFECTED CASES

ENDOSCOPIC FINDINGS	PCR POSITIVE	*CagA* +ve (%)	P VALUE
GASTRITIS	35	16(45.7)	1.0
GASTRODUODENITIS	6	5(83.3)	0.242
GASTRIC ULCER	11	10(90.9)	0.063
DUODENAL ULCER	3	2(66.7)	1.0
OTHERS	1	0(0)	1.0
CARCINOMA STOMACH	0	0	-
NORMAL STUDY	5	2(40)	-
TOTAL	**61**	**35(60.6)**	**0.057**

People with normal findings and patients with carcinoma stomach had no *cagA* positive strains in this study. Percentage of *cagA* positivity among different conditions was 45.7% in gastritis, 83.3% in Gastroduodenitis, 90.9% in gastric ulcer and 66.7% in duodenal ulcer. Statistical analysis by Fisher's exact test revealed that there was no significant association between *cagA* status and any of the endoscopic findings ($p > 0.05$)

TABLE 11: *CagA* AMONG PEPTIC ULCER DISEASE AND NON ULCER DYSPEPSIA

	***cagA* +ve**	***cagA* -ve**	**TOTAL**	**P Value (x^2)**
NUD	21 (50%)	21 (50%)	42	**0.027**
PUD	12 (85%)	2 (14.3%)	14	
TOTAL	**33**	**23**	**56**	

Table 11, shows that *cagA* gene status was distributed equally among patients with non ulcer dyspepsia (Gastritis, Gastroduodenitis, Reflux esophagitis) whereas cag A was positive in 85% of patients with Peptic ulcer disease (Gastric ulcer and Duodenal ulcer). There was a significant association ($p< 0.05$) between *cagA* positivity and peptic ulcer disease. Odds ratio = 6 with 95% confidence interval between 1.19 – 30.1

TABLE 12: CORRELATION BETWEEN ENDOSCOPY FINDINGS AND *vacA* SUBTYPE

ENDOSCOPY FINDINGS	***VacA* s1m1 N (%)**	***VacA* s1m2 N (%)**	***VacA* s2m1 N (%)**	***VacA* s2m2 N (%)**	**P value**
GASTRITIS	12 (34.3)	12 (34.3)	5 (14.3)	6 (17.1)	**0.015**
GASTRODUODENITIS	3 (50)	3 (50)	0(0)	0(0)	**0.012**
GASTRIC ULCER	7(63.6)	4(36.4)	0(0)	0(0)	**0.001**
DUODENAL ULCER	3(100)	0(0)	0(0)	0(0)	**0.018**
OTHERS	0(0)	0(0)	0(0)	1(100)	1.000
CARCINOMA STOMACH	0(0)	0(0)	0(0)	0(0)	-
NORMAL STUDY	0(0)	0(0)	1(20)	4(80)	-
TOTAL	**25 (41%)**	**19 (31%)**	**6 (9.8%)**	**11(18.2%)**	**0.007**

Among the 35 patients who had gastritis s1m1 and s1m2 subtypes predominated with 34.3% each. Gastric ulcer and gastroduodenitis patients shared a similar picture where all the strains were either s1m1 or s1m2 subtypes with no *vacA* s2 subtype at all. All 3 Duodenal ulcer patients had *vacA* s1m1 subtype only. Statistical analysis was done using Pearson Chi-square test and there was a

significant association between the *vacA* subtypes and the above mentioned endoscopy findings ($p < 0.01$)

Table 12 clearly shows that, all 44 *vacA* s1subtype identified were found in patients with pathological findings only where as the s2 subtype was found in both normal as well as diseased patients.

TABLE 13: CORRELATION BETWEEN cagA AND vacA AMONG *H pylori* ISOLATES

vacA / ***cagA***	**s1m1**	**s1m2**	**s2m1**	**s2m2**	**s1**	**s2**	**P value**
cagA+ve	17(68%)	16(84.2%)	1(16.7%)	1(9.1%)	33(75%)	2(11.8%)	**<0.05**
cagA-ve	8(32%)	3(15.8%)	5(83.3%)	10(90.9%)	11(25%)	15(88.2%)	
Total	**25**	**19**	**6**	**11**	**44**	**17**	

About 33 (75%) of the 44 *vacA*s1 isolates were *cagA* positive, while only 2 (11.8%) of the 17 *vacA*s2 isolates were *cagA* positive. Occurrence of *cagA* positivity was 33(75%) out of 44 *vacA*s1 subtype whereas 15 (88.2%) out of 17 s2 subtype were *cagA* negative.

TABLE 14: STATISTICAL SIGNIFICANCE OF *CagA & VacA* GENE AMONG NUD & PUD PATIENTS

Disease / ***cagA &*** ***vacA* status**	**NUD**	**PUD**		**P value**
cagA+ve & vacA s1 +ve	21(70%)	12(85.7%)	33	>0.05
cagA -ve & vacA s1 +ve	9(30%)	2(14.3%)	11	
	30	14		

Among 30 Non ulcer dyspepsia patients with *vacA*s1 subtype, 21 (70%) were *cagA* positive. Among 14 peptic ulcer disease patients with *vacA*s1 subtype, 12 (85.7%) were *cagA* positive. Difference in *cagA* status had no statistically significant association with the disease manifestation in *vacA* s1 positive patients.

TABLE 15: **ANTIBIOTIC SUSCEPTIBILITY OF THE *H pylori* ISOLATES**

ANTIBIOTICS	NO. OF ISOLATES		
	SUSCEPTIBLE	**INTERMEDIATE**	**RESISTANT**
CLARITHROMYCIN	17 (65.38)	7 (27)	2 (7.62)
AMOXICILLIN	20 (76.9)	2 (7.69)	4 (15.38)

Of the 52 isolates stored only 26 were revivable for performing AST for Clarithromycin and Amoxicillin. Of the 26 isolates, 2 (7.62%) were resistant with MIC $\geq$ 1.0 and 17 (65.38%) were sensitive with MIC $\leq$ 0.25 to Clarithromycin. Percentage of amoxicillin resistance and sensitivity were 15.38% (n=4) and 76.9% (n=20) respectively.

