FACTORS ASSOCIATED WITH TREATMENT OUTCOME OF *Helicobacter pylori* AMONG DYSPEPTIC PATIENTS USING CLARITHROMYCIN BASED THERAPY AT MUHIMBILI NATIONAL HOSPITAL.

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By

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A dissertation Submitted in (partial) Fulfillment of the Requirements for the Degree of Master of Medicine at

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CERTIFICATION

The undersigned certifies that, they have read and hereby recommend for acceptance by Muhimbili University of Health and Allied Sciences a dissertation entitled: **"Factors associated with treatment outcome of *H. pylori* among dyspeptic patients using clarithromycin-based therapy at Muhimbili National Hospital**" in partial fulfillment of requirement for the degree of Master of medicine in Internal Medicine Muhimbili University of Health and Allied Sciences.

_____

Dr. Ewaldo Komba

(Supervisor)

Date: _____

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I, **Dalila Mwindadi**, declare that this **dissertation** is my own original work and that it has not been presented and will not be presented to any other University for a similar or any other degree award.

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DEDICATION

I dedicate this dissertation to my beloved parents, my father Dr Mwindadi and my mother Fatuma Hamisi.

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LIST OF ABBREVIATIONS

ACG	American College of Gastroenterology
CagA	Cytotoxin-associated antigen A
CLO	Campylobacter like organism
C. pylori	*Campylobacter pylori*
DupA	Duodenal ulcer-promoting gene A
EIA	Enzyme immunoassay
GERD	Gastroesophageal Reflux Disease
HomB	Helicobacter outer membrane B protein
H. pylori	*Helicobacter pylori*
ICA	Immunochromatography
MALT	Mucosa associated lymphoid tissue lymphoma
MIC	Minimum inhibitory concentration
MNH	Muhimbili National Hospital
MUHAS	Muhimbili University of Health and Allied Sciences
OGD	Oesophago-Gastro-Duodenoscopy
PCR	Polymerase Chain Reaction
PPI	Proton Pump Inhibitor
PUD	Peptic ulcer disease
RUT	Rapid urease test
SAT	Stool Antigen Test
UBT	Urea Breath Test
UGIB	Upper Gastrointestinal Bleeding
VacA	Vacuolating cytotoxin A gene

DEFINITION OF TERMS

Dyspepsia: The Rome IV criteria define dyspepsia as, “any combination of 4 symptoms: postprandial fullness, early satiety, epigastric pain, and epigastric burning that are severe enough to interfere with the usual activities and occur at least 3 days per week over the last 3 months with an onset of at least 6 months in advance.” (1)

Functional dyspepsia: Is a medical condition that significantly impacts on the usual activities of a patient and is characterized by one or more of the following symptoms; postprandial fullness, early satiation, epigastric pain and epigastric burning that are unexplained after a routine clinical evaluation. (1)

Uninvestigated dyspepsia: Refers to patients with new or recurrent dyspeptic symptoms in whom no investigations have previously been done. (2)

Antimicrobial sensitivity: Is a test done to help choose antibiotics that will be most effective against the specific types of bacteria or fungus infection an individual person.(3)

ABSTRACT

Background: *Helicobacter pylori* affects 50 % of the world population, the highest rate of infections seen in developing nations due to poor socioeconomic status and subpar medical services. Although treatment has been available for more than 2 decades, currently failure of treatment with the standard regimen has been noticed. Several factors are seen attributed to the treatment failure, including but not limited to, antibiotic resistance, socio-economic status, diet and many more. Few studies have been done in Africa, and fewer in East Africa to determine the factors that influence treatment outcome of *H. pylori* infection.

Objectives: To determine the factors associated with treatment outcome of *H. pylori* infection with clarithromycin-based therapy among dyspeptic patients at MNH Upanga

Methodology: A cross-sectional study at the Endoscopy unit at MNH Upanga was conducted between July 2022 to February 2023. All dyspeptic patients with stool antigen positive for *H. pylori* were given first line therapy (metronidazole, clarithromycin and PPI), and stool antigen was repeated 6 weeks after completion of the treatment. Biopsies were taken among those with positive stool antigen for *H. pylori* after 6 weeks and culture and AST was done. Data of socio-demographics, endoscopy results, adherence to treatment, previous treatment with triple therapy and failure to eradicate *H. pylori* was tested for after 6 weeks follow up were documented. SPSS version 23 was used for data analysis with statistically significant association from a p-value <0.05.

Results: Cumulatively 385 patients with stool antigen for *H. pylori* were enrolled; mean age (±SD) was 41 (±14) years. Females were more than half of the patients 213 (55.3%), 171 (44.4%) were college graduates, and approximately half of the patients were self-employed 178 (46.2%). Only 11 (3%) were currently smoking, one third reported alcohol use 149 (38.7%).

First line treatment failure as defined positive stool antigen 6 weeks post treatment was observed in 132 (38.3%) of patients. Independent predictors of first line treatment failure were smoking (OR:7.9, 95%CI (0.93 – 6.7), P = 0.03), concomitant herbal medication (OR:6.7, 95%CI (3.9 – 11.6), P <0.001), endoscopy findings (OR:1.7, 95%CI (0.61 – 3.71), P =0.48) and adherence to

treatment (OR:6.2, 95%CI (3.52 – 10.8), P<0.001). From 130 patients 86 (66.15%) were inoculated and AST was done, antibiotic resistant patterns were 30% amoxicillin, 62.8% metronidazole, 30% and for clarithromycin.

Conclusion: About one third of patients showed no eradication from clarithromycin- based therapy which is usually the first line used. This is below the guideline recommended value. Poor adherence, concurrent use of herbal medication and cigarette smoking were seen as significant causes of poor eradication of *H. pylori* eradication. The resistance to all medication used as first line in the management of *H. pylori* are still staggeringly high. Treatment pf *H. pylori* infection should be individualized per patient as not all patients will respond to the same regimen due to increased antibiotic resistance.

CHAPTER ONE

1 INTRODUCTION

1.1 Background

1.1.1 Burden Of *H. Pylori* Infection

Helicobacter pylori is a ubiquitous organism, that is present in half of the world's population. In 1982 to Australian doctors discovered an organism that they named *Campylobacter pylori*. Dr Barry Marshall and Robin Warren are known for discovering the organism that was later renamed as *Helicobacter pylori*. (4) It is a gram negative, microaerophilic, spiral (helical) bacterium usually found in the stomach.

Infection of *H. pylori* has varied geographical distribution locally and on global scale, and there are several factors related to this varied distribution such as socioeconomical status, age, sex, genetic predisposition, sanitation system, ethnicity and education level, both of these determine the incidence and prevalence of this infection in both locally and globally. (5) The most common route of infection of H. pylori is either oral-to- oral or fecal-to- oral contact, the infection is acquired during childhood and persist throughout the life if not treated.

Dyspepsia which refers to a combination of upper gastrointestinal symptoms such as epigastric pain, burning, fullness, early satiety, nausea, vomiting and belching is one of the presenting complain of patients with H. pylori infection. (2) Prevalence of dyspepsia worldwide is ranging from 7% to 34.2% and the prevalence of functional dyspepsia worldwide ranges from 10% to 30%.(6)

1.1.2 Pathogenesis

After acquiring the infection of *H. pylori*, the bacteria colonizes the antral mucosa epithelium of the stomach aided by virulence factors such as dupA, cagA, vacA and homB, this induces the immune response that is despite of its vigor is unable to eliminate the bacterium which may later lead to chronic infection in the gastric.(7)

The chronic infection that results from inadequate immune response to eradicate the bacterium induces gastric damage that is associated with chronic gastritis infection. This usually significantly increases the risk of developing peptic ulcer (gastric or duodenal), gastric

adenocarcinoma and MALT. This infection in most individuals does not cause any symptoms and the infection persist without any clinical evident disease. (6)

1.1.3 Diagnosis Of *H. Pylori*

According to the American and Japanese guidelines indications for testing H. pylori include, low grade gastric mucosa associated tissue (MALT) lymphoma, active peptic ulceration, either duodenal or gastric or a prior history of peptic ulcer if there is no documentation of cure of *H. pylori*, and gastric cancer that is diagnosed at its early stage. (8) Although other indications are more controversial but include uninvestigated dyspepsia in patients <60 years without alarm features, prior history of long term use of non- steroidal anti-inflammatory drugs or chronic use of low dose of aspirin also unexplained iron deficiency anemia in a patient.(9)

Diagnosis of *H. pylori* infection can be made by either invasive (endoscopic) or by non- invasive techniques. To test for active infection of *H. pylori* various methods can be used, this includes, endoscopy- based test, urea breath test and the stool antigen assay. The stool antigen assay can be used to establish initial diagnosis and confirm eradication of H. pylori. Of all the test stool antigen assay is the most cost effective. The test has a sensitivity and specificity of 94% and 97% respectively. (10)

There are two different types of SATs available for the diagnosis of *H. pylori* infection. These are based on enzyme immunoassay (EIA) and another on immunochromatography (ICA). SATs are generally less expensive compared to urea breath test as they do not require expensive chemical agents or specified equipment.(11) Enzyme immunoassay (EIA)– and immunochromatography assay (ICA)–based methods, use either polyclonal antibodies or monoclonal antibodies. Due to the difficulty in obtaining polyclonal antibodies of consistent quality timely monoclonal antibody–based tests show better results. Both the EIA-based and ICA-based tests can be done with monoclonal antibodies but the latter provides more accurate and reliable results. (12)

Prior to the testing for possible *H. pylori* infection, it is required to discontinue the use of proton pump inhibitors for at least 2 weeks, and bismuth/antibiotic use for at least 4 weeks, as these may lead to false negative results. It has been seen that active peptic ulcer bleeding may also

lead to a false negative test, although monoclonal enzyme immunoassay stool assay still has a high sensitivity (94%) in the setting of recent peptic ulcer bleed. (9)

1.1.4 Management of *H. Pylori*

Treatment of *H. pylori* as recommended by Maastricht IV/ Florence report recommends clarithromycin containing triple therapy as the first line management, this includes clarithromycin and amoxicillin/ metronidazole plus a PPI. However currently this combination has showed low efficacy with a cure rate of 70 %, and this is mostly attributed to by antibiotic resistance. Due to this, different alterations have been made with the use of sequential treatment which includes a 5-day period with PPI amoxicillin, followed by a 5-day period with PPI-clarithromycin-metronidazole (or tinidazole). (9)

Geographical locations with high clarithromycin resistance, bismuth-containing quadruple treatments are recommended for first-line empirical treatment.(8) The bismuth- containing quadruple bismuth salts, tetracycline and metronidazole plus a PPI. In case of unavailability of the aforementioned therapy it is recommended to use sequential treatment or a non-bismuth quadruple therapy. (9)

Different guidelines recommend that all patients who are treated for *H. pylori* should be retested to confirm treatment success because of increasing antibiotic resistance. (13) It is recommended, eradication be confirmed with a urease breath test, stool antigen testing, or endoscopy- based testing. In case of persistent *H. pylori* infection after 2 courses of antibiotics, it is recommended to do an endoscopy with biopsy for culture and sensitivity. (9)

Under the Maastricht Florence IV consensus so as to reduce false negatives patients should have completed antibiotics 4 weeks and PPIs 2 weeks prior to retesting for *H. pylori* infection. Serologic testing is not to be used as a confirmatory test as patients may still have antibodies circulating after eradication. (9)

The first complication of *H. pylori* infection is acute gastritis characterized by hypochloridria, chronic infection will lead to atrophic changes if the corpus is affected, If the antrum is affected it will lead to increase acid secretion and peptic or duodenal ulcer formation. Eventually metaplastic changes occur, and it can lead to mucosa associated lymphoid tissue (MALT) lymphoma. It may also complicate to gastric adenocarcinoma, following metaplasia, and dysplasia. (14)

1.2 Problem Statement

Helicobacter pylori was found to infect approximately 4.4 billion individuals worldwide, the infection is more prevalent in developing rather than developed nations with most countries in Africa classified as developing or underdeveloped countries. Africa has the highest prevalence of 79.1%(5). In Tanzania *H. pylori* has been found to be associated with socioeconomic status and infection prevalence has been found as high as 39.1%. (15)

Studies worldwide have indicated reduced clearance of *H. pylori* infection with standard treatment set in guidelines. There has been a tremendous drop in eradication rates that is unacceptable and persistent infection with *H. pylori* that leads to further complications like gastritis, PUD and in some population gastric cancer. Evidenced by studies where healthy individuals where purposefully infected by *H. pylori* can help illustrate best its ability to cause acute gastritis. The evolution of acute to chronic gastritis is with time, however both will have different histological findings(16). There is a clear relationship between the development of peptic ulcer disease in patients infected with *H. pylori*. Infection of *H. pylori* is during childhood and the risk of developing PUD increases with age. (17)

Trends in eradication of *H. pylori* infection through time has changed, eradication rate with standard triple therapy has significantly dropped to unacceptable levels globally, and this has been attributed to by antibiotic resistance, poor compliance, different strains patterns. (13) Initially when triple therapy was the mainstay treatment eradication rates where up to 84%, as time went by there has been a decrease in these rates.

