Helicobacter pylori PREVALENCE AND CULTURE RATE AMONG PATIENTS WITH DYSPEPSIA AT MOI TEACHING AND REFERRAL HOSPITAL, ELDORET, KENYA.

Raymond Churyai

SM/PGM/02/12

A thesis submitted in partial fulfilment of the requirements for the award of the degree of Masters of Medicine in Internal Medicine, Moi University.

Declaration

Student Declaration

This thesis is my original work and has not been presented for a degree in any other University. No part of this thesis may be reproduced without prior permission of the author and/or Moi University.

Dr. Raymond Churyai, MBChB Sign……

SM/PGM/02/12 Date ……

Supervisors' Certification

This thesis has been submitted with our approval as the University supervisors.

Prof. Paul Ayuo, MBChB, M.Med, DLSHTM, MSc Sign.....

Professor, Department of Medicine, Date.....

School of Medicine, Moi University.

Dr. Fatuma Faraj-Some, MBChB, M.Med, MBA Sign.....

Senior Lecturer, Department of Medicine, Date……..

School of Medicine, Moi University

Dedication

I dedicate this thesis to my family and friends whose presence and encouragements during my graduate studies have been valuable.

A special dedication to my loving parents, Augustine and Marsella Sambu whose sacrifices enabled me and my siblings to attend school. I also dedicate it to my brothers and sisters; Odiliah, Norbert, Edina, Appolnary, Norah, Imelda, Immaculate and Beatrice.

I also dedicate this work and give special thanks to my dear wife Cynthia, my daughters Laura and Natalia for their encouragement and being there for me throughout the entire duration of my postgraduate programme.

Helicobacter pylori prevalence and culture rate among patients with dyspepsia at Moi Teaching and Referral Hospital, Eldoret, Kenya.

Abstract

Background: *Helicobacter pylori* (*H. pylori*) infection is associated with upper gastrointestinal diseases including peptic ulcer disease, gastritis, gastric adenocarcinoma and mucosal associated lymphoid tissue lymphoma. *H. pylori* is a fastidious organism and thus difficult to culture especially after prolonged time between sample extraction and start of the culture.

Objective: To determine the *H. pylori* prevalence and culture rate following 20 to 24 hour transportation in normal saline at Moi Teaching and Referral Hospital.

Methods: This was a cross-sectional descriptive study. Participants 18 years and above referred for endoscopy due to dyspepsia were consecutively enrolled until the desired sample size was achieved. Participants underwent endoscopy during which biopsies were taken, two each from the gastric antrum and corpus. Rapid urease test (RUT) for *H. pylori* was done on one sample each from the antrum and corpus. For the samples that tested positive, their pair samples were put in normal saline and packed in ice in a cooler box and sent for *H. pylori* culture within 20 to 24 hours on brain heart infusion agar and subsequent antibiotic susceptibility testing. Data was analyzed using STATA Version 13 SE.

Results: Between April 2014 and February 2015, 634 patients were screened of which 156 were enrolled to the study and subsequently underwent endoscopy, gastric biopsy and RUT. The enrolled participants had a median age of 41 (IQR: 28-58) years; and comprised of 64 (41%) males. The main indication for endoscopy was epigastric pains, seen in 151 (97%) of patients. Eleven (7%) participants had previously been investigated for upper gastrointestinal disease by barium meal 4 (2.7%), endoscopy 2 (1.3%) and serological *H. pylori* testing 5(3.2%). Forty two (27%) of participants had previously received treatment for dyspepsia with either a proton pump inhibitor, histamine receptor type 2 blocker or anti *H. pylori* antibiotics. At endoscopy, frequent abnormal findings included gastritis 123 (79%), oesophagitis and duodenitis with 30(19%) each. These endoscopic findings were not mutually exclusive. Eighty three (53%) had a positive RUT. Culture was done on 69 samples that reached the laboratory within 24 hours. *H. pylori* was isolated in 9 (13%) samples. All the 9 strains of *H. pylori* isolated were resistant to metronidazole.

Conclusion: The prevalence of *H. pylori* by RUT was more than 50% of patients. The culture rate of *H. pylori* following 20 to 24 hour delay was low. All the *H. pylori strains* isolated were resistant to metronidazole.

Recommendations: Patients with dyspepsia should be tested for *H. pylori* and treated as per standard guidelines. Culture of *H. pylori* after 20-24 hour transportation in normal saline is not useful. A comparative study to determine the optimal transportation time and transport media is recommended. A larger study to validate utility of metronidazole in the treatment of *H. pylori* at MTRH is recommended.

Table of Contents

List of tables

List of figures

Acknowledgements

I would like to thank my supervisors, Dr. F. Some and Prof. P. Ayuo for their continued support in the preparation of this thesis. I thank my lecturers and colleagues in the Department of Medicine, Moi University for their input and mentorship. I also thank the staff at Endoscopy unit of Moi Teaching and Referral Hospital (MTRH) for their assistance and cooperation in the execution of the study.

Great gratitude also goes to Dr Anne Mwangi and Mr Alfred Keter the biostatisticians who assisted me in sample size calculation and data analysis respectively.

Also greatly acknowledged are AstraZeneca, National Commission of Science, Technology and Innovation (NACOSTI) for their funding of the study, hence the thesis, and Pathologists Lancet Kenya Limited (PLK) for highly subsidizing the laboratory costs of this study.

List of abbreviations

ACG	-	American College of Gastroenterology
CLSI	-	Clinical Laboratory Standards Institute.
DNA	-	Deoxyribonucleic Acid
EHSG	-	European Helicobacter Study Group
FBS	-	Fetal Bovine Serum
FD	-	Functional dyspepsia
GERD	-	Gastroesophageal reflux disease
H2R	-	Type 2 histamine receptor
IDA	-	Iron deficiency anaemia
IREC	-	Institutional Research and Ethics Committee
ITP	-	Idiopathic thrombocytopenic purpura
KNH	-	Kenyatta National Hospital
MALT	-	Mucosa-associated lymphoid tissue
MIC	-	Minimum Inhibitory Concentration
MTRH	-	Moi Teaching and Referral Hospital
NADPH	-	Nicotinamide Adenine Dinucleotide Phosphate
NSAIDS	-	Non steroidal anti-inflammatory drugs
PCR	-	Polymerase chain reaction
PPI	-	Proton pump inhibitor
PUD	-	Peptic ulcer disease
RUT	-	Rapid urease test
SAT	-	Stool antigen test

UBT	-	Urea breath test
USA	-	United States of America

Definition of terms

Case definition

H. pylori infection was regarded as present if the rapid urease test on gastric biopsy specimens was positive.

Dyspepsia

Dyspepsia was defined according to the Rome III criteria as presence of one or more of the following (Tack et al., 2006):

1. Postprandial fullness (termed postprandial distress syndrome)
2. Early satiation (meaning inability to finish a normal sized meal or postprandial fullness)
3. Epigastric pain or burning epigastric pain (termed epigastric pain syndrome).

Interpretation of susceptibility patterns: Clinical Laboratory Standards Institute (CLSI) definitions will be used (CLSI, 2007);

1. **Resistant isolates:** Isolates not inhibited by the usually achievable concentrations of the agent with normal dosage schedules, and/or that demonstrate zone diameters that fall in the range where specific microbial resistance mechanisms are likely.

2. **Susceptible isolates:** Isolates inhibited by the usually achievable concentrations of antimicrobial agent when the recommended dosage is used for the site of infection.

3. **Intermediate isolates:** Isolates with antimicrobial MICs that approach usually attainable blood and tissue levels and for which response rates may be lower than for susceptible isolates

Minimum inhibitory concentration (MIC): The lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after appropriate incubation period.

CHAPTER ONE: INTRODUCTION

1.1 Background.

Helicobacter pylori (H. pylori) is a microaerophilic gram negative bacterium that colonizes the gastric mucosa (Marshall et al., 1984). Infection with *H. pylori* has been associated with the development of chronic gastritis, peptic ulcer disease, gastric adenocarcinoma, and mucosa associated lymphoid tissue (MALT) lymphoma (Marshall et al., 1985; Parsonnet et al., 1991; Uemura et al., 2001).

It is estimated that more than half of the world's population is infected with *H. pylori* (Goodman et al., 2001). Locally in Kenya, the prevalence of *H. pylori* among patients with dyspepsia has been found to range from 50 to 100% with higher prevalence reported in earlier studies than more recent ones, indicating a general decline in prevalence over time (Kalebi et al., 2007; Mwogi et al 2013; Ogutu et al., 1998; Shmuely et al., 2003).

Several invasive (requiring endoscopy) and non-invasive tests are available for the diagnosis of *H. pylori* infection. Non-invasive tests include urea breath test, stool antigen test and serology while invasive tests are rapid urease test, histology and culture (Chey et al, 2007). The last two are regarded as the gold standard.

Despite its unique role in the management of *Helicobacter pylori*, culture is not used routinely because of various factors: special conditions for transportation, speed required to process specimen, expensive and complicated media, special incubation conditions and duration of culture (Perez-Perez, 2000). These factors contribute to low availability of laboratories that carry out this test. Its low sensitivity, occasioned by low isolation rate due to its fastidious nature, as well as the need for upper gastrointestinal

endoscopy adds to the challenges of using this test (Logan et al., 2001). The availability of other simpler and more sensitive tests for diagnosis adds to the factors limiting its use (Chey et al., 2007).

Although *H. pylori* culture is not routinely used for diagnosis in clinical settings, it is particularly useful because of the prospect of doing antibiotic susceptibility testing in selected patients. Patients who benefit most from culture and antibiotic susceptibility are those who have used two courses of different antibiotic eradication regimes without achieving cure (Chey et al., 2007). Culture and antibiotic susceptibility testing is also useful for new patients prior to any treatment or after initial treatment failure in areas of high antibiotic resistance, and especially that of clarithromycin as this could be cost effective (Malfertheiner et al., 2012; Romano et al., 2003). Outside of clinical settings *H. pylori* culture is also useful in studying growth and metabolism of the bacteria, potential bacterial virulence factors and pathogenesis of infection (Perez-Perez, 2000)

Several factors affect the *H. pylori* culture and isolation rate, thus affecting its sensitivity. Recent use of antibiotic and proton pump inhibitors reduces isolation rate. These drugs need to be stopped 2 to 4 weeks before culture is attempted (Malfertheiner et al., 2012). Prolonged time between specimen extraction and processing for culture also negatively affects the culture rate as is the choice of transport media. Specimens cultured after 6 hours of extraction usually has a low culture yield and thus it is recommended that such specimens should be refrigerated while awaiting culture to be undertaken. This low culture rate after delay is attributable to the organism taking coccoid forms that are difficult to culture (Megraud & et al., 2007; Veenendaal et al., 1993). Samples have been processed after 24 hours of extraction without significant loss to diagnostic yield (Veenendaal et al., 1993). The prospect of doing successful cultures following up to 24 hours delay from sample extraction provides an opportunity

for centres unable to undertake cultures to transport samples for up to 24 hours to central laboratories where cultures can be undertaken.

Several transport media have been used for *Helicobacter pylori* with success. These include: Colombia blood agar, Brain Heart Infusion agar, brucella broth, cysteine albimi (Han et al., 1995; Kimang'a et al., 2010). Normal saline has been used successfully as a transport media without significantly affecting the isolation rate, providing a cheap transport media in resource poor settings (Veenendaal et al., 1993). The choice of culture media also impacts on isolation rate of Helicobacter pylori. In a study that compared the culture media, the isolation rates were: Brain Heart Infusion Agar (96%), trypticase Soy agar (78%), Egg Yolk Agar (64%) and Colombia Blood Agar (32%) (Hachem et al., 1995). Other factors that may affect the isolation rate include number and site of biopsies taken, provision of micro aerophilic environment for culture, duration of culture, use of selective media (Dubois, 1995)

1.2 Statement of the problem

Access to *H. pylori* cultured is poor because few laboratories provide the service. Although upper gastrointestinal endoscopies and biopsies are done at Moi Teaching and Referral Hospital, there are no laboratories within Eldoret and the whole of Western Kenya that have the necessary equipment and expertise to undertake *H. pylori* cultures. Furthermore transport of *H. pylori* usually requires expensive and not easily available transport media. For these reasons *H. pylori* culture is currently not done at MTRH for patients who could benefit from it unless cultures are transported overnight to a central laboratory in Nairobi.

Helicobacter pylori is one of the major causes of upper gastrointestinal diseases associated with dyspepsia.

1.3 Justification.

H. pylori culture is not available in Eldoret and western Kenya and the nearest laboratories that offer these cultures are situated in Nairobi which is over 350 kilometres away and usually requires that samples are transported overnight. There is need to find out if it is useful to send cultures over 20 to 24 hours to a central laboratory in Nairobi that has the equipment and expertise to undertake the culture.

Due to the unavailability and high cost of *H. pylori* transport media, there is also a need to find out if normal saline can be used as a cheap, easily available alternative as a transport media for *H. pylori* for culture.

Findings on current prevalence of *H. pylori* among patients with dyspepsia helps to guide decision on whether or not to test such patients for the infection.

1.4 Research questions

1. What is the prevalence of *H. pylori* among patients with dyspepsia undergoing upper gastrointestinal endoscopy at MTRH?
2. What is the culture isolation rate of *Helicobacter pylori* following a 20 to 24 hour transportation time of samples to a central laboratory?

1.5 Broad objective

To determine the prevalence by RUT and culture rate of *Helicobacter pylori* following 20-24 hour transportation time of samples for patients with dyspepsia at Moi Teaching and Referral Hospital.

1.6 Specific objectives

1. To determine the prevalence of *H. pylori* infection among patients with dyspepsia using the rapid urease test (RUT).

2. To determine the culture rate of *H. pylori* following 20 to 24 hour transportation time using normal saline as transport media.

1.7 Secondary objective

To describe the susceptibility patterns of any isolated *H. pylori* to recommended first line antimicrobial drugs namely; clarithromycin, amoxicillin, metronidazole and tetracycline.

