ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF HELICOBACTER PYLORI ISOLATED FROM PATIENTS WITH DYSPEPSIA IN TWO TERTIARY HOSPITALS IN NAIROBI, KENYA

BY

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DECLARATION

STUDENT DECLARATION

I, **Dr Ann Njeri Kabuthi,** declare that this proposal is my original work and that it has not been presented for the award of a degree in any university.

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I dedicate this book to my daughter, Zahra Kitili, my inspiration to work harder every day.

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LIST OF ABBREVIATIONS

AGA	American Gastroenterology Association
AST	Antimicrobial Susceptibility Testing
CagA	Cytotoxin-associated gene A
CLO	Campylobacter-like Organisms
CLSI	Clinical and Laboratory Standards Institute
DPO	Dual-priming oligonucleotide
E-test	Episilometer strip test
GERD	Gastroesophageal reflux disease
KNH	Kenyatta National Hospital
MALT	Mucosal Associated Lymphoid Tissue
MIC	Minimum inhibitory concentration
NSAID	Non-steroidal anti-inflammatory drugs
OGD	Oesophago-Gastro-Duodenoscopy
PCR	Polymerase Chain Reaction
PPI	Proton pump inhibitors
RFLP	Restriction Fragment Length Polymorphism
TNH	The Nairobi Hospital
VacA	Vacuolating Cytotoxin
WHO	World Health Organization

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ABSTRACT

Background: *Helicobacter pylori* infection is a global problem with prevalence remaining high in developing countries. It has been implicated in the causation of a wide spectrum of gastric pathologies. The emergence of multi-drug resistant *Helicobacter pylori* strains poses a major challenge in the management of the associated diseases in a resource-limited country like Kenya. Therefore, there is a need for local surveillance of antimicrobial resistance, to guide clinicians in their choice of therapy.

Study objective: To determine the current antimicrobial susceptibility profiles of *Helicobacter pylori* isolated from dyspeptic patients at Kenyatta National Hospital and The Nairobi Hospital.

Design: Cross-sectional descriptive study

Method: Participants aged 18years and above with dyspepsia referred for endoscopy at the KNH and TNH endoscopy units were enrolled. Endoscopy was performed and five biopsy samples collected from each patient taken for culture. Antimicrobial susceptibility testing was performed for the samples that tested positive using E-test strips. MIC values $\geq$ 1 for amoxicillin and clarithromycin, $\geq$ 8 for metronidazole and >1 were considered resistant.

Results: A total of 158 patients with dyspepsia at KNH (n=82) and TNH (n=76) were enrolled in the study. These Patients were aged between 18 years and 77 years with a mean age of 43.5 years (SD 13.9). Eighty-five (53.8%) were females. Gastritis was the most common endoscopic finding (79.4%). All the isolates were sensitive to amoxicillin (MIC range 0.016- 0.75ug/ml), 66(97.1%) isolates were sensitive to levofloxacin (MIC range 0.012-2ug/ml) and ciprofloxacin (MIC range 0.016-3ug/ml). Nine (13.2%) isolates were resistant to clarithromycin (MIC range

0.02-2ug/ml). A significant (80.9%, n=55) proportion of the isolates were resistant to Metronidazole (MIC range 3->256ug/ml).

Conclusion: The study demonstrated that the *Helicobacter pylori* isolates were largely sensitive to amoxicillin, levofloxacin and ciprofloxacin. A significant proportion was resistant to metronidazole while 13.2% were resistant to clarithromycin.

CHAPTER ONE

1.1 Background of the study

Helicobacter pylori infection is a global problem that affects fifty per cent of the world's population, with a high prevalence reported in developing countries(1). In 2017, WHO recognized *Helicobacter pylori* as a high priority pathogen, which warrants research and development of new antibiotics, due to increasing clarithromycin resistance (2). Resistance to clarithromycin, metronidazole and levofloxacin has been reported in Algeria, Morocco and Congo in Africa (3) and also in other parts of the world. This resistance is due to the increased consumption of antibiotics (4).

This bacterium has been implicated in the causation of gastritis, peptic ulcer disease, gastric mucosal lymphoid tissue lymphoma and gastric adenocarcinoma. Eradication of this bacteria has been shown to cure and prevent recurrence of peptic ulcer disease (5) and reduce the progression of gastric mucosa-associated lymphoid tissue (MALT) lymphoma (6). WHO classifies *Helicobacter pylori* as a carcinogen that causes chronic inflammation of the gastric mucosa leading to atrophic gastritis and intestinal metaplasia, (7) pre-neoplastic lesions that eventually lead to gastric adenocarcinoma. Eradication of *Helicobacter pylori* causes regression of atrophic gastritis but not metaplasia. In addition, it reduces the risk of developing gastric adenocarcinoma (8). Treatment involves a combination of antimicrobial agents and a proton pump inhibitor. The major cause of treatment failure is antimicrobial resistance to various antimicrobial agents. Studies show that the success rates of standard first-line triple therapy are low in areas with clarithromycin resistance > 15% (9). The standard first-line therapy has been shown to eradicate more than 80% *Helicobacter pylori* isolates susceptible to clarithromycin and this decreases to 20% in clarithromycin resistant bacteria (9). This study aims to investigate the antimicrobial

susceptibility profile of *Helicobacter pylori* isolates to available antimicrobial agents to optimize its eradication and prevent prolonged treatment.

1.2 Problem statement

Antimicrobial resistance of *Helicobacter pylori* to various antimicrobial agents has been reported in various parts of the world including developing countries (3). This necessitates the need for antimicrobial susceptibility tests in the laboratory that will provide valuable information to clinicians when making decisions of what antimicrobial agents to use in patients with dyspepsia arising from *Helicobacter pylori* infection.

1.3 Study Justification

A similar study at KNH done by Lwai-Lume *et al* (2005) showed high rates of sensitivity of *Helicobacter pylori* isolates to tetracycline (98.1%), amoxicillin (95.4%) and clarithromycin (93.6%). However, these isolates were resistant to metronidazole (10). It's more than ten years since this study was done and antimicrobial susceptibility trends are changing. There are no previous similar studies conducted at TNH. Incidences of multidrug-resistant *Helicobacter pylori* have been reported in Africa and other parts of the world. In Africa resistance to clarithromycin was reported in Uganda (2017) (11), Algeria (2017) (12), Morocco (2015) (13) and Congo (2015) (14). High rates of metronidazole resistance were reported in Algeria, Morocco and Senegal while levofloxacin resistance was reported in Morocco and Congo. In addition, studies in western countries show that antimicrobial resistance varies among countries, regions and even periods in the same area. Therefore, regular antimicrobial susceptibility testing is necessary for the appropriate choice of antibiotics.

In February 2017 WHO recognized *Helicobacter pylori* as a high priority pathogen necessitating research and development of new antibiotics (2). This was mainly due to increasing antibiotic resistance rates. The success rate of standard first-line triple therapy is less than 85% in areas with clarithromycin resistance greater than 15% (9). In addition, treatment with standard first-line therapy has been shown to eradicate more than 80% of *Helicobacter pylori* strains susceptible to clarithromycin and this decrease to 20% of clarithromycin resistant strains (9).

Helicobacter pylori affect half the world's population(1). Its prevalence remains high in developing countries. Eradication of *Helicobacter pylori* has been shown to reduce progression to long term complications such as atrophic gastritis and gastric MALT lymphoma (6) and provides a cure and prevent recurrence for peptic ulcer disease (5). It also reduces the risk of gastric adenocarcinoma (8).

This study aimed to investigate the antimicrobial susceptibility profile of *Helicobacter pylori* isolates to available antimicrobial agents to optimize its eradication and prevent prolonged treatment.

1.4 Research Question

What is the antimicrobial susceptibility profile of *Helicobacter pylori* isolated from dyspeptic patients in Kenyatta National Hospital and The Nairobi Hospital?

1.5 Objectives

1.5.1 Broad Objective

To determine the antimicrobial susceptibility profile of *Helicobacter pylori* in patients with dyspepsia referred for upper gastrointestinal endoscopy at Kenyatta National Hospital and The Nairobi Hospital.

1.5.2 Specific Objectives

1. To determine the proportion of *Helicobacter pylori* isolates susceptible to clarithromycin, amoxicillin, metronidazole, ciprofloxacin and levofloxacin.

CHAPTER TWO

2.1 LITERATURE REVIEW

2.2 Dyspepsia

ROME III Criteria defines dyspepsia as having one or more of the following symptoms: epigastric pain, epigastric burning, early satiation and bothersome postprandial fullness. According to the new ROME IV criteria, all the four symptoms above should be determined as bothersome symptoms (15). Dyspepsia can be classified into functional dyspepsia and dyspepsia due to an organic cause such as gastric malignancy, peptic ulcer disease and non-steroidal anti-inflammatory drugs (NSAIDs) use. Functional dyspepsia is due to disorders in gastric motility such as impaired accommodation to a meal and impaired gastric emptying, visceral hypersensitivity and *Helicobacter pylori* infection.

2.2 Helicobacter pylori-associated dyspepsia

2.2.1 Helicobacter pylori

Helicobacter pylori previously known as *Campylobacter pylori* was first described by Barry Marshall and Robin Warren (1983) in Western Australia (16). *Helicobacter pylorus* is a gram-negative curved bacillus that infects the gastric mucosa. It's a fastidious organism that thrives well in a microaerophilic environment.