DISCUSSION

The present study was aimed at determining the prevalence of *H.pylori* infection in dyspeptic patients, to evaluate the usefulness of Rapid urease test, culture and PCR in diagnosing infection, to identify the *cagA and vacA* gene variants and their association with the virulence state and to identify the prevalence of Clarithromycin and Amoxicillin resistant strains in our population using E test method.

Gastritis is the most common cause of dyspepsia in general population. Apart from *H.pylori* infection there are varieties of causes for dyspepsia like consumption of drugs especially NSAIDs, alcohol intake, stress, food habits, etc. The endoscopic finding of the patients included in this study also showed gastritis to be the most common GI disorder in dyspeptics (61.2%) followed by gastroduodenitis (8.4%) and ulcer (3.6%). Similar distribution of findings was seen in other studies too. [104, 111]

Males (64.85%) suffered more from dyspeptic symptoms compared to females (35.15%). Out of the 61 patients who were found to be infected with *H.pylori* infection by PCR, 72% were male and only 28% were female showing an increased prevalence of infection in male patients. These results concure with the findings of Yoosuf H et, Shanthi et al and Archana et al. [112-114] Several foreign studies have results otherwise. Hence sex preponderance may be an unlikely factor in *H.pylori* infection. Increased number of male patients in this study might be one of the reasons for increased prevalence of infection seen in them.

Though *H.pylori* infection rate was said to be high among children, our study population had adults predominantly. Hence most of the patients who suffered from gastrointestinal disorders and those who had the infection were within the age group of 30- 70 years (75.4%). Study by Shanthi et al also showed similar distribution of patients. [113]

Prevalence is known to vary widely across our country. In the current study, the prevalence of *H.pylori* in gastric mucosa of dyspeptic patients visiting OPD for medical attention was 37% in Coimbatore region. This is in contrast to the popular belief in the Western world, stating the prevalence to be 70-90% among Asian population. [90]. Similar results were seen with the studies by Chattopadhyay et al (50%) from Kolkata, Archana et al (48%) from Bagalkot, Tayloor et al (43%) and Mohanprasad et al (22%) from Coimbatore. [8, 114-116]Higher prevalence of 58-70% was seen in studies from Kurnool, Chennai, Amritsar and Chandigarh. [112,113,117-119]

In this study the prevalence of *Helicobacter pylori* infection was maximum in patients with duodenal ulcer (100%) and gastric ulcer (78.6%), followed by gastroduodenitis (54.5%). *H.pylori* infection had a significant association with peptic ulcer disease rather than non ulcer dyspepsia ($p<0.001$). These findings were in accordance with the studies conducted earlier in India. [112-115]

On contrary to the studies from Eastern Asia [10-12], all the 3 carcinoma stomach patients included in this study did not have *H.pylori* in their gastric mucosa. Only 21.7% of the study group with normal endoscopic finding had *Helicobacter pylori* in their gastric mucosa in contrary to the finding by Singh et al which quoted the carriage to be 56% in asymptomatic persons. [113]

Though culture is considered gold standard for confirming any infection, due to the difficulty in cultivation of *H.pylori* and possibility of growth failure due to technical errors, we have considered using PCR as the standard method in this study. RUT and culture were compared against PCR.

Rapid urease test is cheap and easy to perform in day to day practice. Rapid urease test had a better sensitivity (93.4%) and negative predictive value (96%) than culture, making it an ideal screening procedure. Study by Chomvarin et al and Chattopadhyay et al using a similar preparation of Rapid urease test showed the sensitivity 94.1%, 91% and specificity of 94.2 %, 95.6% respectively. [8, 120]

Study by Refaay et al using conventional buffered urease medium gave sensitivity and specificity of 86.7% and 80% respectively.[104] The sensitivity of various RUT tests as primary diagnostic tests is high and has been reported to vary between approximately 80% and 100% and specificity between 97% and 99% [121-124]. Reason for this wide range was the difference in the formulation of the media used for testing. The formulation used in this study was easy to prepare

and had a fairly good sensitivity and specificity. This preparation can be used for routine testing in our Endoscopy units.

RUT of current study showed 10 (15%) false positives and 4 (6%) false negatives. False positive results were mostly due to *Proteus* species found in the specimen. False negative may be due to the patchy distribution of this bacterium in the gastric mucosa or lesser number of bacteria in the sample. Approximately 10^5 bacteria must be present in the biopsy sample for a positive result [125] and anything that reduces the bacterial density may result in false-negative results [126-128]

Isolation rate of the Modified chocolate agar media with Brucella agar base used in this study was 85%, which is better than the results obtained from other studies. Piccolomini et al had isolation rate of 83% with Modified chocolate agar media with Columbia agar base and Refay et al showed isolation rate of 62.5% with Columbia blood agar with Dent supplement. [104, 129] Out of the 61 *H.pylori* positive cases 52 were isolated on culture. Seven biopsies had heavy contamination with other bacteria (Streptococcus sp, Proteus sp & Pseudomonas sp) and Candida species, which might be the reason for failure of *H.pylori* isolation in these samples. Two culture plates had no growth, which could be due to decreased bacterial density in those samples.

Though culture had 100% specificity and 100% positive predictive value, there were a significant number of false negatives (14.8%). Culturing of *H.pylori*

is a cumbersome procedure requiring special media and microaerophilic atmosphere to grow this organism. It also takes 4-5 days on an average for the growth of the organism and its identification. Hence culture can be reserved for cases were antibiotic susceptibility testing is warranted.

It has been suggested that *H.pylori* induced gastroduodenal diseases varied in severity and manifestations depending on the strain virulence. In this study we used *glmM* gene in gastric biopsy samples for identification of patients with *Helicobacter pylori* infection and positive samples were further analyzed for *cagA* and *vacA* variant status. Of the 165 samples 61 (37%) were positive for *glmM* gene by PCR.