CHAPTER TWO: LITERATURE REVIEW

2.1 Prevalence of *H. pylori*

Helicobacter pylori remains one of the most common worldwide human infections with an estimated half of the world's population infected (Goodman et al., 2001). Prevalence varies between developed and developing countries with a higher prevalence found in the developing countries (Khalifa et al., 2010). In a study by Khalifa et al that reviewed different prevalence studies, the average prevalence in a sample of developing countries was 60% compared to 41.9% in developed countries (Khalifa et al., 2010). This phenomenon has been attributed to association of infection of *H. pylori* with low socioeconomic status (Khalifa et al., 2010).

Regionally in Africa, a study in Nigeria using both histology and rapid urease test on antral specimens for diagnosis found a prevalence of 64% among patients with dyspepsia (Jemilohun et al., 2010). Ndip et al in 2008 found a prevalence of 92.2% among patients with upper gastrointestinal pathologies in Cameroon. In Egypt, the prevalence was found to be 88% in Alexandria among pregnant mothers using serological testing (Bassily et al., 1999). Tanih et al in 2010 reported a prevalence of 66.1% among dyspeptic patients in South Africa (Tanih et al., 2010).

In Kenya, in 1991, Lule et al found a prevalence of *H. pylori* infection among patients with dyspepsia to be 70% ,ranging from 50% in those with no endoscopic diagnosis to 87.5% among those with antral gastritis (Lule et al., 1991). In another study where data was collected in 1993 and 1994, Ogutu et al found prevalence of 100% among patients with peptic ulcer disease and 80.5% among patients with dyspepsia and normal endoscopic findings (Ogutu et al., 1998). All the patients in this study were age 12 years and above, and three diagnostic modalities were used to diagnose *H. pylori* i.e

histology, culture and rapid urease. In a seroprevalence study in Nakuru, Shmuely et al found prevalence of *H. pylori* among dyspeptic patients presenting to a provincial public hospital and age and sex marched controls of asymptomatic persons from the community to be 71% and 51% respectively (Shmuely et al., 2003). Another study in 2004 by Lwai-Lume et al found a prevalence of 69% among patients with dyspepsia at Kenyatta National Hospital (KNH), Nairobi (Lwai-Lume et al., 2005). The mean age for patients in this study was 45 years and 48% of them were females. Among the latest published studies, Kimang'a et al in 2010 found the prevalence of *H. pylori* among patients with dyspepsia to be 54.8% among adult patients with dyspepsia presenting to the Aga Khan, a high end hospital in Nairobi (Kimang'a et al., 2010). This indicates a general decline of *H. pylori* compared to the initial data collected more than two decades ago, that we postulate to be due to improving socioeconomic status and widespread use of *H. pylori* eradication regimes.

At Moi Teaching and Referral Hospital in Eldoret, Mwogi et al (Unpublished) found the average prevalence of *H. pylori* among patients with dyspepsia to be 52.3%. It ranged from 0% among those with oesophagitis to 73.7% among those with peptic ulcer disease (Mwogi et al., 2013).

2.2 Microbiology of *H. pylori*

Helicobacter pylori is a gram negative, flagellated microaerophilic spiral or curved bacillus bacteria that resides in the antral gastric mucosa.(Andersen et al., 1987; Marshall et al., 1984) The bacteria may also infect the corpus mucosa of the stomach especially following gastric acid suppression. The flagella of the bacteria aids in propulsion of the organism through gastric secretion to the mucuos layer of the stomach where a few organisms attach to the mucosa through adhesins, the most characterized of which is Bab A adhesin which attaches to Lewis blood group antigens on the host

cells. The bacteria produce several enzymes including urease, catalase, superoxide dismutase and alkylhydroperoxidase which enable the organism to survive in the harsh gastric mucosa (Hazell et al., 1991; Hazell et al., 1986; Spiegelhalder et al., 1993). Under conditions of inhibited growth for example nutrient starvation, antibiotics and proton pump inhibitors, the bacteria can change into coccoid forms (Catrenich et al., 1991).

2.3 *H. pylori* associated medical conditions.

Helicobacter pylori are associated with the development of several diseases of the upper gastrointestinal system. Conditions in which definite association has been established are: chronic gastritis, peptic ulcer disease, non-cardia gastric adenocarcinoma and MALT lymphoma (Marshall et al., 1985; Parsonnet et al., 1991; Parsonnet et al., 1994; Uemura et al., 2001).

Colonization of gastric mucosa by *H. pylori* induces inflammation on the host gastric mucosa leading to an acute gastritis that progresses to subsequent chronic gastritis and /or ulceration. The bacterial virulence factor cagA protein induces inflammatory process from the host. Gastric inflammation also causes destruction of somatostatin producing D cells leading to increased gastrin release. Increased gastrin release leads to increased acid production that subsequently ends up in the duodenum causing gastric metaplasia and subsequent colonization of the duodenum by *H. pylori*. Gastric adenocarcinoma and MALT lymphoma are thought to result from progressive DNA damage and survival of defective epithelial cells (Amieva et al., 2008). The association of *H. pylori* has been classified by WHO as a class 1 carcinogen due to its association with gastric adenocarcinoma and MALT lymphoma.

There is also evidence linking *H. pylori* infection with the aetiology of otherwise unexplained iron deficiency anaemia (Qu et al., 2010).

Inconclusive data also link this infection with functional dyspepsia (FD), idiopathic thrombocytopenic purpura (ITP) and unexplained vitamin B12 deficiency (Malfertheiner et al., 2012; Pellicano et al., 2009)

2.4 Diagnosis of *H. pylori*

There are several tests that can be used for diagnosis of *H. pylori* and deciding which test to use in which situation relies heavily upon whether a patient requires evaluation with upper endoscopy and an understanding of the strengths, weaknesses and costs of individual tests.

For patients who do not require endoscopy, tests include: antibody testing, urea breath tests (UBT) and fecal antigen tests. The antibody test detects serum IgM antibodies against *H. pylori*. This test is not useful in assessing eradication as the antibodies remain in blood for long. The UBT is useful in the detection current infection as well as assessing eradication after therapy (Malfertheiner et al., 2012).

Options for diagnosis for patients who undergo upper endoscopy include rapid urease test (RUT), histology, culture and polymerase chain reaction (PCR). (Chey et al., 2007) Histology and culture are regarded as the gold standard for *H. pylori* diagnosis. PCR is not readily available in the Kenya.

The RUT, utilizes the organism's urease activity. Gastric biopsies are placed into an ager gel or a reaction strip containing urea, a buffer and a pH sensitive indicator. In the presence of *H. pylori's* urease, urea is metabolized to ammonia and bicarbonate leading to a PH increase. A change in colour of the pH sensitive indicator signifies the presence

of active infection. The colour change is seen in 1-24 hours. Pretreatment sensitivity and specificity are more than 90% and 95% respectively (Midolo et al., 2000; Perna et al., 2005). Recent use of medications that reduce the density and/or urease activity of *H. pylori* for example bismuth-containing compounds, antibiotics, or PPIs, can reduce the sensitivity by up to 25% (Midolo et al., 2000).

Culture method is regarded as the gold standard, together with histology, for the diagnosis of *H. pylori* infection, and is also useful in characterization of antimicrobial sensitivities. However, it is not as sensitive as the RUT or histology, and furthermore is expensive and not readily available (Chey et al., 2007; Lehours et al., 2003).

2.5 Benefits of *H. pylori* eradication

Several studies have proven the benefit of *H .pylori* eradication in patients with upper gastrointestinal disease associated with the infection. Eradication of *H. pylori* is beneficial in peptic ulcer healing and prevention of recurrence as compared to acid suppression alone (Ford et al., 2004; Hopkins et al., 1996; Leodolter et al., 2001) . Furthermore in patients with peptic ulcer bleeding, eradication of *H. pylori* is useful in the prevention of recurrence of bleeding (Sharma et al., 2001). In patients with low grade gastric MALT lymphoma, eradication *of H. pylori* induces remission and cure in most patients (Nakamura et al., 2005; Thiede et al., 2001). Benefit of *H. pylori* eradication is also seen in patients with early non-cardia adenocarcinoma of the stomach that has been resected where it prevents recurrence (Uemura et al., 1997). In patients at risk of developing the adenocarcinoma of the stomach, eradication of *H. pylori* can reduce the risk of developing the cancer (Wong et al., 2004).

With these amount of data, the American College of Gastroenterology in its 2007 guidelines recommends a test and treat strategy in patients with active or confirmed

history of peptic ulcer disease, low grade gastric MALT lymphoma, after endoscopic resection of early gastric cancer, and uninvestigated dyspepsia in patients under the age of 55 years with no alarm symptoms to suggest gastric malignancy. It also lists clinical conditions where *H. pylori* eradication for their management is controversial in the United States .These include: functional dyspepsia(FD), gastro-esophageal reflux disease(GERD), persons using non-steroidal anti-inflammatory drugs (NSAIDS) , unexplained iron deficiency anaemia (IDA) and populations at risk of gastric cancer (Chey etal., 2007).

The European Helicobacter Study Group (EHSG) in its 2012 guidelines on the other hand recommends definite eradication of *H. pylori* also in functional dyspepsia, unexplained IDA, idiopathic thrombocytopenic purpura (ITP), unexplained vitamin B12 deficiency, patients starting NSAIDS and patients on aspirin (Malfertheiner et al., 2012).

2.6 Eradication regimes for *H. pylori*

The ACG and the EHSG recommend use of either a clarithromycin based triple therapy, bismuth based quadruple therapy or sequential therapy as first line for eradication of *H. pylori*. The clarithromycin based triple therapy consists of a standard dose PPI, clarithromycin and amoxicillin (or metronidazole) for 10 to 14 days while the quadruple therapy consists of bismuth subsalicylate, metronidazole, tetracycline, and ranitidine (or PPI) for 10 to 14 days. The sequential therapy consists of PPI and amoxicillin for 5 days followed by PPI, clarithromycin, and tinidazole for another 5 days. The guidelines recommend use of bismuth based quadruple therapy or the sequential therapy as first line in areas of high clarithromycin resistance (Chey et al., 2007; Malfertheiner et al., 2012).

The recommended second line therapy after failure of first line is bismuth quadruple therapy where clarithromycin based triple therapy was used as first line, or use of PPI, levofloxacin and amoxicillin combination (Chey et al., 2007; Malfertheiner et al., 2012).

When the second line fails to eradicate the bacteria, it is recommended that the third line be based on susceptibility testing results (Chey et al., 2007; Malfertheiner et al., 2012).

In Kenya, the Ministry of Health, in its 2009 guidelines, recommends the use of clarithromycin, a PPI, and either amoxicillin or metronidazole for 14 days. This guideline, however, does not recommend any specific method for the diagnosis or assessment of success of *H. pylori* eradication (Kenya, 2009).

2.7 Factors affecting treatment outcomes of *H. pylori*

The most important predictors of treatment failure following anti-*H. pylori* therapy include poor compliance and antibiotic resistance (Megraud et al., 2003). Previous treatment with either a macrolide or metronidazole for any reason significantly increases the likelihood of *H. pylori* resistance to these agents (McMahon et al., 2003). Bacterial strains negative for Cag A are associated with increased risk of treatment failure compared to Cag A positive strains (Suzuki et al., 2006). Furthermore, patients with polymorphisms in their cytochrome P450 CYP2C19 enzymes resulting in differences in PPI metabolism could influence treatment outcome (Padol et al., 2006).

2.8 *H. pylori* antibiotic resistance

The triple therapy eradication regime of PPI-clarithromycin and amoxicillin or metronidazole has become universal as was recommended by most guidelines around the world (Chey et al., 2007; Kenya, 2009; Malfertheiner et al., 2012). However, the

most recent data show that this combination has lost some efficacy and often allows the cure of only a maximum of 70% of patients, which is less than the 80% aimed for at the beginning and far below what should be expected for an infectious disease (Graham et al., 2010; Malfertheiner et al., 2012). In a USA multicentre study which collected data between 1993 and 1999, the reported antibiotic resistance rates amongst *H. pylori* strains was 37% for metronidazole, 10% for clarithromycin, 3.9% for both antibiotics and 1.4% for amoxicillin (Meyer et al., 2002). A study in Cameroon showed multidrug resistant strains of *H. pylori*. In this study, the *H. pylori* susceptibility was 56% for tetracycline, 55.3% for clarithromycin, 14.4% for amoxicillin and only 6.8% for metronidazole (Ndip et al., 2008).

A study in Kenya in 1991 had shown that the *H. pylori* strains then were sensitive to metronidazole, tetracycline, erythromycin, ampicillin, chloramphenical, colistin, kanamycin and sulpharazole; but was resistant to nalidixic acid (Sang et al., 1991). However, in a study done more than a decade later in 2004 at Kenyatta National Hospital, Lwai-Lume found susceptibility of 0% to metronidazole, 93.6% to clarithromycin, 95.4% to amoxicillin and 98.1% to tetracycline (Lwai-Lume et al., 2005). In contrast to this finding of absolute resistance to metronidazole, Kimang'a et al in a study done at Aga Khan University Hospital, Nairobi found susceptibility to metronidazole to be 95.4%, while that of clarithromycin and amoxicillin was 100% (Kimang'a et al., 2010). This wide variability in *H pylori* susceptibility to metronidazole could be attributable to the use of different MIC cuts offs and the difficulty of reproducing resistance to metronidazole using the E-Test strips as compared to the agar method (Glupczynski et al., 2002).

Several mechanisms of resistance of *H. pylori* to metronidazole have been found. Most of these mechanisms are mainly explained by the fact that metronidazole is a pro drug

that requires reduction with generation of free radicals that damage deoxyribonucleic acid (DNA) and cause bactericidal activity (Docampo et al, 1986). Some resistant strains of *H. pylori* have been shown to have mutations in a gene that encodes for the oxygen insensitive nitro-reductase Nicotinamide adenine dinucleotide phosphate (NADPH) (Goodwin et al., 1998). Reduced levels of NADPH prevent the reduction of metronidazole to active form resulting in resistance. Inactivation of other reductase genes has also been implicated in resistance to metronidazole. These include flavin oxidoreductase (frxA) and ferrodoxin-like enzymes (frxB)(Kwon et al., 2000). A more recent study has shown that some resistance to metronidazole is mediated by presence of Outer Membrane Efflux Proteins (OEP) in *H. pylori* causes secretion of antibiotics out of the bacteria and thus rendering the drug inactive (Van Amsterdam et al., 2005).