Currently drops with the triple therapy, in developed nations have reached to less than 80 % and in some European countries to 20% (18). In Tanzania, a study conducted at Bugando Hospital successful treatment was recorded in 69% of individuals. In the 30.1 % with treatment failure, clarithromycin resistance was seen in 30.3% of patients with poor adherence also leading to poor eradication. (18)

As shown by the study the low eradication rate cannot all be contributed to by antimicrobial resistance, there are more factors to consider as why the successful treatment of *H. pylori* has

become a problem in Tanzania. In this study we aim to venture to different factors that contribute to poor treatment of *H. pylori* as well as identify locally what is the proportion of treatment success.

1.3 Rationale

The data found from this study will first and foremost provide much needed information of eradication of *H. pylori* infection at the referral hospital in Tanzania, and the factors associated with treatment failure of standard treatment therapy. It will help individualize patient care were *H. pylori* treatment is concerned, this will reduce expenditure on medical management by reducing using ineffective treatment. It will also lead in reduction of complications of *H. pylori* infection, early management of *H. pylori* will avoid the complications that come with its chronic infection.

It is observed that a lot of patients present with many complications, from peptic ulcer disease to malignancy, because of *H. pylori* infection. Policy makers should be able to strategies on how to improve *Helicobacter pylori* treatment, reduce the burden of the disease both financially and health-wise to its citizens.

1.4 Hypothesis

Patients with *H. pylori*, whom are treated with clarithromycin- based therapy may have different outcomes and there are factors that influence these outcomes.

1.5 Research Questions

Overall research question:

What are the factors associated with treatment outcome of *H. pylori* among dyspeptic patients using clarithromycin- based therapy undergoing endoscopy at MNH.

I. What is the treatment outcome of *Helicobacter pylori* infected patients using clarithromycin-based regimen who are undergoing endoscopy at MNH?
II. What are the sociodemographic factors associated with treatment outcome of *Helicobacter pylori* infection in patients undergoing endoscopy at MNH?
III. What are the clinical factors associated with treatment outcome of helicobacter pylori infection in patients undergoing endoscopy at MNH?

1.6 Objective of the Study

1.6.1 Broad Objective

I. To determine the factors associated with treatment outcome among dyspeptic patients with *H. pylori* using clarithromycin-based therapy undergoing endoscopy at MNH.

1.6.2 Specific Objectives

I. To determine the proportion of patients with *H. pylori* eradication following treatment with clarithromycin-based therapy in patients undergoing endoscopy.

II. To identify the socio- demographic factors associated with the treatment outcome of *H. pylori* (age, current smoking, alcohol use, herbal medication use) among dyspeptic patients using clarithromycin-based therapy undergoing endoscopy.

III. To identify the clinical factors (endoscopy findings, adherence to medication) associated with the treatment outcome of *H. pylori* among dyspeptic patients Using Clarithromycin-Based Therapy Undergoing Endoscopy.

1.7 Conceptual Framework

The figure below illustrates risk factors a for developing *H. pylor*i infection and associated factors of *H. pylori* treatment outcome. The factors that contribute to the acquiring of H. pylori infection include low social economic status, overcrowding living condition, improper food handling etc. The factors that are associated with treatment outcome of *H. pylori* include, antimicrobial resistance, advanced age, smoking, alcohol use etc.

Conceptual frame work below:

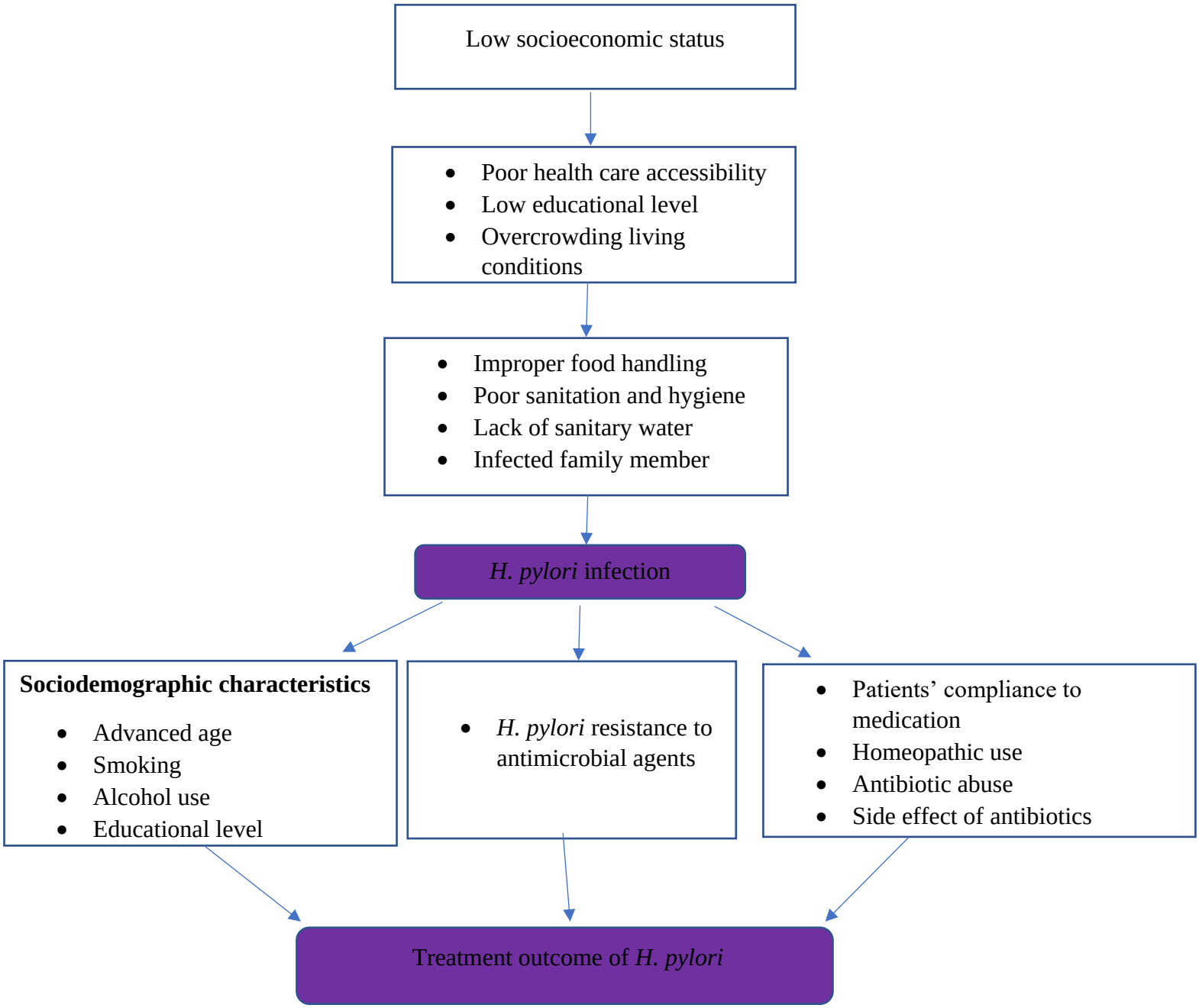


Figure 1: Conceptual framework of the study

CHAPTER WO

2 LITERATURE REVIEW

2.1 Prevalence of *H. Pylori*

Throughout time the rate of *Helicobacter pylori* infection has varied due to different reasons, beginning with age, sex, socio- economic differences, geographical locations, different diagnostic methods and choice of treatment. A meta- analysis conducted by Hooi et al, showed higher prevalence's in developing nations, where regions with the highest reported HP prevalence were Africa 70.1%, South America 69.4%, and Western Asia 66.6%, while regions with the lowest reported HP prevalence were Oceania 24.4%, Western Europe 34.3% and Northern America 37.1%. When comparing prevalence's among rural areas and urban areas in Australia, the prevalence was estimated to be 24.6% among the total population, but was as high as 76.0% in the rural indigenous community in Western Australia .(5)

Pakistan conducted a study among symptomatic dyspeptic patients, the study found that half of the patients had active *H. pylori* infection when screened using the 13C UBT(19). In a systematic review and meta-analysis conducted in Latin America and the Caribbeans, the prevalence of *H. pylori* infection was around 50% among all ages; where in children and adolescents the prevalence was lower than in adults(20). Half of the population in Cameroon were found to have *H. pylori* infection, with an annual decline rate of 6.8%(21).

In Ethiopia it was noted there were fluctuating trends in seroprevalence of dyspeptic patients(22), In a study done in Kenya, the prevalence among patients with gastric ulcer was 46.2%,mostly seen to be higher in the mid-age group, which was found to be 32.4%. In another study conducted in Kenya seropositivity for *H. pylori* was found in 71% symptomatic patients and in 51% asymptomatic participants(23).

A study which took place in Ghana, found the prevalence of *H. pylori* to be 55% among gastric and non – gastric ulcer patient(24). In a study conducted in asymptomatic patients in Libya found the prevalence of 76% *H. pylori* infection among the tested subjects(22). The infection rate of 50 % with *H. pylori*, was found in subjects less than 9 years of age that increased to 84% in teenage subjects and continued to increase with increase age and reaching up to 94% in those

of age greater than 70 years (22). A study conducted in Mwanza showed the seroprevalence of *H. pylori* to be 39.1 %, with drinking unsafe water seen as a significant factor for seropositivity, and infections rate increasing with age.(15)

2.2 Associated Factors of *H. Pylori* Infection

In several studies poor sanitation is consistently identified as a major contributing factor for *Helicobacter pylori* infection. A study conducted in Kazakstan compared two different ethnic groups, the ones ingesting water from the river had higher rate of infections compared to those taking tap water(25).

Alemayehu et al showed that presence of domestic animals, sources of drinking water, toilet type, shared beds with siblings, family size, storing and reusing water and occupational status were variables highly influencing the acquisition of *H. pylori*. In a study conducted in Bugando Hospital in Mwanza Tanzania it concluded; with the risk of acquiring H. pylori infection, patients who ingested untreated water (57%), where twice more at risk of getting infected with *H. pylori* in comparison to individuals using disinfected water (30%).(15)

A study was conducted to ascertain the risk factors for acquiring *Helicobacter pylori* infection in Nigeria revealing, sharing a house with more than three people in close quarters and a prior history of ulcer/gastritis within close family members, plus current use of antibiotics was significantly associated with *H. pylori*.(26)

In another study conducted in Kano north- west Nigeria, revealed a trend of increased prevalence of *H. pylori* more amongst individuals with low socioeconomic status. Factors that influenced high prevalence were poor sanitation in sense of water use, overcrowding in a household and cigarette smoking (which depends on pack years, and whether a current smoker) were significant hazards for *H. pylori* infection(27).

2.3 Testing Of *H. Pylori* Infection

Currently we have invasive and non-invasive methods for testing and diagnosing *H. pylori* infection, different guidelines have stipulated which methods for diagnosing and testing for eradication of *H. pylori* depending on the sensitivity and specificity of the said methods. Cost of the methods are also put in consideration when choosing the most appropriate testing method. It is of importance to consider costs when choosing the testing method especially in developing nations.

In a study done to explore the relationship between different *H. pylori* infection diagnostic methods, and better understand the extent of use of each diagnosis method, a total of 91 patients who underwent OGD were diagnosed using urea breath test, PCR, histopathology, stool antigen and serology. The results concluded that, histology and RUT had the best sensitivities above 95%. The sensitivities of other tests including PCR and stool antigen test were above 90% with serology having 73%. Rapid Urease Test had the best specificity (100%), and the specificities of the other tests, including PCR(95%), stool antigen test (86.7%), histology (77.8%) and serology (55%).(28) Showing serology to have very low specificity compared to other tests.

