SCREENING FOR CARRIAGE AND ANTIMICROBIALRESISTANCE OF *HELICOBACTER PYLORI, ESCHERICHIA COLI AND KLEBSIELLA* SPECIESAMONG PATIENTS PRESENTING WITH ASTRITIS IN KIBERA AND DAGORETTI AREAS IN NAIROBI, KENYA

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Screening for Carriage and Antimicrobial Resistance of *HelicobacterPylori, Escherichia coli and Klebsiella* species among Patients presenting with Gastritis in Kibera and Dagoretti areas in Nairobi, Kenya

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A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Medical Microbiology of the Jomo Kenyatta University of Agriculture and Technology

2023

DECLARATION

This thesis is my original work and has not been presented for a degree in any other University

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DEDICATION

I dedicate this entire thesis work to my dear husband Jacob Ochieng, son Christian Wayne and daughter Victoria Patience who have forever remained my cheer leaders throughout my academics against all odds!

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ABBREVIATION AND ACRONYMS

ABR	Antibiotic Resistance
AFLP	Amplified Fragments Length Polymorphism
AMR	Antimicrobial Resistance
AST	Antimicrobial Susceptibility Test
ATCC	American Type Culture Collection
ATCC	American Type Culture Collection
BHIA	Brain Heart Infusion Agar
Cag PAI	Pathogenicity Island
cagA	Cytotoxin-associated gene A
CagE	Cytotoxic associated gene E
CBA	Columbia Blood Agar
CI	Confidence interval
CLSI	Clinical Laboratory Standards Institute
CMR	Center for Microbiology Research
CTX-M	Cefotaximases
ddNTPs	deodeoxyribonucleic acid triphosphate
DDST	Double Disc Synergy Test
DNA	Deoxyribonucleic acid
dNTPs	deoxy ribonucleotide triphosphate
E. coli	Escherichia coli
ECDC	European Centre for Disease Prevention and Control
ESBL	Extended Spectrum Beta Lactamase
Esokit HP	Esokit Helicobacter pylori Test kit

FBS	Fetal Bovine Serum
FQ	Fluoroquinolone
GDP	Gross Domestic Prodution
GERD	Gastro-Esophageal Reflux Disease
glmM	Malfunction of Glucosamine
H. pylori	Helicobacter pylori
HCW	Health Care Workers
IL	Interleukin
IMViC	Indole. Methyl red, Voges and Citrate
IPM	Imipenem
KEMRI	Kenya Medical Research Institute
KPC	Klebsiella Pnemoniae Carbapenemases
MALT	Mucosa Associated Lymphoid Tissue
MDR	Multi Drug Resistance
MIC	Minimal Inhibitory Concentration
mRNA	Messenger Ribonucleic Acid
NCTC	National Collection of Type Cultures
OR	Odds Ratio
OXA	Oxacillinases
P Value	Prevalence value
PBP	Penicillin Resistance in Pneumococci
PCR	Polymerase Chain Reaction
PH	Potential Hydrogen
PHO	Public Health Officer

PPI	Proton Pump Inhibitor
PUD	Peptic Ulcer Disease
PUD	Peptic Ulcer Disease
RDX	Radixin gene
RL	Sulphamethoxazole Trimethoprim
RNA	Ribo-Nucleic acid
ROC	Republic Of Congo
SERU	Scientific Ethic Review Committee
SHV	Sulfhydryl
SIM	Sulphide Motility Media
SNP	Single Nucleotide Polymorphism
Spp	Species
STEC	Shiga toxin-producing
STEC	Shiga toxin-producing E. coli
TET	Tetracycline
TSI	Triple Sugar Iron agar
UGI	Upper Gastrointestinal Infections
UN	United Nation
UTIs	Urinary Tract Infections
UV	Ultra Violet
Vaca	Vacuolating Cytotoxin gene A
VacA	Vacuolating cytotoxic antigen
WHO	World Health Organization

ABSTRACT

Helicobacter pylori is a global threat to the health sector affecting about a half of the entire population worldwide. In Kenya, 54.8% of people with gastrointestinal complications have been reported, however, this was data from a hospital-based study. Community acquired antimicrobial resistance (AMR) strains have emerged as a threat to public health and are associated with high morbidity and mortality rates. *Escherichia coli* and *Klebsiella species* are known reservoirs of resistant genes including *Extended Spectrum Beta Lactamases* (ESBL) and *Klebsiella Pneumoniae Carbapenemases. H. pylori* also, despite its pathogenicity, is rapidly increasing resistance towards available antimicrobial agents. Despite the threat to public health, limited data exist on prevalence, risk factors and AMR trends of *H. pylori, E. coli,* and *Klebsiella.* This study determined prevalence and AMR patterns of these three bacterial organisms as well as risk factors for *H. pylori* infection. A cross sectional study was conducted among outpatients presenting with gastritis-like symptoms in Mbagathi and Mutuini hospitals in Nairobi. A hundred and seventy-two identified patients were requested to consent to take part in the study. Follow-up visits were made and recruited 237 immediate contacts. Stool samples were collected and aseptically transported to the Mbagathi hospital laboratory for microbiological analysis. All stool samples were tested for *H. pylori* using rapid kit and the positive ones cultured on Columbia agar, and confirmed by PCR. For *E. coli* and *Klebsiella* isolation, all stool samples were cultured on MacConkey. Further identification was by Biochemical tests using standard methods including catalase and urease for *H. pylori* and Indole Methyl red Voges and Citrate for *E. coli* and *Klebsiella*. AMR genes were confirmed by Polymerase Chain Reaction. Risk factors were determined by the Logistic regression model using P-value (P) and odds ratio (OR). Infection was recorded in 83.3% of all participants. Of these, 73.8% were infected with *E. coli*, 28.6% with *H. pylori* and 39.1% with *Klebsiella*. Infection was predominant among Kibera participants (57.7%) when compared to those from Dagoretti (25.7%). Over 90% of patients were infected while 70.1% of contacts had infection. Co-infections among the three bacteria was reported in 25% participants. Antimicrobial resistance was observed in 64.2% isolates. Resistance in *H. pylori* was found against Amoxicillin (1%) Metronidazole (3.5%). Resistance in *E. coli* and *Klebsiella* was high against Sulfamethoxazole Trimethoprim (RL) 80% vs. 71% and Tetracycline (TET) 68% vs. 59% while low resistance was found in Imipenem (IPM), 16% vs 19%. *Klebsiella* exhibited high resistance (55%). Most isolates (25%) were resistant to more than three antimicrobial agents. ESBL was noted in 44% with Oxacillinases (OXA gene) being pre-dominant (65%). A person residing in Kibera was 1.8 more likely to acquire infection (P=0.014, OR:1.8 CI 1.1- 2.9. Similarly smokers were at high risk of infection (P=0.03, OR=1.46 CI 1.13 - 2.88), and in those who had other bacterial infections (P<0.05, OR 1.07 CI 0.88-1.31) Most available antimicrobial agents including broad spectrum are rapidly becoming ineffective in fight against most bacterial isolates. Place of residence, presence of other microbial agents and unhealthy lifestyle were considered potential risk factors associated with *H. pylori* infection.Based on the findings, it can be concluded that symptoms of gastritis needs to be supported with laboratory testing during clinical diagnoses of *H. pylori* patients Mass screening is will be critical in early detection and subsequent treatment to reduce resistance rate. Enforcing the antimicrobial access regulations such as restricting the over counter sale and public awareness about drivers of AMR should be enacted to help fight against infection and AMR in line with vision 2030.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

H. pylori is a gram-negative, helical-shaped, and microaerophilic bacteria mostly associated with gastritis (Oling et al., 2015). The bacterium has become a threat to global health due to delayed clinical symptoms/signs and unreliable diagnostic capacity. This has led to high prevalence in about a half of the population globally, (Negash et al., 2018). Previous studies have reported a prevalence of 69.4% in Africa with Kenya recording a prevalence ranging between 54.8% and 99% (Kimang'a et al., 2010). Colonization with virulent genes such as *vacAs1* and *cagA* presence may cause severe stomach ulceration which if not treated, persist into chronic conditions such as gastritis. Beside these genes some *H. pylori* bacteria may have antimicrobial resistant genes such as *16S rRNA, Pbp* and *AMX* which confers resistance against Metronidazole and Amoxicillin respectively. The human gut hosts a large number of gram-negative commensals beside *H. pylori* especially members of the *Enterobacteriaceae* such as *E. coli* and *Klebsiella* species. These two are known community indicators for presence of multidrug resistant strains (Stanley et al., 2018), both have resistant genes which confer resistance to a broad spectrum of antimicrobial panels. These genes include ESBL genes like *blaTEM, blaOXA blaSHV, and blaCTX-M* for *E. coli* and *KPC* for *Klebsiella* species. These resistant genes can be transmitted across species through mobile genetic elements like integrons or conjugative plasmids (Singh et al., 2019) and through interaction with other commensals. *E. coli* and *Klebsiella* species are also believed to contribute in causing gastroenteritis infection (Foster et al., 2015; Moreno et al., 2007) respectively.

Locally, there is limited data on the prevalence of *H. pylori* and co-infection with *E. coli and Klebsiella* species despite a high burden of infections being attributed to them. This is mainly because the majority of available findings are lone hospital-based and therefore focused mainly on diagnosis and treatment as opposed to co-infections, potential risk factors and possible sources of infection. There is also

limited data of presence of multidrug resistance genes in the general public. Treatment of *H. pylori* in non-diagnosed cases has led to the overuse of anti-ulcer medications and acid-suppressive therapies, which tend to reduce acidic levels, making the stomach more susceptible to colonization by other bacterial species. Infestation with other bacteria is believed to include members of enteric bacteria. Most of these bacterial agents are however inhibited by the high acidic level found in the stomach. However, despite this high acidic environment in the stomach released after perforation, *H. pylori* do survive in such conditions (Bury-Moné et al., 2004), the environment with believed to also have limited availability of nutrients and frequent gastric emptiness, (Brown, 2000). Likewise, despite the increase in stomach inflammation (50% of the stomach related infection worldwide) being attributed to *H. pylori* and members of *Enterobacteraceae* family, currently, there is scarce data on prevalence of infection in community-based research and AMR patterns locally. Additionally, there is also lack of data on co-existence of the three species in Kenya mostly due to the fact that the majority of studies tend to focus on individual species at a time, or else, they focus mainly on diagnosis and treatment therapy as opposed to risk factors and sources of infection. AMR strains may easily transfer resistance genes to other gut microbiota including *H. pylori* strains (Grady et al., 2014), which may thus make it difficult for treatment, prevention/control and also management of infection believed to have been brought by *H. pylori* bacteria. This poses a threat to the public health and calls for more research to come up with evidence-based findings.

The current study adopted both hospital and community-based approaches with an aim of screening for occurrence, antimicrobial resistance trend in *E. coli* and *Klebsiella* species infection and risk factors attributed.

1.2 Problem statement

Despite *H. pylori* infecting about 50% globally and Kenyan above 54% in Kenyan population according to Kimang'a et al., (2010), there is still little existing literature that examines its carriage and resistance pattern in the informal settlement of

Nairobi, Kenya. There is also limited information about general population screening locally.

There is also limited information on carriage and antimicrobial resistance patterns in Nairobi since most studies done are hospital-based hence most likely recruit symptomatic patients hence non-representation of the entire public infection. Inappropriate prescription and misuse of antimicrobials may be the drivers of AMR which has recorded high mortality rates (4.15M) in Africa. This has negatively impacted the Continents' economy. Co-infection of *H. pylori* with *E. coli* and/*Klebsiella* species may transfer antimicrobial resistance genes to *H. pylori* which being a potential pathogen pose serious health issues to the general public and may cause development of drug resistance genes (Gredner, Behrens, Stock, Brenner, & Mons, 2018).

Finally, there is little knowledge on potential risk factors associated with infection which has made it difficult to carb infections that would otherwise be prevented or controlled easily. Researchers have not established the potential risk factors associated with *H. pylori* infection which may lead to poor intervention and prevention of attributable infection. Use of invasive testing techniques like endoscopy is very painful and costly making many to shy away unless they are very sick and recommended for diagnosis prior to treatment, (Marginean, Meliț, & Sasaran, 2022).

1.3 Justification

A major feature of infection related to *H. pylori* is late diagnosis or mis-diagnosis and hence higher risks in terms of mortality and morbidity. This is more often due to the use of invasive ways of detecting the presence of *H. pylori*. Improved diagnosis of *H. pylori* species based on clinical signs guarantees early diagnosis and subsequent treatment reducing chances of adverse infection effects like stomach cancer. This is likely to greatly reduce morbidity and mortality attributed to *H. pylori*. Data availed by this objective will help all stakeholders to safeguard the general public and create awareness in aid to garb infection and ensure prolonged life. Most studies have looked at the prevalence of *H. pylori, E. coli* and *Klebsiella*

bacteria in patients who have been at advanced stages of infections with all indications showing that these patients would actually have infection in question but only performing confirmatory tests. There is limited information on infection in early or initial stages of infection in patients who present with gastritis and also no information of infection in a 'healthy' population composed of people who are neither sick nor show symptoms and signs for infection. In the recent past, antimicrobial resistance has been on the increase with the WHO and Lancet declaring it a public threat killing many daily. Studies have been done but I realized that, locally, we lacked data on the effectiveness of the available antimicrobial agents including the broad spectrum.

Determining carriage of *E. coli* and *Klebsiella* species which are known indicators of presence of antimicrobial resistance and possible pathogenic strains and determining its resistance patterns in Kibera and Dagoretti areas will avail data that will be representative of resistance that is likely to be experience by the general population attributed in Nairobi as a whole. AST & AMR data will promote comprehensive policies that will prevent, control, reduce and mitigate infections and cost impact in sustenance of development goal 2030. It is further expected that the AMR pattern report will serve as a reference to resistance burden/trend, antibiotic use and misuse, administration of antibiotics and streamline the prescription policies including over-counter purchases and better public knowledge on causes of antimicrobial resistance and effective strategies to reduce spread of antimicrobial resistance genes.

This project will link findings of attributable risk factors to public knowledge on potential reservoirs and create awareness on potential risks for infections which will allow duplicates in future to make a sustainable impact on efficient prevention and control of future infections. Detailed information on risk factors in transmission of infection will be of help especially in understanding risk factors attributed to infection of *H. pylori* bacteria and also understanding the possible routes of transmission. This will aid in the establishment of more effective control/prevention measures and general public health management of infections attributed to *H. pylori, E. coli* and *Klebsiella* species.

1.4 Research Questions

1. What is the carriage of *H. pylori, E. coli, and Klebsiella* species in patients presenting with gastritis in Kibera and Dagoretti areas, Nairobi County?
2. What are the Antimicrobial Sensitivity Testing profiles and Antimicrobial Resistance patterns of *H. pylori*, *E. coli* and *Klebsiella* isolates from patients presenting with gastritis in Kibera and Dagoretti areas, Nairobi County?
3. What are the underlying risk factors for the transmission of *H. pylori* infection in Kibera and Dagoretti areas, Nairobi County?

1.5 Objectives

1.5.1 General objective

To screen for carriage of *H. pylori, E. coli and Klebsiella* isolates among patients presenting with gastritis, determine their antimicrobial susceptibility profiles and determine the underlying risk factors for *H. pylori* infection in Kibera and Dagoretti areas, Nairobi, Kenya.

1.5.2 Specific objectives

1. To determine the carriage of *H. pylori, E. coli, and Klebsiella* species in patients presenting with gastritis in Kibera and Dagoretti areas, Nairobi County.
2. To determine the AMR patterns of *H. pylori*, *E. coli* and *Klebsiella* isolates from patients presenting with gastritis in Kibera and Dagoretti areas, Nairobi County.
3. To determine the underlying risk factors for the transmission of *H. pylori* infection in Kibera and Dagoretti areas, Nairobi County.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction to *Helicobacter pylori* infection.

Helicobacter pylori, a Gram negative, spiral-shaped and microaerophilic bacteria, commonly colonizes and invades the stomach mucosa, (L. M. Brown, 2000). It is characterized as a rod with 4-7 flagella that enhances its motility and the ability to establish freely in the stomach's mucus layer, (Zhu et al., 2007). The discovery of *H. pylori* as a major cause of peptic ulcers disease back in 1983 off let it develop into a drug resistant threat (Zaslona et al., 2020). About 20% of the bacteria can adhere to stomach lining along mucus epithelial cells (Baeg et al., 2016). Infection is characterized by *cagA, cagE* and *vacA* genotypes associated with peptic ulcer disease (PUD), chronic gastric carcinoma and MALT Lymphoma (Watanabe et al., 2015). *vacA* and *cagA* contribute to the virulent genes that cause stomach pathogenicity and happen to be the most studied pathotypes associated with severe damage. Resistant genes associated with *H. pylori* include *glmM (ureC)* genes encoding phosphoglucosamine, *rdx* gene, *pbp1A, 23s RNA* and *16s RNA* conferring resistance to metronidazole, amoxicillin, clarithromycin and tetracycline respectively (Diab et al., 2018). Most patients suffering from upper gastrointestinal infections seeking health care have no follow up treatment. In about 60% of those patients investigated, test results rule out peptic ulcer infections, gastro-esophageal reflux, gastric cancer, and non-functional dyspepsia. However, Ddiab and the team noticed that, the benefit of treatment to eradicate *H. pylori* remains controversial and therefore, despite some authors noting that to manage uninvestigated dyspepsia in developed countries requires screening patients, (Khokhar, 2022, Benbassat, 2021 and Valencia & Molano, 2022).

2.2 Prevalence of *H. pylori*

Epidemiologically, *H. pylori* infection has become a public concern hence its consequences and complications of infections attributed to it has to be studied with keen interest. This knowledge is also key in eradication of infections associated with

these bacteria and also in deciding prevention, control and also effective treatment measures and campaigns. Besides, this information will also help in understanding the trends and patterns of antibiotic resistance profiles. Many studies have been done to try and stratify the prevalence of *H. pylori* by gender, age and also socio-economic status. In Pakistan, a study revealed that gender and illiteracy were associated significantly with the rate of *H. pylori* infection (Valliani et al., 2013). The findings indicated a probable sex disparity but recommended for more studies using a larger population.

A similar study by Nabwera et al (2000) demonstrated a high prevalence of *H. pylori* among school going children, (Nabwera, Nguyen-Van-Tam, Logan, & Logan, 2000). The study reported that increase in the number of households, poor sanitation activities, open-air defecation and also female gender and young age were significantly associated with higher prevalence. Nabwera also carried out a study to identify presence of *H. pylori* in children aged 1 to 15 years old which noted that prevalence rates tend to increase with increase in participants' age. The study reported that infections were higher among school going children, those from overcrowded homes and also in those with poor access to safe drinking water and sanitation facilities. Nabwera finally concluded that lack of proper sanitary facilities, lack of safety (Mitonga-Monga, Flotman, & Cilliers, 2016). Looked at the relationship of *H. pylori* with clinical outcomes in patients who presented with dyspepsia in selected hospitals. The study revealed that patients aged 40 years and above had the highest prevalence, followed by those between 29-39 years, while the lowest prevalence was among those under 20 years of age. Female patients had the highest prevalence level compared to male patients who had a slightly lower prevalence. However, subsequent tests revealed that there was no significant relation between *H. pylori* and the respondent's age or sex. A similar study by (Kimang'A et al., 2010), reported that the prevalence of *H. pylori* and antibiotic susceptibility among Kenyans increased among young children with a prevalence of about 73% as compared to adults whose prevalence stood at 73%.

2.2.1 Prevalence of *H. pylori* globally

In a study done by Hooi and team (2017), 14,006 records were presented from databases, of this, 6188 were duplicates, 7611 were dejected for not meeting selection criteria, 22 were not considered for due to inaccessible full text, while 3 records could not be translated for further review. However, a total of 184 papers were selected after full-text review which included 11 from Africa, 75 from Asia, 66 from Europe, 13 from Latin America and Caribbean, 13 from Northern America, and 6 from Oceania. In these records, *H. pylori* prevalence was estimated at 48.5% in 62 countries after 257,768 out of 531, 880 participants tested positive (Hooi et al., 2017). The countries with the highest number of reports were China, Korea, Japan, United States, Germany and Iran. The distribution of papers by regions and categories included age range, sex, and diagnostic methods. Hooi and the team also noted that Indigenous population of the United States and Australia was higher than the general population. In Australia, the pooled *H. pylori* prevalence estimates for the general population was 24.6% (95% CI, 17.2% - 32.1%), a high prevalence same to the 76.0% (95% CI, 72.3% - 79.6%) witnessed in the rural Western Australian indigenous community. Hooi's studies also noted that the pooled HP prevalence estimate for the general population was 35.6% (95% CI, 30.0% - 41.1%), but it was 74.8% (95% CI, 72.9% - 76.7%) in the Alaskan indigenous population. Among aspects studied included modeling, time series, or transmission studies, mortality or survival analyses, diagnostic assay, test performance studies and animal studies (De Brito et al., 2019). Study population that were typically associated with higher prevalence of *H. pylori* like patients with gastric cancer, peptic ulcers. High-risk population groups like migrants, refugees, prisoners, and individuals classified as low socioeconomic status including homeless people, adoptees. Study participants that were restricted to selected age groups either children or elderly,

Globally, Hooi also noted that the highest *H. pylori* burden was in Nigeria 87.7%, Portugal 86.4%, Estonia 82.5%, Kazakhstan 79.5%, and Pakistan 81.0%. Countries which reported the lowest *H. pylori* included Switzerland, Denmark, NewZealand, Australia, and Sweden with infection ranging between 18% and 27%, (Hooi et al., 2017). Regions with the highest reported *H. pylori* infections included were African

70.1%, South America 69.4%, and Western Asia 66.6%.ref On the other hand, regions with the lowest reported *H. pylori* prevalence were Oceania 24.4%, Western Europe 34.3%, and Northern America 37.1%. However, *H. pylori* prevalence after the year 2000 went down in Europe from 48.8% to 39.8%, Northern America 42.7% to 26.6%, and Oceania 26.6% to 18.7%. In contrast, the prevalence was similar in Asia after the year 2000 (53.6% before 2000 vs 54.3% after 2000), and Latin America and the Caribbean (62.8% before 2000 vs60.2% after 2000) according to Hooi *et al*., 2017.

2.2.2 Prevalence of H. pylori infection in Africa

In Africa, the burden of *H. pylori* infection remains high with varying levels of prevalence among children and adults having been reported in different regions of the continent, (Smith et al., 2022). Smith and the team also reported that persistent and non-eradicated infections are seen to result in gastric cancer, although less severe pathological outcomes have also been reported among Africans referring to the so-called 'African enigma.' Infections attributed to *H. pylori* have been grouped under the neglected tropical disease in Africa. This is because other infectious diseases including HIV, malaria, and tuberculosis receive more attention in terms of research funding and grants when compared to *H. pylori* infection.

Yuan et al., (2022) noted that the pathology is not as severe as in Africa as experienced by infected persons in Europe, Asia, and other continents as erosions are seen as the most prevalent manifestation in patients with dyspepsia. The study however noted that prevalence of *H. pylori* varies from one geographical location to another hence it is possible to have different prevalence considering the age, level of literacy, diet, and the location. For example, Yuan and the team noted that in Nigeria, the prevalence of is as high as 87.8% while a prevalence rate of 34.2 and 51.4 (adult population), and 36.3 and 42.6% (children population) have been reported in the southeast and south-south geopolitical zones of the country, respectively. A prevalence of 6.0 and 28% has been reported in children from north central and southwestern parts of Nigeria, respectively. The high prevalence rate of *H. pylori* In Nigeria according to Yuan et al., (2022) has been associated with risk factors such as

low socioeconomic status, unclear water source, overcrowding, cigarette smoking, and increased levels of interferon gamma.

In other parts of Africa, according to Chtourou et al., (2022), the study reported a 70.8% prevalence rate in Burundi, 75% in Rwanda in 2014, with 20.1% of cases having ulcer, 10% gastric obstruction, and 4.5% malignancy. The study also noted 70.41% and 93.1% that was experienced in 2015, respectively, in Togo and Congo Brazzaville, 63.8% in Morocco, 88% in Ghana, and 66.12% in Egypt in 2019. Furthermore, a prevalence of 64.6% in children with risk factors of overcrowding, patronizing of food vendors, and illiteracy was reported in Egypt. In the Republic of Benin, a 71.5% prevalence rate was reported but was not associated with age, sex, marital status, religion, occupation, or education, 73.2% in Cameroon with a significant association with age, socioeconomic status, alcohol, family history, and nonsteroidal anti-inflammatory drugs and similarly anemia, duodenal ulcer, and chronic gastritis have been reported as common in patients with *H. pylori* infection, and 71.43% prevalence rate was published in Algeria. In a research conducted in Ethiopia, 88.9% *H. pylori* prevalence was reported in male individuals, while 82.8% was in female individuals.

These prevalence reports from Africa are higher than reports from other continents according to a study by Moss, (2022). Moss noted that the prevalence of *H. pylori* in Europe (Germany) was 20–40% while North America, Australia and Asia recorded prevalence of 23.1%, 24.6%, and 48.8% respectively.

2.2.3 Prevalence of *H. pylori* in Kenya

In Africa and Kenya in particular, *H. pylori* is referred to as ‘the African enigma’ and prevalence of infection attributed to it is about 65% in immigrants from Africa and 25% of immigrants from Asian immigrants. In Kenya however, 56% of infections from rural parts, 62% of infections from urban Kenya Africans, and also about 58% among urban Kenyan Asians was attributed to *H. pylori* infections, (Kimang’A et al., 2010). Kimang’a also noted that gastro-esophageal reflux disease (GERD) was attributed to about 29% of cases, 1% gastric cancer, 65% gastritis and 2% duodenal ulcers and 3% gastric ulcers. GERD was predominant in female patients (56 -

65.6%) than males. Gastric and duodenal ulcers occur at equal frequency in all racial groups. He also noted that, despite a high prevalence of *H. pylori* in Kenya, its complications were relatively low.

2.3 Escherichia coli infection

2.3.1 Prevalence of *E. coli* infection globally

Escherichia coli, is a Gram-negative bacterium is a rod-shaped and a facultative anaerobe. It is more often found in the human gut and among other warm-blooded animals, (Quaglia et al., 2008). *E. coli* has various pathogenic pathotypes, these include EPEC, EIEC, ETEC, EAEC and STEC. Most of its strains are known to be non-pathogenic except for Shiga toxin-producing *strain* (STEC). STEC is associated with severe foodborne infections since it is easily transmitted via water and food contaminated with infected fecal matter. These foods may include but are not limited to raw and undercooked meat products, contaminated and raw vegetables and sprout and also raw and contaminated milk and milk products (Davis & Kendall, 2012).