Tetracycline is a major antibiotic in the quadruple *H. pylori* eradication regimen and it acts by binding to the 30S ribosomal subunit blocking fixation of the aminoacyl-transfer RNA to the receptor site (Chey et al, 2007; Chopra et al., 2001).

Point mutation at tetracycline primary binding site is a major mechanism *H. pylori* develops resistance to tetracycline (Gerrits, et al., 2003). Other mechanisms of resistance include mutations at secondary binding sites and chemical modification of tetracycline by an NADP dependent oxidoreductase (Chopra et al., 2001; Trieber et al., 1998).

2.9 *H. pylori* culture

2.9.1 Introduction

H. pylori culture on gastric mucosal specimen is a highly specific test for diagnosis of active infection and is one of the options for diagnosis for patients undergoing

endoscopy (Chey et al., 2007). Culture has sensitivity and specificity of 90-95% and 95-100% respectively (Logan et al., 2001).

2.9.2 Role of *H. pylori* culture

Although *H. pylori* culture is not routinely used for diagnosis in clinical settings, it is particularly useful because of the prospect of doing antibiotic susceptibility testing in selected patients. Patients who benefit from culture and antibiotic susceptibility are those who have used two courses of different antibiotic eradication regimes without achieving cure (Chey et al., 2007). Culture and antibiotic susceptibility testing is also useful for new patients prior to any treatment or after initial treatment failure in areas of high antibiotic resistance, and especially that of clarithromycin as this could be cost effective (Malfertheiner et al., 2012; Romano et al., 2003)

Outside of clinical settings *H. pylori* culture is also useful in studying growth and metabolism of the bacteria, potential bacterial virulence factors and pathogenesis of infection (Perez-Perez, 2000).

2.9.3 Challenges of *H. pylori* culture

Although useful in various circumstances, doing Helicobacter pylori culture is not used routinely because of various factors: special conditions for transportation, speed required to process specimen, expensive and complicated media, special incubation conditions and duration of culture (Perez-Perez, 2000) These factors contribute to low availability of laboratories that carry out this test.

The low sensitivity, occasioned by low isolation rate due to its fastidious nature, as well as the need for upper gastrointestinal endoscopy adds to the challenges of using this test (Logan et al., 2001). The availability of other simpler and more sensitive tests for diagnosis adds to the factors limiting its use (Chey et al., 2007).

2.9.4 Factors affecting *H. pylori* culture rate

Several factors affect the *H. pylori* culture and isolation rate, thus affecting its sensitivity. This factors include: recent medication use; time between sample extraction and culture; transport and culture media; number and site of biopsy specimens; microaerophilic environment; use of selective media and duration of culture (Dubois, 1995).

Recent use of antibiotic and proton pump inhibitors reduces isolation rate. These drugs need to be stopped 2 to 4 weeks before culture is attempted (Malfertheiner et al., 2012).

Prolonged time between specimen extraction and processing for culture also negatively affects the culture rate as is the choice of transport media. Specimens which cannot be processed within 6 hours of extraction should be refrigerated (Megraud & Lehours, 2007; Veenendaal et al., 1993). As an example, samples have been processed after 24 hours of extraction without significant loss to diagnostic yield (Veenendaal et al., 1993). In this study patients were included into the study if they had not on any antibiotic or PPI or H2R blockers for at least 3 months prior to enrolment so as to increase the number of bacilli of *H. pylori* thus increasing the success of culture.. Two antral biopsies were used and specimens were transported in only 0.2mls of normal saline. Antrum is where most of *H. pylori* resides and this increases the chance of isolating it, while a little amount of transport media avoid the dilution of the organisms during transport.

Several transport media have been used for *Helicobacter pylori* with good success. These include: Colombia blood agar, Brain Heart Infusion agar, brucella broth, cysteine albimi (Han et al., 1995; Kimang'a et al., 2010). Normal saline has been used successfully as a transport media without significantly affecting the isolation rate,

providing a cheap transport media in resource poor settings (Veenendaal et al., 1993). The choice of culture media also impacts on isolation rate of *Helicobacter pylori*. In a study that compared the following culture media, the isolation rates were: Brain Heart Infusion Agar (96%), trypticase Soy agar (78%), Egg Yolk Agar (64%) and Colombia Blood Agar (32%) (Hachem et al., 1995).

Although there are no specific guidelines for culture of *H. pylori*, several studies as mentioned above have used at least two gastric biopsy specimens (with at least one from the antrum) for culture, transported to the laboratory using brain heart infusion, or other enriched media. These specimens have mostly been transported to the laboratory within 6 hours and cultured under microaerophilic conditions. Samples transported over 24 hours were shown to have a 5 % less yield compared to samples cultured within 2 hours of extraction.

2.9.5 Isolation rates of *H. pylori*

Although it is ideally expected that the sensitivity of culture is 90-95%, as indicated above, several factors affect the isolation rates of Helicobacter pylori cultures (Logan et al., 2001).

Cultures done locally in Kenya have yielded varying isolation rates ranging from 47% to 95% using different types of media (Kimang'a et al., 2010; Lwai-Lume et al., 2005; Sang et al., 1991). All of these studies were done within Nairobi with easy access to laboratory and all samples were processed with 6 hours of collection, resulting in favourable culture rates for most of them.

Cultures have been successfully done elsewhere using normal saline as transport media and after 24 hours of extraction with great success (Veenendaal et al., 1993). The prospect of using normal saline as transport media to achieve acceptable culture rates

offers a cheap option for centres such as Kenya which have no readily available access to expensive enriched media to transport their samples to central laboratories without significantly affective the isolation rates. A literature search of use of normal saline as transport media and sample processing after 24 hours of extraction in Kenya did not yield any articles, published or unpublished hence this study.

CHAPTER THREE: PARTICIPANTS, MATERIALS AND METHODS

3.1 Study design

This was a cross-sectional descriptive study.

3.2 Setting

Patients in this study were recruited at Moi Teaching and Referral Hospital, Endoscopy Unit; while *H. pylori* culture and antibiotic susceptibility where done at Pathologists Lancet Kenya Limited (PLK), Nairobi.

MTRH is a tertiary referral hospital in Eldoret town, Uasin Gishu County, whose catchment area is the whole of western Kenya. It has an endoscopy unit that receives patients for endoscopy from within the hospital as well as referrals from outside the hospital. Over 700 upper gastrointestinal endoscopies are done yearly at MTRH by at least 7 endoscopists.

Pathologists Lancet Kenya Limited is a South African National Accreditation System (SANAS) accredited laboratory on 5th Avenue Office Suites, Upper Hill, Nairobi. It is certified for medical testing laboratory: microbiology, chemistry, haematology and molecular biology (Appendix XIII)

3.3 Participants.

The participants were drawn from adult patients referred for upper gastrointestinal endoscopy at MTRH.

3.4 Sample size determination and sampling technique

3.4.1 Sample size

Fisher's formula for proportions was used to determine the minimum sample size for *H. pylori* culture rate and prevalence on rapid urease test as follows:

$$n = \frac{Z^2_{\left(1-\frac{\alpha}{2}\right)} . P(1-P)}{D^2}$$

Where:

n=sample size

z=z value corresponding to 95% confidence (1.96)

p=estimated proportion of *H. pylori* culture rate

D=level of significance of 0.05

Minimum sample size for Culture attempts

Using an estimated *H .pylori* culture rate culture rate of 96% (based on a study that used the similar culture media as this study; (Hachem et al., 1995), the p value was 0.96.

n, therefore = 60.

Using an adjustment of 5%, (for the effect of delay in culture), (Veenendaal et al., 1993), the minimum number of samples to attempt *H .pylori* culture on following a positive RUT was 64.

Minimum sample size for *H. pylori* prevalence

Using a precision of 5% and estimated prevalence of 91% (from a local study by Ogutu EO, et al),(Ogutu et al., 1998), a minimum sample size of 126 patients for *H. pylori* testing on RUT was obtained.

3.4.2 Sampling technique

Consecutive sampling of patients who met the study criteria was done until the required sample size was achieved for both prevalence and culture rate.

3.5 Eligibility criteria

3.5.1 Inclusion criteria

1. Patients with symptoms of dyspepsia.
2. Age of 18 years and above.
3. Patients not on PPI, H2R blockers or any antibiotic in the preceding two weeks before endoscopy.

3.5.2 Exclusion criteria

1. Failure to intubate the stomach.
2. Declining to consent for the endoscopy and/or the study.

3.6 Study procedure

Patients booked for endoscopy were advised to take a light diet on the day prior endoscopy and to fast from midnight of the day of the procedure. Compliance was assessed before the procedure by a short history. On arrival at the endoscopy unit, the patients were assessed by the investigator or assistant in the unit for eligibility, as per data entry form (Appendix I). Eligible patients were counselled on the process, potential benefits and harm of the endoscopy procedure, the extraction of gastric biopsy samples, the rapid urease testing, *H. pylori* culture testing and antibiotic susceptibility testing. Those who agreed to participate in the study after counselling were requested to give informed written consent for the endoscopy procedure with *H. pylori* testing, and subsequent possible culture and sensitivity testing. Details of consent form were presented in English or Kiswahili as in appendices II and III respectively. Patients who did not consent for the study were excluded, but underwent the routine endoscopy as per hospital routine.

Endoscopies were done between 9: 00am and 1: 00pm as outlined in the appendix IV. The endoscopy procedures were carried out by consultant gastroenterologists or consultant endoscopy surgeons, based on the timetable in the unit. During the procedure, 4 gastric mucosal forceps biopsies (2 from the antrum and 2 from the corpus) were obtained so as to not to miss any possible infection in either part of the stomach. In the standard reporting, gross findings of the upper gastrointestinal tract were recorded.

Two (one each from the antrum and corpus) of the four gastric biopsies were immediately tested for active *H. pylori* infection using the rapid urease test using Esokit Hp Test (Appendix V). Patients whose *H. pylori* test were positive by the urease test while they were still in the endoscopy unit had an additional note in the endoscopy report to the referring physician. *H. pylori* test results that turned positive after patient has left the endoscopy unit, were delivered to the patient or referring physician via the contact details left in the data entry form. This included the recommendation to start standard *H. pylori* treatment protocol and information that more biopsies have been taken for *H. pylori* culture and sensitivity. All results of negative rapid urease tests were delivered to the patient after 24 hours via mobile phone they provided.

For patients whose rapid urease test turned positive within four hours, the other two gastric biopsies harvested were put in one milliliter of normal saline, the volume used as per Kimang'a et al study, and transported overnight in a cooler box to Pathologists Lancet Kenya Laboratory, on 5th Avenue Office Suites, Upper Hill, Nairobi. All the samples were cultured the following day at 9: 00am.

The Brain Heart Infusion Agar culture media in this study was prepared by enriching it with inactivated fetal bovine serum. The selective media was made by further adding a

dent supplement containing vancomycin, trimethoprim, cefsulodin and amphotericin B (Appendix VI).

In the laboratory, *H. pylori* were cultured in Brain Heart Infusion Agar under microaerophilic conditions and its colonies identified. Details of *H. pylori* culture, growth and identification are in appendix VII.

For sensitivity testing, antibiotic Epsilometer (E) test strips were applied for clarithromycin, metronidazole, amoxicillin and tetracycline and incubated for a further 24-36 hours in non-selective culture media before the minimum inhibitory concentrations (MICs) for each antibiotic was read. Appendix III details the antibiotic sensitivity testing procedure.

MIC levels that were interpreted as sensitive were less than or equal to 0.25μg/ml for clarithromycin and amoxicillin, less than or equal to 2μg/ml for tetracycline and metronidazole. On the other hand MIC levels that were interpreted as resistant were equal or more than 1μg/ml for clarithromycin and amoxicillin, equal or more than 4μg/ml for tetracycline and equal or more than 8μg/ml for metronidazole. These MIC breakpoints specifically determine for *Helicobacter pylori* were based on Clinical Laboratory Standards Institute guidelines for clarithromycin and an updated appraisal for the rest of the antibiotics.(CLSI, 2007; Francesco et al., 2011) MIC levels between susceptible and resistant break points for each antibiotic were regarded as intermediate.

Figure 1 below summarizes the algorithm for the study procedure.