2.2.2 Pathogenesis of Helicobacter Pylori

Helicobacter pylori infection can cause both functional and organic dyspepsia. However, the mechanism by which it causes functional dyspepsia is unclear. It is postulated that it causes increased secretion of acid by causing increased release of gastrin and reduced production of somatostatin(17). It also alters the release of ghrelin in gastric mucosa, which is also involved in

gastric acid production (17). El Omar *et al* (1995) showed that the amount of gastrin-releasing peptide was three times more in patients with functional dyspepsia caused by *Helicobacter pylori* infection than the negative controls (18). Eradication of *Helicobacter pylori* has been associated with normalization of acid production and relief of dyspepsia lasting 1 year in patients with functional dyspepsia (19).

Helicobacter pylori have been implicated in the causation of gastritis, peptic ulcer disease, gastric adenocarcinoma and gastric MALT lymphoma. These patients present with dyspepsia.

Upon entering the host's stomach, it produces a urease enzyme which converts urea into ammonia and carbon dioxide making it adapt to the acidic stomach and utilizing two to six sheathed flagella at one polar end, it moves through the mucous layer to the gastric epithelium (20).

Upon reaching the gastric epithelial cells, *Helicobacter pylori* express adhesins that facilitate its attachment to the epithelium. These include blood antigen binding protein A, sialic acid-binding adhesin, heat shock protein 60, lacdinac binding adhesion, *Helicobacter pylori* outer membrane protein Z, Neutrophil associated protein A and adhesion associated protein(*Alp A* and *Alp B* (20).

After adhering to the host epithelial cells, *Helicobacter pylori* releases toxins which include cytotoxin associated gene A (Cag A) and Vacuolating cytotoxin A (VacA) which damage hosts tissues leading to chronic inflammation (20).

2.2.3 Diagnosis of *Helicobacter pylori* in dyspeptic patients

Diagnostic tests for *Helicobacter pylori* include non-invasive and invasive tests, which require endoscopy. Non-invasive tests include urease breath test, stool antigen test, saliva and urinary

assays. Of these, the urease breath test is recommended due to its high sensitivity and specificity of 95% and 95%-100% respectively (9).

Upper gastrointestinal endoscopy is performed to diagnose *Helicobacter pylori*-related pathologies. It allows direct visualization of gastric mucosa and collection of biopsy specimens for rapid urease testing, histology, bacterial cultures which are used for antibiotic susceptibility testing (21).

2.2.3.1 Gastric biopsies

The American Gastroenterologists Association (AGA) 2015 guidelines recommended that biopsies should be taken from the antrum and gastric body in dyspeptic patients undergoing endoscopy (22). These guidelines also recommend the use of the 5-biopsy Sydney System protocol for obtaining biopsies. The 5-biopsy Sydney System involves taking biopsies (one from each site) from the lesser curvature of the antrum, the greater curvature of the antrum, and the lesser curvature of the body, the greater curvature of the body and from the incisura angularis. This has been shown to increase the yield of *Helicobacter pylori*. In addition, more cases of *Helicobacter pylori* will be detected in histology using the routine stains without the need of immune- histochemical stains which are not readily available. All biopsy specimens collected should be placed in the same jar, limiting the cost incurred without interfering with the results (22).

2.2.3.2 Biopsy Urease Test

The biopsy urease test is a rapid test that relies on urease activity. *Helicobacter pylori* produce a urease enzyme that converts urea into ammonia and carbon dioxide on alkaline PH.

Three different types of rapid urease test kits are available in the market including Hpfast (GI supply), Pylori Tec (Serim manufacturers) and CLO (campylobacter-like organism) (Kimberly-Clark Healthcare) test kits. These tests have been shown to have similar specificities of 99-100% and comparable sensitivities of 88%, 89% and 93% respectively at 4 hours (23). The CLO test which is most widely used is a test where a biopsy specimen is placed on an agar plate urea broth and a PH indicator.

All three tests can yield false-negative results in patients with intestinal metaplasia and atrophic gastritis (24) as well as those who were previously on Proton pump inhibitors (PPIs) (25), bismuth salts, and antibiotics (26). False positive tests do rarely occur (21).

To improve on the sensitivity of the rapid tests, tissue biopsies should be taken from the corpus and antrum, this was demonstrated in a study by Uotani *et al.* (2015) (26). Combining biopsy specimens obtained from different sites, taking large biopsies and increasing the number of biopsy specimens collected also enhance the identification of *Helicobacter pylori* (22).

2.2.3.3 Histology

The tissue biopsy is prepared in the laboratory and stained using special stains and viewed under a microscope. It has been considered the gold standard for making the primary diagnosis (27). Histology also provides information about the presence of peptic ulcers, gastritis, gastric malignancies among others.

Modified Giemsa stain can be used for identification of *Helicobacter pylori*, it's highly sensitive and readily available through the best stain to use is an immunohistochemical stain which is most sensitive and specific for helicobacter detection (28).

Proton pump inhibitors enhance the growth of coccoid bacteria which can only be visualized using immuno-histochemical stains which are not readily available and are costly (29). Using multiple tissue biopsies collected from multiple sites has been shown to improve the test sensitivity. Histology could be subjective and this will affect the diagnostic accuracy (27).

2.2.3.4 Molecular Method

In situ hybridization and real-time Polymerase Chain Reaction (PCR) tests can be used to make a diagnosis of *Helicobacter pylori* on tissue biopsies. They are both highly sensitive and specific (30) but are expensive.

2.23.5 Bacterial Culture

Tissue biopsies are homogenized and placed in culture plates containing brain heart infusion agar or Colombia blood agar enriched with *Helicobacter pylori* selective and nutritional supplements and are incubated.

To increase the bacterial yield, antimicrobials should be avoided at least 4weeks and proton pump inhibitors 2weeks before endoscopy because they inhibit the growth of bacteria (31). Biopsy specimen once obtained should be transported to the laboratory and processed within 6 hours and if there's a delay in processing, the specimens should be refrigerated as per UK Standards for Microbiology Investigations (31). Tissue biopsies are then placed in agar plates containing culture medium and incubated under a microaerophilic environment at 37degrees for 7-10 days (31). Bacterial colonies are visible within 3-5 days. The culture medium should be supplemented with antimicrobial agents to inhibit the overgrowth of contaminating bacteria and fungi (31).

After incubation *Helicobacter pylori* are confirmed by gram stain and positive oxidase, urease and catalase tests.

2.3 Antimicrobial Susceptibility Testing (AST)

There are both phenotypic and genotypic methods of performing antimicrobial susceptibility testing. Phenotypic assays include Agar dilution, Broth microdilution, Disc diffusion and Epsilometer strip test (E-test) method. All are culture-based methods.

The Agar dilution method is not routinely done and is considered a reference method for evaluating the accuracy of other phenotypic methods.

Disc diffusion method is the most frequently used method for AST. It's simple and cost-effective. However, it's not recommended for slow-growing bacteria like *Helicobacter pylori* due to the unstable release of antibiotics from the discs (32). An antibiotic coated disc is placed on an agar plate inoculated with the bacteria. The zone of bacterial growth inhibition is then determined. It's a qualitative test, and test results are read as either sensitive or resistant.

The E-test method is a quantitative variant of the Disc diffusion method and is recommended for slow-growing bacteria since it has a stable pattern of antibiotic release and can withstand prolonged incubation. In this method plastic strips calibrated with a predefined concentration of antibiotic are placed on the agar plates inoculated with bacteria. The minimum inhibitory concentration is read from the intersection of the elliptical zone of growth of inhibition. Genotypic assays detect point mutations associated with antimicrobial resistance. These are nucleic acid-based methods and have a sensitivity of 98% and specificity of 92% (33). They include PCR-restriction fragment length polymorphism (RFLP), real-time PCR and dual-priming

oligonucleotide (DPO)-PCR. These methods are fast and available for the detection of clarithromycin, levofloxacin and tetracycline resistance (33).

2.4 Treatment of Helicobacter Pylori

Treatment of *Helicobacter pylori* infection involves the use of a combination of antimicrobial agents and proton pump inhibitors (PPIs).

2.4.1 First Line Therapy

First-line Standard triple therapy consists of a proton pump inhibitor, clarithromycin and amoxicillin for 14 days. A meta-analysis by Zullo *et al* (2013) found sequential therapy, which consists of a PPI and amoxicillin for 5-7 days followed by 5-7 days of a PPI and clarithromycin and metronidazole, superior to a 14-day standard triple therapy (34). Both the 14 days and 10 days regimen offer comparable eradication rates of 90.7%-92.5% and 87% respectively(35).

Treatment failure to this first-line therapy has been attributed to clarithromycin and metronidazole resistance. The Maastricht V/Florence Consensus report recommends the use of bismuth-containing quadruple therapy which contains a PPI, bismuth salicylate, metronidazole and tetracycline, as an alternative first-line in areas where clarithromycin resistance is more than 15%. If not available 14 days of non-bismuth quadruple therapy (concomitant therapy) which consists of a PPI, amoxicillin, clarithromycin and metronidazole should be used (36). This regimen is superior to a 14day triple therapy with eradication rates of 94% (37). Novel concomitant therapy consists of a PPI, amoxicillin, rifabutin and ciprofloxacin for 10 days. For patients with penicillin allergy bismuth is recommended instead of amoxicillin. A study by Tay CY *et al* (2012) showed that the amoxicillin containing regimen attained an eradication rate of 95% while that of bismuth achieved a 94% eradication rate (38).