2.3.1 Prevalence of *E. coli* worldwide

There were 62 studies done which covered a total of 29 872 healthy individuals from the six WHO regions. This gave a global pooled prevalence of ESBL *E. coli* intestinal carriage in the community of 16.5% (95% CI 14.3%–18.7. The highest carriage rates occurred in South-East Asia (27%; 95% CI 2.9%–51.3%), followed by Western Pacific (24.5%; 95% CI 17.6%–31.4%), Africa. 21.4%; 95% CI 12.7%–30.1%) and Eastern Mediterranean (20.6%; 95% CI 10.2%–30.1%). While the lowest pooled prevalence was reported from European studies (6.0%; 95% CI 4.6%–7.5. Based on the country level, the highest community prevalence was reported from Tanzania (76.3%), followed by Vietnam (75.1%), Laos (70.2%), China (58.5%), Thailand (56.1%), Egypt (45.1%) and Lebanon (38.5%). Australia, with a prevalence of1.9%, and the USA (at the rate of up to 3.5%) were among countries with the lowest prevalence. Global trend in prevalence of human intestinal ESBL *E. coli* carriage. The study noted a steady increase in a period of 3 year intervals from 2.6% (95% CI 1.6–4.0) in 2003–05 to 21.1% (95% CI 15.8%–27.0%) in 2015–18,

representing an average increase of 1.2% per year. Similarly, an estimated projection from linear regression analysis revealed a 1.5% yearly increase, with an estimated global prevalence of just under 30% in 2020 (P=0.021). WHO region pooled together data from 30 regions with the highest rate of infection being in South-East Asia, 46%, whereas Europe and the Americas (2%), north (2%), south (3%) and central Europe (3%). Differences in the regional carriage rates compared with our study are most probably due to variation in the study periods and methods (inclusion and exclusion criteria). For instance, our study determined carriage rates specific for ESBL *E. coli* and only confirmed by at least DDST or PCR were included as discussed by (Harrington, Dudley, & Nataro, 2006).

2.3.2 Prevalence of *E. coli* infection in Africa

In Africa, the evidence of Shiga toxin-producing *E. coli* (STEC) O157:H7 infection among the environment, animals, and humans, in general has been reported, (Gambushe et al., 2022). Gambushe and his team noted that, the South African National Veterinary Surveillance and Monitoring Programme for Resistance to Antimicrobial Drugs have shown increased antimicrobial resistance in *E. coli*. These results were similar to results in European countries as noted by Gleaner et al., (2019). Infections with diarrheagenic *E. coli* are likewise more frequent in African countries, mostly in Ethiopia, Nigeria, and South Africa.

According to Onyeka et al., (2022), healthy colonized cattle are the major reservoir of Shiga toxin–producing *Escherichia coli* (STEC) and play a key role in the entry point of the pathogen into the beef chain. He noted that, excretion rates and the concentration of the pathogen in feces influence the epidemiology and transmission of the pathogen within herds and to humans. In their study which sort to evaluate the prevalence and dynamics of fecal shedding of STEC by cattle in a commercial feedlot in Gauteng, South Africa, fecal samples were obtained from 106 randomly selected weaned beef calves on arrival at the feedlot using polymerase chain reaction (PCR) to screen by detecting $stx_1$and stx_2 genes. Subsequently, a longitudinal study was conducted, and 15. STEC-positive and 11 STEC-negative cattle were sampled monthly and followed to slaughter. STEC O157 and non-O157 were enumerated in

samples using commercial chromogenic agar. Initial prevalence of STEC shedding was 27% (29/106; 95% CI [19, 37%]). All 26 cattle shed STEC intermittently or continuously during the study period, all except one were super-shedders (≥4 $\log_{10}$ CFU/g) at one or more samplings, and 19 (73%) were persistent or intermittent super-shedders. Of the 38 STEC isolates recovered, 15 (39%) were serotype able, representing 11 non-O157 serogroups, including O101, O168, O178, and O68. The most frequent virulence combination profile was stx_1 + *eaeA* + *ehxA* (*n* = 12; 32%).

Another similar study identified the presence of genes coding for virulence and phylogroups among *E. coli* isolated from children hospitalized due to diarrhea in Limpopo Province, South Africa, Alfinete et al., (2022). From the 133 isolates tested in the study, 79 were confirmed as *E. coli*. Of these, 19.0% (15/79) were commensals and 81.0% (64/79) were positive for at least one pathotype, of which ETEC was predominant (16.5%, 13/79), followed by EAEC (10.1%, 8/79), EPEC (7.6%, 6/79) and EHEC (2.5%, 2/79). Hybrid pathotypes were also detected and EAEC/ETEC was predominant (25.3%, 20/79). Phylogroup B2 was predominant (30.4%, 24/79), followed by phylogroup B1 (22.8%, 18/79), and phylogroups C and E (both 12.7%, 10/79). Just over 6% (5/79) of isolates were non-typable.

In Morocco, an Extended-spectrum β-lactamase (ESBL) producing *Escherichia coli* was found to be increasingly significant cause of hospital- and community-acquired infections worldwide. Whereas several reports highlighted their increased prevalence also in North African countries, genomic data on isolates associated with these infections are still scarce. This study by Sumbana et al., (2021) aimed to provide data on ESBL-producing *E. coli* isolates from patients with extra intestinal infections at the Military Teaching Hospital Mohammed V of Rabat, Morocco. In the study, the whole-genome sequencing was carried out on 18 ESBL-producing extraintestinal pathogenic *E. coli* (ExPEC) isolates for analysis of phylogenomic evolution, virulence factors and antimicrobial resistance genes. Data were compared with ExPEC lineages from several surrounding countries using multilocus sequence typing (MLST) and single nucleotide polymorphism-based phylogenetic approaches. In the findings, the majority of *E. coli* isolates were ST131 (*n* = 15), followed by ST617 (*n* = 2) and a novel sequence type (ST10703) that is closely related to the

pandemic ST405 clone. All ST131 isolates belonged to the O25b-ST131 pandemic clone. They harbored more virulence genes than their non-ST131 counterparts. IncF plasmid replicons and the *bla*$_{\text{CTX-M-15}}$ β-lactamase gene were identified in all isolates. No ESBL-producing *E. coli* isolates carried any known Carbapenemase gene. The study concluded that the pre-eminence of ST131 as the major factor driving the expansion of ExPEC in the Rabat region while highlighting the potential links with isolates circulating in other neighboring countries.

There are reports of high bacterial resistance to the commonly prescribed antibiotics in sub-Saharan Africa (Elton et al., 2020). However, data on the prevalence of antimicrobial-resistant *E. coli* in the healthy population are scarce in the Republic of Congo. A recent study reported the prevalence of antimicrobial-resistant *E. coli* (48%) in hospitalized patients in Brazzaville, Republic of Congo, (Fils et al., 2019)

2.3.3 Prevalence of *E. coli* infection in Kenya

In Kenya, Wandera et al., (2022), in their study that assessed the impact of water, hygiene and sanitation (WASH), maternal, new-born and child health (MNCH), nutrition and early childhood development (ECD) on diarrhea and microbial quality of water in a resource-constrained rural setting in Kenya. The study took place in Narok County which was noted among the worst health indicators of maternal, newborn and child health (MNCH) in Kenya, under-five mortality 45 per 1000 live births, exclusive breastfeeding 40%, percentage of fully immunized children 54%, delivery at health facilities 18%, access to potable water 20%, latrine coverage 30%, under-five diarrhea prevalence 40%, stunting 33%, wasting 2% and underweight 12%. The pastoral lifestyle, inadequate rainfall and low household food security (availability, accessibility and stability) undermine nutritional indicators for under-fives and pregnant women in Narok County. Furthermore, water scarcity in this area hinders appropriate hygiene practices and insufficient latrine coverage increases susceptibility to diarrhea Through a controlled intervention study, the team tested fecal and water samples collected from both the intervention and control sites before and after the interventions using microbiological, immunological and molecular assays, they also sort to determine the prevalence of diarrheagenic agents and

microbial quality of water. Data from the hospital registers were used to estimate all-cause diarrhea prevalence. The study found that, after the interventions, they observed a 58.2% (95% CI: 39.4–75.3) decline in all-cause diarrhea in the intervention site versus a 22.2% (95% CI: 5.9–49.4) reduction of the same in the control site. Besides rotavirus and pathogenic *E. coli*, the rate of isolation of other diarrhea-causing bacteria declined substantially in the intervention site. The microbial quality of community and household water improved considerably in both the intervention (81.9%; 95% CI: 74.5%–87.8%) and control (72.5%; 95% CI: 64.2%–80.5%) sites with the relative improvements in the intervention site being slightly larger. The integrated WASH, MNCH, nutrition and ECD interventions resulted in notable decline in all-cause diarrhea and improvements in water quality in the rural resource-limited population in Kenya. This indicates a direct public health impact of the interventions and provides early evidence for public health policy makers to support the sustained implementation of these interventions.

Ondiek et al., (2022) sort to determine the course of contamination in household drinking water. The study had presumed that water quality is dependent on a number of determinants which could be arising at the source, during transportation or due to storage and handling practices. According to Ondiek et al, contaminated water is a leading cause of water borne diseases which are a major public health and policy makers concern. The study was designed to investigate the factors affecting household drinking water quality in Kisii Town that has four main zones which include: Mwembe, Jogoo, Nyanchwa and CBD. The study found a significant relationship between household size and water quality in terms of presence of total coliforms. The following hygiene and sanitation factors were found to be having significant relationship with presence of *E. coli* in household drinking water; source of water ($p = 0.002$), transportation container ($p = 0.029$), covering during transportation ($p = 0.012$), storage container ($p < 0.001$), method of drawing from storage container ($p < 0.001$), feces disposal ($p = 0.001$) and garbage disposal method ($p = 0.04$). The conclusion of this study is that good hygiene and sanitation practices are important in ensuring total safety of drinking water at the point of use. There is therefore a need for more capacity building in this region to ensure that

people do not consume contaminated water which is a major contributing factor to water-borne diseases.

In Kenya, the meat value chain (MVC) is an important component of the food supply chain serving as a source of nutrients and income. However, information regarding processing practices, hygiene and equipment use as affecting meat quality still remains unclear despite its relevance for data and for assessment for development of meat quality in the meat trade (Catherine, David and Grace, 2021). Their trio in a cross sectional survey of selected slaughterhouses and butcheries in the Eastern region of Kenya, assessed the postharvest handling practices and meat quality. Forty meat samples were collected from rump, neck, stomach and hind legs cuts of the carcass and analyzed for total viable counts, *Staphylococcus aureus*, *E. coli* and *Listeria monocytogenes*. The findings indicate that over 50% of the meat handlers in slaughterhouses and butcheries have not received any formal training in good hygiene practices for meat handling. Total viable counts ranged from 2.159 to 2.736 log CFU/g, Staphylococcus aureus ranged from 1.112 to 1.324 log CFU/g, Escherichia coli ranged from 1.211 to 1.320 log CFU/g and *L. monocytogenes* ranged from 0.101 to 0.193 log CFU/g in the meat cuts. In conclusion, the study showed poor handling of meat which poses risks to consumers. The study also noted that, the highest percentage of the meat handlers in both the slaughterhouses (68%) and butcheries (55%) was in the age bracket of 35 years and above (Tables 1 and 2), respectively. Most of the operators had attained basic education. Among the slaughterhouse operators, 39% had primary education, 32% secondary education and only 23% had tertiary education. On the other hand, most of the butchery operators (51%) had secondary level education, 39% primary level education, 9% tertiary level education and only 1% had no level of education. In terms of experience, majority of the respondents in the slaughterhouses (68%) and butcheries (46%) had an experience of below 10 years

All the meat handlers (100%) in the slaughterhouse and 97% in the butcheries possess a medical health certificate. Results of the study also show that over 50% of the respondents in the slaughterhouse and butcheries have not been trained in hygienic meat handling. From observations, in all the butcheries, the meat was

hanged in open air for display and purchase by consumers. With regards to cleaning, most of the respondents in the slaughterhouse (93%) said that they cleaned the slaughterhouse after slaughter. On the other hand, all the meat handlers (100%) in the butcheries also indicated that they cleaned their facility after work. However, uncleaned ceilings and white walls with observable dirty spots were noticed. The findings also show that the majority (90 and 84%) of the respondents in the slaughterhouses and butcheries, respectively wear protective clothing while working. However, from observations, most of the dust coats used had changed color from white to brown and the gumboots were not nicely cleaned. Some of the operators were seen handling steel files used for sharpening the knives in their gumboots. When moving meat from the slaughterhouse to the vehicles they carry it on the shoulders of their dirty coats. In addition, infrequent washing of hands was observed and standby hot water baths for sterilizing knives were also not available. They concluded that Meat is an indispensable source of high-quality protein for most populations, hence postharvest handling practices along the meat value chain are critical since they influence the quality and safety of meat.

Finally, Fleming funded study looking at Urinary Tract Infection (UTI), one of the most common reasons for outpatient attendance and antibiotic use worldwide, noted many shortcomings in public-sector microbiology laboratories, including limited professional expertise in the clinical interpretation of urine samples, (Bartonjo and Aiken, 2022). This project aimed to deliver training on identification and AST for staff at five hospital laboratories participating in the Kenyan AMR surveillance network. They made local needs assessments, delivered practical training sessions face-to-face and administered written and practical competency assessments for all participants. According to the findings, after trainings conducted between November 2021 and January 2022 with a total of 13 laboratory staff trained, there was a substantial improvement in written assessment scores from a median of 46/100 pre-training (IQR 36-64) to a median of 90/100 post-training (IQR 85-92). The largest improvements were seen amongst staff with the lowest prior levels of microbiology training (MLS Diploma), though improvements were also seen for staff with BSc and MSc qualifications. Practical assessment included use of standardized organisms – all participants performed well in this practical assessment. They concluded that

training microbiology staff in the accurate processing of urine samples will be an important activity for a Kenyan AMR surveillance system and that, if these training materials are delivered by an experienced trainer, can achieve a clear improvement in knowledge levels and practical competence.

2.4 *Klebsiella* species infection

Klebsiella species is also a rod-shaped Gram-negative bacteria, a member of the genus *Klebsiella* and family *Enterobacteriaceae,* (Yeh et al., 2013). Its biochemical characteristics include being anaerobically anaerobic, oxidase-negative and is a lactose fermenter hence breaks lactose to produce acid and gas. It is more often found in the intestinal tracts in about 5% of healthy humans, (Cellini et al., 2010). In some cases, it has been isolated from the skin and mouth of humans and livestock. Some have a large accessory genome of plasmids and chromosomal gene loci which divides strains into opportunistic, hypervirulent and multidrug resistant hence the three species, *K. pneumoniae*, *K. variicola* and *K. quasipneumoniae* (Holt et al., 2018). Holt and the team also noted that most *Klebsiella* species may act as opportunistic pathogens which infect critically ill and immune-compromised patients and therefore identified as common causes of health-care associated infections including pneumonia, urinary tract infections (UTIs), and bloodstream infections. *K. variicola* and *K. quasipneumoniae* are often not distinguished clinically. Other *Klebsiella* species are hypervirulent, infecting healthy people in community settings which causes severe infections like pyogenic liver abscess, endophthalmitis, and meningitis. *K. pneumoniae* may encode carbapenems becoming highly resistant to antibiotic hence acting as opportunistic species. *Klebsiella* species can also colonize the gastrointestinal tract, and the accessory genome may determine if a colonizing strain remains asymptomatic or progresses to cause disease.

Finally, Ramya, (2022) *Klebsiella* species is a significant pathogen causing more number of community-acquired and hospital-acquired infections. They are one of the leading causes of death in ICU patients worldwide due to an increase in the resistant strain to antibiotics but a very few therapeutic options. It causes roughly 50% mortality in patients with chronic alcoholism and bacteremia. *Klebsiella* species are

one of the primary causes of morbidity and mortality. *Klebsiella* spp causes infections such as respiratory tract infection, urinary tract infection, wound infection, meningitis, peritonitis, and septicemia, *Klebsiella* species strains that are hypermucoviscous have been identified and have been linked to consequences such as liver abscess and metastatic infections. While penicillin and cephalosporins are commonly used for routine treatment, multidrug-resistant gram negative infections are treated with carbapenems, polymyxin B, and colistin.

2.4.1 Prevalence of *Klebsiella* species globally

Klebsiella has been reported as one of bacterial agents fast rising to gain resistance towards available antimicrobial agents hence becoming a worldwide threat, (Scaccaglia et al., 2022). In a study that sort to unravel why NDM-1-producing strains are rendering the last line antibiotics less effective in the republic of Kiribati, six bismuth complexes of general formula $BiLCl_2$, where L is a thiosemicarbazone bearing a quinoline moiety, were synthesized and fully characterized, including their X-ray crystal structures. The synergistic relationship between the compounds and meropenem were tested in a combination therapy in carbapenem-resistant *K. pneumoniae* (NTCT14331) carrying the NDM-1 gene. The team found that, Quinoline-2-carboxaldehyde-N^4-phenyl-3-thiosemicarbazone bismuth dichloride and carbapenem showed synergism in a dose dependent manner with negligible antibacterial activity when used in a monotherapy and could restore antibiotic sensitivity in the strain producing NDM-1 enzyme. In a similar study in India, Yul et al., (2019) also noted a minimum inhibitory concentration (MIC) of meropenem lowered down 128 folds up to 2 μgmL^{-1}, a concentration lower to the sensitivity level. In his team that looked at the effectiveness of the IC_{50} compound against A549 human lung carcinoma cells. The findings showed that HuDe human epithelial tissue was 46.96 ± 16.66 μM and 54.26 ± 9.89 μM respectively. The cytotoxicity against human cells was higher than the effective concentration needed for the synergistic effect in bacterial cells, indicating that a structural optimization of the compounds is needed.

In response to infection with New Delhi metallo-beta-lactamase (NDM)–producing Enterobacteriaceae, combination antimicrobial therapy with ceftazidime/avibactam (CAZ/AVI) plus aztreonam (ATM) according to a study by Rawson et al ., (2022, has been explored. This study evaluated a practical laboratory method of testing for clinically significant synergy between CAZ/AVI+ATM in NDM-producing Enterobacteriaceae. They used minimum inhibitory concentrations (MICs) of clinical NDM-producing isolates to determine ATM while CAZ/AVI+ATM were determined using broth dilution. Restoration of the ATM breakpoint after the addition of CAZ/AVI was explored. A CAZ/AVI Etest/ATM disc method was compared with broth dilution. The study found that, of 43 isolates, 33 (77%) were ATM resistant (median [range] MIC = 56 [16–512] mg/L). Addition of CAZ/AVI restored the ATM breakpoint (MIC <4 mg/L) in 29 of 33 resistant isolates (89%). Overall, the Etest/disc method correlated with the findings from broth dilution in 35 of 43 cases (81%). Etest/disc sensitivity was 77% and specificity 85%. Positive predictive value was 92% and negative predictive value 61%. Rawson and the team determined that CAZ/AVI+ATM demonstrated significant synergy in most ATM-resistant NDM-producing Enterobacteriaceae. The Etest/disc method is a quick, reproducible, and reliable method of testing for clinically relevant synergy in the microbiology laboratory.

Due to a shortage of alternative medicines, multidrug-resistant (MDR) and carbapenem-resistant *Klebsiella* species have become a major therapeutic challenge in various countries. Furthermore, there is a scarcity of novel antibiotics in development, (Ramya, 2022). Ramya reported in a study she did in Fiji that, from a total of 366 *Klebsiella* spp. collected from various clinical samples and subjected to the standard CLSI methods (Kirby-Bauer disc diffusion method), all isolates were screened and confirmed for ESBL/ AmpC β-lactamase/ Carbapenemase producers *Klebsiella* isolates were confirmed as 89% of the total isolates obtained, *Klebsiella* oxytoca (7%), *Klebsiella* aerogenes (4%). Among the 366 isolates, Male predominance was high 63% while female accounted for (37%). Maximum number of *Klebsiella* species were isolated among the age group 60-70 years (19%) followed by 50-60 years of age it contributes about (16%).

Maximum number of isolates were obtained from the pus sample (23%) followed by urine (21%). Highest number of isolates were obtained from Intensive Care Unit it's around (31%) followed by General Surgery ward (20%) *K. pneumoniae* was the most common species isolated from various clinical samples. Highest drug resistance was showed against Amoxicillin clavulanate around (50%) and lowest drug resistance was shown against Tetracycline (12.06%) by the klebsiella isolates obtained from various clinical samples. ESBL producers were most encountered followed by Carbapenemase and AmpC producers. In polymerase chain reaction 40 isolates showed bla NDM gene positive followed by 5 isolates showed positive for bla KPC gene. Ramsya concluded that the widespread use of antibiotics had resulted in an increase in the number of multidrug-resistant *Klebsiella* spp. ESBL producers were the most common among the 366 *Klebsiella* spp. recovered in this study, followed by Carbapenemase and AmpC producers. Infection control practices, as well as antibiotic control policies, play a significant role in limiting the rise in antibiotic resistance in bacteria. The lowest resistance rates to the tetracycline medication were seen in all isolates in our analysis. As a result, it may be regarded as a 9 more effective medicine. Confirmatory tests such as the combination disc diffusion test (ESBL detection), double disc synergy (AmpC detection), and Modified Hodge Test, modified carbapenem inactivation test, EDTA carbapenem inactivation test (Carbapenemase detection) were determined to be the most significant and reliable in our findings, and may be easily performed in routine microbiological testing.

According to Bell et al., (2022), the Australian Group on Antimicrobial Resistance (AGAR) performs regular period-prevalence studies to monitor changes in antimicrobial resistance in selected enteric gram-negative pathogens. The 2021 survey was the ninth year to focus on bloodstream infections caused by Enterobacterales, and the seventh year where Pseudomonas aeruginosa and Acinetobacter species were included. The 2021 survey tested 8,947 isolates, comprising Enterobacterales (8,104; 90.6%), P. aeruginosa (745; 8.3%) and Acinetobacter species (98; 1.1%), using commercial automated methods. The results were analyzed using Clinical and Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints (January 2022). Of the key resistances, resistance to the third-generation

cephalosporin ceftriaxone was found in 12.5%/12.5% (CLSI/ EUCAST criteria) of Escherichia coli and in 6.1%/6.1% of *K. pneumoniae*. Resistance rates to ciprofloxacin were 12.3%/12.3% for E.coli; 7.2%/7.2% for *K. pneumoniae*; 5.4%/5.4% for *Enterobacter* cloacae complex; and 3.7%/8.0% for P. aeruginosa. Resistance rates to piperacillin-tazobactam were 2.8%/6.5%; 2.9%/9.9%; 18.4%/28.1%; and 6.9%/12.8% for the same four species, respectively. Seventeen Enterobacterales isolates from 17 patients were shown to harbour a carbapenemase gene: 12 blaIMP-4; two blaNDM-7; one blaNDM-1; one blaOXA-181; and one blaKPC-2. No transmissible carbapenemase genes were detected among P. aeruginosa or Acinetobacter isolates in the 2021 survey.

Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS), commonly used for microorganism identification, can also be applied for the detection of carbapenemase-producing bacteria by the evaluation of carbapenem hydrolysis, Wilhelm et al., (2021). Since KPC- and NDM-producing bacteria are related to high mortality rates, diagnostic assays for its detection are essential. The aim of this study was to develop and evaluate a method to establish a quantitative measure (hydrolysis index - HI) to detect meropenem hydrolysis by MLADI-TOF MS. bla_{KPC} and bla_{NDM} positive and negative *K. pneumoniae* isolates and *Escherichia coli* ATCC 25922 (control) were incubated in a meropenem solution for 2 h.

Protein extraction from these suspensions were submitted to MALDI-TOF MS analysis. The intensity of peaks at 384 *m*/*z* and 379 m/z of each isolate were used to establish the HI as follows: HI = (Peak intensity$_{384\ Test}$ / Peak intensity$_{379\ Test}$) / (Peak intensity$_{384\ Control}$ / Peak intensity$_{379\ Control}$). Receiver Operating Characteristic curve was used to determine a cutoff value to differentiate carbapenemase-producing from carbapenemase non-producing bacteria. As all carbapenemase-producing *K. pneumoniae* presented HI $\leq$0.55 and all carbapenemase non-producing isolates presented a HI $\geq$0.57, the index of 0.56 was established as a cutoff value to differentiate carbapenemase (KPC and NDM) producing and non-producing bacteria.

2.4.2 Prevalence of *Klebsiella* species in Africa

The extended spectrum β-lactamase producing bacteria are widely spread worldwide (Mohammed et al., 2021). In their study that gene encoding *Klebsiella* species using Molecular techniques in Khartoum state in Sudan, noted that the production of extended spectrum β-lactamase (ESBL) enzymes may lead to bacterial resistance to a wide range of antibiotics. In this study, a total of 100 ESBL *Klebsiella* species were isolated from patients attending Omdurman Teaching Hospital. Full identification of species was performed using analytical profile index (API) protocol. ESBL phenotype was determined by the double disk diffusion test (DDDT) and E-test. When the confirmed species were tested for the existence of CTX M-1 gene using conventional polymerase chain reaction (PCR), none was found positive for CTXM-1 gene. From this study, findings revealed no association between CTXM-1 gene and ESBL producing *Klebsiella* species which is still note conclusive with a need to have further studies.

Abdurehman and Abdurahman (2022) did a cross-sectional study from March 2021 to October 2021 which isolated and identified *Klebsiella* species from the gut of honey bees collected from worker of honey bee (*Apis mellifera*) from hives in Haramaya University bee farm in Ethiopia. The study selected a total of 60 samples of live adult honey bees purposively then live adult worker of the honey bee was individually surface-sterilized and complete alimentary canals of the worker bee were dissected and processed for *Klebsiella* isolation. The study used descriptive statistics determine the occurrence of *Klebsiella* species and the proportion of *Klebsiella* found in the gut was analyzed for the association with study variables by the Pearson chi-square test. The overall prevalence of *Klebsiella* spp. was 50% from samples. The prevalence of *K. pneumoniae* was 26.7% and that of *K. oxytoca* was 23.3% from isolated using bacteriological examined samples.

The duo characterized isolates for the antimicrobial susceptibility test using the disc diffusion method, and among the isolated colonies, *K. pneumoniae* had the highest resistance to ampicillin (84.2%) and showed less resistance to gentamicin and trimethoprim sulfamethoxazole (26.3%). *K. oxytoca* was highly resistant to

ampicillin (54.5%) and erythromycin (54.5%) and showed low and equal resistance to gentamicin and amoxicillin (18.2%). Based on the findings, they concluded that, Molecular characterization should be conducted to identify *Klebsiella* spp. from honey bees and so recommended monitoring antimicrobial effectiveness to tackle the existing problem in apiculture farms, to be able to determine its public health threat to the community by public health professionals. Fatimah et al., 2021 also in a similar study in reported that Extended-spectrum β-lactamase (ESBL) producers are widely spreading worldwide producing resistance to most β-lactam antibiotics, e.g. penicillin and cephalosporin. This study revealed no association between OXA gene and ESBL producing Klebsiella species in Khartoum State (Sudan). Keywords: *Klebsiella* species, extended spectrum β-lactamase, *OXA* gene, PCR.