Comparing the non-invasive diagnostic tests, SAT and UBT have higher diagnostic accuracy than urinary antibody test or serology. Guidelines from the American Gastroenterological Association recommends in patients whom meet the Rome IV criteria for dyspepsia the diagnosis of H. pylori infection can be done by both SAT and UBT.(2)

The UBT has been used for more than 3 decades and is considered to be the most reliable noninvasive test for the diagnosis of H. pylori infection, although it has several setbacks. First it has a high cost compared to SAT. In addition, fasting is required before doing the urea breath test as it is done endoscopically, while is stool antigen tests there is no need of fasting. Furthermore, proton pump inhibitor administration tends to lower urease activity which is detected by UBT hence, false-negative results are noted in patients who have been taking PPIs. PPIs also influence SATs but currently monoclonal antibody-based SATs are available that are not affected by PPIs.(11)

In a study conducted among 302 symptomatic children, between the ages of 1 – 18 years, whom have not undergone any sort of treatment before, recruited at 3 different centers. *H pylori* diagnosis was made by results from culture, histology, and the rapid urease test. The EIA monoclonal stool antigen test was used to locally investigate stool samples. It was brilliant in diagnosing *H pylori* infection in the participant, with a sensitivity 98%, specificity 99%, positive predictive value 98%, and negative predictive value of 98%.(29)

2.4 Eradication of *H. Pylori* Infection

For the past 20 years, the recommended treatment for *H. pylori* eradication is the standard triple therapy, which entails use of a proton pump inhibitor or ranitidine bismuth citrate, in combination with clarithromycin and amoxicillin or metronidazole. (30) The ACG guideline also suggested non-bismuth sequential and non-bismuth hybrid quadruple therapies as first-line regimens. In patients who fail first-line treatment, BQT or levofloxacin-based triple therapy are second-line options that avoid the re-use of clarithromycin; levofloxacin-based triple therapy should only be used second-line if levofloxacin was not used first-line. (8) The Maastricht guidelines recommend culture with susceptibility testing after the failure of second-line treatment. When such testing is not available, BQT or—if a fluoroquinolone had not been used initially—a levofloxacin-based regimen may be attempted. Both high-dose dual therapy (amoxicillin and PPI) and rifabutin-based regimens. (10)

Treatment with first line regimen has shown effectiveness in treating the infection up to more than 90 %. Currently this has decreased to lower than 70 % worldwide, several factors are seen as contributing factors with antimicrobial resistance leading. (31) The low rates of successful treatment are unacceptable under the Maastricht consensus which stipulate that, “rates consistently below 80% by intention-to-treat are not acceptable for treating *H. pylori”*. (9)

2.5 Treatment Outcome of H. Pylori

Several studies have been conducted to determine what is the outcome of treatment of H. pylori with patients on a clarithromycin- based regimen. Jaka et al studied patients attending OGD at Bugando Hospital and treatment failure was seen in 31% of the enrolled patients. (18) A study conducted in Karachi showed treatment failure of 40% when patients were tested after treatment. (32)

In a study conducted in Japan to determine the factors associated with the treatment outcome, the success rate was 71% with patients treated with the first line. (33) in a study a study conducted by Dulciene et al, the treatment outcome *of H. pylori* showed 87%, among patients on the clarithromycin- based regimen. (32)

2.5.1 Sociodemographic Factors

Age has been seen to contribute to successful treatment of *H. pylori* where middle aged patients are seen to have higher successful treatment followed by the elderly and then young individuals. (33) In sociodemographic characteristics, females show a higher tendency in successful eradication rate, and individuals living in rural areas tend to show less success rate in treating *H. pylori* infection. (34) In contrast to this some studies done in Asia have shown reduced success rate among female patients and some professions like farmers tend to also have lower success rate. The female patients with low success rates of eradication were found to be smokers. (35)

Factors such as smoking increases the failure rate in treatment of *H. pylori* infection, where current smokers and high smoking dose (more than 5 cigarettes' per day) has been seen to influence the high failure rate. (36) In a study conducted in Tanzania, poor outcome to treatment with clarithromycin-based regimen was attributed to by poor adherence to medication and resistance to medication, where 22 % of patients with poor adherence and 37% of patients resistant to clarithromycin where unable to clear the infection. (18)

A study was conducted to determine factors associated with *H. pylori* treatment showed no effect of age, sex, smoking, ulcer disease, compliance with therapy, H. pylori colonization density, degree and activity of antral gastritis, or the coexistence of corpus gastritis. (37) A study done in Japan among patients in primary care, young individuals were seen to have failure of treatment outcome due to self- interruption of medication. (33)

2.5.2 Clinical Factors

In a study conducted in rural China to determine clinical factors associated with treatment outcome of *H. pylori*, the eradication rate in patients with duodenal ulcers was higher than that in patients with gastric ulcers (79% vs. 60%). (38) In a study conducted among 180 with positive *H. pylori*, having peptic ulcer disease was associated with treatment success compared to non-ulcer disease, while metronidazole resistance was associated with treatment failure. (39)

A study conducted by Jaka et al at Bugando Hospital, Mwanza, to show predictors of triple therapy of *H. pylori,* concluded one of the major predictors being resistance to antibiotics used in the treatment regimen of *H. pylori.* (18) A study conducted among 111 patients in a high prevalence area, to determine factors associated with treatment failure of *H. pylori*, showed higher rates of failures in patients with gastritis compared to peptic ulcers. (32)

2.6 Complications of *H. Pylori* Infection

Many of *H. pylori* infections are perceived to be asymptomatic, but their symptoms can be synonymous to those of other diseases, such as functional dyspepsia and GERD. Depending on the predisposition of an individual infection with *H. pylori* can lead to development of pathological findings which may be non-malignant or malignant diseases.

Studies done have shown that the underlying cause of functional dyspepsia may be attributed to by many different factors and *H. pylori's'* role is unclear. A large meta-analysis was done, 14 randomized controlled trials were examined, the conclusion that was met, treating *H. pylori* improved dyspeptic symptoms up to 1 year later. (40)

In 1728 a German physician by the name Georg Ernst Stahl first coined the term "gastritis" which was used to describe the inflammation of the inner lining of the stomach, currently known to occur due to mucosal injury. After the discovery of *Helicobacter pylori*, the notion that

gastritis was a histological finding changed and rather started to be perceived as a disease. It has been seen there is a strong association between *H. pylori* infection and chronic gastritis activity. (41)

It is difficult to differentiate PUD either, duodenal or gastric ulceration by history alone, although some indications may be leading to this diagnosis. The cardinal complaint for both gastric and duodenal ulcers is epigastric pain, explained as a gnawing or burning sensation that typically occurs after food intake, occurring within a short period of food intake with gastric ulcers, and a few hours afterward with duodenal ulcers. Studies have shown a possible contribution of peptic ulcer disease with H. pylori mucosal colonization of gastric and duodenum. Mostly seen to cause duodenal ulcer. (42) Another study had similar findings with patients with *Helicobacter pylori* having higher prevalence of peptic ulcer, particularly duodenal ulceration. (43)

It has been proposed that *H. pylori* infection causes acute gastritis, chronic gastritis, dysplasia and gastric cancer especially intestinal type. (6) A prospective study suggested that gastric cancer is 2 – 3 more prevalent in patients infected with *H. pylori.* (44)

GERD relationship with *H. pylori* is of uncertainty, multiple studies done show continued or exacerbation of GERD symptoms and esophageal inflammation even after eradication of the thought offending agent, that is *H. pylori.* However further evaluation in other studies showed improve reflux symptoms post treatment. (16) GERD is a condition which develops when the re-flux of stomach contents causes uncomfortable symptoms and/or could lead to other array of complications. (17)

CHAPTER THREE

3 METHODOLOGY

3.1 Study Design

Prospective cross-sectional study, based within the hospital

3.2 Study Area

This study was conducted at the endoscopy unit at Muhimbili National Hospital, Muhimbili is a referral hospital receiving patients from all over the country. It offers services up to a super specialized level and has state of the art equipment for intervention as well. The endoscopy unit is available for 6 days a week, catering to both insured and non- insured patients. It receives up to 50 patients per day, with procedures such as esophagogastroduodenoscopy and colonoscopy done. Patients are attended by gastroenterologists, master of science fellows in gastroenterology, physicians and residents at the clinic. Those with indication are sent to the endoscopy unit at the pediatric complex, second floor. The endoscopy procedure is performed only by gastroenterologists and master of science in gastroenterology students, and residents only under supervision.

3.3 Study Duration

The study was conducted from July 2022 to February 2023, a total of 8 months, where after enrollment each participant was followed up for 6 weeks to test for treatment outcome after using clarithromycin based triple therapy (clarithromycin, metronidazole and PPI). The antibiotics were used for 2 weeks and PPI for 4 weeks.

3.4 Study Population

All dyspeptic patients, as per ROME IV criteria (appendix 5) whom underwent endoscopy at Muhimbili National Hospital.

3.5 Inclusion Criteria

All consented dyspeptic patients, according to ROME IV criteria, of age ≥ 16years

- With positive test for stool *H. pylori*
- Who were willing to come back to check for eradication

3.6 Exclusion Criteria

All patients presenting with active upper GI bleeding

3.7 Sample Estimation

Calculation of the size of the sample:

$$n = \frac{z^2 p(1-p)}{\varepsilon^2}$$

Where: n = minimum sample size

z = standard normal deviate (1.96)

p = 30.9% (Predictors of triple therapy treatment failure among H. pylori infected patients attending at a tertiary hospital in Northwest Tanzania: a prospective study, Jaka et al)

30.9% were individuals who had a stool *H. pylori* antigen positive 5 weeks after treatment

Ɛ = margin of error 5%

Therefore,

$$n = \frac{1.96^2 \times 30.9\ (100-30.9)}{52}$$

n= 328

The power of the study is 80% with a non- response rate (f) of 10%, with the inclusion of an error due to non- response the final sample size was calculated using the formula:

$$N = \frac{(n \times 100)}{(100 - f)}$$

$$N = \frac{328 \times 100}{100 - 10}$$

N = 364

Therefore, the sample size was 364

3.8 Study Variables

The variables that were used in the study;

3.8.1 Dependent Variables

Outcome of treatment of *Helicobacter pylori* infection. i.e. No eradication of *H. pylori*

3.8.2 Independent Variables

Age, gender, level of education, residence, cigarette smoking, alcohol consumption, herbal medicine use, prior treatment with *H. pylori*, adherence to medication, endoscopy results.

3.9 Sampling Technique and Study Procedure

3.9.1 Technique Used for Sampling

Consecutive enrolment of study participants was used, all consenting adults with dyspepsia (according to ROME IV) undergoing endoscopy were approached for entry into the study. This was conducted on every endoscopy day until the required sample size is reached.

3.9.2 Procedure in the Study

All patients who fit the inclusion criteria were recruited into the study, they were then treated using standard triple therapy (clarithromycin 500mg + metronidazole 400 mg and a PPI) twice daily. A follow up stool for antigen test was done 2 weeks after cessation of PPI, 4 weeks after cessation of antibiotics to determine the treatment outcome. Patients with stool antigen positive after 6 weeks were considered to have treatment failure, and those with stool antigen negative were deemed treatment success.

Patients who had treatment failure after 6 weeks underwent an OGD for biopsy of gastric tissue. The tissue was then used for culture of *H. pylori* and sensitivity to clarithromycin, metronidazole and amoxicillin was done.