On contrary to the Indian studies from Chennai, Calcutta and South east Asian countries where the *cagA* positivity rate was 96% ,92.3% and 95% respectively, current study showed only 60.6% *cagA* positivity in Coimbatore region. However this is in accordance with the western studies (60-70%). [8, 9, 111, 130]

Though *cagA* positivity was high among gastric ulcer (90.9%) and gastroduodenitis (83.3%) patients statistical analysis by Fisher's exact test found no significance ($p>0.05$) attached to the *cagA* status and disease manifestation when compared to patients with normal endoscopic findings. However there was a statistically significant association between presence of *cagA* gene and peptic ulcer disease rather than the non ulcer dyspepsia ($p<0.05$). Odds ratio was 6 with 95%

CI. Samples from peptic ulcer disease (gastric ulcer & duodenal ulcer) cases showed 85% *cagA* positivity while non ulcer dyspepsia (gastritis, gastroduodenitis, reflux esophagitis) cases had an equal distribution of *cagA* status among them. This is in accordance to many Indian studies which showed the same results. [9]

Prevalence of *vacA*s1 variant (72%) was greater compared to s2 variant (28%). None of the *vacA* s1 strains was found in cases with normal endoscopic findings. Similar results were found in other studies too. On contrary to other studies where s2 strains were never associated with the disease, 12 strains were found in patients with gastritis.

There was a statistically significant correlation between the four *vacA* variants (s1m1, s1m2, s2m1 & s2m2) studied and the disease manifestations ($p<0.01$). All the strains from duodenal ulcer cases were *vacA*s1m1 type whereas gastric ulcer patients had 63.6% and 36.4% of s1m1 and s1m2 respectively. *VacAs1* subtype was found in all patients with peptic ulcer disease. Similar results were found in studies from UK, Western Europe, US, Africa and India. [8, 131- 135]

Though few studies showed that multiple *vacA* strains could colonize the stomach of single patient, expressing themselves in PCR [136, 137], none of the patients included in this study were colonized with multiple strains.

The entire normal study group had *VacA* s2 variant only. None of the ulcer disease and gastroduodenitis patients had s2 variants. This clearly shows that *vacA* s1 is a

significant virulence marker and patients harboring s1 strains are more prone to develop ulcers.

Percentage of *vacA* s1 *cagA* positive strain was 54.1 in Coimbatore region. Studies from Calcutta and Middle East countries showed 68% and 86% prevalence respectively. [8,138]

There was a significant association of *cagA* with s1 strain rather than s2, but when the influence of *vacA* s1 *cagA* positive on the diseases manifestations was analyzed, there was no significant difference between PUD and NUD.

Non ulcer dyspepsia patients showed a higher prevalence of *vacA* s1 compared to s2 and this association was significant. *VacA* m subtype was equally distributed among s1 variants and did not have any significance ($p > 0.05$). This was in contrary to the finding from Chennai based study were m2 was found more associated with PUD.[9]

These findings show that the *vacA* s1 *cagA*-positive genotype has an important role in the clinical outcomes. Hence this genotype can be used to identify patients who are at a higher risk for gastroduodenal disease. Patients infected with the strain carrying *vacA* s1 *cagA*-positive genotype should be given more attention. They must be checked for eradication status on completion of antibiotic treatment, to prevent the development of PUD in future.

In this study the resistance to Clarithromycin and Amoxicillin was 7.62% and 15.38% respectively. These figures correlate well with the global resistance rates

as well as resistance in most of the Western countries like Spain, Italy, most of the American states and Columbia.[139- 145] Similar prevalence of Clarithromycin resistance (4.7%) has been observed in Gujarat, India.[146] Lower resistance seen in our setup may be due to decreased use of Macrolide drugs as empirical therapy in common infections and usage of Amoxicillin- Metronidazole combination as first line therapy for *H.pylori* infection. Clarithromycin resistance was assessed to be high ranging from 40-80% in other parts of India. But the overall amoxicillin resistance rate in India (32.8%) is closer to the pattern seen in our study population. [15]

SUMMARY

- Virulence of *H pylori* is variable with different strains and the infections produced by them are an upcoming burden due to the emergence of drug resistant strains. Hence this study was conducted to add on knowledge about the prevalence of various strains and their association with disease as well as drug resistance pattern in this region.
- The study was conducted in the Department of Microbiology, PSGIMS&R.
- Gastric biopsy samples were collected from 165 patients with gastro duodenal diseases undergoing Upper GI Endoscopy in Gastroenterology department in PSG Hospitals, Coimbatore after obtaining a written informed consent.
- Three methods of identification of *H.pylori* infection namely Rapid urease test, Culture and Polymerase Chain Reaction were compared.
- *H.pylori* isolates obtained from culture were tested for Clarithromycin and Amoxicillin susceptibility by E-test method.
- Biopsy samples found positive by PCR for *glmM* gene were further characterized for *cagA* and *VacA* gene variants. Their correlation with disease pattern was analyzed.
- Prevalence of *H.pylori* infection among the dyspeptic patients investigated in our hospital was 37% by PCR method.
- Most of the patients who were infected fall within the age group of 30- 70 years (75.4%)

- Male population, not only suffered from dyspeptic symptoms, but also was found to be infected with *H.pylori* (72%) more often than female (28%), ratio being 3:1.
- *H.pylori* infection had a significant association with peptic ulcer disease rather than non ulcer dyspepsia ($p<0.001$)
- Rapid urease test had better sensitivity of 93.4% compared to culture, but culture had the highest specificity. Culture had the highest positive predictive value (100%) while rapid urease test had a better negative predictive value.
- Rapid urease test is cheap and easy to perform in day to day practice, making it an ideal screening procedure.
- Culture required transport of biopsy sample in Brucella broth and plating in Chocolated Brucella agar with added antibiotic and incubation in microaerophilic atmosphere, in Anoxomat system. Culture, owing to its need for skilled techniques, special media & incubation conditions and time consumption, should be reserved for patients requiring antibiotic susceptibility testing.
- Though PCR is an expensive method compared to RUT, it has the advantage of providing data on the virulence nature of the bacteria in individual patients, making it more informative on decision making grounds like need for treatment, need for follow up, etc.