In West and Central Africa, Mahamat et al. 2021 reported that extended-spectrum *β-lactamase-producing Enterobacteriaceae* (ESBL-E) and carbapenemase-producing Enterobacteriaceae (CPE) are widespread. In their study which used the 'One Health' approach to determine knowledge gaps on ESBL-E and CPE in West and Central Africa, the team searched all articles on ESBL-E and CPE in these African regions published in PubMed, African Journals Online and Google Scholar from 2000 onwards. Among the 1201 articles retrieved 165 studies were selected, 118 from West Africa, and 47 from Central Africa. 136 articles focused only on humans (carriage and/or infection), 6 articles on humans and animals, and 13 on animals, 1 on humans and the environment, 8 on the environment and 1 on humans, animals and environments.

ESBL-E prevalence ranged from 11–72% in humans and 7–79% in aquatic environments (wastewater). In animals, the study noted that ESBL-E prevalence hugely varied, 0% in cattle, 11–36% in chickens, 20% in rats, 21–71% in pigs and 32–75% in dogs. The $bla_{\text{CTX-M-15}}$ gene was the predominant ESBL-encoding gene and was associated with plasmids of incompatibility groups F, H, K, Y, N, I1 and R. CPE were studied only in humans. Class B Metallo-β-lactamases (NDM) and class D *Oxacillinases* (OXA-48 and OXA-181) were the most common Carbapenemases. Their findings show major knowledge gaps, particularly on ESBL and CPE in animals and the environment that might limit antimicrobial resistance management

in these regions. The results also emphasized the urgent need to improve active surveillance programmes in each country and to support antimicrobial stewardship.

Finally, Reddy et al., (2021) in a study that sort to analyze hospital-acquired bloodstream infection (HA-BSI) trends to monitor emerging antimicrobial resistance (AMR) threats and guide empiric antibiotic choices, used a retrospective 10-year review of neonatal HA-BSI on at Tygerberg Hospital's neonatal unit in Cape Town, South Africa. The study reviewed neonatal clinical and laboratory data from 2014 to 2018 (Period 2) comparing it with published data from 2009 to 2013 (Period 1). The study noted that, the neonatal unit's HA-BSI rate declined between periods from 3.9/1000 inpatient-days in Period 1 to 3.3/1000 inpatient-days in Period 2 ($p = 0.002$). Pathogen yield and blood culture contamination rate were unchanged (11.0% to 10.4%, $p = 0.233$; 5.1% to 5.3%, $p = 0.636$ respectively). Gram-negative pathogens predominated 1047/1636, 64.0%), *Klebsiella* species, *S. aureus*, *Serratia marcescens*, *Enterococcus* species and *Acinetobacter baumannii* were the most frequent pathogens. Extended spectrum beta-lactamase production was observed in 319/432 (73.8%) of *Klebsiella* species, methicillin resistance in 171/246 (69.5%) of *Staphylococcus aureus* and extensive drug resistance in 115/137 (83.9%) of *Acinetobacter* species (2009–2018). The crude mortality rate of neonatal HA-BSI episodes increased from Period 1 to Period 2 from 139/717 (19.4%) to 179/718 (24.9%) ($p = 0.014$), but HA-BSI attributable mortality remained unchanged (97/139 [69.8%] vs 118/179 [65.9%], $p = 0.542$).

The *in-vitro* activity of piperacillin-tazobactam and amikacin declined during Period 2 (74.6% to 61.4%; $p<0.001$). They concluded that, although HA-BSI rates declined in the neonatal unit, antimicrobial resistance rates in BSI pathogens remained high.

2.4.3 Prevalence of *Klebsiella* species in Kenya

Miriti et al., (2022) found that respiratory tract infections cause significant morbidity and mortality globally and are the most common infectious diseases in humans. In their study that aimed at determining the prevalence of bacterial respiratory infections and antimicrobial susceptibility profile among outpatients presenting with respiratory tract infections in Meru teaching and referral hospital, the trio found that,

Klebsiella species was 3rd in predominance (19.8) after *Pseudomonas* (30.9%) and *Staphylococcus* (22.2%). The study that was conducted in Meru teaching and referral hospital, Meru County from April 2017 to August 2018 obtained a total of 175 sputum and throat samples aseptically from patients who were clinically suspected to have respiratory infections and cultured in blood agar, MacConkey agar and chocolate agar. Bacterial isolates were identified by colonial morphology, Gram stain and confirmed by biochemical tests. Antimicrobial susceptibility profile was determined using agar disc diffusion method. The study also noted the presence of *pyogenes* (14.8 %) and *Streptococcus pneumoniae* (12.4 %). The susceptibility test showed that both Gram positive and Gram-negativeisolates were highly susceptible to gentamicin, cefuroxime and amikacin while resistance was recorded for amoxicillin and ampicillin. However, resistance to more than two antibiotics was recorded in 54 (66.7 %) of the isolates. The trio concluded that since many of the isolates obtained showed resistance to some antibiotics used hence there is need for a continuous surveillance of antimicrobial resistance in management of respiratory infections in Meru County.

Antimicrobial resistance is a pressing global health issue with limited data that are lacking in detailing the presence and burden of antimicrobial resistance in low and middle-income countries, Drobish et al., (2022). The team did a retrospective descriptive study at Kijabe Hospital, a rural 350-bed teaching hospital, from February 2016 to September 2020. Cultures from blood, urine, and cerebrospinal fluid from all pediatric and adult patients. Data was analyzed and an antibiogram was created using WHONET software. They found that, from January 2016 to September 2020 a total of 3275 distinct isolates were identified, including 1654 positive blood cultures, 1288 positive urine cultures, and 91 positive cerebrospinal fluid cultures. Aggregate gram negative susceptibility to third generation cephalosporins was approximately 41%, with 67% of isolates susceptible to piperacillin-tazobactam, and 93% of isolates susceptible to meropenem. The most frequently identified organism was coagulase-negative Staphylococcus (1534, 47%), followed by *Escherichia coli* (721, 22%), *Klebsiella* species (482, 15%), and Staphylococcus aureus (110, 3.4%). The most common multidrug resistant organism was *Escherichia coli* (664, 20%), followed by *Klebsiella* species (461, 14%). *Acinetobacter baumannii* was found to

be only 57% sensitive to meropenem. *Staphylococcus aureus* was 91% sensitive to cloxacillin. In their conclusion, the high rates of antimicrobial resistance found in this rural referral center were similar to the large urban settings in sub-Saharan Africa. This along with the discovery of multidrug resistant gram negative organisms are of great concern. They recommended the need for continued surveillance, antimicrobial stewardship, and implementation of quality improvement initiatives would be imperative to attempt to curb this burgeoning global problem.

2.5 The Burden of Antimicrobial Resistance

Antimicrobial resistance (AMR) is tracked most closely in clinical settings and high-income countries, (Ikhimiukor et al., 2022). However, resistant organisms thrive globally and are transmitted to and from healthy humans (Amann et al., 2019), animals (Thanner 2016) and the environment (Singer 2016). The overall public health and clinical significance of these transmission opportunities remain to be completely clarified, making it to gain considerable global interest in promoting a One Health view of AMR to enable a more realistic understanding of its ecology. Moolchandani et al., (2017), noted that AMR surveillance outside hospitals remains insufficient hence very challenging to convincingly document transmission at the interfaces between clinical specimens and other niches. In this review, the team described AMR and its transmission in low- and middle-income-country settings, emphasizing high-risk transmission points such as urban settings and food-animal handling. Moolchandani et al., (2017), further observed that the power of genomics to expose transmission channels and hotspots was largely unharnessed, and that existing and upcoming technological innovations need to be exploited towards containing AMR in low- and middle-income settings.

Hart & Kariuki (1998) noted that some patients may miss doses either by mistake or deliberate/abandon their treatment, especially in cases where signs and symptoms begin to subside only to return to the hospital with a recurring infection by a more virulent and resistant strain of the microbe. According to the authors, these actions result in the exposure of surviving pathogens to sub-therapeutic concentrations of antimicrobials leading to increased chances of acquiring resistance. Self-medication

is a common practice in developing countries where patients often get antimicrobials without prescription and through unregulated supply chains. To make the situation even worse, some patients seek their first-line of treatment from traditional healers who provide them with herbal combinations for the treatment of infections. These substances of unknown composition and potency may enhance pathogen fitness and contribute to the development of resistance.

The global emergence of MDRs is increasingly limiting the effectiveness of the existing antibiotic drugs like methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant Enterococci spp (Hancock 2005, Norrby et al. 2005). The development of resistance among the microbes is the result of continuous selection pressure of antibiotics and their surroundings causing genetic alterations (Bush 2004) which, are transferred to the next generation and reach out to the wider range of other geographical regions through the transfer of genetic information exchange between microbes (Amábile-Cuevas 2003). Goossens (2013) reports that increase in AMR among microbes may result in the emergence of new resistant phenotypes and the development of new antimicrobial agents.

Human-animal interactions are increasing, raising the risk for zoonotic infections and the subsequent emergence of resistant pathogens. In many Low and Middle Income Countries, antimicrobials, which are poorly stewarded and easily obtained over the counter, are introduced as food additives for disease prophylaxis, metaphylaxis and growth promotion in aquaculture, poultry and livestock. The quantity of antimicrobials used in animal production is predicted to increase by 11.5% between 2017 and 2030. In animal production operations in LMICs, sanitation, antimicrobial usage, animal overcrowding, lack of human protective gear and other farm management practices are important risk factors. Moser et al., (2018) reported that 429 (69.3%) birds from small-scale farms in Ecuador yielded multidrug-resistant isolates, compared with 58 (15.3%) birds in non-production villages.

Recently, increase in antibiotic resistance has been reported by Nabwera et al., (2000) in developing countries, and a decrease in the development of new drugs according to Luepke et al., (2017). Resistance to antibiotics is always associated with

infections which may pose a high risk in terms of high death rate and increases economic burden worldwide. The World Health Organization (WHO, 2016) has reported an increase in AMR strains globally due to uncontrolled spread resulting from overuse of available antibiotics. Developing countries such as united states, Canada and China are most affected when it comes to drug resistance brought about by lack of diagnosis before drug prescription, misuse of available antibiotics, misdiagnosis due lack of proper diagnosis techniques, use of non-human antibiotics in other livestock, poor quality of drugs, lack of surveillance programs and also general present of contributing socio-economic factors. This has always resulted in below standard healthcare services, recurrent infections associated with bacteria infested but believed to be resistant to the available drugs and also unaffordability of more effective and costly drugs, (Fujimura, Kato, & Kawamura, 2004). Despite, lack of newer drugs may result in resistance which must be contained before options of battling it run out are considered. The World Health Organization's report shows global surveillance of antimicrobial resistance, significant gaps and also testing capacity especially in Africa, South East Asian countries and Eastern Mediterranean region, (Haberecht et al., 2019).

Resistance to antibiotics has failed to neither eradicate nor prevent recurrence of *H. pylori* infections, (Mustafa et al., 2015). Globally, high resistance of *H. pylori* towards most antibiotics has been reported, this has also affected efficacy of current therapeutic regimens, (Kimang'a et al., 2010). Kimang'a further noted that antibiotic resistance varies in different geographical regions and can be mainly attributed to widespread and unregulated use of antibiotics in the general public. Likewise, *K. pneumoniae, one* of the important human and animal pathogens, has over time acquired resistance to antimicrobials, (Ayukekbong, Ntemgwa, & Atabe, 2017). *E. coli* has been noted to cause urinary tract infections high with potentially an alarming increase in resistance against quinolones evidenced by testing environmental and pediatric clinical isolates (Shmuely et al., 2003).

2.6 Antimicrobial Resistance in *H. pylori*

2.6.1 Antimicrobial resistance in *H. pylori* globally

A study in North Carolina in the US reviewed previous studies (the last 6 years) about the *Helicobacter pylori* (*H. pylori*) antibiotic resistance in order to evaluate the trend in antibiotic resistance, Ghotaslou et al., (2015). The study reviewed manuscripts in PubMed, MEDLINE, Science Direct, Google Scholar and Scielo from 2009 to 2014. In their findings, resistance of H. pylori was 47.22% (30.5%-75.02%) for metronidazole, 19.74% (5.46%-30.8%) for clarithromycin, 18.94% (14.19%-25.28%) for levofloxacin, and 14.67% (2%-40.87%) for amoxicillin. The study also noted 11.70% (0%-50%) resistance in tetracycline, 11.5% (0%-23%) for furazolidon and 6.75% (1%-12.45%) for rifabutin. The frequency of tetracycline, metronidazole and amoxicillin resistance was higher in Africa, while clarithromycin and levofloxacin resistance was higher in North America and Asia, respectively. The trio concluded that the most sensitive drug is rifabutin and the lowest sensitive drug is metronidazole in the world. They noted that worldwide *H. pylori* antibiotic resistance to clarithromycin and levofloxacin had increased during the last 6 years. The present systematic review show alarming results and recommended a novel plan towards eradication therapy of *H. pylori* infections.

A study by Suzuki and team (2019) indicated that in Madagascar, rates of resistance were more prominent in metronidazole followed by amoxicillin then clarithromycin with tetracycline presenting the least resistance (Kawai et al., 2014). It was reported that resistance to metronidazole and amoxicillin drugs stood at 1.6% followed by resistance in metronidazole and clarithromycin which was at 5%. Data and information on antimicrobial resistance genes is very important when it comes to decision of treatment measures especially in eradication of *H. pylori* and related infections. Based on the study outcomes, *H. pylori* was reported to be relatively more resistant to metronidazole followed by amoxicillin hence the two antimicrobial agents were recommended as the best first-line drugs in the eradication of *H. pylori* strains.

2.6.2 Resistance of *H. pylori* in Africa

In Africa, 26 articles which had short analysis of resistance of *H. pylori* to metronidazole, to amoxicillin, to tetracycline, clarithromycin and also to quinolones, (Fouz et al., 2020). Low resistance to quinolones was recorded at 17.4% followed by resistance to clarithromycin which recorded a percentage of 29. Tetracycline had a resistance rate of 48.7% while metronidazole stood at 75.8%ref. Amoxicillin had a resistance rate of 72.6% according to the study. It was therefore noted that many strains of *H. pylori* in Africa are more resistant to metronidazole followed by clarithromycin and then amoxicillin in that order. Fouz and the team recommended more surveillance studies to provide more data of *H. pylori* in Africa and also recommended more studies that would determine susceptibility patterns and trends of *H. pylori* bacteria to antimicrobials in Africa for these information would be used in future to help provide more information that would establish effective empirical treatment.

A study in an Egyptian hospital sought to help understand the rate of *H. pylori* strains resistance to antimicrobial agents, these strains were isolated from human subjects and were used in the primary step before commencing treatment (Diab et al., 2018). In a sample of 60 patients, the study revealed that about 25% of *H. pylori* strains were resistant to metronidazole while resistant to amoxicillin was 18.3% respectively. Mutant genes were found to be resistant to clarithromycin by 6.7% and 1.7% to tetracycline. The study also aimed in designing *glmM* primer, a reverse primer that aimed at detecting *glmM* gene by using Polymerase Chain Reaction, PCR. These genes were obtained from clinical samples including gastric biopsies. Commercial u*reA* gene was amplified and used as a positive control. It was noted that these genes were amplified using PCR multiplex in addition to virulence genes *vacA* and *cagA* but nonspecific bands were observed when these primers were used (Tomasini et al., 2003).

Primers obtained from non-multiplexed assays of *glmM* genes are considered because these genes are often highly conserved and therefore mostly used in identification of *H. pylori* obtained from biopsies samples. *glmM* gene is also highly

sensitive and specific with upto 90% as compared to *ureA* gene which has a sensitivity and specificity of 90%. It was recorded that the presence of *H. pylori* strains that turned positive in the total tested biopsies was 44% according to PCR technique using *ureA* and *glmM* genes (Kolar et al., 2014). Another study by Essawi et al., (2013) detected resistance patterns in major four antimicrobial drugs used in eradication of *H. pylori* bugs. These drugs included metronidazole, amoxicillin, clarithromycin and tetracycline. Resistance to these regimes was found to compromise the efficacy of majorly used antimicrobials affecting treatment and intervention of these bacteria. Detected also were mutant genes which were found in about 60% *H. pylori* bacterial strains obtained from patients who went endoscopy testing, (Essawi et al., 2013). Detection was by using rapid test to determine the presence of *16S rRNA* mutant genes in gastric biopsies using conventional PCR technique then sequenced the products. The study found out that genes encoded for resistance to metronidazole are *rdx* genes, amoxicillin *pbp1A* genes. *23S rRNA* gene confer resistance to clarithromycin while *16S rRNA* mutant genes conferred resistance to tetracycline. Detection of *23S rRNA* and *16S rRNA* mutation genes was done by real time PCR procedure, (Zurita et al., 2022)

In Kenya, Vlieghe, et al., (2010) sought to ascertain and establish the sensitivity of *H. pylori* to drugs by use of samples obtained from patients presenting with dyspepsia (Malfertheiner et al., 2007). This study revealed that there was a 100% resistance to metronidazole for all the isolated *H. pylori* strains. *H. pylori* isolates found to be susceptible to clarithromycin accounted for 93.6% while amoxicillin and tetracycline had 95.4% and 98.1% sensitivity rate respectively. A similar study by (Kimang'a et al., 2010) in Nakuru also detected occasional resistance to metronidazole. From the literature reviewed so far, not much work has been done in Kenya on resistance to antimicrobial agents by *H. pylori*, it is therefore recommended for more studies since the outcome will help in adding information to the literature around *H. pylori* resistance in Kenya and probably recommend effective and reliable treatment measures.

2.7 Antimicrobial resistance in *Escherichia coli*

According to Haberecht et al., (2019) *E. coli* bacteria would resist various available drugs, its resistance trends, how virulent these bacteria are and also determine its genetic makeup, (Haberecht et al., 2019). The study isolated *E. coli* bacterial cells from both the community and hospital setting and tested for the presence of urinary tract infections and investigated how bacteria resist plasmid-mediated Quinolone genes. A subset of 18 isolates which were phenotypically resistant to Quinolones were examined. The study revealed that resistance to a suspension of Trimethoprim and Sulfamethoxazole, ampicillin, and a suspension of Ampicillin and Sulbactam reported. Resistance towards Ciprofloxacin, Levofloxacin and Cephalosporin was also observed. More than a half of the isolated strains were resistant to a range of drugs with almost all the Fluoroquinolones resistant strains showing the multidrug resistant phenotype. The infestation of fecal antibiotic resistant *E. coli* among the food handlers in Qatar, showed more than a half of the isolates were found to be resistant to at least one antibiotic while about 27% were resistant to may drugs (Alanis, 2005). About 9% were ESBL producers of which five were resistant to many drugs (Alanis, 2005). The study also concluded that isolates either resistant to a single drug or resistant to many drugs were most common in stool samples among these food handlers in Qatar. Stool samples were obtained from 456 migrant people who were food handlers. These *E. coli* Isolates were tested to ascertain its susceptibility to antibiotics using two tests.

A surveillance study was done in the Netherlands by Friesema et al., (2014) to determine resistance of *E. coli* strains against a number of antimicrobial agents with a similar study in the USA by Dadgostar et al., (2019). Friesema and the team noted that, about 17.1% *E. coli* t produced ESBL genes obtained from waste waters and from water flowing in rivers exhibited resistance to up to three classes of drugs including beta-lactams, tetracycline and aminoglycosides. Another study in Portugal reported that *E. coli* strains were phylogenetically grouped into commensal strains and pathogens (Poirel et al., 2018). Both groups were made up of bacteria which were found to be resistant to first-line antibiotics like Carbapenems which is a known drug for conventional treatment infection in man and animals. Based on this report,

they came up with suggestions to improve on surveillance research and AMR patterns for this would show how resistance spreads within the environmental factors which include water and surface soils. Beta-lactams resistance bacteria include Extended-Spectrum Beta-Lactamase producing bacteria (ESBL) and those which had *K. pneumoniae Carbapenemases* (KPC) enzymes. The study concluded by recommending a thorough application of advanced screening methods in subsequent studies in order to totally characterize antimicrobial resistant genes especially from the environment.

Acquisition of resistance genes may contribute to ESBL and KPC resistance. These genes include *blaCTX-M, blaSHV, blaIMP, blaOXA, and blaTEM, and blaKPC, blaVIM*, and bland (Singh et al., 2019). These genes can be transmitted from one host to another either vertically or horizontally using plasmids. Due to the high potential of horizontal AMR gene transfers to human reservoirs, researchers have increased their attention to the isolation of these resistance genes including those isolated from surface waters and soils according to (Azevedo et al., 2007). These studies will provide information which will help in effective prevention and control of transmission.

In Kenya, a study by Grady et al., (2014) sought to establish the presence of pathogenic *E. coli* in western Kenya and southern Vietnam anti-drug sensitivity profile and the general genetic makeup. Drug sensitivity testing was later done by Kirby-Bauer disk diffusion method. The study revealed that general prevalence across the population was reported as 90.2%. Isolates with diarrheagenic *E. coli* showed strains which were more susceptible to Sulfamethoxazole with a degree of about 97% followed by Co-Trimoxazole which had a susceptibility degree of 96%. Ampicillin had 84% while chloramphenicol 27%. Tetracycline, Kanamycin and Streptomycin had susceptibility degrees of 16%, 10% and 9% respectively. However, most of these *E. coli* strains were sensitive to a single drug gentamicin while approximately a half of the isolates were resistant to multiple antimicrobial agents. The study further revealed that all isolates showed total resistance to Ampicillin which had *Blatem* gene which was an indicator of the possibility of genetic alteration.

2.8 Antimicrobial resistance in *Klebsiella* species

According to Aamer et al., (2019) in Uganda, characterized susceptibility patterns or trends of *E. coli* and *Klebsiella* species to antimicrobials, (Amer et al., 2019). These bacterial cells were obtained from outpatient from both urban and rural districts of Uganda nation. A total of 1448 human subject were selected to participate in this study, of these 985 bacterial cells were obtained from both participants 'stool and urine samples. Most isolates were obtained from about 87% of stool samples collected, of which about 97% of the isolates were *E. coli* while only the remaining 3% were *Klebsiella* species. Increased resistance to antimicrobials were detected among combined isolates of both *E. coli* and *Klebsiella* species isolates. Resistance was high against sulfamethoxazole and trimethoprim which was at 70% followed by mixture of Amoxiclav was at 36%. Chloramphenicol came third with a resistance of 20% then ciprofloxacin and gentamicin followed at 11% each. 4% and 3% isolates showed resistance to Nitrofurantoin and ceftriaxone respectively. Multiple drug resistant was reported in 33% of the total isolates but none of the isolates was resistant to Imipenem. The study concluded that most of the isolates got from Kampala in Uganda were resistant to most of the antimicrobial agents. Frame the sentences to show that organisms showed resistance to drugs

2.9 *Extended-Spectrum β-Lactamase Producing Klebsiella pneumoniae*

Extended-spectrum B-lactamases are enzymes with the ability to hydrolyze penicillin, monobactams, and oxyimino-cephalosporins, inhibited by carbapenems and B-Lactamases inhibitors such as amoxicillin-clavulanate, ticarcillin-clavulanate, ampicillin sulbactam, piperacillin-tazobactam, and cefoperazone-sulbactam. ESBL include, *TEM*, *SHV*, *OXA*, *CTX-M* which are encoded by *blaTEM-1*, *blaSHV-1*, *blaOXA-1*, and *blaCTX-M* respectively. (Bush et al., 2010). The incidence of ESBL-producing strains among clinical *Klebsiella* isolates has been steadily increasing over the past years, which limits its therapeutic possibilities, (Yang et al., 2022). The authors also noted that *Klebsiella* virulence factors have greatly provided new insights into the pathogenic strategies and its pathogenicity attributed to presence of capsules and lipopolysaccharides. *Klebsiella* spp have the SHV gene in the

chromosome which encodes for ampicillin resistance (Babini et al., 2000, & Bialek Davenet et al., 2014). Among plasmid-mediated B-Lactamases is the AmpC enzyme which confers resistance to penicillin, 2nd, and 3rd generation cephalosporins, and cephamycins. AmpC enzymes are mainly present in *K. pneumoniae*. Besides B-Lactamases, other mechanisms include; the alteration of penicillin-binding protein thus reducing B-Lactam affinity (Meroueh et al., 2003) and efflux pumps.

Mahmud et al., (2022) notes that the emergence of virulent caused by Extended Spectrum β-Lactamase producing *K. pneumoniae* (ESBL-KP) including carbapenem-resistant *K. pneumoniae* (CRKP) in hospital-acquired infections has resulted in significant morbidity and mortality worldwide. This study investigated the antibiotic resistance and virulence factors associated with ESBL-KP and CRKP in tertiary care hospitals in Bangladesh and explored their ability to form biofilm. In this study, a total of 67 ESBL-KP were isolated from 285 *K. pneumoniae* isolates from environmental and patient samples, using Enterobacterial Repetitive Intergenic Consensus polymerase chain reaction (ERIC-PCR), 67 isolates were multidrug-resistant (MDR) to different antibiotics at high levels and 42 isolates were also carbapenem-resistant. The most common β-lactam resistance gene was $bla_{\text{CTX-M-1}}$ (91%), followed by bla_{TEM} (76.1%), bla_{SHV} (68.7%), $bla_{\text{OXA-1}}$(29.9%), bla_{GES} (14.9%), $bla_{\text{CTX-M-9}}$ 11.9%), and $bla_{\text{CTX-M-2}}$ (4.5%). The *Carbapenemase* genes blaKPC (55.2%), blaIMP (28.4%), blaVIM (14.9%), blaNDM-1 (13.4%) and blaOXA-48 (10.4%) and the virulence associated genes such as *fimH* (71.6%), *ugeF* (58.2%), *wabG* (56.7%), *ureA* (47.8%) and *kfuBC* (28.4%) were also detected. About 96.2% of the environmental and 100% of the patient isolates were able to form biofilms.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

This purposive study was carried out in two selected areas, a low-income area, Kibera informal settlement and a middle income area, Dagoretti area both in Nairobi County. These areas border each other on the further Nairobi South-west hence experiences the same geographical factors and also most of the residents in these areas attend the same hospitals which would make it easier to collect samples from both residents.