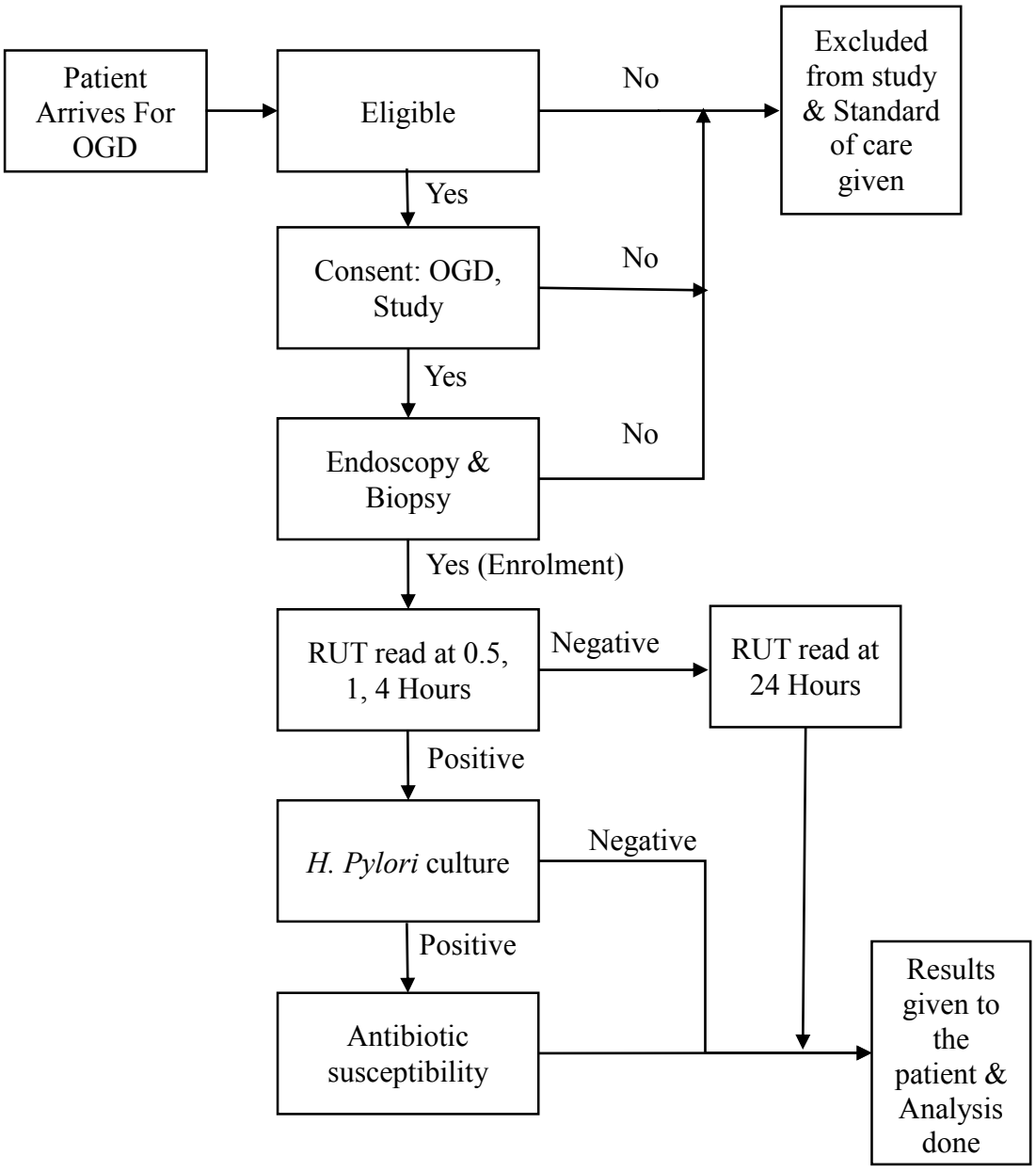

Figure 1: Algorithm of Study Procedure

3.7 Data management

Data was collected by the investigator through an interviewer administered questionnaire and laboratory reports and later these data were dually entered into Access Database and validated. Each data entry form had a unique identifier which was serially determined. Data collected in the data entry form included demographic data, previous investigation and treatment of *Helicobacter pylori,* the gross endoscopic findings and the result of the rapid urease test, results of culture and sensitivity to the specified antibiotics.

3.8 Statistical analysis

Data was analyzed using STATA version 13 special edition. Categorical variables were summarized as frequencies and their corresponding percentages. Normally distributed continuous variables are summarized as mean and corresponding standard deviation while skewed continuous variables are summarized as median and interquartile range. Pearson's Chi Square or Fishers exact tests were used for association between categorical variables. Data presentation are in form of tables.

3.9 Ethical Considerations

Informed consent was sought from all patients before the procedure. For the patients who could not read or write the investigator explained the contents of the consent form in a language they understood, using a translator where necessary, and if the patient consented, used a thumb print as an alternative to a written signature. All patients were informed about the outcome of results and counseled accordingly depending on the outcome. *H. pylori* positive patients were informed about the presence of infection and the need for treatment to be initiated. Participants who left the endoscopy unit had their results communicated to them via the contacts in the data entry form. Participants were

informed of culture and sensitivity results and recommended choice of antibiotics depending on susceptibility testing.

All study costs were paid for by the investigator. Patients were not given any inducements to participate in the study.

All patients who participated in the study did so by free will and those who declined to participate were not discriminated against. They underwent the endoscopy procedure for which they had been referred for as stipulated in the endoscopy procedure (Appendix IV).

The *H. pylori* testing procedure does not, and did not confer additional procedure risks other than those of endoscopy. Instead, there were benefits from the results since it guided the management of the patient significantly.

All information from the procedure was handled confidentially. Only patient codes were used in the computer data entry forms. The referring physicians were informed of the results and the recommended management of the patients.

Partial funding was received for this study from pharmaceutical company, AstraZeneca and National Commission for Science Technology and Innovation (NACOSTI), but they played no role in the study design, execution, and data analysis and presentation of results.

This study was approved by Institutional Review and Ethics Committee (IREC), (Formal Approval Number: FAN: IREC 1048) and Moi Teaching and Referral Hospital management. (Appendices IX-XII)

CHAPTER FOUR: RESULTS

4.1 Patient Recruitment

Between April 2014 and February 2015, 634 patients presenting at the MTRH endoscopy unit for upper gastrointestinal endoscopy were screened of whom 156 were recruited into the study. Four hundred and seventy five were excluded because they did not meet the study criteria for various reasons: 26 were below 18 years, 388 did not meet the Rome Criteria for dyspepsia, while 61 were on antibiotics and or proton pump inhibitor and or histamine receptor type 2 antagonists in the previous two weeks. Two more patients were excluded because they didn't consent for the endoscopy procedure, while one more patient was excluded as the endoscopy procedure failed. (Figure 2)

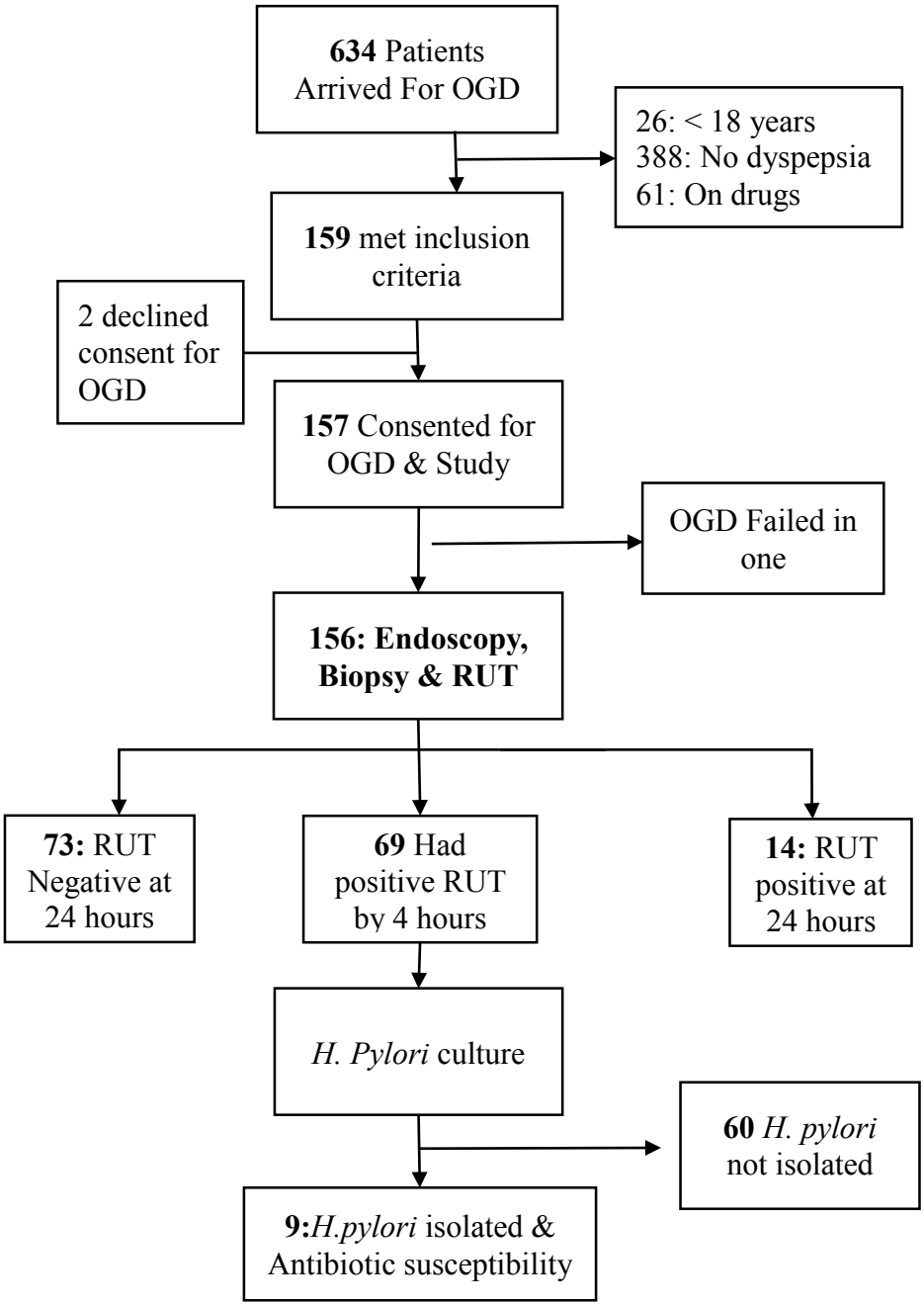

Figure 2: Enrolment Schema

4.2: Demographic Characteristics

The 156 participants enrolled in the study were aged between 18 and 86 years old with a median age of 41 (IQR: 28-58) years. Of this number, 64 (41%) were males. Majority of the participants, 88 (57%), were self-employed. One fifth of the participants were employed (Table 1).

Table 1: Demographic characteristics

Characteristic	**Frequency: n (%) n=156**
Gender	
Male	64 (41%)
Female	92 (59%)
Age (Years)	
18-24	25 (16%)
25-34	34 (22%)
35-44	32 (21%)
45-54	22 (14%)
55-64	20 (13%)
65-74	13 (8%)
>74	10 (6%)
Employment status	
Self employed	89 (57%)
Formal Employment	31 (20%)
Unemployed	36 (23%)

4.3 Clinical Characteristics

Epigastric pain was the commonest symptom being reported by 151 (97%) of the participants followed by 37 (24%) who reported to have had postprandial fullness as the reason for OGD. Early satiety was not a common symptom of dyspepsia, being reported by only 18 (12%) of participants (Table 2).

Table 2: Clinical characteristics

Characteristic	Frequency: n (%) n=156
Indications for endoscopy	
Epigastric pains	151 (97%)
Postprandial fullness	37 (24%)
Early satiety	18 (12%)
Dysphagia	4 (3%)
Previous Investigation of upper GI disease	
Barium meal	4 (2.7%)
Endoscopy	2 (1.3%)
H. pylori serological testing	5 (3.2%)
Previous treatment of Upper GI disease	
Amoxicillin+Bismuth salt+PPI	2 (1.3%
Amoxicillin+clarithromycin+PPI	1(0.6%)
Amoxicillin+metronidazole+PPI	4 (2.7%)
Amoxicillin+PPI	1 (0.6%)
H2R blocker+PPI	1 (0.6%)
PPI	33 (21.1%)

Eleven patients in this study had previous investigation for upper gastrointestinal disease; five participants had serological *H. pylori* testing for which two were positive; four patients had barium meal testing while 2 had done OGD before.

Some participants had received previous treatment for dyspepsia. Thirty three (21.1%) of them had been treated with PPI before, while 7 had received standard *H. pylor*i eradication treatment (Table 2). The participants received these treatments on average 2.5 months (IQR 2-6) prior to presentation for current OGD and for a median treatment duration of 14 days.

4.4 Gross endoscopic findings

There were 30 (19%) participants who had oesophagitis, and 1 (0.6%) who had ulceration. There was no participant with esophageal growth, or varices. Other oesophageal findings were candidiasis and polyp (Table 3).

Gastritis was the commonest endoscopic finding in the stomach occurring in 123 (79%) of the participants. Normal stomach was observed in 23 (15%) of the participants. Other findings included gastric outlet obstruction, hiatus hernia and polyp.

Majority of the participants, 111 (71%), had normal duodenal findings. One fifth, 30 (19%), had duodenitis while 9 (6%) had duodenal ulcerations. Other duodenal findings included deformed pylorus in 2 patients and ampullary mass in one patient. In three participants, the duodenum could not be intubated.

Thirteen (8.3%) of patients had normal upper endoscopic findings.

Table 3: Gross endoscopic findings

Finding	**Frequency n (%) n=156**
Gastritis	123 (79%)
Duodenitis	30 (19%)
Oesophagitis	30 (19%)
Normal	13 (8.3%)
Duodenal Ulcerations	9(6%)
Gastric Ulcerations	7(4%)
Hiatus hernia	6(4%)
Gastric Atrophy	3(2%)
Gastric outlet Obstruction	2(1.3%)
Oesophageal Candidiasis	2(1.3%)
Deformed pylorus	2(1.3%)
Others	5(3.2%)
Not Done	3(2%)

4.5 Prevalence of *H. pylori*

Eighty three (53%) of participants were positive for *H. pylori* on rapid urease test (RUT). Patients who had postprandial fullness as the reason for endoscopy were more likely to be RUT positive as were patients who had duodenitis; p values 0.0449 and 0.0041 respectively (Table 4 below).

Table 4: Association between clinical characteristics and rapid urease test

Clinical Characteristic	Frequency N =156	RUT positive n=83	RUT negative n=73	p-value
Epigastric pains	151 (97%)	80 (96.4%)	71 (97.3%)	1[f]
Postprandial fullness	**37 (24%)**	**25 (30.1%)**	**12 (16.4%)**	**0.0449[c]**
Early satiety	18 (12%)	11 (13.3%)	7 (9.6%)	0.4747[c]
Previous treatment	42 (26.9%)	23 (27.7%)	19 (26.0%)	0.8130[c]
Normal	13 (8.3%)	6 (7.2%)	7 (9.6%)	0.5946[c]
Oesophagitis	30 (19%)	12 (14.5%)	18 (24.7%)	0.1067[c]
Gastritis	123(79%)	67 (80.7%)	56 (76.7%)	0.5405[c]
Gastric Ulcerations	7(4%)	4 (4.8%)	3 (4.1%)	1[f]
Duodenitis	**30 (19%)**	**23 (27.7%)**	**7 (9.6%)**	**0.0041[c]**
Duodenal Ulcerations	9(6%)	6 (7.2%)	3 (4.1%)	0.5033[f]

[f] = Fischers exact [c] = Chi square test

4.6 *Helicobacter pylori* culture

Sixty nine samples that were positive for *H. pylori* within 4 hours on RUT were cultured. The 14 (17%) of samples that turned positive after 24 hours were not cultured because it would have passed the 24 hours under which culture needed to have begun. Culture yield was found in 9 (13%) of the samples. All the 9 positive cultures had satisfactory growth by the 5th day of culture.