- On analysis of virulence genes, *cagA* positivity was 60.6% in our study. There was a statistically significant association between presence of *cagA* gene and peptic ulcer disease rather than the non ulcer dyspepsia ($p<0.05$, odds ratio was 6 with 95% CI).
- Prevalence of *VacA* s1 subtype was 72% and it was distributed only among the patients with pathological findings related to *H.pylori* infection. On the contrary 28% of the s2 strains were more often associated with non specific gastritis and normal endoscopic finding. There was a statistically significant correlation between the *vacA*s1 variant and the disease manifestations ($p<0.01$)
- *VacA*m1/2 variants showed no association with disease manifestation ($p>0.05$)
- None of the patients included in this study were colonized with multiple strain.
- Most of the *VacA* s1 *cagA* positive strains were associated with PUD and gastroduodenitis. This could be used as a virulence marker indicating that the patient harboring these strains need a rigorous eradication therapy and follow up to avoid severe manifestation of *H.pylori* infection.
- In this study the resistance to Clarithromycin and Amoxicillin was 7.62% and 15.38% respectively. These two drugs maybe continued for use as first line therapy in our population

CONCLUSION

With the 37% prevalence of *H pylori* in this region among patients with gastro duodenal diseases, the currently used invasive procedures to detect *H.pylori* infection, the in-house rapid urease test was found to be cheap, easy to perform, quick and a point of care test with good sensitivity. Though cultivation and storage of these fragile organisms was a difficult task, almost 50% of the growths were subjected to AST. The results showed lower prevalence of resistance to Clarithromycin and Amoxicillin 7.62% and 15.38% respectively. Genetic analysis of *VacA* & *CagA* virulent genes and their subtypes turned out to be fruitful, emphasizing the need for their routine testing to identify the severity and administer antibiotics appropriately for eradication.

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APPENDIX

1) GRAM STAIN:

Crystal violet:

Crystal violet	1.0gm
5% sodium bicarbonate	1.0ml
Distilled water	99ml

Grams iodine:

Iodine crystal	2.0gm
Sodium hydroxide	10.0ml
Distilled water	90.0ml

Acetone- 100%

DILUTE CARBOL FUCHSIN- 1 in 10 dilution of strong carbol fuschin.

2) BRUCELLA BROTH:

Brucella broth base (BD)	2.8gm
Distilled water	100ml

Warmed slightly to dissolve the contents and adjusted to pH 7. Dispensed as 3ml each, in screw capped tube and autoclaved at 121^{o}C for 15 minutes at 15 lbs.

2) BRUCELLA CHOCOLATE AGAR:

Brucella agar base with added Hemin and vitamin K(HIMEDIA)	4.31gm
Distilled water	100 ml

The above mixuter is autoclaved at 121^{o}C for 15 minutes at 15 lbs and the cooled to 80^{0}C. Add 7 ml of sterile defibrinated sheep blood and hold at 80^{0}C for 20 minutes in waterbath. Cool to 45-50^{0}C and pour 20ml of this chocolate agar in the petridish to set.

3) BRUCELLA CHOCOLATE AGAR:

Agar preparation is same as the above procedure. Just before the pouring of media, add the following pre-dissolved antibiotics and mix well.

Vancomycin	1mg/100ml
Polymyxin B	250 IU/100ml
Amphotericin B	0.5 mg/100ml.

4) CHRISTENSEN'S UREASE AGAR:

Christensen's urease agar base (HIMEDIA)	2.4gm
Distilled water	100ml
pH	6.8

The above ingredients are mixed by boiling and sterilized by autoclaving at 121^{0} C for 15 minutes.

Urea 40gm

Sterile distilled water 100ml

The above ingredients are mixed and sterilized by membrane filtration.

Mix 5ml of the urea solution in 100ml of autoclaved Christensen's urease agar base when it cools to 50^{o}C. Dispensed in tubes and allowed to cool in a slanted position.

5) CATALASE REAGENT:

Superoxal 10ml

Distilled water 90ml

Store it in dark coloured bottle at 4^{o}C

6) OXIDASE PAPER:

Tetramethyl para-phenylene diamine dihydrochloride 1gm

Distilled water 100ml

Mix well and soak the Whatmann no. 1 filter paper strips in it. Dry the strips at room temperature in dark. Store it in dark coloured bottles.

ANNEXURES

PSG Institute of Medical Sciences & Research
Institutional Human Ethics Committee

Recognized by The Strategic Initiative for Developing Capacity in Ethical Review (SIDCER)
POST BOX NO. 1674, PEELAMEDU, COIMBATORE 641 004, TAMIL NADU, INDIA
Phone : 91 422 - 2598822, 2570170, Fax : 91 422 - 2594400, Email : ihec@psgimsr.ac.in

June 12, 2014

To
Dr J Lavanya
Postgraduate
Department of Microbiology
PSG IMS & R
Coimbatore

The Institutional Human Ethics Committee, PSG IMS & R, Coimbatore -4, has reviewed your proposal on 9th May, 2014 in its expedited review meeting held at IHEC Secretariat, PSG IMS&R, between 10.00 am and 11.00 am, and discussed your study proposal entitled:

"Isolation and characterization of Helicobacter pylori in gastroduodenal disease at a tertiary care hospital"

The following documents were received for review:

1. Duly filled application form
2. Proposal
3. Informed consent forms
4. Data collection tool
5. CV
6. Budget

After due consideration, the Committee has decided to approve the study.

The members who attended the meeting at which your study proposal was discussed are as follows:

Name	Qualification	Responsibility in IHEC	Gender	Affiliation to the Institution Yes/No	Present at the meeting Yes/No
Dr P Sathyan	DO, DNB	Clinician, Chairperson	Male	No	Yes
Dr S Bhuvaneshwari	M.D	Clinical Pharmacologist Member - Secretary	Female	Yes	Yes
Dr Sudha Ramalingam	M.D	Epidemiologist Alt. Member - Secretary	Female	Yes	Yes
Dr Y S Sivan	Ph D	Member –Social Scientist	Male	Yes	Yes

The approval is valid for one year.