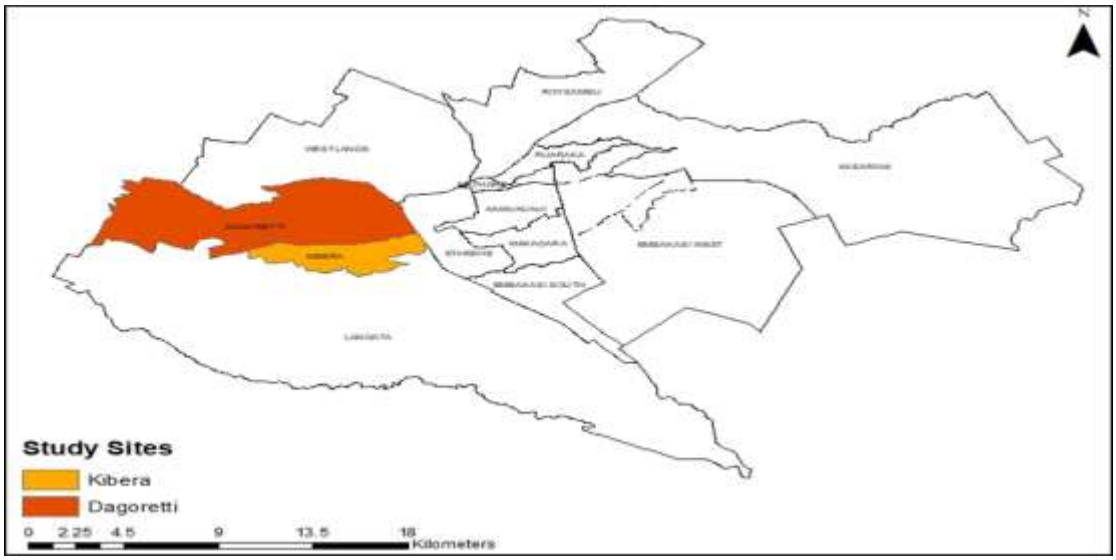


Figure 3.1: Map of Nairobi County showing Kibera and Dagoretti.

Source: (Mahabir, et al., 2017)

3.2 Study design and Sample size and Inclusion/Exclusion criteria

3.2.1 Study design

This was a cross sectional study that recruited outpatients visiting Mbagathi County hospital and Dagoretti sub-county hospital respectively. People of all ages who

presented with gastritis and their first degree relatives or contacts were eligible for the study

3.2.2 Study population and Sample size determination

Sample size was determined by Fisher's exact formula. The sample was calculated with a 95% confidence level (95% – Z score = 1.96), 5 standard deviation, and a margin of error (confidence interval) of +/- 5%.

$$N = \frac{Z^2 PQ}{SE^2}$$

q=0.5 SE=0.05, Z=1.96 and p=0.5

Sample size = ((1.96)2 x 0.5(0.5)) / (0.05)2

(3.8416 x 0.25) /0.0025

0.9604 / 0.0025

384.16 thus, 385 respondents.

n = required sample/population size,

Z = the critical value of normal distribution, t 95 % CI, P = estimated sample proportion, q=standard deviation

d = margin of error (precision) of prevalence estimate at 5 % (0.05), α = level of significance at 5 % and SE is the Margin of error 0.05.

3.2.3 Inclusion and exclusion criteria and community engagement

3.2.3.1 Inclusion criteria

1. Residents of study areas Kibera and Dagoretti area

2. Patients who presented with gastritis-like signs and symptoms or had previous history of *H. pylori*
3. Contacts or people who shared households with patients 1&2 who voluntarily consented or assented for participation.

3.2.3.2 Exclusion criteria

1. Those who failed to consent or assent for participation.
2. Those who didn't understand language being used during recruitment.
3. Those too sick or mentally challenged to provide consent and assent.
4. Those who failed to provide sample

3.3 Sampling techniques

The study recruited 409 human subjects, 258 (63.1%) from Kibera settlement and 151 (36.9%) from Dagoretti area both in the larger Nairobi County. The study recruited outpatients who visited Mbagathi district and Mutuini sub-district hospitals presenting with gastritis related symptoms and signs **(Appendix v).** Follow-up visits were done to recruit people who shared households or residence with the outpatients. People of all walks of life were recruited regardless of their ages, their races and religion and meeting the inclusion criteria were considered for the study. Participation in this study was absolutely voluntary without coercion. All participants aged above 19 years were issued with consent seeking and information form (**Appendix ii),** which they were expected to read, understand before proceeding to sign consent (**Appendix iii**). Children aged between 13 and 18 years were also asked and voluntarily to sign assent forms (**Appendix iv**). Standard study questionnaires **(Appendix i)** were administered to collect data on potential risk factors that may be attributed to *H. pylori* infection. People who were recruited in hospitals were referred to as cases while follow-up participants were referred to as contacts. A total of 409 samples were collected, 258 from Kibera settlement and 151 from Dagoretti area.

3.4 Sampling strategy

Four hundred and nine (409) stool samples obtained were taken to Mbagathi District Hospital laboratory within 2 hours of collection for Microbiological analysis. The study questionnaires administered (**Appendix i**) were used to collect potential risk related factors that would be attributed to *H. pylori* infection since the symptoms and signs used in recruitment were those believed to be attributed to people who have *H. pylori* related complications (Mustafa et al., 2015).

3.4.1 Sampling of outpatient

Outpatients visiting Mbagathi or Mutuini hospitals and residing in Kibera and Dagoretti were identified by facility doctors and or clinicians and referred to the study recruitment desk. Symptoms and signs attributed to gastritis **(Appendix v)** were considered by clinicians and doctors as recruitment inclusion criteria. People of all ages were targeted for the study. A total of 172 patients were recruited in health facilities, 109 patients from Mbagathi (63.3%) and 63 patients (36.7%) were from Mutuini sub-district hospital.

3.4.2 Sampling of Follow-up population (Contacts)

Follow-up samples were first tested by kits right in the field before transporting to the lab for subsequent culture and isolation. Cary Blair transport media was used for transportation as it maintains bacterial viability and also reduces contamination. Sample bags were double packed in absorbent material (paper towels), placed in leak proof zip locks and transported in a cooler box with ice bags. Infant stool samples were collected in plastic wraps spread over toilet seats or inside diaper lining in order to direct urine to run into the diaper and not into the wrap. Stool was prevented from coming in contact with the inside of disposable diapers because the lining often has antibacterial properties which would interfere with the test results.

3.5 Laboratory Procedures

3.5.1 Culture of *H. pylori* isolates

All the 409 samples collected were subjected to stool antigen testing kits. About 10 grams of each stool sample obtained were tested using polyclonal antibodies (One Step *H. pylori* Fecal Antigen test strips; (Negash et al., 2018, Guangzhou Wondfo Biotech, Japan). This kit was routinely applied in participating hospitals as the main diagnostic method for *H. pylori* detection. The stool samples that tested positive by the kit were cultured on Columbia blood agar, (OXOID CM0331 UK) laced with 7% laked horse blood under microaerophilic environment at 37^0 for between 5-7 days. The resulting shiny white colonies were presumed *H. pylori* colonies which were later confirmed by Biochemical testing and Polymerase Chain Reaction. *H. pylori*, ATCC 43504 was used as a positive control while blank plates were incubated alongside as negative control to also confirm sterility of equipment, media and processes.

3.5.2 Culture of *Escherichia coli* and *Klebsiella* isolates

All 409 stool samples collected were directly cultured on MacConkey agar alongside ATCC 25922 for *E. coli*, ATCC KPN 700603 and KPN 13883 for *Klebsiella* isolates. Blank plates were used as negative controls. Inoculated plates were incubated aerobically at 37^0C for 18-24 hours. Growth colonies were macroscopically. Doughnut shaped pink and mucoid moist pink colonies were presumptively considered to be *E. coli* and *Klebsiella* isolates respectively (Amer et al., 2019, Kumaresan & Tiwari, (2015). Purification to obtain monoculture was done by sub-culturing on TSA agar and incubating for 18-24 hours at 37^0.

3.6 Biochemical Identification of Bacterial isolates

In *E. coli* and *Klebsiella* identification, Colonies obtained after 18-24 hour of incubation were picked and introduced to standard Biochemical tests including Indole, Methyl red, Voges proskauer and Citrate (IMViC).These tests were are designed to distinguish among members of the family *Enterobacteriaceae* such as *E.*

coli and *Klebsiella* bacteria. Respective Biochemical reactions were interpreted as described by Emelda, (2012). A positive Indole isolates was indicated by the formation of a red color in the reagent layer on the agar top shortly after introducing the colonies while a yellow or slightly cloudy reagent was interpreted as a negative outcome. Methyl red test was by introducing a few bacterial colonies in a broth medium containing glucose, a positive reaction remained red in color while a yellow color indicated a negative outcome. Voges Proskauer test was done for confirmation of glucose fermentation, a brownish red color was an indicator as a positive sample. A positive citrate test turned blue while a green color was a negative outcome. Biochemical reactions included;

3.6.1 Citrate

This tested for the ability of the organism to utilize citrate as a source of energy. It was prepared as follows; Media powder was added to deionized water at pH. 6.9. Bromothymol blue stain was added and the mixture heated for mixing. The mixture was dispensed into tubes and sterilized at 121 degrees, 15 psi for 15 minutes. The tubes were cooled in slanting posture and refrigerated ready for use. After introduction of the organism by streaking, the cultured media was incubated at 35-37 degrees. Observation was by color change. Results were as follows; *E coli*-No color change (-ve) remains green in color while *Klebsiella* species-color changes to blue (+ve).

3.6.2 Indole

Prepared SIM gar (or peptone water), inoculated the target colony and incubated at 37 degrees for 24 hours. After growth, 1ml of kovacs indole reagent was added and observation made for color change. Expected results *E. coli* colonies are considered +ve for changing color to cherry red while *Klebsiella* species were considered –ve for maintaining yellow color.

3.6.3 Methyl red

Prepared methyl red media, inoculated the target colonies and incubated at 37 degrees for 24-28 hours. After growth, we added 5 drops of methyl red indicator and made color change observations. Expected results, *E. coli* colonies were expected to turn +ve by changing color to red/pink while *Klebsiella* species remained yellow (-ve).

3.6.4 Voges proskauer

We prepared Voges proskauer media, inoculated the target colonies and incubated at 31 degrees for 24-48 hours. After growth, we added 0.5ml of naphthol followed by 0.2ml KOH. The mixture was shaken and exposed to oxygen for 10-15 min and observation made. *E. coli* remained red/brown in color (-ve) while *Klebsiella* species turned black or pale yellow in color (+ve).

3.6.5 Urease

We prepared Christenses urea agar in slanting position, inoculated the target colonies and incubated for 18 hours at 37 degrees. Color change was observed with expected results for H. pylori isolates turning positive rapidly/immediately.

3.6.6 Catalase

Hydrogen peroxide was used to test for catalase positive and catalase negative colonies. Presumptive *H. pylori* isolates were subjected to catalase test and expected to form bubbles (positive). Expected results were *E. coli* (++--) and *Klebsiella* species (--+) for respective tests,

(Kiiru *et al.* 2012) **(Table 3.1)**

Table 3.1: Biochemical Test Interpretation

Bacterial Isolate	`	Positive
E. coli	Indole Motility	Red
	Methyl red	Red
Klebsiella	Voges proskauer	Brown
	Citrate	Blue
Urease	Urease test	Pink
	Catalase test	Bubbles
	Oxidase	Indigo

3.7 Antimicrobial Sensitivity Testing for *Escherichia coli* and *Klebsiella*

Antimicrobial susceptibility testing (AST) was performed by disc diffusion technique according to Kirby-Bauer method (Midolo et al., 1995). Culture was done on Mueller Hinton agar (OXOID, United Kingdom). Test drugs were antimicrobial routinely used in management of most Gram negative bacterial infection, including Ampicillin (AMP

10µg), Amoxicillin-clavulanic acid (AMC, 20/10µg), Cefuroxime (CXM 30µg) Ceftriaxone (CRO, 30µg), (Ciprofloxacin (CIP, 5µg), Cefepime (FEP, 30µg), Nalidixic acid (NAL, 30µg), Ceftazidime (CAZ, 30µg), Cefotaxime (CTX, 30µg), Imipenem (IPM 30µg) Trimethoprim Sulfamethoxazole (RL, 25µg), Tetracycline (TET, 30µg), and Aztreonam (ATM, 30µg). Briefly stocked isolates were revived on Mueller Hinton agar (MHA) and incubated for 18 hours at 37^0 C. Isolates were emulsified in normal saline to attain 0.5 McFarland standard concentration before inoculating the MHA plates. Antimicrobial discs were later introduced and inoculated plates left for 15 minutes undisturbed before incubation for 18-24 hours. The zones of inhibition were measured and results interpreted as susceptible (S), intermediate (I) or resistant (R). Resistance patterns determined using the Clinical Laboratory Standard (CLSI-2019) guidelines.

3.7.1 Determination of *Extended Spectrum Beta-Lactamases* (ESBL)

Presence of potential resistance genes was determined by a double-disk synergy test to which phenotypically detects the likelihood of isolates that potentially carry ESBLs. ESBL target genes included Bla*TEM*, bla*SHV*, and blaCTX-*M*-15. This was performed using disks of 3rd generation Cephalosporin and Cephalosporin inhibitors (Clavulanic acid). KPCs were determined by resistance conferred towards Imipenem, a carbapenem. All tests were done independently of each other alongside standard reference isolates ATCC 25922.

3.7.2 Determination of *Klebsiella Pneumoniae Carbapenemases* (KPC)

All isolates that conferred Resistance towards Iminepem (IMP) were considered to have potential KPC genes including bla*OXA* and KPA. Tests done alongside ATCC KPN 700603 and KPN 13883.

Table 3.2: Antimicrobial breakpoints

Antibiotic classes		Antimicrobial agent	Disc code	S	I	R
1	**Ampicillin**	Ampicillin	AMP-10 µg	>17	14-16	<13
2	**B-lactams**	Amoxicillin/ AmoxiClav	AMC-20/10 µg	>18	14-17	<13
3	**Cephalosporin**	Cefuroxime	CXM-30 µg	>18	15-17	<14
		Cefotaxime	CTX-30 µg	>26	23-25	<22
		Ceftriaxone	CRO-30 µg	>23	20-22	<19
		Ceftazidime	CAZ-30 µg	>21	18-20	<17
		Cefepime	FEP-30 µg	>25	19-24	<18
4	**Monobactam**	Aztreonam	ATM-30 µg	>21	18-20	<17
5	**Carbapenem**	Imipenem	IPM-30 µg	>23	20-22	<19
6	**Tetracycline**	Tetracycline	TET-30 µg	>15	14-15	<11
7	**Quinolones**	Nalidixic Acid	NA-30 µg	>19	14-18	<13
		Ciprofloxacin	CIP-5 µg	>26	22-25	<21
Folate pathways Antagonists		Trimethoprim-Sulfamethox	RL-25 µg	>16	15-16	<10

3.8 Molecular Analysis

3.8.1 DNA Extraction and Polymerase Chain Reaction

DNA extraction for *H. pylori* was done by Qiagen commercial DNA extraction kit (Essawi et al., 2013) while potential ESBL and Carbapenem isolates were revived in readiness for molecular analysis and identification. DNA was extracted by boiling method as described by (Brown et al., 2020), a very convenient and time saving technique for extraction of DNA from Enteric bacteria. Both techniques are believed to yield high quality purified DNA free from contaminants and inhibitors (Mcdonough et al., 2019). However, boiling is not suitable for extracting *H. pylori* DNA which requires a more specialized technique than minimizes degradation. The products were transferred to separate clean cryotubes and temporarily stored at -20^{o} awaiting amplification.

3.8.2 Molecular Identification of *H. pylori.*

Thermocycler used for amplification was type GeneAmpR PCR System 9700 (version 3:12) from Applied Bioscience. Final PCR products were stocked at 4 degrees awaiting visualization by gel electrophoresis genes. Target genes included *PBPs* (conferring resistance against amoxicillin) and *RDX* (conferring resistance against metronidazole) for *H. pylori* and ESBL (Blagens like *TEM, SHV* and *CTX-M*-15) and KPC (*KPC* and bla*OXA*) for *E. coli* and *Klebsiella* respectively, (Alba, Blanco, Alarcón, 2017). About 1 microliter of the DNA was mixed with 12µl of pre-aliquoted Qiagen master-mix, 12µl PCR water, and target primers (forward and reverse primer). Visualization was done on 1.5% agarose gel. Pre-determined resistant genes was displayed at the following base pairs as follows; bla*TEM* 999, bla*SHV* 851, bla*CTX-M* 599 and KPC 390 **(Table 3.3):**

Table 3.3: Identification of Targeted Resistant Genes

Target genes	Primer name	Oligonucleotide Sequence 5'3'	Product size (Base pairs)	Annealing Temp (0C)	References
BlaTEM	*blaTEM-F*	GCGGAACCCCTATTTG	940	62	(Singh et al., 2019)
BlaTEM	*BlaTEM-R*	TCTAAAGTATATATGAGT AAACTTGGTCTGAC			
BlaSHV	*BlaSHV-F*	TTCGCCTGTGATTATCTCCCTG	750	58	(Fils et al., 2019)
BlaSHV	*BlaSHV-R*	TTAGCGTTTGCCAGTGYCG			
BlaCTX-M	*blaCTX-M-F*	ATGTGCAGYACCAG TAARGTKATGGC	899	60	(Amer et al., 2019)
BlaCTX-M	*blaCTX-M-R*	TGGGTRAARTARGTSA CCAGAAYCAGCGG			
BlaOXA	*BlaOXA-F*	GGCACCAGATTCA ACTTTCAAG	882	57	(Fils et al., 2019)
BlaOXA	*BlaOXA-R*	GACCCCAAGTTT CCTGTAAGTG			
BlaKPC	*blaKPC-F*	CGTTGACGCCCAATCC	600	52	(Singh et al., 2019)
BlaKPC	*BlaKPC-R*	ACCGCTGGCAGCTGG			
H. pylori ATCC 43504	ATCC_1F	TTGAATCCATGCCCGATATT	450	58	(Quaglia et al., 2008)
H. pylori ATCC 43504	ATCC_1R	5-GGGATTTTATTGTATGCTACA A			
NCTC *GlmM*	NCTC *GlmM-F*	5-TGCTTGCTTTCTAACACTAAC G	355		(Mirzaei, et al., 2012)
NCTC *GlmM*	NCTC *GlmM-R*	5-TTGATGGCGATGCTGATAGG			
PBP	*PBP-1-F*	5-GCATGATCGTTACAGACACG-3	905	45	(Graham, 1998)
PBP	*PBP-1-R*	5-ATCCACGATTTCTTTACGC-3			
RDX	*RDX-F*	5-GCAACTATCCAATCCCATCAA G	360	51	(Kawai et al., 2014)
RDX	*RDX-R*	5-GCCAGACTATCGCCAAGA			

Thermocycler GeneAmpR PCR System 9700 (version 3:12) from Applied Bioscience, was used for amplification using conventional PCR. Amplification steps were set according to the protocol as indicated in the table below. Primers included blagenes (*blaTEM*, *blaSHV*, *blaCTX-M*) for *E. coli* ESBL isolates, KPC (*KPC* and *OXA*) for *Klebsiella* isolates and *(AMX* and *RDX* genes) for *H. pylori* isolates. (**Table 3.4**)

Table 3.4: PCR Conditions and product Base-pairs (BP)

PCR Condition	*E. coli*		*Klebsiella*			*H. pylon*		
	TEM	***SHV***	***CTX-M***	***OXA***	***KPC***	***GlmM***	***PBPs***	***RDX***
Initial Denaturation	95*5min	94*5min	94*5min	94*5min	95*5min	94*5min	94*5min	94*5min
Final Denaturation	95*1min	94*30sec	94*45sec	94*30sec	94*1min	94*30sec	94*30sec	54*30sec
Annealing	62*1min	58*1min	60*45sec	57*1min	52*1min	58*1min	45*1min	51*1min
	30cycles	30 cycles	30 cycles	30 cycles	30 cycles	30 cycles	30 cycles	30 cycles
Initial Extension	72*2min	72*1min	72*45sec	72*1min	72*1min	72*1min	72*1min	72*1min
Final Extension	72*5min	72*10min	72*10min	72*10min	72*10min	72*1min	72*10min	72*10min
Holding	4	4	4	4	4	4	4	4
Basepairs	940	750	899	882	600	350	289	905

3.8.3 Detection of Resistance genes by PCR products

Approximately 5µl of PCR products plus loading dye were loaded onto horizontal 1.5 % w/v agarose gel and molecular size marker. A 1000 bp DNA ladder was used (Invitrogen, UK) and electrophoresed at 100V for 45 minutes. The gels were stained with SYBR dye (7.5 µl). The DNA bands were then visualized with a UV trans-illuminator (UVP Inc.), and the images were taken using black and white Polaroid film. All runs were done alongside respective primers and *H. pylori* ATCC 43504 sample as positive controls and blanks as negative controls. *PBP* and *RDX* primers were used to detect genes conferring resistance towards Amoxicillin (289bp) and Metronidazole (905bp) respectively.

3.9 Data Management and Analysis

Carriage, AMR and potential risk factors collected by the study questionnaire which included socio-demographic (gender, age, education level and occupation), presence of garden and domesticated animals, geographical location (area of residence), lifestyle and history and laboratory data were entered into Epicollect5 platform and exported to excel sheets and analyzed by STATA version (64bits). Infection

prevalence rate was determined by dividing stool positively identified samples with the total number of collected samples and expressed as percentages. The population variables were summarized as means, and categorical as counts and percentages. Antimicrobial inhibition zones were interpreted based on Clinical and laboratory Standard Institute (CLSI 2019) standards and expressed as either sensitive(S), intermediate (I) or resistance (R). AMR genes were determined after PCR by gel electrophoresis and viewed on a gel viewer. Logistic regression outcome was labeled as binary outcome (Positive/Negative). Associations between various categorical variables were measured using a chi-square test at P=0.05 which tested associations. Binary logistic regression analysis was carried out to generate the adjusted odds ratio with a 95% CI confidence interval. An alpha of less than 0.05 ($P \leq 0.05$) and an odds greater than 1 ($OR \geq 1$) were considered statistically significant.

Laboratory data and information was under controlled access with backup systems put in place. Data validation programs were put in place. Data was collected using the Epicollect5 app and immediately entered into research log books, electronic gadgets and computer data and excel sheets and secondary data storage gadgets like flash discs. Hard Copies data sheets from fields such as data collection forms, consent and assent forms were stored securely for reference purposes. All samples were handled as potential biological hazards. Personal protective equipment and gears were won at all times when handling samples. Specimens from the field had unique barcodes accompanied by sample tracking forms which were required to be fully filled. Samples will be packaged in leak-proof containers and absorbent secondary materials. We ensured sample collection tracking forms were well packed in zip lock bags and accompanied specimens to MDH Microbiology laboratories.

3.10 Approvals, Feedback and Data Dissemination and publishing.

The study was approved by relevant authorities and institutions (Appendices vi-x). Part of the data from this study has been published in a public open journal for open and free access to the public. Results will be used by policy makers in creating awareness and educating the public on various underlying risk factors leading to *H. pylori, E. coli* and *Klebsiella species* infections and how to diagnose and manage

acute infections. All the findings at the end of the study will be sent back to the policy makers for policy making and creating awareness to the general public. The first manuscript covering prevalence of *H. pylori*, *E. coli* and *Klebsiella* and potential risk factors for *H. pylori* infection has been published in an open access journal for the public reference (Appendix xi).

CHAPTER FOUR

RESULTS

4.1 Carriage of *H. pylori, E. coli* and *Klebsiella* species in Kibera and Dagoretti

The study recruited 409 people of whom 341 (83.3%) were infected by either *H. pylori, E. coli* or *Klebsiella* species. A hundred and seventeen (28.6%) isolates tested *H. pylori* positive by stool antigen test while 302 (73.8%) isolates, and 160 (39.1%) isolates were identified by culture and isolation technique as *E. coli* and *Klebsiella* respectively. *E. coli* was the predominant isolate. A hundred and seventy isolates (41.6%) were co-infections, where more than 1 isolates were obtained from the same sample. About 81(19.8%) were co-infected with both *E. coli* and *H. pylori*, 31 (7.6%) had both *Klebsiella* species and *H. pylori* while 58 (14.2%) samples had both *E. coli* and *Klebsiella* species. 38 (9.3%) samples produced all the three bacterial isolates. There was significant statistical association between *H. pylori* and *E. coli* infection, (OR=1.8473, CI=0.2510-0.6091, P=0.0002).

Table 4.1: General Carriage of *H. pylori, E. coli* and *Klebsiella*

	Total		**Infected**	**%**	***H. pylori***	***%***	***E. coli***	***%***	***Klebsiella***	***%***
Kibera	258	63.1	236	57.7	73	17.9	217	53.1	89	21.8
Dagoretti	151	36.9	105	25.7	44	10.8	85	20.8	71	17.4
Total	**409**	**100**	**341**	**83.4**	**117**	**28.6**	**302**	**73.8**	**100**	**39.2**

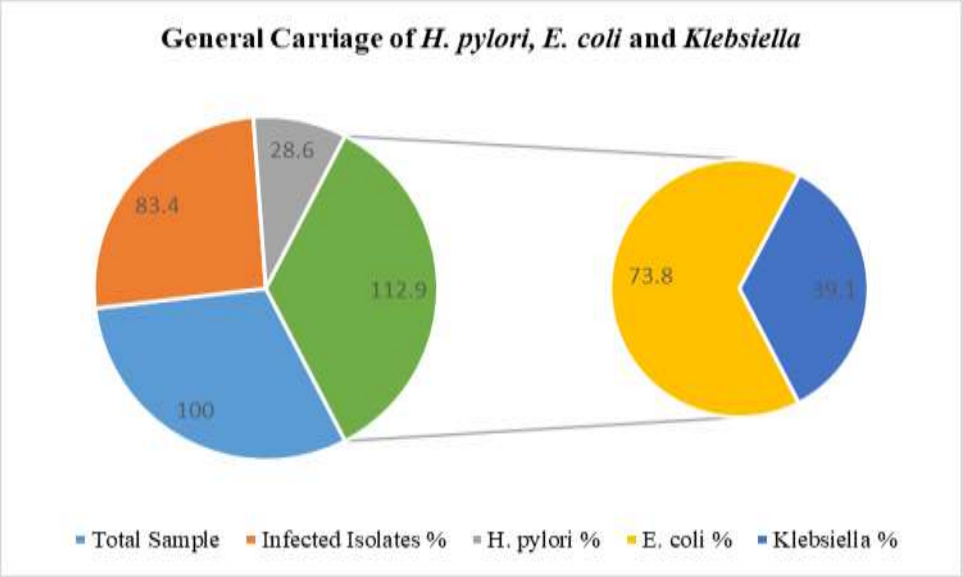


Figure 4.1: Prevalence of *H. pylori, E. coli* and *Klebsiella* species

4.2 Carriage of *H. pylori, E. coli* and *Klebsiella* in Kibera vs. Dagoretti

Two hundred and fifty-eight (63.1%) participants were obtained from Kibera settlement; of these, 236 (91.5%) were infected. The rest 151 (36.9%) were from Dagoretti of which 105 (70%) were infected. Isolates from Kibera made up 57.7% of the total infection with *E. coli* being the predominant species (48.7%). *H. pylori* and *Klebsiella* species accounted for 33.7% and 17.6% of the infection respectively.