Table 5: Association between clinical characteristics and culture rate

Clinical Characteristic	Frequency: n =69	Culture positive n=9	Culture negative n=60	p-value (Fischer exact test)
Epigastric pains	68 (98.6%)	9 (100%)	59 (98.3%)	1
Postprandial fullness	21 (30.4%)	1 (11.1%)	20 (33.3%)	0.2583
Early satiety	7 (10.1%)	2 (22.2%)	5 (8.3%)	0.2244
Previous treatment	20 (29.0%)	3 (33.3%)	17 (28.3%)	0.7120
Normal	4 (5.8%)	0 (0%)	4 (6.7%)	1
Oesophagitis	10 (14.4%)	2 (22.2%)	8 (13.3%)	0.6087
Gastritis	57 (82.6%)	9 (100%)	48 (80%)	0.3422
Gastric Ulcerations	3 (4.3%)	0 (0%)	3 (33.3%)	1
Duodenitis	19 (27.5%)	4 (5.8%)	15 (25%)	0.2469
Duodenal Ulcerations	5 (7.2%)	0 (0%)	5 (8.3%)	1

The mean age of patients with the positive culture was 33.2 years and 5 (56%) of them were male. All of them had epigastric pain as the reason for endoscopy, 2 had early satiety while one had postprandial fullness. None of them had been investigated for upper gastrointestinal disease before. However 2 had received proton pump inhibitor while one had received clarithromycin based triple therapy for *H. pylori* eradication regimes. All of them had gastritis at endoscopy, 4 had duodenitis and 2 had oesophagitis.

There was no significant association between any patient characteristic (including demographics, patients' symptoms, endoscopic findings) and culture rate. (Table 5 above).

4.7 Antibiotic susceptibility testing

The nine positive cultures were each subjected to antibiotic susceptibility. More than half, 5 (56%) were sensitive to clarithromycin. There were no strains resistant to this antibiotic.

Six (67%) samples were sensitive to amoxicillin while 2 (22%) were resistant. All the 9 strains were resistant to metronidazole. Two thirds of the strains were sensitive to tetracycline while a third were resistant (Table 6 below)

Table 6: Antibiotic resistance

Antibiotic	**Sample size**	**Susceptibility level**	**n (%)**
Clarithromycin	9	Sensitive	5 (56%)
		Intermediate	4 (44%)
		Resistant	0
Amoxicillin	9	Sensitive	6 (67%)
		Intermediate	1 (11%)
		Resistant	2 (22%)
Metronidazole	9	Sensitive	0
		Intermediate	0
		Resistant	9 (100%)
Tetracycline	9	Sensitive	6 (67%)
		Intermediate	0
		Resistant	3 (33%)

CHAPTER FIVE: DISCUSSION

5.1 *H. pylori* prevalence

Using the rapid urease test, more than half (53.2%) of patients with dyspepsia undergoing endoscopy at MTRH were infected with *H. pylori*. This high prevalence of *H. pylori* among dyspeptic patients is attributable to its association with development of upper gastrointestinal diseases that present with dyspepsia including gastritis, peptic ulcer disease and gastric adenocarcinoma (Marshall et al, 1984).

Postprandial fullness was more common among patients with *H. pylori* infection (30.1%) than those without the infection (16.4%), p-value 0.0449. This finding is in keeping with studies that have demonstrated that *H. pylori* is associated with delayed gastric emptying and therefore causing postprandial abdominal discomfort (Tucci et al., 1992). Duodenitis was also more common among patients with the infection (27.7%) than those without (9.6%); p-value 0.0041. This finding reflects the pathogenic mechanisms that *H. pylori* plays in causation of duodenitis and subsequent duodenal ulceration.

Our finding of prevalence of *H. pylori* of 53.2% is comparable to a previous study by Mwogi et al in 2010 in the same setting that found a prevalence of 52.3% among adults patients undergoing endoscopy for dyspepsia, as well as another study by Kimang'a et al study at Aga Khan University Hospital Nairobi in 2010 that found a prevalence of 54.8% among (Kimang'a et al., 2010; Mwogi, , 2013). However, studies done locally in Kenya more than a decade ago showed higher prevalence of *H. pylori* among patients with dyspepsia as depicted in the following studies; Lule et al in 1991 found a prevalence of 70% among dyspeptic patients (Lule et al., 1991). In data collected in 1993, Ogutu et al found prevalence ranging from 85.5% in patients with normal endoscopic findings to 100% among patients with peptic ulcer disease (Ogutu et al.,

1998). The study population in this study included patients from older than 12 years and three diagnostic tests namely rapid urease test, histology and culture were used to diagnose *H. pylori* infection. Using serological testing, Shmuely et al in 2003 found prevalence of 71% among patients with dyspepsia in Nakuru provincial general hospital, while Lwai Lume et al found prevalence of 69% in 2004 among patients with dyspepsia aged 15 to 85 years old undergoing endoscopy at Kenyatta National Hospital. This indicates a general trend of decline of *H. pylori* prevalence among patients with dyspepsia. We attribute this decline to two reasons: *H. pylori* is associated with low socioeconomic status, where acquisition mainly occurs in childhood. With the improvement of socioeconomic status in Kenya over the last few decades it's expected that the prevalence will drop (Khalifa et al., 2010; Mitchell et al., 1992). Secondly with the introduction of *H. pylori* eradication regime guidelines, it's expected that *H. pylori* prevalence will drop among patients with dyspepsia as reinfection after successful treatment is rare (Mitchell et al., 1998) .

5.2 *H. pylori* culture rate

In our study we found *H. pylori* culture rate of 13% following a 24 hour transportation time of the samples to a central laboratory.

Several factors could have contributed to the low culture rate observed in this study: first, 28 % of the patients had previously been treated for dyspepsia with PPI, H2R blockers and/or antibiotics. We also attributed this low culture rate to exclusion of patients who were not on any medications for up to two weeks only prior to enrolment as opposed to longer duration. These treatments reduce the culture rate of *H. pylori* by causing the bacteria to assume coccoid forms which are difficult to culture (Catrenich et al., 1991). Secondly, another 27% of patients were not aware whether or not they had previously been treated for *H. pylori*. There is a possibility that some of them could

have previously been treated for *H. pylori* and thus confounding the results. Use of larger volume of normal saline transport media could also have diluted our organisms leading to low culture rate.

We did not find any statistically significant association between patient clinical characteristics (e.g. symptoms, previous treatments, gross endoscopic findings) and positive *H. pylori* cultures. This is probably attributable to the fact that all the patients whose samples underwent *H. pylori* culture were already confirmed as actively infected by *H. pylori*, and therefore were already a highly select group of patients who most likely had similar characteristics.

The culture rate of 13% was much lower than several other studies. In a study by Veenendaal et al in the Netherlands in 1993 in which culture was done after 24 hour delay as in this study, the culture rate was high at 84.6 % while in another study by Hachem et al in which culture was done 2 to 7 days after sample collection on BHIA as in our study the culture rate was 96% (Hachem et al., 1995; Veenendaal et al., 1993). However there are several differences in methodology between the two studies that could account for the disparity of culture rate with our study. First, while we excluded patients who were on antibiotics within the two weeks of presentation for OGD, Veenendaal et al excluded patients who were on antibiotics within three months before presentation. Recent antibiotic and anti-gastric acid secretory drugs reduces yield of *H. pylori* cultures (Malfertheiner et al., 2012). Secondly, Veenendaal et al used two antral biopsy specimens while we used one each from the antrum and corpus. This difference in specimen sampling could determine the difference in culture rate as most of the H. pylori is known to reside more in the gastric antrum than the corpus (Dubois, 1995). Thirdly, our study used 1 ml of normal saline as transport media while Veenendaal et al used 0.2 ml of normal saline. In Hachem et al study, Cysteine Albimi media was used

as transport media instead of normal saline. The larger volume in transport media in our study could have caused dilution of organisms resulting in low culture rate (Lwai-Lume et al., 2005). Fourth, in Hachem et al study, incubation was done up to 14 days while in our study culture was done for up to 7 days. Longer incubation periods have been shown to increase the isolation rate (Dubois, 1995). Furthermore Veenendaal et al study was done several years back (1993) before the widespread use *H. pylori* eradication regimes, which could have an effect on culture rate.

Lule et al in a study done locally in Kenya found a culture rate of 70 % in 1991, while Lwai-Lume et al cultured 69 % in 2004 and Kimang'a et al isolated 92.3% in 2010 (Kimang'a et al., 2010; Lule et al., 1991; Lwai-Lume et al., 2005). The major difference between these studies and ours is that for all of them culture was done within 6 hours of sample collection which is expectedly supposed to give a better yield. Furthermore in Lwai-Lume study patients on antibiotics, PPI and H2R blockers within 3 months before culture were excluded.

5.3 Antibiotic susceptibility patterns

None of the *H. pylori* isolated in our study were resistant to clarithromycin. This finding is consistent with findings by Lwai-Lume et al in 2004 that found only 6.4% of *H. pylori* were resistant and another study by Kimang'a et al that found no clarithromycin resistant *H. pylori* among patients with dyspepsia at Aga Khan University Hospital Nairobi (Kimang'a et al., 2010; Lwai-Lume et al., 2005). These findings indicate that *H. pylori* in Kenya is still largely sensitive to clarithromycin.

The minority, 2 (22%), of isolated *H. pylori* were resistant to amoxicillin. This findings support previous studies that showed that *H. pylori* has not developed significant resistance to amoxicillin. Lwai-Lume in 2004 found only 4.6% of *H. pylori* being

resistant to amoxicillin while Kimang'a et al in 2010 did not find any *H. pylori* strains resistant to amoxicillin (Kimang'a et al., 2010; Lwai-Lume et al., 2005). In these two studies, a higher MIC cut of 2mg/l was used to determine the level above which resistance was defined compared to our study that used the MIC cut off of 1mg/l as currently recommended by the clinical laboratory standards institute (CLSI)(CLSI, 2007). The lower cut off in our study could account for a higher resistance in our study. The higher figure of resistance in our study could also be attributable to our small sample size that tends to exaggerate proportions.

All *H. pylori* strains isolated in our study were resistant to metronidazole. This antibiotic is widely used for diarrheal and other conditions where it's often not indicated and this could explain the total resistance by *H. pylori* (Du Prey, Ford, Bassili, & Zoutman, 2004). Resistance of metronidazole is widespread with 10-50% resistance in developed countries and almost all strains in developing countries(Megraud, 1998). When 5-nitroimidazole based therapy is used for treatment of resistant *H. pylori*, the chance of success is reduced by 20% (Megraud et al., 1999). Our findings on metronidazole resistance were similar to those reported by Lwai-Lume et al in 2004 in which all *H. pylori* were resistant and another by Kimang'a et al in 2010 in which 95.4% were resistant(Kimang'a et al., 2010; Lwai-Lume et al., 2005). However in another study by Sang et al in 1991, no strains of *H. pylori* resistant to metronidazole were found(Sang et al., 1991). Sang et al study was done more than two decades ago probably before widespread use of antibiotics, the use of which raises the incidence of antibiotic resistance (Perez et al., 2002).

Thirty three percent of *H. pylori* isolated were resistant to tetracycline. Our findings were higher that previous studies done locally in Kenya. Sang et al in 1991 did not isolate any *H. pylori* resistant to tetracycline while Lwai-Lume et al in 2004 found only

7.9% of *H. pylori* were resistant to tetracycline (Lwai-Lume et al., 2005; Sang et al., 1991). These studies were done more than a decade ago and we postulate that since tetracycline was not part of *H. pylori* eradication regimes frequently used in Kenya, there was less resistance then compared to our recent study. Our small sample size also has the possibility of exaggerating the proportions.

5.5 Study limitations

Our samples were transported in ice in a cooler box but without any temperature charting. This could have resulted in the break in the cold chain resulting in unviable *H. pylori* in specimens that may have become warmer during transport and therefore reducing the culture yield.

The information on recent antibiotic or anti acid drug use was determined by self-reporting without any documentation. This could have had a potential for recall bias as well as inaccuracy as some patients might not have known the exact drugs they used and the last time they used them. Therefore patients who might have used used PPI, H2R blockers and antibiotics in the preceding two weeks could have been erroneously included in the study if they reported that they were not on the drugs, and therefore reducing the culture rate of *H. pylori.*

Several endoscopists were involved in this study and due to difference in technique and experience, this could have caused a difference in sample collection and size as well as inter-observer difference in reporting of gross endoscopic findings.

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Over half of patients with dyspepsia undergoing OGD at MTRH are infected with *H. pylori*.

The culture rate of *H. pylori* following 20 to 24-hour transportation time of samples to a central laboratory using normal saline as transport media is low.

There were almost no resistant strains of *H. pylori* to clarithromycin while a few strains are resistant to amoxicillin and tetracycline. All *H. pylori* strains isolated were resistant to metronidazole.

6.2 Recommendations

All patients with dyspepsia should be tested for *H. pylori* infection and treated if positive, as recommended by most guidelines.

A comparative study to determine the optimal transportation time and transport media is recommended.

A larger study to validate the susceptibility of *H. pylori* to clarithromycin, amoxicillin, metronidazole and tetracycline is recommended.

References

Amieva, M. R., El-Omar, E. M. (2008). Host-bacterial interactions in Helicobacter pylori infection. *Gastroenterology, 134*(1), 306-323.

Andersen, L. P., Holck, S., Povlsen, C. O., Elsborg, L., Justesen, T. (1987). Campylobacter pyloridis in peptic ulcer disease. I. Gastric and duodenal infection caused by C. pyloridis: histopathologic and microbiologic findings. *Scandinavian Journal of Gastroenterology, 22*(2), 219-224.

Bassily, S., Frenck, R. W., Mohareb, E. W., Wierzba, T., Savarino, S., Hall, E., Clemens, J. (1999). Seroprevalence of Helicobacter pylori among Egyptian newborns and their mothers: a preliminary report. *The American journal of tropical medicine and hygiene, 61*(1), 37-40.

Catrenich, C. E., Makin, K. M. (1991). Characterization of the morphologic conversion of Helicobacter pylori from bacillary to coccoid forms. *Scandinavian journal of gastroenterology. Supplement, 181*, 58-64.

Chey, W. D., Wong, B. C. (2007). American College of Gastroenterology guideline on the management of Helicobacter pylori infection. *The American journal of gastroenterology, 102*(8), 1808-1825.