The use of bismuth-containing quadruple therapy is recommended in areas with resistance to metronidazole and clarithromycin (9) and is the first line of choice in patients with allergy to penicillin (9).

A systematic review and meta-analysis by Lopez-Gongora*et al* (2015) showed that culture guided first-line therapy was to be more effective than the standard therapy efficacy (39).

2.4.2 Second-Line Therapy

It consists of a quadruple therapy that contains bismuth, PPI, metronidazole and tetracycline or levofloxacin based triple therapy (9). Both regimens have comparable cure rates but the levofloxacin based regimen has fewer adverse effects (40). A study by Gisbert J P *et al* (2005) showed that using a quadruple therapy containing both bismuth and levofloxacin provides more than 90% cure rates when used for 14 days (41). In patients with penicillin allergy, a levofloxacin based regimen should be used (41).

2.4.3 Rescue or Third-Line Therapy

When the second line fails, antimicrobial susceptibility testing or molecular determination of resistance should be done in places where it's available (9). Bismuth-based quadruple therapy containing a PPI, bismuth, amoxicillin or tetracycline, furazolidone or metronidazole used for 14 days provides eradications of up to 90% (42).

14 days of levofloxacin-containing sequential therapy, which consists of 7 days PPI and amoxicillin followed by 7 days of PPI, metronidazole and levofloxacin can also be used. A study by Liou JM *et al* (2013) showed that higher eradication rates were achieved using levofloxacin based sequential therapy as compared to tetracycline and clarithromycin therapies (43).

2.5 Antimicrobial resistance of *Helicobacter pylori*

Globally the prevalence of bacterial resistance to antimicrobial agents varies and has been rising in many countries (9). In 2017, WHO recognized *Helicobacter pylori* as a high priority pathogen, which necessitates research and development of new antibiotics, due to increasing clarithromycin resistance (2). A review article by Thung I *et al* (2016) showed that eradication rates of *Helicobacter pylori* have declined globally due to antimicrobial resistance particularly clarithromycin resistance which has been on the rise over the last 10 years (44). In this review, the highest rates were reported in China (50%) and Turkey (40%).

In Europe, resistance to clarithromycin, levofloxacin and metronidazole was reported in a multi-center study (45). In Japan, clarithromycin resistance increased from 19% to 28% in three years (46).

Clarithromycin resistance was also reported in Africa. In Cameroon, Koutcheamabeke *et al* (2019) reported a resistance rate of 13.8% while in Uganda Angol*et al* (2017) reported a resistance rate of 29% (47(11). In Algeria Rauf *et al* (2017) reported resistance rates of 23% and 36% for primary and secondary resistance respectively (12). Clarithromycin resistance was also reported in Morocco (2015) and Congo (2015)(13,14).

Metronidazole resistance on the other hand was reported in Cameroon (2019),(47) , Algeria (2017),(12), Morocco (2015), (13), Senegal (2013), (48), Cameroon (2008),(49) and Kenya (2019, 2005), (50,10) with resistance rates of 40%, 85%,95%,93.2% and 100% respectively. A study by Ndip *et al.* (2008) reported amoxicillin resistance in Cameroon respectively (49). Levofloxacin resistance was reported in Uganda, Morocco and Senegal in studies byAngol*et al* (2017), Bouihat *et al*. (2015) and Seck *et al* (2013) respectively(11),(13),(48). Kiang's *et al.*

(2010) in a study done at the Aga Khan University Hospital, Nairobi, Kenya showed no resistance to the three antibiotics studied (51).

Table 1: Summary of Previous Studies on Antimicrobial Susceptibility of Helicobacter Pylori Isolates in Patients with Dyspepsia

Study	**Country**	**Number of isolates**	**Antimicrobial susceptibility test done**	**Findings** ***H pylori*** **susceptibility rates**
Kouitcheu Maleku *et al* (2019) (47)	Cameroon	140	Disc diffusion	No resistance to IMP, RFB, AZM, LVX, CIP and NOR Resistance to AMX, AMC, AMP and PEN (100%) Resistance to ERY (48%), CLR (13.8%) Resistance to MDZ (98%), TET (0.7%), DOX (2.9%) and MIN (0.71%)
Churyai *et al* (2015) (50)	Kenya	9	E-test	No resistance to CLR Resistance to AMX (22%), MDZ (100%) and (TET 3%)
Anglo et al (2017)(11)	Uganda	21	Real-time PCR	Resistance to LVX and MXF (42%), CLR (29%)
Raaf*et al* (2017) (12)	Algeria	84	Real-time PCR	No resistance to AMX, TET and RIF Resistance to CLR Primary (23%) secondary

				(36%) MDZ resistance Primary (45%), secondary (71%) LVX resistance 1 isolate
Djanne-Hadibi *et al.* (2016) (52)	Algeria	91	Real-time PCR	Resistance to CLR (33%)
OntsiraNgoyi *et al.* (2015) (14)	Congo	63	Real-time PCR	Resistance to CLR (1.7%), TET (2.5%) and LVX (50%)
Bouihat *et al.* (2015) (13)	Morocco	177	E-test and Disc diffusion method	No resistance to AMX, TET and RFB Resistance to CLR (29%), MDZ (40%) and LVX (11%) Both CLR and MDX resistance (2%)
Seck *et al.* (2013) (48)	Senegal	108	E-test	No resistance to CLR ,AMX and TET Resistance to LVX (15%) and MDZ (85%)
Kimanga AN *et al.* 2010 (51)	Kenya	65	E-Test	No resistance to CLR and AMX Resistance to MDZ (4.6%)

Oyedeji KS *et al.* (2009) (53)	Nigeria	186	Disc diffusion and E test	No resistance to OFX, CIP and NOR Resistance to PIP (77%), AMX (66%), TET (100%) and MDZ (95%)
Ndip RN *et al.* (2008) (49)	Cameroon	71	Disc diffusion	Resistance to CLR (44.7%), TET (43.9%), AMX (14.4%) and MDZ (93.2%)
Lwai-lume *et al.* (2005) (10)	Kenya	108	E-Test	Resistance to CLR (6.4 %), AMX (4.6 %), TET (1.9 %) and MDZ (100%)

AMX=amoxicillin; AMC=amoxicillin-clavulanic acid; AMP=ampicillin; AZM=azithromycin; CIP=ciprofloxacin; CLR=clarithromycin; DOX=doxycycline; ERY=erythromycin; IMP=imipenem; LVX=levofloxacin; MDZ=metronidazole; MIN=minocycline; MXF=moxifloxacin; NOR=norfloxacin; OFX=ofloxacin; PEN=penicillin; PIP=piperacillin; RFB=rifabutin; RIF=rifampicin; TET=tetracycline

CHAPTER THREE

3.1 MATERIALS AND METHOD

3.2 Study Sites

The study was conducted at the Kenyatta National Hospital and The Nairobi Hospital endoscopy units.

3.3 Study Design. It was a cross-sectional descriptive study.

3.4 Study Population

The study population comprised of adult patients (≥18 years) with dyspepsia undergoing OGD at KNH and TNH between August 2018 and October 2019

3.4.1 Case Definition for Dyspepsia

Patients with dyspepsia as defined by ROME III criteria: these were patients who had one or more of the following symptoms:

1. Epigastric pain
2. Epigastric burning
3. Early satiation
4. Bothersome postprandial fullness

3.4.2 Inclusion Criteria

1. An adult patient (≥18 years) with a diagnosis of dyspepsia

2. Patients who gave written informed consent to participate in the study

3.4.3 Exclusion Criteria

Patients on antibiotics 4 weeks before endoscopy, those on proton pump inhibitors were not excluded.

3.5 Sample Size Calculation

The interest of this study was to describe the antimicrobial susceptibility profile of *Helicobacter pylori* isolated from dyspepsia patients. The pattern was described in terms of the proportion of *Helicobacter pylori*-positive cultures susceptible or resistant to each type of antibiotic tested. The sample size was therefore calculated based on proportion using Daniel's formula (1999) for finite population

$$n \geq \frac{NZ^2{}_{\alpha/2}P(1-P)}{d^2(N-1)+Z^2{}_{\alpha/2}P(1-P)}$$

Where:

n= minimum sample size required

N=Total estimated accessible population of dyspepsia patients (N=70)

$Z_{\alpha/2}$= Critical value at α-level of significance for a two-sided test (α=0.05, $Z_{\alpha/2}$=1.96)

P=Estimated prevalence of *Helicobacter pylori* susceptibility to clarithromycin among patients at Cameroon (p=55.3%) (68)

d=Margin of error (d=0.05)