Table 4.2: Carriage of *H. pylori, E. coli* and *Klebsiella* in Kibera vs. Dagoretti

	Total		**Infected**	**%**	***H. pylori***	**%**	***E. coli***	**%**	***Klebsiella***	**%**
Kibera	258	100	236	57.7	73	30.9	217	91.9	89	26.7
Dagoretti	151	100	105	25.7	44	41.9	85	80.9	71	53.3
Total	**409**	**100**	**341**	**83.4**	**117**	**34.3**	**302**	**88.6**	**160**	**46.9**

Despite Kibera producing 26.2% more isolates than Dagoretti, its infection rate overall still remained high (91.5%) when compared to infection in Dagoretti (69.1%). Kibera also produced many *H. pylori* (32.2%) as compared to Dagoretti (22.5%), however, there was high rates of Enterobacteraceae isolates in Dagoretti

than Kibera, *E. coli* (82.8% vs. 68.6%) and *klebsiella* (87% vs. 73%) respectively **(Fig.4.2**)

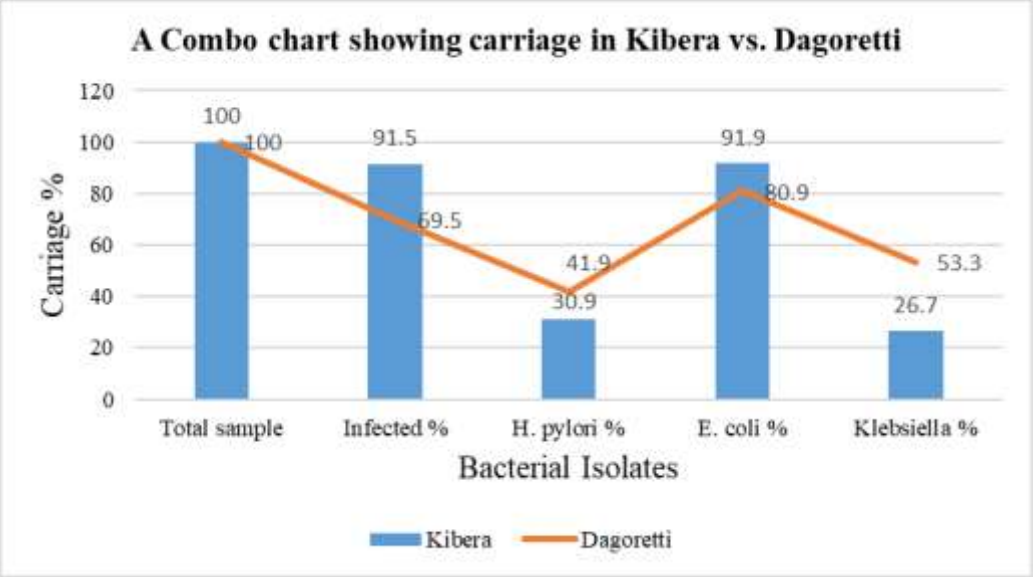


Figure 4.2: A Combo chart showing prevalence of *H. pylori, E. coli* and *Klebsiella* species in Kibera and Dagoretti

4.3 Proportion of infection between patients and contacts.

There was notably high prevalence of infection among contacts 45.2% (185) when compared to prevalence among patients 38.1% (156). Of the isolates obtained, high carriage was also noted in *H. pylori*, 62% and *E. coli*, 63% among close contacts as opposed to infection in *Klebsiella* species which was dominant among the cases (75%). High percentile of non-infected isolates was noted among Dagoretti isolates when compared to Kibera isolates which had competitive positive-negative rates.

Table 4.3: Carriage of *H. pylori, E. coli* and *Klebsiella* in Patients vs. Contacts

	Total samples	**%**	**Total infected**	**Patients Recruited**	**%**	**Patients Infected**	**%**	**Contacts Recruited**	**%**	**Contacts Infected**	**%**
Kibera	258	63.1	236	109	26.6	97	23.7	127	36.4	119	29.1
Dagoretti	151	36.9	105	49	15	59	14.4	56	21.5	66	16
Toatal	**409**	**100**	**341**	**172**	**42.1**	**156**	**90.7**	**237**	**57.9**	**185**	**70.**

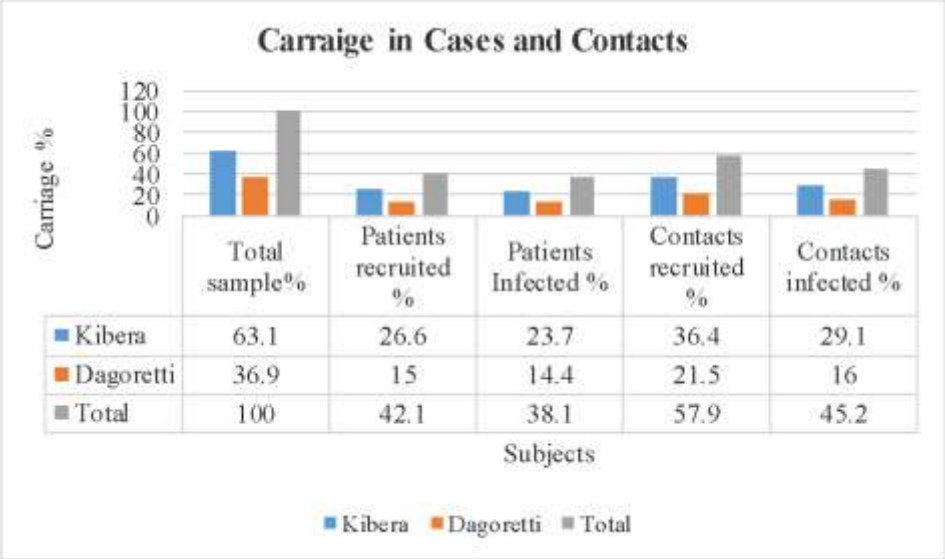


Figure 4.3: Carriage of *H. pylori, E. coli* and *Klebsiella* in Patients vs. Contacts

4.4 Carriage of respective isolates in patients vs. in Contacts.

Patients who were people presenting with symptoms associated with gastritis recorded less carriage of *E. coli* (48.1%) as compared to carriage in Contacts (57.8%) while the contrary was observed in *Klebsiella* where patients recorded high carriage (26.4%) as compared to contacts (11.4%). There was no significant difference of carriage of *H. pylori* in patients as compared to contacts (34%, 34.1%).

Table 4.4: Carriage in Patients vs. Contacts per bacterial isolates

	Patients	**%**	**Infected**	**%**	***H. pylori***	***%***	***E. coli***	***%***	***Klebsiella***	***%***
Kibera	109	100	97	89	31	28	42	39	27	25
Dagoretti	63	100	59	94	23	37	33	52	14	22
Total	**172**	**100**	**156**	**90.7**	**54**	**34**	**75**	**48.1**	**41**	**26.3**
	Contacts	**%**	**Infected**	**%**	***H. pylori %***		***E. coli %***		***Klebsiella %***	
Kibera	149	100	119	80	*43*	*29*	73	49	11	7
Dagoretti	88	100	66	75	20	23	34	39	10	11
Total	**237**	**100**	**185**	**78.1**	**63**	**34.1**	**107**	**57.8**	**21**	**11.4**

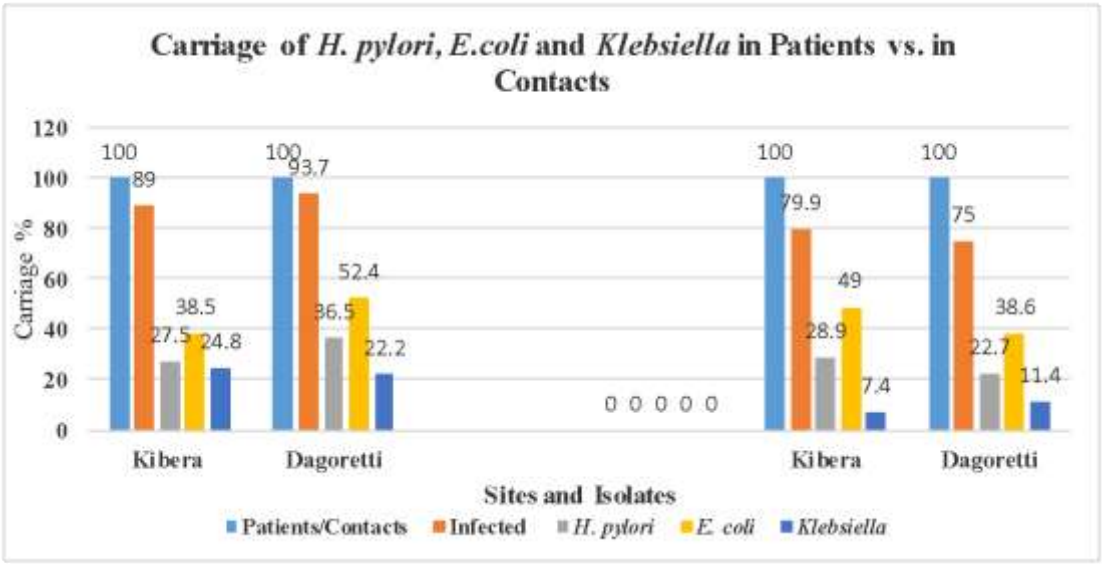


Figure 4.4: Carriage of *H. pylori, E. coli* and *Klebsiella* in Patients vs. Contacts

4.5 Biochemical tests results

Isolates confirmed by the Urease test as *H. pylori* positive were 117, of which 73 isolates were from Kibera while 44 isolates were from Dagoretti. Three hundred and two isolates turned positive by both Indole and Methyl red tests (confirmed *E. coli* isolates). Of these, 217 were isolates from Kibera while 85 were Dagoretti isolates. Likewise, 160 isolates turned positive by both Voges proskuer and citrate methods (confirmed K*lebsiella* isolates), of which 89 were Kibera isolates while 71 isolates came from the Dagoretti area.

Table 4.5: Biochemical test results

	Total	Infected	Urease +ve	Indole/Methyl red +ve	Voges/Citrate +ve
Kibera	258	236	73	217	89
Dagoretti	151	105	44	85	71
Total	**409**	**341**	**117**	**302**	**160**

4.6 Antimicrobial Sensitivity profiles and Resistance genes

Antimicrobial resistance profile was observed in 219 (64.2%) of the 341 isolates obtained. Resistance in *E. coli* isolates was predominant, accounting for 65.7% (146 of 219) of all resistant isolates. *Klebsiella* accounted for 21% (68) of the total resistance while *H. pylori* accounted for only 2.3% of the total resistance with 5 resistant isolates conferring resistance against metronidazole and amoxicillin antimicrobials. At least 8 out of 13 antimicrobial agents were less effective against both *E. coli* and *Klebsiella* isolates. High resistance in both isolates was witnessed in the commonly used antimicrobial agent including Sulfamethoxazole/Trimethoprim, RL and Tetracycline, TET from both study sides.

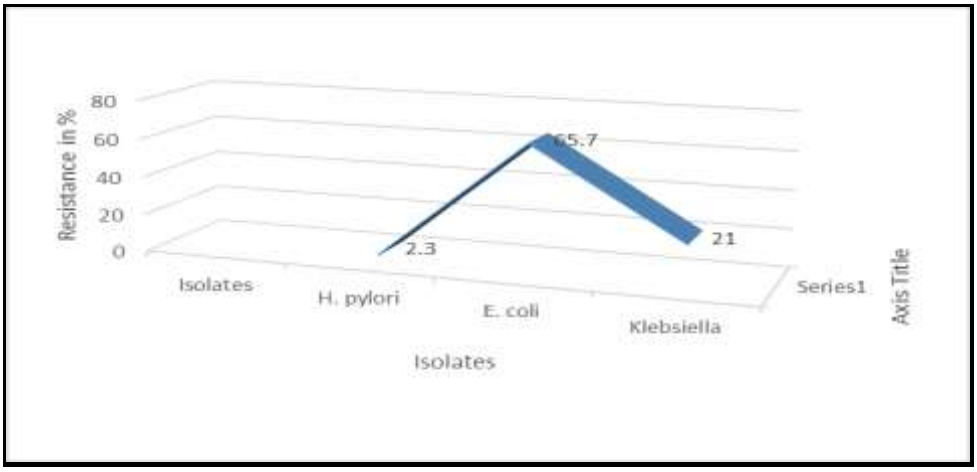


Figure 4.5: Resistance rates by *H. pylori, E. coli* and *Klebsiella*

4.7 Resistance profiles for *H. pylori* isolates

Out of the 409 samples obtained, 117 (28%) turned positive for *H. pylori* by stool antigen test. After culture, 94 samples showed characteristic *H. pylori* colonies of which 57 isolates were successfully confirmed by Biochemical tests and 13 samples subsequently confirmed by PCR with *GlmM* (gene) primers and ATCC 43504 at 289 bp (**Fig. 4.6**). Out of the 13 samples confirmed, 3 isolates had PBP genes that conferred resistance towards amoxicillin while 2 samples confirmed resistance towards metronidazole.

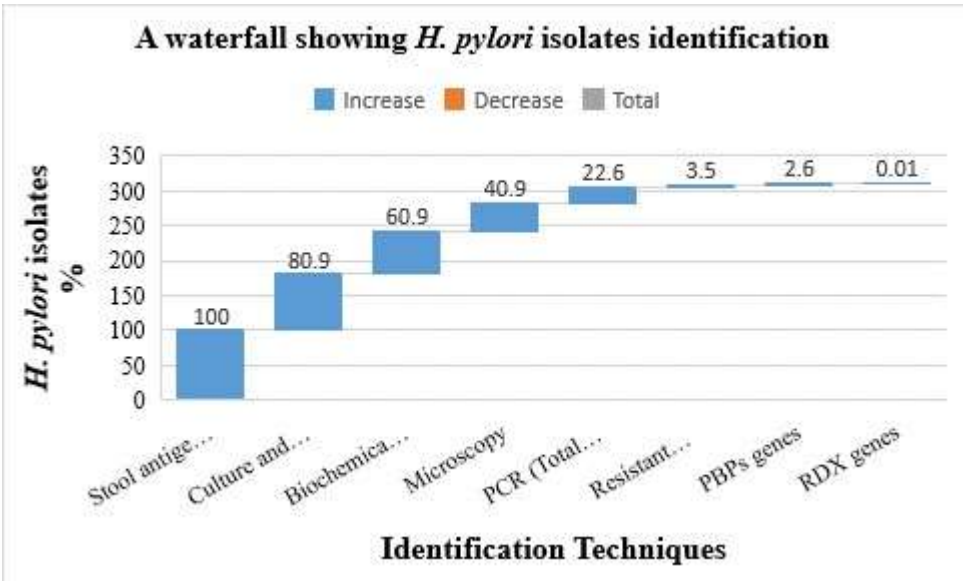
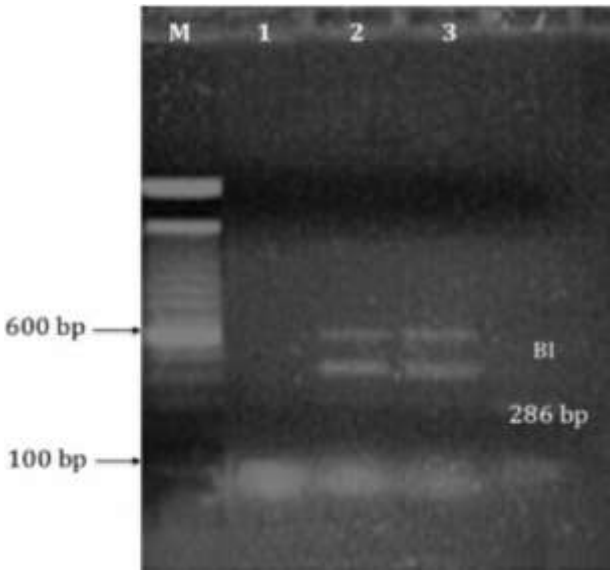


Figure 4.6: A waterfall showing *H. pylori* Identification by different Techniques



(M – DNA Ladder, 1 negative control, 2 the isolate and 3 positive control)

Figure 4.7: A gel showing positively identified *H. pylori* strains using GlmM primers (286 base pairs) using ATCC 43504

4.8 Antimicrobial Sensitivity test profiles for *Escherichia coli*

E. coli isolates accounted for 65.7% of the total resistance. Resistance profiles was determined by isolates being resistant towards Ampicillin (AMP), an Inhibitor

(AMC) and Cephalosporins (CTX, CAZ, CXM, CRO and FEP which form the 2nd, 3rd and 4th generation cephalosporins) with formation of zone of inhibition. 48.3% (146 of 302) of

E. coli isolates subjected to antimicrobial testing exhibited resistance hence potential ESBL producers.

Table 4.6: Antimicrobial Sensitivity profiles of *E. coli* isolates in various Antimicrobial agents

	AMC	AMP	CXM	CRO	CAZ	CTX	FEP	NA	CIP	IPM	ATM	RL	TET	TTL	Ave.	TET
Kibera	62	63	44	36	29	37	19	69	58	17	67	78	69	647	50	69
Dagoretti	56	66	41	28	25	34	20	63	38	15	72	82	67	607	47	67
Patients	66	69	46	40	30	38	21	72	62	20	65	76	72	677	52	72
Contacts	52	60	39	24	24	33	18	60	35	12	74	84	64	579	45	64
Total	118	129	85	64	54	71	39	132	96	32	139	160	136	1254	96	136
Average	**59**	**65**	**43**	**32**	**26**	**36**	**20**	**66**	**48**	**16**	**70**	**80**	**68**	**627**	**48**	**68**

4.8.1 Sensitivity profiles of *Escherichia coli* in Kibera vs. Dagoretti

Overall resistance was high in Kibera as compared to Dagoretti isolates. High resistance generally was noted in Ampicillin (AMP) 63%, Beta-Lactams AMC (62%) and Quinolones, Nalidixic, NA (69%) and Ciprofloxacin, CI (58%). Average resistance in 3rd Generation Cephalosporin was 32%, 26% & 36% respectively for CRO, CAZ and CTX. Resistance in CTX was predominant in both Kibera. Resistance in AMP (66 vs. 63), ATM (72 vs. 67) and RL (82 vs.78) was recorded in Dagoretti when compared to resistance in Kibera.

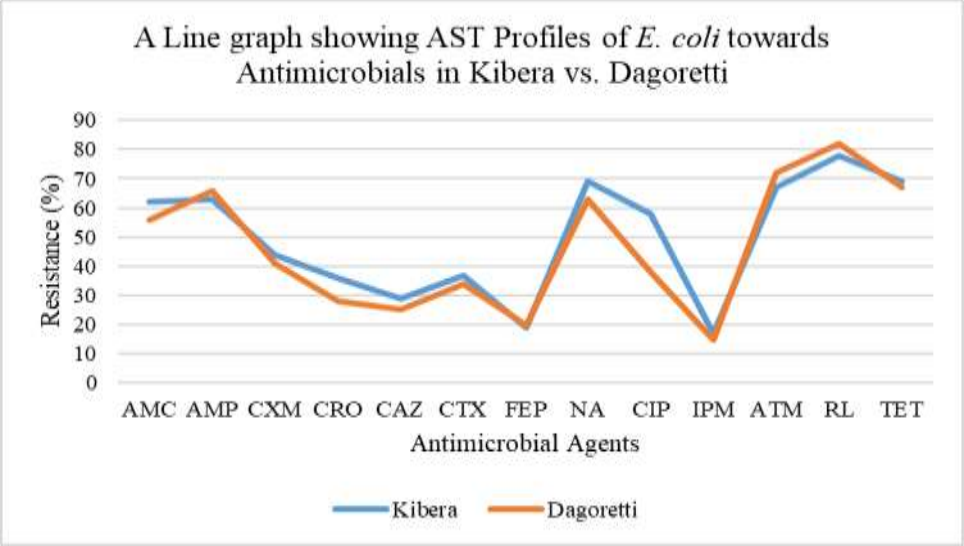


Figure 4.8: Antimicrobial Sensitivity profiles of *E. coli* Isolates to Antimicrobial agents in Kibera vs. in Dagoretti

4.8.2 Antimicrobial Sensitivity profiles of *E. coli* in Patients vs. in Contacts

There was higher resistance in patients, (52.1%) than in contacts, (44.5%). Resistance in contacts was in most commonly used antimicrobial agents including RL (84%) and ATM (74). Patients reported very high resistance recorded AMC in both patients (66%) and contacts (52%) in **(Fig. 4.7).** Significant resistance observed in all 3rd generation cephalosporin in both subjects, Patients vs. Contacts, CTX (46 vs. 39), CRO (40 vs. 24), CAZ (30 vs. 24).

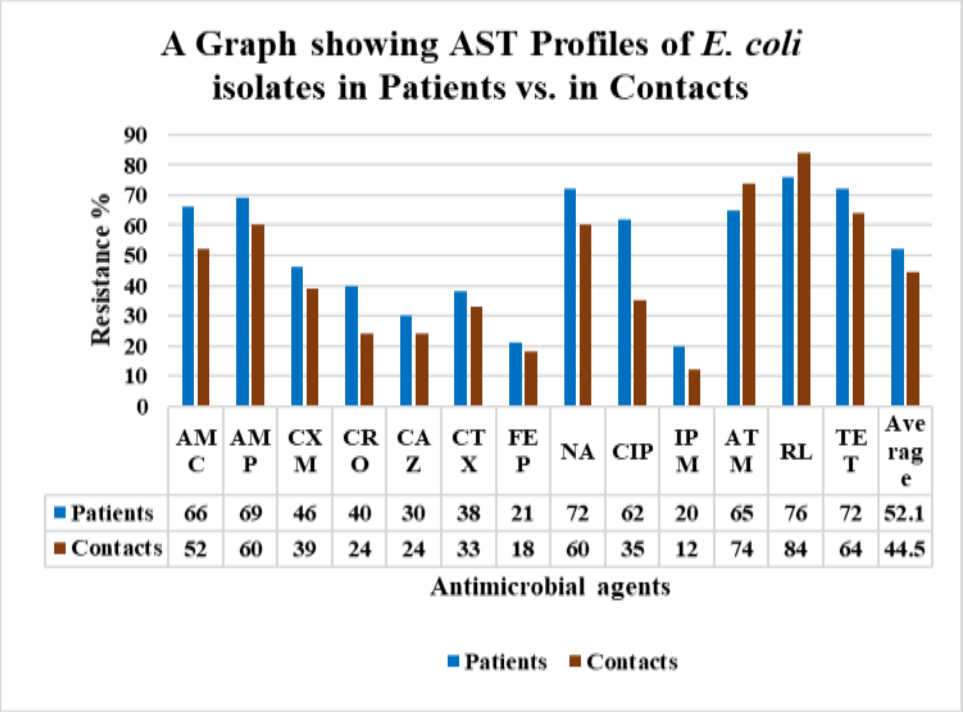


Figure 4.9: AST profiles of *E. coli* isolates in Patients vs. in Contacts

4.9 Antimicrobial sensitivity profiles of *Klebsiella* isolates

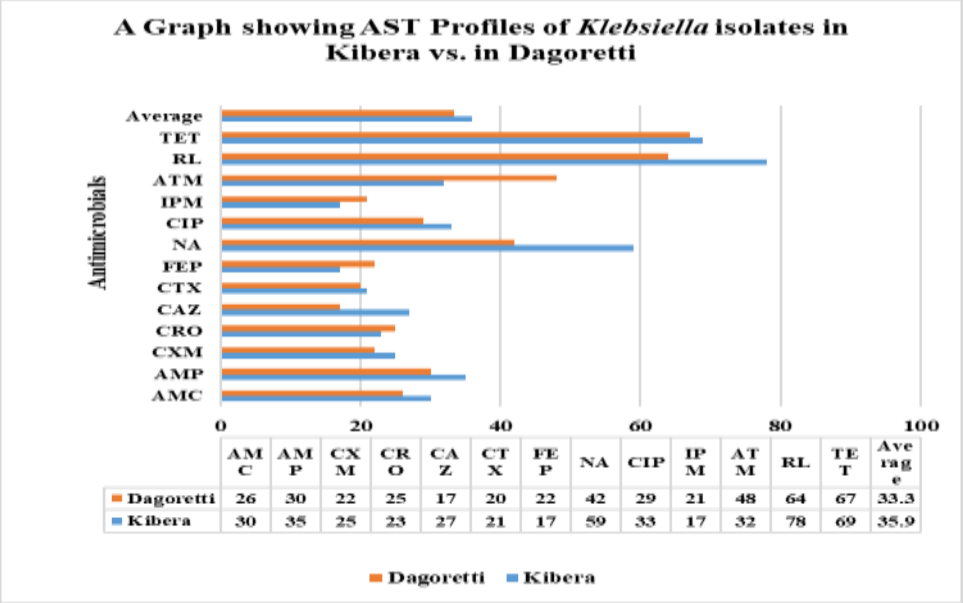
Sixty-eight out of 160 (42.5%) *Klebsiella* isolates were resistant to antimicrobial agents. This made upto 31.1% of the total resistance recorded. Generally, there was high resistance towards commonly used antimicrobial agents as compared to broad spectrum. These included, Sulfamethoxazole, Tetracycline and Nalidixic acid with average resistance of (71%, 59% and 51% respectively). Resistance in Carbapenem (Imipenem, IMP) was alarming (19%) given the strength of the drug and was determinant for a potential *Klebsiella* resistant isolate. (**Table 4.7**)

Table 4.7: Antimicrobial sensitivity profiles of *Klebsiella* isolates

	AMC	AMP	CXM	CRO	CAZ	CTX	FEP	NA	CIP	IPM	ATM	RL	TET	Total	Ave.
Kibera	30	35	25	23	27	21	17	59	33	17	32	78	69	466	36
Dagoretti	26	30	22	25	17	20	22	42	29	21	48	64	67	433	33
Patients	23	35	26	30	28	27	19	47	38	17	35	70	60	455	35
Contacts	33	40	21	17	16	14	20	54	24	21	45	72	58	435	34
Total	56	75	47	47	44	41	39	101	62	38	80	142	118	890	69
Averag	**28**	**38**	**24**	**24**	**22**	**21**	**20**	**51**	**31**	**19**	**40**	**71**	**59**	**445**	**34**

4.9.1 Sensitivity profiles of Klebsiella in Kibera vs. in Dagoretti

There was generally a slightly high resistance (35.9%) in isolates obtained from Kibera as compared to those obtained from Dagoretti (33.3%). Resistant in Imipenem, a determinant for potential *Klebsiella* resistant isolate was high in isolates obtained in Dagoretti (21%) when compared to resistance in isolates from Kibera (17%). Overall high resistance was recorded in RL (71%), TET (59%) and NA (51%).