Chopra, I., Roberts, M. (2001). Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiology and Molecular Biology Reviews, 65*(2), 232-260.

Clinical and Laboratory Standards Institute. (2007). *Perfomance Standards for Antimicrobial susceptibility Testing, 17th Informational supplement*, CLSI document *M100-S17*, Pennsylvania, USA

Docampo, R., Moreno, S. N. (1986). Free radical metabolism of antiparasitic agents. *Fed Proc, 45*(10), 2471-2476.

Du Prey, B., Ford, B. D., Bassili, A. R., Zoutman, D. E. (2004). Antimicrobial recommendations by health care providers in Kenya. *American journal of health-system pharmacy, 61*(3), 302, 304.

Dubois, A. (1995). Spiral bacteria in the human stomach: the gastric helicobacters. *Emerging infectious diseases, 1*(3), 79-85.

Ford, A. C., Delaney, B. C., Forman, D., Moayyedi, P. (2004). Eradication therapy in Helicobacter pylori positive peptic ulcer disease: systematic review and economic analysis. *The American journal of gastroenterology, 99*(9), 1833-1855.

Francesco, V. D., Zullo, A., Hassan, C., Giorgio, F., Rosania, R., Ierardi, E. (2011). Mechanisms of Helicobacter pylori antibiotic resistance: An updated appraisal. *World journal of gastrointestinal pathophysiology, 2*(3), 35-41.

Gerrits, M. M., Berning, M., Van Vliet, A. H., Kuipers, E. J., Kusters, J. G. (2003). Effects of 16S rRNA gene mutations on tetracycline resistance in Helicobacter pylori. *Antimicrobial agents and chemotherapy, 47*(9), 2984-2986.

Glupczynski, Y., Broutet, N., Cantagrel, A., Andersen, L. P., Alarcon, T., Lopez-Brea, M., Megraud, F. (2002). Comparison of the E test and agar dilution method for antimicrobial suceptibility testing of Helicobacter pylori. *European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology, 21*(7), 549-552.

Goodman, K. J., Cockburn, M. (2001). The role of epidemiology in understanding the health effects of Helicobacter pylori. *Epidemiology, 12*(2), 266-271.

Goodwin, A., Kersulyte, D., Sisson, G., Veldhuyzen van Zanten, S. J., Berg, D. E., Hoffman, P. S. (1998). Metronidazole resistance in Helicobacter pylori is due to null mutations in a gene (rdxA) that encodes an oxygen-insensitive NADPH nitroreductase. *Molecular microbiology, 28*(2), 383-393.

Graham, D. Y., Fischbach, L. (2010). Helicobacter pylori treatment in the era of increasing antibiotic resistance. *Gut, 59*(8), 1143-1153

Hachem, C. Y., Clarridge, J. E., Evans, D. G., Graham, D. Y. (1995). Comparison of agar based media for primary isolation of Helicobacter pylori. *Journal of clinical pathology, 48*(8), 714-716.

Han, S. W., Flamm, R., Hachem, C. Y., Kim, H. Y., Clarridge, J. E., Evans, D. G., Graham, D. Y. (1995). Transport and storage of Helicobacter pylori from gastric mucosal biopsies and clinical isolates. *European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology, 14*(4), 349-352.

Hazell, S. L., Evans, D. J., Jr., Graham, D. Y. (1991). Helicobacter pylori catalase. *Journal of general microbiology, 137*(1), 57-61.

Hazell, S. L., Lee, A., Brady, L., Hennessy, W. (1986). Campylobacter pyloridis and gastritis: association with intercellular spaces and adaptation to an environment of mucus as important factors in colonization of the gastric epithelium. *The Journal of infectious diseases, 153*(4), 658-663.

Hopkins, R. J., Girardi, L. S., Turney, E. A. (1996). Relationship between Helicobacter pylori eradication and reduced duodenal and gastric ulcer recurrence: a review. *Gastroenterology, 110*(4), 1244-1252.

Jemilohun, A. C., Otegbayo, J. A., Ola, S. O., Oluwasola, O. A., Akere, A. (2010). Prevalence of Helicobacter pylori among Nigerian patients with dyspepsia in Ibadan. *The Pan African medical journal, 6*(18), 698-764.

Kalebi, A., Rana, F., Mwanda, W., Lule, G., Hale, M. (2007). Histopathological profile of gastritis in adult patients seen at a referral hospital in Kenya. *World journal of gastroenterology :, 13*(30), 4117-4121.

Kenya, M. o. H. (2009). peptic ulcer disease. *Clinical guidelines for management and referral of common conditions at levels 4-6 hospitals., 3*, 55-56.

Khalifa, M. M., Sharaf, R. R., Aziz, R. K. (2010). Helicobacter pylori: a poor man's gut pathogen? *Gut pathogens, 2*(1), 2

Kimang'a, A. N., Revathi, G., Kariuki, S., Sayed, S., Devani, S. (2010). Helicobacter pylori: prevalence and antibiotic susceptibility among Kenyans. *South African medical journal, 100*(1), 53-57.

Kwon, D. H., El-Zaatari, F. A., Kato, M., Osato, M. S., Reddy, R., Yamaoka, Y., Graham, D. Y. (2000). Analysis of rdxA and involvement of additional genes encoding NAD(P)H flavin oxidoreductase (FrxA) and ferredoxin-like protein (FdxB) in metronidazole resistance of Helicobacter pylori. *Antimicrobial agents and chemotherapy, 44*(8), 2133-2142.

Lehours, P., Ruskone-Fourmestraux, A., Lavergne, A., Cantet, F., Megraud, F. (2003). Which test to use to detect Helicobacter pylori infection in patients with low-grade gastric mucosa-associated lymphoid tissue lymphoma? *The American journal of gastroenterology, 98*(2), 291-295.

Leodolter, A., Kulig, M., Brasch, H., Meyer-Sabellek, W., Willich, S. N., Malfertheiner, P. (2001). A meta-analysis comparing eradication, healing and relapse rates in patients with Helicobacter pylori-associated gastric or duodenal ulcer. *Alimentary pharmacology & therapeutics, 15*(12), 1949-1958.

Logan, R. P., Walker, M. M. (2001). ABC of the upper gastrointestinal tract: Epidemiology and diagnosis of Helicobacter pylori infection. *British Medical Journal, 323*(7318), 920-922.

Lule, G. N., Sang, F., Ogutu, E. O. (1991). Helicobacter pylori in peptic ulcer disease in Kenya. *East African medical journal, 68*(5), 324-327.

Lwai-Lume, L., Ogutu, E. O., Amayo, E. O., Kariuki, S. (2005). Drug susceptibility pattern of Helicobacter pylori in patients with dyspepsia at the Kenyatta National Hospital, Nairobi. *East African medical journal, 82*(12), 603-608.

Malfertheiner, P., Megraud, F., O'Morain, C. A., Atherton, J., Axon, A. T., Bazzoli, F., Kuipers, E. J. (2012). Management of Helicobacter pylori infection--the Maastricht IV/ Florence Consensus Report. *Gut, 61*(5), 646-664.

Marshall, B. J., McGechie, D. B., Rogers, P. A., Glancy, R. J. (1985). Pyloric Campylobacter infection and gastroduodenal disease. *The Medical journal of Australia, 142*(8), 439-444.

Marshall, B. J., Warren, J. R. (1984). Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet, 1*(8390), 1311-1315.

Mavumba S.P., L. H. (2013). Determination of clearance of Helicobacter pylori through bacterial density, Nairobi, Kenya. *African Journal of Health Sciences, 26*(3), 189-200.

McMahon, B. J., Hennessy, T. W., Bensler, J. M., Bruden, D. L., Parkinson, A. J., Morris, J. M., Butler, J. C. (2003). The relationship among previous antimicrobial use, antimicrobial resistance, and treatment outcomes for Helicobacter pylori infections. *Annals of internal medicine, 139*(6), 463-469.

Megraud, F. (1998). Epidemiology and mechanism of antibiotic resistance in Helicobacter pylori. *Gastroenterology, 115*(5), 1278-1282.

Megraud, F., Lamouliatte, H. (2003). Review article: the treatment of refractory Helicobacter pylori infection. *Alimentary pharmacology & therapeutics, 17*(11), 1333-1343.

Megraud, F., Lehn, N., Lind, T., Bayerdorffer, E., O'Morain, C., Spiller, R.,Burman, C. F. (1999). Antimicrobial susceptibility testing of Helicobacter pylori in a large multicenter trial: the MACH 2 study. *Antimicrobial agents and chemotherapy, 43*(11), 2747-2752.

Megraud, F., Lehours, P. (2007). Helicobacter pylori detection and antimicrobial susceptibility testing. *Clinical microbiology reviews, 20*(2), 280-322.

Meyer, J. M., Silliman, N. P., Wang, W., Siepman, N. Y., Sugg, J. E., Morris, D., Hopkins, R. J. (2002). Risk factors for Helicobacter pylori resistance in the United States: the surveillance of H. pylori antimicrobial resistance partnership (SHARP) study, 1993-1999. *Annals of internal medicine, 136*(1), 13-24.

Midolo, P., Marshall, B. J. (2000). Accurate diagnosis of Helicobacter pylori. Urease tests. *Gastroenterology clinics of North America, 29*(4), 871-878.

Mitchell, H. M., Hu, P., Chi, Y., Chen, M. H., Li, Y. Y., Hazell, S. L. (1998). A low rate of reinfection following effective therapy against Helicobacter pylori in a developing nation (China). *Gastroenterology, 114*(2), 256-261.

Mitchell, H. M., Li, Y. Y., Hu, P. J., Liu, Q., Chen, M., Du, G. G., Hazell, S. L. (1992). Epidemiology of Helicobacter pylori in southern China: identification of early childhood as the critical period for acquisition. *The Journal of infectious diseases, 166*(1), 149-153.

Mwogi, T. S. (, 2013). *Prevalence of Helicobacter pylori infection and endoscopic findings in patients with dyspepsia at Moi Teaching and referral hospital*. Unpublished. MMed. Thesis, Moi University, Eldoret, .

Nakamura, S., Matsumoto, T., Suekane, H., Matsumoto, H., Esaki, M., Yao, T., Iida, M. (2005). Long-term clinical outcome of Helicobacter pylori eradication for gastric mucosa-associated lymphoid tissue lymphoma with a reference to second-line treatment. *Cancer, 104*(3), 532-540.

Ndip, R. N., Malange T., A. E., Ojongokpoko, J. E., Luma, H. N., Malongue, A., Akoachere, J. F., Weaver, L. T. (2008). Helicobacter pylori isolates recovered from gastric biopsies of patients with gastro-duodenal pathologies in Cameroon: current status of antibiogram. *Tropical medicine & international health, 13*(6), 848-854.

Ogutu, E. O., Kang'ethe, S. K., Nyabola, L., Nyong'o, A. (1998). Endoscopic findings and prevalence of Helicobacter pylori in Kenyan patients with dyspepsia. *East African medical journal, 75*(2), 85-89.

Padol, S., Yuan, Y., Thabane, M., Padol, I. T., Hunt, R. H. (2006). The effect of CYP2C19 polymorphisms on H. pylori eradication rate in dual and triple first-line PPI therapies: a meta-analysis. *The American journal of gastroenterology, 101*(7), 1467-1475.

Parsonnet, J., Friedman, G. D., Vandersteen, D. P., Chang, Y., Vogelman, J. H., Orentreich, N., Sibley, R. K. (1991). Helicobacter pylori infection and the risk of gastric carcinoma. *The New England journal of medicine, 325*(16), 1127-1131.

Parsonnet, J., Hansen, S., Rodriguez, L., Gelb, A. B., Warnke, R. A., Jellum, E., Friedman, G. D. (1994). Helicobacter pylori infection and gastric lymphoma. *The New England journal of medicine, 330*(18), 1267-1271.

Pellicano, R., Franceschi, F., Saracco, G., Fagoonee, S., Roccarina, D., Gasbarrini, A. (2009). Helicobacters and extragastric diseases. *Helicobacter, 14 Suppl 1*, 58-68

Perez-Perez, G. I. (2000). Accurate diagnosis of Helicobacter pylori. Culture, including transport. *Gastroenterology clinics of North America, 29*(4), 879-884.

Perez Aldana, L., Kato, M., Nakagawa, S., Kawarasaki, M., Nagasako, T., Mizushima, T., Asaka, M. (2002). The relationship between consumption of antimicrobial agents and the prevalence of primary Helicobacter pylori resistance. *Helicobacter, 7*(5), 306-309.

Perna, F., Ricci, C., Gatta, L., Bernabucci, V., Cavina, M., Miglioli, M., Vaira, D. (2005). Diagnostic accuracy of a new rapid urease test (Pronto Dry), before and after treatment of Helicobacter pylori infection. *Minerva gastroenterologica e dietologica, 51*(3), 247-254.

Qu, X. H., Huang, X. L., Xiong, P., Zhu, C. Y., Huang, Y. L., Lu, L. G., Yan, W. L. (2010). Does Helicobacter pylori infection play a role in iron deficiency anemia? A meta-analysis. *World journal of gastroenterology*, *16*(7), 886-896.

Romano, M., Marmo, R., Cuomo, A., De Simone, T., Mucherino, C., Iovene, M. R., Nardone, G. (2003). Pretreatment antimicrobial susceptibility testing is cost saving in the eradication of Helicobacter pylori. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association*, *1*(4), 273-278.

Sang, F. C., Lule, G. N., Ogutu, E. O. (1991). Evaluation of culture media and antimicrobial susceptibility of Helicobacter pylori. *East African medical journal*, *68*(11), 865-868.

Sharma, V. K., Sahai, A. V., Corder, F. A., Howden, C. W. (2001). Helicobacter pylori eradication is superior to ulcer healing with or without maintenance therapy to prevent further ulcer haemorrhage. *Alimentary pharmacology & therapeutics*, *15*(12), 1939-1947.

Shmuely, H., Obure, S., Passaro, D. J., Abuksis, G., Yahav, J., Fraser, G., Niv, Y. (2003). Dyspepsia symptoms and Helicobacter pylori infection, Nakuru, Kenya. *Emerging infectious diseases*, *9*(9), 1103-1107.