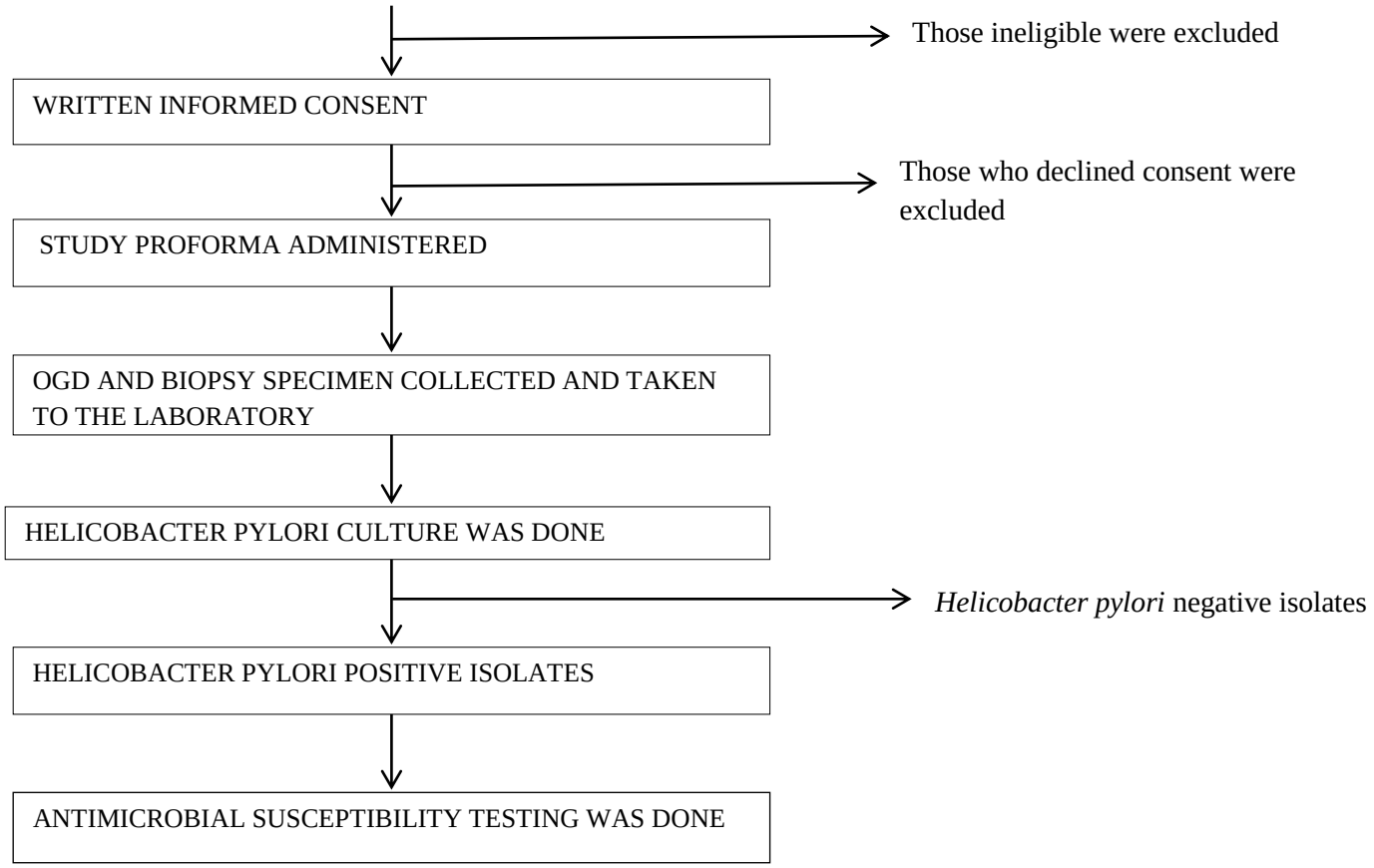
The minimum sample size required was 60 *Helicobacter pylori*-positive patients.

3.6 Sampling Method and Enrollment

Patients were selected consecutively until the required sample size was attained.

The primary investigator (PI) or the research assistant reviewed patients referred for upper gastrointestinal endoscopy to ascertain that they met the inclusion criteria. Those who met the inclusion criteria were given relevant information about the study. Those who gave written informed consent were enrolled. Once consent was obtained, a questionnaire was administered to obtain the demographic data of the study participant. Thereafter endoscopy was performed as planned and biopsy samples were taken to the laboratory for culture. An antimicrobial susceptibility test was then done on the samples where Helicobacter pylori were isolated.

Figure 1: Flow Chart: Patient Recruitment



3.7 Data Collection

3.7.1 Study Variables

Data was collected on the following variables;

- Demographic characteristics of the patient: age, gender, level of education and employment status
- Susceptibility test results for;
    - Clarithromycin
    - Amoxicillin
    - Metronidazole
    - Ciprofloxacin
    - Levofloxacin
- Minimum inhibitory concentrations for the listed antimicrobial agents
- Endoscopic findings

3.7.2 Laboratory Methods

3.7.2.1 Sample Collection

The procedure was explained to the patient. Endoscopy was done using the standard procedure by highly skilled consultant gastroenterologists practicing at KNH and TNH. Five biopsy specimens were taken in five sites; at the greater curvature of the antrum, the lesser curvature of the antrum, the lesser curvature of the corpus, the greater curvature of corpus and at the incisura angularis as per the American Gastroenterologists Association (AGA) 2015 guidelines. Endoscopic findings were then recorded on the study proforma.

3.7.2.2 Transportation and Processing

All the biopsy specimens collected were placed in a sterile specimen bottle containing brain heart infusion broth and transported to the laboratory at the Department of Microbiology, University of Nairobi. Transport and processing of specimens for culture were done within six hours, the time recommended by UK Standards for Microbiology Investigations(54).

The biopsies were transferred to clean sterile tubes using a sterile pipette where they were crushed using the sterile pipette tip and placed on a culture plate containing brain heart infusion agar (Oxoid, UK), inactivated fetal bovine serum, vitox nutritional supplement and dent supplement containing vancomycin (10mg/l), cefsulodin (5mg/l), amphotericin B (5mg/l), and trimethoprim (5mg/l) (Oxoid UK) (Appendix 4). The plate was placed in a jar containing CampyGen gas packs (microaerophilic incubation) and incubated at 37°C for a maximum of 7 days. Also cultured on the plate was a control strain; *Helicobacter pylori* 39500T (Appendix 5).

3.7.2.3 Culture Interpretation

After incubation, *Helicobacter pylori*-positive cultures were confirmed through microscopy as curved gram-negative bacilli, urease positive, catalase-positive, and oxidase-positive respectively.

The positively identified isolates were emulsified in 20% glycerol and stored at - 70 °C until further analysis.

Thereafter, antimicrobial susceptibility testing was done on the *Helicobacter pylori-positive* isolates.

3.7.2.4 Antimicrobial Susceptibility Testing

Isolates identified as *Helicobacter pylori* were tested for susceptibility to clarithromycin, amoxicillin, metronidazole, levofloxacin and ciprofloxacin. We did not include tetracycline because it was not readily available at the beginning of the study.

The isolated bacteria were first thawed at room temperature and then subcultured on Mueller-Hinton agar plates for 24hours. Using the fresh cultures obtained, a bacterial suspension in sterile saline was prepared according to McFarland Turbidity standard 0.5.

Susceptibility testing of the isolated strains of *Helicobacter pylori* was performed using the the Epsilometer strip test (Biomerieux ETEST). Minimum inhibitory concentrations (MIC) of each antibiotic was read from the intersection of the elliptical zones of growth of inhibition and recorded in the study proforma.

3.7.2.5 Interpretation of Results

The MIC of each antibiotic was compared to its MIC breakpoint recommended by Clinical and Laboratory Standards Institute (CLSI) guidelines to determine susceptibility and resistance(55). MIC levels interpreted as resistant were more than or equal to 1ug/ml for clarithromycin and amoxicillin, more than or equal to 8ug/ml for metronidazole and more than 1ug/ml for levofloxacin and ciprofloxacin. Test results were recorded in the study proforma (Appendix 6).

3.8 Quality Control and Assurance

The research assistant was trained by the principal investigator on data collection. Endoscopy was carried out by trained and practicing gastroenterologists using the standard procedure and samples obtained properly labelled. The specimens were delivered to the laboratory promptly. In the laboratory, biopsies were transferred to clean sterile tubes using a sterile pipette where they

were crushed using the sterile pipette tip and placed on a culture plate containing brain heart infusion agar (Oxoid, UK), inactivated fetal bovine serum, vitox nutritional supplement and dent supplement containing vancomycin (10mg/l), cefsulodin (5mg/l), amphotericin B (5mg/l), and trimethoprim (5mg/l) (Oxoid UK). The plate was placed in a jar containing CampyGen gas packs (microaerophilic incubation) and incubated at 37°C for a maximum of 7 days. Also cultured on the plate was a control strain; *Helicobacter pylori* 39500T Growth pattern was compared to the control strain. Each bacterium isolates cultured was gram stained. Other tests done were oxidase, catalase and urease test.

3.9 Data Management and Analysis

Data were entered into Microsoft Excel 2013 sheets. Data cleaning was done to check for completeness, erroneous entries and duplicates in the entered data. For observations with missing information and incorrect entries, reference was done to the study proforma using the unique identifier contained in each study proforma and the missing information filled and erroneous entries corrected.

Data analysis was done using STATA version 13. Univariate analysis was done to describe the demographic characteristics, susceptibility profile and endoscopic findings. Descriptive statistics were reported as follows; for continuous variables e.g. patient age and minimum inhibitory concentrations measures of central tendency (mean/median/mode) and dispersion (SD/IQR) was reported depending on the distribution of the data. For categorical variables e.g. sex and antimicrobial susceptibility test results, frequency and corresponding percentages were reported. Data was presented in form of tables.