	AMC	AMP	CXM	CRO	CAZ	CTX	FEP	NA	CIP	IPM	ATM	RL	TET	Average
Dagoretti	26	30	22	25	17	20	22	42	29	21	48	64	67	33.3
Kibera	30	35	25	23	27	21	17	59	33	17	32	78	69	35.9

Figure 4.10: AST Profiles of *Klebsiella* Isolates in Patients vs. in Contacts

4.9.2 Sensitivity profiles of *Klebsiella* species in Patients vs. in Contacts

Patients showed slightly high resistance in general (35%) when compared to resistance in Contacts (33.5%). However, Contacts isolates exhibited high resistance (21%) from Imipenem, a Carbapenem that shows potential resistance isolates while resistance towards patients isolates was 17%. There was also notably higher resistance towards Ciprofloxacin in patients than in contacts (38% vs. 24%) while high resistance was observed towards ATM in contacts than in patients (45% vs. 35%).

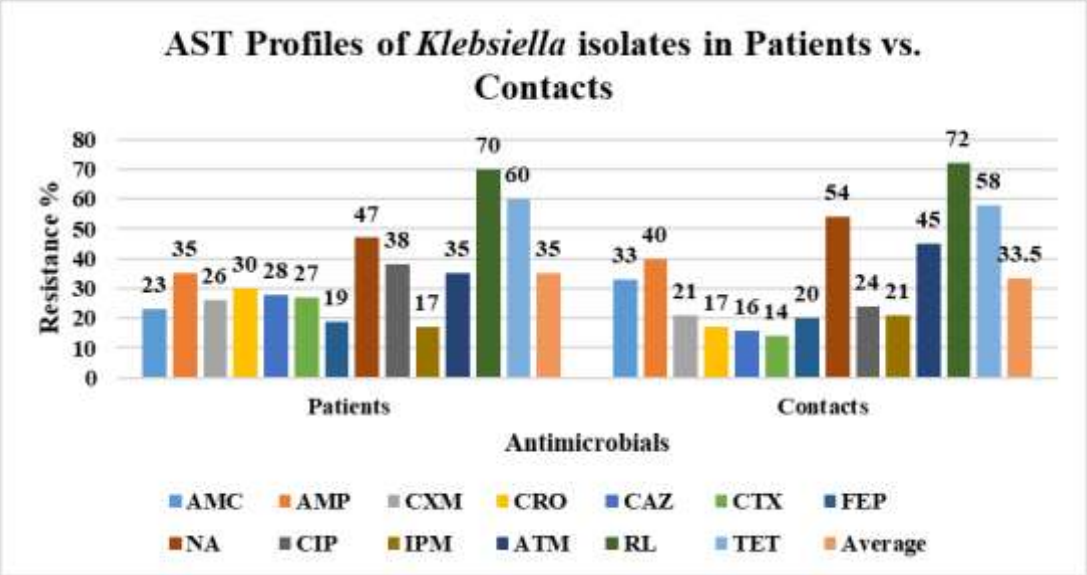


Figure 4.11: Molecular Identification of Resistance genes

4.10 Antimicrobial Resistance Genes Determination

Four *H. pylori* isolates conferred resistance towards Amoxicillin and Metronidazole. Resistance in Metronidazole antimicrobials was predominant in *H. pylori* with three isolates confirming resistance when tested using both rapid kit culture and confirmed by biochemical analysis and PCR. However, resistance towards *E, coli* and *Klebsiella* was alarming with 53% presenting double ghost zone, a characteristic phenotypic appearance for Extended Spectrum Beta-lactamases (ESBL) while 63% were resistant to Imipenem which is a phenotypically for isolates with resistance genes to Carbapenems. Eighteen samples (18) were subjected to ESBL gene testing by PCR. From these samples, 4 samples, (22%), and 8 samples, (44%) showed presence of *blaTEM* and *blaCTX-M* respectively. *BlaOXA* was reported in more than a half of the potentially positive *klebsiella* isolates. No sample reported *blaSHV* genes and KPC genes. Two samples (11%) had both *TEM* and *CTX-M* genes. From the 18 isolates, 10 (56%) were *E. coli* while 8 (44%) isolates were *Klebsiella* species isolates. Eight *E. coli* isolates (80%) had atleast one resistance gene or both. Twenty five 25% of *Klebsiella* species isolates had resistance genes. *CTX-M* genes were predominant resistance genes in *E. coli* isolates.

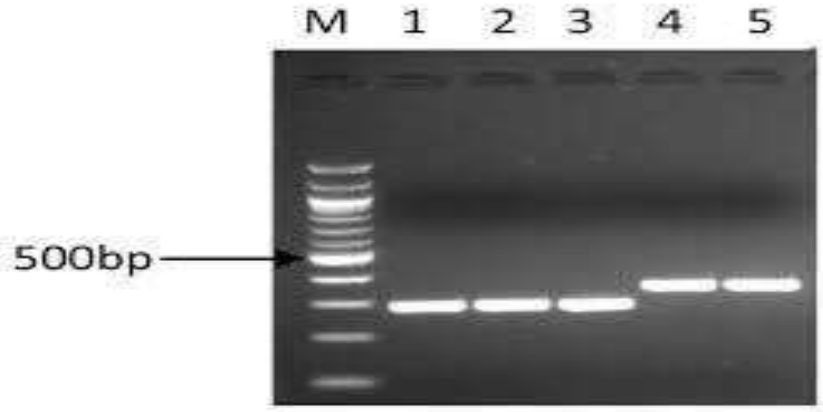
Table 4.8: Resistance genes identified by PCR

Sample	Isolates	AST Profiles	Resistance genes	Study site
MSH029	*E. coli, Klebsiella, H. pylori*	AMP, AMC, CTX, CRO, CAZ, CXM, IMP, MET	*blaTEM, blaCTX-M, blaOXA, PBP-1*	Dagoretti
MSH040	*E. coli*	AMP, AMC, CRO, CAZ, FEP	*blaTEM, blaCTX-M,*	Dagoretti
MDH059	*E. coli*	AMP, AMC, CXM, CTX, CAZ, CRO	*blaTEM, blaCTX-M,*	Kibera
MSH050	*E. coli, H.pylori, Klebsiella*	AMP, AMC, CXM, CTX, CAZ, CRO, IMP, MET, AMX	*blaTEM, blaOXA, blaCTX-M, PBP-1, RDX*	Kibera
MDH073	*E. coli*	AMP, AMC, CXM, CTX, CAZ, CRO	*blaOXA, blaTEM, blaCTX-M,*	Kibera
MDH036	*Klebsiella*	AMP, AMC, CXM, CTX, CAZ, CRO	*blaOXA, blaTEM, blaCTX-M,*	Kibera
MDH063	*E. coli, Klebsiella*	AMP, AMC, CXM, CTX, CAZ, CRO, IMP	*blaOXA, blaTEM, blaCTX-M,*	Kibera
MDH030	*Klebsiella, E. coli*	AMP, AMC, CXM, CTX, CAZ, CRO, IMP	*blaOXAblaTEM, blaCTX-M,*	Kibera
MDH043	*E. coli*	AMP, AMC, CXM, CTX, CAZ, CRO	*blaTEM, blaCTX-M,*	Kibera
MDH053	*E. coli, Klebsiella*	AMP, AMC, CTX, CRO, CAZ, CXM, IMP	*blaTEM, blaCTX-M, blaOXA*	Kibera
MDH041	*E. coli, H. pylori*	AMP, AMC, CRO, CAZ, FEP, MET	*blaTEM, blaCTX-M, PBP-1*	Kibera
MSH035	*E. coli*	AMP, AMC, CXM, CTX, CAZ, CRO	*blaTEM, blaCTX-M,*	Dagoretti
MSH056	*E. coli*	AMP, AMC, CXM, CRO, CTX, CAZ	*blaTEM, blaCTX-M,*	Dagoretti
MSH021	*E. coli, H. pylori*	AMP, AMC, CAZ, CXM, CTX, CRO, AMX	*blaOXA, blaTEM, blaCTX-M, RDX*	Dagoretti
MSH008	*Klebsiella*	AMP, AMC, FEP CXM, IMP, CAZ, CRO	*blaOXA, blaTEM, blaCTX-M,*	Dagoretti
MSH031	*Klebsiella*	AMP, AMC, CXM, CAZ, IMP	*blaOXA, blaTEM, blaCTX-M,*	Dagoretti

4.11 Images of Antimicrobial Resistance gene as seen on gel

4.11.1 *PBP*s genes (*H. pylori*)

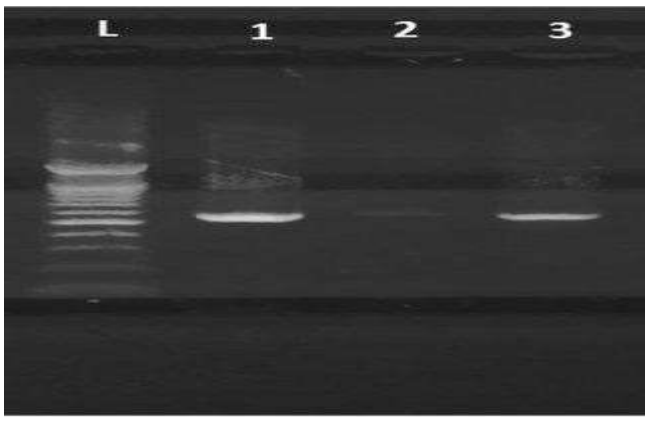
Only 3 *H. pylori* isolates exhibited resistance towards Metronidazole while 1 isolate confirmed resistance towards Amoxicillin (289bps)



(M-DNA ladder, 1-4 – Samples, 5- Positive control)

Figure 4.12.1: *H. pylori* gene PBP1 conferring resistance against Amoxicillin

4.11.2 RDX gene (*H. pylori*)



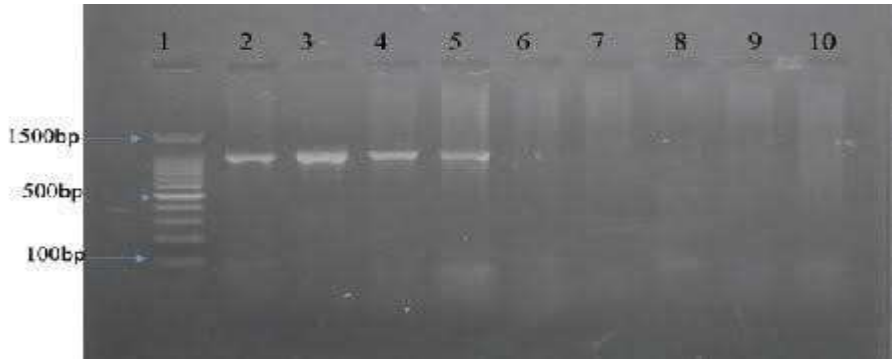
(L-DNA Ladder, 1,2- Samples, 3-Positive control)

Figure 4.12.2: *H. pylori* gene RDX conferring resistance against Metronidazole

Figure 4.12.2 represents Gels showing *E. coli* resistant genes

4.11.3 *BlaTEM* (*E. coli* and *Klebsiella*)

About 22% of the isolates were potential ESBL contained TEM genes believed to degrade third generation Cephalosporin including CTX, CAZ and CRO antimicrobial agents. (Singh et al., 2019)

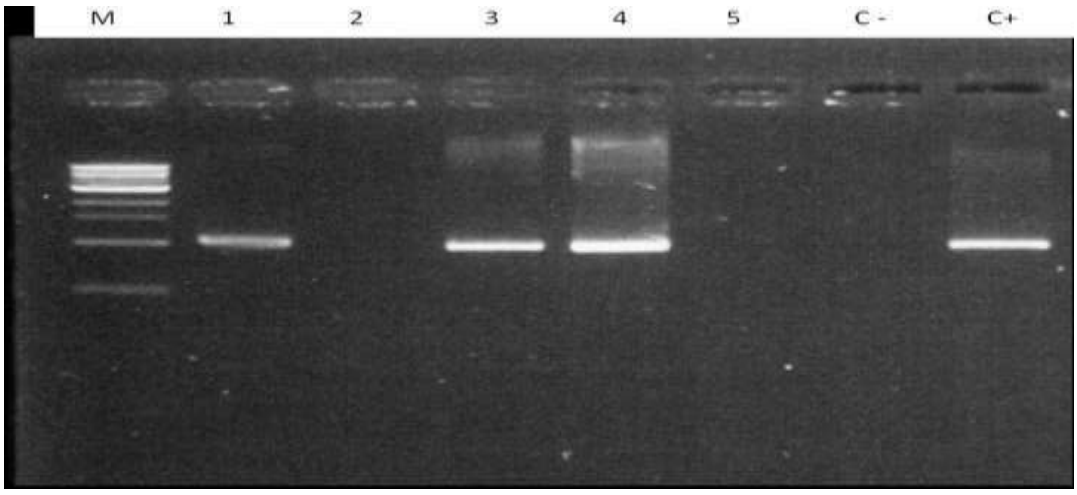


1-DNA Ladder, 1-4- Samples, 5- Positive control)

Figure 4.12.3: *E. coli* gene Bla*TEM* conferring resistance against Cephalosporin

4.11.4 *BlaCTX-M*-15 (*E. coli* and *Klebsiella)*

Forty four (44%) of the suspected ESBL isolates noted presence of blactx-m-15 which are isolates believed to exhibit plasmid mediated resistance which may affect the antimicrobial activity of penicillin and cephalosporin.



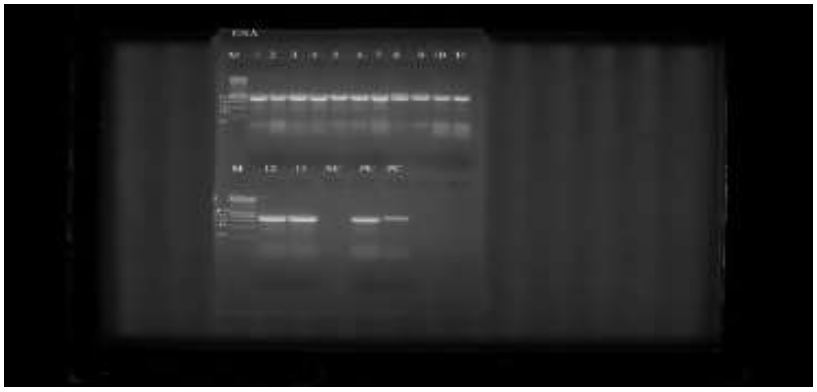
(M- DNA- Ladder, 1-5 Samples, C- Negative control, C+ Positive control)

Figure 4.12.4: *E. coli* gene Bla*CTX-M-15* conferring resistance against Cephalosporin

Fig. 4.12.4 shows Gels showing *Klebsiella* resistant genes

4.11.5 *BlaOXA* (*E. coli* and *Klebsiella*)

More than a half of the isolates that exhibited possible ESBL carriage has *blaOXA* genes which are predominant among the Carbapenem resistant bacteria hence was found in isolates that had shown resistance towards Imipenem (Fils et al., 2019)



(M- DNA Ladder, 1-13 Samples, NC –Negative control, PC-Positive control)

Figure 4.12.5: Bla*OXA* conferring resistance against Carbapenems

4.12 Risk Factors Results

4.12.1 *H. pylori* infection in relation to study site and duration one has stayed in this area

This study analyzed results from two sites, Kibera and Dagoretti. A person in Kibera was 80% more likely to get *H. pylori* than one in Dagoretti. The association between study site and a positive *H. pylori* test outcome was statistically significant (OR =1.8018, CI 1.126285 - 2.882476, P=0.014). Likewise, a positive test outcome was 21% more likely for those who had stayed in study site for more than a year and 30% less likely for participants who had stayed in an area for 7-12 months. (**Table 4.9**)

Table 4.9: *H pylori* carriage vs study area and duration of stay.

Demographic feature		*H pylori* positive	*H pylori* negative	odds ratio	P value	Confidence interval
Study site	Dagoretti	39 (12%)	100 (31%)	1.8018	0.014	1.2628 - 2.8824
	Kibera	78 (24%)	111 (43%)			
Duration of residence in Dagoretti	0 - 6 months	1 (1%)	1 (1%)	0.4299	0.147	0.1374 - 1.3455
	7 - 12 months	3 (2%)	20 (14%)			
	> 1 year	35 (25%)	79 (57%)			
Duration of residence in Kibera	0 - 6 months	1 (0%)	3 (2%)	0.6957	0.354	0.3229 - 1.4987
	7 - 12 months	11 (5%)	20 (11%)			
	> 1 year	66 (35%)	88 (47%)			

4.12.2 Socio-Demographic factors (Gender, Age, Education and Occupation)

A positive test outcome was 29% more likely to occur among male participants in comparison to female participants. Age was categorized into four groups, a positive *H. pylori* test was 2.7 and 1.6 times more likely to occur among participants in the age category of 7-12 years and 13-18years respectively. In education, a positive *H. pylori* test was 21% more likely among those who had attained or in the process of attaining primary school education and17% less likely among those who had attained or in the process of attaining tertiary education. However, none of the associations in the education categories was statistically significant (P>0.05) (**Table 4.10**)

Table 4.10: *H* pylori vs demographic factors

Demographic feature		*H pylori* positive	*H pylori* negative	odds ratio	P value	Confidence interval
Gender	Male	48 (39%)	74 (61%)	1.2878	0.286	0.8095 - 2.0491
	Female	69 (34%)	137 (66%)			
Age (Years)	0 - 6	13 (33%)	27 (67%)	0.8846	0.246	0.7192 - 1.0881
	7 - 12	17 (57%)	13 (43%)			
	13 - 18	12 (43%)	16 (57%)			
	> 18	75 (33%)	155 (67%)			
Occupation	Formal	20 (36%)	36 (64%)	0.9586	0.491	0.8500 - 1.0810
	Informal	43 (33%)	88 (67%)			
	Not work	54 (38%)	87 (62%)			
Education level	Minor	22 (38%)	36 (62%)	0.9038	0.335	0.7359 - 1.1106
	Primary	26 (43%)	36 (57%)			
	Secondary	31(32%)	65 (68%)			
	Tertiary	38 (34%)	75 (66%)			

4.12.3 *H. pylori* carriage in relation to livestock, source of water and a kitchen garden

Individuals that kept livestock had 15% more likely to test positive for *H. pylori* in comparison to those who did not keep livestock. For water sources, none of the categories had significant statistical association with *H. pylori* (P>0.05). A positive *Helicobacter pylori* test was 28% more likely among participants who owned kitchen gardens as compared to those who do not. (OR=1.28, CI= 0.8060-2.0326, P= 0.295). While adjusting for livestock keeping and source of water, participants who owned a garden were 32% more likely to test positive for *H. pylori* in comparison to participants who did not own a garden. There was however no statistical association between keeping animals, water source and having a garden with *H. pylori* test outcome (P>0.05).

Table 4.11: *H. pylori* vs Keeping livestock, water source and kitchen garden

Potential risk		*H pylori* positive	*H pylori* negative	odds ratio	P value	Confidence interval
Keep livestock	No	52 (34%)	101 (66%)	1.148	0.55	0.729-1.807
	Yes	65 (37%)	110 (63%)			
Source of water	Bottled mineral water	2 (2%)	1 (0%)	0.846	0.58	0.465 - 1.537
	Tap water	97 (36%)	175 (64%)			
	Bore hole	18 (34%)	35 (66%)			
Duration of *H. pylori* colonisation signs	< 1 months	32 (10%)	70 (21%)	0.640	0.14	0.354 - 1.158
	1 - 6 months	18 (6%)	36 (11%)			
	> 6 months	28 (9%)	38 (12%)			
Substance use	Non-smokers	75 (31%)	169 (52%)	1.457	0.03	1.044-2.034
	Smokers/ex-sm okers	42 (50%)	19 (6%)			
Garden	Yes	27 (63%)		1.28	0.29	0.806-2.033
	No	51(46%)				

4.12.4 *H. pylori* carriage in relation to personal lifestyle, clinical history

Substance use was categorized into smokers, ex-smokers, hard drug users and non-smokers. There was a statistically significant association between smoking/drug use habits and *H. pylori* infection (P=0.027). Additionally, smokers were 5.6 times more likely to test positive for *H. pylori* in comparison to non-smokers (OR= 5.586, CI= 2.8461-10.9636, P< 0.001). Clinical history was categorized as healthy, burning sensation and cramps, heartburns, general body aches and on medication. The healthy group was the reference category. With all clinical history factors inclusive, a positive *H. pylori* test was 0.01% less likely to occur among participants reporting various clinical symptoms in comparison to those who had none. There was no statistically significant association between a participant's clinical history and *H. pylori* test outcome (OR=0.999, CI=0.8702-1.1468, P=0.989). A positive *H. pylori* test was 72% more likely to occur among participants on medication in comparison to the healthy group although this was not statistically significant, P=0.270. The likelihood of a positive outcome for other categories in comparison to the healthy group were as follows: burning sensation and cramps (23% more likely), heartburns

(43% more likely), and general body aches (12% more likely). The corresponding p-values of these categories however implied no statistical significance (P>0.05

Table 4. 12: ***H. pylori*** **carriage in relation to clinical history**

Demographic factors		n	*H. pylori positive*	H. pylori negative	Odds ratio	P value	Confidence interval
Clinical History	Burning sensation and cramps	*12(8%)*	5(42%)	7 (58%)	1.5765	0.057	0.9868-2.5186
	Burning sensation and	*22(8%)*	10(46%)	12(55%)			
	General body aches	*33(10%)*	13(39%)	20(61%)			
	Healthy	*106(32%)*	39(37%)				
	On medication for long time	*20(6%)*	10(50%)	10(50%)			
	Stomach related complications	*35(41%)*	40(30%)	95(70%)			
Duration of H.pylori colonization signs	< 1 month	*103(31%)*	32(31%)	70(60%)	1.07187		
	1-6 months	*54(17%)*	18(33%)	35(67%)			
	>6 months	*66(20%)*	28(42%)	39(58%)		0.502	0.8751-1.3129

4.12.5 *H. pylori* versus other Isolates and duration symptoms had lasted.

A *H. pylori* positive outcome was 2.7 times more likely to occur with *E. coli* or *Klebsiella* being detected. The association between *H. pylori* and enteric isolates was statistically significant (OR=2.7, CI=1.96-3.66, P<0.001). Duration symptoms were categorized as <1 month, 1-6 months, >6 months. A positive *H. pylori* test was 27% more likely to occur among those with symptoms >6 months as compared to <1 month. There was however no statistical association between duration symptoms and *H. pylori* test outcome, P=0.5020.

Table 4.13: *H. pylori* carriage in relation to presence of other isolates

Logistic Regression					Number of obs = 328
					LR chi ^{2} (1) = 46.29
					Prob > chi^2 = 0.0000
Log likelihood = -190.54856					Pseudo R^2 = 0.1083
H pylori kit test	***Odds Ratio***	***Std. Err***	***Z***	***P>|Z|***	***[95% Conf. Interval]***
Isolates	2.682064	0.4270954	6.20	0.000	1.963011 3.664507
_cons	0.1507541	0.0396245	-7.20	0.000	0.0595528 0.2523482

Table 4.14: Findings with observed statistical difference P<0.05 or with higher odds of income OR<1

Variable	**Odds Ratio**	**Z**	**P**	**[95% Conf. Interval]**
Gender	1.29	1.07	0.29	0.81 - 2.05
Age	0.88	-1.16	0.25	0.72 - 1.09
Study site	**1.8**	**2.46**	**0.01**	**1.13 - 2.88**
Time stayed in site	1.49	1.43	0.15	0.86 - 2.58
Education	0.9	-0.96	0.34	0.74 - 1.11
Occupation	0.95	-0.83	0.41	0.84 - 1.07
Source of water	0.85	-0.55	0.58	0.47 - 1.54
Keeping livestock	1.15	0.6	0.55	0.73 - 1.81
Garden ownership	1.28	1.05	0.3	0.81 - 2.03
Clinical history	1	-0.01	0.99	0.87 - 1.15
Duration of symptoms	1.07	0.67	0.5	0.88 - 1.31
Presence of other Isolates	2.68	6.2	0	1.96 - 3.67
Smoking/drug use	1.46	2.21	0.03	1.04 - 2.03

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 Discussion

This was a comparative study in Nairobi which determined the prevalence of *H. pylori,* an enteric species associated with gastroenteritis among slum dwellers and low class estate occupants. High prevalence (52.8%) was noted in Kibera informal settlement when compared to prevalence noted in Dagoretti. This was attributed to a number of factors including socio-economic factors like, population density among other factors. Attributing high prevalence of infection Kibera to its characteristic dense population and compromised water and sanitation program (WASH) concurs with findings of Kotilea et al., (2020), and Alanis, (2005), whose studies also attributed presence of infections to overcrowding. This however disagrees with McNicholas et al., (2000), they noted that overcrowding doesn't have any association with infection. Kibera slums has got a generally unsafe environment with presence of open sewer lines, open defecation areas and low socio-economic factors including illiteracy. Brown et al., (2020, Campioli et al., (2021), and Ozbey et al., (2017) also found that most infections are as a result of lack of knowledge and awareness of associated risk factors and ignorance, factors which this study noted with 62% of Kibera dwellers not having idea about bacterial infections and dangers these bacteria pose to the general public.

Some urease-producing bacteria such as *Klebsiella* species according to Pérez-Pérez & Hanson, (2002), and *E. coli* (Orth et al., 2006) are thought to play a role in *H. pylori* symptoms through bacteria-host-bacteria interactions. The journal (Foster et al., 2015) also noted *E. coli* and *Klebsiella species'* association with gastroenteritis placing *E. coli* as the main cause of gastroenteritis in infants. However, colonization and co-infections are also affected by other multiple factors such as environmental and host factors. Our finding (19.8) also noted aspects of co-infections between pathogenic *E. coli* and *H. pylori* bacteria hence the need for prescribed medication to reduce chances of administering a single antibiotic in scenarios with multi-infection.

There was high prevalence of *H. pylori* which is pathogenic among contacts when compared to patients who were symptomatic. Based on this finding, gastritis should not be conclusive in determining infection. This finding also calls for the need to roll-out mass screening to unravel infection rate and reduce possibility of spread of bacterial species to unsuspecting individuals. In other findings, (Nyerere et al., 2015) noted 45.2% of *H. pylori* infection among symptomatic contacts, this study reported 35.4% infection in the same group. This reveals 40% possibility of having *H. pylori* infection if you have related gastrointestinal symptoms. Infections attributed to *H. pylori* believed to be characterized with either delayed or lack of symptoms and signs, to this cause, public awareness, clinician education and strategized policy making should be ideal in fighting this impending pandemic.