We request you to intimate the date of initiation of the study to IHEC, PSG IMS&R and also, after completion of the project, please submit completion report to IHEC.

PSG Institute of Medical Sciences & Research
Institutional Human Ethics Committee

Recognized by The Strategic Initiative for Developing Capacity in Ethical Review (SIDCER)
POST BOX NO. 1674, PEELAMEDU, COIMBATORE 641 004, TAMIL NADU, INDIA
Phone : 91 422 - 2598822, 2570170, Fax : 91 422 - 2594400, Email : ihec@psgimsr.ac.in

This Ethics Committee is organized and operates according to Good Clinical Practice and Schedule Y requirements.

Non-adherence to the Standard Operating Procedures (SOP) of the Institutional Human Ethics Committee (IHEC) and national and international ethical guidelines shall result in withdrawal of approval (suspension or termination of the study). SOP will be revised from time to time and revisions are applicable prospectively to ongoing studies approved prior to such revisions.

Kindly note this approval is subject to ratification in the forthcoming full board review meeting of the IHEC.

Yours truly,

12.6.14

Dr S Bhuvaneshwari
Member - Secretary
Institutional Human Ethics Committee

SECRETARY
PSG IMS&R
COIMBATORE-641004.
INSTITUTIONAL HUMAN ETHICS COMMITTEE

PSG Institute of Medical Science and Research, Coimbatore
Institutional Human Ethics Committee
INFORMED CONSENT FORMAT FOR RESEARCH PROJECTS

(strike off items that are not applicable)

I **Dr J Lavanya** am carrying out a study on the topic: ***Isolation and characterization of Helicobacter pylori in Gastroduodenal disease at a tertiary care hospital'***

as part of our research project being carried out under the aegis of the Department of microbiology

(*Applicable to students only*): My / our research guide is: **Dr J Jayalakshmi**

The justification for this study is: Helicobacter pylori is a rising burden in developing countries due to increased prevalence and upcoming drug resistance.

The objectives of this study are:
PRIMARY OBJECTIVE:

1. Study the prevalence of virulence factors of *H pylori* and their association with disease pattern among patients in a tertiary care hospital.

SECONDARY OBJECTIVES:

1. To isolate and characterize *H pylori* from biopsy samples of patients .
2. To compare various detection methods such as Rapid Urease test , Staining, and PCR against culture *"the gold standard method*
3. To Identify the prevalence of drug resistant strains in our population
4. To identify the CAG and VAC genes and their association with the virulence state

Sample size: **165**

Study volunteers / participants are (specify population group & age group**): Patients undergoing Upper GI Endoscopy in Gastroenterology department.**

Location: **___PSG Hospital____.**

We request you to kindly cooperate with us in this study. We propose collect background information and other relevant details related to this study. We will be carrying out:

Data collected will be stored for a period of **__5___** years. We will not use the data as part of another study.

Clinical examination (Specify details and purpose**): NA**

Biopsy sample collection:
Number of biopsy samples collected: **3 bits for this study apart from routine**

No. of times it will be collected: **All 5 Gastric biopsy samples will be collected in a single Endoscopy**.

Whether biopsy sample collection is part of routine procedure or for research (study) purpose:

1. Routine procedure - **1-3 samples**

2. Research purpose - **3 samples**

Specify **purpose**: **For Bacterial culture and PCR**.

Whether sample collected will be stored after study period: **No, it will be destroyed**

Whether sample collected will be sold**: No**

Whether sample collected will be shared with persons from another institution: **No**

Benefits from this study: **Early and effective management of patients with H.pylori infection.**

Risks involved by participating in this study: **Endoscopy procedure related risks only. No specific risk concerned to this study**

How the **results** will be used: **For Dissertation purpose**

If you are uncomfortable in answering any of our questions during the course of the interview / biological sample collection, **you have the right to withdraw from the interview / study at anytime.** You have the freedom to withdraw from the study at any point of time. Kindly be assured that your refusal to participate or withdrawal at any stage, if you so decide, will not result in any form of compromise or discrimination in the services offered nor would it attract any penalty. You will continue to have access to the regular services offered to a patient. You will **NOT** be paid any remuneration for the time you spend with us for this interview / study. The information provided by you will be kept in strict confidence. Under no circumstances shall we reveal the identity of the respondent or their families to anyone. The information that we collect shall be used for approved research purposes only. You will be informed about any significant new findings - including adverse events, if any, – whether directly related to you or to other participants of this study, developed during the course of this research which may relate to your willingness to continue participation.

Consent: The above information regarding the study, has been read by me/ read to me, and has been explained to me by the investigator/s. Having understood the same, I hereby give my consent to collect gastric biopsy samples from me. I am affixing my signature / left thumb impression to indicate my consent and willingness to participate in this study (i.e., willingly abide by the project requirements).

Signature / Left thumb impression of the Study Volunteer / Legal Representative:

Signature of the Interviewer with date: Witness:

Contact number of PI: **8754564994**

Contact number of Ethics Committee Office**: 0422 2570170 Extn.: 5818**

ஒப்பதல் படிவம்

தேதி

மரு.ஜெ.லாவண்யா ஆகிய நான் பி.எஸ்.ஜி மருத்துவக்கல்லூரியின் நுண்ணுயிரியல் துறையின் கீழ் "இரைப்பை மற்றும் குடல் சம்பந்தமான நோய் உள்ளோரில் ஹெச். பைலோரி என்னும் கிருமியை கண்டறிதல் மற்றும் இயல்பாராய்தல் என்ற தலைப்பில் ஆய்வு மேற்கொள்ள உள்ளேன்

என் ஆய்வு வழிகாட்டி : மரு. ஜெ.ஜெயலட்சுமி

ஆய்வு மேற்கொள்வதற்கான அடிப்படை :

இந்தியா போன்ற வளரும் நாடுகளில் ஹெச். பைலோரி பரவலாக உள்ளது. மற்றும் இக்கிருமி மருந்து எதிர்ப்பு தன்மை கொண்டது. இப்பகுதி மக்களில் இக்கிருமியின் பாதிப்புத்தன்மை இதுவரை கண்டறியப்படாததால் இவ்வாய்வு மேற்கொள்கிறோம்.