3.10 Ethical Considerations

The study was carried after approval by the Department of Clinical Medicine and Therapeutics, the University of Nairobi, Kenyatta National Hospital/University of Nairobi Research Committee (Approval reference P19/01/2018) and The Nairobi Hospital Bioethics and Research Committee (Approval reference TNH/ADMIN/CEO/22/07/19). Patients were asked to consent to study participation. Only patients who gave consent were enrolled in the study. Those who did not consent were not discriminated against in any way. An invasive procedure (OGD and biopsy) was done as per the standard procedure. Patients were explained for the risks anticipated during biopsy collection including minimal bleeding at the biopsy site, minimal pain and throat irritation.

Study participants were assigned a unique number at enrollment that was used to identify the participant's specimen. Therefore, there was no possibility of the study team identifying the study participants. All information gathered from study participant was kept confidential. Results were communicated to the patient. Those who were found to have *Helicobacter pylori* colonization were referred to the attending physician and offered treatment.

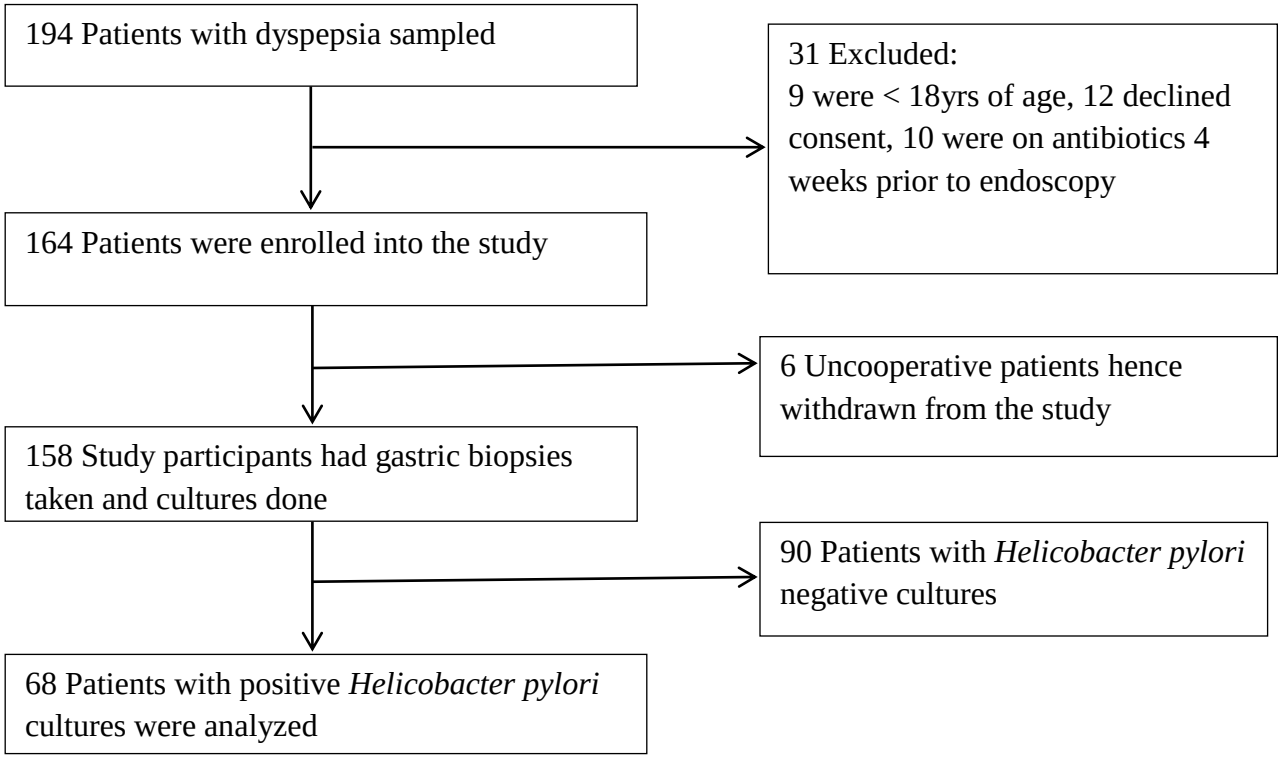
CHAPTER FOUR

RESULTS

4.1 Patient Recruitment

Between August 2018 and October 2019, a total of 194 patients with dyspepsia at KNH (n=106) and TNH (n=88) referred for endoscopy were recruited for the study. Thirty-one patients who did not meet the inclusion criteria were excluded from the study. Gastric biopsies were taken from 158 patients and *Helicobacter pylori* cultures done. Six participants were uncooperative and their biopsies not taken hence excluded from the study. Of the 158 samples taken, *Helicobacter pylori* were isolated in 68 samples. Ninety biopsies tested negative for *Helicobacter pylori.*

Figure 2: Study Flow Chart



4.2 Patients' Sociodemographic Characteristics

A total of 158 patients with dyspepsia were enrolled in the study. These Patients were aged between 18 years and 77 years with a mean age of 43.5 years (SD 13.9). Eighty-five (53.8%) were female. One hundred and six (67.1%) of the study participants were married. Eighty-four per cent (n=113) of the participants had attained secondary level education. One hundred and thirty-five (79.4 %) were employed.

Table 2: Patients Socio-demographic Characteristics

Socio-demographic characteristics	Frequency (n=158) n (%)
Gender (n=158)	
Female	85 (53.8)
Male	73 (46.2)
Age (years)(n=158)	
18-24	11 (7)
25-34	35 (22.1)
35-44	44 (27.9)
45-54	34 (21.5)
55-64	18 (11.4)
65-74	12 (7.6)
>75	4 (2.5)
Marital status (n=158)	
Single	43 (27.2)
Married	106 (67.1)
Divorced	5 (3.2)
Widowed	4 (2.5)
Level of education (n=158)	
None	4 (2.5)
Primary	21 (13.4)
Secondary	44 (27.8)
Tertiary	89 (56.3)
Employment status (n=158)	
Self-employed	81 (51.3)
Formal employment	54 (34.2)
Unemployed	23 (14.5)

4.3 Medication History

Drug use four weeks before endoscopy was reported in sixty-nine (44%) patients. Of the sixty-nine patients, use of proton pump inhibitors (PPI) was noted in sixty-eight (43%). Esomeprazole and omeprazole were the commonly used PPIs. Use of antacids was reported in 8.9% and these patients were also on PPIs, either omeprazole or esomeprazole. None of the patients was on bismuth. Patients on antibiotics four weeks before endoscopy were excluded.

Table 3: History of Drug Use

Drug use before endoscopy	Frequency (n=68) n (%)
Omeprazole	34 (21.5)
Esomeprazole	34 (21.5)
Others	
Antacids	14 (8.9)

4.4 Patients' Endoscopic Findings

As shown in Table 4, one hundred and four (65.8%) were found to have gastritis. Other findings reported were; normal OGD (22.8%), duodenitis (9.5%), gastric ulcers (5.7%), duodenal ulcers (3.2%), reflux esophagitis (7%), gastric polyps (3.2%) and gastric cancer (1.9%).

Table 4: Summary of endoscopic findings

Endoscopic findings	Frequency (n=158) n (%)
Normal OGD	36 (22.8)
Gastritis	104 (65.8)
Duodenitis	15 (9.5)
Gastric ulcer	9 (5.7)
Duodenal ulcer	5 (3.2)
Reflux esophagitis	11 (7)
Gastric polyp	5 (3.2)
Gastric cancer	3 (1.9)

4.5 Antimicrobial Susceptibility Profile of *Helicobacter Pylori* Isolates

Helicobacter pylori isolate isolated from the 68 patients were subjected to five antimicrobial agents for susceptibility testing. All the isolates were sensitive to amoxicillin (100%); 97.1% (n= 66) isolates were sensitive to levofloxacin and ciprofloxacin. Nine (13.2%) were resistant to clarithromycin, while eleven (16.2%) had intermediate resistance. A significant (80.9%, n=55) proportion of the isolates were resistant to Metronidazole. Overall, 14.7% of the isolates were susceptible to all five antibiotics.

Table 5: Susceptibility Profile of 68 Helicobacter Pylori Isolates

Antimicrobial agent tested	Susceptibility profile	Frequency (n=68) n (%)
Clarithromycin	Sensitive	48 (70.5%)
	Intermediate	11 (16.2%)
	Resistant	9 (13.2%)
Amoxicillin	Sensitive	68 (100%)
	Resistant	0 (0%)
Metronidazole	Sensitive	11 (16.2%)
	Resistant	55 (80.9%)
Levofloxacin	Sensitive	66 (97.1%)
	Resistant	2 (2.9%)
Ciprofloxacin	Sensitive	66 (97.1%)
	Resistant	2 (2.9%)

4.6 Minimum Inhibitory Concentrations of the 5 Antimicrobial Agents Tested

All isolates were sensitive to amoxicillin (MIC range 0.016 to 0.75ug/ml); the mean MIC was 0.165ug/ml (SD 0.21). 97.1% were sensitive to levofloxacin (MIC range 0.012 to 2ug/ml), the mean MIC was 0.218ug/ml (SD 0.36) and 97.1% sensitive to ciprofloxacin (MIC range 0.016 to 3ug/ml) with a mean MIC of 0.25ug/ml (SD 0.52). 13.9% of *Helicobacter pylori* isolated were resistant to clarithromycin (MIC range 0.02 to 2ug/ml), the mean MIC was 0.36ug/ml (SD 0.50) and 80.9% resistant to metronidazole (MIC range 3 to >256ug/ml) with a mean MIC of 40.8ug/ml (SD 58.7).