3.10 Flow Chart of The Study

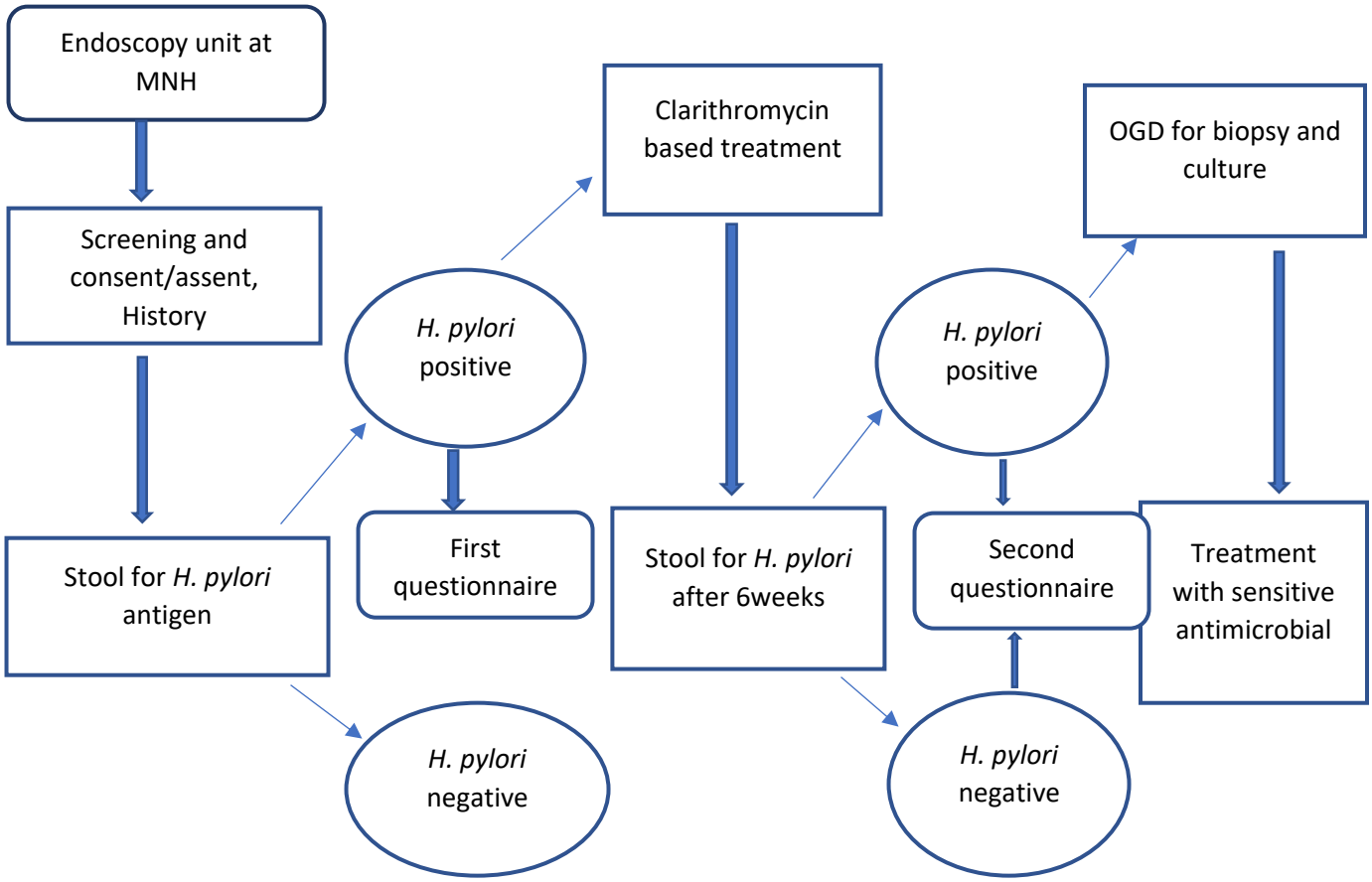


Figure 2; Flow chart of the study

3.11 Data Collection Procedure

A structured questionnaire was used to collect data in both the recruitment and follow up phases. Patients sociodemographic and medical information was collected during the first encounter and drug utilization, adherence and added-on-therapy was assessed during the follow up period. The questionnaire included sections of demographic data, clinical characteristics of the patient, adherence to medication, history of being diagnosed with PUD previously, history of using triple therapy before, OGD and/or stool antigen test for H. pylori results.

Adherence to medication was assessed using a MMAS questionnaire (Morisky Medication Adherence Scale), which has a total of 8 questions with a total value of 8. A score of 6 and above denoted good adherence and below 6 denoted poor adherences.

3.12 Stool For Antigen Test Procedure

Patients were instructed to collect a fresh, random stool using a special spatula which was given together with a collecting container (Note: watery and diarrhea stool are inappropriate for testing hence not used). Patients were told to avoid urine and toilet water in specimens which may cause dilutional factor. Specimen were collected by using a plastic, leakproof container. Patients were instructed to present the specimen 2 hours after collection, if delay was anticipated then the specimen were refrigerated.

In interpreting results, the reference range used was negative or positive stool antigen for *Helicobacter pylori*, and the results were anticipated after 24 hours of specimen collection. The methodology used was polyclonal antibody enzyme immunoassay.

The results of the test were recorded in a designed data collection tool. Patient came to collect their results, and others were informed through the phone in the upcoming days (day 2 up to a week) and for those with positive results after 6 weeks had gastric and duodenal biopsies collected.

3.13 OGD Procedure

All patients for OGD were counselled on the endoscopy procedure and explanation was given on each step and complications that pertain to the procedure. Instructions on a 6 hour fast was explained and patients who had to take insulin injections for DM management were given priority and for those whom cannot tolerate the fast. Upon consenting to undergo the procedure the consent form was then signed.

On the day of the procedure, confirmation of fasting was verified. If need be, for sedation arises propofol was administered. Three specimens were collected two from antrum and 1 from corpus for culture, with a minimum of 3 specimen collected per endoscopy, and placed in a falcon tube containing normal saline and stored in a cooler box with ice blocks to maintain the temperature at 4 degrees centigrade.

After the procedure patient were taken to the recovery room. After waking up fully with no procedure complication, patients were allowed to go home and the date for tests result was provided.

3.14 Culture Procedure

Optimal growth requirement for H. pylori isolation is microaerophilic and moist condition maintained at 5% to 7% oxygen and 5% to 10% carbon dioxide environment at a temperature of 35°C to 37°C. (3) A candle jar was used to maintain a microaerophilic environment. (44) Blood agar was used for the isolation of H. pylori. The specimen used were gastric biopsy taken from the antrum and corpus during the endoscopy procedure. The specimen were placed in a sterile falcon tube containing a small amount of isotonic saline 0.9% to maintain moisture and transferred to the laboratory in an ice bag container. (45)

In the laboratory, a Columbia base agar was supplemented with sheep blood. *H. pylori* are resistant to various antibiotics such as vancomycin, trimethoprim, sulphonamide, these antibiotics together with amphotericin B were added to the media to inhibit the growth of other organisms. Using a swab containing the biopsy each blood agar plate was inoculated and the specimen was incubated in a microaerophilic moist chamber at temperature 35°C to 37°Cfor for 7 days. Culture read was done on days 4, 5, and 7. After isolation detection of *H. pylori* was

done by using gram staining in which gram-negative straight rods on microscopy which is oxidase, urease, and catalase-positive was considered as *H. pylori* isolate. (3)

Seven days after the procedure culture results were reported. The positive result was reported as *H. pylori* growth while the negative result was considered as no growth. All the isolates were stored in STGG storage media in a refrigerator maintained at -80° C degree centigrade for susceptibility pattern later. (45)

3.15 Antimicrobial Susceptibility Pattern

Due to the importance of antimicrobial susceptibility testing for *H. pylori*, choosing a testing method that has high levels of accuracy for clinical settings is very important. The clinical and Laboratory Standards Institute (CSLI) recommends the agar dilution method as a gold standard for testing antimicrobial susceptibility patterns for *H. pylori*. (46) Despite being the gold standard, the agar dilution method is very difficult to perform in routine activities and E-test has been approved to be the best alternative for performing antimicrobial susceptibility pattern for *H. pylori,* but because of its cost implication (approximately US $2.6/strip), it was economically impractical to our study. The disc diffusion method was preferred in this study because it is simple to perform, economically friendly and it has been shown to have an excellent correlation with E-test in testing antimicrobial susceptibility pattern for *H. pylori*.

The antimicrobial susceptibility of *H. pylori* isolates was assessed using the disc diffusion method against three antimicrobial agents (disks (6-mm diameter, Hi-Media, India)) including; amoxicillin (30 μg), metronidazole (5 μg), and clarithromycin (15 μg). Under the microaerophilic condition, the antimicrobial discs were aseptically placed on the dried surface of Columbia agar with 5% sheep blood incubated at 37 °C for 72 hours. Antimicrobial susceptibility testing to determine zones of inhibition was performed with reference to previous studies with slightly modification to CSLI, where a zone size < 17mm was evaluated resistant for amoxicillin, $\leq$16 mm for metronidazole resistance, < 17 mm and <18 mm for clarithromycin resistance. (47)

3.16 Outcomes

3.16.1 Primary Outcome

The outcome was recorded as treatment outcome of *Helicobacter pylori* i.e. stool *H. pylori* antigen positive at 6 weeks

3.16.2 Secondary Outcome

Factors associated with treatment outcome of *Helicobacter pylori*

3.17 Case Definition

Our case definition was the treatment outcome of *Helicobacter pylori* with standard treatment and factors contributing to the outcome.

3.18 Data Collection Tool Pre Testing

The data collection tools were pretested on a small group of patients attending OGD to ensure validity and consistency of the study. This was conducted for 2 weeks prior to the start of the study and was done in the same manner as the main study.

3.19 Data Processing and Analysis

Data was entered into EPI data version 3.1; to be checked for consistency, validity and ensure no unavailable information. Analysis was done using statistical package for social sciences (SPSS) version 23.0. The main outcome in this study was treatment failure which was determined by positive stool antigen after 4 weeks treatment with antibiotics. The categorical variables such as sex, residence, alcohol use, smoking, concurrent use of herbal medication was summarized as proportions, while continuous variables such as age were summarized as medians with interquartile range.

Proportions were used to determine poor versus good treatment outcome after 4 weeks of treatment with antibiotics. The univariate analysis was done which described the relationship between individual factors and treatment outcome. Furthermore, multivariate analysis was done on variables with $p < 0.2$ to eliminate confounders, and conclude factors independently associated with treatment failure, odds ratios (OR) with respective 95% confidence intervals (CI) were computed.

A sub group analysis was done to determine the proportion of growth of *H. pylori* among patients who were deemed to have treatment failure and resistance pattern to clarithromycin, metronidazole and amoxicillin in those who had inoculation of the bacteria.

3.20 Ethical Approval

Prior to commencing the data collection ethical clearance was requested from the MUHAS research ethical committee. Informed consent and assent (for participants younger than 18 years) was sought from all participants. The patients who willingly agreed to partake in the study were enrolled and confidentiality was maintained. Patients who did not agree to participate in the study were assured that their decision not to partake in the study will not affect their management in anyway.

All patients diagnosed to have *Helicobacter pylori* infection were counselled and managed according to the standard of care. Also, patients were counseled about endoscopy procedure that is invasive and biopsy will be taken and there are minor complications that can happen during the procedure such as gastric bleeding, over sedation and sometimes gastric perforation. Patients were counseled on the possibility of a second biopsy depending on the treatment outcome on standard clarithromycin regimen. Patients found to have resistance at 6 weeks were counseled to return to their primary physician with the results for further management.

Confidentiality was maintained throughout the whole process. All patients were identified by numbers so as protect participants identity and all data collected was kept in a device protected by a password and secured in a closed filing cabinet.

CHAPTER TWO

4 RESULTS

4.1 Flow Diagram of Participants

There were 413 dyspeptic patients attending the endoscopy unit at Muhimbili National Hospital were approached for recruitment into the study. A total of 385 patients who tested positive for *H. pylori* stool antigen were included into the study. Out of the 385 who were *H. pylori* stool antigen positive, 253 (65.7%) vs 132 (34.2%) had *H. pylori* stool antigen negative and stool antigen positive respectively after treatment with clarithromycin- based therapy for 4 weeks. Out of the 132 patients who were still *H. pylori* stool antigen positive, 2/132 (1.5%) were lost to follow up. A total of 130 patients were followed to completion and had biopsy for culture taken. The inoculated *H. pylori* 86/130 (66%) were used to determine antibiotic resistance pattern.