ஆய்வின் பிரதான நோக்கம் :

இரைப்பை திசுமாதிரிகளில் உள்ள ஹெச். பைலோரி கிருமியின் இயல்பாராய்தல் நச்சுக் காரணிகள் மற்றும் நோய் உண்டாக்கும் தன்மையை கண்டறிதல்.

நோக்கங்கள் :

1. இரைப்பையில் உள்ள இக்கிருமியை பகுத்து ஆராய்தல்
2. இக்கிருமியை கண்டறியும் பல்வேறு முறைகளை ஒப்பிடுதல்.
3. மருந்து எதிர்ப்பு தன்மையின் சதவிகிதம் அறிதல்.
4. இக்கிருமிகளின் நச்சுக் காரணிகளான சிஏஜி மற்றும் விஏசி என்ற மரபணுக்களை கண்டறிதல்

ஆய்வில் பங்கு பெறும் நபர்களின் எண்ணிக்கை : 165

ஆய்வு மேற்கொள்ளும் இடம் : **பி.எஸ்.ஜி மருத்துவமனை, நுண்ணுயிரியல் துறை**

ஆய்வின் பலன்கள் :

குறைந்த காலத்தில் கிருமியை கண்டறிவதன் மூலம் தகுந்த சிகிச்சைகளை தக்க காலத்தில் பெறும் லாபங்கள்.

ஆய்வினால் ஏற்படும் அசௌகரியங்கள் / பக்க விளைவுகள்

என்டோஸ்கோபி சம்பந்தமான அசௌகரியங்கள் மட்டுமே இவ்வாய்வினால் தனிப்பட்ட பக்க விளைவுகள் ஏதுமில்லை.

இந்த ஆய்வில் கிடைக்கும் தகவல்கள் 5 வருடங்கள் பாதுகாக்கப்படும். இவை வேறு எந்த ஆய்விற்கும் பயன்படுத்தப்படமாட்டாது. எந்த நிலையிலும் உங்களைப் பற்றிய தகவல்கள் யாருக்கும் தெரிவிக்கப்படமாட்டாது. அவை இரகசியமாக வைக்கப்படும்.

இந்த ஆய்வில் பங்கேற்க ஒப்புக் கொள்ளுவதால் எந்தவிதமான பலனும் உங்களுக்கு கிடைக்காது. எந்த நேரத்தில் வேண்டுமானாலும் ஆய்விலிருந்து விலகிக் கொள்ளும் உரிமை உங்களுக்கு உண்டு.

இந்த ஆராய்ச்சிக்காக உங்களிடம் சில இரைப்பை திசு மாதிரிகள் என்டோஸ்கோபி எடுக்கப்படும்.

- வழக்கமான பரிசோதனைக்காக எடுக்கப்படும் திசு மாதிரிகளின் எண்ணிக்கை–3
- இவ்வாய்விற்காக எடுக்கப்படும் திசு மாதிரிகளின் எண்ணிக்கை – 2
- இவ்வாய்விற்காக பயன்படுத்தப்படும் திசுமாதிரிகள் அவைகளில் உள்ள ஹெச். பைலோரி கிருமியை செயற்கைச் சூழலில் வளர்க்கவும் பிசிஆர் செய்முறை வழியாக கிருமியின் மரபணுவை ஆராயவும் பயன்படுத்தப்படும்.
- உங்கள் திசு மாதிரிகள் சேமிக்கப்படாது. வேறு ஆய்வுகளுக்கு பயன்படுத்தப்படாது.
- ஆய்வின் முடிவுகள் ஆய்வறிக்கைக்கு மட்டுமே பயன்படுத்தப்படும்.

மேலும் இந்த ஆய்வில் பங்கு கொள்வது உங்கள் சொந்த விருப்பம். இதில் எந்த விதக் கட்டாயமும் இல்லை. நீங்கள் விருப்பப்பட்டால் இந்த ஆய்வின் முடிவுகள் உங்களுக்குத் தெரியப் படுத்தப்படும்.

ஆய்வாளரின் கையொப்பம் :

தேதி :

ஆய்வுக்குட்படுபவரின் ஒப்புதல்

நான் இந்த ஆராய்ச்சியின் நோக்கம் மற்றும் அதன் பயன் பாட்டினைப் பற்றி தெளிவாகவும் விளக்கமாகவும் தெரியப்படுத்தப்பட்டுள்ளேன். இந்த ஆராய்ச்சியில் பங்கு கொள்ளவும் இந்த ஆராய்ச்சியின் மருத்துவ ரீதியான குறிப்புகளை வரும் காலத்திலும் உபயோகப்படுத்திக் கொள்ளவும் முழு மனதுடன் சம்மதிக்கிறேன்.