Spiegelhalder, C., Gerstenecker, B., Kersten, A., Schiltz, E., Kist, M. (1993). Purification of Helicobacter pylori superoxide dismutase and cloning and sequencing of the gene. *Infection and immunity*, *61*(12), 5315-5325.

Suzuki, T., Matsuo, K., Sawaki, A., Ito, H., Hirose, K., Wakai, K., Tajima, K. (2006). Systematic review and meta-analysis: importance of CagA status for successful eradication of Helicobacter pylori infection. *Alimentary pharmacology & therapeutics*, *24*(2), 273-280.

Tack, J., Talley, N. J., Camilleri, M., Holtmann, G., Hu, P., Malagelada, J. R., Stanghellini, V. (2006). Functional gastroduodenal disorders. *Gastroenterology, 130*(5), 1466-1479.

Tanih, N. F., Okeleye, B. I., Ndip, L. M., Clarke, A. M., Naidoo, N., Mkwetshana, N., Ndip, R. N. (2010). Helicobacter pylori prevalence in dyspeptic patients in the Eastern Cape province - race and disease status. *South African medical journal, 100*(11), 734-737.

Thiede, C., Wundisch, T., Alpen, B., Neubauer, B., Morgner, A., Schmitz, M., Neubauer, A. (2001). Long-term persistence of monoclonal B cells after cure of Helicobacter pylori infection and complete histologic remission in gastric mucosa-associated lymphoid tissue B-cell lymphoma. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology, 19*(6), 1600-1609.

Trieber, C. A., Burkhardt, N., Nierhaus, K. H., Taylor, D. E. (1998). Ribosomal protection from tetracycline mediated by Tet(O): Tet(O) interaction with ribosomes is GTP-dependent. *Biological Chemistry, 379*(7), 847-855.

Tucci, A., Corinaldesi, R., Stanghellini, V., Tosetti, C., Di Febo, G., Paparo, G. F. (1992). Helicobacter pylori infection and gastric function in patients with chronic idiopathic dyspepsia. *Gastroenterology, 103*(3), 768-774.

Uemura, N., Mukai, T., Okamoto, S., Yamaguchi, S., Mashiba, H., Taniyama, K., Kajiyama, G. (1997). Effect of Helicobacter pylori eradication on subsequent development of cancer after endoscopic resection of early gastric cancer. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology, 6*(8), 639-642.

Uemura, N., Okamoto, S., Yamamoto, S., Matsumura, N., Yamaguchi, S., Yamakido, M., Schlemper, R. J. (2001). Helicobacter pylori infection and the development of gastric cancer. *The New England journal of medicine, 345*(11), 784-789.

Van Amsterdam, K., Bart, A., van der Ende, A. (2005). A Helicobacter pylori TolC efflux pump confers resistance to metronidazole. *Antimicrobial agents and chemotherapy, 49*(4), 1477-1482.

Veenendaal, R. A., Lichtendahl-Bernards, A. T., Pena, A. S., Endtz, H. P., van Boven, C. P., Lamers, C. B. (1993). Effect of transport medium and transportation time on culture of Helicobacter pylori from gastric biopsy specimens. *Journal of clinical pathology, 46*(6), 561-563.

Wong, B. C., Lam, S. K., Wong, W. M., Chen, J. S., Zheng, T. T., Feng, R. E., Ching, C. K. (2004). Helicobacter pylori eradication to prevent gastric cancer in a high-risk egion of China: a randomized controlled trial. *The journal of the American Medical Association, 291*(2), 187-194.

Appendices

Appendix I: Data Entry Form

1) Patient identification and contacts

Patient Number ☐ Telephone ☐

2) Demographic data

Age in years ☐ Sex ☐

Residence ☐ Occupation ☐

3) Medical history

A. Which of the following symptoms does the patient have as the reason for referral for endoscopy? (Tick where appropriate)

☐ Epigastric pain
(Upper abdominal pain)

☐ Dysphagia
(Difficulty in swallowing)

☐ Postprandial fullness
(Upper abdominal fullness after eating)

☐ Early satiety
(Feeling full earlier than expected after

B. Previous diagnosis of upper gastrointestinal disease

I. Have you ever been investigated for upper gastrointestinal disease before?

Yes ☐ No ☐

II. If yes what was the date, mode of investigation and results?

Method : Endoscopy ☐ Barium studies ☐ *H.pylori* testing ☐

Date of the testing/Procedure ☐

Results ☐ Don't know ☐

If *H. pylori* were tested, which method was used?

Serology ☐ UBT ☐ SAT ☐ RUT ☐

Histology ☐ Culture ☐ Don't know ☐

What were the results of *H. pylori* testing?

Positive ☐ Negative ☐ Don't know ☐

C. Previous treatment of *H. pylori*

I. Have you ever been treated for *H. pylori* infection? Yes ☐ No ☐

Don't know ☐

II. If yes, which of these drugs were used?

PPI ☐ H2R blocker ☐ Bismuth salt ☐ clarithromycin ☐

Amoxicillin ☐ Metronidazole ☐ Tetracycline ☐ Tinidazole ☐

Fluoroquinolone ☐ Don't know ☐

III. How long ago?(months) ☐ Don't know ☐

IV. Duration of treatment in days ☐ Don't know ☐

D. Recent medication use.

Have you been on any of the following medications in the last two (2) weeks?

(Tick where appropriate)-From patient prescription notes or from patient history

Proton pump inhibitors ☐ H2 receptor blocker ☐ Antibiotics ☐

☐ ☐ ☐

Antifungals Bismuth salts DON'T KNOW

4). Endoscopic findings

A) Esophagus (Tick where appropriate and indicate level of lesion in centimeters)

Normal ☐ Erosions ☐ ulceration ☐ growth ☐

varices ☐ Others (Describe)

B) Stomach (Tick where appropriate and indicate the site after the tick)

Normal ☐ erosions ☐ ulcerations ☐ growth ☐

atrophy ☐ Others (Describe)

C) Duodenum (Tick where appropriate and indicate the site after the tick)

Normal ☐ Erosions ☐ Ulcerations ☐

Others (Describe)

5) Rapid urease test results.

Negative at 24 hrs ☐

Positive at: 30mins ☐ 1hr ☐ 4hrs ☐ 24hrs ☐

6) Culture results

A. *H. pylori* cultured ☐ *H. pylori* not cultured ☐

B. Duration to identification of satisfactory culture of *H. pylori*

3 days ☐ 5 days ☐ 7 days ☐

7) **Antibiotic sensitivity results (MIC in µg/ml)**

clarithromycin ☐ amoxicillin ☐ metronidazole ☐ tetracycline ☐

Appendix II: Consent Form-English version

Patient Number | | Date | |

I, DR CHURYAI RAYMOND, am student in the department of medicine, Moi University and I am carrying out a study to determine the culture rate, prevalence and antibiotic sensitivity of *Helicobacter pylori* infection among patients undergoing endoscopy at Moi Teaching and Referral Hospital. *Helicobacter pylori* is an organism that infects the stomach and is known to cause peptic ulcer disease and other diseases of the stomach and upper intestines and could be responsible for your reason for referral to undergo endoscopy.

The study is not the endoscopy procedure that you have been referred to undergo. It involves a collection of your personal details like name, age and address. During the endoscopy procedure, a small biopsy of your stomach lining will be taken and tested if you have Helicobacter pylori infection. If the results turn positive within 4 hours, your sample will be sent to the laboratory for culture and antibiotic sensitivity testing. This will further confirm the infection with *H. pylori* and will determine whether the antibiotics you will be given will be effective. If the *H. pylori* test doesn't turn positive within 4 hours, the test will be read after 24 hours and results given to you by phone or in person, although your samples will not be taken for further test of antibiotic susceptibility because it will be beyond the recommended time. If it turns positive within 24 hours, you will be treated for *H. pylori* infection. You will not be charged any additional fee for the test *H. pylori* testing, culture and sensitivity. You will be informed of the test results at every stage as soon as they are received. There is the inconvenience that the endoscopy procedure will take longer and a small risk of stomach lining bleed during endoscopy. If sedation is used during endoscopy, you may feel some drowsiness for a few minutes after the procedure.

You are free to withdraw from the study at any time before or after the procedure. Your withdrawing from the study does not interfere with you undergoing the endoscopy and receiving the usual standard of care. All your personal information and results will be treated with confidentiality.

I agree to participate in the study,

NAME_____ SIGN_____

(Patient/Guardian)

WITNESS_____ SIGN_____

(Nurse/Doctor)

Appendix III: Consent Form-Kiswahili version

Nambari ya mgonjwa | | Tarehe | |

Mimi, DR CHURYAI RAYMOND, ni mwanafunzi katika Chuo Kikuu Cha Moi kitengo cha Internal Medicine,na ninafanya utafiti ili kujua kama chembe chembe cha H.pylori inaweza kukuzwa kwa mahabari baada ya kusafirishwa kwa masaa 24.Pia utafiti utaonyesha kiwango cha wagonjwa walioambukizwa chembe chembe cha H. pylori na kama inaweza kumalizwa na madawa tunayoitumia kukitibu katika wagonjwa wanaofanyiwa endoscopy hapa Moi Teaching and Referral Hospital. Helicobacter pylori ni chembe chembe cha bacteria inayoambukiza tumbo na kusababisha vidonda vya tumbo kando na magonjwa mengine ya tumbo na matumbo na inaweza kua ni chanzo cha sababu umetumwa hapa kufanyiwa endoscopy.

Utafiti huu sio endospcopy uliotumwa kufanyiwa. Utafiti utajumuisha kuulizwa maswali kukuhusu na kuhusu ugonjwa wako na kupimwa kwa sampuli kidogo ya tumbo.Wakat wa endoscopy, sampuli kidogo itatolewa kutoka kwa tumbo lako na kupimwa hapa kama umeambukizwa ugonjwa wa H.pylori.Majibu ya kuambukizwa yatasomwa baada ya nusu saa, saa moja, masaa manne,na masaa ishirini na nne.Kama majibu baada ya masaa manne yataonyesha kuwa umeambukizwa, sampuli hiyo itapelekwa mahabara kupimwa kama itamea, na kama itamea itapimwa kama inaweza kumalizwa na madawa ambayo huwa tunaitumia kuitibu. Lakini kama majibu baada ya masaa manne yataonyesha kuwa hujaambukizwa,itasomwa tena baada ya masaa ishirini na nne lakini sampuli hiyo haitapelekwa mahabara kwa sababu masaa yanayohitajika yatakua yamepita.

Tutakupigia simu kukujulisha juu ya majibu yatakayotokea baada ya wewe kutoka hapa .Ukipatikana na ugonjwa huu, utapewa dawa inayostahili. Hautalipishwa ada zozote

zaidi kwa vitu vyote vitakavyofanwa kwa sababu ya utafiti huu. Kuna uwezekano ya kuwa endoscopy utachukua muda zaidi kidogo na kutokwa na damu kidogo tumboni.Kama madawa ya kutuliza yatatumiwa, utaweza kusikia kusinzia kidogo mpaka dakika chache baada ya endoscopy.

Una ruhusa kukataa kushiriki katika utafiti huu wakati wowote bila kukatazwa kufanyiwa endoscopy au kupewa matibabu yote yanayostahili.

Ninakubali kushiriki katika utafiti huu,

JINA_____ SAHIHI_____

(mgonjwa/msimamizi)

SHAHIDI_____ SAHIHI_____

(Daktari/msaidizi)

Appendix IV: Rapid urease test

(Adopted from ESOKIT® Hp test)

Test Kit Contents

Each test kit consists of a twin well cartridge containing a substrate tablet in each well and an ampoule. The substrate tablet contains urea, phenol red and buffer salts in tablet form and an ampoule of buffer.

Principle of the test

If the urease enzyme of *Helicobacter pylori* is present in a biopsy specimen, the rise in pH associated with the hydrolysis of urea causes a change in color from yellow to pink/red.

Method

The cartridge lid is opened and each well filled with the buffer to a level marked on the well. All buffers are noted to ensure they are colorless before proceeding. If after addition of the buffer the reaction well in pink/red in color, the test kit is discarded. The lid is then closed and then agitated to dissolve the tablet in each well.

During endoscopy, a biopsy specimen, two to three millimeters in size, one from from the prepyloric antrum is added to well one. Another biopsy specimen of same size from the corpus is added to well two. Well two can be used as the negative control if no specimen is added. The lid is then closed immediately afterwards. On the label, the patients number, date and time the specimens were inserted are noted.

Pink/red coloration in the reaction wells indicates a positive reaction and therefore confirms the presence *of Helicobacter pylori*. Both wells do not have to give the same

color change for it to be regarded as positive. Any color change to pink/red is regarded as positive. A positive result will be obtained within 30 minutes. If the result is negative after 30 minutes, further color checks are made at 1 hour, 4 hour and 24 hours. Color changes after 24 hours will be regarded as negative.

Test Specificity and Sensitivity

The rapid urease test has sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy as 98%, 100%, 100%, 98% and 99% respectively.

False positive and negative results

False positive tests are unusual. However, false negative results can occur in patients with recent gastrointestinal bleeding or with the use of Proton pump inhibitors, H2 receptor antagonists, antibiotics, or bismuth-containing compounds. Obtaining tissue samples from the antrum and the fundus increases the sensitivity of the test.

Choice of RUT kit

Esokit Hp Test is the available RUT in our setting. Although we could not find studies comparing this brand and other brands, it has been previously been used in other studies here at MTRH and in Nairobi.(Mavumba S.P., 2013; Mwogi, , 2013) .

Appendix V: The Upper Gastrointestinal Endoscopy Procedure

Preparation

Patients are usually informed about the procedure and an informed consent obtained. Patients are usually advised to fast from midnight of the day of the procedure, ensuring 8 hours diet free period.

The Procedure

Upper gastrointestinal endoscopy is usually performed on an outpatient basis unless the patient being referred is an inpatient. It is carried out at the MTRH endoscopy unit by consultant gastroenterologists or endoscopy surgeon. On arrival at the endoscopy unit, the patient is assessed for compliance with the preparation and whether the patient is fit to undergo the procedure.