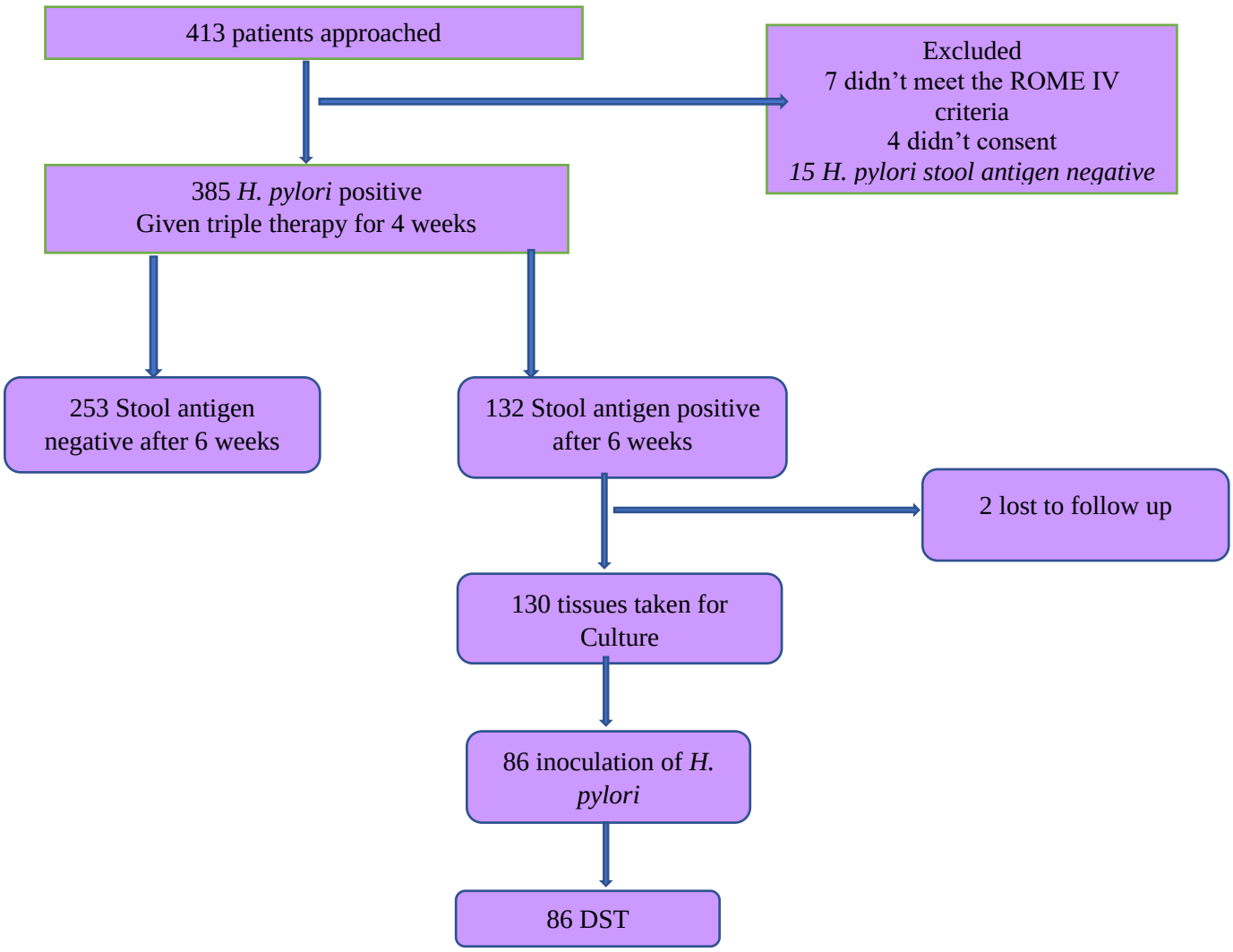


Figure 3; Consort diagram showing flow of participants

4.2 Baseline Characteristics of Study Population

Total of 385 participants were included in the analysis. The mean age of the study population was 41 years with a standard deviation of 14 years with majority being female constituting 55.3%. Only 3 % reported history of smoking while 38.7% of participants reported current use of alcohol. Other characteristics are been shown in the table 1 below.

Table 1: Social demographic characteristics of the study population.

Variable	Frequency (%) N=385
Age (mean ± SD)	41 ± 14
< 60	343 (89.1)
≥ 60	42 (10)
Sex	
Male	172 (44.7)
Female	213 (55.3)
Residence	
Urban	302 (78.4)
Rural	83 (21.6)
Education level	
No education	29 (7.5)
Primary	63 (16.4)
Secondary	122 (31.7)
College/ university	171 (44.4)
Marital Status	
Single	104 (27.0)
Married	213 (55.3)
Divorced	29 (7.5)
Cohabiting	12 (3.1)
Widow/widower	27 (7.0)
Occupation	
Unemployed	72 (18.7)
Formal employment	135 (35.1)
Self employed	178 (46.2)
Smoking	
Yes	11(2.9)
No	374 (97.1)
Alcohol Use	
Yes	149 (38.7)
No	236 (61.3)

4.3 Outcome of *H. Pylori* Treatment

At the end of six weeks, a total of 253 (65.7%) vs 132 (34.2%) participants had a negative stool antigen test and positive stool antigen respectively for *H. Pylori*, after treatment with clarithromycin based triple therapy. This implies that the proportion of treatment failure for H. pylori is 34.2% at six weeks following treatment of a positive stool antigen test for *H. pylori.* Figure 3, illustrates the treatment outcome of H. pylori among previously infected individuals.

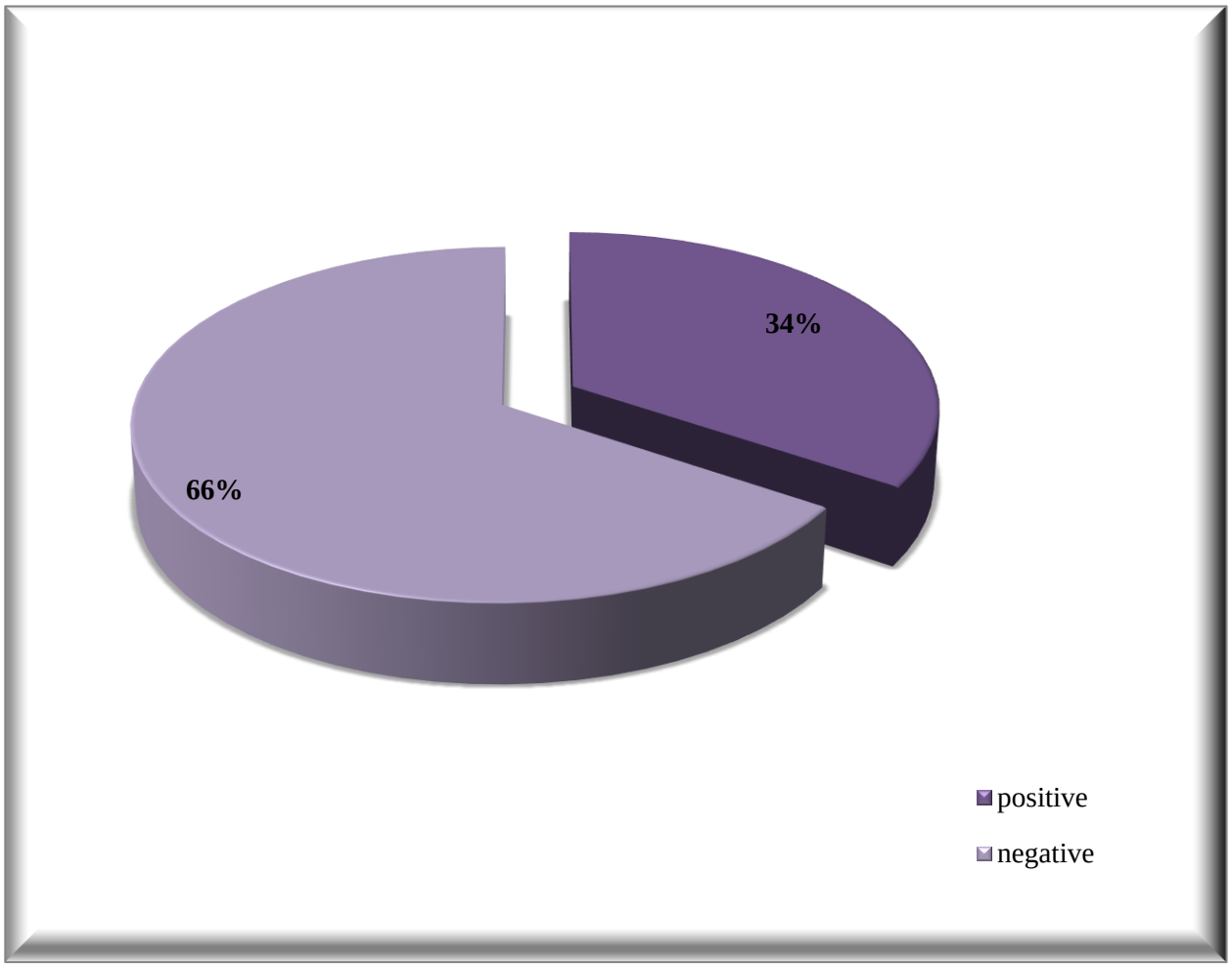


Figure 4 ; Stool antigen for H.pylori results after 6 weeks N= 385

4.4 Factors Associated with Outcome of *H. Pylori* Treatment

Factors that were found to be significantly associated with a successful *H. Pylori* treatment outcome at six weeks were good adherence (79%) to medication. Ninety percent (90%) of current smokers and fifty nine percent (59%) of patients who concurrently used herbal medication were found to have a significant association with treatment failure. Age, sex, current alcohol use and presence of peptic ulceration were not found to have any association with treatment outcome.

Table 2; Factors associated with treatment outcome of H. pylori infection N =385

Factors	**Treatment outcome n (%)**		**P P-value VALUE**
	Success n= 253	Failure n =132	
Age			
< 60	223 (65)	120 (35)	0.41
≥ 60	30 (71)	12 (29)	
Sex			
Male	107 (62.2)	65 (37.8)	0.19
Female	146 (68.5)	67 (31.5)	
Alcohol use			
Yes	91 (61.1)	58 (38.9)	0.12
No	162 (68.6)	74 (31.3)	
Current smoker			
Yes	1 (9.1)	10 (90.9)	< 0.001
No	252 (67.4)	122 (32.6)	
Concurrent use of herbal medication			
Yes	53 (40.1)	77 (59.2)	< 0.001
No	200 (78.4)	55 (21.6)	
Pre - treatment OGD results			
No Ulceration	234 (67.2)	114 (32.8)	0.06
PUD	19 (51.4)	18 (48.6)	
Adherence to medication			
Good	200 (79)	53 (21)	< 0.001
Poor	53 (40)	79 (59)	

4.5 Factors Associated With Poor Treatment Outcome Of H. Pylori By Logistic Regression

Multivariate logistic regression model revealed the same factors associated with treatment failure as those from bivariate analysis. Patients who were current smokers were more likely to have *H. pylori* stool antigen positive 6 weeks post treatment (OR: 7.9, 95% CI;(0.93 – 6.7) P =0.03) compared to those who did not smoke. In addition, patients with concurrent use of herbal medication (OR: 6.7, 95% CI;(3.9 – 11.6) P < 0.001) were also more likely to have *H. pylori* stool antigen positive 6 weeks post treatment. These findings coincided with presence of an ulcer on endoscopy (OR:1.7, 95% CI; (0.61 – 3.71), P = 0.48) and poor adherence to medication (OR: 6.2; 95% CI; (3.52 – 10.8) P < 0.001) as shown in table below.

Table 3; Logistic Regression analysis to determine factors associated with treatment failure of *H. pylori* infection

Variables	Univariate Analysis Crude OR (95%CI)	P- value	Multivariate Analysis aOR (95% CI)	P-value
Sex				
Male	0.7 (0.36 – 1.50)	0.42	1.0 (0.62 – 3.70)	0.91
Female	Ref		Ref	
Current smoker				
Yes	2.0 (0.01 – 0.40)	0.004	7.9 (0.93 – 6.7)	0.03
No	Ref		Ref	
Alcohol Use				
Yes	0.7 (0.46 -1.12)	0.1	0.7 (0.53 – 1.631)	0.8
No	Ref		Ref	
Previous H. pylori treatment				
Yes	1.1 (0.71 – 1.73)	0.6	0.9 (0.55 – 1.59)	0.81
No	Ref		Ref	
Concurrent use of herbal medication	3.7 (0.12 – 0.29)	< 0.001	6.7 (3.9 – 11.6)	< 0.001
Yes	Ref		Ref	
No				
Adherence to medication				
Poor	5.6 (3.50 – 8.90)	<0.001	6.2 (3.52 – 10.80)	< 0.001
Good	Ref		Ref	
Pre-treatment OGD results				
PUD	0.51 (0.26 – 1.08)	0.056	1.7 (0.61 – 3.71)	< 0.48
No ulcer	Ref		Ref	

4.6 Subgroup Analysis, To Determine Culture Of H. Pylori Among Patients With Poor Treatment Outcome

Subgroup analysis was done for the patients with poor treatment outcome (N = 130), where from figure 4 shows culture results from gastric biopsies. Growth of *H. pylori* was observed in 66% of the biopsies. In figure 5 the resistance patterns of the inoculated *H. pylori* were 30% for amoxicillin, 62.8 % for metronidazole and 30 % for clarithromycin.