ஆய்வுக்குட்படுபவரின் பெயர், முகவரி :

கையொப்பம் :

தேதி :

ஆய்வாளரின் தொலைபேசி எண் :

மனித நெறிமுறைக் குழு அலுவலகத்தின் தொலைபேசி எண் 0422-2570170 Extn. 5818

S.No	AGE	SEX	ENDOSCOPIC FINDING	RUT	CULTURE	PCR FOR *glmM* GENE	*CagA* STATUS	*VacA* SUBTYPE
1	72	M	Gastritis	-	-	-		
2	53	M	Gastritis	+	+	+	-	s1m1
3	32	F	Gastritis	+	-	-		
4	37	F	Normal study	-	-	-		
5	36	M	Gastritis	-	-	-		
6	55	F	Normal study	-	-	-		
7	29	F	Gastritis	+	-	-		
8	30	M	Gastroduodenitis	+	+	+	+	s1m1
9	62	M	Gastritis	-	-	-		
10	38	M	Grade A reflux esophagitis	-	-	-		
11	46	F	Tropical sprue	-	-	-		
12	37	M	Gastritis	-	-	-		
13	27	F	Gastritis	-	+	+	-	s2m2
14	67	M	Gastritis	+	+	+	+	s1m2
15	40	M	Gastritis	-	-	-		
16	47	F	Gastritis	-	-	-		
17	60	F	Gastroduodenitis	-	-	-		
18	70	M	Duodenal Ulcer	+	+	+	+	s1m1
19	37	M	Gastritis	-	-	-		
20	21	M	Gastritis	-	-	-		
21	29	M	Gastritis	-	-	-		
22	59	M	Gastritis	-	-	-		
23	50	F	Gastritis	+	+	+	+	s1m2
24	34	M	Gastritis	-	-	-		
25	75	M	Gastritis	-	-	-		
26	33	M	Normal study	+	-	-		
27	80	M	CA Stomach	+	-	-		
28	27	M	Gastritis	+	+	+	-	s1m1
29	36	M	Gastritis	-	-	-		
30	19	F	Gastritis	+	+	+	-	s2m1
31	66	M	Gastritis	-	-	-		
32	40	M	Gastritis	+	+	+	+	s1m2
33	43	F	Gastritis	-	-	-		
34	31	M	Normal study	-	-	-		
35	57	F	Gastritis	-	-	-		
36	53	F	Gastritis	-	-	-		
37	50	M	Gastritis	-	+	+	+	s1m1
38	50	M	Duodenal Ulcer	+	+	+	-	s1m1
39	56	F	Gastritis	+	+	+	-	s2m2
40	15	M	Gastroduodenitis	+	+	+	-	s1m1
41	20	M	Reflux esophagitis	+	-	+	-	s2m2
42	18	M	Normal study	-	-	-		
43	36	F	Gastritis	-	-	-		
44	54	M	Gastritis	-	-	-		

45	37	M	Duodenal Ulcer	+	+	+	+	s1m1
46	57	M	Gastritis	-	-	-		
47	75	M	Normal study	-	-	-		
48	30	F	Normal study	-	-	-		
49	67	M	Gastritis	+	+	+	+	s1m1
50	70	F	Gastritis	-	-	-		
51	55	M	Gastritis	-	-	-		
52	75	M	Gastritis	+	+	+	-	s2m1
53	35	M	Gastric polyp	-	-	-		
54	60	M	Gastritis	+	-	+	-	s2m2
55	37	F	Gastritis	-	-	-		
56	31	F	Normal study	-	-	-		
57	52	M	Normal study	-	-	-		
58	50	F	Gastritis	+	-	+	-	s2m2
59	59	M	Gastritis	-	-	-		
60	30	M	Gastritis	-	-	-		
61	73	M	Gastritis	-	+	+	+	s1m1
62	35	F	Normal study	+	-	+	-	s2m2
63	63	M	Gastritis	-	-	-		
64	40	M	Gastroduodenitis	+	+	+	+	s1m1
65	43	M	Gastritis	+	+	+	+	s1m2
66	66	M	Gastroduodenitis	-	-	-		
67	41	F	Gastroduodenitis	+	+	+	+	s1m2
68	53	F	Gastritis	+	-	-		
69	52	M	Gastritis	+	+	+	-	s2m2
70	32	F	Gastric Ulcer	+	+	+	+	s1m1
71	61	M	Gastroduodenitis	+	+	+	+	s1m2
72	46	F	Gastritis	-	-	-		
73	20	M	Gastritis	-	-	-		
74	52	F	Gastritis	-	-	-		
75	60	F	Gastric Ulcer	+	+	+	+	s1m1
76	23	M	Gastroduodenitis	-	-	-		
77	30	F	Normal study	-	-	-		
78	79	M	Gastritis	+	-	+	-	s1m1
79	48	M	Gastritis	-	-	-		
80	65	M	Gastroduodenitis	+	+	+	+	s1m2
81	56	M	Gastritis	-	-	-		
82	47	M	Gastritis	-	-	+	+	s1m1
83	90	M	Esophageal diverticula	-	-	-		
84	58	M	Gastritis	+	+	+	-	s2m1
85	70	M	Duodenal polyp	-	-	-		
86	57	F	Gastritis	+	-	-		
87	55	F	Gastritis	-	-	-		
88	44	F	Normal study	-	-	-		
89	59	M	Gastritis	-	-	-		
90	60	M	Gastritis	+	+	+	-	s2m1
91	42	M	Gastritis	-	-	-		
92	59	M	Gastritis	+	+	+	-	s2m2