H. pylori isolate demonstrated resistance towards both Amoxicillin and Metronidazole which are the most prescribed drugs in treatment and management of people presenting in gastritis in Mbagathi and Mutui-ini hospitals. *H. pylori* being pathogenic bacteria, this outcome should be treated with utmost consideration given the fact that if it does spread to the public, it may pose serious and adverse health issues or even be a death threat. *E. coli* and *Klebsiella* isolates demonstrated great resistance towards folic pathways antagonists like Trimethoprim/sulfamethoxazole (RL) (70, 65), and Tetracycline (64, 55), which were considered because of its extensive administration and use in most hospital pharmacies and chemists despite having been found to have high resistance in *E. coli* use strains in uncomplicated cystitis according to (Nyerere et al., 2015), but is believed to be effective in UTIs. Trimethoprim also is a better option in treatment of acute infections as a third line drug in place of fluoroquinolones and nitrofurantoin. An overall resistance of 33.58% (436.6/13 is a threat to the communities given the fact that most of the isolates came from asymptomatic individuals. This indicates fast spread of these genes across populations without signals. Cephalosporin retained high resistance rates throughout the tests without significant changes. Among the third generation agents, CTX demonstrated greater resistance as compared to CAZ and CRO. Fourth generation cephalosporin cefepime is rapidly gaining resistance while second generation is showing some decreased resistance percentages opposed to the trend witnessed in a pooled study summarized by Mengya wang *et al.,* in 2020.

Carbapenemes also demonstrated significant percentages of non-efficacy towards both *E. coli* and *Klebsiella* species isolates. *Enterobacteriaceae* resistance has been found to have adverse clinical and economic outcomes, this was attributed to antibiotic use and the length of hospital stay. Less resistance was noted in FEP and Imipenem (IMP), however, being a Carbapenem, IMP was considered a stronger antibiotic as compared to Cephalosporin. The people who had kept livestock were at high risk of contracting bacterial infections as compared to those who don't rare/keep domesticated animals. Likewise, *Klebsiella* species recorded high resistance to antibiotic agents if compared to *E. coli* isolates.

This study noted smoking as one of the risk factors (OR = 1.46, P = 0.03). Smoking is believed to cause a ruinous effect on the gastric mucosa lining, elevating the risk of *H. pylori* colonization. These findings agree with an Ethiopian study which reported a significant association between infection and smoking (P<0.05 (Seid & Demsiss, 2018). This however, contradicts Omulo et al., (2010) whose study found that infection depends entirely on the presence of causal agents and ineffective WASH programs and not lifestyle. Children of school going age (7-18) were more affected than <6 years and >19 years. This might be a case of cross infection between them while at school where they take more hours. These however shouldn't be conclusive findings unless noted by subsequent comparative studies.

5.2 Conclusion

Based on the study finding, gastritis is not a determining factor in *H. pylori* carriage hence should not be conclusive for treatment of infection attributed to *H. pylori* or other strains believed to affect the stomach unless proper diagnosis is done. Most of those with *H. pylori* bacteria were asymptomatic hence treatment should only be determined by laboratory diagnosis. The study also found that presence of *E. coli* and *Klebsiella* may be an indicator of possible presence of multidrug resistance strains in a given area. Most of the asymptomatic people may be carriers of even serious antimicrobial resistance strains hence screening should be often done to reduce the chances of spread of these bacterial strains across communities.

Uncontrolled prescription and over-counter purchases of antibiotic agents has become a hindrance to fight against AMR hence an aspect that requires all stakeholders to come up with a consensus and policies put in place to streamline the whole process. First, community over counter outlets for drugs and medicines should be put on track and understand the need of prescribed treatment only. This will control the buyers to obtain only prescribed drugs from this chemist and pharmacies. The use of these drugs for empirical therapy in uncomplicated infections should be advised only if the antimicrobial agent of involved has resistance prevalence of <10% to <20% but where the resistance exits such rates, an alternative agent need to be considered, Otherwise, the situations may present a risk for treatment and should be closely monitored and treated.

According to the study findings and existing literature, I concluded that compromise socio-economic factors, lack of proper water and sanitation programs and lack of training and awareness programs are the key drivers for most bacterial infections especially species associated with contamination. If these factors are readily considered, this will restore good health in more than 80% slum dwellers, reducing mortality rate by 30%. Smoking and life threatening practices should be prohibited and educational activities put in place for public awareness. Policy makers come up with more guiding strategic plans to prevent, control and manage the spread of *H. pylori* throughout the world.

5.3 Recommendation

Increased coverage of surveillance of health care associated infections and mass screening to generate more data on infections.

Total adherence laboratory diagnosis and prescribed treatment to help fight the impending antimicrobial resistance pandemic.

More studies and research to unravel risk related factors associated with various bacterial infections.

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APPENDICES

Appendix I: Study Questionnaire

Demographic Information

Interviewer's name…….

Respondent's name……..

Study site……..Study number……

Respondent's gender.

- Male
- Female

What is the age of the respondent?

- 0-6 years
- 7-12 years
- 13-18 years
- Adult

Personal socio-economic information

What is the level of education of the respondent?

- Completed primary school education
- Completed secondary school education
- Completed tertiary college
- University graduate

What is your occupation/ what do you do for a living? (For adult only)

- Self-employed

- Health sector
- Farmer
- Education sector
- Business
- Unemployed

Do you smoke cigarettes or use hard drugs?

- Yes, I smoke cigarette
- I use hard drugs (Name it)
- I am a non-smoker nor have I used any hard drugs
- I am an ex-smoker

Criteria for Enrollment; Where do you stay in Nairobi? (Ask outpatient at the hospital facility)

- Kibera
- Dagoretti

How long have you stayed at your present resident?

- 0-6 months
- 6-12 months
- More than 12 months

Is the subject a patient or a contact?

- Patient
- Contact

General Household information

Do you keep any domesticated livestock or birds in your house?

- Yes
- No

What is your source of drinking water?

- I use tap water
- I buy water from venders (Not sure where they get it from)
- I draw water from bore hole
- I use stored rain water
- I use free flowing water
- All of the above

Clinical History

Have you ever experienced a case of stomach ailment/aches?

- Yes I have had stomach ache for the last 3 weeks
- Yes I have had stomach ache for the last 1-3 months
- Yes I have had stomach ache for the last 3-6 months
- Yes I have had stomach ache for more than 6 months
- No I have never had stomach aches

If yes, what was the experience?

- Severe
- Moderate
- Mild

Did you consider seeking medical attention from a hospital when you had stomach aches?

- Yes
- No.

If yes, which medication were you given? Name it.

If no, what made you not visit? I took traditional herbs

- I bought drugs from chemist/pharmacy

- I didn’t have money for medication
- Hospital is too far from my place
- Hospital medicine did not help me the last time I took them

History on hospital visits

- Hospital admissions due to *H. pylor*i related infections
- Previous stay with a person who was a primary case
- Diagnosed with stomach ulcers
- Stomach cancer patients

What clinical signs did you have that made you visit a hospital

- Gastrointestinal Bleeding
- Abdominal Pain and discomfort
- Discomfort and fullness
- Nausea
- Loss of appetite
- Heartburn
- Peptic ulcers
- Postprandial fullness,
- Inability to finish a normal sized meal,
- Burning sensation in the abdomen and bloating

Additional Comments

……
……
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……
……
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Study Title: Screening for carriage and antimicrobial resistance profiles of *Helicobacter pylori* strains and selected Gram-negative co-isolates using a community-based one-health approach in Nairobi, Kenya.

Principal Investigator (PI): Sophia Kuve.

Address: P.O BOX 52428-00200, Nairobi.

Telephone: +254 726982140/+254 732242512

E. mail: sophikuve@yahoo.com

Institution: JKUAT in collaboration with KEMRI Graduate School (ITROMID).

Appendix II: Consent seeking and information form for adult aged 18+

Title of the Research Study: Screening for carriage and antimicrobial resistance of *Helicobacter pylori* strains and selected Gram-negative co-isolates using a community-based one-health approach in Nairobi, Kenya.

Who is conducting this study?

Principal investigator: Sophia Atingo Kuve, MSc. student, JKUAT/KEMRI Graduate School.

Collaborators:

……
……

Dr. John M. Njeru, Research Scientist, KEMRI.

Dr. Kimang'a A. Nyerere, CoD Medical Microbiology, JKUAT.

Study location: The study will be carried out in a slum settlement, Kibera and in a middle class estate, Dagoretti.

What are we investigating?

We aim to obtain information regarding germs that cause stomach discomfort which persist into untreated cases to become stomach ulcers and other stomach malignancies. These germs are referred to as *Helicobacter pylori*. The study will look at the presence of *H. pylori* and known community drug resistant indicator strains which are *Escherichia coli* and *Klebsiella.* These germs are believed to be transmitted across humans, livestock and the environment. *H. pylori, E. coli and Klebsiella* affects all ages and gender, it is attributed to a number of social-economic factors like lifestyle, occupation among others

Many people with *H. pylori* may not know in early stages until it persists into chronic stage when the symptoms and signs are chronic. This makes them remain

untreated for a long period, hence a threat to public health since they may pass the germs to other households' members, animals and even the environment. Some of the risk factorsattributed to infection include poor personal hygiene, Illiteracy, congetion, unhealthy lifestyle, livestock, water and soil contamination, Previous results on related studies shows that *H. pylori*, *E.coli* and *Klebsiella* germs are becoming untreatable with existing antibiotic agents hence these germs have become fatal. Our study will help provide information on carriage and AMR to policy makers that can reduce deaths and complications related to *H. pylori*, *E. coli* and *Klebsiella* strains.

What are we intending to do?

1. Detection of *H. pylori* germs is normally difficult, even in hospitals. One of our main aims is to improve detection criteria and help hospitals treat infections effectively based on symptoms and signs.
2. We want to determine the prevalence of carriage of *H. pylori, E. coli* and *K. pneumonia* among the general public in Nairobi County.
3. We will determine the AMR patterns among *H. pylori*, *E. coli* and *K. pneumoniae* strains isolated and the reason why some people fail to seek treatment even when they suspect that they have *H. pylori*.
4. To determine the underlying community-based risk factors that may promote infections within a community and also determine transmission pathways.
5. We want to investigate the microbial relatedness between *H. pylori, E. coli* and *K. pneumonia* which will help in understanding transmission pathways.

What is your role in this study?

Based on your doctor's analysis, we believe that it is important to screen for *H. pylori* germs in your system and also get a stool sample for further analysis in our laboratory. We will also ask you some questions that will allow us to understand and estimate your risk for *H. pylori* germs. Such questions will include your treatment history, use of treatment drugs, age, lifestyle etc. It could take us up to 15

minutes to complete this question and answer session. We will then request you to allow us to visit your residence to also reach-out to other members of your household and collect stool sample from them and fecal matter or rectal samples from your animals.

We will also ask your family more questions regarding their sickness history, use of medicines, lifestyle and eating habits, handling of animals, access to water and toilets, level of education and age. This homestead questionnaire will help us understand and assess the risk for your family and suggest methods to minimize them. Once you give us the stool sample, we will analyze for the presence of these germs and should we find that you are not infected, we will inform you. If we find you infected, we will contact you and your healthcare provider (the facility doctor or clinician) to advise on the next action.

What procedures shall we use to obtain samples and data from you?

1. You will be instructed on how to collect your own stool and that of your child, especially those below the age of 6. School-going age children will be instructed on how to obtain their own samples
2. A trained officer will collect fecal samples from your animals when we visit your home
3. The questions we will ask you will be recorded on a phone and you will have authority to see what data we record. This data will not be sent to anyone who is not part of this study or to any of your family members.
4. After taking your samples and recruiting you, we will give you information that will help you explain to your family the reasons that will make us visit your family
5. We will contact you and get consent or permission from you before visiting your family.

What additional procedures will there be for patients who help with research?

In order to obtain stool specimens, we shall give you plastic containers and a clean cotton swab. We will give you instructions on how to obtain and pack your samples and that of your children below age 6.

What shall we do with the samples we obtain from you and from your family?

1. We will try to isolate *H. pylori*, *E. coli* and *K. pneumonia* germs from your samples and assess if you have stomach infections. Should this be found to be true, you will be contacted and referred to your doctor who will advise or treat you.
2. We will preserve a small portion of your stool samples and that from your animals for future analysis. These samples will be preserved for research and analysis only not for any other profit.

What are the risks associated with sample collection and participation in this study?

The procedures we intend to use will not cause pain to you. You will in fact collect your own samples. However,

1. Some questions we will ask about your hygiene and illness may not be comfortable or even embarrassing therefore the interviewer will ensure he/she conducts the interview in an environment that will ensure privacy.
2. Information that will be collected will be treated as confidential not to be accessed by an unauthorized person.
3. You may find it embarrassing to collect and submit stool specimen both yours or your child's but our study officers will give you sufficient instructions to help you do this comfortably

Your rights are as below;

1. There are no penalties for declining participation in this study
2. You and your children can withdrawal from this study at any time
3. You may decline to answer any of our questions
4. Besides your consent to allow your child to participate, we shall obtain the child's permission (assent) to voluntarily participate if he/she is aged between 13 and 18 years of age.

What permission are we requesting from you?

1. Let us interview you and record your answers which will help us ascertain if you and your family could be at risk of *H. pylori.*
2. Allow us to obtain stool samples from you and your family members and your animals once we visit.
3. Let us ship your samples to our laboratory for testing. We intend to keep the germs or the DNA obtained from your sample indefinitely so that we can conduct further studies related to the current or in the future.
4. To call you or contact you and then arrange to visit your home in order to sample your relatives and animals.
5. Allow us to use your specimen in the present study and in any other related study in future that may generate data that may be beneficial in improving strategies for effective prevention, control measures, treatment and management of similar infections.

What is your potential benefit for participation?

1. There are no monetary gains for participating in this study
2. Since these germs may go unnoticed, the data that we will generate will help us identify people who may be infected but they don't know.
3. If we find infection in your sample and that of your households, we shall help you identify the best way to avoid being infected. If we find germs such as

those that cause other diseases, especially stomach discomfort in your stool, we will assist your doctor with information that will help him treat you.

Who is eligible to access this information?

Your information will only be accessed and read by authorized personnel, labeled using special private/barcode numbers so that the participant cannot be recognized by people not directly involved in the study. Coded information will be held on computers protected by passwords known to the research team only.

In-case you have any questions or want to know more about the research in future you can contact the below address and or researchers.

The Committee Chairperson, KEMRI Scientific and Ethics Review Unit, P. O. Box 54840-00200, Nairobi; Telephone numbers: 020-2722541, 0717719477.

Email address: seru@kemri.org. Or

Any of the following people at KEMRI, Centre for Microbiology Research (CMR) and

JKUAT

PO Box 19464-00202, Nairobi.

1. Sophia A. Kuve 0732242512
2. Dr. John M. Njeru 0721994526
3. Dr. Andrew K. Nyerere 0722689073

Appendix III: Consent signing form for adults

I hereby confirm that I have read and understood the information provided for this study and that

I have had the opportunity to ask questions. I understand that participation in this study is voluntary and that I am free to withdraw from this study at any time without giving reasons and without my medical care or legal rights being affected. I therefore consent

to;

- Take part in participation in the study and provide the necessary specimenfor analysis.
- Allow my children and those under my care to participate in the study after individual assent forms and for the researchers to collect fecal samples from them.
- Allow other members of my households to take part in the study and provide fecal samples after individual consenting.
- Allow the team to collect rectal samples from my domesticated animals.
- To transport my sample to the lab and keep it indefinitely for future studies.

Name of consenting adult _____ Sign _____ Date _____

(Please print name)

Right/Left hand Thumb Print impression for those who cannot sign

I certify that the above has been verbally explained to me, and that I understand the nature and purpose of the study and therefore consent to participate in the study. I

have given the research team an opportunity to ask questions which have been answered satisfactorily.

Witness' signature: _____ Date_____

Witness' name: ___ _____ (Please print name)

Right/Left hand Thumb Print impression for those who cannot sign

Researcher's Signature _____ Date_____

Researcher's Name: _____ (Please print name)

Appendix IV: Assent signing form for children aged between 13-18 years

I confirm that I have understood the information provided for this study and have had the opportunity to ask questions. I understand that I can withdraw my participation in this study and we will not be punished for refusal. I will also not be denied treatment for not participating I also understand that I have a right to refuse to participate even if my parent or guardian wants me to be a part of this study. I therefore agree to;

- Participate in the study by offering my stool samples for analysis
- Allowing the research team to visit my resident after my parents/guardian consent.
- Allowed the research team to store my sample and germs obtained from them for
- future research

Name of assenting child _____ Sign _____ Date _____

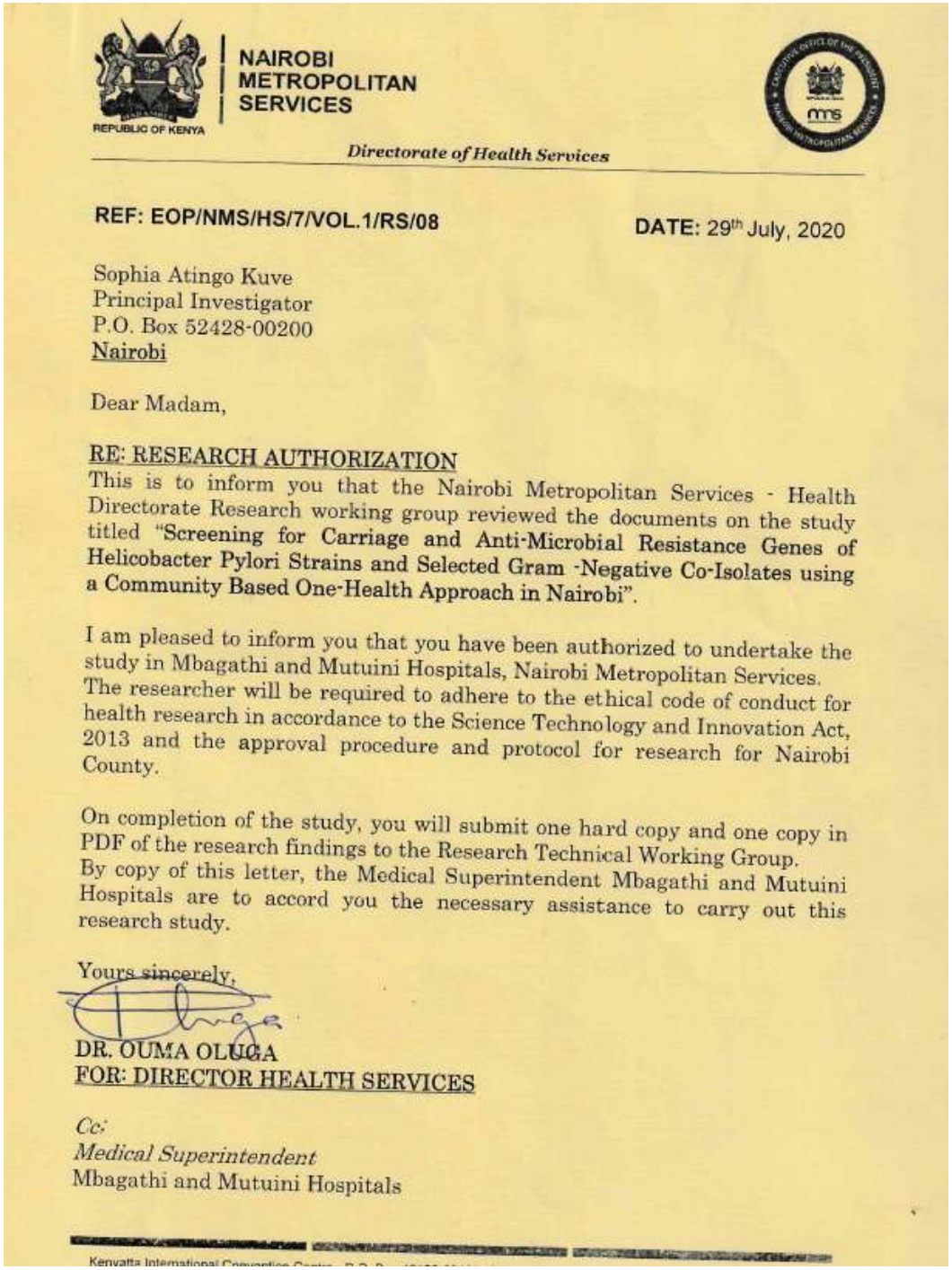
(Please print name)

Right/Left hand Thumb Print impression for those who cannot sign

Researcher's Signature _____ Date_____

Researcher's Name: _____ (Please print name)

Appendix V: Nairobi Metropolitan Services Research Authorization (NMS)



NAIROBI METROPOLITAN SERVICES

REPUBLIC OF KENYA

Directorate of Health Services

REF: EOP/NMS/HS/7/VOL.1/RS/08 **DATE:** 29th July, 2020

Sophia Atingo Kuve
Principal Investigator
P.O. Box 52428-00200
Nairobi

Dear Madam,

RE: RESEARCH AUTHORIZATION

This is to inform you that the Nairobi Metropolitan Services - Health Directorate Research working group reviewed the documents on the study titled "**Screening for Carriage and Anti-Microbial Resistance Genes of Helicobacter Pylori Strains and Selected Gram -Negative Co-Isolates using a Community Based One-Health Approach in Nairobi**".

I am pleased to inform you that you have been authorized to undertake the study in Mbagathi and Mutuini Hospitals, Nairobi Metropolitan Services. The researcher will be required to adhere to the ethical code of conduct for health research in accordance to the Science Technology and Innovation Act, 2013 and the approval procedure and protocol for research for Nairobi County.

On completion of the study, you will submit one hard copy and one copy in PDF of the research findings to the Research Technical Working Group. By copy of this letter, the Medical Superintendent Mbagathi and Mutuini Hospitals are to accord you the necessary assistance to carry out this research study.

Yours sincerely,

DR. OUMA OLUGA
FOR: DIRECTOR HEALTH SERVICES

Cc:
Medical Superintendent
Mbagathi and Mutuini Hospitals

Kenyatta International Convention Centre

Appendix VI: Center for Microbiology Research Scientific Committee Approval Letter (CMR/CSC)

KENYA MEDICAL RESEARCH INSTITUTE

Centre for Microbiology Research, P.O. Box 19464 - 00202, NAIROBI - Kenya,
Tel: (254) (020) 2720794, 2720038, Nairobi. Website: www.kemri.org

Tuesday, 28th January 2020,

Sophia Atingo Kuve, The Principal Investigator

RE: KEMRI/CMR/P00134/12/2019: Screening for Carriage and Antimicrobial Resistance of *Helicobacter Pylori* Strains and Selected Gram-Negative Co-Isolates using a Community-Based One-Health Approach in Nairobi, Kenya.

This is to inform you that during the full meeting of the **Centre for Microbiology Research, Scientific Committee** (CMR, CSC) held on Thursday, December 13th 2019, the above referenced application was discussed. After careful consideration, the committee found that more information was necessary on the following issues before a final decision on the study can be reached:

1. Format your references properly, i.e. use similar referencing style
2. Use an appropriate referencing tool
3. Attach your questionnaire and informed consent as part of your proposal. They should appear as appendices after your references
4. Chapter three is much disorganized. I would expect description of all lab processes to be before ethical consideration, possible risks, data management etc.
5. The proposal generally has a very poor formatting especially when it comes to numbering and spacing, could be very tiring to read through
6. The subtopic Antimicrobial resistance lacks content and should be redone
7. The abstract should not contain paragraphs
8. Microbiological processes stated on the abstract to be done should be mentioned briefly i.e culture and identification, biochemical tests, serological tests not just a bulk phrase 'microbiological procedures will be done.
9. Proper grammar should be used i.e. distinction between do and does, don't and doesn't
10. Revise the title to reflect the two study arms; clinical and community based arms
11. Please specify how the study selected these health facilities. Was it purposive?
12. Please explain how the study will mitigate the risk of breaching participant's privacy
13. Revise inclusion/exclusion criteria to include households without livestock and kitchen garden
14. Give units to be used in estimating the total weight of each sample collected
15. Since the study uses cross-section design, studying risk factor would be difficult. Please consider determining "associated factors" instead.
16. Please provide Assent and consent, and questionnaire for review
17. If 96 respondents are chosen, how many stool, animal, soil etc samples are there all together?
18. Please provide Budget justification in the protocol.
19. Please revise the timeline to reflect the current situation

Appendix VI: National Commission for Science, Technology and Innovation Research License (NACOSTI)

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: 568570

Date of Issue: 18/November/2020

RESEARCH LICENSE

This is to Certify that Ms., Sophia Atieno Kuve of Jomo Kenyatta University of Agriculture and Technology, has been licensed to conduct research in Nairobi on the topic: Screening for Carriage and Antimicrobial Resistance genes of Helicobacter Pylori Strains and Selected Gram-Negative Co-Isolates using a Community-Based One-Health Approach in Nairobi, Kenya. for the period ending : 18/November/2021.

License No: NACOSTI/P/20/7e03

568570

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

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Appendix VII: Mbagathi Hospital Research Authorization

THE PRESIDENCY
EXECUTIVE OFFICE OF THE PRESIDENT
NAIROBI METROPOLITAN SERVICE

Telephone: (020) 311 808, 2724712, 2725791

E-mail: mbagathihosp@gmail.com

Mbagathi Hospital
P.O Box 20723-00202
NAIROBI

26th August 2020

Sophia A. Kuve

RE: RESESEARCH AUTHORIZATION

This is in reference to your application for authority to carry out a research on *"Screening for Carriage & Anti-Microbial Resistance Genes of Helicobacter Pylori Strains & Selected Gram Negative Co-Isolates using a Community Based One-Health Approach in Nairobi."*

I am pleased to inform you that your request to undertake research in the hospital has been granted.

On completion of the research you are expected to submit one hard copy and one soft copy of the research report/ thesis to this office.

Dr. David Kimutai
Chairman – Research & Training Committee
Mbagathi Hospital.

28 AUG 2020

Appendix VIII: Mutuini Hospital Research Authorization

NAIROBI METROPOLITAN SERVICES

REPUBLIC OF KENYA

MUTUINI HOSPITAL

REF: DSDH/RES/002/020 DATE: 22ND OCTOBER 2020

SOPHIA A. KUVE,
PRINCIPAL INVESTIGATOR.