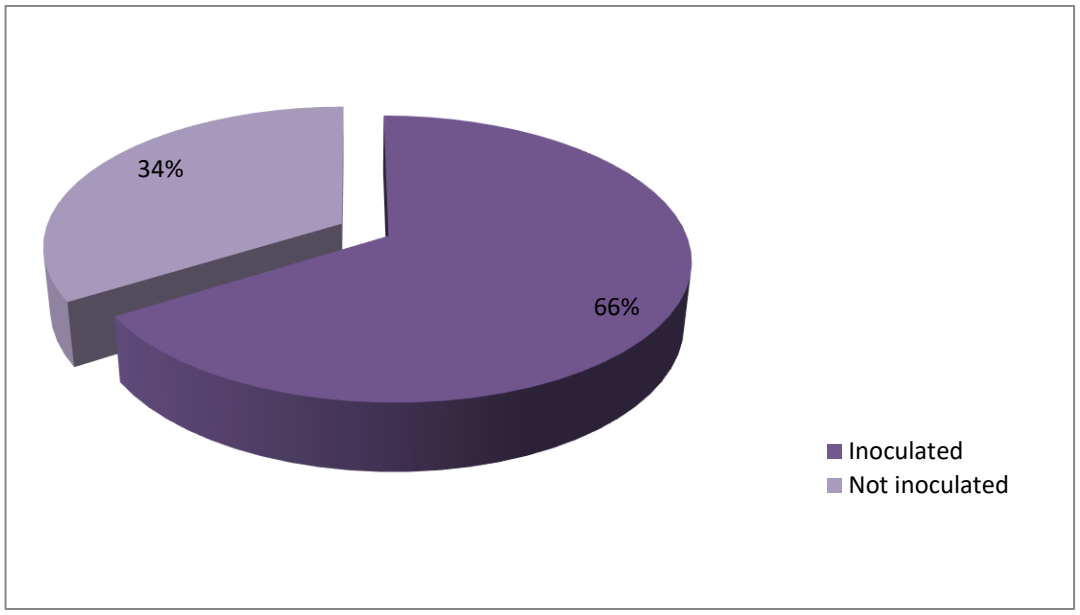


Figure 5: Culture results for patients with treatment failure N=130

4.7 Subgroup Analysis, To Determine Antibiotic Susceptibility Pattern in The Isolated H. Pylori from Culture

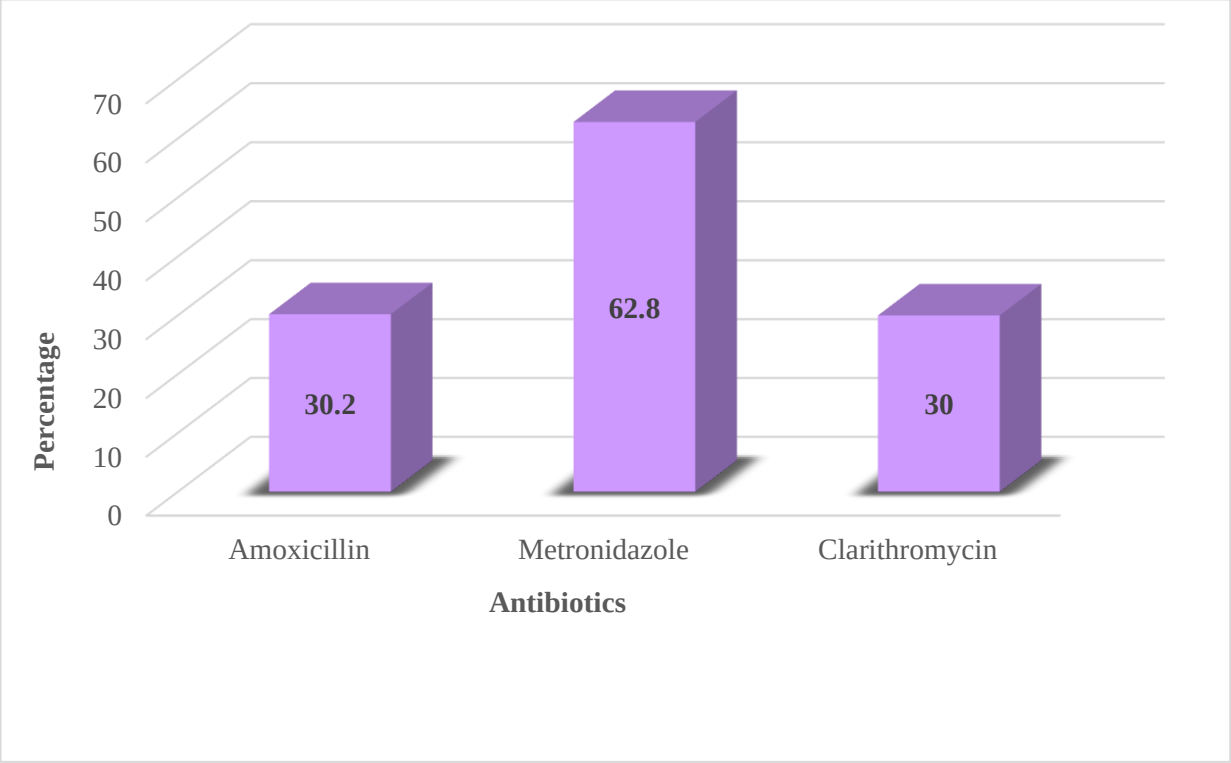


Figure 6: Antibiotic resistance pattern for inoculated H. pylori

CHAPTER FIVE

5 DISCUSSION

Helicobacter pylori prevalence varies greatly between different populations in different countries, worldwide the prevalence is estimated around 4.4 billion people in 2015. (5) In Tanzania a study conducted in 2014 found the prevalence of H. pylori to be 39.1%. (15) Dyspeptic symptoms may be appreciated in patients with H. pylori infection, patients with these symptoms are suspected to have H. pylori infection and appropriate management is to follow. Standard treatment of H. pylori is triple therapy, and this has been the treatment of choice for many years. (18)

Unfortunately, through the years the efficacy of triple therapy has declined and sequential, concomitant and hybrid therapy has replaced it. As evidenced by various studies the efficacy of triple therapy which is still guideline recommended first line has declined, and its efficacy has decreased to < 80%. (47) This is very far from the guideline indicated eradication rate of > 90%. (8) Some studies have shown decreased eradication rate of less than even 80% but, here we report an alarming treatment failure of over 30%, among patients seeking medical care in the referral Hospital in Tanzania. The data from this study coincides with the study done in Bugando Mwanza, were treatment failure of H. pylori on standard triple therapy was 30.9%. (18) The difference being the study conducted in Bugando only included dyspeptic patients who were not on antibiotics for the past 30 days, while in this study the patients included were those with dyspepsia without considering prior use of antibiotics. A study done in Egypt had similar results, where eradication rate was 67% in patients treated with standard clarithromycin based triple therapy for 14 days. (48)

As it is no secret that efficacy of clarithromycin based triple therapy for the treatment of H. pylori has plummeted below acceptable levels, this study also elucidated different reasons for this staggering decline. Among the factors observed was smoking while using triple therapy management. In our study 90% of the patients who smoked had treatment failure as opposed to those who did not smoke, and smoking was significantly associated with treatment failure (OR: 7.9 95% CI; (0.93 – 6.7) P= 0.03). This is in keeping with a meta- analysis done which showed

patient who smoked while on treatment had increased failure rate on eradicating H. pylori. (36) As time passes this conclusion still stands, in a meta- analysis of 2005 similar results were found and a conclusion was smoking increases the treatment failure rate of H. pylori eradication. (49) Nicotine reduces blood flow to the gastric mucosa and increases the gastric acid secretion and pepsin which might reduce delivery of the antibiotics to the gastric mucosa. Smoking may also affect activity of CYP2C19, increasing the metabolism of PPI which are cleared by this system. As PPIs are needed to aid antibiotics by reducing the gastric acidity. (50)

In addition to smoking, use of herbal medication with triple therapy is seen to have increased rate of treatment failure among the studied patients. Out of the 385 patients130 reported to be using herbal medication while on triple therapy. Some of the herbal medication mentioned was ash from charcoal, this was said to reduce the dyspeptic symptoms. The nigella sativa seed (black seed) in combination with honey was also widely used among patients to supplement antibiotics for the treatment of H. pylori infection. Studies have been done to show use of herbal medication to be successful to treat H. pylori. (51) In our study we looked at the relationship between use of herbal medication concurrently with use of triple therapy and the results showed it independently led to treatment failure (OR:6.7 95% CI; (3.9 – 11.6) P= <0.001). Further studies are needed to determine if use of some herbal medication alone can lead to treatment of Helicobacter pylori and to pinpoint which exact herbal medication may be used for treatment of H. pylori.

Good adherence to medication was found to increase the rate of having treatment success of H. pylori infection. Majority of the patients with good adherence to medication as assessed by the Morisky Medication Adherence Scale (MMAS) had treatment success after 4 weeks of treatment. Similar results were observed in a study conducted by Jaka et al. (18) Poor adherence to medication always leads to sub- therapeutic levels of medication resulting in a poor clinical outcome. Guideline approved first- line treatment of H. pylori consists of 2 antibiotics, clarithromycin based being the most used with an addition of a PPI. The duration of this treatment is usually 28 days, with 14 days of all 3 medication and the remaining 14 days with only PPIs.(52) Most patients tend to forget using the medication because of the long duration of

treatment. A study reported overall good adherence in patients using triple therapy, and for those who are non-adherent forgetting to take medication was found to be the main reason. (53)

In our study patients with endoscopy results of a peptic ulceration had poor treatment outcome as compared to those with non- ulcer findings. The reason for this is not clearly understood, gastric ulceration usually leads to increase gastric acid secretion and inactivation of antibiotics and that is one hypothesis. (38) Comparing to a different study which found treatment failure to be higher in duodenal and peptic ulcer as compared to non-ulcer patients. (38) These results differ in the study conducted on clinical trial participants, were they found higher success rates in patients in duodenal ulcer vs non ulcer patients. However, they did not consider gastric ulceration in their study which was considered in ours, and other factors such as resistance to the antibiotics was not explored in both groups. (54) In a study conducted in , the eradication rate of H. pylori among patients with both duodenal and gastric ulcer was 71% while in chronic gastritis was 77%, hence eradication rate was lower in peptic ulceration. In our study eradication rate was higher in non-ulcer patients (67%) vs peptic ulcer disease 51% endoscopic findings. (38) It has been suggested in other studies to consider different treatment approach for patients having H. pylori infection if they do, or do not have peptic ulceration. (54)

Initially resistance to antibiotic used in treatment of H. pylori was low, but as time rise in resistance first line treatment has been seen. Many studies have identified high resistance patterns to clarithromycin which is in the first line management of H. pylori. In the earlier days pre-treatment clarithromycin resistance ranged from 0.8% - 18%. In developing nations such Australia resistance to clarithromycin was noted between 6.1% to 7.8 %), with France having as prevalence as high as 18%. Metronidazole resistance had similar trends with prevalence reaching as high as 60% in Australia. This was attributed to increase use of metronidazole in other infections. (55)

In 2018, a pooled systemic meta-analysis was conducted to determine the magnitude of resistance to antibiotic of H. pylori infection in Africa. Results from 2085 isolates were tested for metronidazole, amoxicillin, clarithromycin, tetracyclines and quinolones, and overall pooled proportion of resistance were 75.8%, 72.6%, 29.2%,48.7% and 17.4 % respectively. This study concluded that prevalence to first line management i.e., clarithromycin, amoxicillin and metronidazole is high in Africa. (56) Jaka et al found the presence of clarithromycin resistant mutation was independently associated with first line treatment failure. (18) In our study, resistance patterns proportion among the first- line antibiotics for treatment of Helicobacter pylori was 30%, 62.8% and 30% for amoxicillin, metronidazole and clarithromycin respectively.