93	34	M	Gastritis	+	-	-		
94	54	M	Gastritis	+	-	-		
95	53	M	Gastroduodenitis	-	-	-		
96	51	M	Gastritis	+	+	+	-	s2m1
97	47	M	Duodenal Ulcer	+	+	+	+	s1m1
98	38	F	Gastritis	-	-	-		
99	60	F	Gastritis	-	-	-		
100	36	F	Gastritis	+	+	+	+	s1m2
101	49	M	Normal study	-	-	-		
102	23	F	Normal study	+	+	+	+	s2m2
103	57	F	Gastritis	-	-	-		
104	47	M	Gastritis	-	-	-		
105	50	F	Gastric Ulcer	+	+	+	+	s1m2
106	49	M	Gastritis	+	+	+	+	s1m1
107	62	M	Barret esophagus	-	-	-		
108	34	M	Gastric Ulcer	+	+	+	+	s1m2
109	39	F	Gastritis	-	-	-		
110	73	M	Gastritis	-	-	-		
111	59	M	Normal study	-	-	-		
112	70	M	Gastritis	-	-	-		
113	66	M	Gastritis	-	-	-		
114	54	M	Normal study	-	-	-		
115	40	F	Gastritis	-	-	-		
116	62	M	Duodenal Ulcer	+	+	+	+	s1m2
117	63	F	Gastritis	-	-	-		
118	27	F	Gastritis	-	-	-		
119	54	M	Gastritis	-	-	-		
120	35	M	Gastritis	-	-	-		
121	36	F	Gastritis	-	-	-		
122	45	M	Gastritis	+	+	+	+	s1m2
123	60	F	Normal study	-	-	-		
124	37	F	Gastritis	-	-	-		
125	44	M	Gastritis	-	-	-		
126	54	M	Normal study	-	-	-		
127	56	M	Gastric Ulcer	+	+	+	+	s1m1
128	54	F	Gastritis	+	+	+	-	s1m1
129	55	M	Gastritis	-	-	-		
130	48	F	Gastritis	+	+	+	+	s1m2
131	67	M	Gastritis	-	-	-		
132	39	M	Normal study	+	+	+	+	s2m1
133	48	F	Gastritis	-	-	-		
134	15	M	Gastritis	+	+	+	-	s1m1
135	46	M	Gastritis	-	-	-		
136	70	F	Gastric Ulcer	+	-	+	+	s1m2
137	52	F	Gastritis	-	-	-		
138	31	M	Normal study	-	-	-		
139	78	F	Gastritis	+	+	+	-	s1m2
140	77	M	Gastritis	-	-	-		
141	33	F	Gastritis	-	-	-		

142	56	M	Gastric Ulcer	+	-	-		
143	37	F	Gastritis	+	+	+	+	s1m2
144	49	M	Gastric Ulcer	+	+	+	+	s1m1
145	64	M	Gastritis	+	-	+	+	s1m1
146	64	M	Gastritis	+	-	-		
147	20	M	Gastritis	+	-	+	-	s1m2
148	23	M	Gastritis	-	-	-		
149	21	M	Normal study	+	+	+	-	s2m2
150	71	M	CA Stomach	-	-	-		
151	45	F	Gastric Ulcer	-	-	-		
152	77	F	Gastric polyp	-	-	-		
153	57	M	Gastritis	+	+	+	-	s1m2
154	61	F	Gastritis	-	-	-		
155	62	M	Gastric Ulcer	+	+	+	-	s1m1
156	22	M	Normal study	-	-	-		
157	70	M	Gastric Ulcer	-	-	-		
158	53	M	Gastric polyp	-	-	-		
159	70	F	Gastritis	-	-	-		
160	65	F	Gastritis	+	+	+	+	s1m1
161	65	M	Gastritis	+	+	+	+	s1m2
162	44	M	Normal study	+	+	+	-	s2m2
163	60	M	CA Stomach	-	-	-		
164	36	F	Fundal polyp	-	-	-		
165	60	M	Gastric Ulcer	+	+	+	+	s1m1

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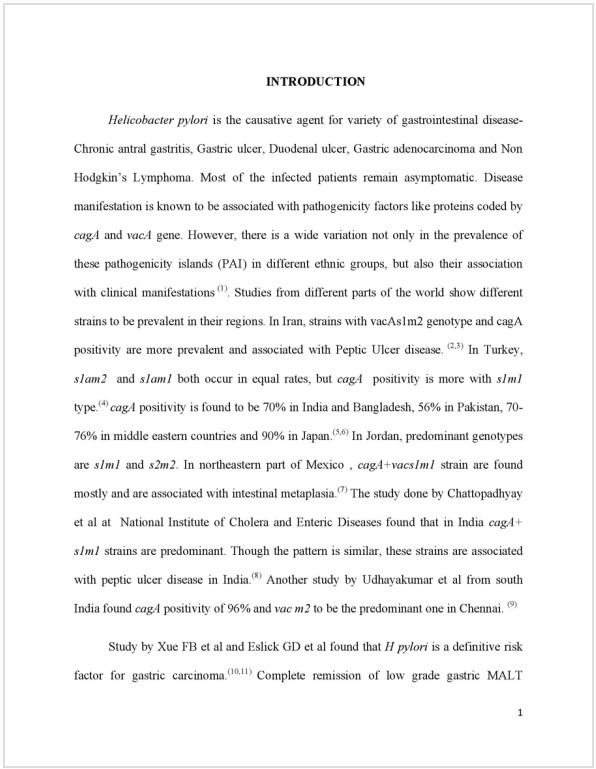

INTRODUCTION

Helicobacter pylori is the causative agent for variety of gastrointestinal disease- Chronic antral gastritis, Gastric ulcer, Duodenal ulcer, Gastric adenocarcinoma and Non Hodgkin's Lymphoma. Most of the infected patients remain asymptomatic. Disease manifestation is known to be associated with pathogenicity factors like proteins coded by *cagA* and *vacA* gene. However, there is a wide variation not only in the prevalence of these pathogenicity islands (PAI) in different ethnic groups, but also their association with clinical manifestations [1]. Studies from different parts of the world show different strains to be prevalent in their regions. In Iran, strains with vacAs1m2 genotype and cagA positivity are more prevalent and associated with Peptic Ulcer disease. [2,3] In Turkey, *s1am2* and *s1am1* both occur in equal rates, but *cagA* positivity is more with *s1m1* type.[4] *cagA* positivity is found to be 70% in India and Bangladesh, 56% in Pakistan, 70-76% in middle eastern countries and 90% in Japan.[5,6] In Jordan, predominant genotypes are *s1m1* and *s2m2*. In northeastern part of Mexico , *cagA+vacs1m1* strain are found mostly and are associated with intestinal metaplasia.[7] The study done by Chattopadhyay et al at National Institute of Cholera and Enteric Diseases found that in India *cagA+ s1m1* strains are predominant. Though the pattern is similar, these strains are associated with peptic ulcer disease in India.[8] Another study by Udhayakumar et al from south India found *cagA* positivity of 96% and *vac m2* to be the predominant one in Chennai. [9]

Study by Xue FB et al and Eslick GD et al found that *H pylori* is a definitive risk factor for gastric carcinoma.[10,11] Complete remission of low grade gastric MALT

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