RE: PERMISSION TO CARRY OUT RESEARCH AT MUTUINI HOSPITAL

TITLE: SCREENING FOR CARRIAGE AND ANTIMICROBIAL RESISTANCE GENES OF HELICOBACTER PYLORI STRAINS AND SELECTED GRAM-NEGATIVE CO-ISOLATES USING A COMMUNITY-BASED ONE-HEALTH APPROACH IN NAIROBI, KENYA

Your request for permission to carry out the above named study has been approved. Permission granted is subject to compliance with the following requirements:-

- Only approved documents will be used
- All changes are submitted for approval by a Research Review Board before implementation and permission for this sought from the Hospital Administration
- Death and life threatening problems and severe adverse events are reported to the hospital within 24 hours
- Submit an executive summary report within 30 days upon completion of the study
- Provide reports on the study progress every 3 months
- Indemnify the hospital against any claim that may arise from the research

On completion submit a soft copy of the study findings to the hospital records department c/o Office of the Medical Superintendent

MEDICAL SUPERINTENDENT

DR JOSEPHINE WAMBUI NGURI

MEDICAL SUPERINTENDENT

Kenyatta International Convention Centre P.O. Box 49130-00100, GPO, Nairobi, Kenya
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Appendix IX: Publication (East African Medical Journal)

East African Medical Journal Vol. 99 No. 3 March 2022

PREVALENCE AND RISK FACTORS FOR THE TRANSMISSION OF *HELICOBACTER PYLORI, ESCHERICHIA COLI* AND *KLEBSIELLA* SPECIES AMONG PATIENTS PRESENTING WITH GASTRITIS IN NAIROBI, KENYA

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Box 62000-00200 Nairobi, *Kenya*, John Njeru Mwaniki, KEMRI Graduate School, Kenya Medical Research Institute. P.O Box 54840-00200, *Nairobi*, Kenya, Centre for Microbiology Research, Kenya Medical Reearch Institute. P.O. Box 19464-00202, Nairobi, Kenya.

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PREVALENCE AND RISK FACTORS FOR THE TRANSMISSION OF

***HELICOBACTER PYLORI, ESCHERICHIA COLI* AND *KLEBSIELLA* SPECIES**

AMONG PATIENTS PRESENTING WITH GASTRITIS IN NAIROBI, KENYA

S. A. Kuve, A. K. Nyerere and J. N. Mwaniki

ABSTRACT

Objective: This study aimed at determining prevalence of *H. pylori, E. coli* and *Klebsiella* species, and identifying risk factors associated with *H. pylori*. *Setting*: The study was cross sectional done in Kibera and Dagoretti areas in Nairobi, Kenya.

Subjects: Outpatient visiting Mbagathi and Mutuini hospitals presenting with gastritis were identified by clinicians and follow-up visits done to recruit immediate contacts.

Interventions: Stool samples were collected and subjected to *H. pylori* stool antigen and cultured on MacConkey for *E. coli* and *Klebsiella* isolation. Only stool that turned positive by kit were cultured on Columbia for *H. pylori* isolation and Biochemical test done.

Outcome measures: Potential risk factors were collected using a pre-tested questionnaire. Prevalence data was summarized as means, counts and percentages. Assessment was by measuring associations using logistic regression at 95% CI and observing significant differences, $P<0.05$.

Results: H. pylori, E. coli and *Klebsiella* were found in 34%, 49% and 18% participants respectively. A high prevalence (82%) of bacterial infection was found among persons residing in Kibera, and among close contacts (76%). Co-infections for targeted bacterial organisms was found in 25% of subjects. Place of residence, presence of other microbial agents and smoking were identified risk factors associated with *H. pylori* infection, ($P\leq0.05$, $OR.\geq1$). No significant associations were found

between *H. pylori* infection and other socio-demographic factors, presence of livestock, and source of water (P>0.05).

Conclusion: Bacterial infections is on the rise hence more data should be shared for policy purposes and creation of awareness about this silent killer infection.

INTRODUCTION

H. pylori is a gram-negative, helical-shaped, and micro-aerophilic bacterium mostly associated with gastritis (1). The bacterium has become a threat to global health due to delayed clinical symptoms and signs and unreliable diagnostic capacity. *H. pylori* is more endemic in developing countries estimated in about a half of the population (2). Previous studies have reported a prevalence of 69.4% in Africa with Kenyan prevalence ranging between 54.8% and 99% (3). The human gut hosts a large number of gram-negative commensals beside *H. pylori* especially members of the *Enterobacteriaceae* such as *E. coli* and *Klebsiella* species. The two species are believed to contribute in causing gastroenteritis infection (4) and (5) respectively. These two are also known community indicators for the presence of multidrug-resistance strains (6).

Locally, there is limited data on the prevalence of *H. pylori* and co-infection with *E. coli and Klebsiella* species despite high burden of infections being attributed to it. This is mainly because the majority of available findings are hospital-based and therefore focused mainly on diagnosis and treatment as opposed to coinfections, potential risk factors and possible sources of infection. This has led to the overuse of anti-ulcer medications and acid-suppressive therapies, which tend to reduce acidic levels, making the stomach more susceptible to colonization by other bacterial species, including members of enteric bacteria. Despite of most bacteria being susceptible to high acidic environment in the stomach released after perforation by *H. pylori*, a few species including *H. pylori* do survive in such conditions (7).

The spread of *H. pylori*, *E. coli*, and *Klebsiella* species has been associated with a number of factors (8), these factors included lack of access to clean water, contaminated environment, over-crowding, and presence of domesticated animals (9). Findings from this study will be able to influence some change in policy making and create public awareness.

MATERIALS AND METHODS

Study site and Participants: The study was carried out in low-income Kibera informal settlement and middle income Dagoretti area in Nairobi County. The study targeted outpatients of all ages visiting Mbagathi District Hospital and Mutuini sub-district hospital presenting with gastritis. These were referred to as cases while those who stayed with cases recruited during follow-up visits were referred to as close contacts. Inclusion criteria for cases were clinical history of acid reflux, abdominal pain, heartburn, vomiting, bloating, and lack of appetite. Identification of these patients was done by facility clinicians who referred them to the study recruitment desk. Consents and questionnaires were obtained.

Collection of socio-demographic data: A standard questionnaire was administered to obtain geographical data including area of residence and duration one has spent at the present area, socio-demographic data such as age, gender, education, and occupation, lifestyle including smoking, presence of livestock and garden and also one's clinical history.

Sample collection and transport: A stool sample was collected from participants who consented, in sterile stool containers. The sample was immediately transported to Mbagathi hospital microbiology laboratory for bacteriological analysis. Sample from close contacts was obtained during follow-up visits and transported to the laboratory within two hours of collection.

Testing and isolation for *H. pylori:* All stool samples collected were initially screened for *H. pylori* antigen using a stool antigen test kit (One Step *H. pylori* Feces Antigen test strips; Guangzhou Wondfo Biotech, Japan). Only stool samples that turned positive by the test kit was later cultured on Columbia blood agar as described by Kimang'a *et al.,* 2010. Briefly, stool was emulsified in normal saline in a sterile container, a loopful was transferred to Columbia agar base (OXOID CM0331 UK) supplemented with 7% laked horse blood. This was done through a filter membrane that only allowed minute microbes of less than 0.1 mm including *H. pylori* bacteria to

pass through. Inoculated media was incubated at 37 degrees for 5-7 days in a micro-aerophilic environment. Characteristic tiny whitish bacterial colonies were presumptively considered as *H. pylori*. The potential *H. pylori* isolates were then confirmed using urea test.

Isolation and identification of E. coli and Klebsiella species: Each stool sample was cultured on MacConkey agar and incubated aerobically at 37 degrees for 18-24 hours. *E. coli* and *Klebsiella* species are both lactose fermenters hence the colonies appeared pink in color. However, presumptive *E. coli* colonies were reddish pink colonies or doughnut shaped pink while mucoid dark pink colonies were presumed to be *Klebsiella* species.

These colonies were identified and subcultured on TSA media for purification. Positive control used were *E. coli* (ATCC 25922) and *Klebsiella* species (ATCC 700603).

Questionnaire and laboratory data were entered into Epicollect5 data-gathering platform and exported to excel app 2013. Data analysis and presentation was by STATA13 and SPSS. The population variables were summarized as means, and categorical as counts and percentages. Logistic regression outcome was labelled as binary outcome (Positive/Negative). Interpretations were done based on odds ratios OR$\geq$1 at 95% CI and associations between various categorical variables were measured using a chi-square test at P$\leq$0.05.

RESULTS

Part 1: Carriage and prevalence data

Prevalence of H. pylori, E. coli and Klebsiella species

The study recruited 328 people of whom 234 (71%) tested positive for either *H. pylori*, *E. coli* or *Klebsiella* species. During microbiological analysis 346 isolates were recovered of which *E. coli* isolates were predominant 49% (166). *H. pylori* came second with 34% (117) while Klebsiella species isolates were 18% (63). Eighty-one (25%) of the participants were coinfected with both *E. coli* and *H. pylori* while 31 (9%) had both *Klebsiella* species and *H. pylori*.

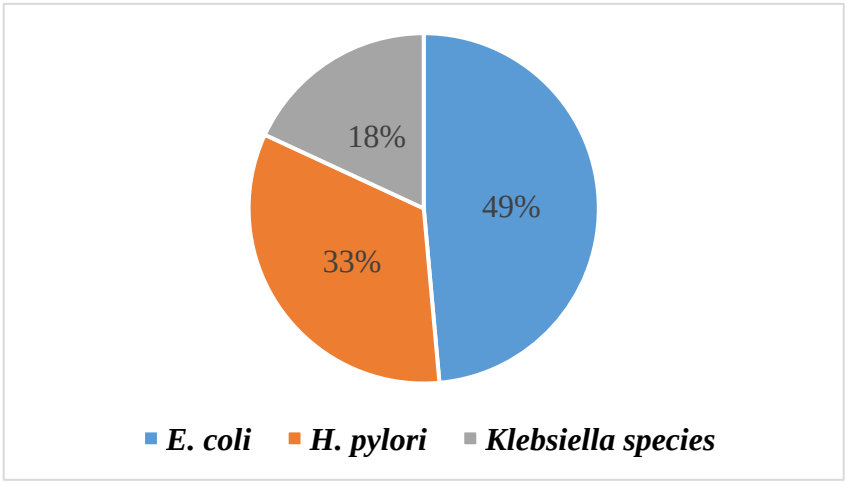
Eleven people (3%) had all the three species.

those without infection.

Proportions of infection in cases and close contacts
The study reported a high prevalence among close contacts 76% as compared to prevalence among cases 65%. From the 346 isolates recovered during analysis, 65%(225) isolates were recovered from follow-up samples. *H. pylori* 62% and *E. coli* 63% were predominant among the close contacts while *Klebsiella* was dominant among the cases (75%).

In figure 2 below, 'cases' represent outpatients while 'Close contacts' represents follow-up population. Likewise, exposed group represents those with infections while unexposed represents

Fig. 1.1: A Pie chart showing prevalence of H. pylori, E. coli and Klebsiella species



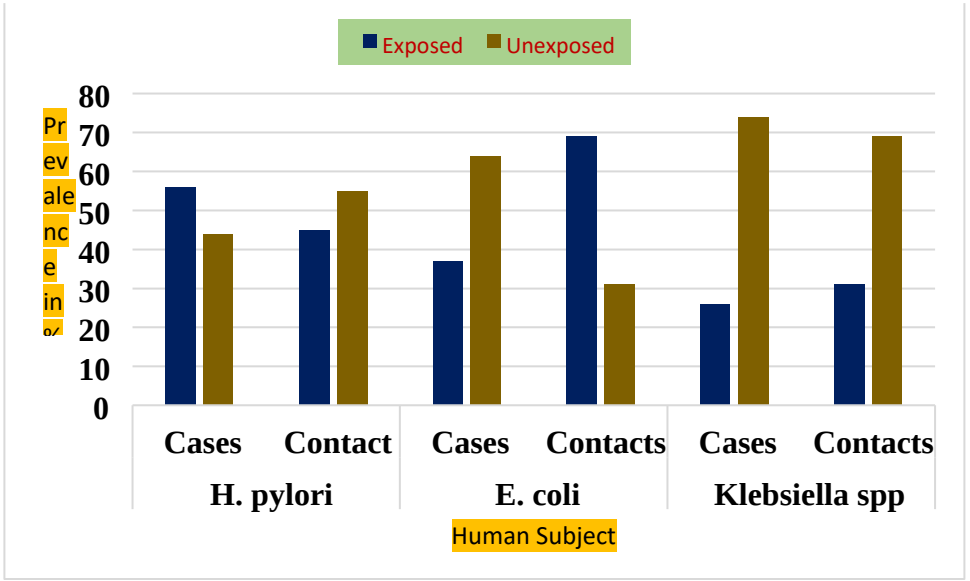


Fig. 1.2: A graph showing proportions of infection in cases and close contacts

Table 1

Prevalence of bacterial isolates in Kibera and Dagoretti area

	Pos.	**%**	**Neg.**	**%**	**Total**
Kibera	154	82	35	18	189
Dagoretti	79	54	60	46	140

Among the participants from Kibera settlement, 82% tested positive for either of the three isolates while from Dagoretti, 54% tested positive. Out of the total isolates obtained, 67% were from Kibera. Predominant species was *E. coli* with 55% followed by *H. pylori* 42% and *Klebsiella* 27%. The study noted high prevalence of infection associated with *E. coli* 62%, *H. pylori* 67%, and *Klebsiella* 79% in Kibera if compared to prevalence recorded in

Dagoretti.

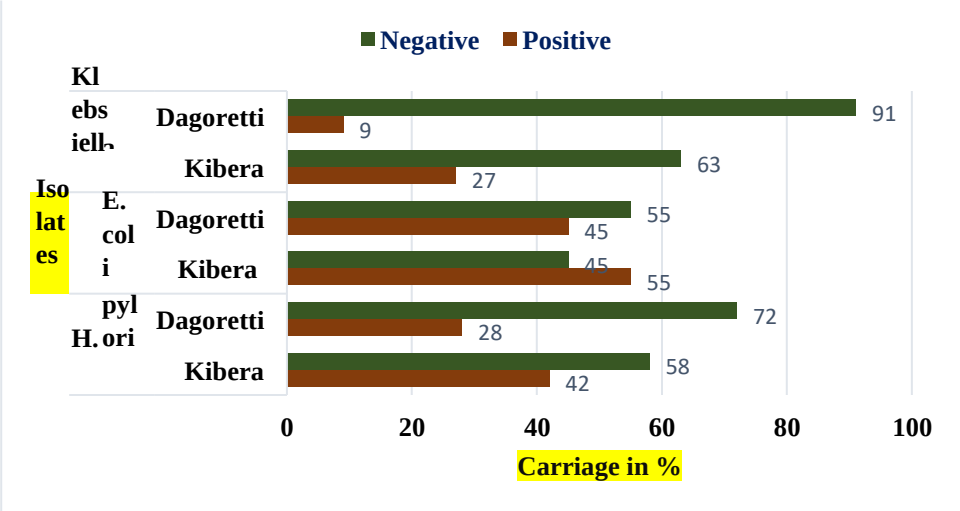


Figure 3: A graph showing proportions of bacterial isolates

Part 2: Potential risk factors associated with factors and *H. pylori* carriage. The test results

***H. pylori* infection.** were considered statistically significant at

The table below shows logistic regression and P<0.05 while P>0.05 was deemed as a nonodds ratio outcomes between presumed risk significant association.

Table 2

Logistic regression and odds ratio of risk factors associated with H. pylori infection

Variable	Odds Ratio	Z	P	[95% Conf. Interval]
Gender	1.29	1.07	0.29	0.81 2.05
Age	0.88	-1.16	0.25	0.72 1.09
7-12 years	2.72	2.00	0.05	1.02 7.23
13-18 years	1.56	0.87	0.39	0.57 4.23
Adults	1.00	0.01	0.99	0.49 2.06
Study site	1.80	2.46	0.01	1.13 2.88
Time stayed in site	1.49	1.43	0.15	0.86 2.58
7-12 months	0.70	-0.39	0.70	0.12 4.25
More than a year	1.21	0.22	0.83	0.23 6.72
Education	0.90	-0.96	0.34	0.74 1.11
Primary	1.22	0.52	0.60	0.58 2.53
Secondary	0.78	-0.71	0.48	0.39 1.54
Tertiary	0.83	-0.51	0.61	0.41 1.69
University	0.82	-0.44	0.66	0.33 2.01
Occupation	0.95	-0.83	0.41	0.84 1.07
Unemployed	0.43	-2.17	0.03	0.20 0.92
Self-employed	0.53	-1.86	0.06	0.28 1.03
Business	0.44	-2.17	0.03	0.21 0.92
Farming	3.19	1.63	0.10	0.79 12.80
Education sector	1.06	0.15	0.88	0.48 2.34
Health sector	0.27	-2.24	0.03	0.08 0.85
Source of water	0.85	-0.55	0.58	0.47 1.54
Tap water	0.28	-1.04	0.30	0.03 3.10
Other water sources	0.26	-1.08	0.28	0.02 3.03
Keeping livestock	1.15	0.60	0.55	0.73 1.81
Garden ownership	1.28	1.05	0.30	0.81 2.03
Clinical history	1.00	-0.01	0.99	0.87 1.15

Burning sensations	**1.23**	**0.33**	**0.74**	**0.37 4.13**
Heartburns	**1.43**	**0.76**	**0.45**	**0.57 3.62**
Stomach complications	**0.72**	**-1.17**	**0.24**	**0.42 1.24**
General body aches	**1.12**	**0.27**	**0.79**	**0.50 2.49**
On medication	**1.72**	**1.10**	**0.27**	**0.66 4.49**
Duration of symptoms	**1.07**	**0.67**	**0.50**	**0.88 1.31**
<1 month	**0.79**	**-0.82**	**0.41**	**0.44 1.40**
1-6 months	**0.86**	**-0.43**	**0.67**	**0.43 1.71**
>6 months	**1.27**	**0.74**	**0.46**	**0.68 2.37**
Isolates	**2.68**	**6.20**	**0.00**	**1.96 3.67**
Klebsiella	**10.28**	**5.18**	**0.00**	**4.26 24.84**
E. coli	**11.20**	**6.04**	**0.00**	**5.12 24.52**
Smoking/drug use	**1.46**	**2.21**	**0.03**	**1.04 2.03**
Smokers	**5.59**	**5.00**	**0.00**	**2.85 10.96**
Ex-smokers	**1.18**	**0.35**	**0.72**	**0.46 3.04**
Hard drug users	**1.61**	**0.54**	**0.59**	**0.28 9.13**

H. pylori infection in relation to study site and duration one has stayed in this area

This study analyzed results from two sites, Kibera and Dagoretti. A person in Kibera was 80% more likely to get *H. pylori* than one in Dagoretti. The association between study site and a positive *H. pylori* test outcome was statistically significant (OR =1.80, CI 1.13 - 2.88, P=0.02). Likewise, a positive test outcome was 21% more likely for those who had stayed in study site for more than a year and 30% less likely for participants who had stayed in an area for 7-12 months.

Socio-Demographic factors (Gender, Age, Education and Occupation)

A positive test outcome was 29% more likely to occur among male participants in comparison to female participants. Age was categorized into four groups; a positive *H. pylori* test was 2.7 and 1.6 times more likely to occur among participants in the age category of 7-12 years and 13-18years respectively. In education, a positive *H. pylori* test was 21% more likely among those who had attained or in the process of attaining primary school education and17% less likely among those who had attained or in the process of attaining tertiary education. However, none of the associations in the education categories was statistically significant (P>0.05)

H. pylori carriage in relation to Keeping livestock, source of water and having a kitchen garden

Individuals that kept livestock had 15% more likely to test positive for *H. pylori* in comparison to those who did not keep livestock. For water sources, none of the categories had significant statistical association with *H. pylori* (P>0.05). A positive *Helicobacter pylori* test was 28% more likely among participants who owned kitchen gardens as compared to those who do not. (OR=1.28, CI= 0.81-2.03, P= 0.3). While adjusting for livestock keeping and source of water, participants who owned a garden were 32% more likely to test positive for *H. pylori* in comparison to participants who did not own a garden. There was however no statistical association between keeping animals, water source and having a garden with *H. pylori* test outcome (P>0.05). Substance use was categorized into smokers, ex-smokers, hard drug users and non-smokers. There was a statistically significant association between smoking/drug use habits and *H. pylori* infection (P=0.03). Additionally, smokers were 5.6 times more likely to test positive for *H. pylori* in comparison to non-smokers (OR= 5.59, CI= 2.85-10.96, P< 0.001).

H. pylori carriage in relation to personal lifestyle, clinical history and duration symptoms had lasted.

Clinical history was categorized as healthy, burning sensation and cramps, heartburns, general body aches and on medication. The healthy group was the reference category. With all clinical history factors inclusive, a positive *H. pylori* test was 0.01% less likely to occur among participants reporting various clinical symptoms in comparison to those who had none. There was no statistically significant association between a participant's clinical history and *H. pylori* test outcome (OR=0.99, CI=0.87-1.15, P=0.99). A positive *H. pylori* test was 72% more likely to occur among participants on medication in comparison to the healthy group although this was not statistically significant, P=0.27. The likelihood of a positive outcome for other categories in comparison to the healthy group were as follows: burning sensation and cramps (23% more likely), heartburns (43% more likely), and general body aches (12% more likely). The corresponding p-values of these categories however implied no statistical significance (P>0.05).

Duration symptoms lasted were categorized as <1month, 1-6 months, >6months. A positive *H. pylori* test was 27% more likely to occur among those with symptoms >6months as compared to <1month. There was however no statistical association between duration symptoms lasted and *H. pylori* test outcome, P=0.50. *H. pylori* versus other Isolates.

A *H. pylori* positive outcome was 2.7 times more likely to occur with *E. coli* or *Klebsiella* being detected. The association between *H. pylori* and enteric isolates was statistically significant (OR=2.7, CI=1.96-3.66, P<0.001).

DISCUSSION

This was a comparative study in Kenyan urban which determined the prevalence of *H. pylori*, and enteric species associated with gastroenteritis among slum dwellers and middle-class estate occupants. High prevalence was noted in Kibera informal settlement than it was in Dagoretti. This was attributed to a number of factors including socio-economic factors like overcrowding, cultural factors especially primitive lifestyle and also environmental factors like presence of open sewer lines, compromised sanitation and hygiene. This findings concurs with other research findings (10), and (11) that associated slum setting with increased risk to infection. Although *E. coli* was predominant it is treated as a normal flora which inhabit freely. 41% of *H. pylori* prevalence is considered significant being pathogenic.

Some urease-producing bacteria such as *Klebsiella* species (12), and *E. coli* (13) are thought to play a role in *H. pylori* symptoms through bacteria-host-bacteria

(14) also noted *E. coli* and *Klebsiella's* association with gastroenteritis placing *E. coli* as the main cause of gastroenteritis in infants. However, colonization and co-infections are also affected by other multiple factors such as environmental and host factors. Our finding also noted aspect of co-infections between enteric and *H. pylori* hence need for prescribed medication to reduce chances antimicrobial resistance.

There was high prevalence of infection noted among close contacts who were asymptomatic. Based on this finding, gastritis should not be conclusive in determining infection. This finding also calls for need to roll-out mass screening to unravel infection rate and reduce possibility of spread of bacterial species to unsuspecting individuals. In 2018, (15), noted

45.2% of *H. pylori* infection among symptomatic contacts, this study reported 35.4% infection in the same group. This reveals 40% possibility of having *H. pylori* infection if you have related gastrointestinal symptoms. Infections attributed to *H. pylori* believed to be characterized with either delayed or lack of symptoms and signs, to this cause, public awareness, clinician education and strategized policy making should be ideal in fighting this impending pandemic.

Risk factors for infection according to this study included over-crowding, smoking and presence of other bacterial isolates. This findings agrees with Ethiopian study (16) which reported a significant association between infection and smoking ($P<0.05$. Smoking is believed to cause a ruinous effect on the gastric mucosa lining, elevating the risk of *H. pylori* colonization.

Children of school going age (7-18) were more infected than <6years and >19years. This might be a case of cross infection between them while at school where they take more hours. These however shouldn't be conclusive findings unless noted by subsequent comparative studies. Most studies have weakly convincing fact of determining prevalence conclusion based on stool antigen testing and culture hence encouraging researchers to vouch for need for further diagnostic techniques especially molecular analysis.

Overcrowded areas were concluded as possible home for most infections.

Asymptomatic infections call for prescribed medication to avoid drug resistance. Smoking and life threating practices should be prohibited, and educational activities put in place for public awareness. Policy makers to come up with more guiding strategic plans to prevent, control and manage the spread of *H. pylori* throughout the world. Increased coverage of surveillance of health care associated infections and mass screening to generate more data on infections. Total adherence to prescribed treatment to help fight the impending antimicrobial resistance pandemic.

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Appendix X: Scientific Ethical Review Unit (SERU)

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel: (254) 2722541, 2713349, 0722-205901,0733-400003, Fax: (254) (020) 2720030
Email: director@kemri.org, info@kemri.org, Website. www.kemri.org

KEMRI/RES/7/3/1 **March 25, 2020**

TO: **SOPHIA A. KUVE,**
PRINCIPAL INVESTIGATOR.

THROUGH: **THE DIRECTOR, CMR,** 13th May, 2020
NAIROBI.

Dear Madam,

RE: **PROTOCOL NO. KEMRI/SERU/CMR/P00134/3989 *(RESUBMISSION OF INITIAL SUBMISSION)*: SCREENING FOR CARRIAGE AND ANTIMICROBIAL RESISTANCE GENES OF *HELICOBACTER PYLORI* STRAINS AND SELECTED GRAM-NEGATIVE CO-ISOLATES USING A COMMUNITY-BASED ONE-HEALTH APPROACH IN NAIROBI, KENYA.**

Reference is made to your undated application. The KEMRI Scientific and Ethics Review Unit (SERU) acknowledges receipt of the following revised study documents on March 13, 2020;

i. Protocol
ii. SERU submission form
iii. CSC approval letter
iv. Response letter to SERU reviewers' comments
v. Response letter to CSC reviewers' comments
vi. The investigators' ethics training certificate
vii. CV of non-KEMRI investigator
viii. Translation certificate

This is to inform you that the issues raised during the 296th Committee B meeting of the KEMRI Scientific and Ethics Review Unit (SERU) held on **February 19, 2020** have been adequately addressed.
Consequently, the study is granted approval for implementation effective this day, **March 25, 2020** for a period of **one (1) year.** Please note that authorization to conduct this study will automatically expire on **March 24, 2021.** If you plan to continue with data collection or analysis beyond this date, please submit an application for continuation approval to SERU by **February 11, 2021.**
You are required to submit any proposed changes to this study to SERU for review and the changes should not be initiated until written approval from SERU is received. Please note that any unanticipated problems resulting from the implementation of this study should be brought to the attention of SERU and you should advise SERU when the study is completed or discontinued.

You may embark on the study.

Yours faithfully,

ENOCK KEBENEI,
THE ACTING HEAD,
KEMRI SCIENTIFIC AND ETHICS REVIEW UNIT.