It has been recommended that for clarithromycin to be used as a first line treatment in a geographical area, resistance to clarithromycin has to be < 15% in that area, in this study clarithromycin resistance is double the value. (57) It is of paramount importance to consider change in our local management of H. pylori from clarithromycin based to bismuth based quadruple therapy and if not available to use concomitant therapy each for 14 days. As first line treatment a study compared 14 days and 10 days BQT, revealing high efficacy (96% vs 95%). Another study was conducted for concomitant regimen for10 days and eradication rates were as high as 96%. (52)

Metronidazole has been the commonest and highest resistant antibiotic in almost all regions. In America and Europe resistance rates where 42.1% and 38.9% respectively and African rates went as high as 75.8 %. (52) It is noted that resistance to metronidazole can be overcome by increasing doses to at least 1500 mg/24 hrs for 14 days. (58) Metronidazole is widely used in Tanzania for other infections and this could be the reasons for such high number of resistance used. It usually taken as an over-the-counter medication for various gastrointestinal causes. In a study conducted in Northern Tanzania metronidazole was the second most self-prescribed antibiotic after amoxicillin, self-medication has a multitude of hazardous effects and 96% of antibiotics self-medication can result to resistance. (59)

As of currents Tanzanian practice is prescription of macrolides, mostly azithromycin for urinary tract infection. In some of this cases OTC use of macrolides are used when symptoms that are thought to be in keeping with UTI occur, this can be as vague as headaches. A study in children alone proved prevalence of UTI to be up-to 40%, (60) and most of these patients are treated with macrolides. Due to this trend, it gives a clue as to why there has been a rise in clarithromycin resistance in our settings. It is important to advocate for reduction in use of macrolides for undiagnosed infections, public education is needed to ensure reduction in OTC use of this antibiotics so as to avoid the crises of the rise in antibiotic resistance. In addition to all of this, the emergence of COVID-19 pandemic led to the wide use of macrolides for its treatment. This can also attribute to the rise of clarithromycin in recent years.

In this study age and gender where not independently associated with the treatment outcome. Although in some studies being female was associated with treatment failure. (38) Age also had an association in other studies were, either being old was associated with treatment failure, attributed to atrophy of mucosal lining, or being young to treatment failure reason being reduced adherence to medication. (33)

The data from this study suggest a switch in management regimen, of either having antibiotic sensitivity test before initiation of H. pylori treatment, or first line regimen being switched to bismuth quadruple therapy due to increase in clarithromycin resistance to < 15%. Public education needs to be advised on avoidance of use of over-the-counter antibiotics, and ensure only diagnosed infection is treated according to how guidelines recommend. Factors that were found to be independently associated with treatment failure of H. pylori include concurrent smoking while on treatment, concurrent use of herbal medication, having a peptic ulcer disease and poor adherence to medication.

5.1 Strength of the Study

This was a large hospital-based cross-sectional study, effectively powered to accurately determine the treatment outcome of *H. pylori* infection with clarithromycin-based regimen among dyspeptic patients attending the endoscopy unit at MNH. Also, this study tested the antimicrobial susceptibility pattern against *H. pylori* by using the three, first-line antibiotics, which can benefit in suggesting alternative antibiotics in eradication therapy.

5.2 Limitation of the Study

In our study, adherence was patient reported and this could have led to imprecise judgement of adherence to the medication. In addition, stool antigen for *H. pylori* was used to determine eradication and this could have led to some false positive results. The study was conducted at a referral Hospital and hence might not be generalizable to the population. We also improvised the culture method by maintaining the microaerophilic environment required for *H. pylori* growth by using a candle jar which may lead to the microaerophilic environment not being attained. This could explain why we got few numbers of isolates. In testing the antimicrobial susceptibility pattern, we used the disc diffusion method instead of the recommended agar dilution and Etest which can overestimate or underestimate the susceptibility results

5.3 Conclusion

About one third of patients showed no eradication from clarithromycin-based therapy which is usually the first line used management for *H. pylori* infection.

Poor adherence, concurrent use of herbal medication and cigarette smoking were seen as significant causes of poor eradication of *H. pylori* eradication.

The resistance to all medication used as first line in the management of *H. pylori* are still staggeringly high.

5.4 Recommendations

Strengthening adherence counseling before and during treatment is crucial in ensuring treatment success. More effort is needed to ensure individual tailored treatment basing on the susceptibility patterns is practiced. Due to increased resistance to clarithromycin, first- line management should be shifted to bismuth based, or determination of sensitivity to antibiotics prior to initiation should be implemented.

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Appendix 1: Questionnaire

Factors associated with treatment outcome of *H. pylori* among dyspeptic patients using clarithromycin-based therapy at MNH

Sociodemographic characteristics of study participants

1. Study number: _____
2. Date of enrolment: _____

3. Sex: ☐ M ☐ F
4. Age: _____
5. Address: _____
6. Mobile number: _____
7. Marital status:
   a. Single ☐
   b. Married ☐
   c. Divorce ☐
   d. Cohabiting ☐
   e. Widow ☐
   f. Widower ☐

8. Level of education:
   a. No education ☐
   b. Primary incomplete ☐
   c. Primary complete ☐
   d. Secondary ☐
   e. College/ University ☐

9. Occupation:
   a. Unemployed
   b. Formal employment
   c. Self- employed (business owner)
   d. Casual laborer
   e. Peasant

10. Place of residence:
    a. Rural
    b. Urban

11. Do you currently smoke cigarettes? ☐ Yes No ☐
    a. If no:

12. Have you ever smoked cigarettes?
    in the past? Yes ☐ No ☐

13. Do you currently consume alcohol? Yes ☐ No ☐
    a. If no:

i. Have you ever consumed alcohol?
   in the past? Yes ☐ No ☐

CLINICAL CHARACTERISTICS

14. History of PUD diagnosis?
    Yes ☐
    No ☐

15. Was it treated?

Yes ☐

No ☐

16. Modality of treatment

a. PPI alone
b. Antibiotics alone
c. Triple therapy

17. Any use of PPI within the past 2 weeks?

Yes ☐

No ☐

18. Any history of using homeopathic medication.

Yes ☐

No ☐

TEST RESULTS

21. Stool antigen test after 4 weeks

a. Positive
b. Negative

22. Culture results

a. Growth

b. No growth

19. Tested drugs for susceptibility testing of *H. pylori*

	Resistant	Not resistant
i. Clarithromycin		
ii. Metronidazole		
iii. Amoxycillin		

20. Endoscopy results:

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Appendix 2: Assessment of Adherence to Medication Using MMAS

	YES	NO
1. Do you sometimes forget to take your medication?		
2. People sometimes miss taking their medications for reasons other than forgetting. Over the past 2 weeks, were there any days when you did not take your medication?		
3. Have you ever cut back or stopped taking your medication without telling your doctor because you felt worse when you took it?		
4. When you travel or leave home, do you sometimes forget to bring your medication?		
5. Did you take all your medication yesterday?		
6. When you feel like your symptoms are under control, do you sometimes stop taking your medication?		
7. Taking medication every day is a real inconvenience for some people. Do you ever feel hassled about sticking to your treatment plan?		
8. How often do you have difficulty remembering to take all your medication? Never/Rarely……..... Once in a while……... Sometimes…….. Usually…… All the time……..		

Appendix 3. Data Collection Tool (English)

Appendix 2: Consent Form

Consent to participate in research

Read Informed Consent

Factors associated with treatment outcome of *H. pylori* infection among dyspeptic patients using clarithromycin-based therapy at Muhimbili National Hospital

Introduction and Purpose

My name is Dalila Mwindadi, I am a 2nd year resident at Muhimbili University of Health and Allied Sciences (MUHAS) together with my supervisors Dr. Ewaldo Komba and Dr. Mtebe Majigo we are conducting a research study on factors associated with treatment outcome of H. pylori infection among dyspeptic patients using clarithromycin-based therapy at Muhimbili National Hospital. We are requesting for your participation.

Why are we conducting this study; this is due to the fact that H. pylori infection is common worldwide and almost half of the world population is affected. Moreover, H. pylori infection is associated with GI complication such as PUD, gastric cancers and lymphoma. Hence, the information which will be gathered from this study will be useful in determining the magnitude of H. pylori infection in Tanzania and what reasons are associated with treatment outcome with standard regimen. Your participation is vital and will be highly appreciated.

Participation in research is voluntary. You are free to decline to take part in the study. You can decline to answer any questions. You are free to stop taking part in the research study at any time. There will be no penalty to you or loss of services if you do decide not to answer any questions or decide to stop being in the study.

Procedures

If you agree to be part of this research, I will conduct an interview with you now. The interview will involve some questions and investigations, I will collect your stool sample before the OGD

procedure and treatment will be given to you depending on the results (which will be present within 24 hours) of the sample taken. We will kindly request to see you two weeks after cessation of your medication for H. pylori infection, where your stool sample will be collected for retesting for infection. In case of a positive results after treatment we will request for a second OGD procedure for biopsy and culture which will guide us on the proper management.

Benefits

The results of this study will help us to improve care of our patients.

Risks/Discomforts

Some of the research questions may be uncomfortable to you, you are free to refuse to answer any questions, or to stop the interview at any time. As with all research, there is a small chance that your privacy could be breached; however, we are taking precautions to minimize this risk. After taking biopsy patient may experience pain, anti-pain such paracetamol will be given to all patients in need.

Confidentiality

Your study data will be handled with maximum confidentially. If results of this study are published or presented, individual names and other individual information will not be used.

All study records will be stored on a password protected, encrypted computer. All paper survey forms will be kept in a locked cabinet at the study office, which is not at the clinic.

Do you have any questions for me? (If yes, note the questions)

You may ask questions about this study at any time. If you have questions after you leave the hospital, please ask me.

Concern

If you have any concern, you may contact

Dr. Dalila Mwindadi

Principal Investigator

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PO Box 65000, Dar es Salaam, Tanzania.
Mobile phone: +255 713200171.
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In case of any inquiries on your rights as a participant in this study kindly contact:

The Chairperson of Research and Publication Committee
Muhimbili University of Health and Allied Sciences (MUHAS)
P.O. Box 65001 Dar es Salaam, Tanzania
Tel: +255-022-2152489, Fax: +255-022-2152489, Email: drp.muhas.ac.tz.

Consent

I have understood the above information and I willingly agree to participate in this study with full understanding of the study purpose.

Signature of the participant: _____ **Date**: _____

Thank you

_____ ____/____/_____

Signature of interviewer **Date:**

Certifying that informed consent has been given verbally by respondent

Appendix 4: Informed Consent (Swahili Version)

Soma mbele ya mgonjwa

Fomu ya ridhaa ya kushiriki katika utafiti huu

Sababu zinazohusishwa na matokeo ya matibabu ya maambukizi ya Helicobacter pylori kwa wagonjwa wenye kingulia, maumivu ya tumbo juu ya kitovu, tumbo kufura na kushiba kwa haraka wanaotibiwa na dawa zenye msingi wa clarithromycin katika hospitali ya taifa ya Muhimbili

Dibaji na Lengo la utafiti

Jina langu ni Dalila Mwindadi, daktari ninayesomea masomo ya uzamili katika chuo kikuu cha Sayansi na Tiba Muhimbili (MUHAS). Tunafanya utafiti wa kuangalia sababu zinazohusishwa na matokeo ya matibabu ya maambukizi ya bacteria H. pylori, hivyo nakuomba uweze kuwa mshiriki katika utafiti huu. Tunafanya utafiti huu kwa sababu inakadiriwa nusu ya watu duniani kote wana maabukizi ya hawa bacteria. Kwa maana hiyo, matokeo ya utafiti huu yatatusaidia kuona ukubwa wa tatizo hili na sababu husishi ya matoke mabaya ya matibabu katika nchi yetu kwa wagonjwa ambao wanahudhuria matibabu katika Hospitali ya Taifa Muhimbili.

Ushiriki wako ni wa muhimu sana katika utafiti huu. Ushiriki katika utafiti huu ni wa hiari. Uko huru kukataa kushiriki kwenye utafiti huu,na endapo umekubali kuanza kushiriki na baadaye ukaghairi unaruhusiwa kuacha. Hakutokuwa na adhabu yoyote kama ukikataa kushiriki katika utafiti hu una kukataa kwako hakutaathiri utaratibu wowote kwenye matibabu yako.