Table 6: Minimum Inhibitory Concentrations of Five Antimicrobial Agents against Helicobacter Pylori

Antimicrobial agent	MIC range (ug/ml) Overall	MIC indicative of resistance
Clarithromycin	0.016 -2	≥1
Amoxicillin	0.016 - 0.75	≥ 1
Metronidazole	3->256	≥8
Levofloxacin	0.012-2	>1
Ciprofloxacin	0.024-3	>1

Table 7: Means of the MICS of Five Antimicrobial Agents

Antimicrobial agent	Mean MIC (SD)
Clarithromycin	0.36 (0.50)
Amoxicillin	0.165 (0.21)
Metronidazole	40.8 (58.7)
Levofloxacin	0.218 (0.36)
Ciprofloxacin	0.25 (0.52)

CHAPTER FIVE

5.1 DISCUSSION

In this study, 68 *Helicobacter pylori* isolates were obtained and subjected to clarithromycin, amoxicillin, metronidazole, levofloxacin and ciprofloxacin for susceptibility testing. Nine (13.2%) *Helicobacter pylori* isolates were resistant to clarithromycin. This antibiotic is used for the management of *Helicobacter pylori* in combination with a proton pump inhibitor and a second antibiotic, as well as other infections such as atypical pneumonia and it's also readily available as an over-the-counter medication. Widespread use of other macrolides including erythromycin and azithromycin for the treatment of communicable disease could also contribute to this due to macrolide cross-resistance. In this study, the clarithromycin rate (13.2%) is higher than that found in previous studies in Kenya using the same methodology. Churyai *et al*. (2015) (50) found four out of nine isolates intermediate resistant and none resistant to clarithromycin while Kimanga *et al* (2010) (51) found no resistant isolates and by Lwai-Lume *et al* (10) found a resistant rate of 6.4%. The high rate could be due to the emergence of *Helicobacter pylori* resistant strains to clarithromycin which is a key component to treatment. Our current rate is similar to that in Cameroon in a study by Kouitcheu Maleku et al (2019) (47) and lower than that in Cameroon (2008) (47.7%) (49). However, these two studies used the disc diffusion method to determine antimicrobial susceptibility. Disc diffusion method is a qualitative test, test results are read as either sensitive or resistant, has high rates of major errors (56). In this study we used the E-test method which is an accurate method for testing *Helicobacter pylori*, test results compare with the agar dilution method which is the gold standard (56). Our clarithromycin rate was higher than that in Senegal (1%) (48), in a study done by Seck *et al* using the same method as ours, Congo (1.7%) (14) in a study done by Ontisira Ngoyi *et al* using

real time PCR and the overall clarithromycin resistant rate in Africa (29.2%) reported in a systematic review and meta-analysis by Jaka et al (2018) (3). The variation in resistance rates may reflect differences in the use of clarithromycin in the different countries.

All *Helicobacter pylori* isolates were sensitive to amoxicillin. Similar studies done previously in Kenya showed high sensitivity to amoxicillin. Churyai *et al* in 2015 (50) found six out of nine isolates sensitive to amoxicillin, while Kiman'ga *et al* in 2010 (51) found no resistance and Lwai-Lume found resistance in 4.6% (10). The high sensitivity means that amoxicillin resistance is not a major challenge in the treatment of *Helicobacter pylori* in our setup. In East Africa, an overall resistance rate of 0-6 % has been reported (37). Bouihat *et al.* (2015) (13) in Morocco used both E-test and disc diffusion methods and found high sensitivity rates of up to 100%. This is similar to what Seck *et al.* (2013) (48) found in Senegal using E-test method. Low resistant rates have also been reported in Europe (0.35%), North America (2%) and South America (6.6%) (3).

A significant proportion (80.9%) of the isolates was resistant to metronidazole. The highest resistance (97.2%) reported at KNH. This antibiotic is inexpensive and widely used for the management of diarrheal diseases, dental, parasitic and gynecological-related infections in many public hospitals such as KNH. These findings are similar to what Churyai *et al.* (2015) (50)and Lwai-Lume *et al.* (2005) (10) found in Kenya. However, Kiman'ga *et al.* (2010) (51) found a resistance rate of 4.6%. This study was done in 2010 and since then the overall rate of metronidazole resistance has increased worldwide from 26.7% in 2010 to 47.22% in 2015 (3). The overall metronidazole resistance rate in Africa is 75.8% which is higher than that reported in South America (52.8%), Asia (46.6%) and Europe (31.2%)(57). This high resistance in Africa

has been attributed to the use of metronidazole for the treatment of endemic diseases such as diarrhoea and protozoal infections.

The majority (97.1 %) of isolated *Helicobacter pylori* were sensitive to levofloxacin and ciprofloxacin. These two drugs are not commonly used for the treatment of *Helicobacter pylori* in our set-up and are usually reserved for those who fail first-line therapy. Low resistance rates have also been observed in other similar studies done in Africa. Bouihat *et al.*(13) (2015) in a study done in Morocco found a resistance rate of 11% while Seck *et al*(48) (2013) in Senegal found a levofloxacin resistance rate of 15%. Both Oyedegi *et al.* (53) in Nigeria and Tanih *et al.* in South Africa found no resistance to ciprofloxacin (58).These two studies used E-test method for antimicrobial susceptibility testing. Jaka *et al* in a systematic review and meta-analysis of 26 articles documented an overall fluoroquinolone resistance rate of 17.4% in Africa(3) . Low resistance rates were also observed in other continents in descending order; Asia 25.3%, South America 21%, North America 19% and Europe 14% (59).

5.2 Study Limitations

The history of recent drug use was determined by self-reporting without any supporting document. This could have had a potential for recall bias as well as inaccuracy since patients who had used antibiotics two weeks before the study could have been erroneously included in the study if they reported they were not on the drugs.

This study was conducted at two centers, with a limited sample size implying that the results obtained will limit the extent of generalization of the finding since the majority of the patients were from Nairobi and its environs.

CHAPTER SIX

6.1 CONCLUSIONS AND RECOMMENDATIONS

6.2 Conclusions

The *Helicobacter pylori* isolates were largely sensitive to amoxicillin, levofloxacin and ciprofloxacin. A significant proportion was resistant to metronidazole while 13.2% were resistant to clarithromycin.

6.3 Recommendations

Metronidazole should not be considered for *Helicobacter pylori* eradication in Nairobi and its environs.

A sustainable surveillance program for antibiotic resistance is recommended to monitor emergence and changes in resistance, particularly resistance to clarithromycin.

A larger study on resistance patterns is recommended in future to inform clinical guidelines.

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APPENDICES

APPENDIX 1: CONSENT INFORMATION FORM

Introduction

My name is Dr. Ann Njeri Kabuthi, a post graduate student pursuing Masters in internal medicine at the University of Nairobi. The purpose of this consent form is to give you information about the study am carrying out.

Purpose of this study

Patients participating in this study have or had a previous history of dyspepsia and have been referred for endoscopy. One of the causes of dyspepsia is a bacteria called *Helicobacter pylori* .The purpose of this study is to establish whether this bacteria responds to the current treatment available here at Kenyatta National Hospital. This will enable better treatment for those found to have the infection.

Benefits of the study participant

You will not be charged for the samples taken from you to the laboratory. The information obtained from the study will be shared with attending your physician to aid in the management of the illness. There will be no monetary benefits for the study participants.

Risk

Endoscopy is a fairly safe procedure. You may experience throat irritation, minimal pain and bleeding.

Procedure

If you consent to participate, you will be enrolled in the study. You will fill a questionnaire, there after endoscopy will be performed as planned and by standard procedure and biopsy samples taken. These are the samples we will use for the study. The samples will then be taken to the laboratory for analysis. After analysis the bacteria obtained from your specimen will then be frozen and stored long term for further medical research.

Confidentiality

Confidentiality will be maintained at all times. You will be assigned a unique study number that will be used for identification of your specimen and data analysis. Any information collected will remain completely confidential, and your name will not be linked directly to the test results. You are allowed to withdraw from the study without loss of benefits or penalty. Your participation in this study is voluntary and will highly be appreciated.

In case of any questions about the study, please contact Dr. Ann NjeriKabuthi through 0720710041.

In case of any ethical concerns, kindly contact:

The chairman, KNH/UON- Ethics and Research Committee,

Hospital Road along Ngong Road,

P.O.BOX 20723- 00202

NAIROBI.

Tel: 020 – 2726300 ext 44355

APPENDIX 2: PATIENTS CONSENT FORM

I …… consent / decline to participate in this study and to storage of the samples for future analysis. This study has been explained to me. All the questions were we answered satisfactorily, and in case I have questions about the study later, I can ask the investigator. I confirm that the isolates obtained from my sample may be stored beyond the present study for further medical research.

Signed …….. Date ……..

Witness ……. (PI/ASSISTANT) Date.....