The patient is positioned in the left lateral position; the throat is anesthetized by a spray or liquid 10% lidocaine. Intravenous sedation with diazepam 5-10mg is usually given to relax the patient, deaden the gag reflex and cause short-term amnesia. If it is a therapeutic procedure, intravenous propofol or fentanyl may be used. For some individuals who can relax on their own and whose gagging can be controlled, the exam is done without intravenous sedation.

The endoscope is then gently inserted into the upper esophagus. The patient can breathe easily throughout the exam. Other instruments can be passed through the endoscope to perform additional procedures if necessary. A polyp or tumor can be removed using a thin wire snare and electro cautery. Mucosal biopsy is taken using biopsy forceps, 2 each from the antrum and the corpus. The exam takes from 15 to 30 minutes, after

which the patient is taken to the recovery area. There is no pain with the procedure and patients seldom remember much about it.

Results

The results are then written on a standard endoscopy form indicating the findings throughout the upper gastrointestinal tract. Comments and recommendations are also indicated.

Side effects and Risks

A temporary, mild throat irritation sometimes occurs after the exam. Serious risks with upper GI endoscopy, however, are very uncommon. One such risk is excessive bleeding, especially with removal of a large polyp. In extremely rare instances, a perforation, or tear, in the esophagus or stomach wall can occur.

These complications may require hospitalization and, rarely, surgery. Quite uncommonly, a diagnostic error or oversight may occur since this is largely user dependent. Due to the mild sedation, the patient should not drive or operate machinery following the exam. For this reason, someone else should be available to drive the patient home.

Appendix VI: Culture media preparation

Inactivation of fetal bovine serum (FBS)

FBS to be used for culture media preparation is completely thawed at room temperature, transferred to a water bath at 56^0C and stirred manually every 10 minutes. When the FBS reaches 56^0C (indirectly measured by the temperature of the water bath), it is incubated for 30 minutes, cooled to room temperature, dispensed in aliquots in 35 ml into tubes labeled 'Inactivated FBS', and stored at -20^0C. Sterility control is performed by incubating an aliquot of the FBS at 37^0C for 48 hours.

H. pylori selective supplement (Dent) and nutritional' supplement (Vitox)

To a bottle of Dent, 2 ml of sterile distilled water is added and mixed gently. The solution is used the same day. Vitox is prepared by mixing the provided solvent and the Vitox powder. All are prepared according to the manufacturers' instructions.

Non-selective culture media: (BHIA + *7%* FBS)

BHIA (23.5+0.1 g) (Oxoid, UK) were each weighed and put in 500 ml bottles. The media is suspended in 500±0.5 ml sterile distilled water and fully dissolved by boiling. These bottles are labeled 'BHIA+7% FBS' and autoclaved at 121 °C for 15 minutes. After autoclaving, the media are cooled to 45°C in a water-bath and gently mixed with 35 ml aliquots of the inactivated FBS and the Vitox supplement. The media are poured (approximately 25 ml per plate), left to solidify and dried at room temperature for 2 hours before being stored at 8"'C in a refrigerator until use. The plates are used within 1 week from the

preparation date. Sterility control on 2 plates from every 20 plates are done at 37°C for 24 hours, 1 under aerobic and 1 under micro-aerophilic conditions.

Selective culture media: BHI agar + 7% FBS + Dent plates (BHIA D + 7% FBS)

(BHIAD = brain- heart- infusion agar with Dent selective supplement containing, vancomycin, trimethoprim, cefsulodin and amphotericin B.)

BHI A (23.5±0.1 g) (Oxoid, UK) are weighed and put in 500±0.5 ml of sterile bottles. The media are suspended in 500±5 ml of sterile distilled water and are fully dissolved by boiling. These bottles are labeled 'BHIAD + 7% FBS' , autoclaved at 121^0C for 15 minutes, cooled down to 45 ^{0}C in a water-bath, and gently mixed with the inactivated FBS; thereafter, Dent and Vitox are added and mixed by gently rolling the bottle. The media are poured (approximately 25 mi per plate), left to solidify and dried at room temperature for 2 hours before being stored at 8°C in a refrigerator until use. The plates are used within 1 week from the preparation day. Sterility control at 37°C for 24 hours on two plates from every pack of 20 plates is done; 1 under aerobic and 1 under micro-aerophilic conditions.

Appendix VII: Procedure for *H. pylori* culture

The biopsies are transferred by sterile 1 000µl pipette to clean sterile tubes, where they are completely crushed using the pipette tip; 200 µl of BHI broth, enriched with 5% FBS, are added and uniformly mixed. Next, 100 µl of the ruptured and homogenised material are aseptically transferred to each of the appropriately labelled plates, one selective and the other non-selective, using a disposable 10 µl sterile inoculating loop. The inoculum is aseptically spread, on the surface of the plates. Inverted, the plates are placed in a 2.5 1 jar. A Campy Micro-aerophilic kit is added, and the jar immediately closed, and incubated undisturbed at 37°C for 3 days. After 3 days, the plates are removed and visually inspected. When the colonies appear too small, a new Campy Micro-erophilic kit is placed in the 2.51 jar and the plates re-incubated immediately for a further 2 days. If growth is not satisfactory after 5 days, further incubation is done up to 7 days with a new Campy Micro-aerophilic kit until the colonies are well visible. *H. pylori* identification is confirmed by colony morphology, Gram-stain, and oxidase, urease and catalase positivity. Old cultures are a mixture of short rods and coccoids, while young cultures are curved rods. Subcultures are done on antibiotic-free media. Pure culture are harvested by transferring 200 µl phosphate-buffered saline (PBS) onto the plate. The pure bacteria growth on the plate is emulsified in the PBS using a sterile inoculating loop. The suspension is then transferred to cryotubes containing 500 µl BHI broth enriched with 20% glycerol and 5% FBS, and stored at -80^0C

Appendix VIII: Procedure for antibiotic sensitivity testing.

A frozen vial of the correctly identified *H. Pylori* is thawed at room temperature and mixed by pippeting up and down with a micropipette. This sample is used for subculture. Young, pure cultures are harvested by transferring 200 µl PBS onto the cultures plate and bacteria emulsified into a suspension using a sterile inoculating loop. The suspension is collected into a sterile tube and a McFarland's turbidity standard no 3 made for use in susceptibility testing. The inoculum is aseptically spread over the entire portion of the surface of the sensitivity test plate, and allowed to stand at room temperature for 10 minutes before the antibiotic E-test strips for clarithromycin, amoxicillin and tetracycline (range 0.016-256µg/ml) and metronidazole (range 0.002-32µg/ml.amoxicillin) are applied. Inverted inoculated plates are then placed into the jar. One envelope of Campy Micro-aerophic gas kit is put into the 2.51 jar following the manufacturer's instructions. The jar is incubated at 37^0 for 24 hours, and then removed and inspected for satisfactory *H. pylori* growth, if the growth are insufficient, the plates are immediately put back into the jar with a new CampyGen Micro-aerophilic gas kit, and incubation continued at 37^0 for a further 12 hours. When growth is satisfactory, the minimum inhibiting concentration (MIC) for each antibiotic is read as recommended by the National Committee on Clinical Laboratory Standards / Clinical and Laboratory Standards Institute (NCCLS/CLSI) standards.

Appendix IX: IREC Approval of amendment

INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)

MOI TEACHING AND REFERRAL HOSPITAL
P.O. BOX 3
ELDORET
Tel: 33471/2/3

MOI UNIVERSITY
SCHOOL OF MEDICINE
P.O. BOX 4606
ELDORET
Tel: 33471/2/3

Reference
11th March, 2015

IREC/2013/140
Approval Number: 0001048

Dr. Churyai Raymond,
Moi University,
School of Medicine,
P.O. Box 4606-30100,
ELDORET-KENYA.

INSTITUTIONAL RESEARCH & ETHICS COMMITTEE
11 MAR 2015
APPROVED
P. O. Box 4606-30100 ELDORET

Dear Dr. Churyai,

RE: APPROVAL OF AMENDMENT

The Institutional Research and Ethics Committee has reviewed the amendment made to your proposal titled:-

"Helicobacter Pylori Culture Isolation rate following 24 Hour Transportation Time".

We note that you are seeking to make an amendment as follows:-

1. To change the title as above from: ***"Helicobacter Pylori Antibiotic Susceptibility among Patients with Dyspepsia at Moi Teaching and Referral Hospital".***

The amendment has been approved on 11th March, 2015 according to SOP's of IREC. You are therefore permitted to continue with your research.

You are required to submit progress(s) regularly as dictated by your proposal. Furthermore, you must notify the Committee of any proposal change(s) or amendment(s), serious or unexpected outcomes related to the conduct of the study, or study termination for any reason. The Committee expects to receive a final report at the end of the study.

Sincerely,

PROF. E. WERE
CHAIRMAN
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

cc: Director - MTRH
Principal- CHS
Dean - SPH
Dean - SOD
Dean - SOM
Dean - SON

Appendix X: IREC formal approval

INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)

MOI TEACHING AND REFERRAL HOSPITAL
P.O. BOX 3
ELDORET
Tel: 33471/2/3

MOI UNIVERSITY
SCHOOL OF MEDICINE
P.O. BOX 4606
ELDORET

Reference: IREC/2013/140
Approval Number: 0001048

29th August, 2013

Dr. Churyal Raymond,
Moi University,
School of Medicine,
P.O. Box 4606-30100,
ELDORET-KENYA.

Dear Dr. Churyal,

RE: FORMAL APPROVAL

The Institutional Research and Ethics Committee have reviewed your research proposal titled:-

"Helicobacter Pylori Antibiotic Susceptibility among Patients with Dyspepsia at Moi Teaching and Referral Hospital".

Your proposal has been granted a Formal Approval Number: ***FAN: IREC 1048*** on 29th August, 2013. You are therefore permitted to begin your investigations.

Note that this approval is for 1 year; it will thus expire on 28th August, 2014. If it is necessary to continue with this research beyond the expiry date, a request for continuation should be made in writing to IREC Secretariat two months prior to the expiry date.

You are required to submit progress report(s) regularly as dictated by your proposal. Furthermore, you must notify the Committee of any proposal change (s) or amendment (s), serious or unexpected outcomes related to the conduct of the study, or study termination for any reason. The Committee expects to receive a final report at the end of the study.

Sincerely,

03/09/2013
DR. W. ARUASA
DEPUTY-CHAIRMAN
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

APPROVED
29 AUG 2013

cc Director- MTRH Dean - SOM Dean - SON
Principal- CHS Dean - SPH Dean - SOD

Appendix XI: IREC Continuing approval

INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)

MOI TEACHING AND REFERRAL HOSPITAL
P.O. BOX 3
ELDORET
Tel: 33471/2/3

MOI UNIVERSITY
SCHOOL OF MEDICINE
P.O. BOX 4606
ELDORET
Tel: 33471/2/3

Reference: IREC/2013/140
Approval Number: 0001048

29th August, 2014

INSTITUTIONAL RESEARCH & ETHICS COMMITTEE
29 AUG 2014
APPROVED
P. O. Box 4606-30100 ELDORET

Dr. Churyal Raymond,
Moi University,
School of Public Health,
P.O. Box 4606-30100,
ELDORET-KENYA.

Dear Dr. Churyal,

CONTINUING APPROVAL

The Institutional Research and Ethics Committee has reviewed your request for continuing approval to your study titled:-

"Helicobacter Pylori Antibiotic Susceptibility among Patients with Dyspepsia at Moi Teaching and Referral Hospital".

Your proposal has been granted a Continuing Approval with effect from 29th August, 2014. You are therefore permitted to continue with your study.

Note that this approval is for 1 year; it will thus expire on 28th August, 2015. If it is necessary to continue with this research beyond the expiry date, a request for continuation should be made in writing to IREC Secretariat two months prior to the expiry date.

You are required to submit progress report(s) regularly as dictated by your proposal. Furthermore, you must notify the Committee of any proposal change (s) or amendment (s), serious or unexpected outcomes related to the conduct of the study, or study termination for any reason. The Committee expects to receive a final report at the end of the study.

Sincerely,

PROF. E. WERE
CHAIRMAN
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

cc:
Director	-	MTRH
Principal	-	CHS
Dean	-	SOM
Dean	-	SPH
Dean	-	SOD
Dean	-	SON

Appendix XII: MTRH approval

MOI TEACHING AND REFERRAL HOSPITAL

Telephone: 2033471/2/3/4
Fax: 61749
Email: director@mtrh.or.ke
P. O. Box 3
ELDORET

Ref: ELD/MTRH/R.6/VOL.II/2008

29th August, 2013

Dr. Churyai Raymond,
Moi University,
School of Medicine,
P.O Box 4606-30100,
ELDORET-KENYA.

RE: APPROVAL TO CONDUCT RESEARCH AT MTRH

Upon obtaining approval from the Institutional Research and Ethics Committee (IREC) to conduct your research proposal titled:-

"Helicobacter Pylori Antibiotic Susceptibility among Patients with Dyspepsia at Moi Teaching and Referral Hospital".

You are hereby permitted to commence your investigation at Moi Teaching and Referral Hospital.

DR. J. KIBOSIA
DIRECTOR
MOI TEACHING AND REFERRAL HOSPITAL

CC - Deputy Director (CS)
- Chief Nurse
- HOD, HRISM

Appendix XIII: Pathologists Lancet Kenya Accreditation

sanas
South African National Accreditation System

CERTIFICATE OF ACCREDITATION

LANCET KENYA LIMITED
UPPERHILL NAIROBI LABORATORY
Co. Reg. No.: C168507

Facility Accreditation Number: **M0462**

is a South African National Accreditation System accredited laboratory provided that all SANAS conditions and requirements are complied with

This certificate is valid as per the scope as stated in the accompanying schedule of accreditation, Annexure "A", bearing the above accreditation number for

MEDICAL TESTING LABORATORY
CHEMISTRY, ENDOCRINOLOGY, HAEMATOLOGY, MICROBIOLOGY AND MOLECULAR BIOLOGY

The facility is accredited in accordance with the recognised International Standard

ISO 15189:2012

The accreditation demonstrates technical competency for a defined scope and the operation of a laboratory quality management system

While this certificate remains valid, the Accredited Facility named above is authorised to use the relevant SANAS accreditation symbol to issue facility reports and/or certificates

Dr E Steyn
Acting Chief Executive Officer
Effective Date: 23 December 2014
Certificate Expires: 22 December 2018