Manufaa ya utafiti

Ni matumaini yetu kuwa matokeo ya utafiti huu yatasaidia kuboresha huduma zetu kwa wagonjwa ambao watakutwa na maambukizi ya hawa bacteria.

Adha ya utafiti huu

Baadhi ya maswali utakayoulizwa yanaweza kukuudhi/kukukela,ni hiari kujibu au kukataa kujibu swali lolote utakaloulizwa, au kukataa kuendelea na mahojiano hayo,pia tutajitahidi kutunza taarifa zako kama siri.

Usiri

Taarifa zako zote zitakuwa siri na ,kama matokeo ya utafiti huu yatachapishwa taarifa zako binafsi hazitajumuishwa. Taarifa zako zote zitatunzwa kwa usiri wa hali ya juu.

Maswali

Kama una swali lolote kuhusiana na haki zako kama mshiriki katika utafiti huu waweza kuwasiliana na;

Dr. Dalila Mwindadi

Principal Investigator

Muhimbili University of Health and Allied Sciences (MUHAS)

Department of Internal Medicine

P.O. Box 65001

Dar es Salaam.

Tel. +255 743238813

AU

Dr. Ewaldo Komba, msimamizi mkuu wa utafiti huu,

MUHAS, PO Box 65000, Dar es Salaam, Tanzania.

Simu namba: +255 713200171.

Barua pepe: kombaewaldo@gmail.com

Kwa mawasiliano Zaidi kuhusiana na haki zako kwenye utafiti huu kama mshiriki tafadhari wasiliana na;

Mwenyeketi wa tume ya tafiti na uchapishaji wa tafiti

Muhimbili University of Health and Allied Sciences (MUHAS)

P.O. Box 65001

Dar es Salaam

Tel. 022-2152489

Sahihi ya mhojiwa: _____ **Tarehe ya mahojiano**: _____

Asante sana!_____ ____/____/_____

Sahihi ya mhojaji:_____ Tarehe ya mahojiano_____

Kuthibitisha kuwa ridhaa imetolewa kwa mdomo na mgonjwa

Appendix 5: **Rome Iv Diagnostic Criteria For Dyspepsia**
Provides criteria for diagnosis of dyspepsia.

- It is used in patients with recurrent upper GI symptoms on average once weekly in the last three months with symptoms onset >6months and no abnormalities on diagnostic testing, including upper endoscopy.

- It is not used in patients with alarm symptoms such as GI bleeding, unexplained iron deficiency anemia, unintentional weight loss, palpable abdominal mass, family history of colon cancer or symptoms onset >50 years of age and not yet screened for colon cancer or sudden onset of new change in bowel habit.

- Patient must not have evidence of organic, systemic, structural disease likely to explain the symptoms

- Patient must have one or more of the following symptoms for 3 months prior with onset of symptoms for > 6 months

 Bothersome postprandial fullness

 Bothersome early satiety

 Bothersome epigastric pain

 Bothersome epigastric burning

Appendix 5: Ethical Clearances

UNITED REPUBLIC OF TANZANIA

MINISTRY OF EDUCATION, SCIENCE AND TECHNOLOGY

MUHIMBILI UNIVERSITY OF HEALTH AND ALLIED SCIENCES

OFFICE OF THE DIRECTOR - RESEARCH AND PUBLICATIONS

Ref. No.DA.282/298/01.C/1471 Date: 01/12/2022

MUHAS-REC-12-2022-1471

Dalila Mwindadi
MMed in Internal Medicine,
School of Medicine,
MUHAS

RE: APPROVAL FOR ETHICAL CLEARANCE FOR A STUDY TITLED: TO DETERMINE FACTORS ASSOCIATED WITH TREATMENT OUTCOME OF HELICOBACTER PYLORI AMONG DYSPEPTIC PATIENTS USING CLARITHROMYCIN BASED THERAPY AT MUHIMBILI NATIONAL HOSPITAL.

Reference is made to the above heading.

I am pleased to inform you that the Chairman has on behalf of the University Senate, approved ethical clearance of the above-mentioned study, on recommendations of the Senate Research and Publications Committee meeting accordance with MUHAS research policy and Tanzania regulations governing human and animal subjects research.

APPROVAL DATE: 01/12/2022
EXPIRATION DATE OF APPROVAL: 30/11/2023

STUDY DESCRIPTION:
Purpose:
The purpose of this hospital based observational, cross-sectional study is to determine the factors associated with treatment outcome among dyspeptic patients with H. pylori using clarithromycin-based therapy undergoing endoscopy at MNH.

The approved protocol and procedures for this study is attached and stamped with this letter, and can be found in the link provided:
https://irb.muhas.ac.tz/storage/Certificates/Certificate%20-%2014021.pdf and in the MUHAS archives.

9 United Nations Road; Upanga West; P.O. Box 65001, Dar Es Salaam: Tel. G/Line: +255-22-2150302/6; Ext. 1038; Direct Line:+255-22-2152489; Telefax:+255-22-2152489;E-mail:drp@muhas.ac.tz;Web:https://www.muhas.ac.tz

The PI is required to:

1. Submit bi-annual progress reports and final report upon completion of the study.
2. Report to the IRB any unanticipated problem involving risks to subjects or others including adverse events where applicable.
3. Apply for renewal of approval of ethical clearance one (1) month prior its expiration if the study is not completed at the end of this ethical approval. You may not continue with any research activity beyond the expiration date without the approval of the IRB. Failure to receive approval for continuation before the expiration date will result in automatic termination of the approval for this study on the expiration date.
4. Obtain IRB amendment (s) approval for any changes to any aspect of this study before they can be implemented.
5. Data security is ultimately the responsibility of the investigator.
6. Apply for and obtain data transfer agreement (DTA) from NIMR if data will be transferred to a foreign country.
7. Apply for and obtain material transfer agreement (MTA) from NIMR, if research materials (samples) will be shipped to a foreign country,
8. Any researcher, who contravenes or fail to comply with these conditions, shall be guilty of an offence and shall be liable on conviction to a fine as per NIMR Act No. 23 of 1979, PART III section 10 (2)
9. The PI is required to ensure that the findings of the study are disseminated to relevant stake holders.
10. PI is required to be versed with necessary laws and regulatory policies that govern research in Tanzania. Some guidance is available on our website https://drp.muhas.ac.tz/.

Prof. Bruno Sunguya
Chairman, MUHAS Research and Ethics Committee

c.c Director of Postgraduate Studies
MUHAS

9 United Nations Road; Upanga West; P.O. Box 65001, Dar Es Salaam: Tel. G/Line: +255-22-2150302/6; Ext. 1038; Direct Line:+255-22-2152489; Telefax:+255-22-2152489;E-mail:drp@muhas.ac.tz;Web:https://www.muhas.ac.tz

Appendix 6: Introduction Letter

UNITED REPUBLIC OF TANZANIA
MINISTRY OF EDUCATION, SCIENCE AND TECHNOLOGY
MUHIMBILI UNIVERSITY OF HEALTH AND ALLIED SCIENCES
OFFICE OF THE DIRECTOR – POSTGRADUATE STUDIES

In reply quote;
Ref. No. HD/MUH/T.87/2020 07th December, 2022

The Executive Director,
Muhimbili National Hospital,
P.O. Box 65000,
DAR E SALAAM.

Re: INTRODUCTION LETTER

The bearer of this letter is Dalila Mwindadi, a student at Muhimbili University of Health and Allied Sciences (MUHAS) pursuing MMed. Internal Medicine.

As part of her studies she intends to do a study titled: "***To Determine Factors Associated with Treated Outcome of Helicobacter Pylori Among Dyspeptic Patients Using Clarithromycin Based Therapy at Muhimbili National Hospital.***"

The research has been approved by the Chairman of University Senate.

Kindly provide her the necessary assistance to facilitate the conduct of her research.

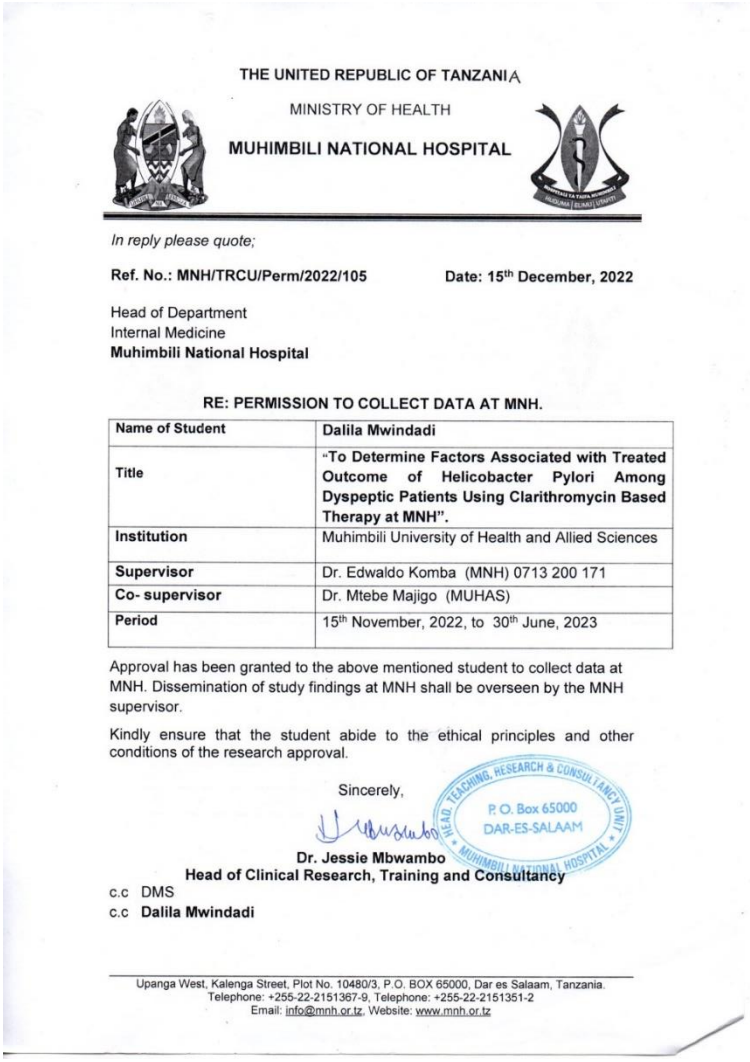
We thank you for your cooperation.

Victoria Mwanilwa
For: DIRECTOR, POSTGRADUATE STUDIES

c.c. Dalila Mwindadi

9 United Nations Road; Upanga West; P.O. Box 65001, Dar Es Salaam: Tel. G/Line: +255-22-2150302/6; Ext. 1015; Direct Line:+255-22-2151378;Telefax:+255-22-2150465;E-mail:dpgs@muhas.ac.tz;Web:https://www.muhas.ac.tz

Appendix 7: Permission Letter



THE UNITED REPUBLIC OF TANZANIA

MINISTRY OF HEALTH

MUHIMBILI NATIONAL HOSPITAL

In reply please quote;

Ref. No.: MNH/TRCU/Perm/2022/105 **Date: 15th December, 2022**

Head of Department
Internal Medicine
Muhimbili National Hospital

RE: PERMISSION TO COLLECT DATA AT MNH.

Name of Student	**Dalila Mwindadi**
Title	**"To Determine Factors Associated with Treated Outcome of Helicobacter Pylori Among Dyspeptic Patients Using Clarithromycin Based Therapy at MNH".**
Institution	Muhimbili University of Health and Allied Sciences
Supervisor	Dr. Edwaldo Komba (MNH) 0713 200 171
Co- supervisor	Dr. Mtebe Majigo (MUHAS)
Period	15th November, 2022, to 30th June, 2023

Approval has been granted to the above mentioned student to collect data at MNH. Dissemination of study findings at MNH shall be overseen by the MNH supervisor.

Kindly ensure that the student abide to the ethical principles and other conditions of the research approval.

Sincerely,

HEAD, TEACHING, RESEARCH & CONSULTANCY UNIT
P. O. Box 65000
DAR-ES-SALAAM
MUHIMBILI NATIONAL HOSPITAL

Dr. Jessie Mbwambo
Head of Clinical Research, Training and Consultancy

c.c DMS
c.c **Dalila Mwindadi**

Upanga West, Kalenga Street, Plot No. 10480/3, P.O. BOX 65000, Dar es Salaam, Tanzania.
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