CONTACT INFORMATION

For further information you can contact any of the following:

Dr. Ann N Kabuthi,

P.O.BOX 5022- 10100

NYERI

Mobile number: 0720710041

Kenyatta National Hospital, University of Nairobi Ethics and Research review committee

P.O.BOX 20723,

NAIROBI

Tel 020-726300

FOMU YA MAELEZO NA KUKUBALI KUJIUNGA NA UTAFITI

Kielezo

Jinalanguni Dkt. Ann Njeri Kabuthi, mwanafunzi wa shahada ya juu katika idhaaya matibabu ya watu wazima, katika chuo kikuu cha Nairobi. Madhumuni ya fomu hii ya kuomba idhini ni kukufahamisha kuhusu swala ninalolifanyia uchunguzi na kukuomba ujiunge na utafitihuu. Ukohurukuu lizamaswaliamakuombamaelezo Zaidi kuhusu sehemu yoyote ambayo hujaelewa.

Mintarafuyauchunguzihuu

Wagonjwa watakao shiriki kwenye utafiti huu wanaugua au wamewahikuuguakiungulianawamependekezewakufanyiwa Endoskopi. Mojabaadhiyasababuzaki ungulianiviinivya bacteria inayofahamikakama. Nia ya utafiti huu ni kudadisi kwa mbama dawa yaliyopona yanayotumika kutibu maradhi haya yanayosababishwa na viini vya bacteria, bado yana uwezo na nguvu ya kufanya hivyo. Matokeo ya zoezi hili yatawezesha matibabu bora kwa watakaopatikana na maradhi haya.

Manufaa ya utafiti huu kwa mshiriki

Hautatozwamalipoyoyotekwasampulizitakazotolewakwakonakupelekwakwenyemaabarakwauch unguzizaidi.Ufahamuutakaopatikanakwenyezoezihiliutamfikiatabibu/daktariwakoilikuboreshama tibabuyako.Hakutakuanamanufaayakifedhakwawagonjwawatakaoshirikizoezihili.

Athari ya Utafiti Huu

Endoskopi ni zoezi linaloweza sababisha kuvuja damu kidogo kwa tumbo na maumivu kidogo.

Kanuni yazoezi hili

Ukipeana idhini yako utajumuishwa kwenye utafiti huu. Utajazafomuyamaswalimachache, kishaendoskopiitafanywakulingananakanunizinazohitajika, Sampuli ya nyama kidogo itachukuliwa na kupelekwa kwenye maabara ili kufanya uchunguzi zaidi.

Usiri

Usiri utadumishwa nyakati zote. Utapati wa namba ya kipekee kwenye zoezi hili, itakayotumiwa kuweka alama kwenye sampuli yako kwenye uchunguzi huu na pia kujumuisha matokeo.Unaruhusiwa kujiondoa kwenye zoezi hili. Hautapoteza manufaa yoyote wala kutozwa faini yoyote. Kushiriki kwako ni kwa hiari.

Kama kuna maswali yoyote kuhusu zoezi hili, tafadhali wasilianana Dkt. Ann Njeri Kabuthi kupitia nambari ya simu 0720710041. Ukiwa na maswali kuhusu kanuni za zoezi hili, tafadhali wasiliana na:

Mwenyekiti,

KNH/UoN – Ethics and Research Committee

Hospital Road along Ngong Road,

P.O. Box 20723-00202

NAIROBI.

TEL 020-2726300 EXT 44355

FOMU YA IDHINI

Mimi ……. Nimekubali/kata kujiunga na utafiti huu ambao umeelezwa kwa ukamilifu kwangu. Pia nimekubali kuwekwa kwa sampuli yangu kwa maabara itakayotumika kwa uchunguzi zaidi, utafiti huu utakapokwisha. Nimesoma na kuelewa maelezo yote. Maswali yangu yote yamejibiwa kwa ukamilifu na mtafiti.

Sahihi ……. Tarehe ……..

Shahidi ……(mtafitim kuu/msaidizi) Tarehe …….....

MAWASILIANO

Ukiwa na maswali yoyote ya ziada, unaweza wasiliana na wafuatao:

Dkt Ann N Kabuthi

S L P 5022, 10100,

NYERI

Simu: 0720710041

Kamati La Maadili Ya Hospitali Ya Kenyatta Na Chuo Kikuu Cha Nairobi

S. L. P 20723,

NAIROBI

Simu: 020-726300

APPENDIX 3: STUDY PROFORMA

CURRENT ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF HELICOBACTER PYLORI ISOLATED IN PATIENTS WITH DYSPEPSIA AT KNH TO AVAILABLE ANTIBIOTICS

BIODATA

Study number ……

Name (initials) ……..

Physical address …….

Tel no …….

Date of enrollment …….

DEMOGRAPHIC DATA

Age ……..

Gender (tick one) Male…….. Female ……

Marital status (tick one) Single..... Married ……. Divorced ……. Widowed …….

Level of education (tick one) None.……. Primary ……. Secondary ……. Tertiary ……

Employment status (tick one) Self-employed ……..Formal employment…… Unemployed

MEDICATION HISTORY

In the last 4 weeks have you been on the following medication (tick one)

1. Amoxicillin Yes…… No…….
2. Metronidazole Yes…… No…….
3. Clarithromycin Yes …… No…….
4. Ciprofloxacin Yes……. No …….
5. Levofloxacin Yes…….. .. No …….
6. Omeprazole Yes…… No……..
7. Esomeprazole Yes…….. No…….
8. Other If yes, which one…….. No……

ENDOSCOPIC FINDINGS …….....

LABORATORY RESULTS

1. Antimicrobial susceptibility test result:

Amoxicillin	…….	……..	……...
Clarithromycin	……	……..	……..
Metronidazole	……	……..	……..
Levofloxacin	……	……..	.. ……..
Ciprofloxacin	……	……..	……..

2. Minimum inhibitory concentrations

Amoxicillin …….

Clarithromycin ……

Metronidazole ……

Levofloxacin ……

Ciprofloxacin ……

APPENDIX 4: CULTURE MEDIA PREPARATION

Inactivation of Fetal Bovine Serum

Fetal bovine serum used in the preparation of culture media was thawed at room temperature, transferred to a water bath at 56°C and stirred manually every ten minutes. When the fetal bovine serum reached 56°C (this was indirectly measured by the temperature of the water bath), it was incubated for thirty minutes cooled to room temperature, dispensed in aliquots of 35mls into tubes labelled inactivated fetal bovine serum. These were stored at -20°C. Sterility control was performed by incubating an aliquot of the fetal bovine serum at 37°C for 48hrs.

Helicobacter pylori selective supplement (Dent) and nutritional supplement (Vitox)

2 mls of distilled water was added to a bottle of Dent (containing vancomycin (10mg/l), cefsulodin (5mg/l)amphoterin B (5mg/l) and trimethoprim (5mg/l)) and mixed gently. The solution was used the same day.Vitox was prepared by mixing the Vitox powder and provided solvent. Both supplements were prepared as per manufacturers insrtuctions

Selective culture media: Brain heart infusion agar + inactivated fetal bovine serum+ Dent and Vitox supplements

Brain heart infusion agar was weighed and put in a 500mls bottle.The media was suspended in 500mls $\pm$ 0.5 ml of sterile distilled water, fully dissolved by boiling and then autoclaved at 121°C for fifteen minutes, cooled down at 45°C in a water bath and gently mixed with inactivated fetal bovine serum. Thereafter Dents and Vitox supplement were added and mixed gently by rolling the bottle. The media was then poured (approximately 25mls per plate), left to solidify, dried at room temperature for two hours and refrigerated at 8 °C until use. The plates were used within one week from preparation day. Sterility control on two plates from each pack of twenty was done at 37°C for twenty four hours, one under aerobic and one under microaerophilic conditions.

APPENDIX 5: PROCEDURE FOR HELICOBACTER PYLORI CULTURE

The biopsies were transferred to clean sterile tubes using a sterile pipette where they were crushed using the sterile pipette tip. 200 ul of brain heart infusion broth enriched with 5% fetal bovine serum was added and uniformly mixed. Using a 10ul inoculating loop, 100ul of the homogenized material was transferred to appropriately labelled culture plateplates containing selective media. The inoculum was aseptically spread on the surface of the plates. Also cultured on the plate was a control strain, *Helicobacter pylori* 39500T. The plate was placed inverted in a jar containing a CampyGen gas pack and incubated at 37°Cfor a maximum seven days (microaerophilic incubation). Helicobacter pylori identification was confirmed through microscopy and by use of gram stain and, urease, catalase and oxidase tests as curved gram negative bacilli, urease positive, catalase positive and oxidase positive respectively. The positively identified isolates were submerged in 20% glycerol and stored at -70°C.

APPENDIX 6: ANTIMICROBIAL SUSCEPTIBILITY TESTING PROCEDURE

Isolates identified as *Helicobacter pylori* were tested for susceptibility or resistance to clarithromycin, amoxicillin, metronidazole, levofloxacin and ciprofloxacin.

The isolated bacteria was thawed in room temperature and sub-cultured on Mueller - Hinton agar plates. Using the fresh culture obtained, a bacterial suspension using sterile saline was prepared according to McFarland Turbidity standard 0.5 (approximately 1.5×10^8 cfu /ml). For each test a swab was dipped into the suspension, and after draining off the excess, the swab was used to confluently seed the medium surface. The plate was allowed to stand at room temperature for ten minutes. This was done for each isolate and for each antimicrobial agent tested. E-test strips (Biomereiux ETEST) for clarithromycin, amoxicillin, metronidazole, levofloxacin and ciprofloxacin were placed onto the medium surface. The test plates were placed inverted in jars containing CampyGen gas packs and incubated at 37°C for upto five days after which susceptibility readings were determined from the E-test strips. This was indicated by an ellipse-shaped inhibition zone intersecting the graded test strip at the inhibitory concentration (ellipse-shaped zone of inhibition) of the antimicrobial used. The MIC of each antibiotic was compared to its MIC breakpoint recommended by Clinical and Laboratory Standards Institute (CLSI) guidelines to determine susceptibility or resistance. MIC levels $\geq$ 1ug/ml for clarithromycin and amoxicillin, $\geq$8ug/ml for metronidazole and > 1 for levofloxacin and ciprofloxacin were indicative of resistance. Test results were recorded in the study proforma.

KENYATTA NATIONAL HOSPITAL
APPROVED
14 FEB 2018
KNH/UoN-ERC
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Ref: KNH-ERC/A/66

14th February, 2018

Dr. Kabuthi Ann Njeri
Reg. No.H58/79849/2012
Dept.of Clinical Medicine and Therapeutics
School of Medicine
College of Health Sciences
University of Nairobi

Dear Dr. Kabuthi

RESEARCH PROPOSAL; CURRENT ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF HELICOBACTER PYLORI ISOLATED FROM PATIENTS WITH DYSPEPSIA AT KENYATTA NATIONAL HOSPITAL (P19/01/2018)

This is to inform you that the KNH- UoN Ethics & Research Committee (KNH- UoN ERC) has reviewed and **approved** your above revised proposal. The approval period is from 14th February 2018 – 13th February 2019.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH-UoN ERC before implementation.
c) Death and life threatening problems and serious adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH-UoN ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH- UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
f) Submission of an *executive summary* report within 90 days upon completion of the study. This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/ or plagiarism.

For more details consult the KNH- UoN ERC website **http://www.erc.uonbi.ac.ke**

Yours sincerely,

PROF. M. L. CHINDIA
SECRETARY, KNH-UoN ERC

c.c. The Principal, College of Health Sciences, UoN
The Deputy Director, CS, KNH
The Chairperson, KNH-UON ERC
The Assistant Director, Health Information, KNH
The Dean, School of Medicine,UoN
The Chair, Dept. of Clinical Medicine and Therapeutics,UoN
Supervisors: Prof. G.N. Lule, Dr. Edna Kamau,Dr.Anne Maina, Dr.John Mwaniki

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Ref. No.KNH/ERC/R/58

April 9, 2019

Dr. Kabuthi Ann Njeri
Reg.No.H58/79849/2012
Dept.of Clinical Medicine and Therapeutics
School of Medicine
College of Health Sciences
University of Nairobi

Dear Dr. Kabuthi

Re: Approval of Annual Renewal – Current Antimicrobial Susceptibility profile of Helicobacter Pylori isolated from patients with Dyspepsia at Kenyatta National Hospital (P19/01/2018)

Refer to your communication dated March 23, 2019.

Upon review of your communication, the KNH-UON ERC hereby grants you annual extension approval for ethics research protocol **P19/01/2018.**

The approval dates are 14th February 2019 – 13th February 2020.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH/UoN ERC before implementation.
c) Death and life threatening problems and severe adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH/UoN- ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH/UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
f) Clearance for export of biological specimens must be obtained from KNH/UoN-Ethics & Research Committee for each batch of shipment.

g) Submission of an *executive summary* report within 90 days upon completion of the study
This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/or plagiarism.

For more details consult the KNH- UoN ERC website **http://www.erc.uonbi.ac.ke**

Yours sincerely,

PROF. M.L. CHINDIA
SECRETARY, KNH-UON ERC

c.c. The Principal, College of Health Sciences, UoN
The Director CS, KNH
The Chairperson, KNH-UoN ERC

Protect to discover

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Ref: KNH-ERC/ 01/MISC/154

9th April, 2019

Dr. Kabuthi Ann Njeri
Reg. No. H58/79849/2012
Dept. of Clinical Medicine and Therapeutics
School of Medicine
College of Health Sciences
University of Nairobi

Dear Dr. Kabuthi

Re: Approval of modifications– Current Antimicrobial Susceptibility profile of Helicobacter pylori isolated from patients with Dyspepsia at Kenyatta National Hospital (P19/01/2018)

Your communication of 28th March 2019 refers.

The KNH-UoN ERC has reviewed and **approved** inclusion of Nairobi Hospital endoscopy unit as an additional study site.

These changes are incorporated in the revised proposal and are acceptable.

Yours sincerely,

PROF. M.L. CHINDIA
SECRETARY, KNH- UoN ERC

cc. The Principal, College of Health Sciences, UoN
The Director, Clinical Services, KNH
The Chairperson, KNH-UoN ERC
The Dean, School of Medicine, UON
The Chair, Dept. of Clinical Medicine and Therapeutics, UON
Supervisors: Prof.G.N. Lule, Dr. Edna Kamau, Dr.Anne Maina, Dr. John Mwaniki

THE NAIROBI HOSPITAL

Our Ref. TNH/ADMIN/CEO/22/07/19

22 July 2019

Dr. Ann N. Kabuthi
P. O. Box 5022 - 10100
Nairobi

Dear Dr. Kabuthi,

RE: CURRENT ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF HELICOBACTER PYROLI ISOLATED FROM PATIENTS WITH DYSPEPSIA AT THE KENYATTA NATIONAL HOSPITAL & THE NAIROBI HOSPITAL

Reference is made to your request to carry out the above study at The Nairobi Hospital. We are pleased to advise that approval has been granted.

In line with the Research Projects Policy, you will be required to submit quarterly update reports of the study to the Committee. You are also required to submit a copy of the final findings for the Committee's records.

Do note that information/data collected and potential findings shall not be in conflict with the Hospital's Confidentiality Clause which states that "You will not without consent of the Association disclose any of its secrets or other confidential matters to anyone who is not authorized to receive them".

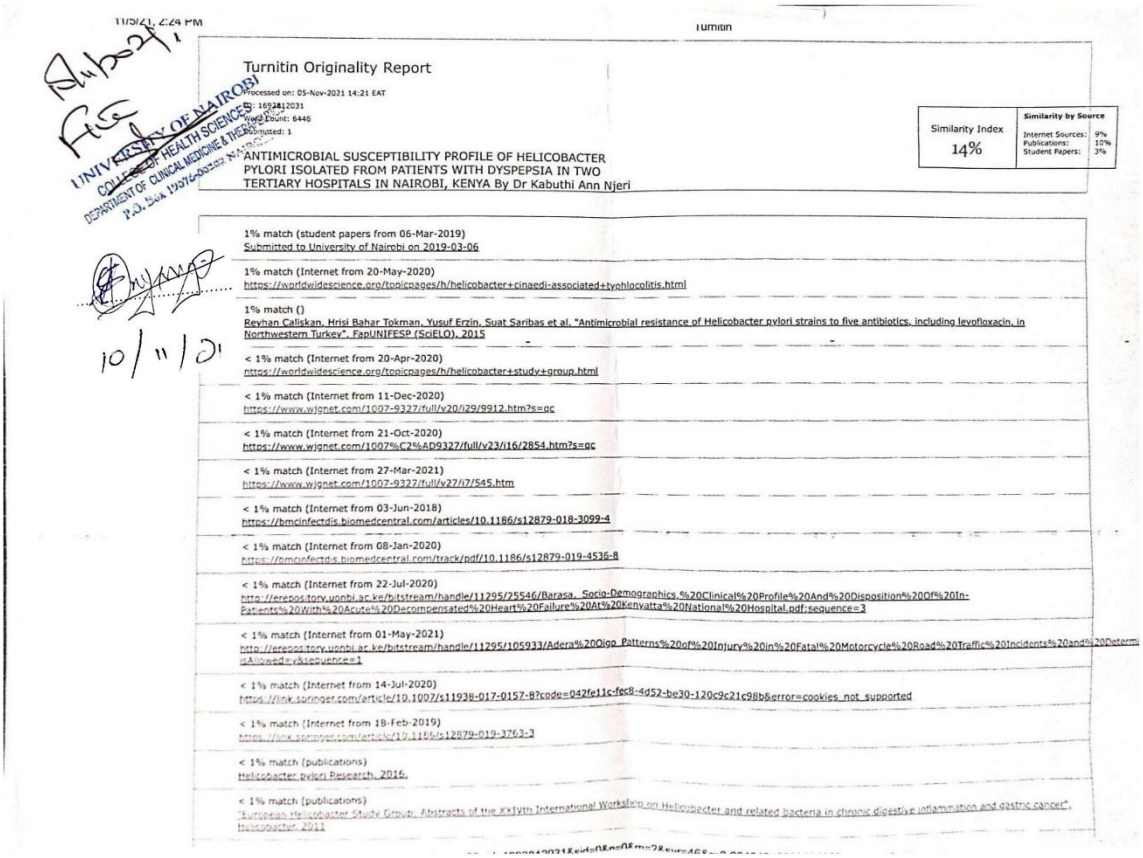
Please note that this approval is valid for the period July 2019 to July 2020, if an extension is required, a fresh application should be done before proceeding with the study.

Yours sincerely,
FOR: THE NAIROBI HOSPITAL

Christopher Abeid M.D.
AG. CHIEF EXECUTIVE OFFICER

C.c. Chairman - Bioethics & Research Committee

TURNiTIN REPORT



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